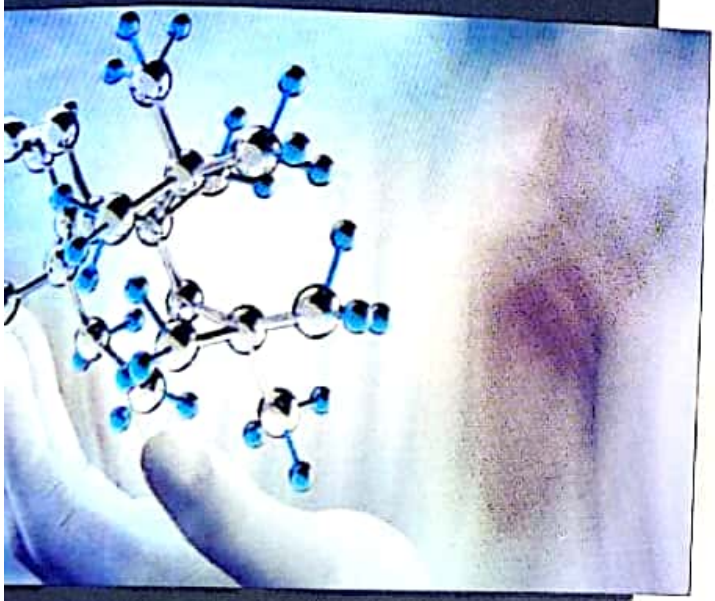
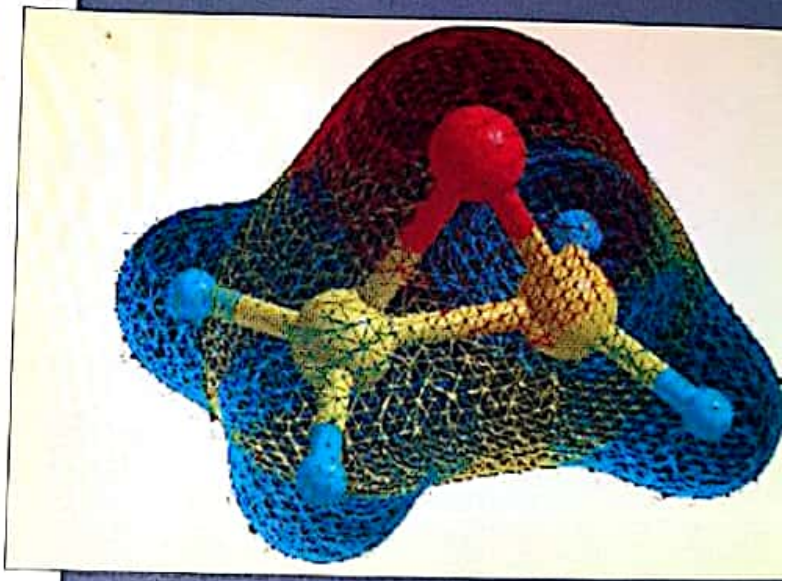
AS PER PCI REGULATIONS
THIRD YEAR B. PHARM.
SEMESTER-VI

MEDICINAL CHEMISTRY-III

Dr. K. G. BOTHARA





NIRALI PRAKASHAN
ADVANCEMENT OF KNOWLEDGE

Raksha C-#

A TEXT BOOK OF

MEDICINAL CHEMISTRY-III

As Per PCI Regulations

For
Third Year B. Pharm.
Semester - VI

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PREFACE

In the last two decades, the phases of ever-growing volume and ever-changing nature of the drug information in the field of antibiotics, antibacterials and antifungal agents are witnessed. This is mainly due to an increase in the rate of introduction of new drugs and an increase in the number and depth of published work on both, new as well as existing drugs. Above facts necessitated addition of all recent information wherever it deserves, while presenting the first edition of this book.

The book was appreciated in all corners of the profession. It has now attained the reputation as a class-room text book for undergraduate and post-graduate students of pharmacy. However, our aim remains the same as to present a review of basic principles of medicinal chemistry and to explain the effects of structural modifications of the lead nucleus on the selectivity of action, duration of action and on the intensity and frequency of adverse-effects.

Each chapter is revised thoroughly to meet the needs of future facts and fantacies. Since this book is written basically for degree students, a backbone understanding in basic disciplines is assumed.

I wish to place on record my sincere thanks to the publisher Mr. D. K. Furia for his kind cooperation. I am greatly indebted to my colleagues for their generous help and criticism. I also wish to acknowledge indebtedness to all who have assisted for the completion of the book.

Suggestions from all corners of the profession are welcome. I am responsible for any deficiencies or errors that might have remained and would be grateful if readers would call them to my attention.

Author

UNIT IV (08 Hrs.)

Antifungal agents:

Antifungal antibiotics: Amphotericin-B, Nystatin, Natamycin, Griseofulvin. Synthetic Antifungal agents: Clotrimazole, Econazole, Butoconazole, Oxiconazole Tioconozole, Miconazole*, Ketoconazole, Terconazole, Itraconazole, Fluconazole, Naftifine hydrochloride, Tolnaftate*.

Anti-protozoal Agents: Metronidazole*, Tinidazole, Ornidazole, Diloxanide, Iodoquinol, Pentamidine Isethionate, Atovaquone, Eflornithine.

Anthelmintics: Diethylcarbamazine citrate*, Thiabendazole, Mebendazole*, Albendazole, Niclosamide, Oxamniquine, Praziquantal, Ivermectin.

Sulphonamides and Sulfones

Historical development, chemistry, classification and SAR of Sulfonamides: Sulphamethizole, Sulfisoxazole, Sulphamethizine, Sulfacetamide*, Sulphapyridine, Sulfamethoxaole*, Sulphadiazine, Mefenide acetate, Sulfasalazine.

Folate reductase inhibitors: Trimethoprim*, Cotrimoxazole.

Sulfones: Dapsone*.

UNIT V (07 Hrs.)

Introduction to Drug Design

Various approaches used in drug design.

Physicochemical parameters used in quantitative structure activity relationship (QSAR) such as partition coefficient, Hammet's electronic parameter, Tafts steric parameter and Hansch analysis.

Pharmacophore modeling and docking techniques.

Combinatorial Chemistry: Concept and applications chemistry: solid phase and solution phase synthesis of combinatorial

❖❖❖

CONTENTS

UNIT I

Chapter...1

ANTIBIOTICS

♦ SYNOPSIS ♦

1.1 INTRODUCTION

The term chemotherapy can be defined as 'the treatment of diseases caused due to infective parasites or organisms without causing destruction of their animal host'. Modern chemotherapy began with the work of Paul Ehrlich (1854 - 1915). Due to his pioneer discoveries in this field, he is regarded as "Father of Chemotherapy".

The second phase of revolution emerged in the 1930's (following the discovery of the British bacteriologist Alexander Fleming when he tested the filtrate of a broth culture of a *penicilium mold* for its antibacterial activity.

The term antibiotic has its origin in the word antibiosis (i.e., against life); the latter being first time used by Vuillemin in 1889 in an attempt to describe the concept of survival of the fittest. Although the discovery of penicillin is named after Sir Fleming in 1928, it was not until 1940 at Oxford that Florey and Chain and their associates isolated it and described its properties in detail, and thus turning Fleming's discovery to practical significance.

Among the many attempts to define the term antibiotic, the most appropriate one may be stated as "An antibiotic is a chemical compound derived from or metabolically produced by microorganism and that in high dilution antagonizes the growth and/or the survival of one or more species of microorganisms". The probable points of differences amongst the antibiotics may be physical, chemical and pharmacological properties, antibacterial spectra and mechanism of action.

1.2 CLASSIFICATION

(i) Depending upon clinical effectiveness, spectrum of activity and degree of selectivity, those inhibiting only one group of microorganism are called as 'narrow spectrum antibiotics' e.g. nystatin and bacitracin. These antibiotics exhibit a high degree of selectivity. A few antibiotics inhibit both gram-positive and gram-negative bacteria and/or other intracellular organism may be termed as broad spectrum antibiotics e.g. chloramphenicol and tetracyclines.

(ii) Depending upon the sources from which antibiotics are obtained they can be classified as follows:

(a) Natural: These antibiotics are obtained from the large scale fermentation of microorganisms. e.g. bacitracin and polymixin are obtained from some bacilli while streptomycin, tetracyclines etc. from streptomyces species.

(b) Semisynthetic: The observation that 6-aminopenicillanic acid can be obtained from cultures of *P. chrysogenum* that were depleted of side chain precursors led to the development of this class. For example, during the commercial production of benzyl penicillin (Penicillin G), phenylacetic acid is added to the medium in order to achieve predominance of the product.

(c) Synthetic: This class includes antibiotics which are having purely synthetic origin. For example, Chloramphenicol, a broad spectrum antibiotic initially isolated from a fermented media in 1947 and later was produced synthetically on a commercial basis.

(iii) The third basis of classification involves the differences in mechanism of action, accordingly these agents can be divided as:

(a) Drugs that interfere with the biosynthesis of bacterial cell-wall e.g. Penicillins, Cephalosporins, Cycloserine, Bacitracin and Vancomycin.

(b) Drugs that interfere in the functioning of cytoplasmic membrane e.g. Polymixins, Amphotericin B and Nystatin.

(c) Drugs that interfere with the protein biosynthesis e.g. Erythromycin, Lincomycins, Tetracyclines, and Chloramphenicol and

(d) Drugs that interfere with the nucleic acid biosynthesis e.g. Actinomycin, Griseofulvin and Rifampin.

(iv) Antibiotics can be in general classified as:

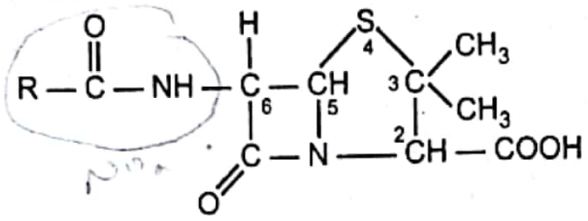
(A) β-lactam antibiotics
(B) Aminoglycoside antibiotics
(C) Tetracycline antibiotics
(D) Peptide antibiotics
(E) Macrolide antibiotics
(F) Lincomycins
(G) Unclassified antibiotics

1.3 β-LACTAM ANTIBIOTICS

(i) Penicillins:

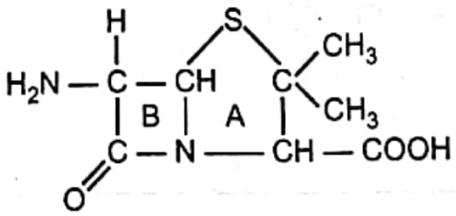
Even though penicillin had been discovered in 1928, and is a member of β-lactam antibiotics, the term β-lactam antibiotics had to wait till 1942 to get registered in the dictionary of medicinal chemists. Thanks to Prof. Howard W. Florey and Dr. Ernst B. Chain, working at that time at the William Dunn School of Pathology, Oxford with their sincere efforts, isolated and characterised the basic structure of the penicillins. This work was supplimented by the efforts of the chemists Dr. Abraham and Dr. Heatley. The clinical effectiveness of penicillin was first tested on 12 February, 1941 in the form of a sodium salt.

Thus, long after the antibiotic projected its appearance on the screen of research, the structure of penicillin was determined.



Penicillins

The penicillins can be considered as the amido derivatives of the 6-aminopenicillanic acid.



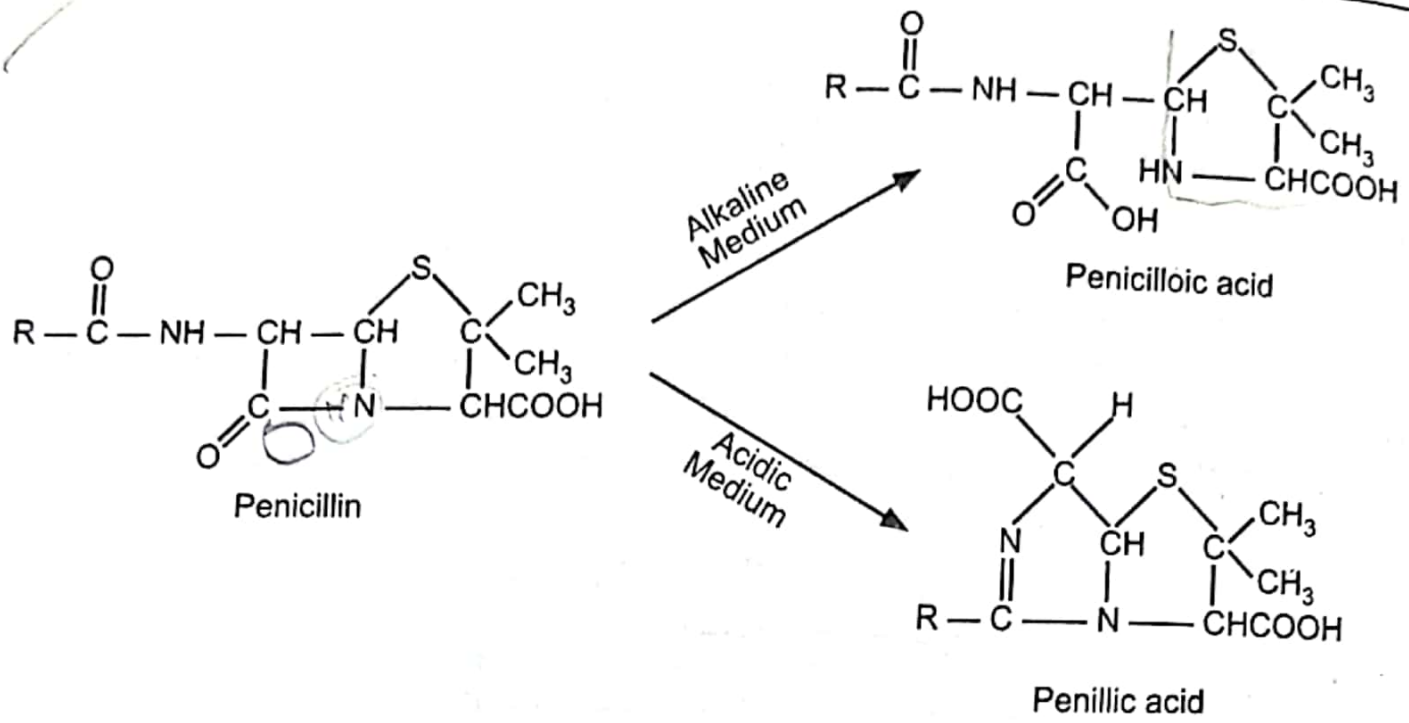
6-Aminopenicillanic acid (6-APA)

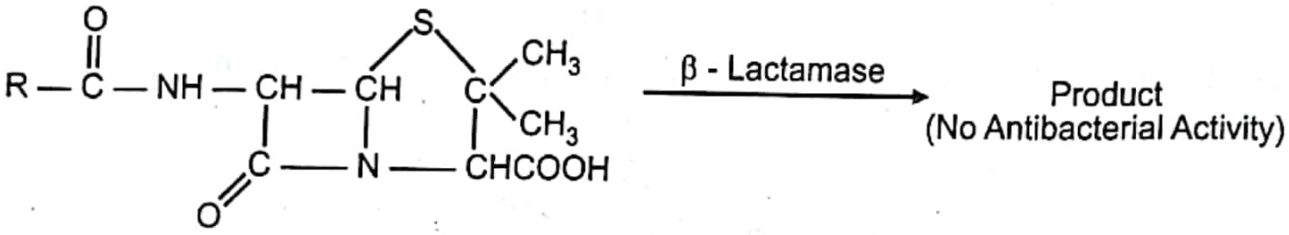
In the basic skeleton, a thiazolidine ring (A) is fused with a beta-lactam ring (B) which is a four membered cyclic amide. The penicillins differ from each other in antibacterial and pharmacological characteristics due to variation in the structure of acid moiety of the amide side-chain at C - 6. For example, penicillin G (where, R = $C_6H_5CH_2$ –) after about 45 years of clinical use, remains an extremely effective and is the only natural penicillin used clinically. Acylation of 6-APA with appropriate carboxylic acids resulted in new penicillins, some of which are broad-spectrum antibiotics.

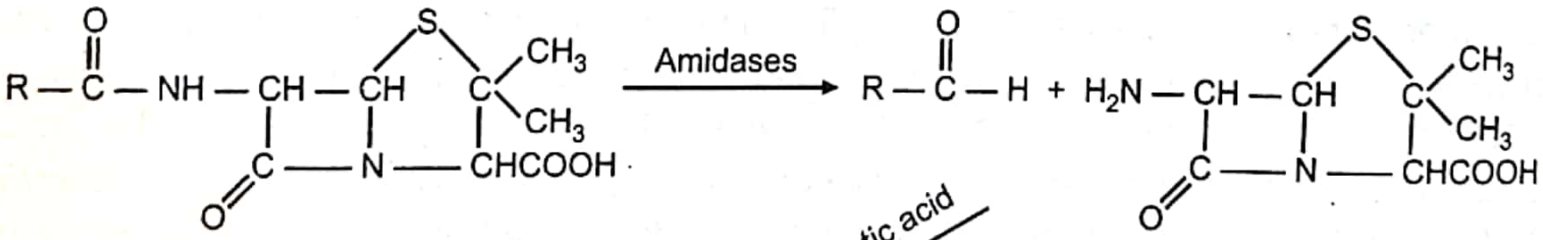
Degradation Products of Penicillins:

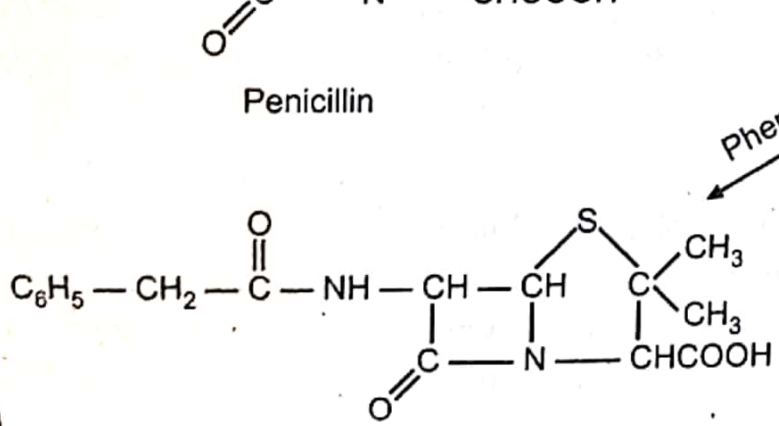
Natural penicillins are acid and base unstable. Instability in acid media logically precludes their oral administration due to the highly acidic pH in stomach. At acidic pH, a sort of molecular rearrangement results. The compound is known as penillic acid and has no activity. Similarly at basic pH, penicillin molecule gets converted to penicilloic acid which is again an inactive form.

Certain strains of microorganisms can destroy beta-lactam antibiotics enzymatically. The enzymes are more popularly known as penicillinases or β-lactamases can open the β-lactam bond. The difference in the susceptibility to the β-lactamase enzymes depends upon the nature of the amide side-chain at C - 6. It also depends upon the bacterial strain involved.


Alkaline Medium
Penicilloic acid
Penicillin
Acidic Medium
Penillic acid


β - Lactamase
Product
(No Antibacterial Activity)
Penicillin


Amidases
Penicillin
6-Aminopenicillanic acid


Phenyl acetic acid
Benzyl Penicillin

SAR of β-lactam Antibiotics:

All β-lactam antibiotics contain a four membered β-lactam ring which is fused through the nitrogen and tetrahedral carbon atom to a second heterocyclic ring. Difference in the structure of this second heterocyclic ring leads to sub-divisions of β-lactam antibiotics.

For example,

(A) Penicillins consist of β–lactam ring fused with thiazolidine.

(B) Thienamycins consist of β–lactam fused with pyrroline ring.

(C) Clavulanic acid consists of β–lactam fused with oxazolidine ring and

(D) Cephalosporines consist of β–lactam fused with a six membered dihydrothiazine ring.

A carbonyl group attached to the lactam nitrogen is a common feature of all above classes.

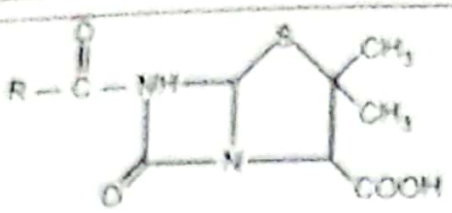
Since, penicillin after its clinical application in second world war proved to be a wonder drug in healing the wounds and preventing the infections, extensive chemical studies were undertaken either individually or through co-operation of both, industrial as well as government laboratories. Soon after, the scientists seemed disappointed due to relative unstability of natural penicillin in acidic or basic medium. For example, benzyl penicillin was found to be a relatively narrow spectrum antibiotic. It is susceptible to degradation under acidic or basic conditions; certain strains of microorganisms carry β lactamase enzymes that inactivate the drug by hydrolysis, and many patients may allergic to it. Many analogues had been synthesized in order to overcome these clinical deficiencies prevailing in natural penicillins. The main principles behind this drug design was the manipulation of polar amide side-chains. Variations in this moiety resulted in differences in antibiotic potency and in chemical-physical properties including stability.

Introducing chemical inducers in the culture medium by varying the nutritional composition of the growth medium, by including mutational change in the strain of microorganism are but few tools employed to increase both quality and quantity of antibiotics.

e.g. (1) 6-Aminopenicillanic acid is produced in large quantities with the aid of an amidase from Penicillium chrysogenum and the culture medium is fed with the chemical inducers (e.g. Phenylacetic acid) in order to achieve the predominance of the desired antibiotic.

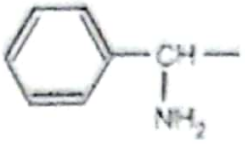
(2) The stability of benzyl penicillin can further be increased by substitution of an electron withdrawing group at α-position of benzyl penicillin. e.g. the α–aminobenzyl, α-halobenzyl and phenoxy-methylpenicillin are significantly more stable than benzylpenicillin towards acid catalysed hydrolysis.

(3) Some bacteria, for example, many species of gram negative bacilli are naturally resistant to the action of penicillins. Other normally sensitive species are capable of developing penicillin resistance. This resistance is mainly arising due to the production of β-lactamase. After the commercial production of 6-APA began, synthesis of numerous

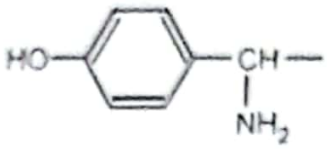


Penicillin

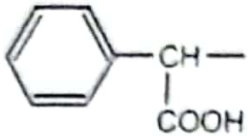
(1) Ampicillin ⟶ R =



(2) Amoxicillin ⟶ R =



(3) Carbenicillin ⟶ R =



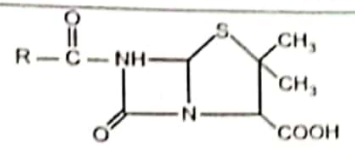
semisynthetic penicillins were undertaken by manipulating the structure of polar amide chain at C-6. The SAR studies of these analogs revealed that (a) increasing the steric hindrance at the α-carbon of the acyl group increased the resistance to Staphylococcal β-lactamase with quaternary substitution leads to maximum resistance, (b) an aromatic (phenyl or naphthyl) or heteroaromatic (e.g. 4 - isoxazoyl) systems can be incorporated utilizing α-acyl carbon as a part of this ring system. (c) the ring substitution at the ortho positions (methacillin) or at 2 position in 1-naphthyl system (nafcillin) confer increased β-lactamase resistance due to increased steric hindrance of acyl group. All these efforts oriented to increase the steric hindrance of the acyl group indicates the possibility of the side chain amide carbonyl oxygen atom participation in β–lactam ring opening to form penicillenic acid. This fact is confirmed by the presence of bulky substituents in oxacillin, cloxacillin, fluoxicillin which require substituents at both 3 and 5 positions for effectiveness against β-lactamase producing Staphylococcus aureus.

(4) The incorporation of an ionized or polar group or an acidic substituent at the α-position of the side chain benzyl carbon atom of benzyl penicillin imparts clinical activity against gram-negative bacilli. Carbenicillin is found to be effective against both β-lactamase producing and non-β-lactamase producing strains of gram-negative bacteria.

(5) All of the natural penicillins are strongly dextrorotatory.

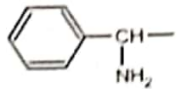
(6) The free penicillin not suitable for oral or parenteral administration. They are utilized hence in the form of their sodium and potassium salts. For the depot preparations, penicillins are treated with organic bases like, procaine, benzathine or hydrabamine. These organic salts have limited water solubility and hence can release the drug over a long period.

(7) Along with the older ring systems, some new basic skeletons have appeared through fermentation screening programs.

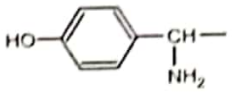


Penicillin

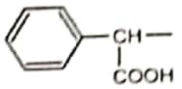
(1) Ampicillin → R =



(2) Amoxicillin → R =



(3) Carbenicillin → R =



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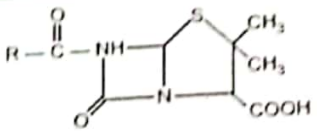
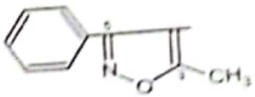
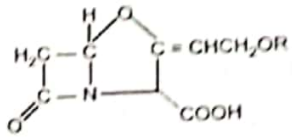


Penicillins

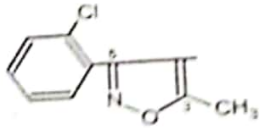
(i) Oxacillin ; R =

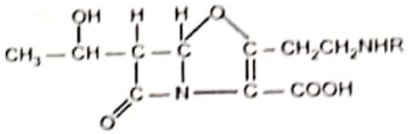




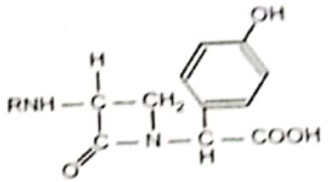
Clavulanic acids

(ii) Cloxacillin ; R =



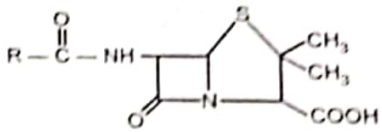


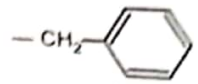
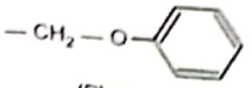
Thienamycins



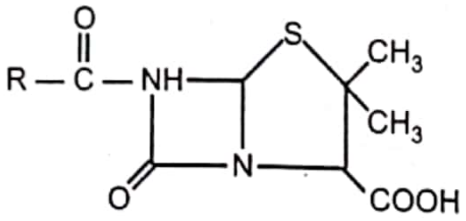
Nocardicins

Table 1.1

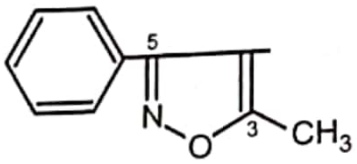


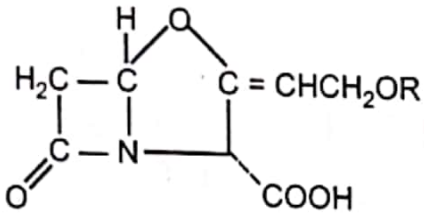
Name	Nature of Substituent, R	Resistance to Penicillinase
(A) Penicillinase-Susceptible Penicillins:		
(i) Penicillin G	 (Benzyl)	No
(ii) Penicillin V	 (Phenoxymethyl)	No

Conti

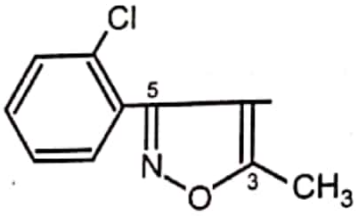


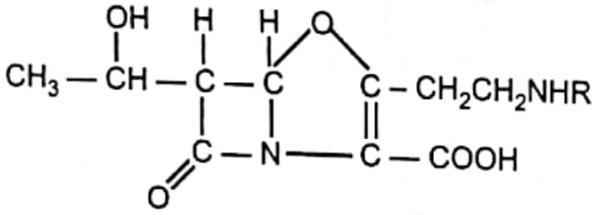
Penicillins

(i) Oxacillin ; R = 

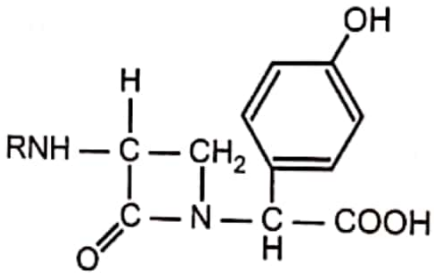


Clavulanic acids

(ii) Cloxacillin ; R = 



Thienamycins



Nocardicins

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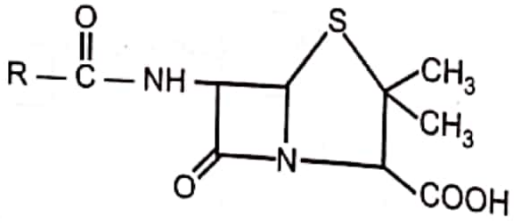

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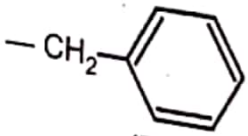
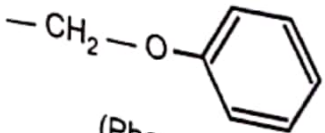
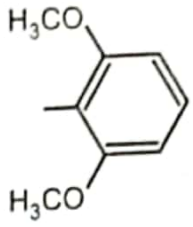
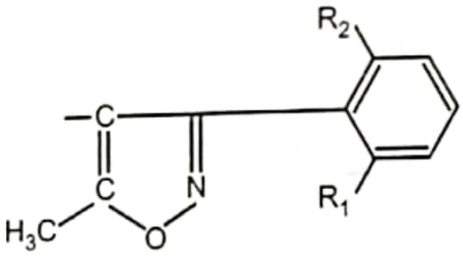
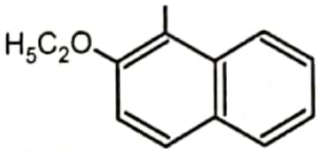
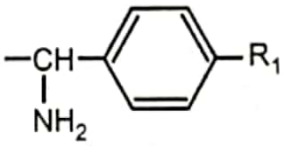
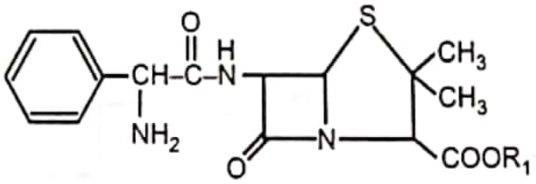
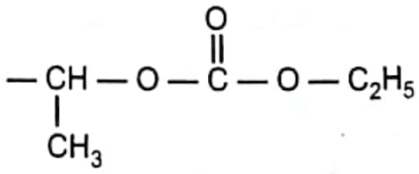
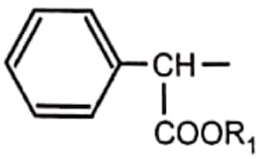
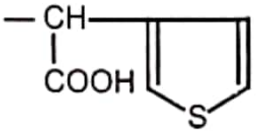
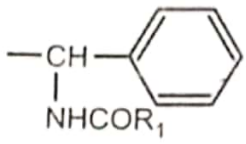
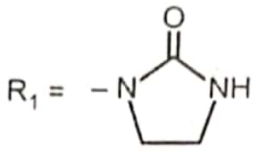
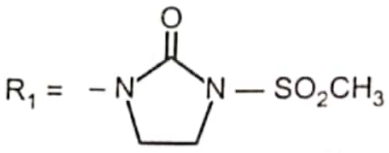
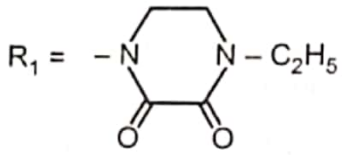
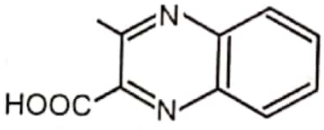
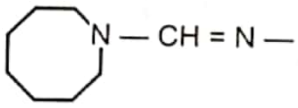
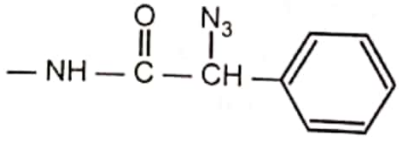
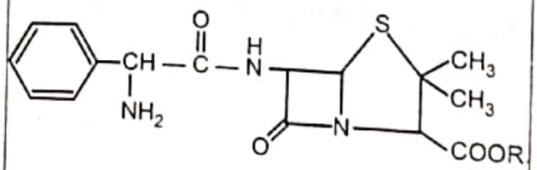
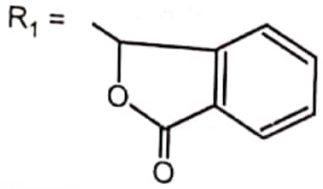
(i) Penicillin G	 (Benzyl)	No
(ii) Penicillin V	 (Phenoxymethyl)	No

(B) Penicillinase-Resistant Penicillins:		
(i) Methicillin		Yes
(ii) Oxacillin ($R_1 = R_2 = H$)		Yes
(iii) Cloxacillin ($R_1 = H$; $R_2 = Cl$)		Yes
(iv) Dicloxacillin ($R_1 = R_2 = Cl$)		Yes
(v) Floxacillin ($R_1 = F$; $R_2 = Cl$)		Yes
(vi) Nafcillin		Yes
(C) Aminopenicillins:		
(i) Ampicillin ($R_1 = H$)		No
(ii) Amoxicillin ($R_1 = OH$)		No
(iii) Bacampicillin		No
	$R_1 =$ 	
(D) Carboxypenicillins:		
(i) Carbenicillin ($R_1 = H$)		No
(ii) Carfecillin (R_1 = – phenyl)		
(iii) Indanyl carbenicillin (R_1= 5-indanol)		No
(iv) Ticarcillin		No

Contd.

(E) Ureidopenicillins:		
(i) Azlocillin		No
(ii) Mezlocillin		
(iii) Piperacillin		
(F) Miscellaneous Penicillins:		
(i) Quinicillin		Yes
(ii) Amidinopenicillins (Mecillinam)		No
(iii) Azidocillin		No
(iv) Talampicillin	 	No

Evolution of the Series of β-lactam Antibiotics: The β-lactam antibiotics are cell-wall inhibitors towards susceptible bacteria. It just means that they cannot kill or inhibit all bacteria e.g., Penicillin G (Benzyl Penicillin) has a fairly narrow antibacterial spectrum. In particular, fungi and many gram-negative bacteria are relatively insensitive to this agent. It is readily hydrolysed by the enzyme penicillinase. The pronounced hydrolytic susceptibility of the β–lactam bond to penicillinase, hindered early progress in working with penicillins.

Following the realization that the presence of phenylacetic acid in the fermentation leads to predominance of the product, benzylpenicillin, wide variety of other acids were added to the growing culture. Thus, the second generation known as 'semi-synthetic penicillins' was born. The proper design of the side - chains thus accessible has served not only to overcome many of the drawbacks of the early penicillins (like enzyme susceptibility, acid liability and lack of oral activity) but also helped to develop broad spectrum antibiotics. The involvement of acyl carbon of the side - chain in the hydrolytic clevage of β–lactam bond was recognised. Improvement in clinical qualities was then achieved by creating steric hindrances to this acyl carbon, thus making it less reactive. The fact was attested by attachment of an aromatic or heterocyclic ring directly to the amide carbonyl, thus affording antibiotics with increased penicillinase resistance e.g. Methicillin, oxacillin family and nafcillin.

One of the most successful penicillin candidates is Ampicillin which is prototype of third generation β–lactam antibiotics. It is characterised by increased oral activity than its precursor. The early observation that drugs acylated by amino acids had somewhat greater oral activity turned to be an inspiration behind its development. Thus, addition of an amino group to benzylpenicillin also leads to broadening of spectrum of activity alongwith increased oral activity. The next congener is Amoxicillin. The aminopenicillins still suffer from the major drawback, i.e. susceptible to β-lactamase enzymes and thus are ineffective for most staphylococcal infections. In an attempt to improve further, the pharmacodynamic characteristics, prodrug development program of Ampicillin was undertaken. As a result bacampicillin, hetacillin and talampicillin appeared on the screen.

The fourth generation of β–lactam adds to the list of chemotherapeutic agents in the rm of carboxypenicillins. Carboxylic acid group if introduced into the primary amine oiety of ampicillin and other allied skeleton may significantly affect the biological pectrum of the lead, was noted seriously and served as an impulse in the generation of this class. The examples are carbenicillin family and Ticarcillin.

A parallel observation is also registered stating that acylation of the amino group of ampicillin broadens the antimicrobial spectrum of the prototype drug. Azlocillin is a front-line drug in this series which was named under ureidopenicillins. Recently, new impetus has been added to the chemotherapy by the discovery of new ring systems in fermentation liquors.

New ureidopenicillin derivatives show an expanded gram-negative spectrum and increased potency against many enteric bacilli. All the ureidopenicillins possess significant stability to β-lactamase.

Despite the enormous efforts expanded during the past three decades, the β–lactam antibiotic field remains still a field of severe competition within itself. The new drugs coming up differ from benzyl penicillin (natural penicillin) in one or more of four properties - acid sensitivity, susceptibility to inactivation by penicillinase, antibiotic potency and spectrum of antibacterial activity.

When the acid stability of penicillin is increased, the drug is not destroyed by gastric acid and thus can be orally administered. In general, the acid stable penicillins are less active agents on W/W basis than benzylpenicillin. The penicillinase resistant penicillins are not hydrolysed by the enzymes produced by Staphylococcus aureus and hence they are effectively used to treat the infections caused by resistant strains of microorganisms.

Bio-chemical Mechanisms of Bacterial Resistance to β-lactam Antibiotics:

It should be noted that penicillin resistance may not always be due to penicillinase production, even among the Staphylococci. Certain other mechanisms of resistance can be operative, like

(a) A change in antibiotic target site, which may not be vital for microbial survival, thus resulting into drug resistance.

(b) Inability of the agent to penetrate to its site of action.

(c) A reduction in cellular permeability to the antibiotic.

(d) The antibiotic agent, instead of attacking the microorganisms, may be utilized to antagonize a biochemical intermediate released by microbes.

(e) A sensitive strain may undergo mutational change and thereafter acquire resistance to antibiotic agent.

Thus, penicillin resistance developes into sensitive strains of microbes due to a single or sometimes due to overlapping of one or more mechanisms mentioned above.

1.4 HYPERSENSITIVITY OR ALLERGIC REACTIONS

Hypersensitivity reactions may occur with any dosage form of penicillin. In some cases, the reaction is mild and disappears even while the use of drug is continued. While in others, reactions may persist for 1 or 2 weeks or longer after therapy has been stopped. These reactions include immediate or delayed type skin allergies, fever, bronchospasm, serum sickness and anaphylactic reactions.

These manifestations may be due to the following reasons:

(a) A breakdown product, penicilloyl moiety results due to opening of β–lactam ring. This fraction is considered to be the most important antigenic intermediate of penicillins.

(b) Certain other contaminants (mycelial residues) of high molecular weight originating from fermentation process may serve as a cause of manifestations.

(c) A non-protein polymer of unknown origin may also be present in penicillin and may be antigenic.

(d) The degradation products, penicillanic acid and / or penicilloate may interact with sulfhydryl / amino groups present in vital tissue proteins. The resulting complexes may serve themselves as penicillin antigens. Thus above mentioned reactions are responsible for releasing foreign proteins in the body which ultimately leads to the generation of allergic reactions to penicillins.

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1.5 CEPHALOSPORINS

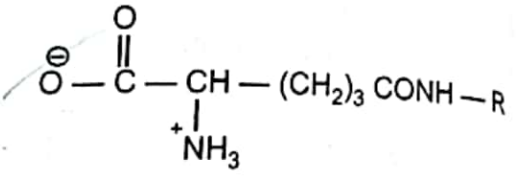
The concept that certain antibiotic producing fungi may occur in soils and further environments rich in bacteria led to a worldwide examination of soils, sewage sludges and related material for new antibiotic. The concept crystallised out with some signal success. A species of Cephalosporium, isolated near a sewage outfall of the Sardinian coast by Brotzu in 1948 was studied at Oxford. The mould produced three antibiotics which were named as

(1) Cephalosporin N: It has a penicillin like structure being a derivative of 6-aminopenicillanic acid.

(2) Cephalosporin P: An acidic antibiotic, which is steroidal in nature.

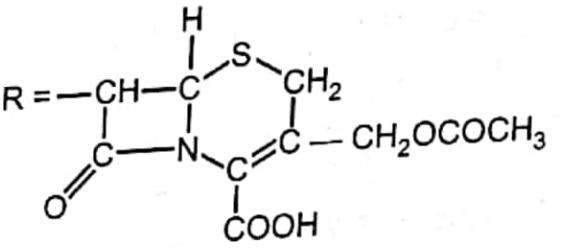
(3) Cephalosporin C: It is a true cephalosporin and is a derivative of 7 amino cephalosporanic acid. The latter served as a lead nucleus for the development of totally new series of compounds, Cephalosporins.

Widespread clinical acceptance continues to be accorded to the cephalosporins and the field is extremely active in searching for new drugs adding better oral activity and broader antimicrobial activity.

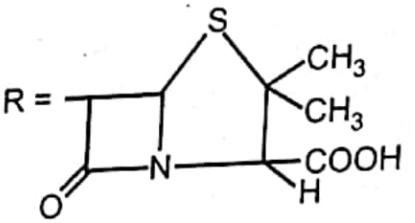


Generalised formula for cephalosporins

(i) In cephalosporin C,

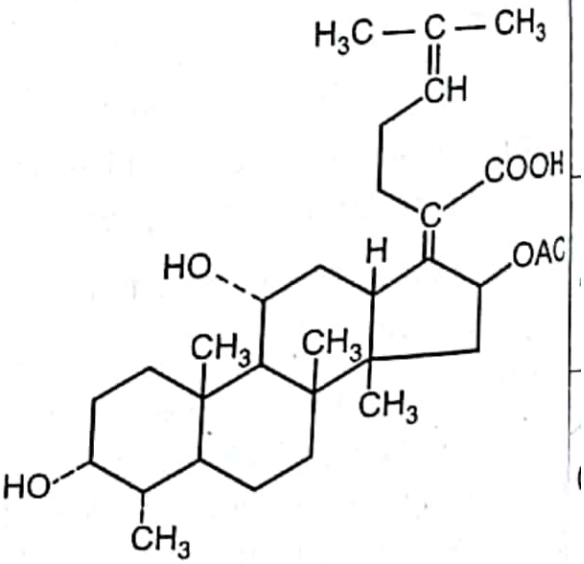


(ii) In cephalosporin N,



Penicillanic acid

Cephalosporin C contains a side-chain derived from D-α-aminoadipic acid, which is attached to 7-amino cephalosporanic acid or chemical name - a dihydrothiazine beta - lactam ring system. Regardless of the structure of the side-chains, cephalosporins are relatively more acid-stable and penicillinase resistant than Penicillin family. A compound structurally similar to cephalosporin P is fusidic acid, an antibiotic produced by the mould *Fusidium coccineum*. Fusidin is the sodium salt of fusidic acid. Both the antibiotics i.e. cephalosporin P and fusidic acid are steroidal in structure.



Fusidic acid

Classification of Cephalosporins:

Options are open to classify cephalosporins on the basis of their chemical structure, clinical pharmacology, antimicrobial spectrum or penicillinase resistance. The one suggested by O' Callaghan in 1979 is based upon the clinical pharmacology of cephalosporins. Thus, they are classified as:

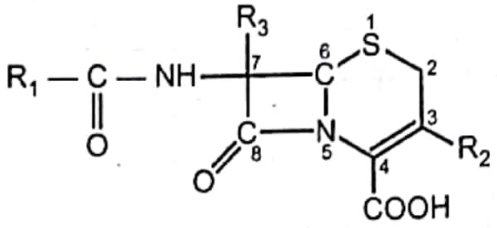
∴ **(a) Orally administered:** Cephalexin, cephradine and cefaclor.

(b) Parenterally administered: Cephalothin, cephapirin, cephacetrile, cephaloridine and cefazedone. These agents are sensitive to β - lactamase.

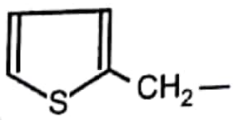
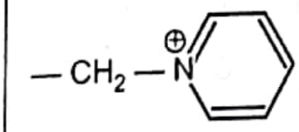
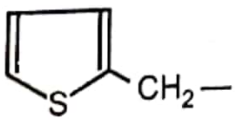
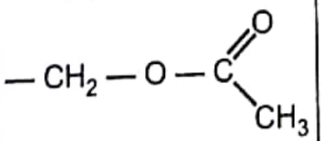
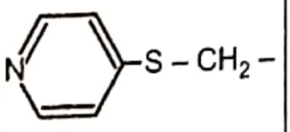
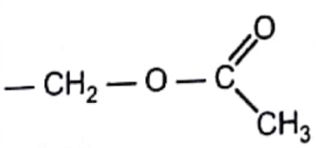
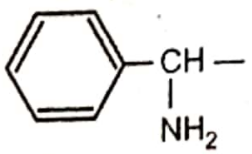
(c) Resistant to β-Lactamase and parenterally administered: Cefuroxime, cefamandole, cefoxitin.

(d) Metabolically unstable: Cephalothin and cephairin.

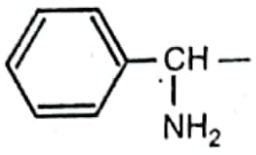
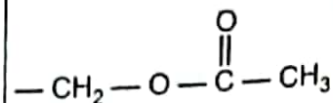
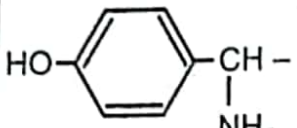
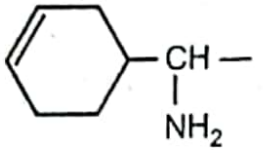
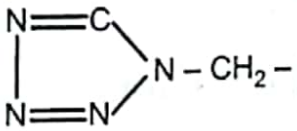
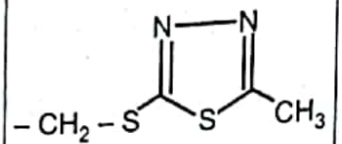
Table 1.2: Clinically used cephalosporins



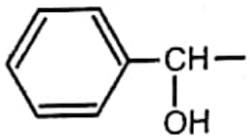
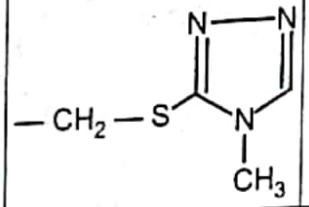
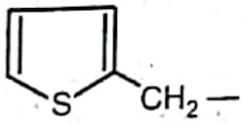
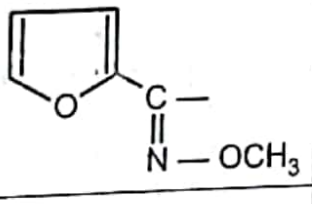
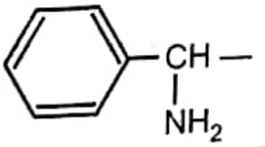
Cephalosporins

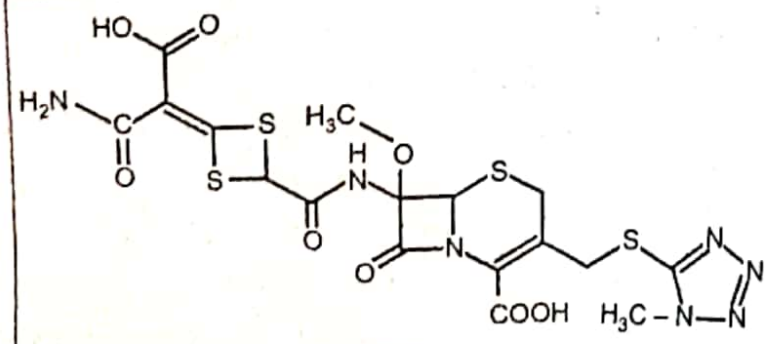
Name	R_1	R_2	R_3	Plasma half life (hr)	Protein binding %	% metabolism	Lactamase susceptibility
(A) First Generation Cephalosporins: cephalexin, cephradoxil							
(i) Cephaloridine			H	1-1.5	10 - 30	5 - 10	Yes
(ii) Cephalothin			H	0.5-0.7	65	30-35	Yes
(iii) Cephapirin			H	0.5-0.7	45-50	40	Yes
(iv) Cephalexin		$-CH_3$	H	1.0	10-15	5-10	No

Contd...

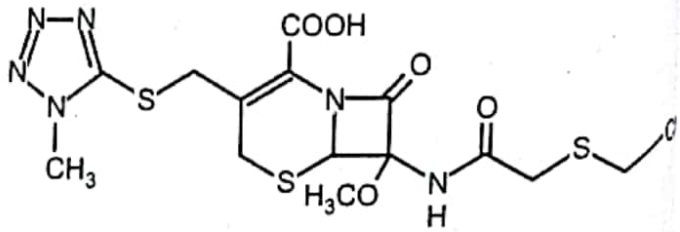
(v) Cephaloglycine			H	1.5	15-25	90	No
(vi) Cefadroxil		$-CH_3$	H	1.2-2.0	20	5-10	No
(vii) Cephradine		$-CH_3$	H	0.8	6-20	5-10	No
(viii) Cefazolin			H	1.8-2.0	80	5-10	Yes

(B) Second Generation Cephalosporins:

(i) Cefamandole			H	0.6-0.8	70	5-10	No
(ii) Cefoxitin		$-CH_2OCONH_2$	$-OCH_3$	0.8	70-75	5-10	No
(iii) Cefuroxime		$-CH_2OCONH_2$	H	1- 2	50	5-10	No
(iv) Cefaclor		Cl	H	0.6-0.9	25	30	No

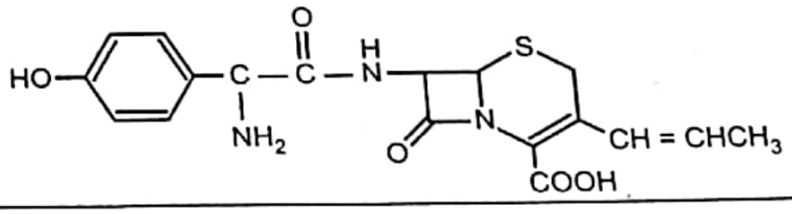


Cefotetan

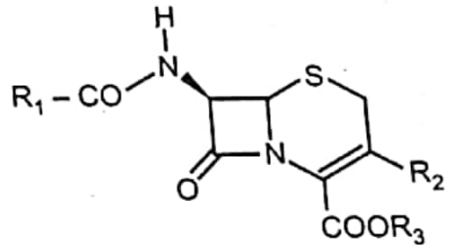


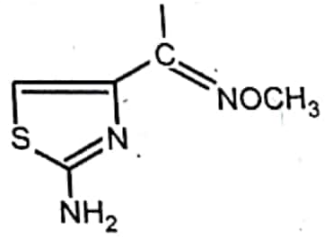
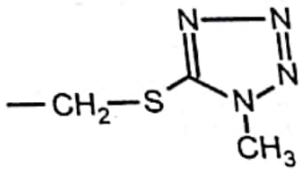
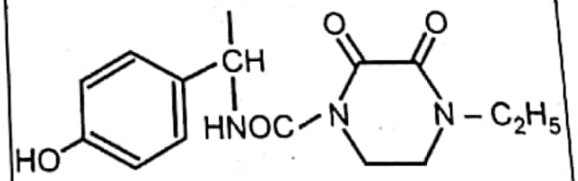
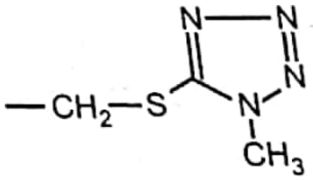
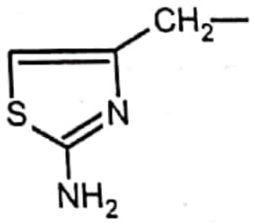
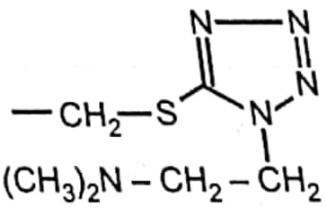
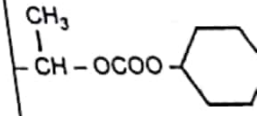
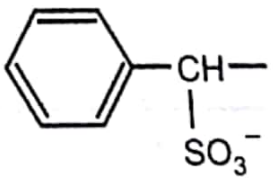
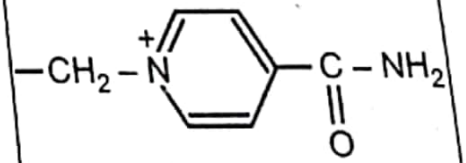
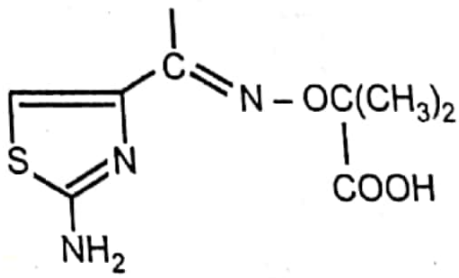
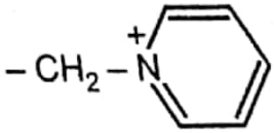
Cefmetazole

Cefprozil: It is a second generaction cephalosporin used to treat bronchitis, ear infection and skin infections.



(C) Third Generation Cephalosporins:



Compound	R_1	R_2	R_3
Cefmenoxime			H
Cefoperazone			H
Cefotiam			
Cefsulodine			H
Ceftazidime			H

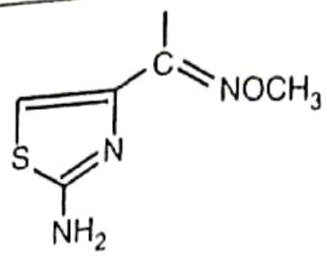
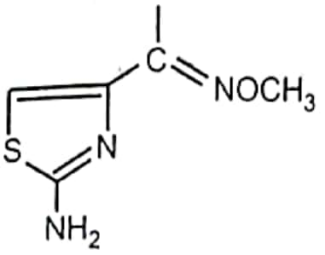
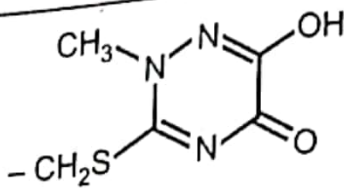
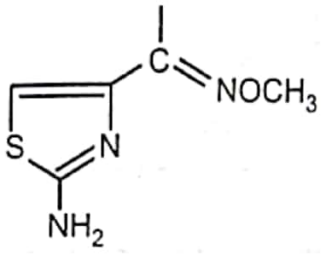
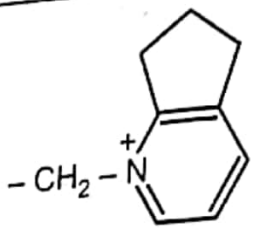
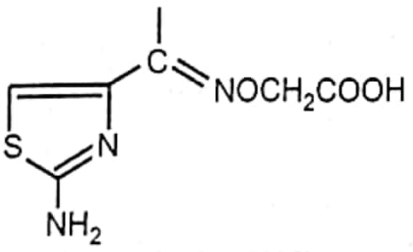
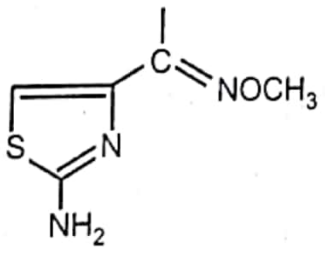
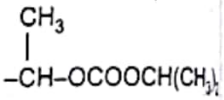
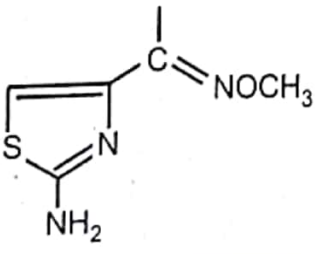
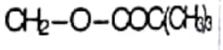
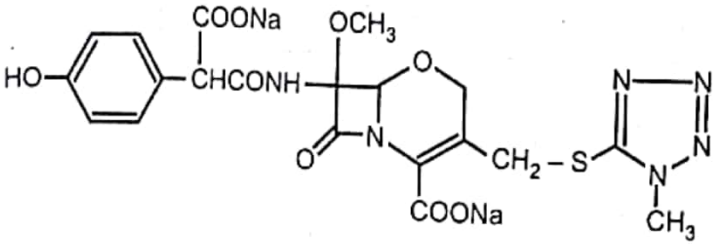
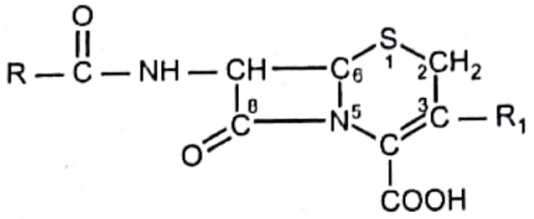
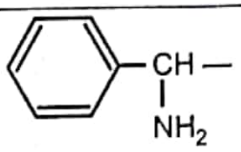
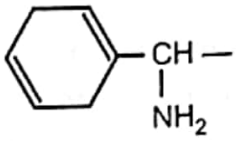
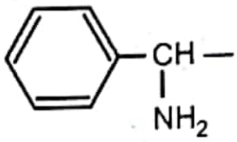
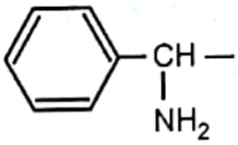
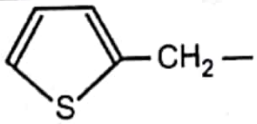
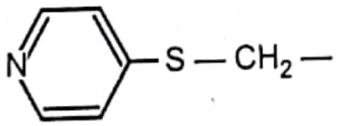
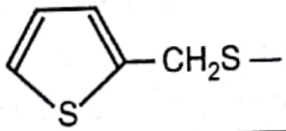
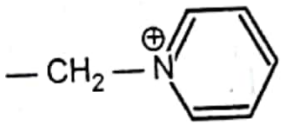
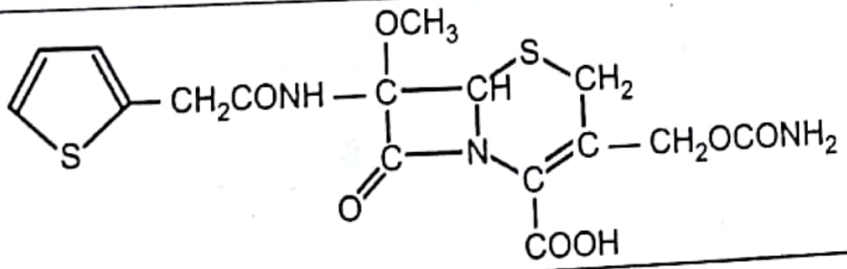
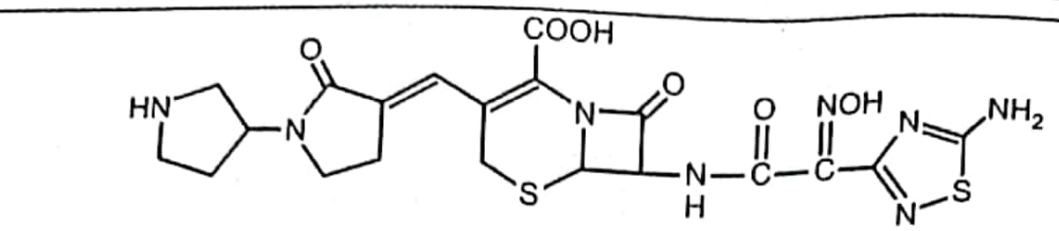
Ceftizoxime		H	H
Ceftriaxone			H
Cefpirome			H
Cefixime		$-CH=CH_2$	H
Cefpodoxime		$-CH_2-OCH_3$	
Cefetamet		$-CH_3$	
Moxalactam	 (Total structure)		

Table 1.3: Cephalosporins

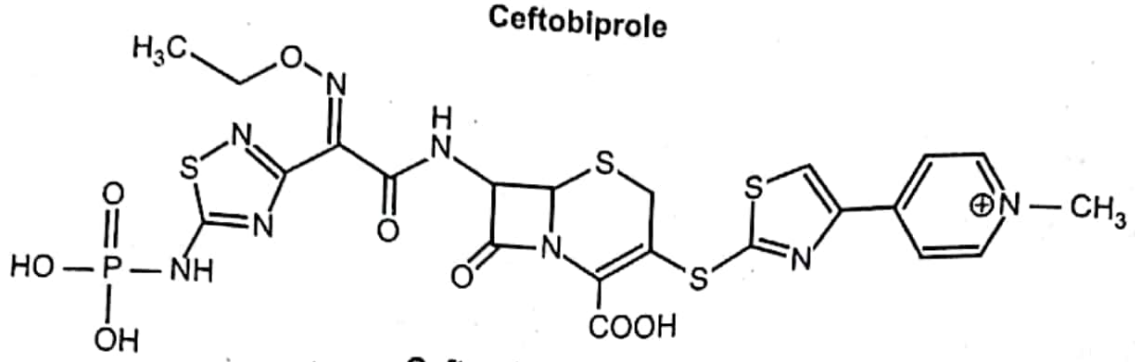


Cephalosporins

Name	R	R_1
(A) Oral Cephalosporins:		
(i) Cephalexin		$-CH_3$
(ii) Cephradine		$-CH_3$
(iii) Cefaclor		$-Cl$
(iv) Cephaloglycin		$-CH_2OCOCH_3$
(B) Parenteral Cephalosporins:		
(i) Cephalothin		$-CH_2OCOCH_3$
(ii) Cephapirin		$-CH_2OCOCH_3$
(iii) Cephaloridine		
(C) Resistant to β-lactamase: (Parenteral Cephamycin)		
(i) Cefoxitin		
(D) Metabolically Unstable:	e.g. Cephalothin and Cephapirin	



Ceftobiprole



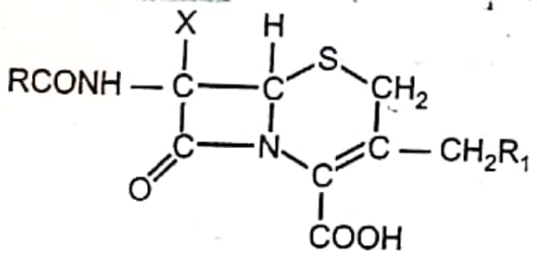
Ceftaroline fosamil

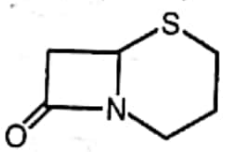
SAR of Cephalosporins:

The low potency of cephalosporin C soon made it clear that the natural product itself is unsuitable as a clinical antibiotic. The structure would have to be modified in the laboratory to give a more potent semi-synthetic analog. The semi-synthetic cephalosporins are obtained by attaching a side-chains to 7-amino cephalosporanic acid just as penicillins are made from 6-aminopenicillanic acid. Hence, the SAR among the cephalosporins appear to remain parallel with those among the penicillins in as far as the acyl group is concerned.

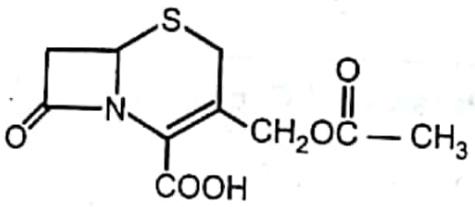
The basic skeletons involved in cephalosporin chemistry are:

(1) Cephalosporins X = – H (2) Cephamycins X = – OCH_3



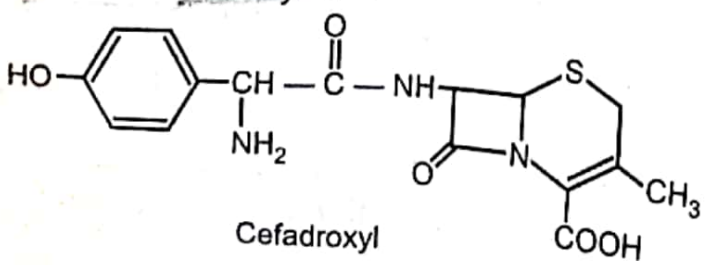


Cepham



Cephalosporanic acid

1. The cephalosporins are considered as broad spectrum antibiotics with patterns of antibacterial effectiveness similar to ampicillins.
2. Cephalosporins are significantly less sensitive than β - lactamase resistant penicillin to hydrolysis by the enzymes from *Staphylococcus aureus* and *Bacillus subtilis*. This may be due to the bicyclic cephem ring system rather than the acyl group.
3. Drug design in cephalosporin series followed the prominant footprints marked in penicillin evolution. Cefadroxyl is an example. The design of this drug seems to have derived from the succcss of amoxycillin.



Cefadroxyl

4. Similar to penicillin series, phenylglycine moiety if attached to 7 - amino cephalosporanic acid, affords a compound with increased oral activity; example is cephaloglycin.
5. The allylic acetoxy group at C - 3 is apparently not necessary for antibiotic activity e.g. cephalexin, cephradine do not contain this group.
6. Analogous to azlocillin - mezlocillin, acylation of the amino group of 2 - phenylglycine containing cephalosporins (e.g. cephaloglycin) is consistent with antipseudomonal activity e.g. cefoperazone.
7. A sulfonic acid moiety if present in acyl side-chain, confers antipseudomonal activity to certain penicillins. The analogy works with cephalosporins as well, resulting into Cefsulodin.
8. While screening for β–lactam antibiotic stable to β–lactamases, a strain of Streptomyces lactamdurans is found to release certain agents containing 6 - α - methoxy group whose electronic and steric properties protect the antibiotic from enzymatic attack. This generates a new series of compounds, known as 'cephamycins'. Cefoxitin is one of the clinically useful agents from this class.
9. Several cephalosporins penetrate into CSF in sufficient concentration to be useful for the treatment of meningitis. For example cefuroxime, cefotaxime and moxalactam.
10. Cephalosporins are still useful as alternatives to penicillins for a variety of infections in patients who cannot tolerate penicillins. These include Streptococcal and Staphylococcal infections.

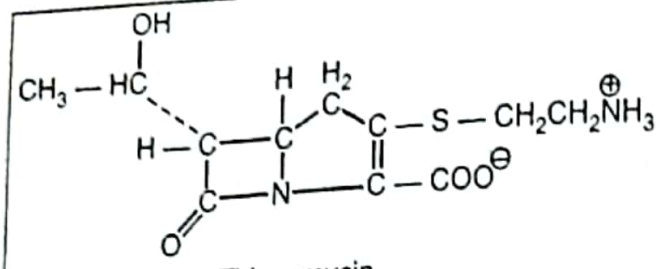
Other β-lactam Antibiotics:

The story of β-lactam antibiotics which began in 1929, had propogated through two distinct phases, one marked by penicillin analogs and second phase dominated by cephalosporin family. The day to day research is still adding new entities to antibiotic field and exposing one or more clinical deficiencies perceived in existing drugs. The new basic skeletons encountered would either reach the market place or add to the volumes of dead stock in the literature is yet uncertain. Recently three new classes of β-lactam antibiotics have come up. They are (1) Thienamycins, (2) Nocardicins and (3) Clavulanic acid.

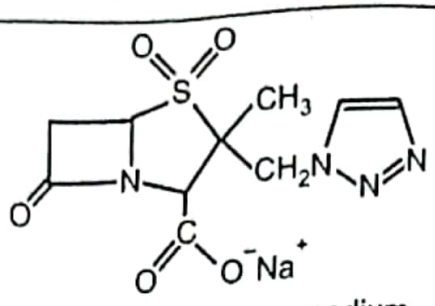
(1) Thienamycins:

The research groups at Merck were the first, to isolate and characterize this antibiotic rom Streptomyces cattleya. Like penicillins and cephalosporins, it contains a fused bicyclic ing system containing β-lactam and a 3-carboxylic group but instead of β-acylamido side - hain, it has α-1 hydroxyethyl group.

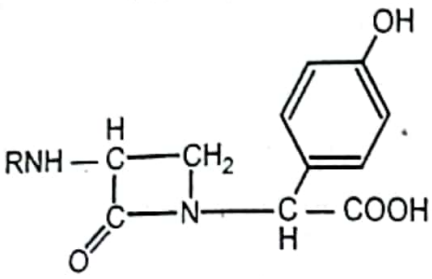
Two distinct features of thienamycin and cephalosporins are its broad spectrum of ctivity and its β-lactamase resistant property which make it effective against many strains esistant to pencillins and cephalosporins.



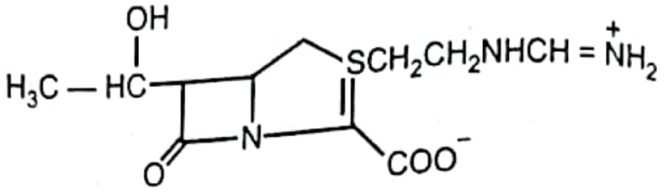
Thienamycin



Tazobactam sodium



Nocardicins



Imipenem

(2) Nocardicins:

It is a group of about seven antibiotics isolated from various Nocardia species. Here we do not observe a fused bicyclic ring-system which is characteristic of β-lactam antibiotics. Nocardicin A is a narrow spectrum antibiotic and their status in future clinic still remains to be established.

(3) β-lactamase inhibitors:

These agents exhibit weak broad spectrum antibacterial activity and therefore are not entitled to be used as effective antibiotics. However, they have an affinity for β-lactamases and serve as potent irreversible inhibitors of many β-lactamases produced by gram-positive and gram-negative bacteria.

Due to these pharmacological features, they are not used as primary antibiotics but are combined usually with the conventional β-lactam antibiotics that are substrates for these enzymes. This usually results into potentiation of activity of β-lactam antibiotics.

Examples from this category are:

(a) Clavulanic acids: These are produced by *Streptomyces clavuligerus*. (The same actionomycete which produced cephamycin).

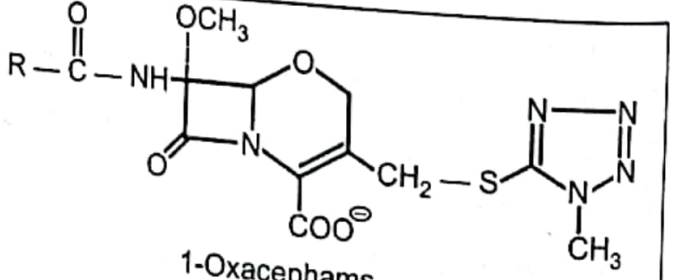
(b) Salbactam: Similar in structure to clavulanic acid.

(4) Imipenem:

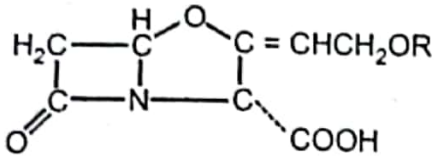
It is the N-formimidoyl derivative of thienamycin. It is having a β-lactam structure and is very resistant to most of the β-lactamases.

(5) 1-Oxacephems:

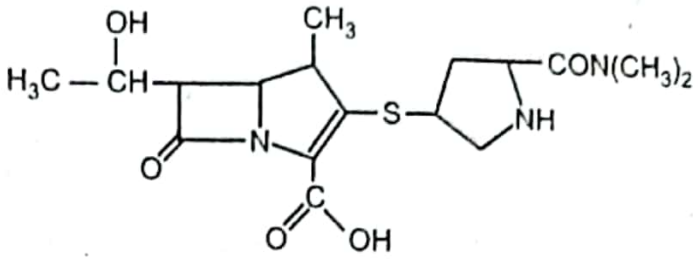
In these compounds, at one hand as the oxygen atom unstabilizes the molecule, the introduction of 7 α-methoxy group works as a compensation. Moxalactam, an example from this class is a broad spectrum antibiotic, having β-lactam resistance property.



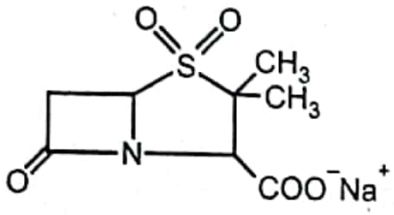
1-Oxacephams



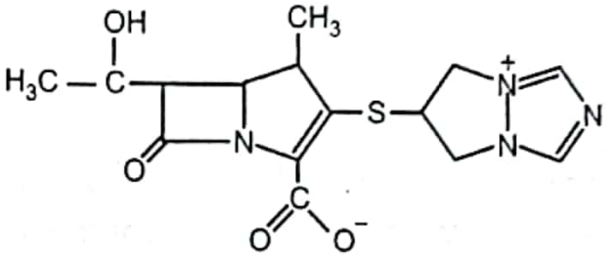
Clavulanic acid



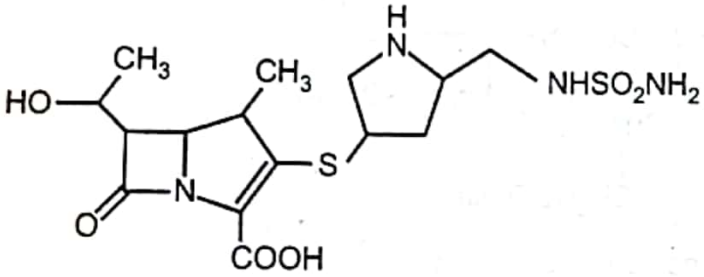
Meropenem



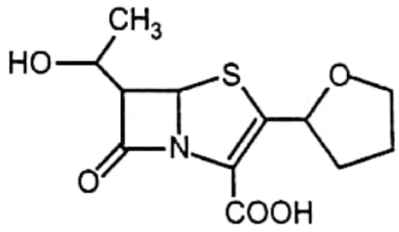
Salbactam sodium



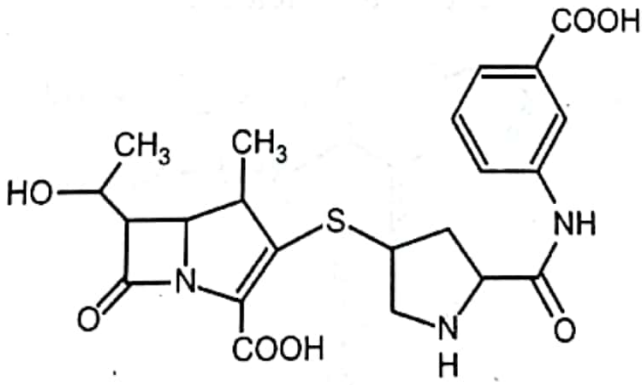
Biapenem



Doripenem



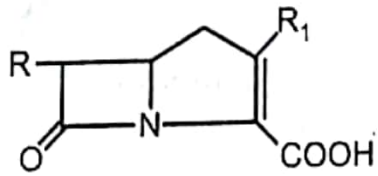
Faropenem



Ertapenem

(6) 1-Carbapenems:

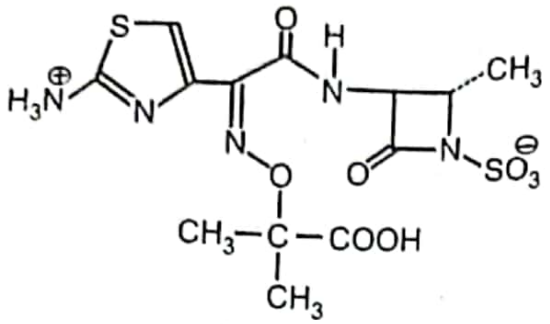
They are structural analogs of penicillins where sulphur atom is replaced by carbon. Olivanic acids stand as example of this class. They are broad spectrum antibiotics isolated from *Streptomyces olivaceus*.



1-Carbapenems

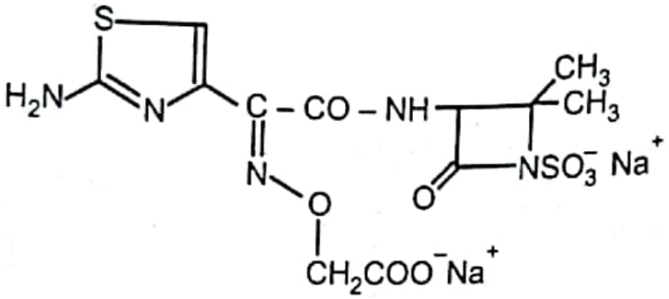
(7) Monobactams:

These are monocyclic β-lactam antibiotics isolated from *Chromobacterium violaceum*. The basic nucleus is 3-aminomono bactamic acid (3 - AMA).



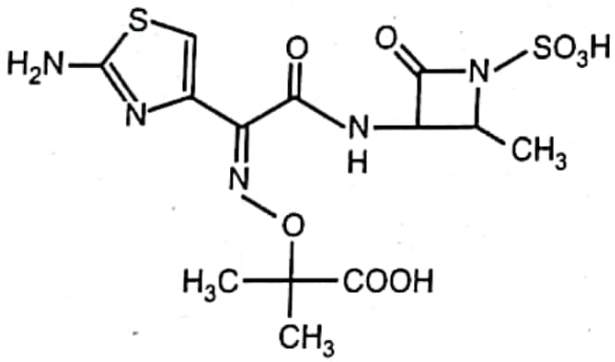
Azactam

Azactam is an example of this class which is active against the most of the gram negative bacteria and resistant to the most of the β-lactamase enzymes.



Tigemonam disodium

Aztreonam: It is a synthetic monocyclic β-lactam antibiotic with the skeletal (monobactam) isolated from *Chromobacterium violaceum*.



Cilastatin: It is a dipeptidase inhibitor that specifically inhibits dehydrodipeptidase 1, a renal membrane bound glycoprotein involved in the hydrolytic metabolism of penem and carbapenem β-lactum antibiotics. It maintains the antibiotic activity of imipenem through inhibiting dipeptidase activity.

1.6 AMINOGLYCOSIDE ANTIBIOTICS (Aminocyclitol Antibiotics)

This series includes streptomycin, gentamycin, neomycin, kanamycin, tobramycin, amikacin, netilmicin, spectinomycin and framycetin. These consist of aminosugars linked as glycosides. They are all mixture of water soluble, basic carbohydrates that are closely related

chemically. They inhibit the growth of gram-positive, gram-negative and mycobacteria. Except for the gentamycins, all are the products of species of Streptomyces. They are characterized by the presence of atleast one aminohexose but some prefer pentose lacking an aminogroup (e.g. Streptomycin). A highly substituted 1, 3-diaminocyclohexane central ring (aminocyclitol) is present in the form of deoxystreptamine in all members except streptomycin and dihydrostreptomycin. Both contain streptidine, whereas spectinomycin is also an aminoglycoside but has no amino sugar.

The aminoglycosides are used primarily in infections caused by gram-negative bacteria. They are mostly ineffective against anaerobic bacteria, fungi and viruses. These are very poorly absorbed if given orally. The systemic use is also restricted due to oto - and nephrotoxicity. Serious toxicity is the major limitation where each member measures same spectrum of toxicity. The development of strains of Enterobacteriaceae resistant to aminoglycosides led to detailed study of mechanisms of resistance.

Atleast nine different aminoglycoside inactiviating enzymes are identified which differ from each other due to

(a) Having different target functional groups (sites of attack) in aminoglycosides and

(b) Steric factors.

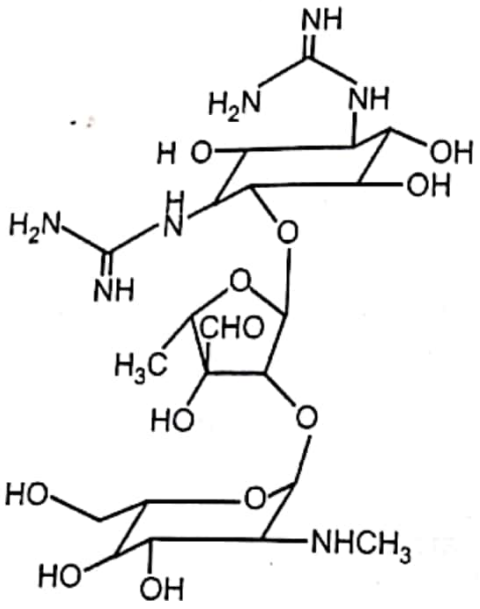
Examples of such enzymes are acetylase, adenylase and phosphorylase.

Table 1.4

Sr. No.	Antibiotic	Source	Year of Introduction
1.	Streptomycin	*Streptomyces griseus*	1944; Waksman
2.	Neomycin	*S. fradiae*	1949; Waksman
3.	Kanamycin	*S. kanamyceticus*	1957; Limezawa
4.	Gentamicin	*Micromonospora purpurea*	1964
5.	Tobramycin (nebramycin)	*S. tenebrarius*	1967; Higgins
6.	Framycetin, (soframycin)	*S. decaris*	
7.	Paromomycin	*S. rimosus formoparamomycinus*	1959
8.	Amikacin	Semisynthetic product from kanamycin	1972; Kawaguchi
9.	Sisomicin	M. inyoensis	1980
10.	Netilmicin (N-ethyl sisomicin)	Semisynthetic product from sisomicin	1963; Weinstein

SAR of Aminoglycoside Antibiotics:

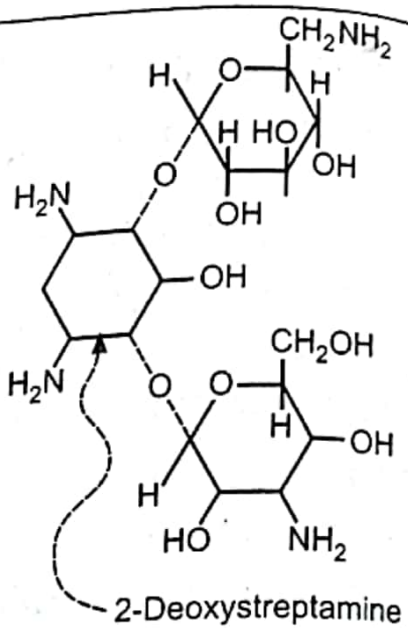
The aminoglycosides consist of two or more aminosugars joined in glycoside linkage to a highly substituted 1, 3-diaminocyclohexane (aminocyclitol) centrally placed ring. This ring is 2-deoxystreptamine in all aminoglycosides except streptomycin and dihydrostreptomycin where it is streptidine.

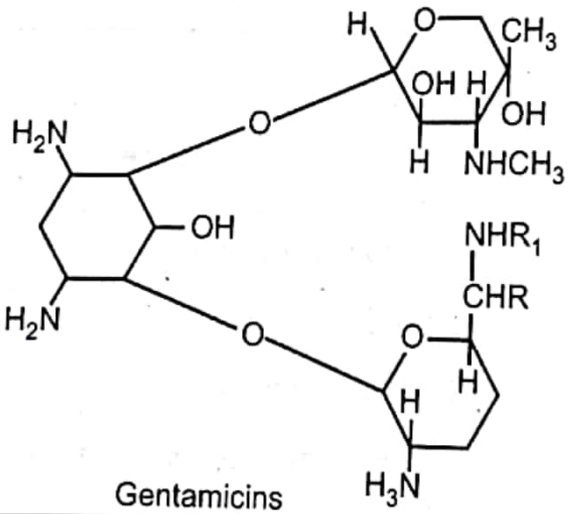


Streptidine

L-Streptose

N-Methyl-L-Glucosamine





Thus,

(1) In Kanamycin and gentamicin families, two aminosugars are attached to 2-deoxy-streptamine.

(2) In Streptomycin, two aminosugars are attached to Streptidine.

(3) In Neomycin family, there are three aminosugars attached to 2-deoxystreptamine.

So in summary, the aminoglycoside antibiotics contain two important structural features:

(1) Aminosugar portion and

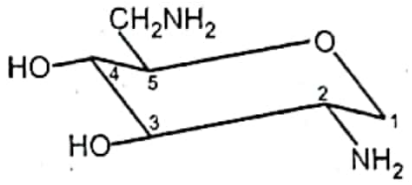
(2) Centrally placed hexose ring either 2-deoxystreptamine or streptidine.

(1) SAR of aminosugar portion:

(i) The amino function at C-6 and C-2 serve as major target sites for bacterial inactivating enzymes.

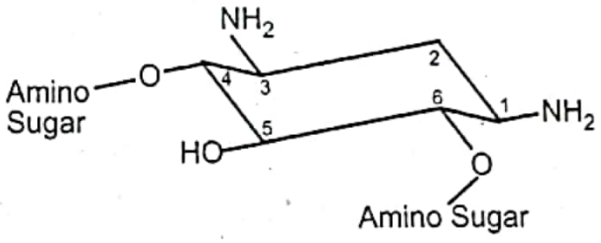
(ii) Methylation at C-6 position does not decrease the activity; instead increases enzyme resistance.

(iii) Clevage of 3-hydroxyl or the 4-hydroxyl or both groups does not affect the activity.



(2) SAR of centrally placed hexose ring (aminocyclitol ring):

(i) Various modifications at C-1 amino group have been tested. The acylation (e.g. amikacin) and ethylation (e.g. 1-N-ethylsisomicin) though not increases the activity, helps to retain the antibacterial potency.



(ii) In sisomicin series, 2-hydroxylation and 5-deoxygenation results in increased inhibition of bacterial inactivating enzyme systems.

Thus, very few modifications of the central ring are possible which do not violate the activity spectrum of aminoglycosides.

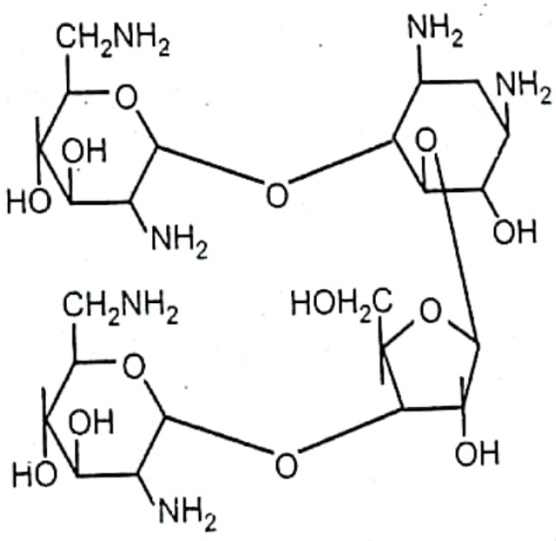
Biological Evolution of Aminoglycosides:

(1) Streptomycin and dihydro-streptomycin:

The organism Streptomyces griseus was isolated in 1943 by Waksman et al as a result of a well - planned scientific research covering more than ten thousand organisms. Other substances like, hydroxystreptomycin, mannisidostreptomycin and cycloheximide are also released by the organism but do not reach upto the required activity/potency level. The development of resistant strains of bacteria and chronic toxicity constitutes major drawbacks of these category.

(2) Neomycins:

Neomycin, kanamycin, gentamycin and paromomycin are among the most stable antibiotics known. Neomycin is a group of six antibiotics (A, B, C, D, E, and F) which are produced by certain strains of Streptomyces fradiae. While Neomycin A is a degradation product of Neomycin B and C, Neomycin B is still utilized as topically employed antibiotic. It has a broad spectrum of activity. However, it causes severe renal toxicity and ototoxicity when administered parenterally. It is also known for its effect on the bowel flora.



Neomycin B

(3) Kanamycins:

It is produced by *S. kanamyceticus* and has properties and actions similar to streptomycin and neomycin. The mixture consists of three related structures i.e. kanamycins A, B, and C. The kanamycins do not possess D-ribose molecule that is present in neomycins and paromomycins. The use of kanamycins is restricted to infections of the intestinal tract and to systemic infections which are not responding to other antibiotics. Due to the toxicity and development of resistant strains of bacteria, kanamycins are largely replaced by the newer agents from this class.

(4) Gentamicins:

These are the metabolic products of gram-positive bacteria, *Micromonospora purpurea* and constitute a group of closely related broad spectrum aminoglycosides (C_1, C_{1a}, and C_2) that have structures similar to those of neomycins and kanamycins. Gentamicin is effective in the treatment of various skin infections and urinary tract infections caused by gram - negative bacteria. The high potency, relatively low toxicity and a virtual absence of resistant strains highlight the clinical utility of these agents. It works by binding the 30S subunit of the bacterial ribosome, interrupting protein synthesis.

(5) Paromomycins:

The isolation of drug was first reported in 1956. Paromomycin is similar to neomycin except that it contains D-glucosamine instead of the 6-amino - 6 deoxy - D - glucosamine found in neomycin B. In addition to inhibiting gram-positive, gram-negative and myco-bacteria, it is very active against *Entamoeba histolytica*. The antibiotic mixture consists of Paromomycin I and Paramomycin II. It is employed in the treatment of amoebic dysentery and for the elimination of tapeworms.

Novobiocin: It was isolated from *Streptomyces* species and was reported in 1995 as a product of *S. spheroids* and *S. niveus*. Currently, it is produced from cultures of both. Its chemical identity was established by Shunk et al and Hoeksema et al and was confirmed by Spencer et al. It has a unique structure possessing a glycosidic sugar moiety. Its action is largely bacteriostatic. It inhibits bacterial protein and nucleic acid synthesis. It binds to the subunit of DNA gyrase and interferes with DNA super coiling and energy transduction in bacteria. Its effectiveness is confined to gram-positive bacteria. Its low activity against gram-negative bacteria is due to poor cellular penetration.

(6) Semisynthetic aminoglycosides:

Introduced into clinical practice in the 1970's these antibiotics are categorised into semi-synthetic aminoglycosides. Biologically derived as one of several components of a complex of aminoglycoside mixture released by *Streptomyces tenebrarius*, chemically tobramycin is characterised as 3'-deoxykanamycin B.

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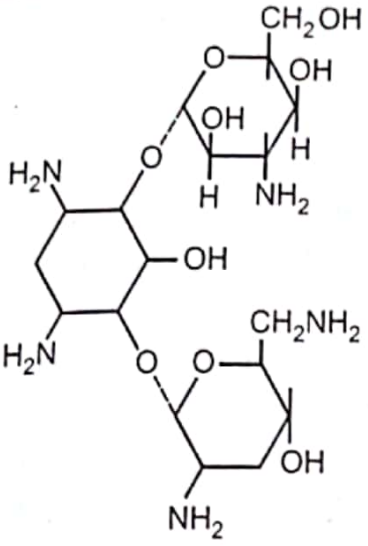
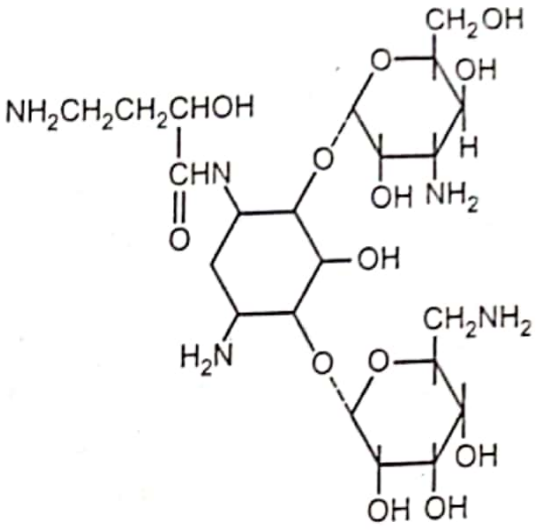
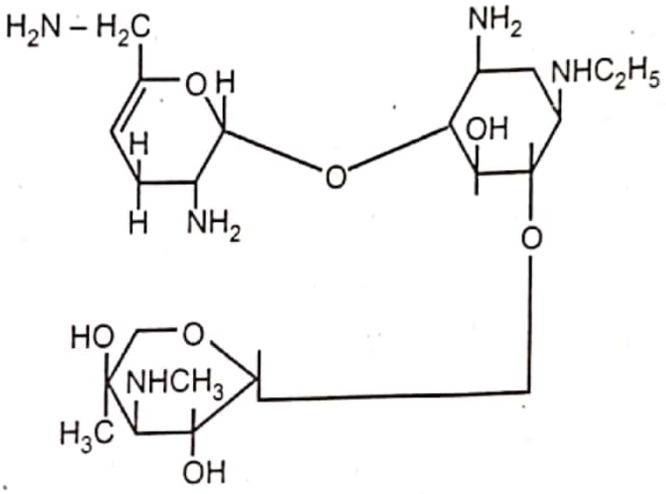


Tobramycin



Amikacin



Netilmicin

It resembles the gentamicins in its activity and toxicities. Amikacin, on the other hand, has a substituted amino-butyryl in the C-1 amino group and is considered as a derivative of Kanamycin. It was identified by Kawaguchi et al in 1972. Netilmicin is a semisynthetic N- ethylsisomicin.

1.7 BACTERAL RESISTANCE AND RECENT TRENDS IN DRUG DESIGN OF AMINO GLYCOSIDE ANTIBIOTICS

Based upon the common structural features an antibiotic shares, these are grouped as under:

(1) Streptomycin family: It includes streptomycin and dihydrostreptomycin.

(2) Neomycin family: It includes neomycins and paramomycins.

(3) Kanamycin family: It includes kanamycin A, kanamycin B, amikacin and tobramycin.

(4) Gentamicin family: It includes gentamicins (C_1, C_{1a}, and C_2), sisomicin and netilmicin.

A new wave of hope propagated in medical profession with the introduction of amino-glycoside antibiotics. They have broad spectrum of activity. They are featured by projection of polar groups (like hydroxyl or amino groups) upon a basic carbohydrate skeleton. These structural features unfortunately then served as a platform for enzymatic deactivation of

antibiotics resulting into development of microbial resistance to therapy. These polar groups may be phosphorylated, adenylated or acetylated by microbial enzymes present in the periplastic space of gram-negative bacteria. Unfortunately the resistance can be transmitted from one generation to another due to extrachromosomal R - factors which are self-replicative and transferred by direct contact. The R factor genetically controls the biosynthesis of microbial enzymes. Strains that carry R-factor are resistant to streptomycin, kanamycin and other aminoglycoside antibiotics.

Attempts are made to find out or synthesize such antibiotics in which target site or functional group is either removed or sterically hindered. Examples can be quoted, like gentamicins do not possess the specific target sites at which streptomycin and kanamycins are deactivated. Amikacin, the 1-N-r-amino-α butyrylderivative of Kanamycin A, presents another example of antibiotic resistant to most of microbial enzymes. In this case the susceptible site in kanamycin is sterically hindered. The same principle is stretched and set as a guideline for future trends.

1.8 TETRACYCLINE ANTIBIOTICS

A clear cut division of work is observed amongst previous two classes of antibiotics, penicillins being active against gram-positive organisms while Streptomycin family being incharge of gram-negative affairs. Generation of the tetracyclines was the result of a need to develop such antibiotics which can effectively cover both microbial faculties.

The series consists of about eight members: 7-chlortetracycline discovered in 1948 by Duggar. They are obtained either as metabolic by-products from various species of Streptomyces or as semisynthetic derivatives of the natural products. Tetracyclines are all yellow amphoteric compounds forming salts with either acids or bases. They exist as zwitter ions at pH 7. Although they are not completely absorbed from GIT, tetracyclines are known for their oral use. Exception is rolitetracycline which is given parenterally. Epimerization at C-4 is witnessed with tetracyclines in the solutions of intermediate pH range. Epitetracyclines, as these isomers are known by, exhibit much less activity than the neutral isomers.

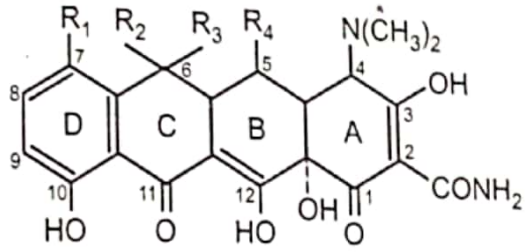
Resistance to the tetracyclines develops relatively slowly, cross-resistance (i.e. an organism resistant to one drug shows resistance to all other members of the series) is also reported. Most of the members safely escape the metabolic degradation after absorption but chlortetracycline and doxycycline do not exhibit this tendency.

Tetracyclines in Pregnancy:

Tetracyclines chelate easily many metals like calcium, magnesium, aluminium and iron. The chelates formed are insoluble in water thus render the drug - absorption. The deposition of tetracyclines in newly forming teeth and bones is favoured by chelation process. The tetracycline calcium orthophosphate complex formed is characterized initially by a yellow fluorescence on teeth which may develop into brown discolouration over a period of time. Their untoward effects on calcium present in newly forming teeth and bone black-listed their use in pregnancy or in children under 8 years of age.

ucture-Activity Relationship in Tetracyclines:

The tetracyclines are truely broad - spectrum antibiotics with the broadest spectrum than any of the presently available antibiotics. The basic nucleus common to all tetracyclines is a polycyclic naphthace-necarboxamide which is comprised of four fused, six-membered rings A, B, C and D. The group name tetracycline thus describes the pattern of backbone skeleton. A tetracyclic backbone skeleton is essential for activity.



Tetracyclines

The enolized system present at carbons 1 to 3 must be intact for good activity.

The amide function at C-2 is essential for the activity.

Epitetracyclines are very much less active than neutral isomers.

Substitution at C-6 decreases chemical stability e.g. oxytetracycline is chemically less stable than doxycycline.

In general, C-6 methylated analogs achieve higher blood levels.

C-7 substitution results in increased potency and the drug may sometimes be active against resistant microbial strains.

Strong acid dehydrates tetracyclines utilizing a 6-hydroxyl group and the 5α-hydrogen. This route led to development of 6-deoxytetracycline.

. A cis type fusion between A/B with an α-hydroxyl group at 12a is necessary for retention of activity.

.0. Electron withdrawing groups and electron donating groups both are equally effective at C-7, e.g. chlortetracycline contains an electron withdrawing group at C-7 and minocycline possesses an electron releasing (dimethyl amino) group at C-7.

11. The SAR of 8 - substituted analogs is yet not documented.

12. The SAR of positions 5, 6, 7 and 9 can be modified by various substituents resulting into retention and in some cases, improvement of antibiotic activity.

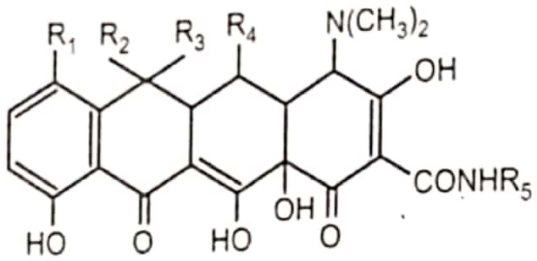
13. 6-thiatetracyclines in a preliminary report are showing excellent superior pattern of activity. They contain a sulphur atom at C - 6. A recent derivative thiacycline, is found to be more active than minocycline against tetracycline - resistant bacteria.

14. Tetracyclines have low solubility in water which may be overcome by aminoalkylation at carboxamido group using Mannich reaction. The clinically effective mannich bases are rolitetracycline (pyrrolidinomethyltetracycline), Iymecycline (Tetracycline-L-methylene-lysine) and clomocycline (N-methylol-7-chlortetracycline).

15. Semisynthetic analogs have also been obtained in an attempt to achieve advances in chemotherapy. Methacycline, doxycycline and minocycline are some results of such efforts. For example, chlortetracycline through a catalytic dehalogenation can be converted to tetracycline. Methacycline is obtained from oxytetracycline while hydrogenation of methacycline offers doxycycline.

Minocycline is a tetracycline group of antibiotic with good deal of anti-inflammatory activity. It is therefore considered to be the drug of choice for inflamed acne.

Table 1.5



Tetracyclines

Name	R_1	R_2	R_3	R_4	R_5	Source	Year of Intro
7-chlortetracycline	Cl	CH_3	OH	H	H	*S. aureofaciens*	194
Oxytetracycline	H	CH_3	OH	OH	H	*S. rimosus*	1950
Tetracycline	H	CH_3	OH	H	H	Semi-synthetically from chlortetracycline	
Demeclocycline	Cl	H	OH	H	H	Mutant strain of *S. aureofaciens*	1952
Methacycline	H		$=CH_2$	OH	H	Semi-synthetically from oxytetracycline	1959
Doxycycline	H	CH_3	H	OH	H	Semi-synthetically from oxytetracycline	1961
Minocycline	$N(CH_3)_2$	H	H	H	H	Semi-synthetically from oxytetracycline	1966
Rolitetracycline	H	CH_3	OH	H	X	Semi-synthetically from tetracycline	1972
Lymecycline	H	CH_3	OH	H	Y	Mannich base of tetracycline	
Sancycline	H	H	H	H	H		
Clomocycline	Cl	CH_3	OH	H	CH_2OH	Semi-synthetically from chlortetracycline	

In rolitetracycline X = $-CH_2N$ (pyrrolidine)

In lymecycline Y = $-CH_2NH-CH(COOH)-(CH_2)_4-NH_2$

UNIT II

Chapter...2

ANTIBIOTICS

♦ SYNOPSIS ♦

2.1 MACROLIDE ANTIBIOTICS

The research glamour created by the discovery of penicillins still, continued its way resulting into identification of a totally new class i.e. macrolide antibiotics. Isolated from actinomycetes, it is a group of chemically related compounds distinguished by three common structural features as:

1. a many membered large lactone ring (hence the name, macrolide)
2. various ketonic and hydroxyl functions and
3. glycosidically linked 6-deoxy sugars.

More often the lactone ring has 12, 14, or 16 atoms in it and is partially unsaturated with the presence of a double bond in conjugation with a ketone function. At present more than 70 such antibiotics are reported. The presence of a dimethylamino group on the sugar moiety imparted basic properties to the macrolides.

These antibiotics are called as penicillin substitutes. Members of this class are azithromycin, clarithromycin, carbomycin, erythromycin etc. They are employed to treat infections due to penicillin resistant organism or where patient feels allergic towards penicillin analogs. They are, generally effective against gram-positive bacteria, both cocci and bacilli. They exhibit low spectrum of activity against gram-negative organisms.

They are not influenced by penicillinase enzymes but organisms may develop resistance by other route. Examples and their microbial source are quoted in the following table.

Table 2.1

Sr. No.	Antibiotic	Microbial Source	Sr. No.	Antibiotic	Microbial Source
1.	Carbomycin A	*Streptomyces halstedii*	5.	Josamycin	*S. kitasatoensis*
2.	Chalcomycin		6.	Leucomycin A_1	*S. kitasatoensis*
3.	Erythromycin	*S. bikiniensis*	7.	Tylosin	*S. fradiae*
4.	Oleandomycin	*S. erythreus*	8.	Spiramycin I	*S. ambofaciens*

(1) Erythromycin:

It received the widest clinical acceptance amongst the macrolides. Its isolation was reported by McGuire et al in 1952 from *Streptomyces erythreus*. It is treated as a drug of choice for the treatment of variety of upper respiratory and soft - tissue infections due to gram-positive bacteria.

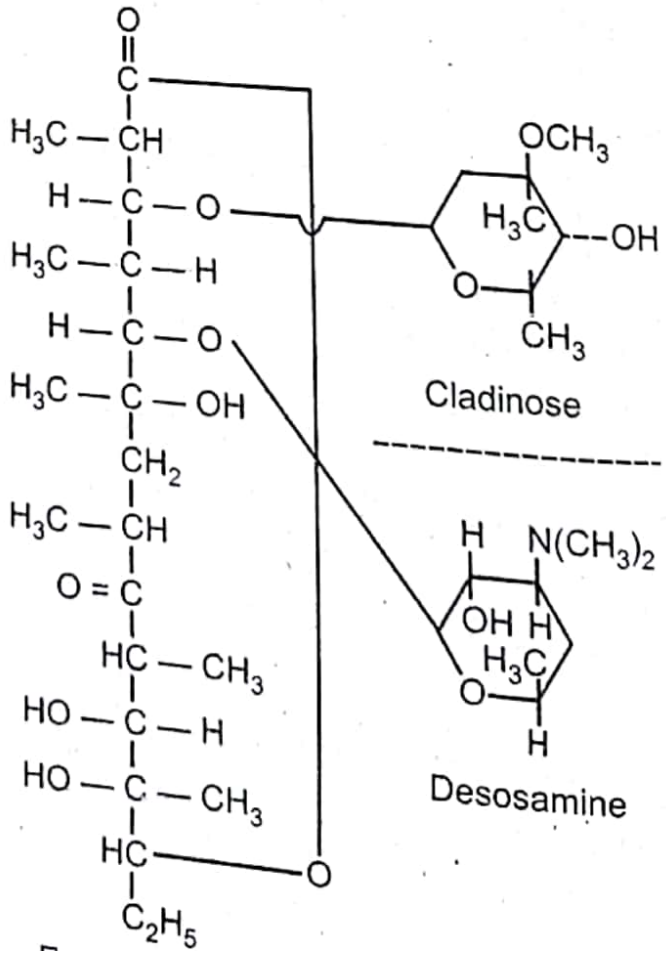
The aminosugar, desosamine is attached to C - 5 while another carbohydrate skeleton i.e. cladinose is linked glycosidically to C - 3. The large lactone structure is known as erythronolide. Two structures closely related to erythromycin have been isolated from *S. erythreus*. They are identified as Erythromycins B and C. The B analog does not possess C-12 hydroxyl group and is more stable but less active than erythromycin A. Erythromycin C lacks cladinose methoxy group and is equipotent with A. The clinical grade erythromycin contains 90% erythromycin A and about 10% erythromycin B with minute quantity of analog C.

Erythromycin is active against - Neisseria, *H. influenzae* and *Legionella pneumophila* but not against the Enterobacteriaceae; its activity shows pH dependence, increasing with pH upto about 8.5.

A number of derivatives are designed to improve:

1. either its water or lipid solubility necessary to develop more acceptable dosage form,
2. its acid stability to increase oral absorption, and
3. the acceptance by masking its bitter taste.

The basic nature of the dimethylamino group of the desosamine moiety was utilized to prepare its acid salts, like the lactobionate, glucoheptonate and the stearate, and esters of the 2' - hydroxyl group of the desosamine, including the ethyl carbonate, ethyl succinate and the estolate e.g. Erythromycin estolate being more acid stable, promotes high oral absorption. Due to good water solubility, lactobionate and glucoheptonate forms are used in parenterals. The 2'-esters as such do not possess antibiotic activity and hence efficacy of particular ester depends upon the *in-vivo* rate of ester hydrolysis to release the free - base.



Bacterial resistance gradually develops to erythromycin. In resistant strains, the affinity of drug to bacterial ribosomal binding sites is modified towards negative side. The latter then, no longer binds erythromycin.

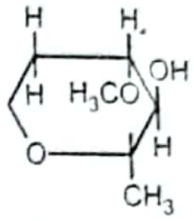
(2) Oleandomycin:

It bears similar structural features as that of erythromycin and stands as an alternative to erythromycin for limited indications. It was obtained from *S. antibioticus* by Sobin et al. It loses its claim in gram-positive bacterial treatment due to a bitless activity and high incidence of side effects. It differs chemically from erythromycin in having:

(a) L-oleandrose instead of cladinose moiety, and

(b) A 14-membered lactone ring possessing an exocyclic methylene epoxide on C-8, designated as oleandolide instead of erythronolide.

The position of linkage of both the sugars remain same i.e. desosamine at C-5 and L-oleandrose at C-3.



L-Oleandrose

The triacetyl ester derivative, a more preferred form of oleandomycin is prepared by acetylating three hydroxyl groups one in each of the sugars and one in the oleandolide. The preference is due to (a) retention of *in-vivo* antibiotic activity, (b) superior pharmacokinetic properties and (c) its tasteless nature.

(3) Spiramycin and Josamycin:

They are well established clinically in Europe and Japan and now are clinical newcomers in United States. They are mainly indicated for the gram-positive bacterial infections. Both have similar range of activity as erythromycin but are less active. Resistance develops very gradually. However cross - resistance among above four discussed members is reported.

To overcome the drawbacks of erythromycin, a number of semisynthetic macrolides have been produced of which Roxithromycin, Clarithromycin, Azithromycin are some of the examples.

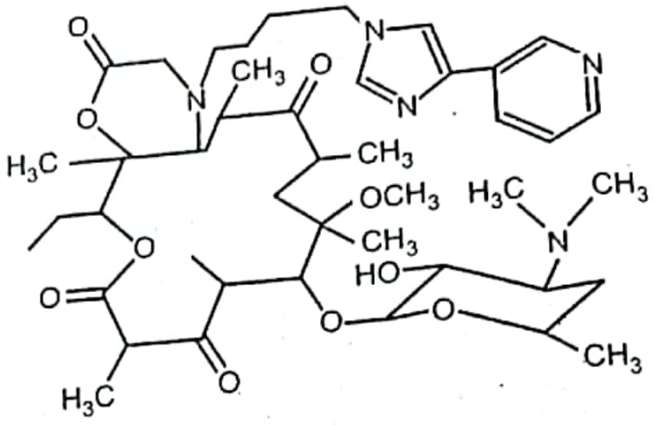
(a) Roxithromycin: It is a semisynthetic long acting acid stable macrolide whose antimicrobial spectrum resembles closely with erythromycin.

(b) Azithromycin: This new azalide congener of erythromycin has an expanded spectrum, improved pharmacokinetics, better tolerability and drug interaction profiles. It is more active than other macrolides against *H. influnenzae* but less active against gram positive cocci.

The improved pharmacokinetic properties are acid stability, rapid oral absorption, marked tissue distribution, and intracellular penetration. Due to higher efficacy, better gastric tolerance, it is now preferred over erythromycin in many infections like pneumonia, trachomatis, tonsillitis, sinusitis and pharyngitis.

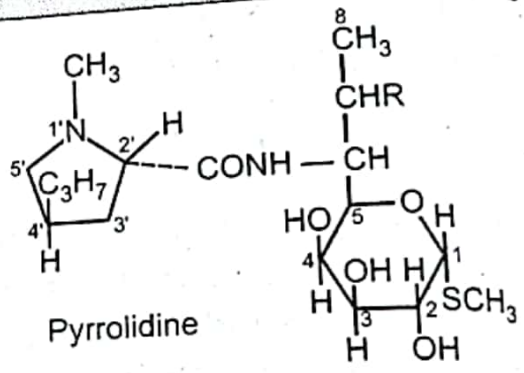
(c) Clarithromycin: It is a semisynthetic macrolide antibiotic derived from erythromycin. It exerts bactericidal effect by inhibiting bacterial protein synthesis. It is used to treat throat infection, pneumonia, skin infections and *H. pylori* infection. It can also be used in combination with anti-ulcer medications to treat certain types of stomach ulcers. It should not be used to treat viruses such as common cold. Side effects include nausea, vomitting, diarrhea and stomach pain.

Telithromycin (2001): It is a semi-synthetic erythromycin derivative. The methoxy group at C_6 improves acid stability and prevents internal hemiketalization. The 3-keto function avoids resistance induction and $C_{11/12}$ carbamate side chain improves ribosome binding. It shares the same basic 14-membered lactone ring structure of erythromycin. It is the first ketolide antibiotic to enter clinical use.



2.2 LINCOMYCINS

The antibiotic Lincomycin is obtained from actinomycete, Streptomyces lincolnensis (so named because it was found in Lincoln, Nebraska) stood as the first example of sulphur - containing antibiotics. Released in 1963, the antibiotic is followed by its synthetic derivative clindamycin in 1967. Latter has an improved antibacterial and pharmacokinetic profile. Lincomycins, in general, possess similar topological, antibacterial spectrum, biochemical mechanism of action, pattern of bacterial-resistance and cross resistance with that of Erythromycin analogs. They exert bactericidal action on gram-positive bacteria particularly *Staphylococcus aureus*. The activity shows concentration dependence pattern.



Pyrrolidine

Lincomycin: R = – OH

Clindamycin: R = – Cl

Variation of the substituents on pyrrolidine portion and C - 5 side-chain affects the activity. e.g.

(1) N-demethylation imparts activity against gram-negative bacteria.

(2) Increase in chain length of the propyl substituent at 4 position in pyrrolidine moiety upto n-hexyl increases *in-vivo* activity about 1.5 times than parent compound.

(3) The thiomethyl ether of α-thiolincosamide moiety is essential for activity.

(4) Structural modifications at C - 7, like introduction of 7S chloro or 7R - OCH_3 changes the physicochemical parameters of the drug (i.e., partition coefficient) and thus alters activity spectrum and pharmacokinetic properties.

The ability of lincomycin to penetrate into bone, adds to its qualities and it gets promoted in chemotherapy of bone and joint infections by penicillin resistant strains of *Staphylococcus aureus*.

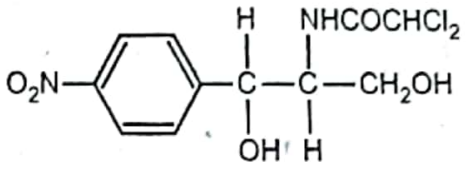
The usual side-effects include skin rashes, nausea, vomiting and diarrhoea. A number of patients developed gastrointestinal complaints ranging into severity from diarrhoea to pseudo-membranous colitis. The latter characterised by diarrhoea, abdominal pain, fever and mucous and blood in the stools, may turn out to be lethal.

2.3 CHLORAMPHENICOL

These antibiotics retain their reputation in the chemotherapy, though do not retain common structural features with any other antibiotic. Due to their high clinical utility, they deserve special attention.

(1) Chloramphenicol:

Originally isolated from *Streptomyces venezuelae* by Ehrlich et al in 1947, chloramphenicol (chloromycetin) is now produced totally by a synthetic route. It contains chlorine and is obtained from an actinomycete and thus named as chloromycetin.



Chloramphenicol has a spectrum of activity resembling that of the tetracyclines except that it exhibits a bit less activity against some gram-positive bacteria. It is specifically recommended for the possesses treatment of serious infections caused by *Hemophilus influenzae*, *Salmonella typhi* (typhoid), *Streptococcus pneumoniae* and *Neisseria meningitidis*. It is marked effectiveness against several gram-negative bacterial and also exhibits antirickettsial activity. Its ability to penetrate into CNS presents an alternative therapy for meningitidis.

SAR of chloramphenicol can be studied under the following headings:

(1) SAR of p-nitrophenyl group:

(a) Replacement of the nitro group by other substituents leads to reduction in activity.

(b) Shifting of the nitro group from the para position also reduces the antibacterial activity.

(c) Replacement of phenyl group by the alicyclic moieties results in less potent compounds.

(d) The p-nitrophenyl group may be replaced by other aryl structures without appreciable loss of activity.

(2) SAR of dichloracetamido side-chain:

(a) Other dihaloderivatives of the side - chain are less potent though major activities are retained.

(b) While in case of trihaloderivatives, Hansch et al in the light of QSAR calculations claimed that the 2 $NHCOCF_3$ derivative would be about 1.7 times as active as the chloramphenicol.

(3) SAR of 1, 3 - propanediol:

The primary alcoholic group on C - 1 atom if modified, results in a decrease in activity hence the alcoholic group seems to be essential for activity.

Of the four stereoisomers of chloramphenicol the antibacterial activity resides in only D-threo compound. Other isomers are inactive compounds.

Chloramphenicol is a broad-spectrum antibiotic. Unfortunately instances of serious hematological abnormalities resulting in aplastic anemia occurred which established the needs to reaudit the clinical position of it. The nitrobenzene moiety of the chloramphenicol molecule is supposed to depress the bone marrow and to affect the elements of blood resulting into a fatal outcome.

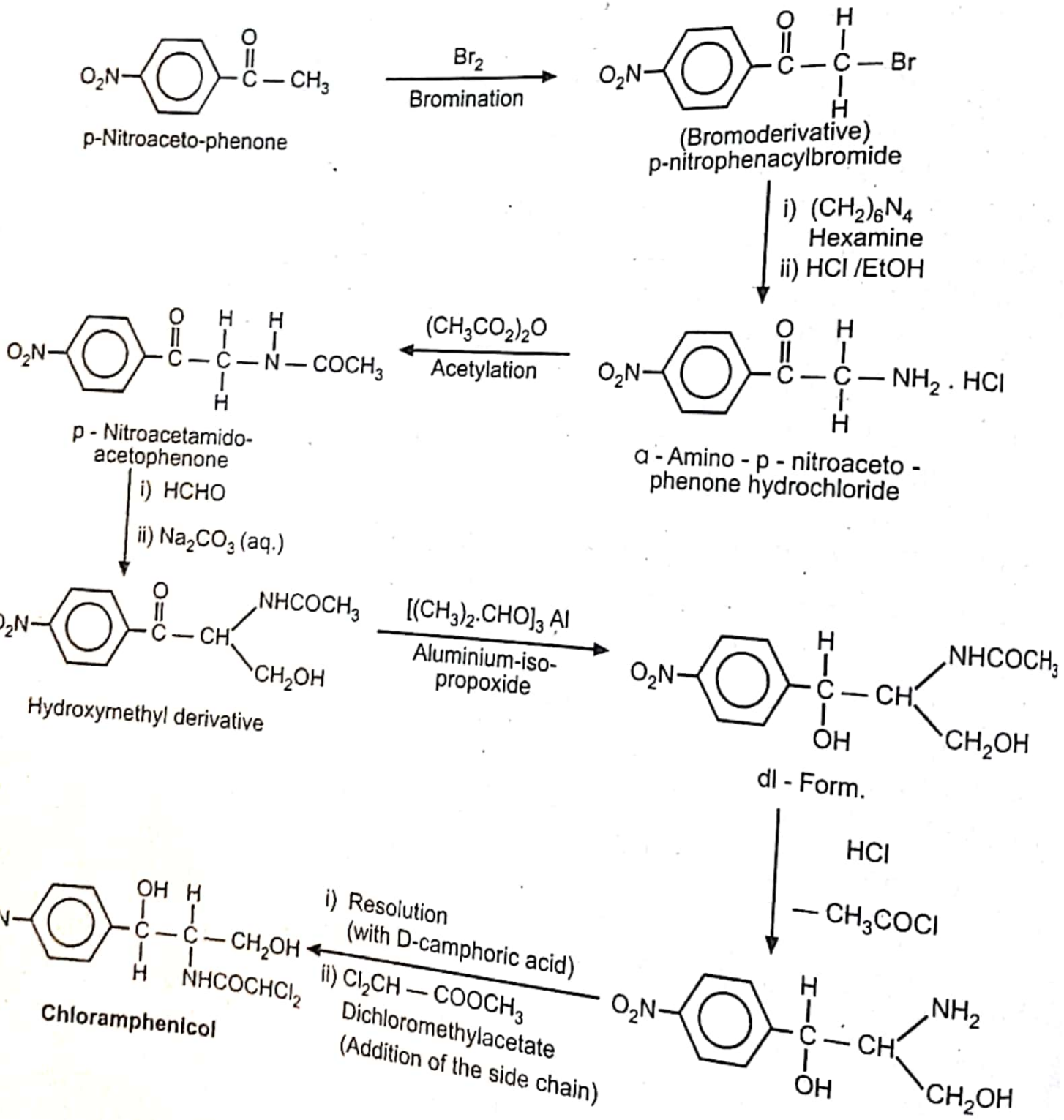
Chloramphenicol offers three reaction sites open for microbial inactivating enzyme resulting into:

(1) reduction of nitro group

(2) hydrolysis of the amide linkage and by chloramphenicol acetyltransferase, acetylate the hydroxyl groups in the 1, 3-propandiol portion to produce initially, 3-acetox chloramphenicol and finally 1, 3, diacetoxychloramphenicol. Both metabolites ar devoid of antibacterial activity.

Synthesis

(i) Chloramphenicol :



Chapter...3

MODES OF ACTION OF ANTIBIOTICS

♦ SYNOPSIS ♦

3.1 INTRODUCTION

The term chemotherapy can be defined as 'the treatment of diseases caused due to infective parasites or organisms' without causing destruction of their host cells. Modern chemotherapy began with the work of Paul Ehrlich (1854 - 1915). Due to his pioneer discoveries in this field, he is regarded as 'Father of Chemotherapy'.

The second phase of revolution emerged in the 1930's following the discovery of the British bacteriologist, Alexander Fleming when he tested the filtrate of a broth culture of a penicillium mold for its antibacterial activity.

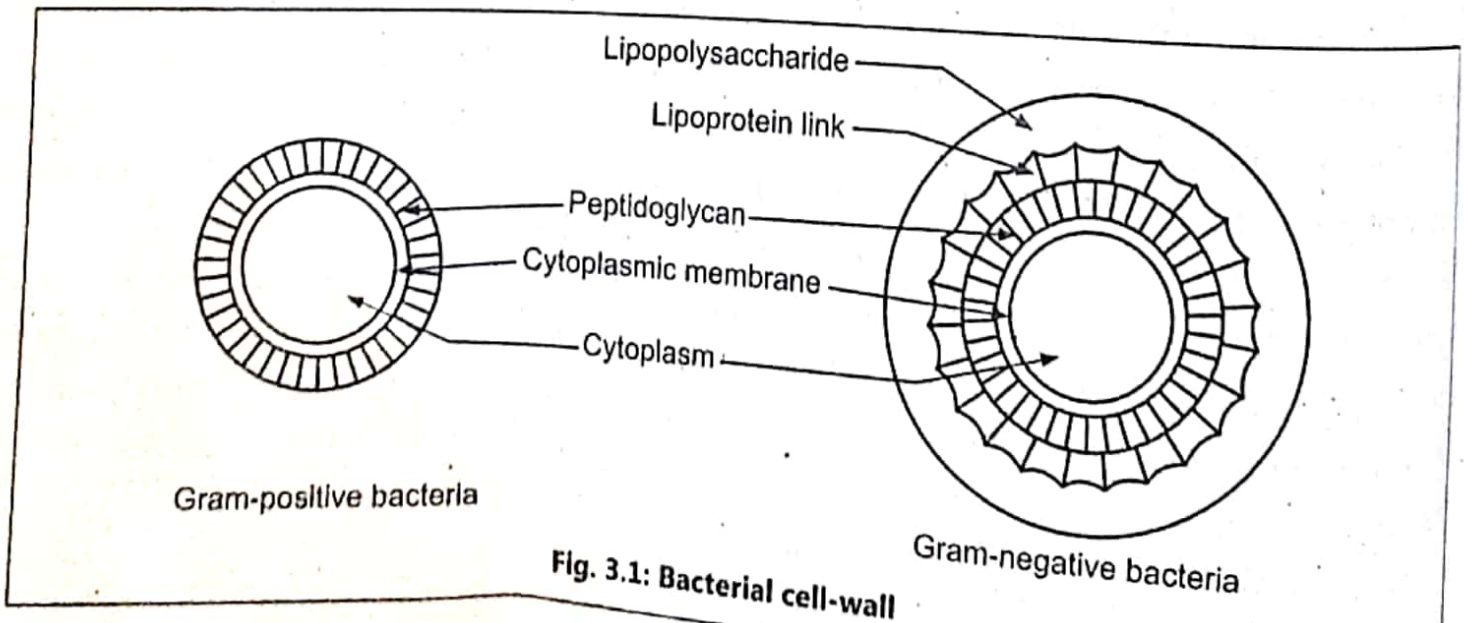
The term antibiotic has its origin in the word, antibiosis (i.e. against life); the latter being first time used by Vuillemin in 1889 in an attempt to describe the concept of survival of the fittest. Although the discovery of penicillin is named after Sir Alexander Fleming in 1928, it was not until 1940 at Oxford that Florey and Chain and their associates isolated it and described its properties in detail and thus turning Fleming's discovery to practical significance. Among the many attempts to define the term, antibiotic, the most appropriate one may be stated as "*Antibiotic is a chemical agent derived from or metabolically produced by microorganism and that in high dilution it antagonizes the growth and/or the survival of one or more species of microorganisms.*" The probable points of differences amongst antibiotics include physical, chemical and pharmacological properties, antibacterial spectra and mechanism of action.

Table 3.1: Landmarks in the field of antibacterial agents

Scientists	Discovery	Period
1. Paul Ehrlich	Father of chemotherapy	1854 - 1915
2. Sir Alexander Fleming	Penicillin	1929
3. Domagk	Prontosil and sulphonamides	1932 - 1935
4. Florey	Penicillin isolation	1939
5. Waksman	Streptomycin	1944
6. Steinberg	Amphotericin B	1956
7 Gentiles	Griseofulvin	1958
8. Prusoff	Clinical use of iodoxuridine	1959
9. Bauer	Clinical use of β-isatin thiosemicarbazone	1962

Park et al in 1952, reported the isolation of first nucleotide linked precursor of peptidoglycan from *Staphylococcus aureus* grown in the presence of benzylpenicillin. They were later identified as uridine diphosphate-acetylmuramyl pentapeptide and its breakdown products like uridine-5'-pyrophosphate and N-acetylmuramic acid. Park nucleotides as they are known, were already shown to be an integral part of the bacterial cell-wall structure. This key observation by Park, led the foundation of the concept that penicillin interfers in the biosynthesis of bacterial cell-wall.

In all bacteria, the cell-wall offers protection to the cytoplasmic membrane against changes in osmotic pressure. The cytoplasmic membrane is a fragile structure and is the site of most of the biological processes of the cell. Any defect in the cell-wall, therefore, may cause the lysis of cytoplasmic membrane and will indirectly impair protein synthesis in bacteria.



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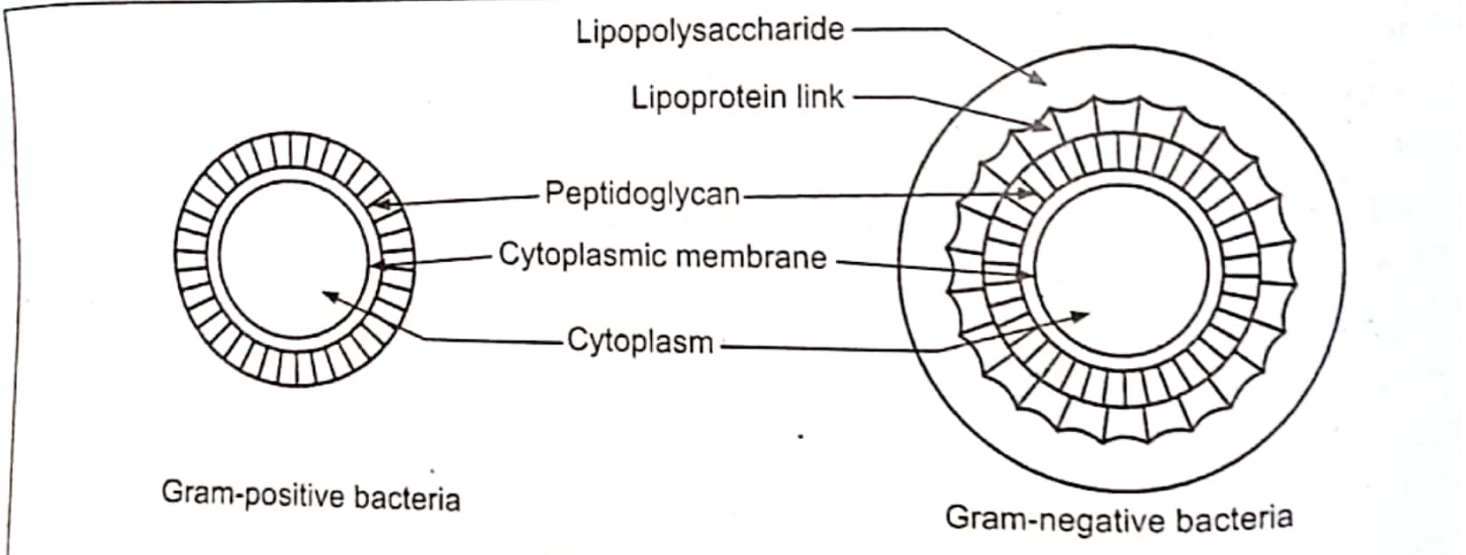


Fig. 3.1: Bacterial cell-wall

The walls of all bacteria contain peptidoglycan as their main structural components. This cell-wall polymer protects the bacterium from the lysis in a hypotonic environment. In gram-positive bacteria, peptidoglycan is the major constituent of the cell-wall and may account for as much as 80% of total weight of the wall. However, in gram negative bacteria, it represents only a minor component of the cell-wall. Peptidoglycan is thus a vital component of bacterial cell wall which offers a network of high tensile strength and rigidity. It is formed through the cross-linking of the glycan chains. Some glycan chains may remain uncross-linked and may get interspread through the peptidoglycan network.

Due to the simplified structure of gram-positive bacteria, they are highly susceptible to the action of β–lactam antibiotics due to the freely permeable nature of their cell-wall. The bacterial cell-lysis occurs due to the activation of autolytic peptidoglycan hydrolases, by β-lactam antibiotics. In gram-negative bacteria, however, above the peptidoglycan, a complex outer membrane is present which contains lipoproteins (linked covalently with the peptidoglycan), phospholipids, lipopoly-saccharide and other components. Through its selective permeable nature, the outer membrane effectively controls the composition of periplasmic fluid that lies between the outer membrane and the cytoplasmic membrane. The permeability of outer membrane greatly varies among gram-negative bacteria, being generally high in Neisseria, intermediate in Enterobacteriaceae and very low in Pseudomonads. Periplasmic fluid is the main site where the β-lactamase enzymes are present in the higher concentration and they decrease the ability of β-lactam antibiotic to enter the gram-negative bacteria. Thus, the selective permeability of the outer membrane and the presence of β-lactamases in the periplasmic fluid are the main factors that lower the effectiveness of β-lactam antibiotics against gram-negative microorganisms.

3.2 PENICILLIN-BINDING PROTEINS (PBP)

The bacterial cell wall consists of specific binding sites for β-lactam antibiotics. These membrane-associated binding sites for antibiotics are known as penicillin-binding proteins. These are nothing but the enzymes (i.e., transpeptidases and carboxypeptidases) which are actually involved in the terminal stages of peptidoglycan biosynthesis. Inactivation of these enzymes occurs mainly through the formation of penicilloyl ester linkages. This results into immediate biochemical defects in the synthesis of cell-wall peptidoglycan.

In gram-negative bacteria, penicillin binding proteins are present in the periplasmic fluid. Due to the high concentration of β-lactamases in this region, it becomes difficult for β-lactam antibiotic to bind with these proteins and to produce biochemical defects in the synthesis of cell-wall. Moreover the impressive variety of β-lactamases that gram-negative bacteria are able to produce, helps the organism to hydrolyze both penicillin and cephalosporins. The highly permeable nature of gram-positive bacteria allows the easy passage of not only all β-lactam antibiotics but also the penetration of larger antibiotics (e.g., bacitracin, vancomycin, etc.) which otherwise remain ineffective against gram-negative bacteria due to their poor penetration ability.

Table 3.2: Bacteria where resistance develops through plasmid mediated mechanisms

Gram-positive bacteria	Gram-negative bacteria
(1) *Bacillus species*	*Citrobacter species*
(2) *Clostridium perfringens*	*Enterobacter species*
(3) *Staphylococcus aureus*	*Escherichia coli*
(4) *Staphylococcus epidermidis*	*Haemophilus influenzae*
(5) *Streptococcus faecalis*	*Neisseria gonorrhoeae*
(6) *Streptococcus pyogens*	*Pseudomonas species*
(7) *Streptococcus pneumoniae*	*Salmonella species*

Most of the bacteria produce β-lactamase enzymes. These enzymes hydrolyze the β-lactam ring of different β-lactam antibiotics including penicillins and cephalosporins. It involves the formation of an acylenzyme intermediate that is probably important in all β-lactamase reactions. Studies with several different β-lactamases have implicated a serine residue as a site for acetylation. After some time, deacetylation occurs to release hydrolyzed β-lactam molecule and a normal β-lactamase enzyme.

The number of penicillin-binding proteins present varies between 10^3 to 10^4 per bacterial cell. Depending upon the molecular weight, these proteins can be broadly catagorised as:

(i) abundant but relatively low molecular weight penicillin-binding proteins, and

(ii) less but relatively high molecular weight penicillin-binding proteins.

3.3 BACTERIAL RESISTANCE TO THE ANTIBIOTICS

The emergence of microbial strains that are resistant to the antibacterial agents obviously limits therapeutic value of these agents. Various mechanisms have been put forward to explain the development of resistant strains of microorganisms.

(a) The production of β-lactamase enzymes will continue to be an extremely important mechanism of resistance to β–lactam antibiotics. The presence of β–lactamases lower down the concentration of antibiotic and decreases the binding of antibiotic to the penicillin-binding proteins present in the bacterial cell-wall.

(b) Resistance to the antibiotic action also developes due to modifications in penicillin-binding proteins through the mutational-effects. This leads to reduction in the penetration ability of the antibiotic. Such mutational changes in binding proteins are reported to occur in methicillin-resistant strains of *S. aureus*, pneumococci and gonococcus and in moxalactam created gram-negative bacilli. Mutation generally occurs at a frequency of 1 in 10^5 - 10^{10} cell division. Resistance to streptomycin by enteric bacilli constitutes the example of spontaneous single step mutation while resistance to penicillin by gonococci exemplifies the development of slow step-wise resistance. Such resistance developed through genetic mechanisms are transformed from resistant cell to the sensitive cell in bacteria by transduction, transformation or conjugation process.

(c) The penetration of the antibiotic in the bacterial cell is governed by permeability of the cell-wall and energy supplied for the transportation. The transport of antibiotic may be hampered due to lack of energy. This may be induced by mutational or a plasmid coded product that interferes in the synthesis of ATP. Moreover alterations in the structural features of the antibiotic result into reduced transportation and decreased affinity of antibiotic to its binding sites. This specifically is reported to occur with aminoglycosides.

Nalidixic acid group and polymyxins are the only exceptional examples of antibacterial agents to which plasmid resistance is not reported to develop.

(d) Sometimes the microorganisms may acquire resistance to the action of antibiotic by developing a totally separate pathway resistant to the attack of antibiotic. Thus, resistance develops due to bypassing the antibiotic-sensitive metabolic step. In these bypass mechanisms, a plasmid provides a cell with an enzyme that is refractory to the action of antibiotic. For example, *E. coli* when treated, with trimethoprim develops resistance due to designing of an altered dihydrofolate reductase enzyme.

In certain cases resistance may be acquired by reducing the demand of the metabolite which is influenced by the anti-bacterial agent. For example, sulphonamide-resistant strains of Neisseria require less thymine than the sensitive strains.

(e) The antibiotic agent, instead of attacking the microorganism, may be utilized to antagonise a biochemical intermediate that is released by microorganism itself.

Table 3.3: Pathogenic microorganisms causing infections in humans

Microorganism	Possible infection
(A) Gram-positive cocci	
1. *Staphylococcus aureus*	abscesses, bacteremia, cellulitis, endocarditis, meningitis, osteomyelitis, pneumonia etc.
2. *Streptococcus bovis*	bacteremia, endocarditis
3. *Streptococcus pyogenes*	bacteremia, cellulitis, erysipelas, otitis media, pharyngitis, acute pneumonia, scarlet fever, sinusitis etc.
4. *Streptococcus agalactiae*	bacteremia, meningitis, septicemia etc.
5. *Streptococcus faecalis (Enterococcus)*	bacteremia, endocarditis and urinary tract infections
6. *Streptococcus viridans*	bacteremia, endocarditis
7. *Streptococcus pneumoniae*	arthritis, endocarditis, meningitis, otitis media, pneumonia, sinusitis etc.
8. *Streptococcus (anaerobic)*	bacteremia, endocarditis, localized abscesses, sinusitis etc.

(B) Gram-negative cocci	
1. *Neisseria gonorrhoeae*	gonorrhea and other infections of genitalia
2. *Neisseria meningitidis (meningococcus)*	bacterimia, meningitis
(C) Gram-positive bacilli	
1. *Bacillus anthracis*	anthrax, pneumonia
2. *Clostridium botulinum*	botulism
3. *Clostridium perfringens (welchii)*	gas gangrene
4. *Clostridium tetani*	tetanus
5. *Corynebacterium diphtheriae*	diphtheria
6. *Listeria monocytogenes*	bacteremia, endocarditis, meningitis
(D) Gram-negative bacilli	
1. *Aeromonas hydrophilia*	osteomyelitis, septicemia, wound infections etc
2. *Bacteroides species*	otitis media, sinusitis, oral infections etc.
3. *Bacteroides fragillis*	bacteremia, endocarditis and tissue abscesse mainly in brain, intra-abdominal region and
4. *Bordetella pertussis*	lung
5. *Brucella abortus*	whooping cough
6. *Campylobacter fetus*	brucellosis
7. *Citrobacter species*	bacteremia, enteritis
8. *Enterobacter aerogenes*	infections of respiratory and urinary tracts.
9. *Escherichia coli*	urinary tract infections
10. *Flavobacterium meningosepticum*	bacteremia, urinary tract infections
	meningitis
11. *Fusobacteriae*	empyema, genital infections, lung abscess ulcerative pharyngitis etc.
12. *Haemophilus ducreyi*	chancroid
13. *Haemophilus influenzae*	bronchitis, meningitis, otitis media, pneumonia sinusitis, respiratory tract infections etc.
14. *Haemophilus vaginalis*	vaginitis, urethritis
15. *Klebsiella pneumonia*	pneumonia, urinary tract infections.
16. *Pasteurella multocida*	bacteremia, abscesses, meningitis etc.
17. *Proteus mirabilis*	urinary tract infections
18. *Providencia*	infections of urinary and respiratory tracts
19. *Pseudomonas aeruginosa*	bacteremia and infections of urinary and respiratory tracts
20. *Salmonella typhi*	typhoid fever
21. *Salmonella species*	bacteremia, gastroenteritis, paratyphoid fever typhoid fever, etc.

22. *Shigella species*	gastroenteritis
23. *Vibrio cholerae*	cholera
24. *Yersinia pestis*	plague
25. *Treponema pallidum*	syphilis
(E) Acid - fast bacilli	
1. *Mycobacterium leprae*	leprosy
2. *Mycobacterium tuberculoseae*	renal, meningeal, miliary and pulmonary tuberculous infections.
(F) Miscellaneous organisms	
1. *Borrelia recurrentis*	relapsing fever
2. *Actinomyces israelii*	abdominal, cervicofacial and thoracic lesions
3. *Chalamydia trachomatis*	inclusion conjunctivitis, pneumonia, trachoma and urethritis
4. *Mycoplasma pneumoniae*	atypical pneumonia
5. *Urea plasma urealyticum*	urethritis

3.4 PATHOGENIC MICROORGANISMS

In 1877, Pasteur and Joubert reported for the first time the pathogenic potential of microorganisms. Thereafter the era of chemotherapy began. The term chemotherapy, first coined by Paul Ehrlich, can be defined as "*treatment of infection caused by pathogenic microorganisms leaving the host unaffected.*" Microorganisms may gain entry to the tissues of the body; may release poisonous substances (toxins); may establish themselves in the organ and may alarm fatal reactions in the host. The whole process can be described as an infection. From the sites of initial infections, organisms may pass into the circulating blood and accommodate themselves at suitable places to release their next generations. The antibacterial agents were thus desired which will be selectively toxic against microorganisms but with much reduced toxicity towards the host. People tried to find out the biochemical differences between the infective organism and the host. Selective toxicity of the chemotherapeutic agents against pathogenic organism could be achieved by just then exploiting these differences.

3.5 CLASSIFICATION OF ANTIBIOTICS

There are various ways by which antibiotics can be classified. The probable points of differences regarding chemical and pharmacological properties, antibacterial spectra and mechanism of action serve as the basis of classification.

(i) Depending upon clinical effectiveness, spectrum of activity and degree of selectivity, the antibiotics that inhibit only certain groups of micro-organisms are called as 'narrow spectrum antibiotics'. Examples include nystatin and bacitracin. These antibiotics exhibit a high degree of selectivity. Many antibiotics inhibit both gram-positive and gram-negative bacteria and/or other intracellular organisms, which are called as 'broad spectrum antibiotics'. Examples include chloramphenicol and tetracyclines.

(ii) Depending upon the sources from which antibiotics are derived, they can be categorised as follows:

(a) Natural:

These antibiotics are naturally obtained from the large scale fermentation of micro organisms. For example, bacitracin and polymixin are obtained from some bacilli while streptomycin is obtained from Streptomyces griseus.

(b) Semisynthetic:

The observation that 6-aminopenicillanic acid can be obtained from the cultures of *P. chrysogenum*, led to the development of this class. For example, during the commercial production of benzyl penicillin, the phenylacetic acid is added as the side-chain precursor to the medium in order to achieve predominance of the product.

(c) Synthetic:

This class includes antibiotics which are of purely synthetic origin. Chloramphenicol, for example, initially was isolated from a fermented media in 1947 and later was produced synthetically on the commercial basis.

(iii) The general scheme of classification include following different categories of antibiotics:

(a) β-lactam antibiotics

(b) aminoglycoside antibiotics

(c) tetracycline antibiotics

(d) peptide antibiotics

(e) macrolide antibiotics

(f) lincomycins, and

(g) unclassified antibiotics

(iv) The fourth basis of classification of antibiotics is their mechanisms of action. Accordingly these agents can be categorised as:

(a) Drugs that interfere with the biosynthesis of bacterial cell-wall. Examples include penicillins, cephalosporins, cycloserine, bacitracin and vancomycin.

(b) Drugs that interfere with the functioning of cytoplasmic membrane of bacteria or fungi. Examples include polymyxins, amphotericin β, colistin and nystatin.

(c) Drugs that interfere with the protein biosynthesis in microorganisms. Examples include erythromycin, lincomycins, tetracyclines and chloramphenicol.

(d) Drugs that interfere with the nucleic acid biosynthesis in microorganisms. Examples include actinomycin, griseofulvin and rifampin, and

(e) Drugs that antagonise the essential metabolic processes in microorganisms. Examples include sulphonamides, trimethoprim and most of anticancer drugs.

3.6 MECHANISMS OF ACTION OF ANTIBIOTICS

Today hundreds of antibiotics are clinically available and future prospects about new additions are also bright. The new additions can easily be accommodated in the present frame of classification because antibiotics follow certain common tracks to exert their antibacterial activity. All presently available antibiotics can be classified according to the target sites they prefer to exert their action. Following are major routes through which antimicrobial activity is exerted.

(a) Inhibition of cell-wall synthesis,

(b) Inhibition of protein biosynthesis,

(c) Cisorganisation of cytoplasmic membrane

(d) Interference in nucleic acid biosynthesis, and

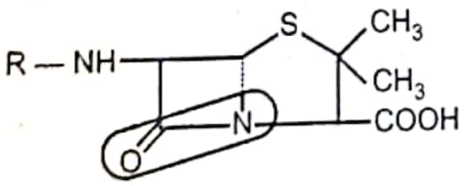
(e) Inhibition of biosynthesis of tetrahydrofolate.

(a) Inhibition of Bacterial Cell-wall Synthesis:

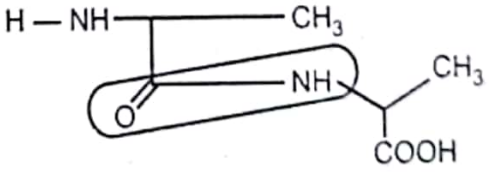
(i) UDP-acetylmuramyl - pentapeptide - UDP - N-acetylmuramic acid - $(amino\ acid)_5$ is called as 'Park nucleotide'.

The benzyl penicillin induced accumulation of Park nucleotides in the growth medium of staphylococcus alongwith the presence of shorter peptide chain indicated that penicillin affects state IV (i.e., cross-linking) of bacterial cell-wall synthesis. This results due to the inhibition of transpeptidation reactions. The cell-wall network loses its rigidness and the cell ruptures by osmotic lysis.

The cell-wall is essential for the growth and survival of bacteria. Rigid stability of cell-wall is provided by a highly cross-linked lattice like structure, composed of peptidoglycans. There is a close structural similarity between penicillins or other β-lactam antibiotics and the D-alanyl-D-alanine end of the polypeptide side-chain of peptidoglycan.



Penicillins



D-alanyl-D-alanine

The labile CO-N bond in the β-lactam ring of penicillin lies in the same position of peptide bond involved in transpeptidation. Due to this similarity, penicillin binds to the transpeptidase enzyme through covalent bonding instead of D - alanyl - D alanine end of polypeptide. Thus, the enzymes necessary for transpeptidation reaction (or for cross-linking of polypeptides) are occupied by the antibiotic. This irreversible inactivation of enzymes by penicillin results into the formation of faulty and weak cell-wall. Bacteria fail to divide. They may swell and then rupture with exudation of the cell-contents.

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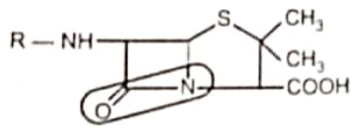
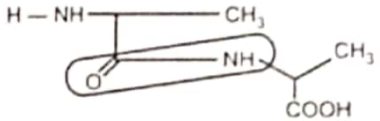


Penicillins



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The reversible enzyme-penicillin complex undergoes an irreversible change resulting the opening of the β-lactam bond. Thereafter the enzyme is released at a very slow from the inactive penicilloyl complex, and can not carry out its assigned functions.

(ii) The peptidoglycan hydrolase (autolysins) enzymes play a key role in the lysis bacteria. Penicillins are reported to decrease the availability of the inhibitors of autolys The uninhibited enzyme then performs its duty without any hesitation. However, autolys are not necessarily present in all bacteria. For example, *Staphylococcus aureus* and *Strep coccus pneumoniae* lack these enzymes.

(iii) In bacterial cell, the cell-membrane is generally present inside the cell-wall. So antibiotics may change or alter the permeability of the cell membrane, leaving the cell-w undisturbed. For example, novobiocin, nystatin and polymyxins interfere with the integ and functioning of cell membranes of microorganisms.

(iv) Unlike penicillins, vancomycin and ristocetin form the stable 1 : 1 complexes wi D-alanyl-D alanine end of polypeptide and interfere in the peptidoglycan synthes Bacitracin complexes with membrane lipid pyrophosphate protein of peptidoglycan a interferes in cell-wall synthesis.

(v) L-alanine is converted to D-alanine by the action of alanine racemase. Such tw molecules get condensed to form D-alanyl-D-alanine by the action of D-alanyl-D-alanin synthetase enzymes. The substances which are unique to cell-wall of bacteria and oth microorganisms include diaminopimelic acid, muramic acid, teichoic acid, amino sugar amino acids, carbohydrates and lipids. Amino acids that are important for the cell-wa synthesis include L-alanine, D-alanine, D-glutamic acid, L-lysine, meso-diamin-opimelic aci glycine, L-serine, L-threonine and D-aspartic acid. Due to the close structural similarity wit D-alanine, D-cycloserine competitively inhibits both, alanine racemase and D-alanyl -D alanine synthetase enzymes. This results into impairment in the synthesis of cell-wall.

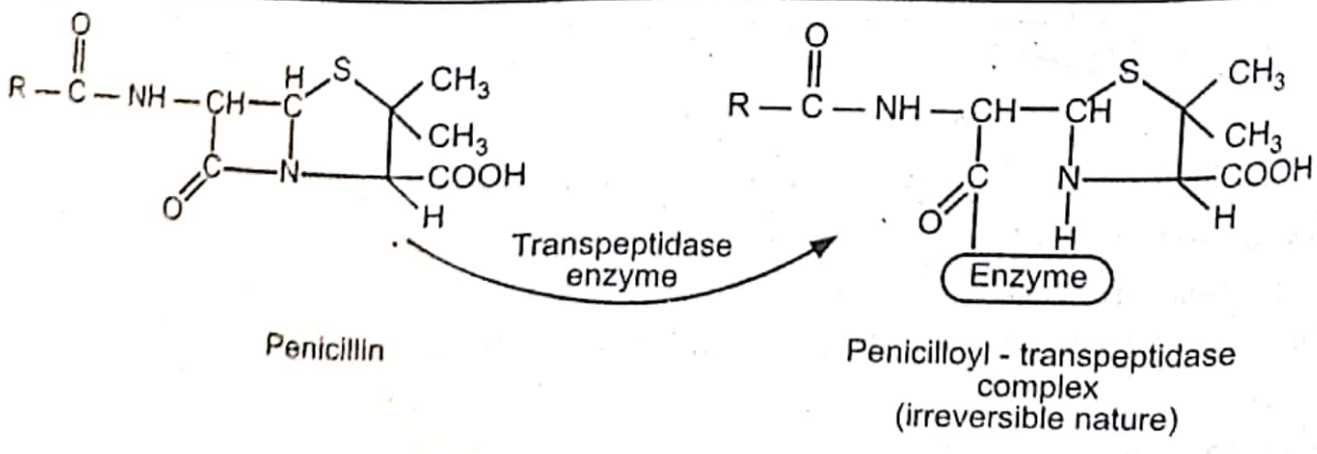

Fig. 3.2: Inactivation of transpeptidase enzymes

(b) Inhibition of Bacterial Protein Synthesis:

Protein biosynthesis is perhaps one of the important processes that provide peptides. These may be assembled as per the needs of the organism, in the proper sequence to biosynthesis various enzymes and/or nucleic acids.

The important events in the protein biosynthesis can be outlined as:

(i) amino acid activation (ii) formation of aminoacyl-t-RNA

(iii) peptide bond formation (iv) translocation.

The antibacterial activity results due to the attack of the drug on one or more of the above events occurring on the ribosomal (r-RNA) surface.

The bacterial ribosomes differ from mammalian ribosomes. The difference has been figured out by their sedimentation coefficients. For example, bacterial ribosomes have the sedimentation coefficient of 70 (70 S) with two subunits 30 S and 50 S. While the mammalian cytoplasmic ribosomes are 80 S and give rise to 40 S and 60 S subunits. Mitochondria contain similar (but not identical) ribosomes to that of the bacterial ribosomes. Hence the degree of selectivity of an antibiotic will define its clinical effectiveness. For example, erythromycin does not bind to the mammalian ribosomes. It selectively inhibits bacterial protein synthesis by binding to the 50 S ribosomal subunits of sensitive strains of microorganisms. Protein synthesis in microorganisms is affected by many antimicrobial as well as anticancer agents. Chloramphenicol, macrolides and lincomycin bind to 50 S ribosomes while tetracyclines block the reaction between amino acid -t-RNA and ribosome on m-RNA. All these antibiotics destabilize ribosomes by inhibiting transpeptidation on polyribosomes by inducing conformational changes in ribosomes. They also interfere with translocation reactions.

It seems that the antibiotic bound ribosomal subunit still can offer the space and activity sufficient to produce small chain peptides. Thus, the ribosome cycle continues but polypeptide elongation is prevented. Similarly tetracyclines and streptomycin also bind to and inhibit the attachment of the 30 S subunits to the m-RNA.

(c) Disorganisation of the Cytoplasmic Membrane:

Next to the cell-wall, cytoplasmic membrane serves the purpose of protecting the vital bacterial cell constituents from damage. If this membrane is disorganised due to any reason, it results into rapid killing of that microorganism. In contrast to bacteria, the fungal membrane contains sterol as the membrane constituent, which is mainly ergosterol.

Antibiotics like polymyxins, may damage the integrity of the cytoplasmic membrane by disorienting the lipophilic groups present in the membrane. This leads to the leakage of intracellular components. Amphotericin B and nystatin have a high affinity for sterols present in fungal membrane. Hence, these antibiotics possess a potent antifungal activity. They combine with the membrane sterols and thus create pores or channels in the fungal membranes. They are ineffective against bacteria since bacteria do not have sterols as their membrane constituents.

Unfortunately mammalian cell membranes also consist of sterols. This is the reason why the margin of safety of antifungal antibiotics is quite narrow.

(d) Interference in the Bacterial Nucleic Acid Synthesis:

Nucleic acid synthesis is affected mainly by anticancer agents. Nucleic acids (i.e., DNA and RNA) are the vital ingredients of the microbial cell. DNA governs both the quality and quantity of RNA synthesis, while RNA is a key instructor that read out the message on which the synthesis of various proteins and enzymes, necessary for overall growth of microorganism depends.

RNA molecules are synthesized by the polymerization of ribonucleoside triphosphates under the influence of DNA-dependent RNA polymerase enzyme. DNA template serves as a platform upon which the transcription occurs. Thus, the sequence of peptides in RNA being synthesized, is nothing but the copy of the sequence in that particular DNA - template on which, RNA has been synthesized. Antibiotics may affect the nucleic acid biosynthesis in two different ways:

(i) By interacting specifically with the enzyme (i.e., DNA dependent RNA - polymerase) which catalyses the polymerization of ribonucleoside triphosphates.

(ii) Or by interacting with the DNA template and thus disturbing the whole process.

Rifampicin represents an example of drugs acting through first mechanism. If it is added to the culture medium where the enzymes are synthesizing RNA chains, the enzyme process is not immediately inhibited. The bio-synthesis of new chains stops but partly formed RNA chains continue the process till completion. In brief, rifampicin does not interfere in chain elongation and termination but inhibits the chain initiation processes. Such sense of selectivity of action is not uniformly observed in the antibiotics which act through second mechanism. For example, rifampicin does not affect mammalian cell but actinomycin (which interferes with DNA template) is unselective in action, affecting both the host cells and bacteria. It binds specifically to helical double-stranded DNA and does not interact at all with RNA synthesis.

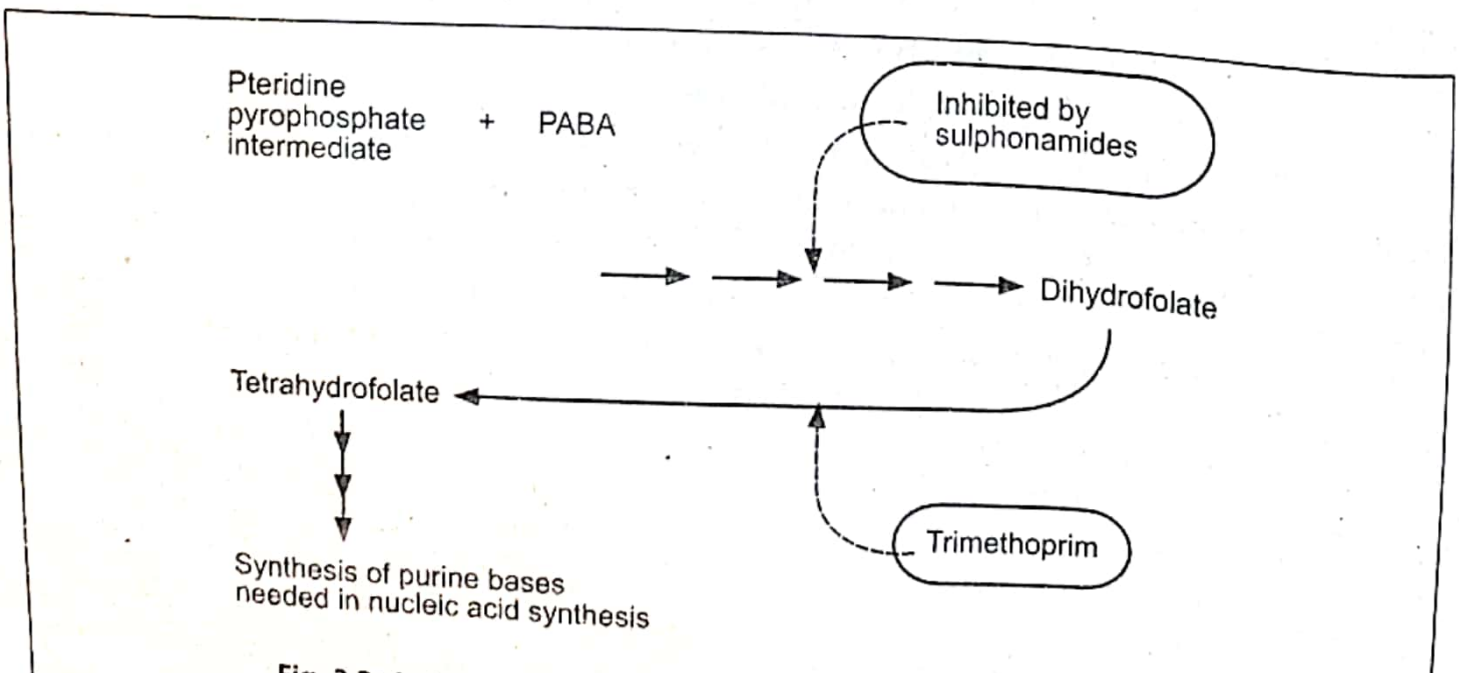

Fig. 3.3: Antagonism of essential metabolic processes in bacteria

(e) Inhibition of the Tetrahydrofolate Biosynthesis:

Sulphonamides are structural analogs of para-amino benzoic acid which is an essential metabolite in the bacterial cell and acts as a precursor of folic acid. Both in the host cells and microorganisms, the tetrahydrofolate serves as an essential co-factor in the transfer and reduction of 1-carbon fragment and for the production of nucleic acid via synthesis of methionine, thymine and other purine bases.

Due to the structural similarity with PABA, the sulphonamides interfere with the reaction between pteridine pyrophosphate intermediate and PABA. While trimethoprim selectively attacks and inhibits dihydrofolate reductase enzyme that catalyzes the reduction of dihydrofolate to tetrahydrofolate product.

The dietary folic acid is sufficient to meet the metabolic demands for tetrahydrofolate in the case of mammalian cells. Micro-organisms, however, are unable to utilize preformed folic acid and have to rely upon their own machinery to biosynthesize it from pteridine pyrophosphate intermediate and para aminobenzoate.

3.7 NEPHROTOXIC REACTIONS OF ANTI-MICROBIAL AGENTS

In the earlier paragraphs, we have discussed some important mechanisms of action by which antimicrobial agents bring about their therapeutic effectiveness. In many drugs, the adverse effects associated with the clinical use can be explained by considering them as the extension of their therapeutic effects. Nephrotoxicity is the main adverse effect associated with the use of most of antimicrobial agents. It occurs mainly due to the mechanism of action of these drugs and due to the fact that the urinary excretion serves as the dominating route for the elimination of major fraction of administered dose. The prominant nephrotoxic reactions of antimicrobial agents include:

(a) **Hypersensitivity induced glomerular damage:** It is reported to occur with penicillins, sulphonamides, amphotericin etc.

(b) **Tubular necrosis at proximal tubule:** It is caused by cephalosporins, polymyxins, aminoglycosides etc.

(c) **Distal tubular damage** is characterised either by distal tubular acidosis (e.g. amphotericin) or hypokalemic alkalosis (e.g. penicillins, carbenicillin, ticarcillin).

(d) **Renal blood vessel damage** is characterised either by decreased renal blood flow (e.g., amphotericin) or due to anaphylaxis reaction caused by penicillins, cephalosporins or sulphonamides.

(e) **Interstitial nephritis:** It is reported to occur with penicillin or cephalosporin administration.

(f) **Obstruction in collecting duct** is induced by sulphonamides while demethyl chlortetracycline causes nephrogenic diabetes insipidus.

3.8 NOVEL β-LACTAM ANTIBIOTICS

With the introduction of penicillins in the early 1940s, various microbial infections caused due to strains of staphylococci, streptococci, *H. influenzae* and *Neisseria gonorrhoeae* were soon brought under control. However, in the past several decades resistance to β-lactam has become an increasingly serious problem of concern. To deal with the problem of β-lactamase enzymes, attempts were made to synthesize new β-lactam antibiotics with increased stability to this enzymatic hydrolysis. The classic β-lactam antibiotics include two prominant classes like penicillins and cephalosporins. The non-classic β-lactam antibiotics are mostly derived after 1970. The prominant members of this category are reported in the table 3.4. Carbapenems possess a potent broad spectrum antibacterial activity in addition to β-lactamase inhibitory activity. They exhibit a high degree of activity against Gram-positive and Gram-negative bacteria but lack significant activity against Pseudomonas aeruginosa.

Oxacephems result due to substitution of sulphur atom in dihydrothiazine nucleus by an oxygen atom while carbacephems were developed by replacing sulphur atom in dihydrothiazine nucleus by a carbon atom.

3.9 β-LACTAMASE INHIBITORS

The emergence of antibiotic-resistance strains of microorganisms proved to be a major limitation to the clinical utility of antibiotics. β-lactamases can hydrolyze the β-lactam ring of different β-lactam antibiotics including penicillins, cephalosporins, carbapenems and monolactams. Various measures were then undertaken to develop such inhibitors that will bind or inactivate β-lactamases present in the microorganisms.

In a microorganism, β-lactamases may be present at extracellular as well as intracellular sites. These enzymes when released into external environment, will prevent the access of antibiotic towards the microorganisms by rapidly inactivating the drug. The membrane bound intracellular β-lactamase will protect the organism from the residual antibiotic that escapes from the attack of extracellular enzyme.

Inactivation of β-lactam antibiotics is brought about by these enzymes through the cleavage of CO-N bond present in the β-lactam ring. Enzymes form a sort of irreversible complex with the carbonyl group. Studies with several different β-lactamases have implicated a serine residue as acylation site. Regeneration of the active enzyme from this complex then occurs through hydrolysis of the acyl linkage.

By providing false substrates having very high affinity for β-lactamase enzyme with long term occupying capacity (i.e. very slow rate of deacylation), we can effectively increase potency of β-lactamase sensitive antibiotics. Such substrates are known as β-lactamase inhibitors. However, such agents must be able to inhibit not only extracellular β-lactamases but also should penetrate the bacterial cell-wall at adequate concentration to inhibit intracellular β-lactamases. They should also have the broad spectrum of activity covering β-lactamases present in both, gram-positive and gram-negative bacteria.

Table 3.4: Non-classical β-lactam antibiotics

Examples	Year of introduction
1. 7 α-methoxy cephalosporins (cephamycins)	1971
2. Amidinopenicillins	1972
3. Nocardicins	1976
4. Clavulanic acid	1976
5. Carbapenems	
(a) Olivanic acids	1976
(b) Thienamycins	1978
(c) Epithienamycins	1977
(d) Asperenomycins	1982
(e) Pluracidomycins	1982
(f) Carpetimycins	1981
6. Oxacephems	1978
7. Carbacephems	1984
8. Monobactams	1981

Examples of clinically used β-lactamase inhibitors include clavulanic acid, sulbactam, olivanic acids and halogenated sulfone derivatives. These inhibitors in general possess weak antibacterial activity of their own.

3.10 SYNERGISTIC ANTIBIOTIC COMBINATIONS

Many antibiotics are effective only against gram-positive microorganism. Some agents show antibacterial activity only against gram-negative pathogens. Such a narrow spectrum of activity exhibited by these agents imposes limitation to their clinical utility. Moreover the emergence of antibiotic resistant strains of microorganisms has had a marked influence on chemotherapy. In certain cases, relatively high concentrations are required when a single antibiotic is used. Such high concentrations of the drug expose the patient to the high risk of serious adverse effects. All the above circumstances emphasizes the need of using the combination therapy of such antibiotics having synergistic anti-bacterial activity. When used concurrently, antimicrobial drugs may exhibit additives, antagonistic or synergistic effects.

The combination antibacterial therapy not only broadens the antibacterial spectra but also reduces the probability of emergence of antibiotic - resistant strains of microorganisms. The combination therapy provides such a useful approach for both - to enhance the clinical efficacy of the antibiotics and to lower down the risk of serious adverse effects of the drugs at the same time.

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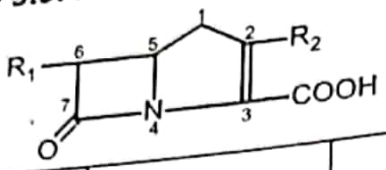
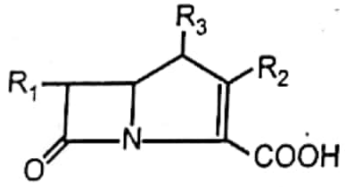
Table 3.5: Some novel carbapenems



Name	Source	R_1	R_2	Year of Introduction
1. Thienamycin	*S. cattleya*	$-CH(CH_3)OH$	$-SCH_2CH_2NH_2$	1979
2. Northienamycin	*S. cattleya*	$-CH_2OH$	$-SCH_2CH_2NH_2$	1979
3. Carpetimycin A	*S. griseus*	$-C(CH_3)_2$- OH	$-SOCH=CHNHCOCH_3$	1980
4. Carpetimycin B	*S. griseus*	$-C(CH_3)_2OSO_3H$	$-SOCH=CHNHCOCH_3$	1980
5. Pluracidomycin A	*S. sulfonafaciens*	$-CH(CH_3)OSO_3H$	$-SO_3H$	1982
6. Pluracidomycin B	*S. pluracidomyceticus*	$-CH(CH_3)OSO_3H$	$-SOCH_2COOH$	1982
7. Pluracidomycin C	*S. pluracidomyceticus*	$-CH(CH_3)OSO_3H$	$-SOCH(OH)_2$	1982
8. Asparenomycin A	*S. tokunonensis*	$=C(CH_3)CH_2OH$	$-SOCH=CHNHCOCH_3$	1982
9. Asparenomycin B	*S. argenteolus*	$=C(CH_3)CH_2OH$	$-SOCH_2CH_2NHCOCH_3$	1982
10. Asparenomycin C	*S. argenteolus*	$=C(CH_3)CH_2OH$	$-SOCH=CHNHCOCH_3$	1982

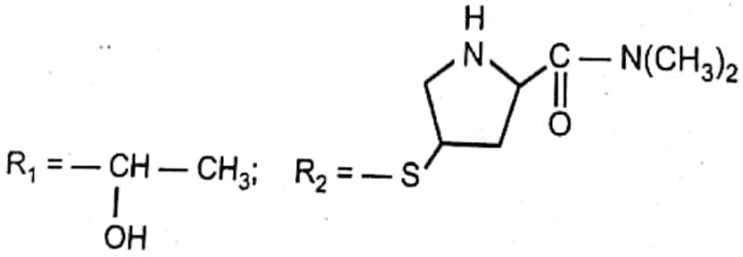
Table 3.6: Some examples of non-classical β-lactam antibiotics derived after 1970

(i) Carbapenem:



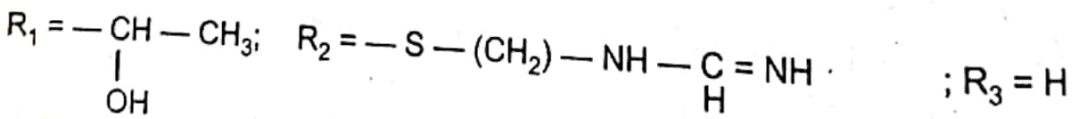
($R_1 = R_2 = R_3 = -H$)

(ii) Meropenem:



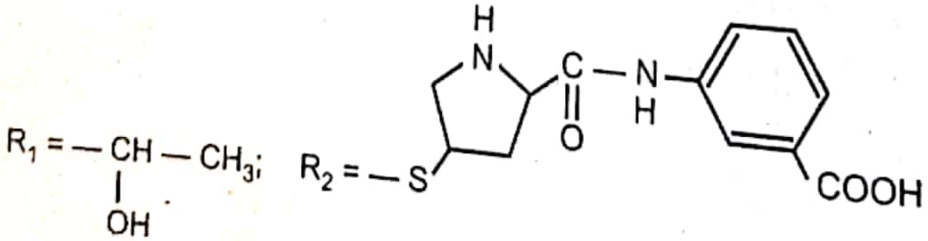
; $R_3 = -CH_3$

(iii) Imipenem:



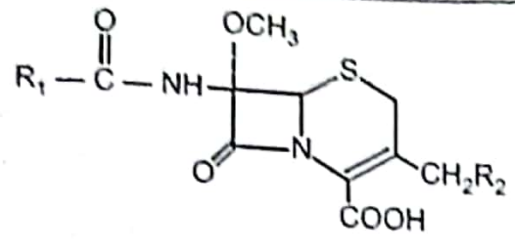
; $R_3 = H$

(iv) Ertapenem:



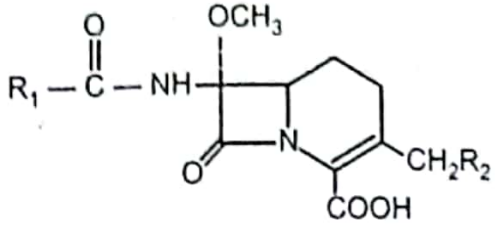
; $R_3 = -CH_3$

(a)



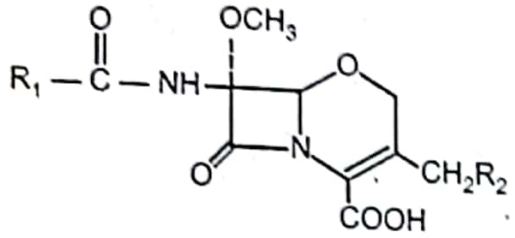
Cephamycins

(d)



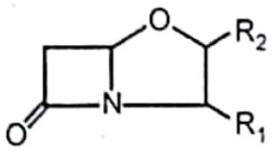
Carbacephems

(b)



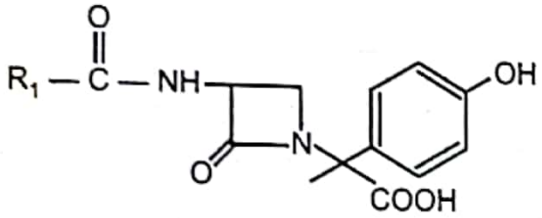
Oxacephem

(e)



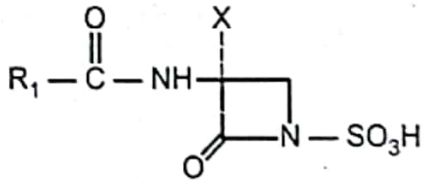
Clavam

(c)



Nocardicins

(f)



Monobactam

Many newer β-lactam antibiotics are commonly used in combination with monoglycoside to provide a broader spectrum of antibacterial activity. In this, β-lactam antibiotics probably facilitate the penetration of monoglycosides in the bacteria.

Other examples of effective antibiotic combinations include gentamicin and cephalosporin in the treatment of gram-negative bacteremic shock and carbenicillin with gentamicin to delay the emergence of resistant strains in tuberculosis and in severe pseudomonas aeruginosa infection.

Such antibiotic combinations comprised of

(i) drugs having similar mode of action, and

(ii) bactericidal drug and bacteriostatic drug should never be used.

Several classes of agents are available for the treatment of bacterial infections (Fig. 3.4). These include the β-lactams and the glycopeptides which target the peptidoglycan cell wall, the tetracyclines and macrolides, which target bacterial protein synthesis and the quinolines whose mode of action is to bind the complex formed between DNA gyrase and DNA, thereby forming a bactericidal ternary complex.

3.11 SUPRAINFECTION (SUPERINFECTION)

It is the emergence of a new strain or a new species of pathogenic microorganisms or fungi (usually Candida albicans) in the patient receiving antimicrobial treatment, especially of broad-spectrum antibiotic.

A normal non-pathogenic bacterial flora present in the human body include more than 300 different species of organisms. Antibiotic therapy (especially with broad spectrum antibiotic), depresses this normal bacterial flora that leads to emergence of drug-resistant pathogenic microorganisms in the body. Suprainfection usually affects gastrointestinal and genital tracts.

The common symptoms of suprainfections include oral burning, xerostomia, black hairy tongue, stomatitis, glossitis, cheilosis, enteritis, colitis, diarrhoea and pruritus ani. Some members of normal bacterial flora of the human body are infective in nature but they remain silent because of strong cellular immune and phagocytic system of the normal adult. Suprainfection due to such members may also occur in patients with impaired cellular immune and phagocytic defence mechanisms.

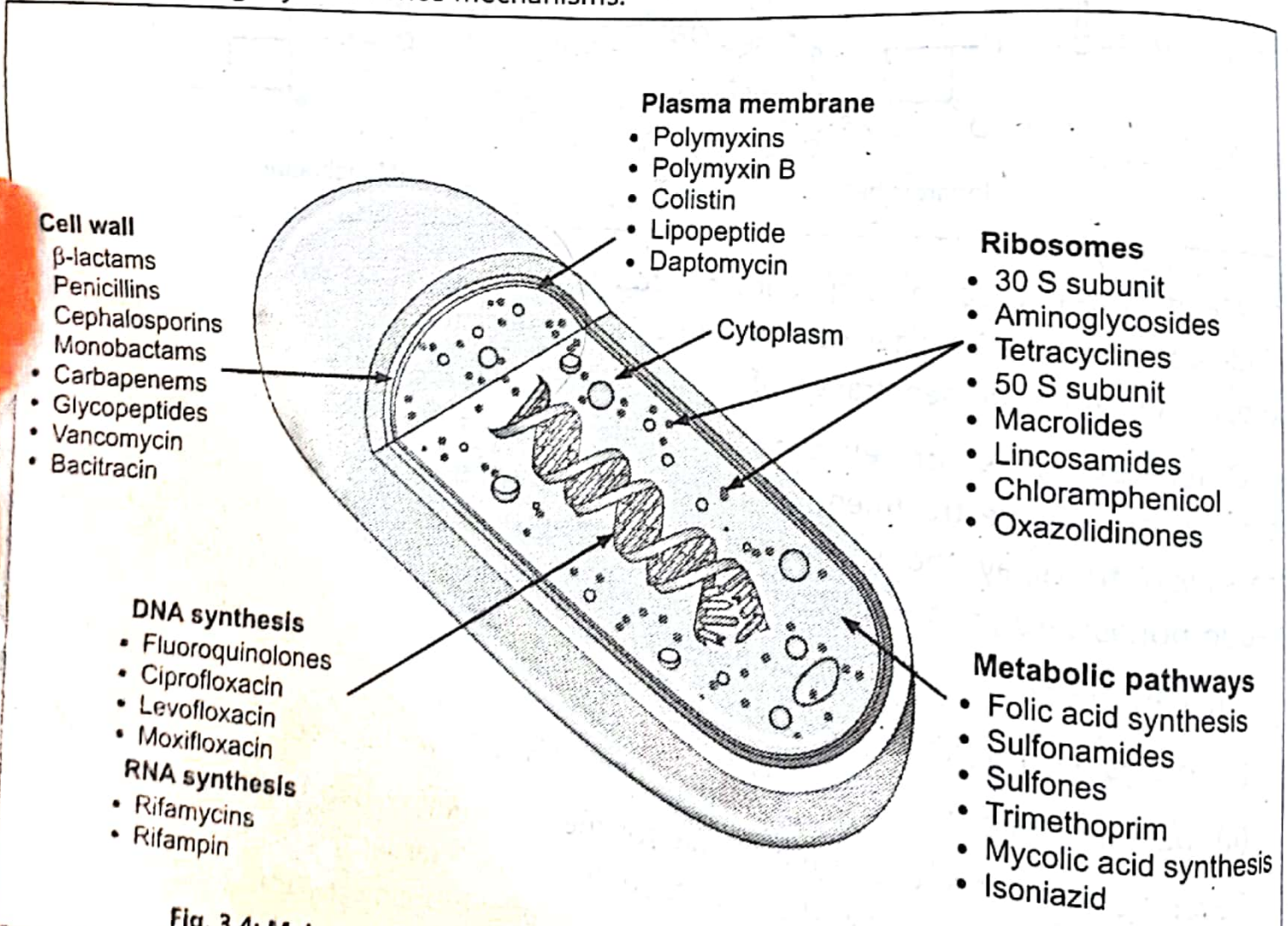


Fig. 3.4: Major classes of systemic antibacterial agents and their tar

Chapter...4

PRODRUGS

♦ SYNOPSIS ♦

4.1 PRODRUG DESIGNING

Prodrug is a chemically modified form of a drug which has a superior delivery property. The term prodrug was coined by Albert.

In 1958, another similar term 'drug latentiation' proposed by Harper (1959) is defined as the chemical modification of a biologically active compound to form a new compound which, upon *in-vivo* enzymatic attack, will liberate the parent compound. It refers to a pharmacologically inactive compound that is converted to an active drug by a metabolic biotransformation at appropriate time and place in the body without substantial direct elimination or untoward metabolism. This activation may occur at any time during absorption, distribution or metabolism. For instance, castor oil is a laxative because it is hydrolyzed intenstinally to the active, ricinoleic acid. Another classical example is conversion of prontosil to sulfanilamide.

Prodrug thus may be considered as drug containing specialized non-toxic protective group utilized in a transient manner to alter or eliminate undesirable properties in the parent drug.

Prodrug designing is required to overcome many formulation, pharmacokinetic or pharmacodynamic drawbacks. The prominent drawbacks include:

(i) unpleasant taste or odour (gastric irritation),
(ii) a wide range of adverse effects,
(iii) shorter duration of action,
(iv) instability.
(v) site non-specificity,
(vi) poor absorption or distribution,
(vii) poor water solubility,
(viii) some compounds are more active but unable to reach the site of action (e.g., GABA).

4.2 TYPES OF PRODRUG

(a) Non-intentional prodrug:

Sometimes, after administration of the drug the metabolic studies indicate the prodrug nature of drug. It becomes accidentally evident that the activity of a drug is because of its metabolite and not because of the parent drug. Example includes the anti-inflammatory agent, sulindac.

(b) Carrier-linked prodrug:

It is a compound that contains an active drug linked to a carrier group that can be removed enzymatically, such as an ester which is hydrolysed to an active carboxylic acid containing drug. The carrier group must be non-toxic and biologically inactive when detatched from the drug. It should be removed easily to allow the active drug to be released efficiently *in-vivo*. The most common reaction for activation of carrier-linked prodrugs is hydrolysis.

A simple hydrolysis reaction cleaves the transport moiety at the adequate rate (e.g., progabide, bacampicillin).

Targetting of Drugs: The side effects associated with a drug are the outcome of non-specific distribution of the drug administered. Targetted drug delivery not only decreases the side effects but also helps to lower down the therapeutic dose of the drug by enhancing the selectivity of attack. This can be achieved by selecting pharmacologically inert, ready to degrade and non-immunogenic carriers to convey the drug molecules selectively towards their target cells. The link between the drug and its carrier should remain inert and stable in the blood stream and extracellular spaces but should be sensitive to the enzymes present in or around the target sites. Nanoparticles, microspheres, lisosomes, glycolipids, antibodies and peptide hormones are being evaluated as carriers for a variety of medicinally active agents. At present targetted drug delivery systems have been utilized for the chemotherapy of cancer and protozoal diseases. It may also be extended in the chemotherapy of intracellular infections such as those caused by protozoa and viruses.

(c) Bioprecursor:

The bioprecursor does not contain a temporary linkage between the active drug and a carrier moiety, but designed from a molecular modification of the active principle itself.

It is a compound that is converted to active drug through metabolic biotransformation. For example, if the drug contains a carboxylic acid group, the bioprecursor may be a primary amine which is metabolized by oxidation to the aldehyde which is further metabolized to the carboxylic acid drug. e.g. fenbufen, phenylbutazone / oxyphenbutazone, acetanilide (Paracetamol), imipiramine (demethylimipramine).

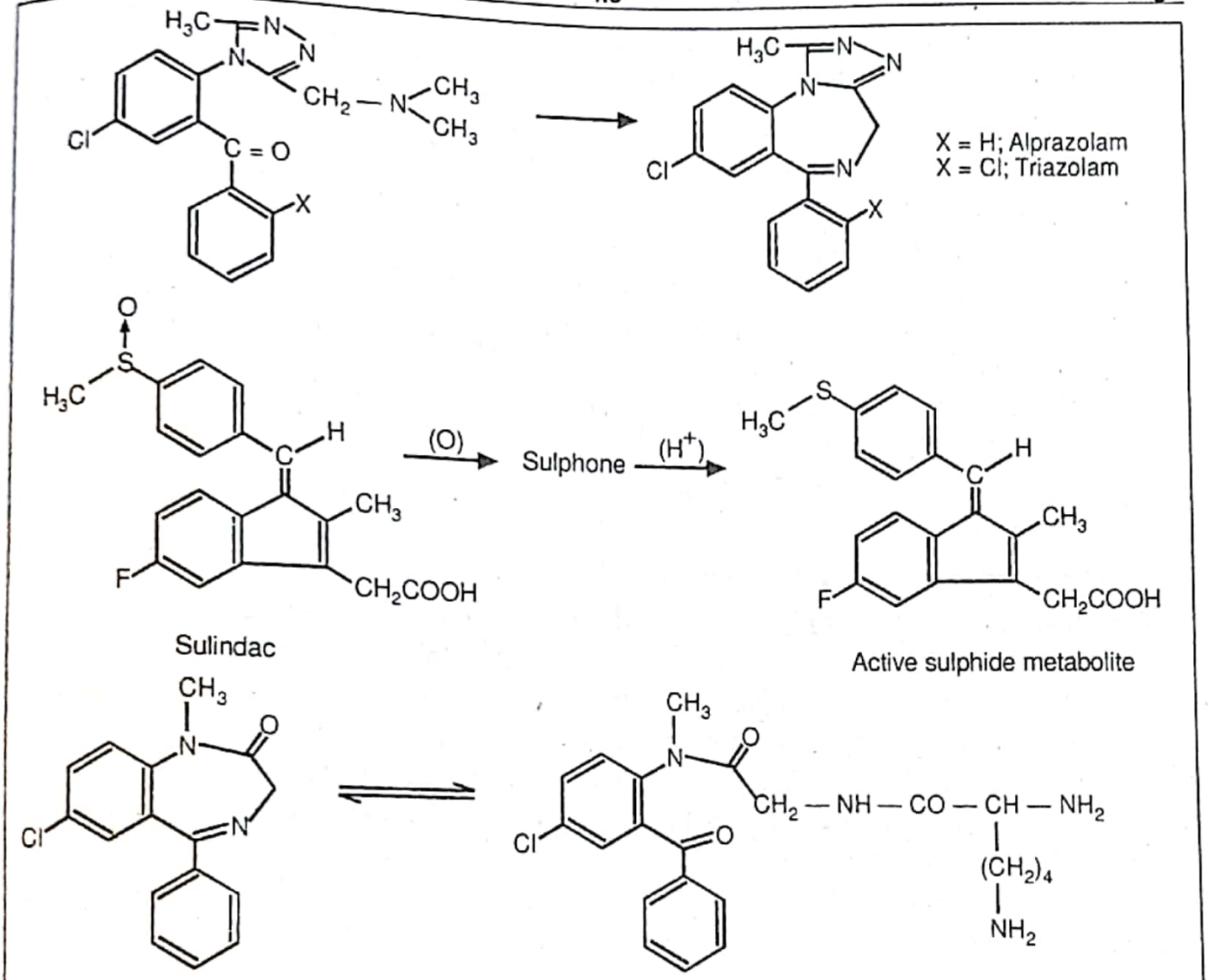


Fig. 4.1: In-vivo Conversion of Diazepam Prodrug to Diazepam

Similarly, pyrrolines are the bioprecursors of GABA and its analogs. N-alkylaminobenzophenones are designed to get in-vivo benzodiazepines by N-dealkylation of tertiary amine and ring closure. The linkage between the drug substance and the transport moiety is usually a covalent bond.

Sulindac, a non-steroidal anti-inflammatory bioprecursor, gets converted to the sulphide metabolite (active drug) via sulphone.

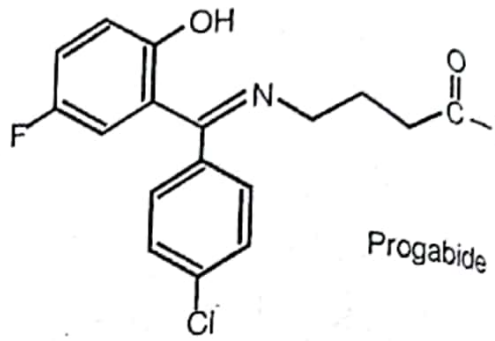
4.3 CARRIER-LINKED PRODRUGS

Alcohol-containing drugs can be acylated with aliphatic or aromatic carboxylic acids to decrease water-solubility (increase lipophilicity) or with carboxylic acids containing amino or additional carboxylate group to increase water solubility. Conversion to phosphate or sulphate esters also increase water solubility. Thus, by changing the degree of water-solubility, we can impart desirable absorption and distribution properties to the drug molecule. Succinate esters can be used to accelerate the rate of hydrolysis by intramolecular catalysis. If a hydrolysis is too slow, addition of electron withdrawing groups on the alcohol

part of the ester can increase the rate. If a slower rate of ester hydrolysis is desired, long chain aliphatic or sterically hindered esters can be used.

Activated amides, generally of low basicity amines or amides of amino acids are more susceptible to enzymatic cleavage. Phenyl carbamates (R $NHCO_2Ph$) can also be used as prodrugs because of their susceptibility to the attack of plasma enzymes.

The anticonvulsant agent progabide is a prodrug form of γ-aminobutyric acid, an important inhibitory neurotransmitter. Its lipophilicity helps it to cross blood-brain-barrier. Once it enters the CNS, it is hydrolyzed to GABA. Some drugs may contain an aldehyde or ketone functional group. The carbonyl group may be converted to Schiff base, oxime, acetal (ketal), enol ester, oxazolidine or thiazolidine.



Progabide

(a) Prodrugs for increased water solubility:

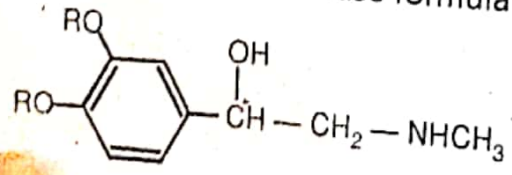
The hydrophilic characteristic of a drug can be improved by formation of hemisuccinates, hemiglutarates, hemiphthalates or metasulphobenzoates which then serve as a site of formation of water soluble sodium, potassium or amine salts. Phosphates have been used to prepare the hydrophilic carrier prodrugs in the fields of steroids and vitamins. Water solubility of 1, 4-benzodiazepines may be elevated by the formation of a peptide bond between the drug and L-lysine. Similarly, β-glycosidation helps to get a non-irritating water-soluble derivative of menthol.

Prednisolone and methylprednisolone are poorly water soluble corticosteroid drugs. Prednisolone phosphate (PO_3Na_2) is a water soluble prodrug for prednisolone that is activated in-vivo by phosphatases. Methylprednisolone sodium succinate is a water soluble prodrug of methylprednisolone. Since, amidase catalyzed hydrolysis occurs rapidly in human serum, water soluble amide prodrug forms of benzocaine can be prepared with various amino acids.

(b) Prodrugs for improved absorption and distribution:

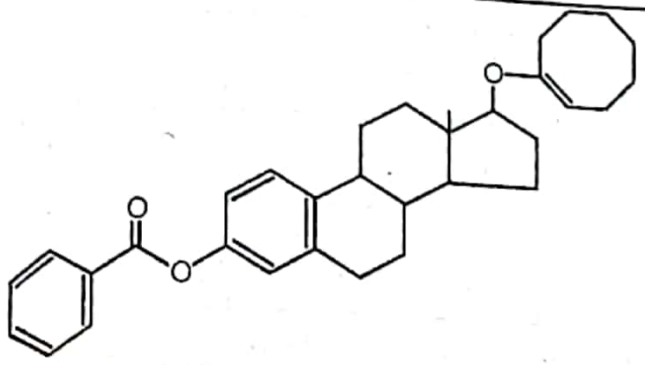
Drugs applied to the skin are poorly absorbed. Corticosteroids for the topical treatment of inflammatory, allergic and pruritic skin conditions can be made more suitable for topical absorption by esterification or acetonidation. Once absorbed through the skin, an esterase can release the drug.

Examples include fluocinolone acetonide and fluocinonide. Dipivaloylepinephrine (dipivefrin), a prodrug for epinephrine, has better cornea penetration rate than epinephrine and is used in the treatment of glaucoma. Similarly, estradiol-3-benzoate-17-cyclooctenyl ether was designed for a sustained release formulation of oestradiol.

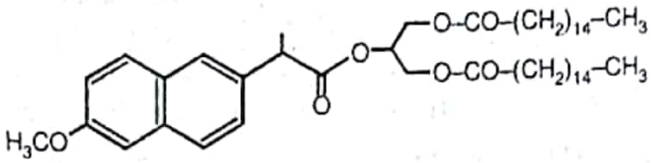


(i) Epinephrine: R = H

(ii) Dipivefrin: R = $(CH_3)_3$ CCO



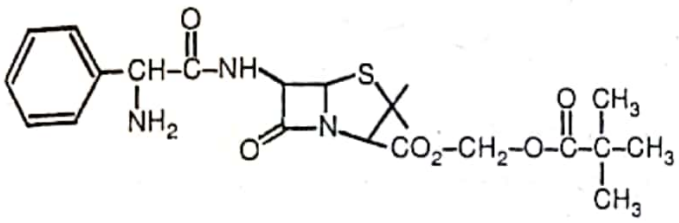
Estradiol-3-benzoate-17-cyclooctenyl ether



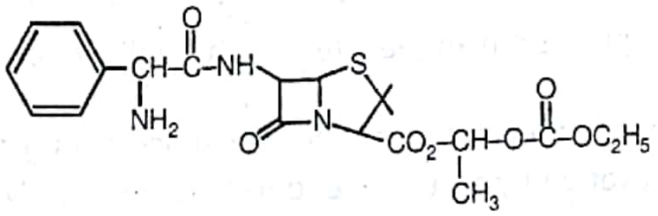
Naproxen - 2- glyceride
(less gastric irritation and higher plasma level)

Fig. 4.2: Lipophilic Carrier Prodrugs

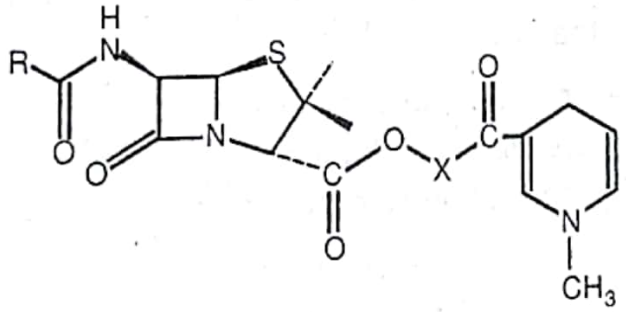
Similarly ampicillin, when administered orally only about 40% of dose is absorbed. Hence, ampicillin, when presented in the form of its esters, has increased oral absorption, e.g., bacampicillin, pivampicillin.



Pivampicilline



Bacampicilline

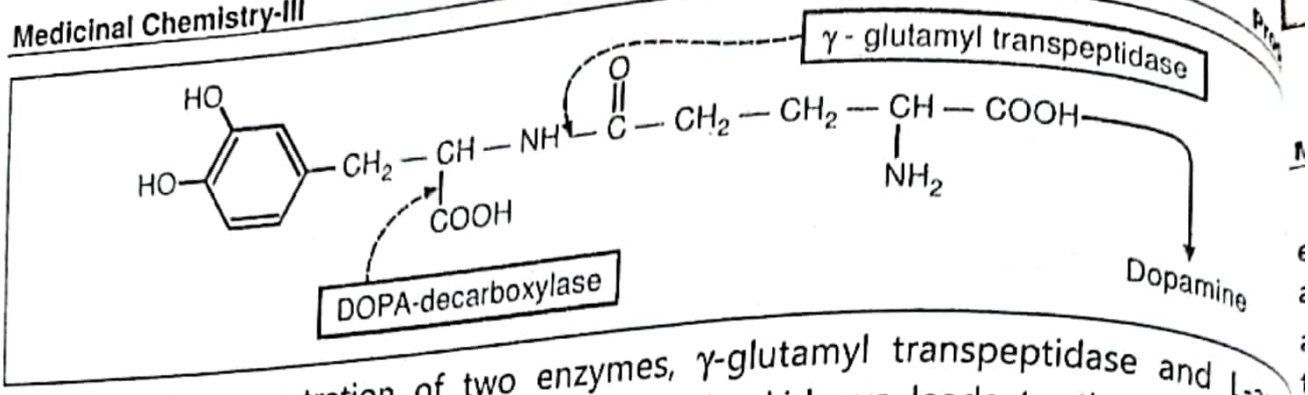


(c) Prodrugs for site specificity:

The designing of centrally acting drugs need ability to cross the blood-brain-barrier. The approach is based on attaching a lipophilic carrier to the hydrophilic drug in a loosely bound form. The complex releases hydrophilic drug in the CNS.

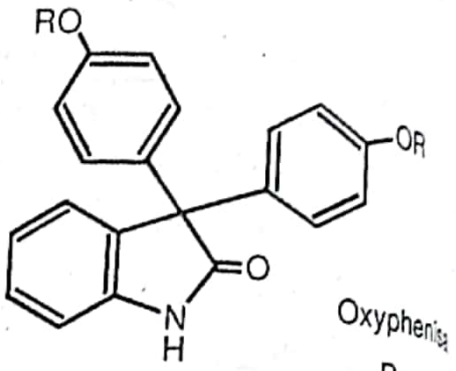
For example, β-lactam antibiotics may be used in the treatment of bacterial meningitis. Since, the β-lactam antibiotics are hydrophilic, they enter the brain very slowly, but they are actively transported back into the blood. Bodor and co-workers have synthesized dihydropyridine-penicillin prodrugs that deliver β-lactam antibiotic in high concentrations into the brain.

The phosphoamidases are abundant in neoplastic cells than in normal cells and hence, cyclophosphamide is developed by phosphorylating the nitrogen mustard. The drug might be hydrolysed in tumour cells by the enzyme phosphoamidases.



The high concentration of two enzymes, γ-glutamyl transpeptidase and L-aro amino acid (DOPA) decarboxylase present in the kidneys leads to the developme γ-glutamyl-DOPA as a selective renal vasodilator.

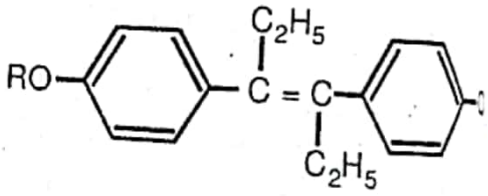
Another example is progabide which is a prodrug of an inhibitory neurotransmitter, GABA. Oxyphenisatin (R = H) is a bowel sterilant that is active only when rectally given. An orally active prodrug can be designed (R = CH_3CO) which releases an active drug, oxyphenisatin, in the intestine through hydrolysis.



Oxyphenis
R = –H

Another approach for site specific drug delivery is to design a prodrug that requ activation by an enzyme found predominantly at the desired site of action. Diethylstilbes diphosphate (R = PO_3^{--}) was designed for site-specific delivery of diethylstilbestrol prostatic carcinoma tissues, since tumour cells were found to have higher concentration phosphatases and amidases than the normal cells.

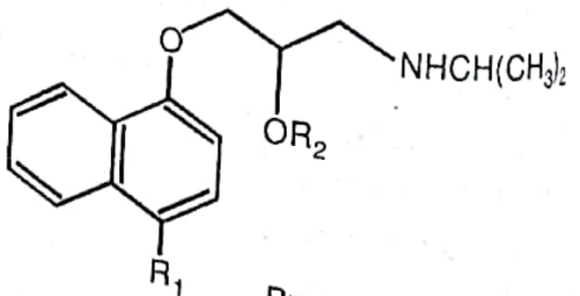
Yet another example of site-specific delivery prodrug design is the conversion of dopamine to L-dopa and preparation of aliphatic and steroidal esters of GABA.



(d) Prodrugs for stability:

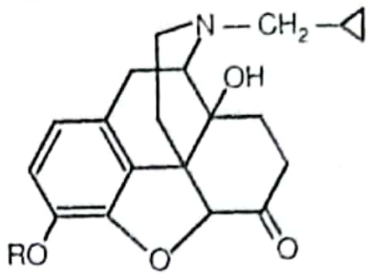
Extensive first-pass metabolism in liver is the most important cause that restricts o effectiveness of many drugs. Metabolic studies of drugs provide such clues as how to ma the drug resistant to first-pass metabolism and to increase oral effectiveness.

For example, the major metabolites of propranolol were found to be propranolol -o-glucuronide (R_1 = –H, OR_2 = glucuronide), p-hydroxy-propranolol (R_1 = –OH, R_2 = –H) and its o-glucuronide (R_1 = –OH; OR_2 = glucuronide). Hence, oral administration of propranolol hemi-succinate (R_1 = H, R_2 = $COCH_2$ CH_2 COOH) elevates plasma levels of propranolol about 8 times.



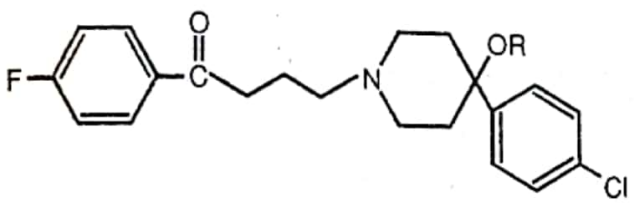
Propranolol
$R_1 = R_2 = H$

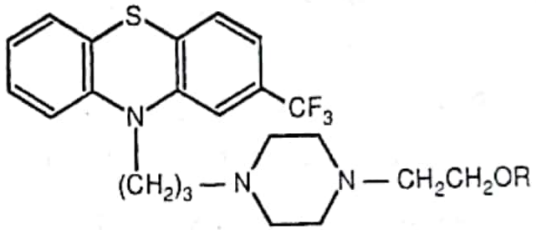
Similarly, naltrexone (R = H), undergoes extensive first-pass metabolism. When its ester analogs, namely anthranilate (R = CO -o- NO_2Ph) and the acetylsalicylate (R = CO -o- ACOPh) used, the bioavailability of naltrexone was found to be increased to 45 and 28-times respectively.



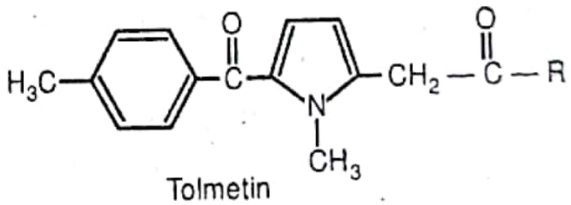
(e) Prodrugs for slow and prolonged release:

It can best be achieved by making a long chain aliphatic ester because these esters hydrolyze slowly. This principle has been well elaborated in the drug designing of sex hormone derivatives e.g.; progestins or androgens. Examples include haloperidol decanoate [R = $CO(CH_2)_8CH_3$] which when injected intramuscularly as a solution in sesame oil, its activity lasts for about one month in comparison to haloperidol (R = H) as such (2-6 hrs). Similarly, another antipsychotic agent, fluphenazine enanthate [R = $CO(CH_2)_5CH_3$] and decanoate [R = $CO(CH_2)_8$-CH_3] have duration of action of about a month in comparison to plain fluphenazine (6-8 hrs).





When a glycine conjugate (R = NH CH_2COOH) of anti-arthritis drug, tolmetin sodium (R=O–Na^+) is used, both potency and duration of action are prolonged because of the slow hydrolysis of the prodrug amide linkage.



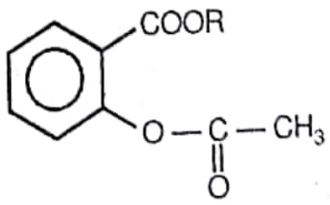
Tolmetin

Among local anaesthetics, procaine is an ester, and is therefore easily hydrolyzed by esterases. By conversion of the ester into an amide (lidocaine), the duration of action is increased by several folds.

(f) Prodrugs to lower toxicity profile:

Examples include the use of prodrug dipivaloylepinephrine (R = Me_3CCO) instead of epinephrine (R = H) in the treatment of glaucoma.

Similarly, the side effects associated with the use of aspirin are gastric irritation and bleeding. Esterification of aspirin (R = alkyl) greatly suppresses gastric ulcerogenic activity.

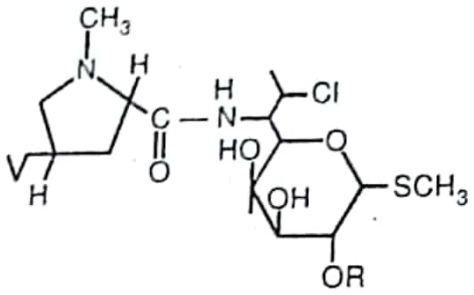


Aspirin (R = –H)

(g) Prodrugs to improve patient acceptance:

Clindamycin (R = H) has a bitter taste, so it is not well accepted by children. It was found that by increasing the chain-length of 2-acyl esters of clindamycin, the taste improved from

bitter (acetate ester) to a non-bitter taste (palmitate ester). Bitter taste results from compound dissolving in the saliva and interacting with a bitter taste receptor in the tongu Esterification with long-chain fatty acids makes the drug less water-soluble, resulting in non-bitter taste. Yet another example from this category is chloramphenicol palmitate.



Clindamysin; R = – H

Thus in summary, prodrug concept may be utilized to improve the undesirab properties of the drug. Such undesirable properties may include,

(a) Physico-chemical properties: e.g. poor solubility, instability, unpleasant taste odour.

(b) Pharmacokinetic properties: e.g. poor bioavailability due to incomplete absorpti or shorter duration of action due to high rate of metabolism.

(c) Toxicities or side-effects: e.g. gastric irritation. Sometimes drug may be m active but unable to reach its site of action. In other cases, due to large volume distribution, drug may get distributed to other sites alongwith its site of action. This leads appearance of side-effects, because of drug concentration of unintended sites. In all su cases prodrug concept can be applied. However toxicity testing of prodrug is also necessa

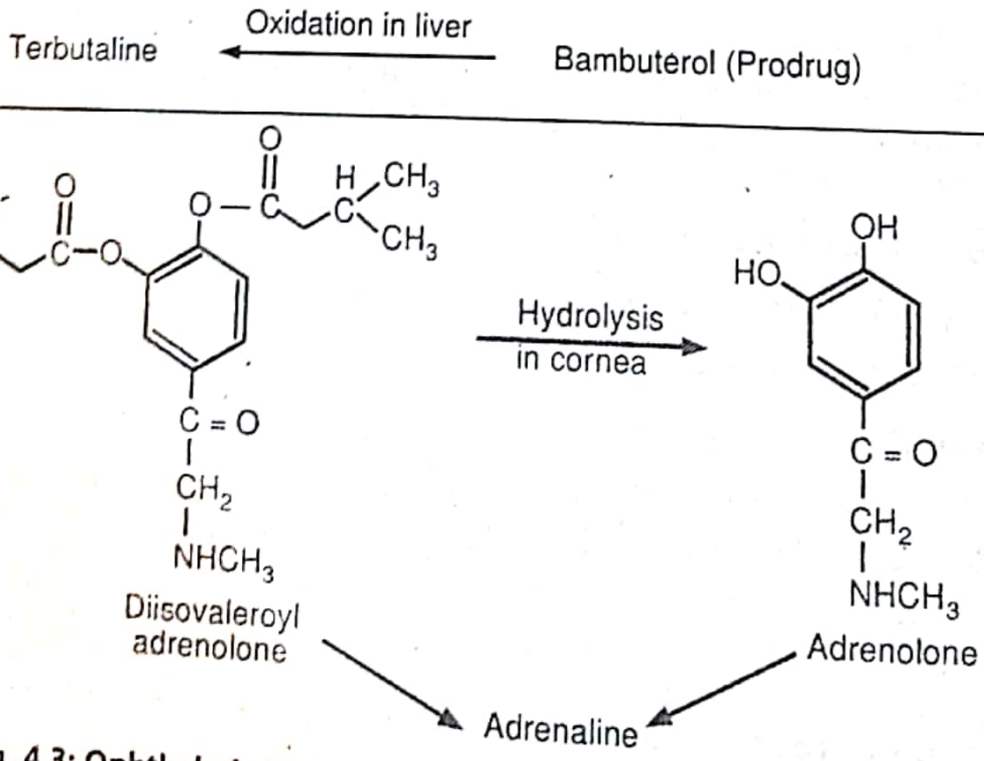
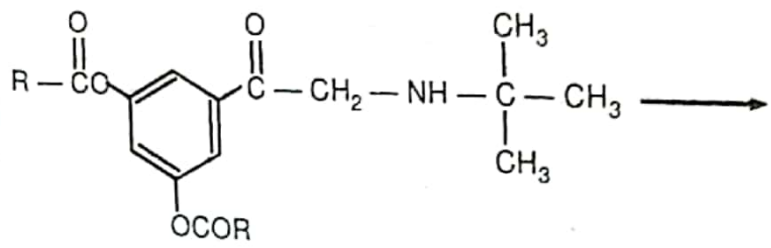


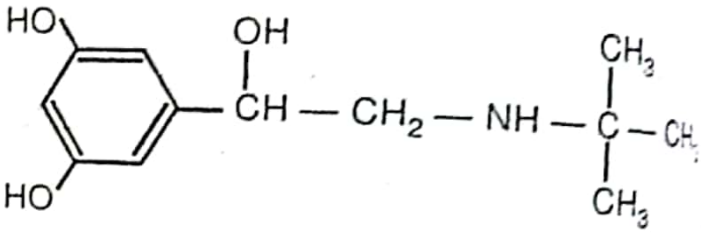
Fig. 4.3: Ophthalmic Delivery of Drugs (Adrenaline Diesters)

Table 4.1: Clinically used Prodrugs

Prodrug	Active form	Prodrug	Active form
Levodopa	Dopamine	Proguanil	Proguanil triazine
Enalapril	Enalaprilat	Prednisone	Prednisolone
Alpha methyl dopa	Alpha methylnorepinephrine	Bacampicillin	Ampicillin
Dipivefrine	Epinephrin	Sulfasalazine	5-ASA
Sulindac	Sulfide metabolite	Cyclophosphamide	Aldophosphamide, Phosphoramide mustard, Acrolein
Hydrazide (MAO inhibitor)	Hydrazide derivative	Primidone, phenobarbitone	Phenobarbitol
Mercaptopurine	Methylmercaptopurine ribonucleotide	Bambuterol	Terbutaline
Valaciclovir	Aciclovir	Dipyridamole	Adenosine
Psilocybin	Psilocin	Fosphenytoin	Phenytoin
Heroin	Morphine	Midodrine	Desglymidodrine
Nabumetone	6-MNA	Melagatran	Ximelagatran
Lovastatin and simvastatin	Active-hydroxyl derivatives	Alatrovafloxacin	Travafloxacin
Phenacetin	Acetaminophen	Famciclovir	6-deoxypenciclovir
Chloramphenicol palmitate	Chloramphenicol	Tenofovir disoproxilfumarate	Tenofovir
Azathioprine	Mercaptopurine	Dipivefrin	Epinephrine
Bacampicillin	Ampicillin	Enalapril	Enalaprilat
Benonrylate	Aspirin + Paracetamol	Levodopa	Dopamine
Cortisone	Hydrocortisone	Proguanil	Proguanil triazine
Cyclophosphamide	Aldophosphamide	Sulindac	Sulfide metabolite
Sulfasalazine	5 Aminosalicylic acid	Zidovudine	Zidovudine triphosphate



Ketone-diester of t-butaline



t-butaline

Applications of Prodrug Concept:

(a) Increasing absorption of drugs:
e.g. ampicillin esters.

(b) Improve site specific drug delivery; e.g. epinephrine.

(c) Prolongation of drug action:
e.g. testosterone.

(d) Decrease side-effects and toxicity: e.g. NSAID.

(e) Improve taste and odour:
e.g. chloramphenicol palmitate.

(f) Delivery to brain: e.g. dopamine to L-dopa. L-dopa to its methyl ester GABA to its aliphatic and steroid esters.

4.4 DRAWBACKS OF PRODRUG APPROACH

The prodrug concept may become a potential source of toxicities if,

(a) the prodrug generates toxic metabolites which are not generated by the parent drug;

(b) increased consumption of glutathione during the conversion of prodrug to active metabolite may leave vital cell constituents unprotected;

(c) the inert carrier moiety could not remain inert and leads to formation of toxic metabolites.

(d) the prodrug or/and carrier moiety generate such metabolites which alter the pharmacokinetic features of the parent drug by either inducing metabolic enzymes or by competing the active drug for binding with plasma-proteins.

4.5 SOFT DRUG CONCEPT

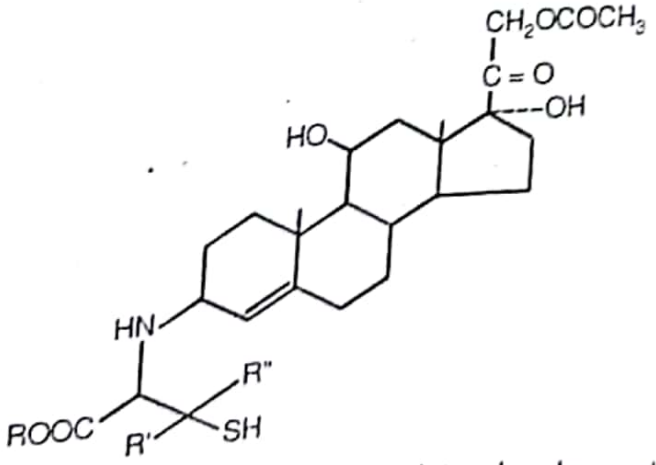
Prodrugs are designed in such a way that the active drug is generated by the major metabolic pathway. Prodrugs might effectively eliminate some toxicities by protecting the drug from unwanted degradations, particularly those occurring in GIT prior to and during absorption or possibly during the first passage through the liver. The application of the concept of 'soft drugs' is necessary to overcome and to improve (a) pharmacokinetic insufficiencies, (b) transportability, and (c) site specificity.

The soft drugs are defined as therapeutically beneficial agents characterized by a predictable and controllable in-vivo metabolism to non-toxic moieties, after they achieve their therapeutic role. The site-specific delivery via chemical modifications involves the design of a soft drug from an inactive metabolite. The designed drug is then transformed by facile and predicted routes of metabolism ultimately resulting in the delivery of the active drug at the expected sites of action.

The concept was successfully applied to local delivery of steroids, drugs acting on specific areas in the eye, brain and testes. If it is possible to deliver potent drugs exactly at the site of action, very less dose will be required, which will not cause unexpected toxicities.

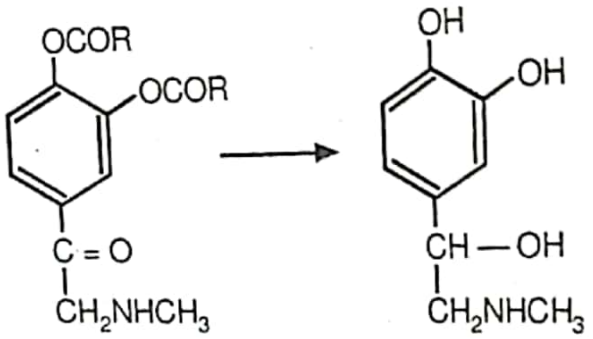
For example, increased separation of activity from toxicity (i.e. improved selectivity) may be achieved by using 3-spirothiazolidine derivative of hydrocortisone. Unlike hydrocortisone, it lacks specific hydrocortisone binding and affinity properties, even if it is absorbed systemically during topical administration.

This is because, the 4, 5-unsaturated 3-ketone group is absent in this derivative which is slowly generated in dermal cells by stepwise hydrolysis of thiazolidine ring to deliver the active 3-keto compound. Using similar approach, an antiacne topical progesterone preparation was developed containing the cystein 3,20-bisthiazolidine derivative of progesterone.



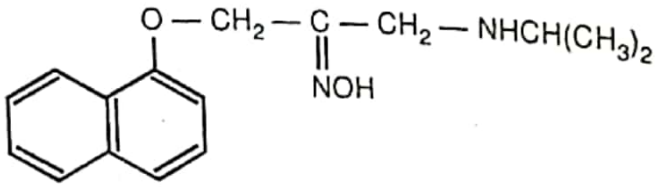
The concept of 'soft drug' may also be applied to develop selective and safer ocular drug delivery systems for the treatment of glaucoma, ocular inflammations and infections. It was found by Bodor et al. in 1978 that diester derivatives of adrenolone have a high level of ocular sympathomimetic activity due to the conversion of former to adrenaline via a combined reduction-hydrolysis process in the eyes.

Using same approach, tertbutaline was generated selectively in the iris-ciliary tissues by the action of reductases and esterases on ketone-diester precursors of tertbutaline.



Diester of adrenolone — Adrenaline

Similarly propranolol is generated at the iris-ciliary body by the action of esterases and reductases on the topically applied keto-oxime derivative of propranolol.



The 'soft drug' concept was utilized to develop loteprednol etabonate, a topical anti-inflammatory and anti-allergic agent.

It is locally potent but systemically safe.

Chapter...5

ANTIMALARIALS

♦ SYNOPSIS ♦

5.1 INTRODUCTION

Atleast 45,000 species of protozoa have been described, out of which many species are parasitic. Examples of such parasitic species include, a single celled protozoa, amoeba; helminthes etc. Parasitic infections pose a major world-wide health problem. Moreover, the development of resistance by these parasites to drugs and of mosquitoes to the insecticides, make the problem more complicated. Many factors contribute towards the spread of these parasitic diseases.

Infections with helminthes and protozoa are the most common causes of human diseases. The occurrence of these infections is much more in the poor, undeveloped or developing countries than in developed countries. Population crowding, poor sanitation and negligence to provide health education are some of the important causes behind it. These diseases are prominently seen in tropical countries.

5.2 ANTIMALARIAL DRUGS

In developing countries, the paramount needs are still related to nutrition, communicable diseases and poverty. The messianic call for "health for all by the year 2000", however emotionally attractive, is very difficult to achieve, in the surroundings of hard realities. Unhealthy economic system influences the standards of both, health and education in the country. Malaria is one of such diseases whose appearance may be related to the socio-economic status of the society. It is mainly a disease of tropic and sub-tropic countries. Though on large scale malaria eradication programme was initiated since from 1957, this disease still affects about 200 million people and causes atleast 2 million deaths per year.

Malaria in humans is caused by the infection with protozoan parasites of the genus, Plasmodium. These parasites spend an asexual phase in a man and a sexual phase in female Anopheles mosquitos. Out of several hundred known Anopheles species, four species infect man.

The parasitic diseases are easy to acquire, difficult to eradicate and are prone to relapse. Table 5.1 illustrates commonly occurring parasitic diseases.

Table 5.1: Commonly occurring parasitic diseases

Infection	Parasitic protozoa	Effective drugs used
1. Amoebiasis	*Entamoeba histolytica*	Chloroquine, emetine, iodoquinol, diiodohydroxyquine, metronidazole, dehydroemetine, diloxanide, tetracycline, paromomycin, nimorazole, tinidazole.
2. Balantidiasis	*Balantidium coli*	Metronidazole, tetracycline, paromomycin
3. Giardiasis	*Giardia lamblia*	Metronidazole, nimorazole, quinacrine, tinidazole
4. Leishmaniasis	*Leishmonia donovali* *Leishmonia tropica*	Amphotericine B, sodium stibogluconate, meglumine antimoniate, pentamidine
5. Malaria	*Plasmodium falciparum* *Plasmodium vivax* *Plasmodium malariae* *Plasmodium ovale*	Amodiaquine, chlorguanide, chloroquine, primaquine, pyrimethamine, quinine, sulfadiazine, sulfisoxazole
6. Enterobiasis		Mebendazole, piperazine, pyrantel, pyrvinium pamoate, thiabendazole
7. Pneumocystosis	*Pneumocystis carinii*	Pyrimethamine, sulfadiazine, sulfisoxazole, trimethoprim-sulfamethoxazole pentamidine
8. Trichomoniasis	*Trichomonas vaginalis*	Metronidazole, nimorazole, tinidazole
9. Trypanosomiasis	*Trypanosoma cruzi*	Melarsoprol, nifurtimox, pentamidine suramin sodium, diminazene aceturate
10. Ascariasis		Bephenium, mebendazole, piperazine, pyrantel pamoate, thiabendazole, levamisole

These include:

1. ***Plasmodium falciparum*:**

It causes malignant tertian or subtertian form of malaria which may cause death by invading the CNS. About half of the cases of malaria are caused by this species. In this type, the attacks are more severe and relapses do not occur.

The parasitic diseases are easy to acquire, difficult to eradicate and are prone to relapse. Table 5.1 illustrates commonly occurring parasitic diseases.

Table 5.1: Commonly occurring parasitic diseases

Infection	Parasitic protozoa	Effective drugs used
1. Amoebiasis	*Entamoeba histolytica*	Chloroquine, emetine, iodoquinol, diiodohydroxyquine, metronidazole, dehydroemetine, diloxanide, tetracycline, paromomycin, nimorazole, tinidazole.
2. Balantidiasis	*Balantidium coli*	Metronidazole, tetracycline, paromomycin
3. Giardiasis	*Giardia lamblia*	Metronidazole, nimorazole, quinacrine, tinidazole
4. Leishmaniasis	*Leishmonia donovali* *Leishmonia tropica*	Amphotericine B, sodium stibogluconate, meglumine antimoniate, pentamidine
5. Malaria	*Plasmodium falciparum* *Plasmodium vivax* *Plasmodium malariae* *Plasmodium ovale*	Amodiaquine, chlorguanide, chloroquine, primaquine, pyrimethamine, quinine, sulfadiazine, sulfisoxazole
6. Enterobiasis		Mebendazole, piperazine, pyrantel, pyrvinium pamoate, thiabendazole
7. Pneumocystosis	*Pneumocystis carinii*	Pyrimethamine, sulfadiazine, sulfisoxazole, trimethoprim-sulfamethoxazole pentamidine
8. Trichomoniasis	*Trichomonas vaginalis*	Metronidazole, nimorazole, tinidazole
9. Trypanosomiasis	*Trypanosoma cruzi*	Melarsoprol, nifurtimox, pentamidine suramin sodium, diminazene aceturate
10. Ascariasis		Bephenium, mebendazole, piperazine, pyrantel pamoate, thiabendazole, levamisole

These include:

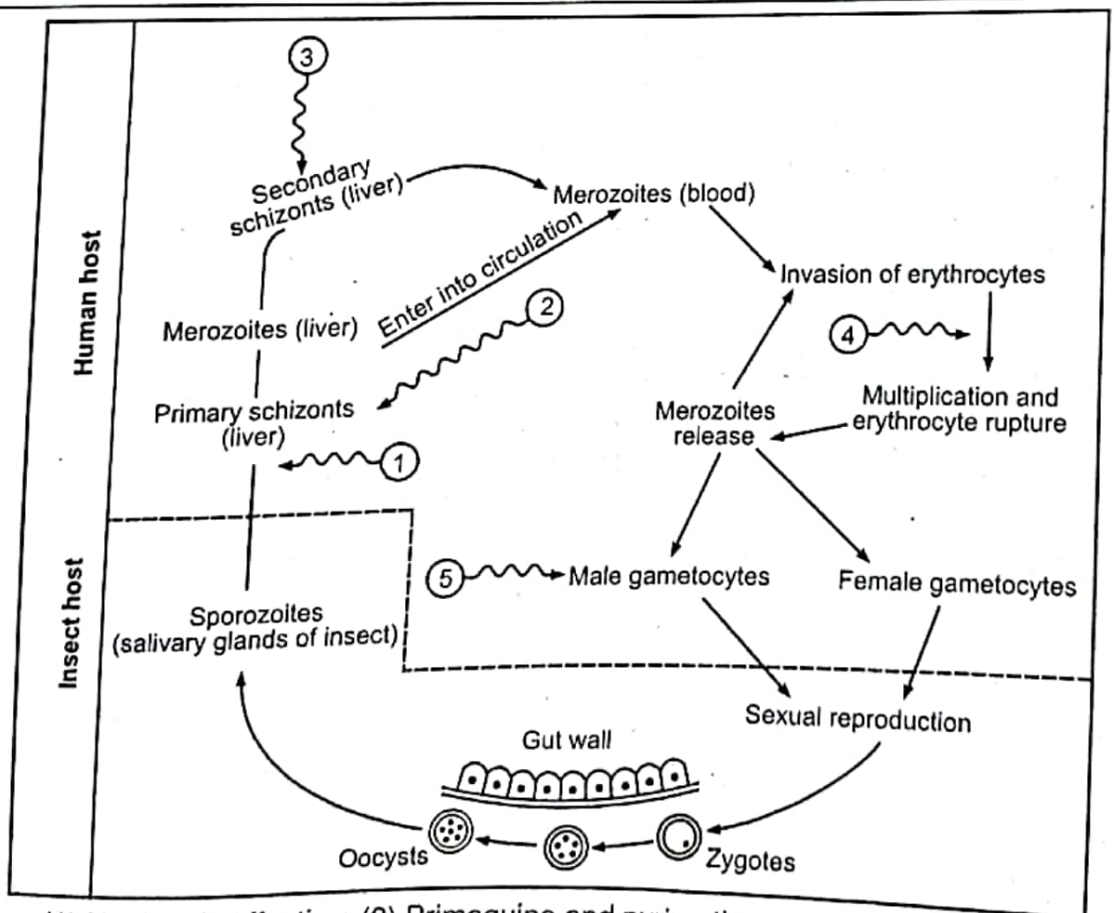
1. ***Plasmodium falciparum*:**

It causes malignant tertian or subtertian form of malaria which may cause death by invading the CNS. About half of the cases of malaria are caused by this species. In this type, the attacks are more severe and relapses do not occur.

2. ***Plasmodium vivax*:**
   It causes benign tertian malaria in which fever revisits patient every 48 hours or on the third day. About 40% of the cases of malaria are caused by this species.
3. ***Plasmodium malariae*:**
   It is responsible for the occurrence of quartan malaria in which fever repeats after every 72 hours. It is a milder form of infection. *Plasmodium falciparum* and *P. malariae* do not persist within the liver cells after erythrocytes have become infected.
4. ***Plasmodium ovale*:**
   It is responsible for the mild tertian malaria which is most commonly seen in West Africa. Except *P. falciparum*, all three species of Anopheles have a secondary exo-erythrocytic stage. Plasmodium vivax and *P. ovale* do persist within the liver cells even after erythrocytes become infected and may produce true relapses months or even years after.

Life-cycle of Malarial Parasite:

The female Anopheles mosquito feeds on vertebrate blood. Malaria infection is initiated through the bite of infected female Anopheles mosquito which releases motile sporozoites into the human blood stream. Within 1 - 2 hours the sporozoites get entry into the parenchyma cells of the host liver. Through repeated nuclear divisions sporozoites multiply and develop into schizonts. After the period of 10 - 16 days, liver cells rupture due to multiple repeated divisions of schizonts. This results in the release of approximately 20,000 merozoites into circulation. This stage is known as pre-erythrocytic or exo-erythrocytic phase of infection.



(1) No drug is effective, (2) Primaquine and pyrimethamine, (3) Primaquine
(4) Chloroquine, amidoquine and sulphonamides, (5) Primaquine

Fig. 5.1: Diagrammatic representation of life cycle of plasmodium

Merozoites now enter into the circulation and invade erythrocytes. Some merozoites invade fresh liver cells and repeat erythrocytic cycle. Erythrocytes are invaded by merozoites for the following reasons:

(1) The plasma constituents and haemoglobin serve as a source of several amino acids necessary for the survival of the parasite.

(2) For rapid multiplication of merozoites, the purine bases (i.e., adenine and guanine) are obtained from erythrocytes which are then utilized to synthesize parasitic DNA and RNA molecules.

(3) Pentoses and phosphates are necessary for nucleic acid synthesis. The protozoal parasites do not have any means to get these raw materials. Obviously, it is the host who is going to suffer.

Inside erythrocytes, the merozoites continue to grow. In erythrocytes, the merozoites undergo asexual multiplication which results into formation of daughter cells, schizonts. Due to the repeated multiplication of the latter, erythrocyte ruptures and releases about 6-24 merozoites into the circulation. Each merozoit again invades fresh erythrocyte and the cycle of asexual multiplication is repeated again. This stage is known as schizogony phase of infection. It continues for 48-72 hours. Febrile clinical manifestations are witnessed due to schizogony phase.

After this phase, some of the erythrocytic merozoites develop into male and female gametocytes by some unidentified mechanisms. Such infected blood when ingested by female mosquito, the sexual forms (i.e., gametocytes) undergo reproduction within the gut of the insect. The resulting zygote, through various stages of development gives rise to the infective sporozoite. The latter gets localized in the salivary glands of the insect and enters the host blood circulation when the infected mosquito bites a healthy person. The story thus goes on repeating.

Symptoms of Malaria:

The life-cycle of malarial protozoa is dependent on the erythrocytes of the human host, where the parasite undergoes main morphological changes. The symptoms of malaria however, are reported to occur about 12 - 16 days after the mosquito bite. It means that the pre-erythrocytic phase is free of any symptoms. It is the erythrocytic phase which is responsible for the occurance of various symptoms of malaria. These symptoms can be grouped together as:

(1) Symptoms like nausea, vomiting, severe chills, delirium and fever may reappear after every 3 to 4 days depending upon the species of protozoa and hence upon the type of malaria.

(2) As the erythrocytic phase continues, increasing number of erythrocytes undergo destruction, resulting into severe form of anemia, and

(3) To provide necessary amino acids for the multiplication of parasite, haemoglobin undergoes breakdown process. If this process continues, it leads to jaundice because of accumulated bilirubin.

5.3 CHEMOTHERAPY OF MALARIA

Two different attempts have been made to design new or modern antimalarial drugs. After studying the life-style of malarial parasite in human, many drugs were designed and their target selectivity was tested for various phases of plasmodium life-cycle. For example,

(a) Drugs effective against primary tissue schizonts:

These drugs are also known as prophylactic agents. Since no drug is effective at this stage, true prophylaxis does not exist for malarial parasites.

(b) Drugs active against erythrocytic phase:

These drugs are also known as schizontocidal agents. Examples include, amodiaquine, chloroquine, mefloquine, quinine, pyrimethamine etc.

(c) Drugs active against all malarial parasites:

These drugs are also known as gametocytocidal agents. Examples include primaquine. Gametocytes of *P. falciparum* may remain in the circulation for prolonged period, even after the patient receives the treatment with chloroquine. These gametocytes are rapidly killed by a single dose of 79 mg of primaquine phosphate.

In second approach, the host-parasite relationship was thoroughly examined to point out biochemical differences. For example, mammalian cells are capable of utilizing preformed folates while bacteria and protozoa are unable to do so. Hence, they must synthesize folates of their own. Hence, such drugs that selectively inhibit folate biosynthesis in the protozoa by blocking the enzymes involved therein, can be used for suppression or radical cure of malaria.

Certain antimalarial drugs are not capable of destroying plasmodium merozoites. They just inhibit the erythrocytic stage of development of malarial parasite and thus prevent the onset of symptoms. The treatment with such drugs is known as suppressive treatment. It may be used to prevent maturation of the erythrocytic infection but it may not have any effect on the stages in liver cells. It serves as a prophylactic measure before entering the area susceptible to malarial infection. Drugs commonly employed in suppressive treatment are chloroquine, amodiaquine, pyrimethamine and proguanil. Some antimalarial drugs completely destroy the plasmodium merozoites and thus terminate the malarial attack. Such agents are said to provide clinical cure of the disease.

Examples include chloroquine, and amodiaquine. However, the patients treated with these agents may show the relapse of the disease due to the presence of gametocytes in the circulation for prolonged period even after the drug treatment. Radical cure is the third category in which a combination therapy is generally used to eradicate both, the developed parasites and those still developing in the erythrocytes and other tissues. Generally primaquine is used in combination with chloroquine or amodiaquine.

4 CLASSIFICATION OF ANTIMALARIAL AGENTS

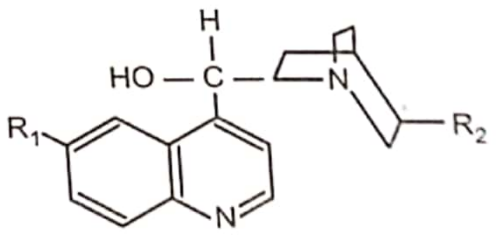
Early discoveries of Paul Ehrlich with organic dyes and organoarsenicals gave new mensions to the traditional methods of treating malaria. Presently available various ntimalarials are the direct outcome of Ehrlich outstanding pioneering efforts. On the hemical basis, antimalarial drugs are classified as:

1) Quinolines
   (a) Cinchona alkaloids,
   (b) 4-Aminoquinolines, and
   (c) 8-Aminoquinolines
(2) 9-Aminoacridines
(3) 2, 4-Diaminopyrimidines
(4) Biguanides
(5) Sulfones and sulphonamides, and
(6) Miscellaneous agents.

(1) Quinolines:

(a) Cinchona alkaloids:

Cinchona bark contains a mixture of more than 20 alkaloids. Four major alkaloids are isolated from it, which are effective against erythrocytic merozoites and constitute a part of suppressive treatment of malaria. All four are derivatives of 4-quinolinemethanol which is nked with a substituted quinuclidine moiety.



Cinchona alkaloids

1. Quinine:
   $R_1 = -OCH_3$; $R_2 = -CH = CH_2$ (–) isomer
2. Quinidine:
   $R_1 = -OCH_3$; $R_2 = -CH = CH_2$ (+) isomer
3. Cinchonine:
   $R_1 = -H$; $R_2 = -CH = CH_2$ (+) isomer
4. Cinchonidine:
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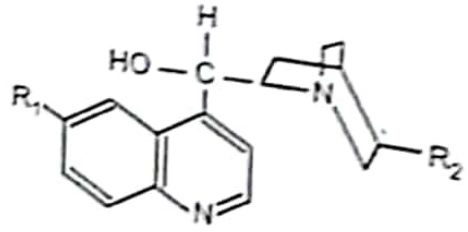

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4. Cinchonidine:
   $R_1 = -H$; $R_2 = -CH = CH_2$ (–) isomer

Quinine and its d-isomer, quinidine are the only cinchona alkaloids currently in the use. Quinine is the most active antimalarial ingredient of the cinchona bark and is present in highest concentration to the extent of about 5.0%. It is schizonticidal and gametocytocidal for *P. vivax* and *P. malariae*. It is orally active in the form of sulfate while quinine dihydrochloride may be used for intravenous administration. The antimalarial activity is mainly associated with the laevorotatory form. Subcutaneous or intramuscular administration is not usually recommended due to local tissue damage.

SAR of quinolines:

Quinoline compounds have long been used for the treatment of malaria. The SAR studies of quinine led to the potent and inexpensive 4-aminoquinolines (chloroquine). The search of highly active drug agonist chloroquine-resistant strains of *P. falciparum* led to the development of mefloquine. Most of these quinolines retain the 7-chloroquinoline nucleus but vary in the length and nature of their basic amono side-chain.

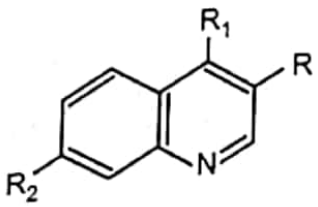
SAR studies of quinine nucleus revealed the importance of secondary alcohol group in the structure. The methoxy (R_1), vinyl (R_2) moieties were found to be not necessary for antimalarial activity.

It is extremely useful in treating chloroquine resistant *P. falciparum* infections. High doses of quinine may produce a quinidine like depressant effect on the heart. It causes vasodilation and may cause hypotension. Since it antagonises the actions of physostigmine on the skeletal muscles by exerting curare-like effect, it may be beneficial in the symptomatic relief of nocturnal muscle cramps or myotonia congenita. Toxic doses of quinine may induce abortion. It has analgesic, antipyretic and local anaesthetic properties.

Due to its low therapeutic index, quinine is not used alone. It can be used alongwith primaquine, pyrimethamine or a sulphonamide in the combination therapy.

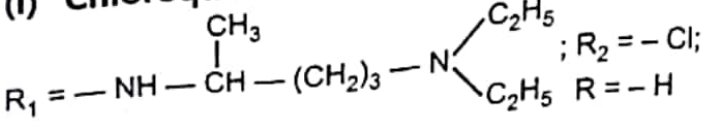
(b) 4-Aminoquinolines:

During the period 1940-1944, a limited number of antimalarials were available in the market. They were associated with a high toxicity profile and a low therapeutic index. To overcome this situation, 4-aminoquinolines were investigated in United States through a research programme. Chloroquine, hydroxychloroquine and amodiaquine are the most important members of this series.

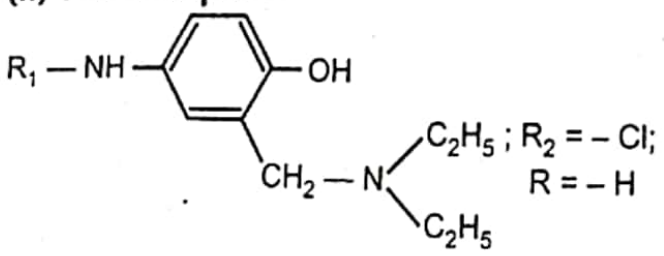


4 - aminoquinolines

(i) Chloroquine:



(ii) Amodiaquine:



Chloroquine is found to be concentrated in parasitized red cells where it binds to double-stranded DNA. This results into inhibition of the functions of DNA and RNA polymerases. It inhibits several enzyme systems and binds to lysosomes resulting into their stabilization. This exerts an inhibitory influence on the cell growth of bacteria and protozoa. However erythrocytes affected by chloroquine-resistant strains of plasmodium, are less permeable to the drug. Hence chloroquine is accumulated less readily and to a lesser extent in such parasitized erythrocytes.

Chemically chloroquine is 7 – Chloro-4 (4-diethylamino- 1-methylbutylamino) quinoline. Due to the presence of asymmetric carbon in the side – Chain it exists as isomers. The chemical name of amodiaquine is 7 - chloro- 4 - (3-diethylamino- 4 - hydroxyanilino) quinoline. Both these drugs are effective against a sexual erythrocytic form of all four plasmodium species.

SAR studies indicated that 7 – Chloro group, the tertiary amine and diaminoalkyl side chain are the essential features for antimalarial activity. Principal metabolites include, desethyl – Chloroquine, bisdesethyl – Chloroquine and a carboxylic acid analog which are excreted in the urine along with 52-53% dose in unchanged form.

Chloroquine and amodiaquine have some depressant effects on the bone marrow. Both these agents, cause hemolysis in patients with glucose-6-phosphate dehydrogenase deficiency. The toxicity profile of these agents is less severe and include nausea, vomiting, headache, blurred vision and dermatitis. Chloroquine is more prone to cause photoallergic dermatitis since it accumulates in the skin to a greater extent than amodiaquine. Hence, it must be used with caution in children.

Chloroquine also has anti-histaminic and anti-inflammatory properties. It is used to treat hepatic amebiasis, rheumatoid arthritis, discoid lupus erythematous, cutanea tarda, solar urticaria and polymorphous light erruptions. It is a drug of choice for the suppressive prophylaxis and for the treatment of acute clinical attacks in all types of malaria except chloroquine-resistant falciparum strains. For the treatment of chloroquine-resistant falciparum malaria, a combination therapy comprising of quinine, pyrimethamine and sulfadiazine may be given. The combination of a 2-day course of quinine and a single dose of mefloquine is even more effective.

In chloroquine sensitive strains, response in adults is rapid when 1.5 g of chloroquine is given over 2 days. If parenteral administration is required, the intramuscular route should be preferred. However, to prevent relapsing malaria, a single dose of 79 mg of primaquine phosphate may also be given to the patient. Amodiaquine hydrochloride and hydroxy chloroquine sulfate are other clinically used members of this group having uses and adverse effects similar to chloroquine.

(c) 8-Aminoquinolines:

Primaquine was the first synthetic antimalarial agent to be introduced into the clinical practice in 1929. Principal agents from this class include primaquine, pamaquine and quinocide.

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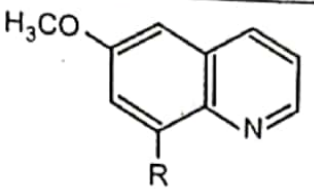
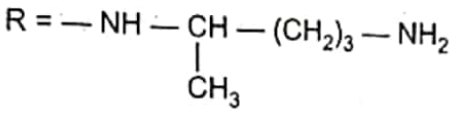
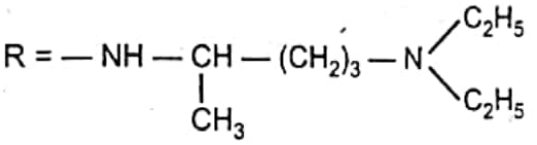


8 - Aminoquinolines

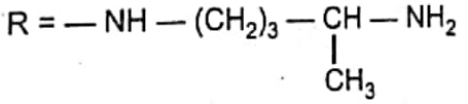
(i) Primaquine:



(ii) Pamaquine:



(iii) Quinocide:

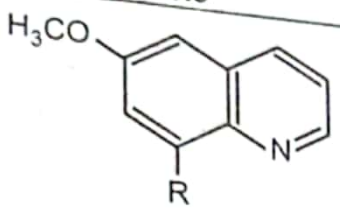


In contrast to 4-aminoquinolines, these agents lack activity against erythrocytic merozoites. In fact, they attack both, the pre-erythrocytic phase of the disease and also show gametocidal activity to some extent. Obviously then, if used alone, they can only be used for prophylactic purposes. They produce radical cure of vivax malarias when they are used alongwith chloroquine.

Their toxic effects are much more severe than those of 4-aminoquinolines and are related principally to the central nervous system and circulatory system. These include, nausea, vomiting, anorexia, headache, hemolytic anemia, leukopenia and methemoglobinemia. Because of its relative safety and lower toxicity, primaquine is the only member of this series which is clinically used.

In the form of its diphosphate, primaquine is completely absorbed by oral route. It is rapidly metabolized to various metabolites that include 5-hydroxyprimaquine, 5-hydroxy-6-desmethyl primaquine and 8-(3-carboxyl-1-methyl propylamino)-6-methoxyquinoline. They all appear in the urine along with small amount of unchanged drug. The parenteral form of primaquine is not available.

The antimalarial activity of primaquine is due to its ability to interfere with protein synthesis, with enzymes and with erythrocyte phospholipid metabolism in the parasites. It is usually given along with a 4-aminoquinoline schizontocide (e.g., chloroquine) to prevent relapses in *P. vivax* and *P. ovale* infections and to reduce the chances of development of chloroquine resistant strains of plasmodium. It may also be used against primary exo-erythrocytic forms of P. falciparum. Besides this, it also depresses myocardial excitability and possesses antiarrhythmic actions.



8 - Aminoquinolines

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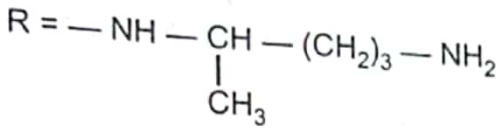
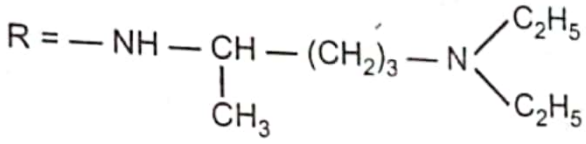
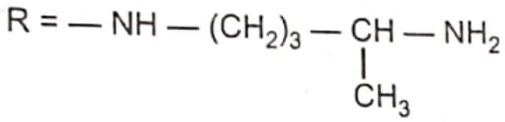


(ii) **Pamaquine:**



(iii) **Quinocide:**



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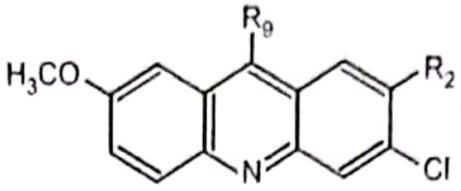
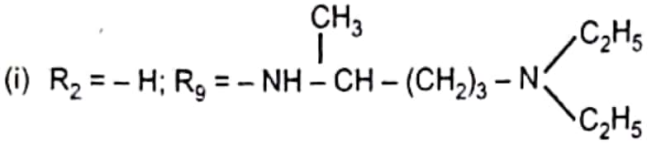
(2) 9-Aminoacridines:

Quinacrine, acriquine and aminoacrichin are the clinically useful agents from this class

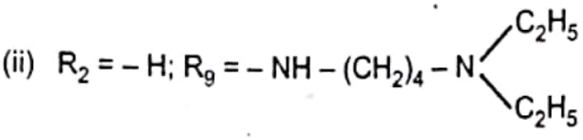


9 - Aminoacridines

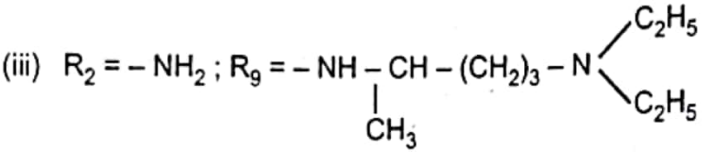
(i) Quinacrine:



(ii) Acriquine:



(iii) Aminoacrichin:



Quinacrine is the most active compound of the series. In general, these derivatives po a high degree of risk with low activity profile. Hence after the development 4-aminoquinolines, they are used rarely for the treatment of malaria.

Yellow pigmentation of the skin and yellow colour appears in the urine during t treatment with quinacrine. These signs disappear with the discontinuation of therap Nausea, vomiting, headache, convulsions, aplastic anemia and psychotomimetic reactio are the adverse effects associated with these drugs.

(3) 2, 4-Diaminopyrimidines:

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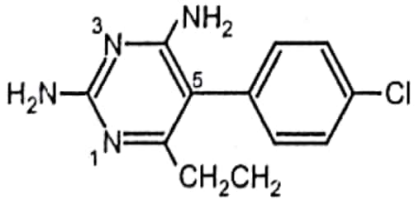
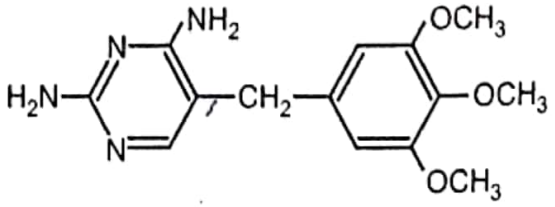


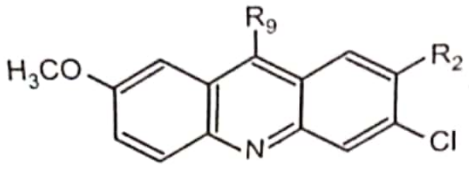
Pyrimethamine



Trimethoprim

(2) 9-Aminoacridines:

Quinacrine, acriquine and aminoacrichin are the clinically useful agents from this class.



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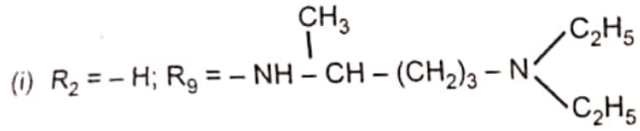
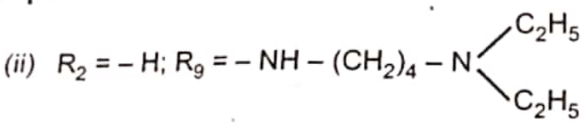


(i) $R_2 = -H$; $R_9 = -NH-CH(CH_3)-(CH_2)_3-N(C_2H_5)_2$

(ii) Acriquine:



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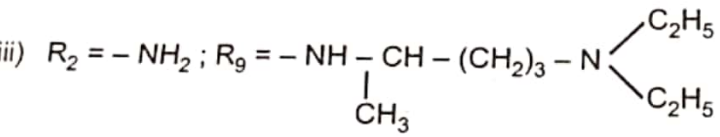

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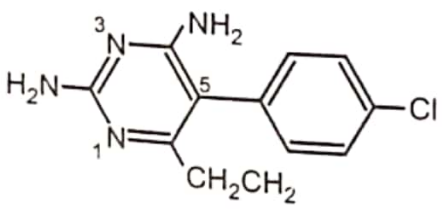
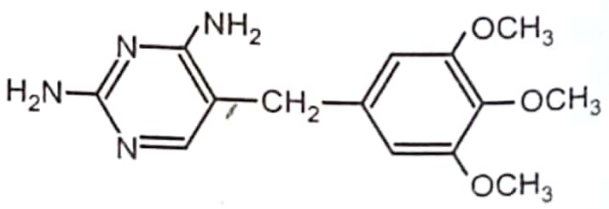


Pyrimethamine



Trimethoprim

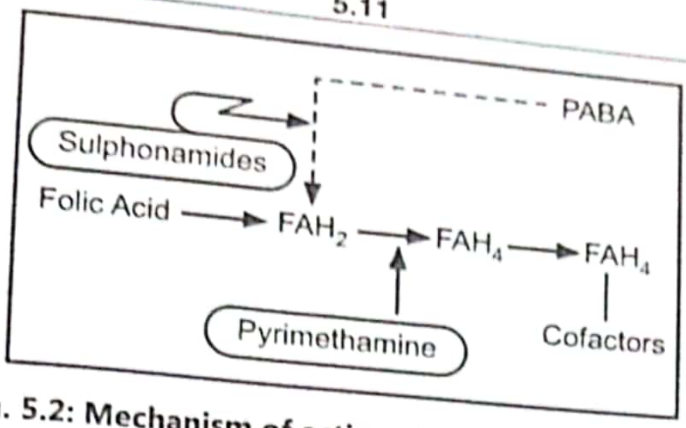


Fig. 5.2: Mechanism of action of Diaminopyrimidines

Pyrimethamine is very effective in the chemoprophylaxis and treatment of chloroquine-resistant falciparum malaria. It is also used in the treatment of toxoplasmosis and pneumocystosis. Diaminopyrimidines, biguanides and dihydrotriazines are the drugs which are designed through the studies of biochemical differences between the host and parasites.

Tetrahydrofolate (FAH_4) is required for the biosynthesis of purines, pyrimidines and certain amino acids (needed for protozoal DNA synthesis). It is obtained by the reduction of dihydrofolate (FAH_2) catalyzed by the enzyme, dihydrofolate reductase. Mammalian cells are permeable to folates whereas bacteria and protozoa are unable to transport preformed folates. Hence, they must synthesize their own folates. Thus, any attempt to inhibit protozoal biosynthesis of FAH_2 (e.g., sulphonamide) or a selective inhibition of the protozoal enzyme, dihydrofolate reductase (e.g., pyrimethamine) leads to the disturbances in the protozoal DNA synthesis and subsequently death of protozoal cells.

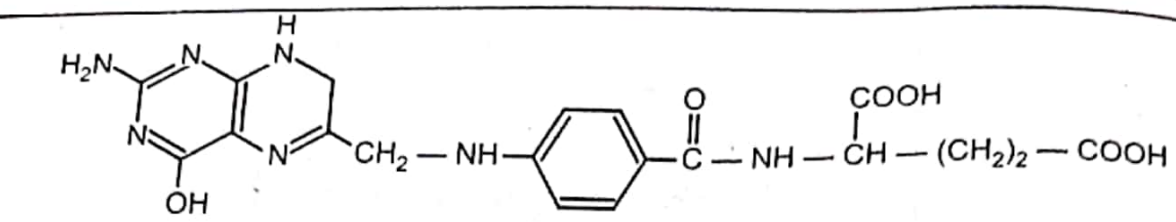
Pyrimethamine inhibits malarial dihydrofolate reductase at concentration far lower than needed to inhibit the mammalian enzymes.

Due to the structural similarity with part of FAH_2 structure, pyrimethamine competitively tries to block the action of dihydrofolate reductase enzyme. However, parasites can develop drug resistance due to utilization of alternative metabolic pathways. Sulphonamides inhibit the conversion of folinic acid to dihydrofolate. Hence, the combination of pyrimethamine with a long acting sulpha drug (e.g., sulfadoxine, sulphamethoxazole etc.) gives a supradditive therapeutic effect and reduces the chances for developing drug-resistant strains. Pyrimethamine is more potent antimalarial agent than chloroguanide and has a much longer duration of action because of its slow rate of excretion.

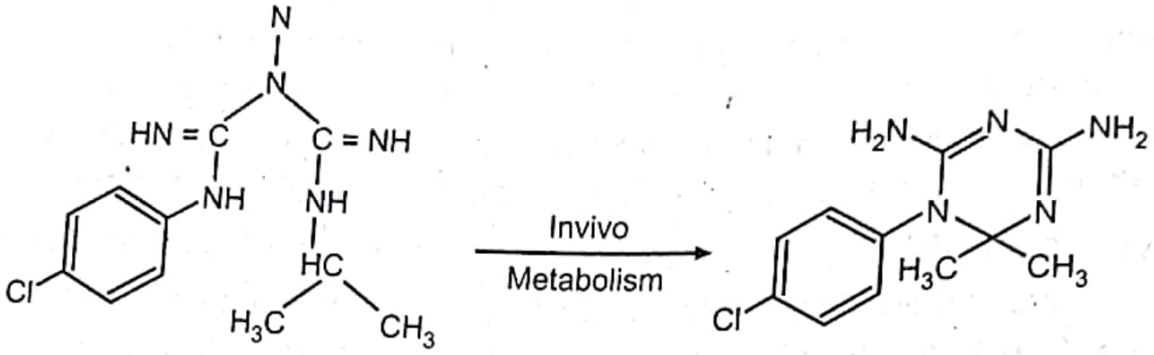
SAR studies found an increase in antimalarial activity if:

1. An electron releasing substituent is present at position six.
2. Chlorine atom is present at para position.
3. Two rings are not separated by carbon or other atom.

Due to non-selectivity in bacterial enzyme inhibition and shorter half life, trimethoprim is never used alone in the treatment of malaria. It is usually combined with another antimalarial agent.



Dihydrofolic acid (FAH_2)



Chloroguanil (Proguanil) Cycloguanil

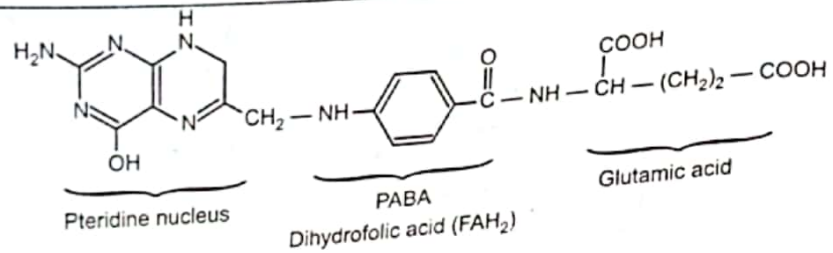
(4) Biguanides:

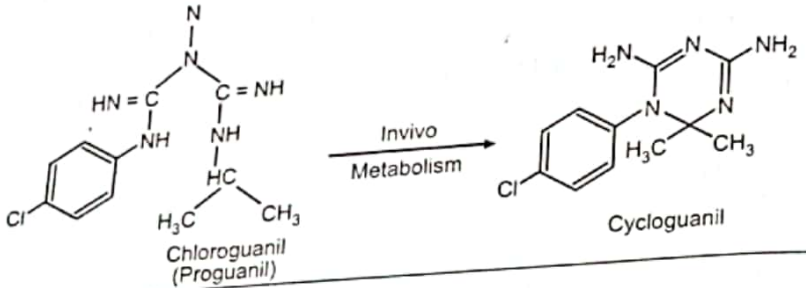
A large number of biguanides and dihydrotriazines have been synthesized and tested for their antimalarial activity. Biguanides are largely prodrugs and are not active until they are metabolized in-vivo to the dihydrotriazine derivatives. Proguanil was first synthesized in Britain in 1945. Chloroguanil is metabolized to the active triazine ring compound cycloguanil which is responsible for its antimalarial activity.

The antimalarial activity of cycloguanil is due to its structural and functional similarity with pyrimethamine. Due to this similarity, the parasites that are resistant to the action of pyrimethamine, also exhibit resistance to the action of chloroguanide. It inhibits dihydrofolate reductase enzyme and interferes in the folic acid metabolism. This leads to inhibition of nuclear division in malarial parasites. Cycloguanil has a duration of action of several weeks. The drug damages gametocytes so that they fail to complete their cycle in mosquito.

Chloroguanide acts slowly and is effective against susceptible strains of plasmodium. However, the development of drug resistant strains limits its clinical utility. In the form of hydrochloride, it is used orally, while in the form of cycloguanil embonate or pamoate, it is used intramuscularly. It provides a longer duration of action.

SAR studies revealed the importance of halogen atom in the phenyl ring. An additional chloro-substitution in the proguanil structure (chloroproguanil) increases activity with simultaneous increase in toxicity.





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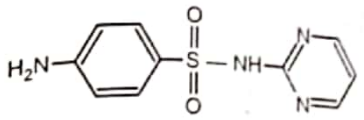
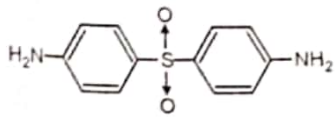
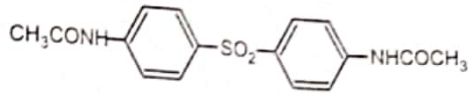
(5) Sulphones and sulphonamides:

Though the antimalarial potency of sulphonamides was proved long back in 1943, they were neglected because of their low therapeutic index. Later on, due to the development of chloroquine resistant strains of *P. falciparum*, long acting sulphonamides were tried in combination with pyrimethamine or trimethoprim. Dapsone, in combination with pyrimethamine can also be effectively used as a chemoprophylactic agent against drug-resistant strains of malarial parasites. Effective members of this class include:


Sulphadiazine

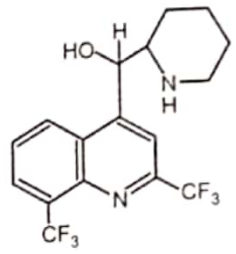

Dapsone


Diacetyl dapsone

Dapsone is found to possess mild toxicity and prolonged duration of action. Both, sulphonamides and sulphones are active only against erythrocytic stages of malarial parasite. They are ineffective in the treatment of *P. vivax* infections.

(6) Miscellaneous agents:

(a) Mefloquine: It bears some degree of structural similarity with quinine. Chemically it is a 4-quinoline-carbinolamine. It is marketed in the form of its racemic mixture where the erythro form is more active than threo-isomer. It is an orally active derivative of 4-quinoline - methanol. About 95 - 98% of administered dose is bound to the plasma-proteins. It has a plasma half-life of 17 days. Principal metabolites include, 2, 8-bis-trifluoromethylquinoline - 4- methanol and a carboxylic acid analog. They are excreted in the faeces. Presence of trifluoromethyl moiety at position 2, lowers down the rate of metabolism and precludes the phototoxic effects commonly associated with other carbinolamines.


Mefloquine

Very little is known about its mechanism of action. It is predicted that it may be acting at erythrocytic stage in the life-cycle of plasmodium. It may affect the ring stages of *P. falciparum* and *P. vivax* by inducing morphological changes. To potentiate its spectrum of activity it may also be used in combination with pyrimethamine, trimethoprim or sulfadoxine. However, it can not be used in infants, children and during pregnancy.

It can be used in both, chemosuppressive and radical cure of infections caused by resistant strains of parasites. Adverse effects are mild and mainly include its effects on CVS and pulmonary systems.

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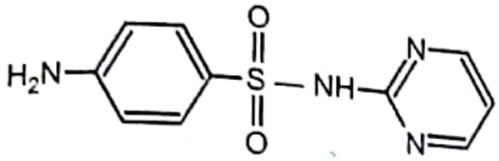
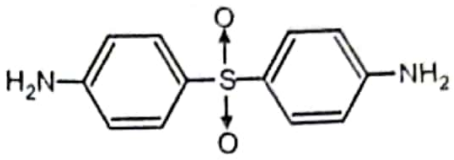


Sulphadiazine



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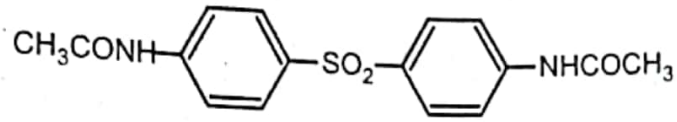

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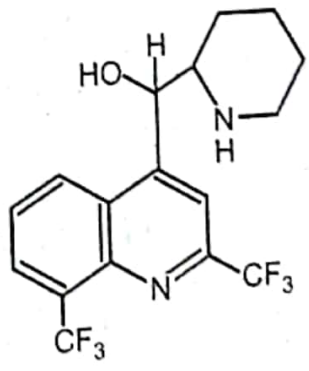

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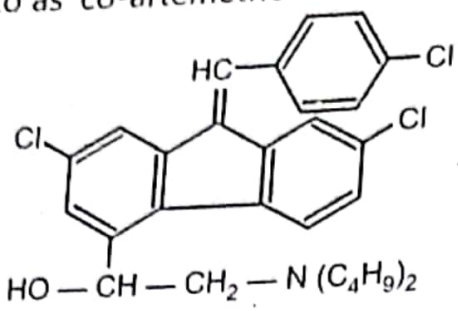
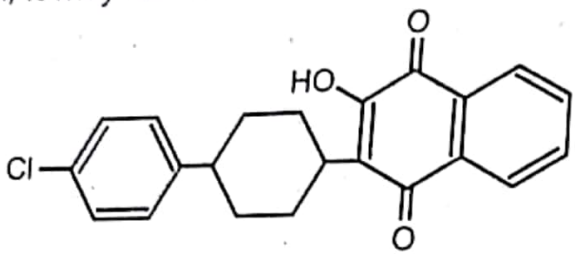
It can be used in both, chemosuppressive and radical cure of infections caused by resistant strains of parasites. Adverse effects are mild and mainly include its effects on CVS and pulmonary systems.

Lumefantrine: It is an antimalarial used only in combination with artemether. T combination is often referred to as 'co-artemether'.



Atovaquone: It is an analogue of ubiquinone with antipneumocystic activity. I combination with proguanil, it may be used in the treatment of malaria.



Halofantrine: It is an antimalarial drug containing a substituted phenanthrene. I structure is related to quinine and lumefantrine. It binds to plasmpesin, a haemoglobi degrading enzyme unique to malarial parasites.

(b) Other antibacterials like tetracyclines, clindamycin, lincomycin and chloramphenicol are found to possess antimalarial activity. A combination of quinine and tetracycline has been used to treat clinical attacks of chloroquine-resistant *P. falciparum* infection. However, the use of antibiotics produces antibiotic-resistant pathogenic bacteria if continued for long-term.

Shortly after the discovery of mefloquine, a new drug, halofantrine has been developed as a promising alternative to mefloquine.

Inactivated, parasitized red blood cells or their fractions and more recently extracellular erythrocytic merozoites have been tested as vaccines in various forms of experimental procedures. In 1976, Trager and Jensen became successful in demonstrating immunogenicity of small amounts of merozoit antigen isolated in high yield and relative purity from cultured parasites. It was a milestone in the history of malariology.

Red blood cells however, are used for culture which exposes the risk of inclusion of red blood cells antigens in the vaccine. This may develop a severe autoimmune haemolytic anaemia in patients receiving this vaccine.

5.5 DRUG RESISTANCE

Over 16,000 agents were synthesized and tested for their antimalarial potential just between 1941-46. Similarly, 250,000 compounds were screened out during the decade 1968-78. Due to the extensive and liberal use of currently available antimalarial drugs, resistance to most of these drugs has developed in the strains of *P. falciparum* (this species

s responsible for about 85% cases of human malaria). The progressive development of drug-resistance in Plasmodium strains may overburden the research programmes to yield new synthetic antimalarials.

Beside the chloroquine resistant strains, incidences of resistance development to pyrimethamine sulfadoxine combinations are also accumulating. The increased number of cases of appearance of multi-drug resistant strains of *P. falciparum* and failure of quinine to re-exhibit its clinical potency are the problems of severe concern. It is for this reason, mefloquine should be reserved only for the treatment of multi-drug resistant strains and it should not be over exposed. The parasites would not need much time to develop mefloquine-resistant strains, if misuse of this drug is permitted.

Unfortunately the underlying principles of acquired resistance to antimalarial drugs still remain unclear and demand further investigations.

Synthesis

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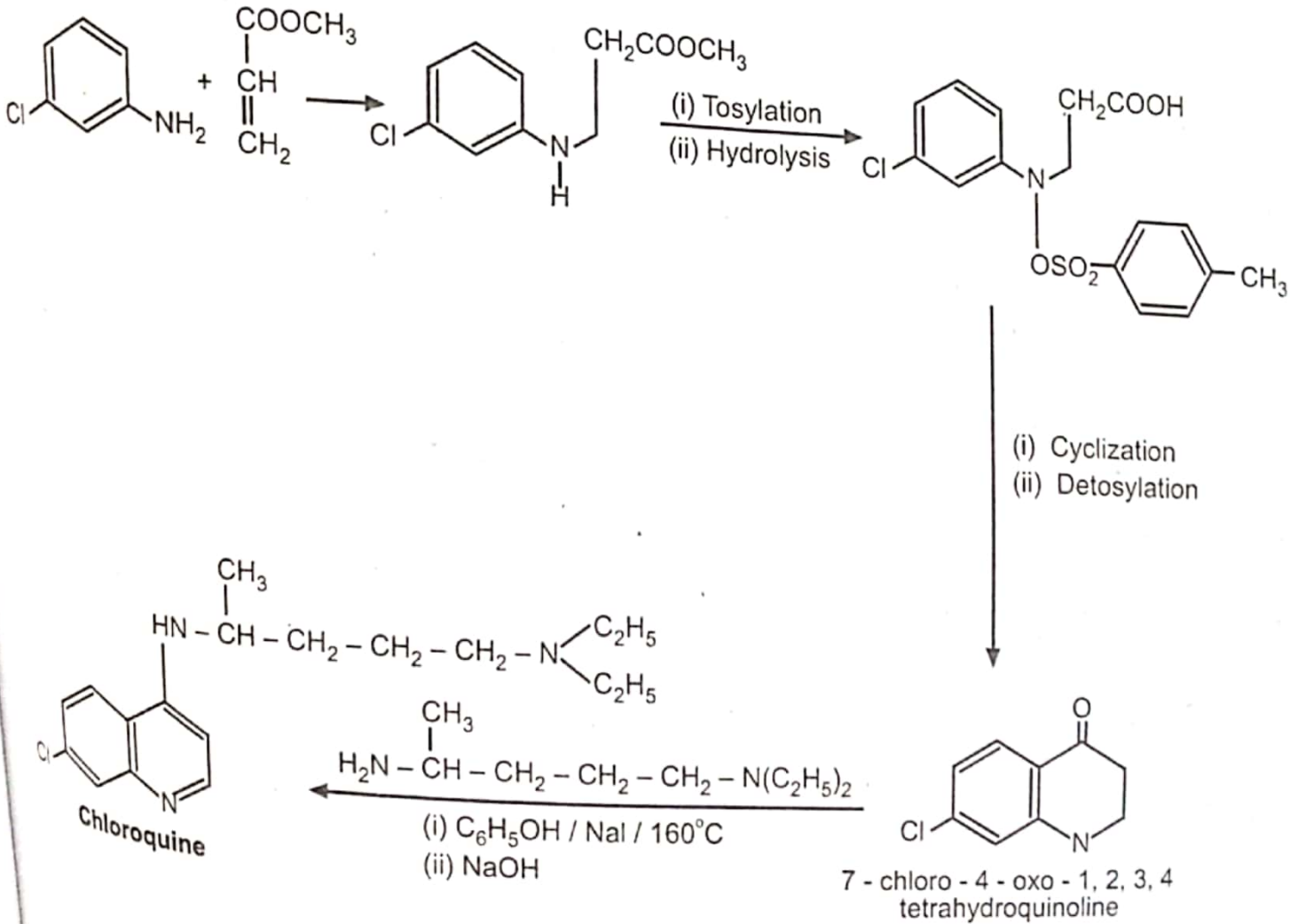

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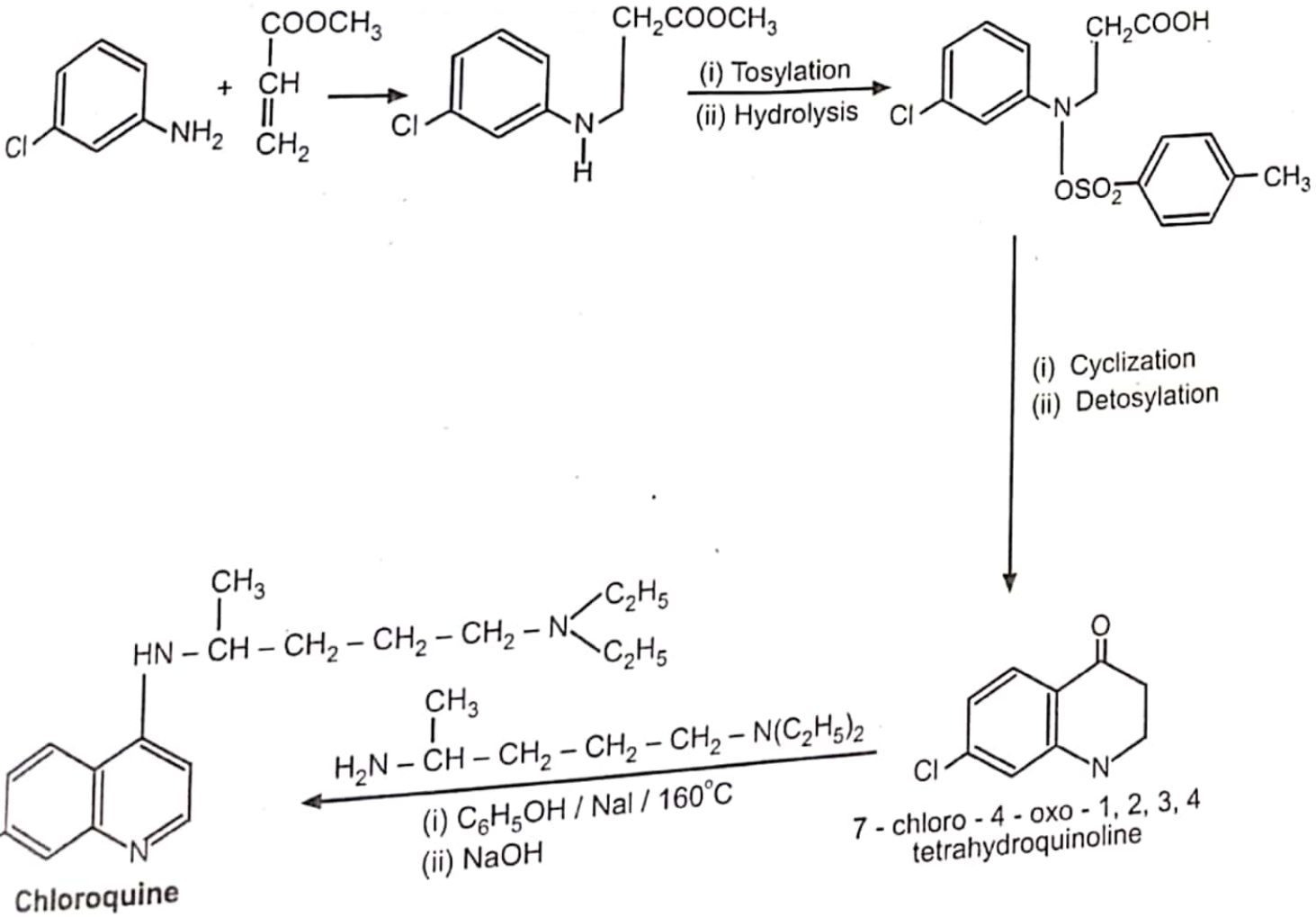
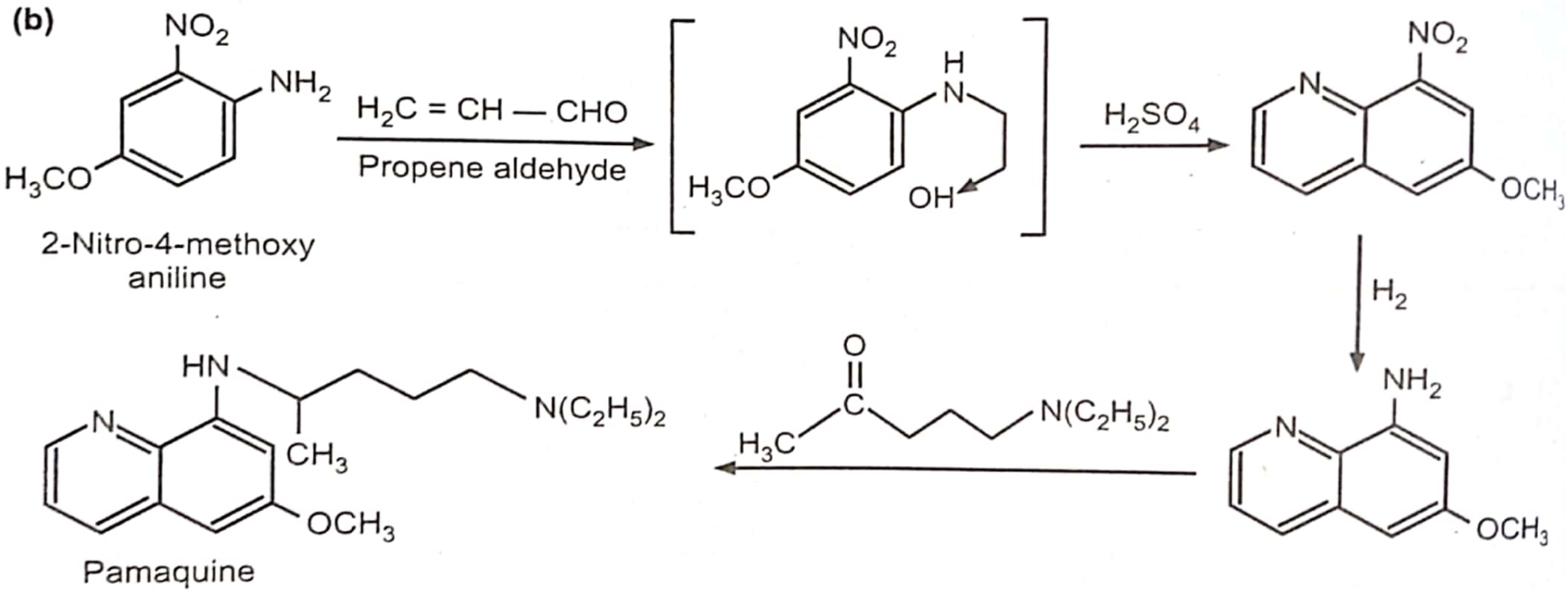


(ii) Pamaquine:

(a) $H_2C(OH)-CH(OH)-CH_2OH$ (Glycerol) $\xrightarrow[\text{Dehydration}]{\text{Conc. } H_2SO_4}$ $H_2C=CH-CHO$ (Propene aldehyde)

(b)



Chapter...6

UNIT III

ANTI-TUBERCULAR AGENTS

♦ SYNOPSIS ♦

6.1 INTRODUCTION

Tuberculosis and leprosy are the diseases caused by Mycobacteria species. Tuberculosis is caused by either *Mycobacterium tuberculosis* (in man) or by *Mycobacterium bovis* (in animals) while leprosy is caused by *Mycobacterium leprae*.

Tuberculosis is a disease of respiratory transmission. A person gets infected when he comes in contact with the environment contaminated with viable tubercle bacilli. These bacilli are expelled by coughing, sneezing, shouting and singing of a patient with active tuberculosis. When these bacilli are inhaled by a person, they are inoculated into his respiratory bronchioles and alveoli usually towards the apex of the lung. When these microorganisms are multiplied to the sufficient extent, an antigen-antibody interaction is evoked by the cell-mediated T-lymphocytes. Tubercles (Ghon foci) are then formed due to accumulation of macrophages at the site of infection. This may lead to either permanent suppression of the infection or some microbes may survive in the foci and may become the source of postprimary infection when these foci breaks down under the conditions of weak host defence mechanisms. This may occur immediately or months or years later. The hilar lymph nodes may get easily infected due to spreading of some macrophages containing active bacilli. The released microorganisms from the foci are circulated through lymph and blood to different parts of the body and infect (i) reticuloendothelial system (e.g., liver, spleen and lymph nodes), (ii) serosal surfaces and (iii) sites with high oxygen pressure (e.g. apices of lungs, renal cortex and epiphyses of growing bones). Due to the multiplication of microorganisms at these sites, numerous small foci develop throughout the body. This type of wide-spread infection is known as 'miliary tuberculosis'.

In some patients, the formation of Ghon foci leads to temporary suppression of the infection. Microorganisms may still be present in the foci. During coughing, the caseous material containing live microorganisms is expelled out leaving cavities in the lungs.

These active bacilli may then either be:

(i) swallowed by the same patient resulting into infection of his alimentary tract, or

(ii) inhaled by a healthy adult resulting in an infection of his trachea, larynx or bronchi.

Such cases are more possible in the conditions of overcrowding and poor personal and public hygiene. Infections of oropharynx, larynx and tracheobronchial tree respond fairly well to anti-tuberculosis therapy while infections in gastrointestinal tract, urinary tract or lymph nodes respond partially to the drug-treatment.

Tubercles are formed in the infected organs during the course of the disease. Hence, the disease is known as 'tuberculosis'. The main symptoms are cough, tachycardia, cyanosis and respiratory failure. Depending upon the site of infection, the disease is known as:

(i) pulmonary tuberculosis (respiratory tract),

(ii) genitourinary tuberculosis (genitourinary tract),

(iii) tuberculous meningitis (nervous system), and

(iv) miliary tuberculosis (a wide-spread infection)

A wide variety of drugs are clinically available for the treatment of tuberculosis. Efforts began in 1938 when sulfanilamide and dapsone did not satisfy the clinical requirements of antituberculosis therapy. The major breakthrough was given by Waksman and his co-workers by the introduction of streptomycin in 1944. This was followed by the introduction of p-amino salicylic acid, isoniazid, ethambutol and rifampin.

Chemotherapy of tuberculosis faced some special problems because of slow growth rate of mycobacteria and their intracellular location. Since, the disease is chronic by its nature, the therapy needs to be continued for atleast about 1 - 2 years in most of the cases. In such a chronic treatment, if only single drug is used, the risk of development of drug-resistant strains of Mycobacteria is always high. This is coupled with the risk of drug toxicity due to high doses of a single drug needed. The obvious solution to this problem is to use combination therapy. When two or more effective drugs are used in combination, resistance will not develop. However, drugs with similar toxicological profiles should not be used together.

The drugs used in the combination therapy are usually selected from ethambutol, isoniazid and rifampin. The choice is dependent upon the type of disease and some patient-related factors.

For the sake of clinical convenience, these drugs are categorised into:

(i) First-line agents:

These are the drugs having high activity and reduced or minimal toxicities. Examples include, streptomycin, isoniazid, ethambutol and rifampin.

(ii) Second-line agents:

These are the drugs having less efficiency and significant toxicity. Examples include, pyrazinamide, ethionamide, amino salicylic acid, kanamycin, capreomycin, viomycin, amikacin and cycloserine. These drugs are relatively toxic. Hence, they should be used only when the organism develops resistance to the first-line agents.

The above scheme of classification of antituberculosis agents is made by taking into account efficacy, organism susceptibility and spectrum of adverse reactions of the drug.

Table 6.1: Clinically used anti-tuberculosis agents

Drug	Route of administration	Plasma protein bound (%)	Plasma half-life (hour)	Principal metabolites	Prominant adverse effects
(I) First-line agents:					
1. Isoniazid	orally parenterally	Nil	1 - 1.5	Acetylisoniazid, isonicotinic acid and its glycine conjugate, isonicotinyl hydrazones, and N-methylisoniazid	Hepatitis, peripheral neuropathy and hypersensitivity
2. Streptomycin	intramuscularly intrathecally	50 - 60	5 - 7	Nil metabolism	Ototoxicity and nephrotoxicity
3. Ethambutol	orally	20 - 30	3 - 4	An aldehyde and dicarboxylic acid derivative	Optic neuritis and hypersensitivity
4. Rifampin	orally	90	3.5 - 4.0	25 - 0 - desacetyl - rifamycin and 3-formyl-rifamycin	Hepatitis, fever, thrombo-cytopenia
(II) Second-line agents:					
1. p-Amino salicylic acid	orally	60	1.0	N-Acetyl derivative	GI-intolerance, hepatotoxicity, hypersensitivity, fluid retention
2. Kanamycin	intramuscularly	1 - 3	2.5	Nil metabolism	Ototoxicity and nephrotoxicity
3. Ethionamide	orally	10	1 - 1.5	Carbomoyl, thiocarbomoyl and 5-oxocarbomoyl derivative	GI-intolerance, hepatotoxicity and hypersensitivity
4. Pyrazinamide	orally	50 - 60	9 - 10	Pyrazinoic acid and 5-hydroxy-pyrazinoic acid	Hepatotoxicity and hyperuricemia
5. Cycloserine	orally	–	–	Minor metabolism	Rash, seizures, and psychoses
6. Capreomycin	intramuscularly	–	–	Minor metabolism	Ototoxicity and nephrotoxicity
7. Viomycin	intramuscularly	–	–	Minor metabolism	Ototoxicity and nephrotoxicity

6.2 FIRST-LINE AGENTS

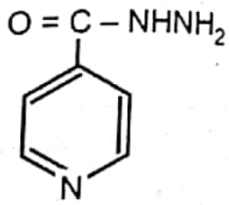
(a) Streptomycin:

Amikacin, kanamycin and streptomycin are aminoglycoside antibiotics which are having *in-vitro* bactericidal and *in-vivo* bacteriostatic activity against *Mycobacterium tuberculosis*. All these agents exhibit more or less similar pharmacological and toxicological properties. Streptomycin reported in 1944, was the first clinically effective anti-tuberculosis agent. Its introduction radically changed the handling and prognosis of patients with these diseases. It is administered by I.M. route and occasionally by intrathecal route. Initially, it was given in large doses. The development of drug-resistant strains and incidences of severe adverse effects then aroused the awareness about its dose-calculations. Presently, it is given in the combination with other drugs. It is a more preferred agent in the treatment of tuberculous meningitis than that of pulmonary tuberculosis. It helps to suppress the disease but does not help to eradicate it. This preventive action may result due to its inventory action on the bacterial protein synthesis.

It may cause nephrotoxicity, ototoxicity and blood dyscrasias in patients. Its potential to cause these adverse effects is related to dose and duration of the treatment. Streptomycin resistant strains are usually treated with kanamycin. If resistance develops to kanamycin then viomycin may be tried.

(b) Isoniazid:

Introduced in 1952, isoniazid is an extremely effective and safe antimycobacterial agent. Chemically it is a hydrazide of isonicotinic acid. It exhibits bacteriostatic action on the resting bacilli. Though its single agent therapy is approved, rifampin-isoniazid combination is the most favoured anti-tuberculosis therapy.



Isoniazid

It is an orally active agent and its oral absorption is reduced by the presence of food and antacids. It does not bind to plasma proteins. It has a plasma half-life of 1-1.5 hours. It is well distributed to different body tissues and fluids including cerebrospinal fluid. Because of its wide-spread distribution in the body, it is equally effective against all types of tuberculosis. It undergoes significant first pass hepatic metabolism. The principal metabolites include acetylisoniazid, isonicotinic acid and its glycine conjugate, isonicotinyl hydrazones and N-methyl-isoniazid. They are excreted in the urine alongwith 10 - 25% dose in unchanged form.

Mechanism of Action:

Isoniazid inhibits mycolase synthetase, an enzyme necessary for the biosynthesis of mycolic acids. The latter are the important constituents of mycobacterial cell-wall. Since, mycolic acids are present only in mycobacteria, isoniazid exhibits such a high degree of anti-mycobacterial action.

Because of its ability to complex essential metals such as copper or iron present in mycobacterial enzyme, it interferes with various enzyme systems requiring pyridoxal phosphate as a cofactor. This results in changes in the metabolism of lipids, proteins and carbohydrates. Nucleic acid synthesis is also affected.

Adverse Effects:

Most of the isoniazid undergoes metabolism by N-acetylation process in the liver. The latter depends on the transfer of acetyl group from coenzyme A by an N-acetyl-transferase. Since the rate of acetylation is under genetic control, patients may be categorised as slow acetylators and rapid acetylators. In slow acetylators, the rate of isoniazid metabolism is slow resulting into more prolonged plasma levels of isoniazid than in rapid acetylators. Hence, slow acetylators are more susceptible to the adverse effects than do rapid acetylators.

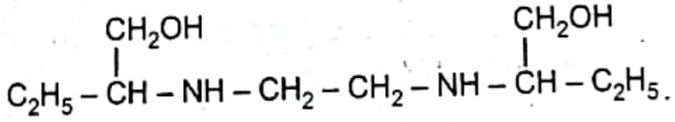
The most common adverse effects of isoniazid include dryness of mouth, epigastric distress, allergic reactions, peripheral neuritis, mental abnormalities, methaemoglobinemia, and hepatotoxicity.

The most significant adverse reaction is hepatotoxicity that may arise due to acetyl-hydrazine, a toxic metabolite of acetylisoniazid. It is characterized by fatigue, malaise, weakness, anorexia, fever arthralgias and increased Serum Glutamic-Oxaloacetic acid Transaminase (SGOT) level.

The neurotoxicity specifically is seen in malnourished patients, chronic alcoholics or slow acetylators. It usually occurs in the form of peripheral neuritis which is characterized by numbness and tingling in lower extremities and paresthesias in the hands and fingers. A supplimentary dose of pyridoxal phosphate (vitamin B_6) of 10 mg daily (for 50-100 mg isoniazid dose) corrects the neurotoxic effects of isoniazid.

(c) Ethambutol:

It is an orally effective bacteriostatic agent active against most strains of *M. tuberculosis*, *M. kansas* and *M. marinum*. However, *M. avium* and *M. intracellulare* are usually resistant to its action.



Ethambutol

Chemically it is ethylene diamino-di-1-butanol. Activity is stereospecific. Dextro isomer is having the maximum antimycobacterium activity. Upon oral administration, it is well distributed in most of the body tissues and fluids except cerebrospinal fluid. Because of drug-retention ability, erythrocytes may serve the function of slow drug releasing depots. About 20 - 30% administered dose is bound to the plasma-proteins. It has a plasma half-life of about 3 - 4 hours. Major metabolites include an aldehyde and a dicarboxylic acid derivative which are excreted in the urine alongwith about 70% dose in unchanged form. SAR studies indicated that the nature of branching, distance between two nitrogen and extent of N-alkylation are the activity governing factors.

Little is known about its mechanism of action. It probably interferes in the synthesis of proteins and nucleic acids by acting as an antimetabolite. Its complex forming ability is also a contributing factor to its bacteriostatic activity.

Adverse effects include nausea, vomiting, abdominal pain, optic neuritis, headache, drug fever, malaise, diminished visual activity, dermatitis, pruritus, joint pain, dizziness, confusion, hallucination and peripheral neuritis. It is contraindicated in pregnancy and in children below 13 years of age. Monthly eye-examination of patients is necessary.

It is the most favoured drug used in combination therapy with rifampin or isoniazid against streptomycin-resistant strains of tubercle bacilli.

(d) Rifampin:

It is an orally active bactericidal semi-synthetic derivative of rifamycin B, a macrocyclic antibiotic produced by *Streptomyces mediterranei*. Its oral absorption is impaired in the presence of food and p-amino salicylic acid. It is well distributed to almost every body tissue and fluid including cerebrospinal fluid. About 90% administered dose is bound to the plasma-proteins. It has a plasma half-life of 3.5 - 4.0 hours. The principal metabolites include 25 desacetylrifamycin (active) and 3-formylrifamycin (inactive). They are excreted in the urine alongwith 7-10% dose in unchanged form. About 60-65% dose appears in the faeces through the bile circulation.

Mechanism of Action:

DNA - dependent - RNA polymerase is an enzyme necessary for RNA synthesis. Rifampin acts on B-subunit of this enzyme resulting into formation of stable complex. This in turn, causes inhibition of bacterial RNA synthesis. However, mammalian enzymes are not affected by this drug.

Adverse effects include nausea, vomiting, headache, erythema, nervousness, restlessness, emotional disturbances, tremors, pulmonary edema, hyperglycemia, hypokalemia, increased cardiac output and cardiac arrythmias.

Rifampin is a first-line agent. Since bacterial resistance develops rapidly if rifampin is taken alone, its combination with either isoniazid or ethambutol are preferably used. However, combined use of isoniazid and rifampin may increase the risk of hepatotoxicity.

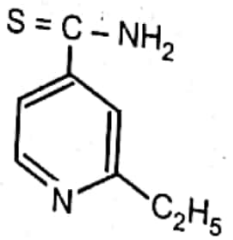
6.3 SECOND-LINE AGENTS

A number of second-line agents are available that may be used specifically when bacterial resistance or severe drug toxicity develops with first-line agents.

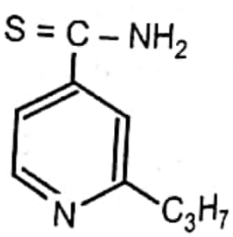
(a) Ethionamide:

Prothionamide and ethionamide are the congeners of thioisonicotinamide having anti-mycobacterial activity.

Ethionamide was synthesized in 1956 with an aim to improve further antimycobacterial activity of the thioureas and thiosemicarbazones. Chemically it is 2-ethylthioisonicotinamide. It is a thioamide analoge of isoniazid.



Ethionamide



Prothionamide

It is an orally effective agent. However to minimize mucosal irritation, it is usually given in the form of enteric-coated capsules. It is well distributed to various body tissues and fluids including cerebrospinal fluid. About 10% administered dose is bound to the plasma-proteins. It has a plasma half-life of 1 - 1.5 hours. Principal metabolites include carbomoyl, thiocarbomoyl and 5-oxocarbomoyl analogs which are excreted in the urine alongwith < 1% dose in unchanged form.

Mechanism of Action:

(i) It may interfere in peptide synthesis by acting as antimetabolite and inhibiting the incorporation of sulfur-containing amino acids.

(ii) It may inhibit mycobacterial mycolic acid synthesis.

(iii) It may affect dehydrogenase systems in tubercle bacilli, and

(iv) It may form a substituted isonicotinic acid derivative that may interfere with NAD-dependent systems.

Adverse effects include nausea, vomiting, anorexia, diarrhoea, headache, skin rashes, blurred vision, drowsiness, depression, asthenia, olfactory disturbances, restlessness, tremors, impotence and postural hypotension. Because of structural similarity, it blocks hepatic acetylation of isoniazid by acting as alternative substrate for acetylation.

It is used in combination with other antimycobacterial agent in the treatment of pulmonary tuberculosis resistant to isoniazid.

(b) p-Amino Salicylic Acid:

Because of its sour taste and irritant properties, this drug is mainly used in the form of its sodium, potassium or calcium salts. In the salt form, it is more water-soluble and less irritant to gastrointestinal mucosa. Moreover, aluminium hydroxide is usually included in its formulation to further reduce GIT-irritation caused by the drug. It is also available in the form of its phenyl and benzoyl esters.

It is an orally active and is widely distributed in various body tissues and fluids. About 60% administered dose is bound to the plasma-proteins. It has a plasma half-life of 1.0 hour. The principal metabolites include acetylated derivative, free and acetylated p-amino salicyluric and 2, 4-dihydroxy benzoic acid which are excreted in the urine alongwith about 40% dose in unchanged form. Probenecid prolongs its duration of action by inhibiting its tubular excretion.

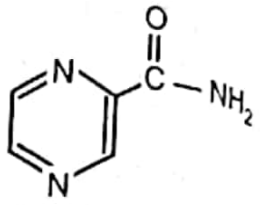
Adverse effects include nausea, abdominal distress, diarrhoea, anorexia, eosinophilia, leukopenia, agranulocytosis, thrombocytopenia, hemolytic anemia and allergic reactions. Crystalluria may develop due to poor solubility of free drug and its metabolites in acidic urine. In children, it develops acidosis because of its strongly acidic nature.

Its structural similarity with PABA suggests its possible role in folate biosynthesis. It interferes with the transfer of one carbon unit. It however is effective against only certain mycobacteria. Like ethionamide, it inhibits isoniazid metabolism by competing for hepatic enzymes involved in isoniazid acetylation. Hence, it elevates the serum isoniazid level when

concomittantly administered. It also delays the development of bacterial resistance t streptomycin and isoniazid.

(c) Pyrazinamide:

It is a pyrazine analog of nicotinamide. It is a drug of low potency and constitutes part of short term (six months) multiple drug therapy alongwith streptomycin and isoniazid. Almost all structural modifications of pyrazinamide resulted in the decrease in the activity.



Pyrazinamide

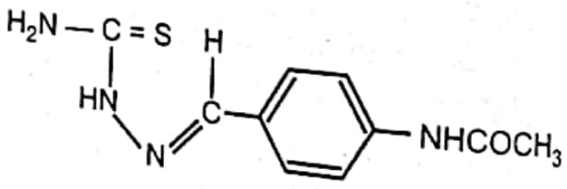
It is an orally effective agent. About 50 - 60% administered dose is bound to the plasma proteins. It has a plasma half-life of 9 - 10 hours. Principal metabolites include pyrazinoi acid and 5 hydroxypyrazinoic acid. They appear in the urine alongwith 4 - 14% dose i unchanged form.

Adverse effects include nausea, vomiting, urinary retention, anorexia, dysuria, drug rash fever, malaise, arthralgias, jaundice, hepatic necrosis and decreased urate excretion. Th pyrazinoic acid metabolites decrease the renal tubular excretion of urate and may induc hyperuricemia and acute gouty arthritis. The concomittant administration of p-amino salicylic acid was found to prevent or delay the appearance of hyperuricemia. The drug is contraindicated in patients with gouty conditions or hepatic dysfunction.

Its mechanism of action is still unclear. It acts probably by suppressing bacterial protein synthesis. Pyrazinamide is reserved mainly for the treatment of resistant strains of tubercle bacilli, where it is used alongwith isoniazid.

(d) Thiacetazone (Amithiozone):

Based upon some encouraging results with sulphonamides and sulphones, Domagk and co-workers designed this drug in 1948. It was found to be effective in tuberculous laryngitis and enteritis but not in acute miliary or meningeal tuberculosis.



Thiacetazone

Chemically, it is a thiosemicarbazone. Because of the structural similarity with heterocyclic sulphonamides, it may have sulphonamide like action. It forms a copper complex that interferes with the biochemical carriers for copper in Mycobacteria.

It is orally effective and is excreted primarily in the urine. Adverse effects include nausea, omiting, skin rashes, leukopenia, hemolytic anemia, thrombocytopenia, hepatotoxicity and enal damage.

It is now rarely used to treat pulmonary tuberculosis and tuberculoid leprosy. It is owever effective in delaying the emergence of isoniazid resistant tubercle bacilli. It may so be used in some combination regimens for leprosy. The drug is given in the daily dose 50 mg which may then gradually be increased to 300 mg and then is continued for many onths.

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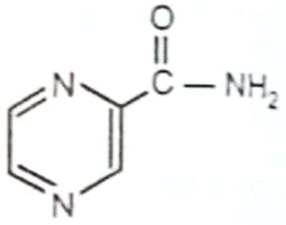

Pyrazinamide

It is an orally effective agent. About 50 - 60% administered dose is bound to the plasma-proteins. It has a plasma half-life of 9 - 10 hours. Principal metabolites include pyrazinoic acid and 5 hydroxypyrazinoic acid. They appear in the urine alongwith 4 - 14% dose in unchanged form.

Adverse effects include nausea, vomiting, urinary retention, anorexia, dysuria, drug rash, fever, malaise, arthralgias, jaundice, hepatic necrosis and decreased urate excretion. The pyrazinoic acid metabolites decrease the renal tubular excretion of urate and may induce hyperuricemia and acute gouty arthritis. The concomittant administration of p-amino salicylic acid was found to prevent or delay the appearance of hyperuricemia. The drug is contraindicated in patients with gouty conditions or hepatic dysfunction.

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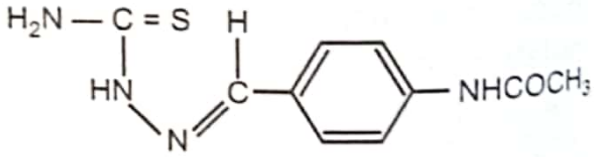

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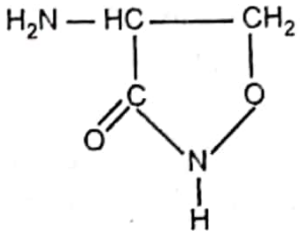
It is now rarely used to treat pulmonary tuberculosis and tuberculoid leprosy. It is however effective in delaying the emergence of isoniazid resistant tubercle bacilli. It may also be used in some combination regimens for leprosy. The drug is given in the daily dose of 50 mg which may then gradually be increased to 300 mg and then is continued for many months.

(e) Cycloserine:

It is an analog of D-alanine having broad spectrum antimicrobial profile. It inhibits the growth of some gram-positive and gram-negative bacteria.



Cycloserine

It is obtained from *Streptomyces orchidaceus*. Chemically it is D - 4 - amino - 3 - isoxazolidone. It is orally effective drug. It is widely distributed in various body tissues and fluids, including cerebrospinal fluid. It is excreted in the form of its metabolites and 50% unchanged drug in the urine.

Adverse effects include headache, visual disturbances, nervousness, irritability, depression, confusion, tremors and psychoses. The concomittant administration of vitamin B_6 (100 mg three times a day) reduces its neurotoxicity.

Because of structural similarity with D-alanine, cycloserine prevents the synthesis of cross-linking dipeptide which is necessary in the formation of bacterial cell-wall.

Cycloserine is now rarely used in the treatment of tuberculosis. It is however used in the treatment and long-term suppression of urinary-tract infections.

(f) Capreomycin and Viomycin:

Both these antibiotics are strongly basic peptides having close structural resemblance. Capreomycin is produced by *Streptomyces capreolus* while viomycin is produced by *Streptomyces pumiceus*. Both share similar pharmacological and toxicological properties. Capreomycin is more potent but less toxic antimycobacterial agent than viomycin. The crude capreomycin is a mixture of four cyclic polypeptides, IA, IB, IIA and IIB. The clinically used drug consists mainly IA and IB.

Like all other polypeptide antibiotics, these agents are given parenterally. Injections of these drugs are painful. Adverse effects include skin rashes, drug fever, blood dyscrasias, ototoxicity, nephrotoxicity and severe pain at the site of injection. Nephrotoxicity is characterized by pyuria, proteinurea, hematuria, nitrogen retention, and electrolyte disturbances. Viomycin is rarely indicated in children.

Both these agents act by binding to both 30 S and 50 S ribosomal subunits resulting into inhibition of bacterial protein synthesis.

Capreomycin is mainly used in combination with ethionamide or ethambutol against Streptomycin resistant strains of *M. tuberculosis*. Sometimes isoniazid or amino salicylic acid may also be used in the combination with capreomycin.

6.4 ANTI-TUBERCULAR ANTIBIOTICS

(i) Rifampicin

It is an orally active bactericidal semi-synthetic derivative of rifamycin B, a macrocylic antibiotic produced by *Streptomyces mediterranei*. Its oral absorption is impaired in the presence of food and p-amino salicylic acid. It is well distributed to almost every body tissue and fluid including cerebrospinal fluid. About 90% administered dose is bound to the plasma-proteins. It has a plasma half-life of 3.5 - 4.0 hours. The principal metabolites include 25 desacetylrifamycin (active) and 3-formylrifamycin (inactive). They are excreted in the urine alongwith 7-10% dose in unchanged form. About 60-65% dose appears in the faeces through the bile circulation.

Mechanism of Action:

DNA - dependent - RNA polymerase is an enzyme necessary for RNA synthesis. Rifampin acts on B-subunit of this enzyme resulting into formation of stable complex. This in turn causes inhibition of bacterial RNA synthesis. However, mammalian enzymes are not affected by this drug.

Adverse effects include nausea, vomiting, headache, erythema, nervousness, restlessness, emotional disturbances, tremors, pulmonary edema, hyperglycemia, hypokalemia, increased cardiac output and cardiac arrythmias.

Rifampin is a first-line agent. Since bacterial resistance develops rapidly if rifampin is taken alone, its combination with either isoniazid or ethambutol are preferably used. However, combined use of isoniazid and rifampin may increase the risk of hepatotoxicity.

(ii) Rifabutin (1992):

Rifabutin is a bactericidal antibiotic drug used to treat tuberculosis in those who can not tolerate rifampin. For active tuberculosis it is used with other antimycobacterial medications. It is used as first line treatment for latent (drug-resistant) tuberculosis. Common side effects include nausea, abdominal pain, headache, muscle pain and low blood neutrophil levels.

(iii) Cycloserine:

It has already been discussed on page 6.9.

(iv) Streptomycine:

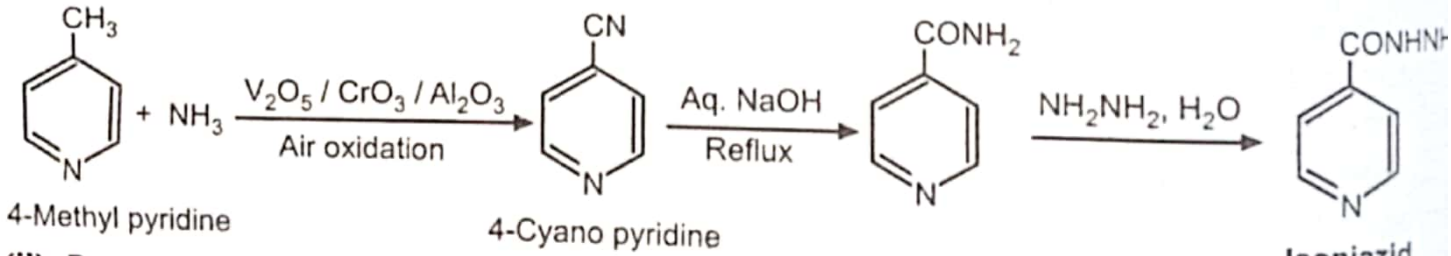
It has already been discussed in chapter 1 under aminoglycosides, page no. 1.23.

(v) Capreomycin sulphate:

It has already been discussed on page 6.10.

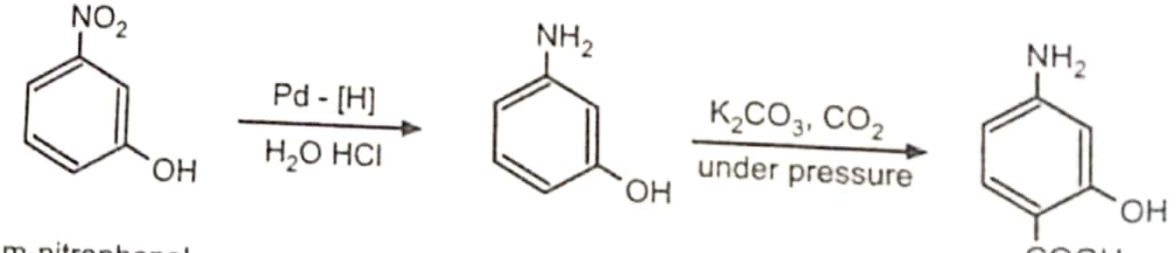
Synthesis

(i) Isoniazid:



4-Methyl pyridine — 4-Cyano pyridine — **Isoniazid**

(ii) Para amino salicylic acid:



m-nitrophenol — m-aminophenol — **p-amino salicylic acid**

Mechanism of Action:

DNA - dependent - RNA polymerase is an enzyme necessary for RNA synthesis. Rifampin acts on B-subunit of this enzyme resulting into formation of stable complex. This in turn, causes inhibition of bacterial RNA synthesis. However, mammalian enzymes are not affected by this drug.

Adverse effects include nausea, vomiting, headache, erythema, nervousness, restlessness, emotional disturbances, tremors, pulmonary edema, hyperglycemia, hypokalemia, increased cardiac output and cardiac arrythmias.

Rifampin is a first-line agent. Since bacterial resistance develops rapidly if rifampin is taken alone, its combination with either isoniazid or ethambutol are preferably used. However, combined use of isoniazid and rifampin may increase the risk of hepatotoxicity.

(ii) Rifabutin (1992):

Rifabutin is a bactericidal antibiotic drug used to treat tuberculosis in those who can not tolerate rifampin. For active tuberculosis it is used with other antimycobacterial medications. It is used as first line treatment for latent (drug-resistant) tuberculosis. Common side effects include nausea, abdominal pain, headache, muscle pain and low blood neutrophil levels.

(iii) Cycloserine:

It has already been discussed on page 6.9.

(iv) Streptomycine:

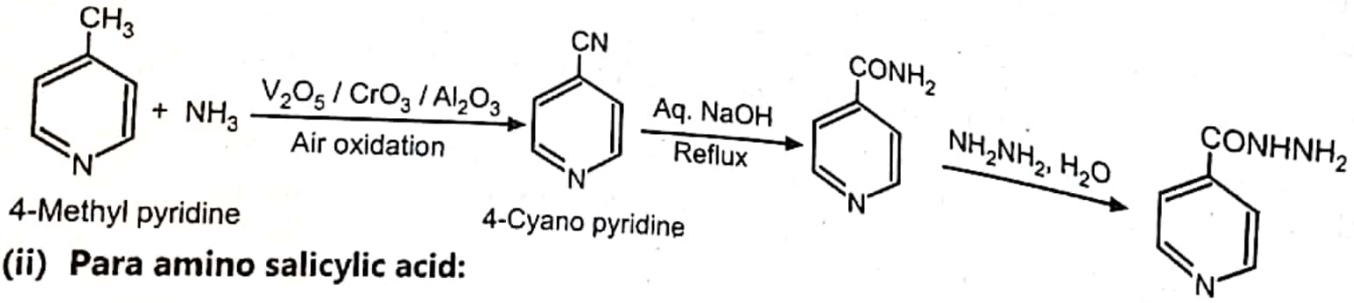
It has already been discussed in chapter 1 under aminoglycosides, page no. 1.23.

(v) Capreomycin sulphate:

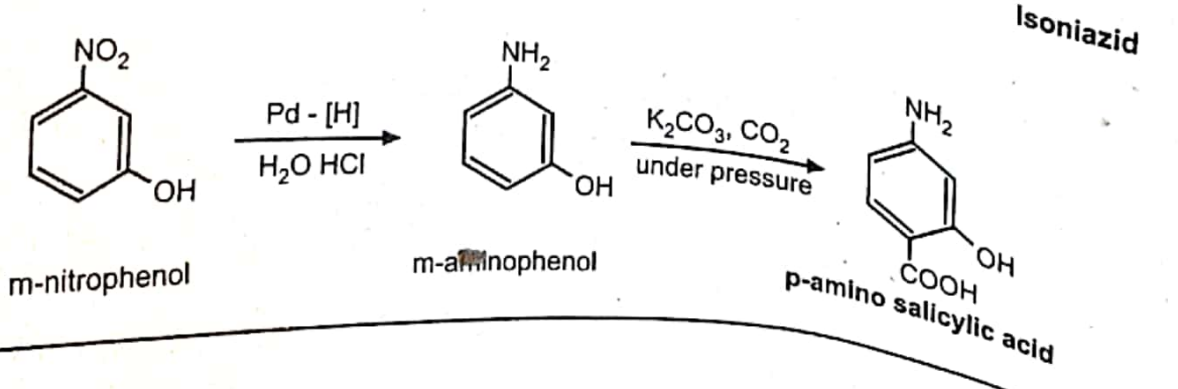
It has already been discussed on page 6.10.

Synthesis

(i) Isoniazid:



(ii) Para amino salicylic acid:



Chapter...7

URINARY TRACT ANTI-INFECTIVE AGENTS

♦ SYNOPSIS ♦

7.1 INTRODUCTION

7.2 QUINOLONES ANTIBIOTICS

7.1 INTRODUCTION

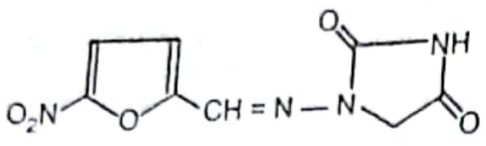
Many drugs are used to control urinary tract infections mainly because of their ability to develop concentration in urinary bladder, adequate to inhibit the growth of infective organisms. Examples include nitrofurantoin, nalidixic acid, oxolinic acid, resoxacin, norfloxacin, cinoxacin and methenamine. Several other four-quinolones, such as ciprofloxacin, amifloxacin, enoxacin etc. are under clinical investigations.

(a) Nitrofurantoin: It is an orally active nitrofuran antibacterial agent effective against various strains of *E. coli*, Klebsiella, Proteus species and *S. faecalis*. Chemically it is N-(5-nitro-furfurylidine) - 1 - aminohydantoin.

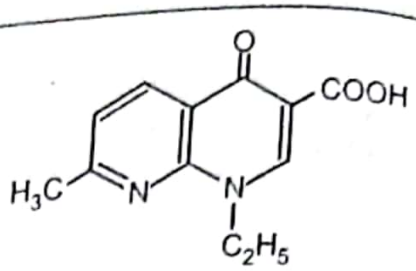
The antibacterial activity is due to the presence of 5-nitro group which helps to generate the superoxide and other toxic oxygen compound by undergoing conversion to nitro anion. The latter undergoes interaction with molecular oxygen leading to formation of superoxide and the original nitro group. The former interferes with the carbohydrate metabolism in bacteria. It does not affect the functioning of human cell because of its low serum concentration and rapid rate of drug metabolism in liver. Antibacterial activity can be potentiated in acid pH range. Alkalinization of urine enhances the rate of excretion in urine and also lowers down its antibacterial efficacy. It is a bacteriostatic in low concentrations while exerts bactericidal effect at higher concentrations.

It exhibits antibacterial activity against most of the organisms that cause lower urinary tract infections. It may also be used to prevent recurrences of these infections. It may also be used to prevent bacteriuria after prostatectomy. Resistance in bacteria may develop during the treatment. This may be because of formation of nitroso and hydroxyamine metabolites which act as mutagens. It is contraindicated in children of below one month of age and in patients with pregnancy or renal dysfunction.

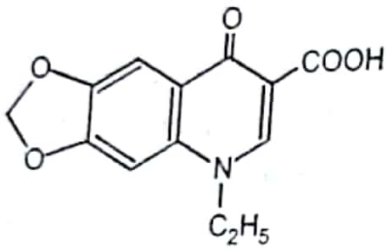
Table 7.1: Drugs used in the treatment of urinary tract infections



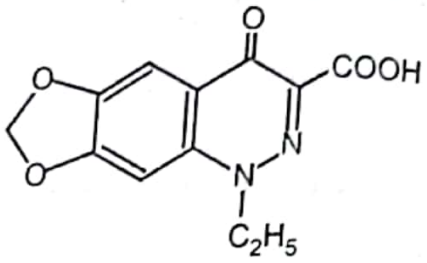
Nitrofurantoin



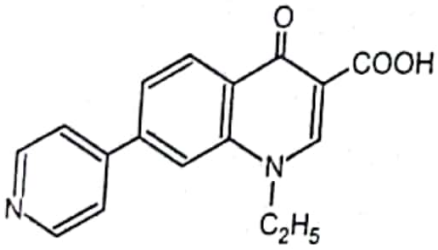
Nalidixic acid



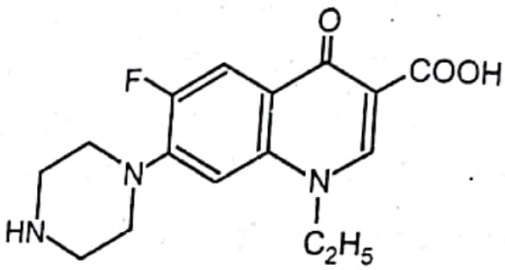
Oxolinic acid



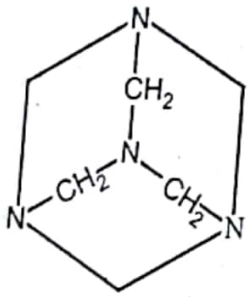
Cinoxacin



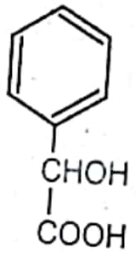
Rosoxacin



Norfloxacin



Methenamin



Mandelic acid

(b) Nalidixic acid: It is an orally active antibacterial agent belonging to 4-quinolone series. Its introduction in 1962 was followed by development of other agents from this class. These include cinoxacin, oxolinic acid, resoxacin, norfloxacin, ciprofloxacin, amifloxacin. All these are found to be effective against most of gram staphylococci and *P. aeruginosa*.

Nalidixic acid is effective against *E. coli*, *P. mirabilis*, Klebsiella, Enterobacter, coliform bacteria and some shigellae. Hence, it is used as a bactericidal agent in the treatment of urinary tract infections caused by all the above gram-negative bacteria. It acts by inhibiting DNA - gyrase enzyme that is responsible for unwinding of supercoiled bacterial DNA prior to its replication and transcription. Bacteria, however can readily develop resistance to its action.

About 93 - 97% of administered dose is bound to the plasma-proteins. It has a plasma-half-life of 8 hours. Active hydroxynalidixic acid metabolite is excreted as conjugated form in the urine alongwith 2 - 3% dose in unchanged form. About 4 - 5% dose also appears unchanged in the faeces.

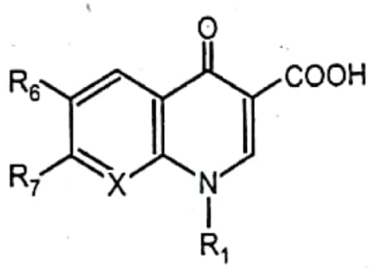
Adverse effects include nausea, vomiting, abdominal pain, urticaria, eosinophilia, photo-sensitivity, headache, vertigo, dizziness, weakness, drowsiness, visual disturbances, hallucinations, hemolytic anemia, leukopenia and thrombocytopenia. It should not be used in infants under 3 months of age because of their inability to metabolize or excrete nalidixic acid efficiently.

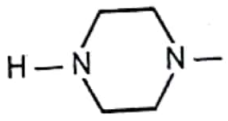
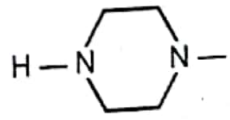
It may also be useful in the treatment of mild to moderate forms of gastroenteritis.

Cinoxacin is an orally active antibacterial agent having structural similarity with nalidixic acid. About 63% of administered dose is bound to the plasma proteins. It has a plasma-half-life of 1.5 hours. About 60% dose appears in urine in unchanged form alongwith 30 - 40% dose as inactive metabolites.

Oxolinic acid is yet another 4-quinolone having potent antibacterial activity. It is extensively metabolized in the liver. Inactive metabolites in the form of glucuronides appear in the urine alongwith 5% dose in unchanged form. Adverse effects and therapeutic uses are similar to those of nalidixic acid.

Table 7.2: Effective antibacterial quinolone derivatives



Name	X	R_6	R_1	R_7
Nalidixic acid	N	H	CH_2CH_3	$-CH_3$
Enoxacin	N	F	CH_2CH_3	
Pipemidic acid	N	H	CH_2CH_3	

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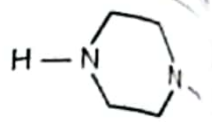
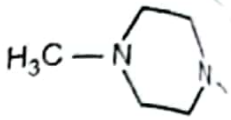
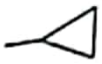
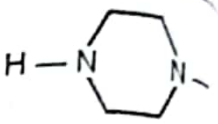
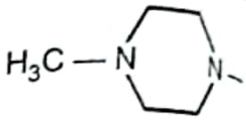
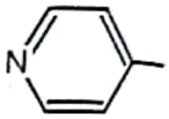
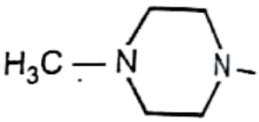
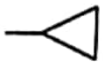
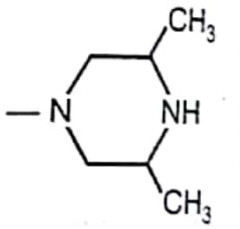
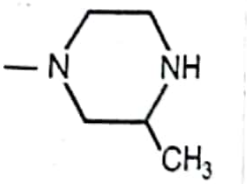
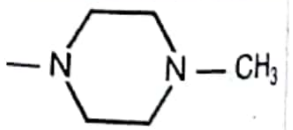
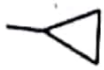
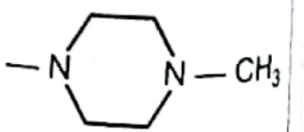
Norfloxacin	CH	F	CH_2CH_3	
Pefloxacin	CH	F	CH_2CH_3	
Ciprofloxacin	CH	F		
Amifloxacin	CH	F	$-NHCH_3$	
Rosoxacin	CH	H	$-CH_2CH_3$	
AM - 833	CH	F	$-CH_2CH_2F$	
Sparfloxacin	—CF—	F		
Lomefloxacin	—CF—	F	$-C_2H_5$	
Fleroxacin	—CF—	F	$-CH_2CH_2F$	
Tefloxacin	—CH—	F		

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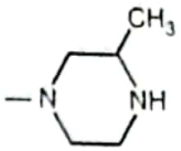
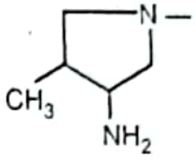
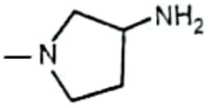
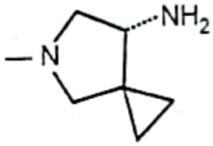
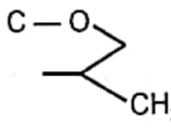
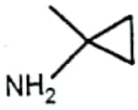
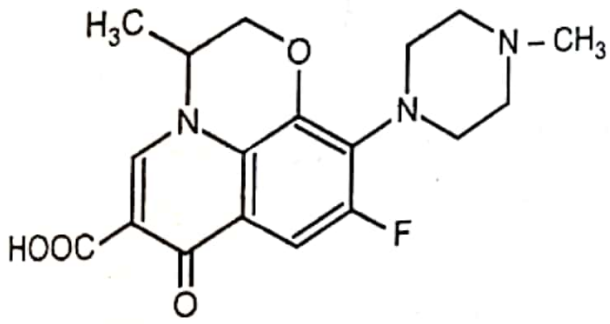
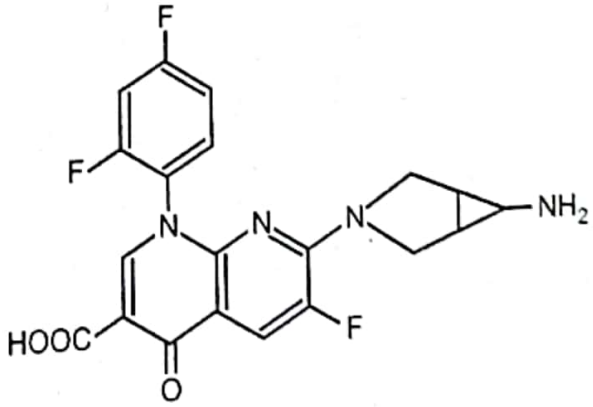
Gatifloxacin (AM-1155)	$-COCH_3-$	F		
AM – 1174	$-COCH_3-$	F		
Clinafloxacin	$-CCl-$	F		
Sitafloxacin	$-CCl-$	F		
Pazufloxacin	$-CH-$	F		

7.2 QUINOLONE ANTIBIOTICS



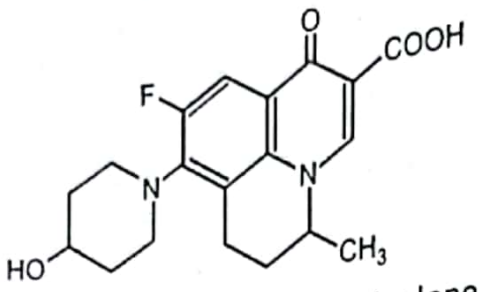
Levofloxacin
(s-enantiomer of ofloxacin)



Trovafloxacin
(withdrawn due to hepatotoxicity)

(a) Ciprofloxacin and norfloxacin are the examples of other orally active quinolone antibacterial agents used in the treatment of urinary tract infections. Rosoxacin is the member of same class but it is not used in the treatment of urinary tract infections. It is extremely effective against penicillin-resistant strains and is preferred in the treatment of gonorrhoea.

(b) Nadifloxacin: It is a topical fluoroquinolone used to treat acne vulgaris and other skin infections.



(c) Enoxacin: It is an oral broad-spectrum fluoroquinolone antibacterial agent used in the treatment of urinary tract infections and gonorrhea. It is active against many gram-positive bacteria. It inhibits bacterial DNA gyrase and topoisomerase IV. The inhibition of these enzymes prevent bacterial DNA replication, transcription, repair and recombination.

Enoxacin can be used to treat a variety of infections, particularly gastroenteritis, respiratory tract infections, gonorrhea and urinary tract infections. Insomnaia is a common adverse effect.

(d) Ofloxacin: It occurs as a racemic mixture, which consists of 50% levofloxacin (the biologically active isomer) and 50% of dextrofloxacin. First reported in 1980, ofloxacin is used in the treatment of a number of bacterial infections. Common side-effects include nausea, vomiting, diarrhea, headache and insomnia.

(e) Lomefloxacin: Approved in 1989 for medical use, lomefloxacin is a fluoroquinolone antibiotic used to treat bronchitis and urinary tract infections. It is associated with phototoxicity and CNS-related adverse effects.

(f) Sparfloxacin: Approved for medical use in 1993, sparfloxacin is indicated for treating acute sinusitis, chronic bronchitis and community acquired pneumonia. Phototoxicity and insomnia are the most common adverse effects.

(h) Gatifloxacin: It is an antibiotic of the fourth generation fluoroquinolone family. Approved for medical use in 1999, gatifloxacin is banned in India in 2011 because of its adverse side-effects.

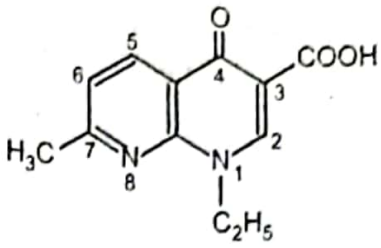
(i) Moxifloxacin: Approved for medical use in 1999, it is an antibiotic used to treat pneumonia, endocarditis, sinusitis, conjunctivitis and tuberculosis. It is used by an oral, intravascular route and as an eye drop.

SAR of Quinolones:

With the introduction of nalidixic acid (the first clinically used drug from quinolone series) in 1962, almost after 10 years, the addition of a fluorine at position C6 of the pharmacophore led to introduction of 'fluoroquinolones' in 1972.

The narrow clinical indication of nalidixic acid was a consequence of (a) Poor serum and tissue concentration achieved after oral administration and (b) narrow spectrum of activity, restricted primarily to the Enterobacteriaceae.

Over 10,000 fluoroquinolones were synthesized to get compounds having broader spectrum of antibacterial activity, improved pharmacokinetic profile with least adverse effects.



Nalidixic acid

The important SAR points to quinolones include:

(i) A cyclopropyl moiety (e.g., ciprofloxacin and sparfloxacin) at position 1 imparts significant activity against gram-negative bacteria. Similarly the 2, 4-difluorophenyl group confers increased activity against anaerobes.

(ii) At position 2, a hydrogen atom is optimal. Any large moiety may create a steric hindrance to the binding interactions of position 3 and 4 (i.e. carboxylic acid and oxygen) with bacterial DNA bases.

(iii) Electron releasing small functional groups like $-NH_2$ (sparfloxacin) or $-CH_3$ (grepafloxacin) at position 5 improve activity against gram-positive bacteria.

(iv) Placing fluoro moiety at position 6 leads to broader spectrum of activity (ciprofloxacin) and excellent tissue distribution (ofloxacin).

(v) Attaching a five or a six-membered nitrogen hetero cycle (like aminopyrrolidines or piperazines) at position 7 led to potent drugs with optimum pharmacokinetic features. The aminopyrrolidine moiety tends to confer better activity against gram-positive bacteria whereas piperazine moiety helps to improve activity against gram-negative bacteria.

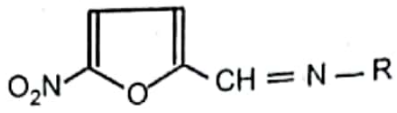
(vii) The replacement of 'N' at position 8 by –CH (ciprofloxacin), –CF (lomefloxacin), –CCl (sparfloxacin) or by $-COCH_3$ moiety led to marked improvements in activity against anaerobic bacteria.

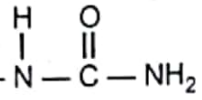
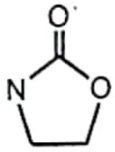
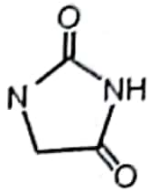
Miscellaneous:

(a) Methenamine (Hexamine or hexamethylenetetramine): It is an orally active urinary tract antiseptic agent used in the form of either mandelate or hippurate. If administered in the form of normal tablet, a considerable amount (about 10–30%) of methenamine decomposes in the stomach due to acidic pH. Hence, it is to be supplied in the form of enteric coated tablet. In circulation it remains unmetabolized. In acidic urine of pH = 5.5 or lower, it spontaneously decomposes to ammonia and formaldehyde. The latter agent (alkylating agent) denatures bacterial protein and acts as a non-specific antibacterial agent effective against both gram-positive and gram-negative bacteria. Acidic urine is must for the liberation of formaldehyde. However, some bacteria (e.g., Proteus species) prevent normal urinary acidification by releasing ammonia from urea. Hence methenamine is usually administered as a salt of mandelic, hippuric or ascorbic acid to impart acidic pH to the urine. Beside this function, all these acids also exert antibacterial property.

Adverse effects include nausea, stomach upset, epigastric distress, bladder irritation, skin rash, pruritus, albuminurea, crystalluria and painful and frequent micturition. It is contraindicated in renal insufficiency.

(b) Furan derivatives: Nitrofurazone (Furacin) is the most effective anti-infective agent in the class. The essential features of this class include a nitro group at 5 position and an enamine group at 2 position. The effective members of this class include.



1. Nitrofurazone; R = 
2. Nifuroxime; R = — OH
3. Furazolidone; R = 
4. Nitrofurantoin; R = 

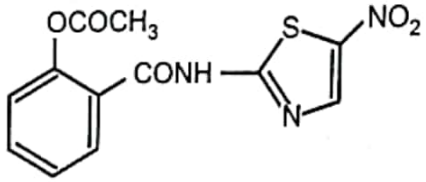
First studied in 1944, nitrofurazone is used topically in the form of a 0.2% cream, solution, ointment and powder on superficial wounds and for surgical dressings. It is highly effective in the treatment of burns. It is effective against a wide variety of gram-positive and gram-negative bacteria and some protozoa. It lacks fungistatic properties.

The bactericidal action of these furan derivatives may be due to:

(i) cessation of cell-division due to the blockage of energy transfer processes,

(ii) inhibition of bacterial respiratory enzymes.

Nitazoxanide: It is a nitrothiazolyl salicylamide derivative used as antiprotozoal agent.

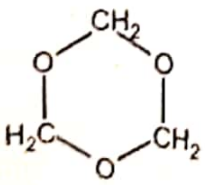


(c) Aldehydes: Several aldehydes possess bactericidal, sporicidal and virucidal activities. Following are some of the examples of effective anti-infective agents.

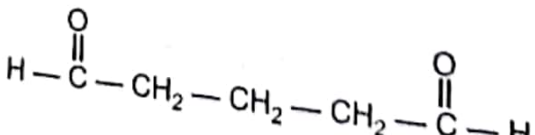
HCHO
Formaldehyde

$(CH_2O)_n$
Paraformaldehyde

$(CH_2)_6N_4$
Methenamine



Trioxymethylene



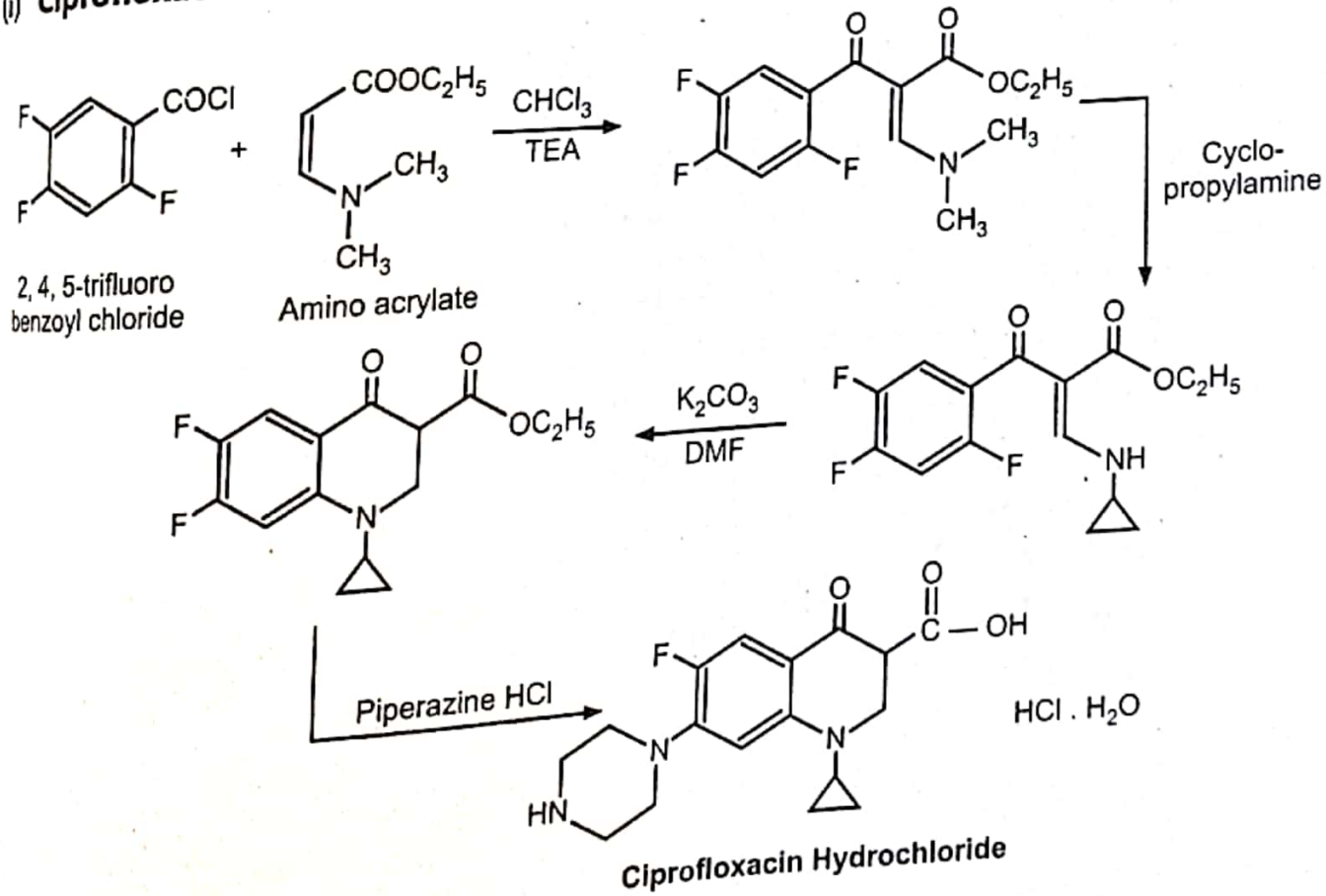
Glutaraldehyde

Formaldehyde is an excellent germicide but having unpleasant odour. It is highly effective antimicrobial agent and is used as a vapour or as a aqueous solution in the concentration range of 1 - 10%. Because of its irritant nature and poor penetrability in the tissues it can not be used as an antiseptic. Hence, it is widely used as a disinfectant for surface sterilization. For example, rooms can be sterilized by the use of formaldehyde gas.

The mechanism of action involves denaturation of proteins by the replacement of labile H-atoms in amino, carboxyl, hydroxy or thiol groups of component amino acids. Ethylene oxide also acts as anti-infective agent by the same mechanism. It reacts with water and chloride to form ethylene glycol and ethylene chlorohydrin. Both are active germicides. Because of its explosive nature, ethylene oxide should not be used in the concentration above 3%. It is mainly used for sterilization of plastic equipments. It is mutagenic, carcinogenic and causes irritation to eyes and mucous membranes. Glutaraldehyde (Gluteral) is effective dialdehyde against almost all microorganisms when its 2% aqueous solution buffered at pH = 7.5 - 8.0 is employed. It has the broad spectrum antimicrobial and sporicidal activities. It is mainly used to sterilize equipments, surgical instruments and surfaces contaminated with hepatitis virus. Beside this, succinic dialdehyde is also sometimes used as a disinfectant.

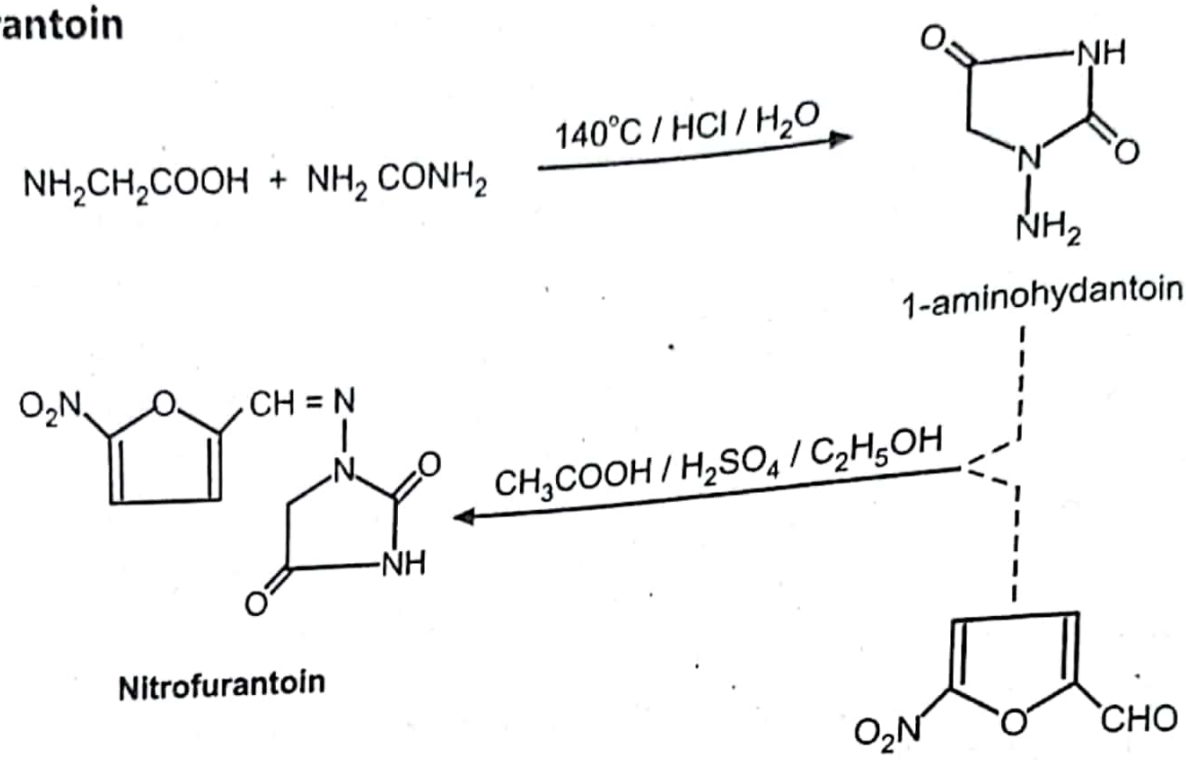
Synthesis

(i) Ciprofloxacin



Ciprofloxacin Hydrochloride

(ii) Nitrofurantoin



Chapter...8

ANTIVIRAL AGENTS

♦ SYNOPSIS ♦

8.1 INTRODUCTION

Viruses represent a separate and unique class of infectious agents. The smallest viruses possess a diameter of not more than 20 mm while in the large viruses, diameter may go upto 300 mm. The constitution of a virus is much more simple as compared to the bacteria. According to Lwoff, an infectious agent:

1. possesses simple chemical composition.
2. lacks the metabolic enzyme machinery.
3. lacks the protein-synthesizing system.
4. contains only one type of nucleic acid (i.e., either DNA or RNA), and
5. possesses a host-cell dependent machinery of multiplication can be named as virus.

Unlike bacteria, viruses do not possess cell-wall. Viruses consist of one or more strands of a linear or helical strands of either DNA or RNA enclosed in a shell of protein known as the capsid. The capsid is composed of several sub-units known as capsomeres that decide the shape of the capsid. Though often be spherical, capsid may possess different shapes. In certain cases, capsid may be surrounded by an outer protein or lipoprotein envelope. This encircling membrane may be called as an 'envelope'.

Since the biology of viral replication is dependent on the host cell metabolic machinery (e.g., protein synthesis, various enzyme systems), unlike bacteria, viruses will not grow on the nutrient media. They can replicate only in the host cell which may be a bacteria, animal or plant cell. Hence, viruses are considered as obligatory intracellular parasites that utilize many of biochemical machinery and products of host cell to sustain their viability.

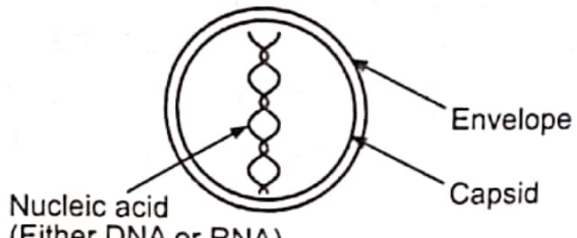


Fig. 8.1: Virus

Viral diseases include, influenza, smallpox, rabbies, poliomyelitis, yellow fever, ornithosis, psittacosis and lymphogranuloma venereum.

The viral replication may be outlined as under:

1. **Adsorption:**

The virion invades the host cell-membrane. The reactive sites on the capsid firmly bind with their complimentary sites on the host cell. The viral particle is encapsulated by host cell cytoplasm, forming a vacuole.

2. **Uncoating:**

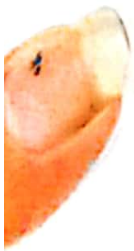
The genetic material or viral genome (DNA or RNA) passes into the host-cell, leaving the capsid covering outside the host cell. Sometimes uncovering of viral genome occurs within the host-cell. This step is referred to as penetration into host cell. Only the viral genome is infectious to the host cell and the capsid of the protein coat determines the site of the attacks of virus within the host. The viral genome is different from the host nucleic acids and hence it is infectious. Sometimes due to its proteineous nature, capsid contents may turn to be antigenic to the host cell and initiates a number of immunological reactions to the host.

3. **Synthesis of viral components:**

Viral genome enters the cytoplasm or nucleoplasm and directs or utilises the host nucleic acid machinery for the synthesis of new viral protein and the production of more viral genome. Thus, it not only consumes the actual material for its own use from the host-cell but also enjoys the services of the biochemical systems of the host to get incorporated this material into the several proteinous subunits needed for its replication.

4. **Release of the virus:**

Viral nucleic acid and capsid protein materials are synthesized in different parts of the host cell by the host-cell ribosomes. The m-RNA is synthesized from the viral genome. The host cell machinery, however, fails to differentiate between viral and cell directed orders. The large number of newly synthesized viral particles then have to be brought together for assembling into new virions. The latter are released from the cell by budding process. They may acquire the lipoprotein envelope at the time of their release. The new virions then invade fresh host-cell and repeat the whole process. Since the host cell machinery is totally utilized for production of new virions, the normal cell function ceases at the time of replication. Some viruses induce the production of toxic intermediates that adds to their pathogenicity. Viruses are composed of one or more strands of a linear or helical nucleic acid core, consisting of either DNA or RNA, but not both. Viruses thus can be classified as per the type of nucleic acid present in them. For example,



(A) DNA viruses: This class includes pox viruses, papoviruses, adenoviruses and herpes viruses.

(B) RNA viruses: This class includes, arboviruses, myxoviruses, picornaviruses and rhinoviruses.

8.2 DESIGN OF ANTIVIRAL AGENTS

Viral chemotherapy is still in the phase of infancy. Although a great deal of work has been done, it has resulted in the development of only 3-4 clinically used agents. Presently immunization, public health measures and physical and chemical disinfection procedures play a vital role in the prevention of spread of viral diseases.

Viruses are essentially intracellular parasites. Unlike bacteria, the viral replication is totally dependent upon the energy, proteins and enzymatic machinery of the host cell. Bacteria have self contained biosynthetic machinery. Hence, the drug in bacterial chemotherapy enjoy the advantage of selective attack on bacteria due to many metabolic and molecular differences between the pathogen and the host cell. Since viruses literally take over the metabolic machinery of the infected human cell, a close relationship exists between the multiplying virus and the host cell. Virus replication is intimately dependent upon the host cell metabolism. This fact severely limits the usual opportunities to design antiviral agent having selective effect on the viral cell. Attempts to inhibit viral growth without damaging the host-cells became fruitless.

When the host cell is infected by a virus, its presence is felt so late that extensive viral multiplication and tissue damage has been already occurred. The late diagnosis and recognition of the disease state projects almost negligible chances for effective therapy. The drugs become useless, even if they are made available.

The important key events in the viral replication are diagrammatically shown in the Fig. 8.2.

It is apparent that various steps involved in the viral replication could be successfully utilized as the basis of designing antiviral agents. For example, the following sites in viral replication offered promising points for the attack of antiviral agents.

1. Adsorption of virus on the host cell.
2. Penetration of virus in host cell, and
3. Synthesis of viral genetic material.

Amantadine hydrochloride inhibits viral penetration and prevents influenza while methisazone and idoxuridine inhibit viral DNA synthesis. Due to one or more reasons (e.g., narrow activity spectra or toxicity) not a single drug in this area, enjoyed clinical popularity. The development of antiviral agent having selective toxicity to viral cells leaving host cells unaffected still remains a dream for the medicinal chemists.

Antiviral agents have often been proved to be disappointing due to the following problems in their development:

1. lack of satisfactory experimental models.
2. use of wrong virus in the laboratory.
3. narrow spectrum of activity.
4. limitations on uses due to their toxicity.
5. difficulties in their clinical assessment.

These disappointing features of antiviral therapy force us to accept vaccines as the best prophylactic agents in the treatment of viral diseases. There are four forms of antigen used in vaccines. These include,

(a) attenuated living
(b) killed by the chemicals, such as formalin,
(c) toxoids (i.e., toxins which through the application of heat or formalin are converted to non-toxic).
(d) subunits prepared by purifying important antigens of microorganisms.

Vaccination can be effectively used to prevent measles, rubella, mumps, poliomyelitis, yellow fever, smallpox and hepatitis B. They have only prophylactic utility. They remain ineffective once the infection has occurred and spreaded within the host.

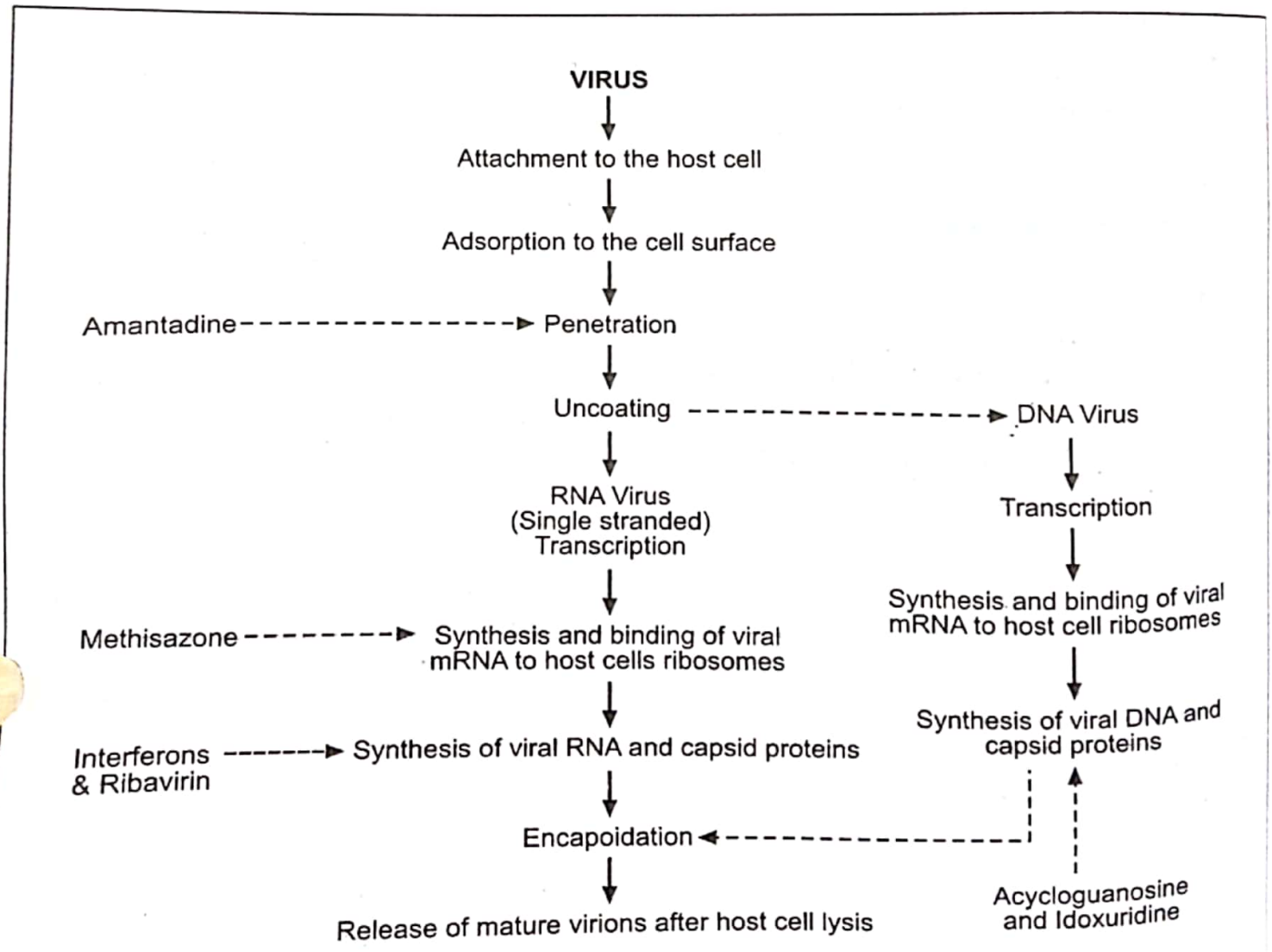


Fig. 8.2

8.3 CLASSIFICATION OF ANTIVIRAL AGENTS

Various antiviral agents so far introduced, are found to possess very narrow spectrum of activity. For example, agents which are effective against DNA viruses are usually ineffective against RNA-viruses and drugs which show activity against RNA-viruses remain silent against DNA-viruses. During the course of antiviral therapy, the drug used receives very fast acknowledgement from the viruses, resulting into rapid development of drug resistant strains of viruses.

The currently available antiviral drugs can be chemically classified as:

(A) Purine nucleosides and nucleotides
(B) Pyrimidine nucleosides and nucleotides
(C) Thiosemicarbazones
(D) Benzimidazoles
(E) Adamantane amines
(F) Interferons
(G) Miscellaneous agents

(A) Purine Nucleosides and Nucleotides:

Acyclovir triphosphate (Acycloguanosine):

It is a synthetic purine nucleoside analog in which a linear side-chain (i.e., $-CH_2OCH_2CH_3$) is attached at 9-position instead of the cyclic sugar present in the guanosine molecule.

Acyclovir undergoes phosphorylation process with the help of viral thymidine kinase enzymes to form acyclovir triphosphate. The latter selectively inhibits herpes virus DNA polymerase. The faulty transcription in viral DNA leads to inhibition of virus replication process. The affinity of viral thymidine kinase enzymes for acyclovir is about 200 times greater than that of mammalian enzymes for the drug. This explains the selectivity of attack on the viruses.

It can be used orally, topically and intravenously. About 15% of administered dose is bound to the plasma proteins. It has plasma half-life of about 2.5 hours. It is excreted in urine mostly in unchanged form alongwith minor amount of inactive metabolite, 9-carboxy methoxy methylguanine.

Adverse effects include nausea, headache, amnesia, hypotension, tremors and coma. Local irritation, ulceration and burning may occur when it is applied to genital lesions.

It is effective against herpes virus infections, herpes zoster infections and infections caused by varicella zoster and cytomegalovirus. It may be used orally for the treatment of both initial and recurrent episodes of genital herpes. A 3% acyclovir ointment may be used for the treatment of ocular herpetic infection and herpes keratoconjunctivitis. It may also be used intravenously in the treatment of herpes simplex encephalitis. However, development of resistance to acyclovir may limit its clinical utility.

(B) Pyrimidine Nucleosides and Nucleotides:

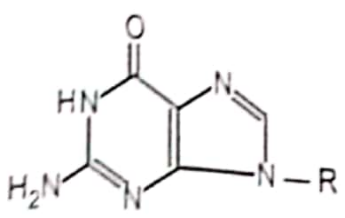
(i) Idoxuridine: It was discovered in 1959 by Prusoff. It is a topically used antiviral agent chemically related to trifluridine and is effective against vaccinia and herpes simplex virus, pseudorabies, B. virus, myxoma virus, polyoma virus and some papovaviruses.

Idoxuridine is chemically very similar to thymidine, a compound which in the normal cell undergoes phosphorylation and then get incorporated into DNA molecule. Due to the structural similarity with thymidine, with the help of thymidine kinase and thymidine monophosphate kinase enzymes, it is converted to the active triphosphorylated derivative. The latter blocks DNA polymerase enzyme resulting into interference in nucleic acid and protein synthesis in DNA-viruses. It also prevents the assembling of viral components by inhibiting the synthesis of a protein required for their assembling. Since the host cell-DNA synthesis is also affected, it produces host cytotoxicity. Hence, its systemic use is not recommended. It is thus usually given topically. Hence, serious side-effects rarely attend its use.

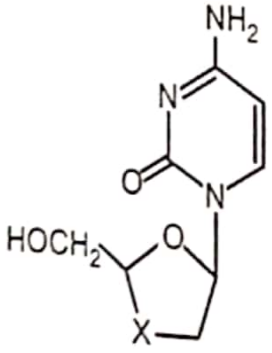
It is available in the form of ophthalmic ointment (0.5%) and ophthalmic solution (0.1%). It gets rapidly inactivated by deaminases or nucleotidases into 5-iodouracil and inorganic iodides. Adverse effects include pain, irritation, pruritus, inflammation or edema of conjunctiva and eyelids, corneal vascularization, lacrimation, stomatitis, neutropenia and thrombocytopenia. It is effective in the treatment of herpes simplex infections of eyelid, conjunctiva and cornea.

(ii) Trifluridine: It is a halogenated thymidine analog used as a topical antiviral agent. It is effective against herpes simplex virus, vaccinia and adenovirus. It is converted to the active 5-monophosphate form. Due to the structural similarity with thymidine, it gets incorporated, instead of thymidine phosphorylated form, into replicating viral DNA. This results into wrong expression of genetic information due to abnormal base pairing.

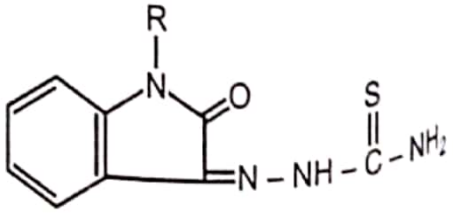
Table 8.1: Currently used antiviral agents



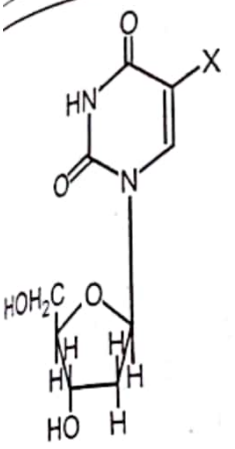
Acyclovir : R = — $CH_2OCH_2CH_2OH$
Ganciclovir : R = — $CH_2OCH(CH_2OH)_2$
Penciclovir : R = — $CH_2CH_2CH(CH_2OH)_2$
Famciclovir : R = — $CH_2CH_2CH(CH_2OCOCH_3)_2$



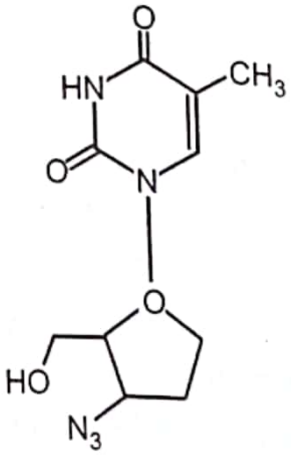
Zalcitabine : X = C
Lamivudine : X = S



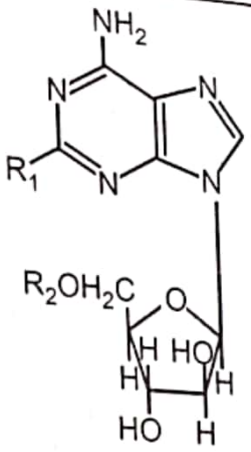
Methisazone; R = CH_3
Isatin - β - thiosemicarbazone; R = H



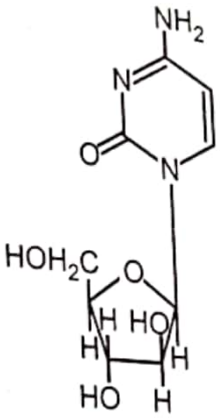
Idoxuridine ; X = I
Trifluridine ; X = CF_3



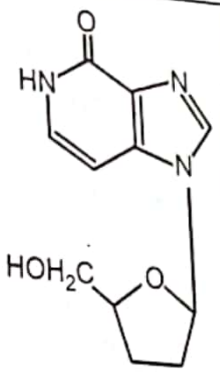
Zidovudine



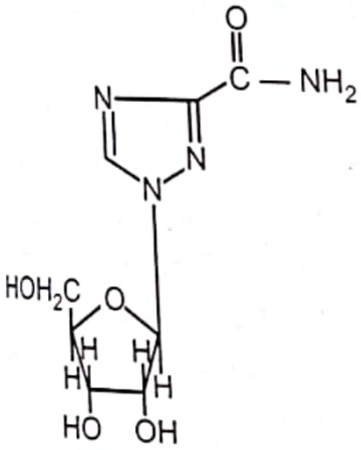
Vidarabine
($R_1 = R_2 = H$)
Fludarabine;
$R_1 = F_1$; $R_2 = HOPO_2$



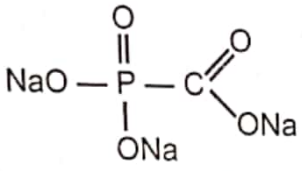
Cytosine arabinoside



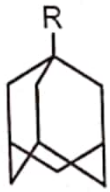
Didanosine



Ribavirin



Foscarnet sodium



Amantadine; R = NH_2

Rimantadine; R = — CH(CH_3)(NH_2)

1-Adamantyl; guanidine R = — C(NH_2)(=NH)

It also blocks thymidylate synthetase and deoxythymidine kinase enzymes in the viruses. The overall biological effects may get reflected into:

(i) increased number of errors in viral protein synthesis,

(ii) increased rate of mutation, and

(iii) inhibition of viral replication process.

In the form of 1% ophthalmic solution, it is used to treat certain ocular infections (e.g., ocular kercititis and keratoconjunctivitis). It exhibits effectiveness in cases where idoxuridine treatment fails.

(iii) Vidarabine (Adenine arabinoside) (Ara - A):

It is a purine nucleoside. This antiviral agent is isolated from the bacteria, Streptomyces antibioticus. It is an analog of adenosine originally developed for the treatment of leukemia. It is converted *in-vivo* enzymatically to ara-ATP. The latter impairs early steps in viral DNA synthesis, presumably by inhibiting viral DNA polymerase. It exhibits considerable host toxicity due to part inhibition of cellular DNA polymerase enzymes.

It is effective against vaccinia, herpes simplex virus, cytomegalo virus, varicella zoster, pseudorabies and myxoma virus. It may be used topically as a 3% ophthalmic ointment or as I.V. infusion. About 20-30% administered dose is bound to the plasmaproteins. It has a plasma half-life of 1.5 hours. In the cornea and in the plasma, it metabolizes to arahypoxanthine (active; half-life is 3.3 hours) by xanthine oxidase enzyme through a process of deamination.

It is used to treat herpes simplex infections in neonates. It is also used to treat herpes simplex encephalitis and herpes zoster infections in immuno compromised patients. It may have activity in cytomegalovirus infections and in type B virus hepatitis. In the form of 3% ophthalmic ointment, it may be used topically to control recurrent epithelial keratitis and kerato-conjunctivitis. However, it remains ineffective against bacterial, fungal and adenovirus infections. Its antineoplastic potential is under investigations .

Vidarabine-5'-monophosphate and vidarabine hypoxanthine-5' monophosphate are the vidarabine derivatives that are currently under investigation for their utility in antiviral therapy.

(iv) Ribavirin: It is a synthetic nucleoside chemically related to inosine, guanosine and xanthosine. It is a broad spectrum antiviral agent effective against nearly all major viruses. It has virustatic properties. It is *in-vivo* converted to ribavirin-5'-monophosphate which acts as a competitive inhibitor of inosine 5' monophosphate dehydrogenase. This results into inhibition of guanine monophosphate synthesis, followed by inhibition of viral RNA synthesis. It has a plasma half-life of 9 hours. It is extensively metabolized in the liver. Principal metabolites include mono, di and triphosphate derivatives, tricarboxylic acid analog and 1, 2, 4-triazole carboxamide metabolite. Deribosylation and breakdown of triazole ring is also reported to occur.

It is an investigational antiviral agent used in the treatment of infections due to respiratory viruses. It is used in the form of aerosol to treat severe lower respiratory tract infections due to respiratory syncytial virus (RSV). It is also undergoing evaluation in the treatment of human immunodeficiency virus (HIV) infections, like AIDS.

Adenine arabinoside and cytosine arabinoside are other examples from this category. Cytosine arabinoside (cytarabine) is effective against herpes viruses. It is presently used in cancer chemotherapy.

(C) Thiosemicarbazones:

In 1947, Domagk (sulfonamide fame) reported that some derivatives of benzaldehyde thiosemicarbazone protected laboratory animals against tuberculosis. This initiated an extensive investigation of the thiosemicarbazones during which the activity of methisazone (N-methylisatin - β thiosemicarbazone) was discovered. It is effective against a variety of poxviruses and some RNA viruses.

The antiviral action of thiosemicarbazone is perhaps due to the formation of metal chelate with various metal ions including Cu, Zn, Ni, ferrous and manganese. Methisazone thus acts by interacting with metalloenzymes that are necessary for the replication of certain

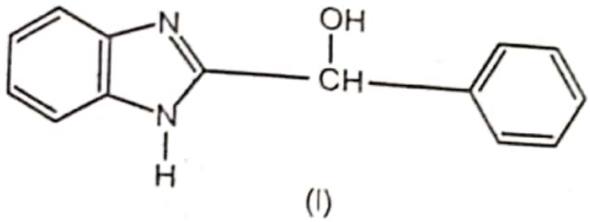
viruses. The thiosemicarbazones may also react directly and specifically with viral nucleic acid.

Methisazone prevents replication of vaccinia viruses. It is poorly absorbed by oral route. Major metabolic pathways include demethylation, replacement of sulphur by oxygen in the side-chain and hydroxylation in the aromatic ring. Isatin - 3 - thiosemicarbazone is the active metabolite retaining half the activity of parent drug.

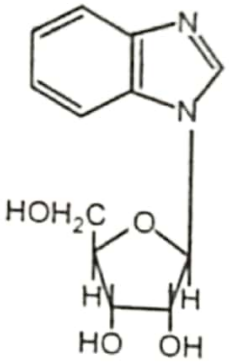
It is used as a prophylactic agent against small pox and for the prevention and treatment of generalised vaccinia or vaccinial encephalitis.

(D) Benzimidazoles:

Following two compounds of this family are found to show promising results in trials.



(I)



(II)

Both these agents do not interfere with the viral adsorption, penetration and uncoating. They appear to act by inhibition of viral RNA synthesis.

(E) Adamantane Amines:

Amantadine hydrochloride:

It was first reported by Davies et al in 1964. It is a synthetic antiviral agent effective against infections caused by influenza A viruses including H_3N_2, Hsw $1N_1$ and H_1N_1 subtypes. In higher concentrations, it is also effective against influenza B, rubella and other viruses.

Amantadine allows viral adsorption to the host cell but inhibits the uncoating of the influenza virus and prohibits penetration of viral genome into the host cell. Because the virion remains adsorbed to host cell surface, it becomes susceptible to attack by host antibodies.

Besides its antiviral potential, it may also be employed in the therapy of parkinsonism.

Rimantadine is the better tolerated and less toxic analog of amantadine which is currently undergoing clinical evaluation. It has similar actions and uses.

(F) Interferons (Antiviral Proteins):

The presence of interferons was first reported in 1957 by scientists Issaes and Lindermann at the National Institute for Medical Research, U.K. The term is applied to a class of glycoproteins of molecular weights from about 20,000 to 50,000. Each contains approximately 150 amino acids. Interferons are produced from the host cell when it is

infected or is exposed to an inactive virus. The endogenous synthesis of interferons is under the control of host cell RNA. There are three major types of human interferons that are designated α, β and γ according to antigen specifications. The preferred abbreviation of interferon is IFN. Interferons can also be designated as per the source. For example, human (Hu IFN), bovine (Bov. IFN), murine (Mu IFN), etc. Currently major source of human interferons is from white blood cells that have been cultured and then exposed to appropriate viruses. In body, after their release, they may attach to surface receptors of the adjacent cells and initiate the production of additional interferons.

Viruses differ in their ability to induce the synthesis and / or liberation of interferons. The adsorption of a virus to the host cell or the full infected condition of the host cell induces the formation and release of interferons. This is a sort of immunization process. The release of interferon imparts resistance to the person against the attack of viral infection. Generally, a person once infected with one virus, develops a resistance against other viral infections. This phenomenon is known as viral interference. In non-infected cells, it can induce the formation of second inhibitory protein which has an ability to prevent the transcription of any viral m-RNA that might subsequently be produced in that cell.

Interferons are characterized by the following features:

1. non-toxic substances to the host cell.
2. exhibit antiviral activity in extremely low concentrations, and
3. do not possess antigenic activity.

Interferons appear very soon and sometimes within seconds after the viral attack. However, they lack specificity. Once released from the infected host cell, interferons induce the synthesis of translational inhibitory proteins in other non-infected cells. These proteins impair the translation of viral m-RNAs and thus interfere in the viral replication. The synthesis of viral RNA polymerase and viral thymidine kinase is inhibited. The transcription of viral genome is also found to be inhibited.

Interferon preparations may contain more than one type. These exhibit a unique broad spectrum of antiviral activity *in-vitro* and show promising effects when used in combination with vidarabine. Interferon preparations are effective against infections caused by varicella zoster, encephalomyocarditis virus, vesicular stomatitis virus, rabies, vaccinia, influenza B and cytomegalovirus.

Interferons are not orally absorbed. They are usually given by I.V. infusion and have plasma half life of about 15 - 20 minutes. Metabolic pathways for interferons are not still known.

Adverse effects mainly include fever, reticulo-cytopenia, neutropenia and thrombocyto-penia. They are clinically effective against chronic active hepatitis B viral infection, cytomegaloviral infection, congenital rubella infections and respiratory viral infections. Interferons are also showing promising results in the therapy of some cancers like,

melanoma, multiple myeloma, osteogenic sarcoma, certain leukemias and breast cancer. Their use in organ transplantation is also being extensively studied.

Interferon synthesis and liberation in animals can be induced by:

1. **Biological inducers:**

These include human leukocytes, fibroblasts or lymphoblastoid cells, and

2. **Chemical inducers:** These include:

(a) Polyriboinosinic polyribocytidilic acid which is a complex of polyinosinic and polycytidilic acids. Structurally, this complex is a synthetic analog of double stranded RNA,

(b) A new group of 6-phenylpyrimidine derivatives inducing the host cell to produce interferons, and

(c) Tilorone hydrochloride.

All these interferon inducers including viral infections produce diminished response upon re-exposure. It was proved difficult to make interferon in sufficient quantities for trials due to limitations of human tissue culture techniques. Recently recombinant DNA technology has been employed as an alternative method of producing large quantities of interferon in prokaryotic (bacteria) cells. In this technique, genes for human interferon are inserted into *E. coli* genome. The interferon thus produced could be used to perform extensive clinical trials against a variety of human viral infections.

The major drawbacks of interferon therapy are:

(1) Interferon will have to be obtained from human subjects if it is to be used in the treatment of human viral disease.

(2) In human subjects, its serum half-life, is not more than 15 - 20 minutes. The brief survival of interferon in body may be attributed to its rapid distribution and excretion from the body.

(3) Numerous clinical trials of interferon utility suggested that it is not as useful in the therapy of viral infections as initially suspected.

(G) Miscellaneous Agents:

(i) Ureas and thioureas: Members of this class exhibited good antiviral activity but they could not be evaluated further because of their immunosuppresive side effects.

(ii) Guanidines and biguanidines: Guanidine hydrochloride shows selective inhibition of enteroviruses and more specifically polio viruses. But probably due to its rapid urinary excretion, the drug failed to achieve a clinical status. The mode of action involves the selective inhibition of viral RNA polymerases. An extensive investigation in this class is still going on.

(iii) Heterocyclic dyes: The members of this series having antiviral potential include proflavin and neutral red. The herpetic lesions are painted with 0.1% solution of these dyes and then the painted area is exposed to 16 - 30 W fluorescent light for 15 minutes. This results in breakdown of viral DNA and death of the virus. The painted area may be re-exposed to the light at the interval of 6 - 8 hours.

(iv) Gamma globulin (Immuno globulin): It is the antibody-rich fraction of the plasma which contains a variety of antiviral antibodies and is thought to act by preventing the penetration of virus into the host cell. It is orally ineffective. After a single injection, it offers protection against viral infection for about 2 - 3 weeks.

These immunoglobulins are derived from β lymphocytes and plasma cells in very low amounts. Usually only one class of immunoglobulins is initially synthesized by a given immunocyte and a clone of immunocytes synthesizes a specific type of immuno globulins.

Details about the fate of immunoglobulins are not available. Liver serves as a major site where they are degraded. Rarely immunoglobulines are eliminated through the urine.

(v) Antibiotics: A number of antibiotics of diversified structures have demonstrated antiviral activity to less or more extent. Extensive studies in this regard are yet to be made in this field. Examples of antibiotics possessing significant antiviral activity include rifamycin, bleomycin, gliotoxins and clistamycin A. However, the doses for antiviral activity of these antibiotics are usually higher than the dose needed for antibacterial activity. Hence, the use of these drugs as antiviral agents is always accompanied by the high risk of adverse effects.

The antiviral activity of these antibiotics may be due to their ability to inhibit:

(a) assembling of particles into a mature virion, and

(b) viral envelope formation.

Various polymerase enzymes involved in either RNA and DNA synthesis in viruses are the principle target sites of action of these antibiotics.

(vi) During the next decade, in 1959 Idoxuridine, another antiviral agent was discovered, by Prusoff and actively used against vaccinea Herpes virus.

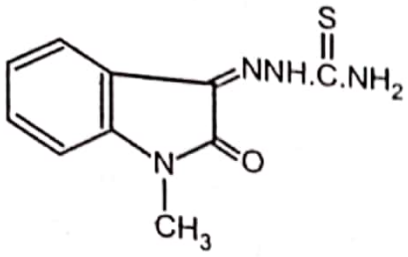
Idoxuridine (Herpid, Kerecid) competes with thymidine during synthesis of DNA. It interferes with the multiplication of herpes simplex virus which needs DNA, but, of course, it also interferes with mammalian cells, especially bone marrow.

In the form of solution in dimethyl sulphoxide, idoxuridine penetrates the skin and can be effective by topical application in cutaneous herpes zoster.

(vii) Other investigational antiviral agents: These include phosphonoformic acid, bromovinyldeoxyuridine, 2 - (3, 4 - dichloro-phenoxy) - 5 - nitrobenzenitrile and 4, 6 - dichloroflavan. The former two agents are found to be effective against herpes virus infections while the latter two are effective against infections caused by enteroviruses or rhinoviruses.

8.4 MECHANISM OF ACTION AND STRUCTURE-ACTIVITY RELATIONSHIPS

(1) Thiosemicarbazones:



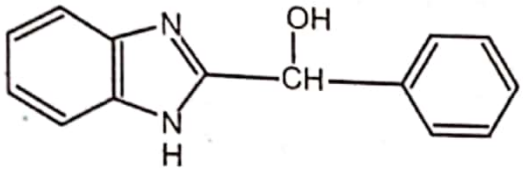
Methisazone

The antiviral action of the thiosemicarbazones appears to form *the co-ordination compounds with metal ions like copper, zinc, nickel and manganese.*

A potential side-effect of methisazone is a suppression of the host immune system.

(2) Benzimidazole:

2-(α-hydroxybenzyl benzimidazole) or HBB



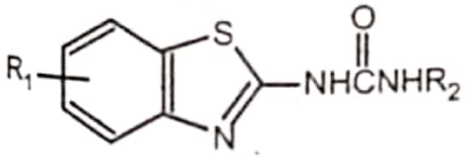
Hydroxybenzyl benzimidazole (HBB)

HBB has no effect on virus adsorption, penetration and uncoating. The primary site of action of this antiviral agent appears to be *inhibition of viral RNA synthesis.*

It is interesting that 5, 6-dichloro-*1*-B-d ribofuranozyl benzimidazole is *an enhancer of interferon induction,* a possible result of inhibition of *interferon RNA synthesis* and of the shut off of interferon production. The benzimidazoles have varying degrees of immuno suppressant activity.

(3) Urea and Thioureas:

Mechanism of action is same as that of benzimidazole.

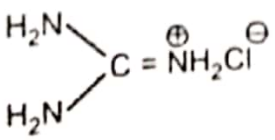


1. R_1 = H, R_2 = 4-$NO_2C_6H_5$
2. R_1 = 5, 6 di CH_3, R_2 = 1 - $C_{10}H_7$

These are 1-(Benzothiazol-2-yl)-3-substituted ureas.

(4) Guanidines and Biguanides:

Guanidine HCl



They inhibit the viral RNA synthesis. The initiation of viral RNA chain is blocked by the compound possibly by the interaction between viral RNA polymerase and guanidine.

The compound is a specific inhibitor of the synthesis of viral RNA polymerase.

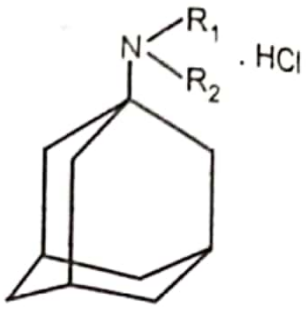
(5) Adamantane Amines and Related Compounds:

Amantidine is clinically active antiviral drug. It is active against number of RNA and DNA viruses *in-vitro*.

Its major effectiveness is against influenza A-group viruses.

Amantidine and related compounds prevent the viral nucleic acid from initiating new viral growth by preventing penetration of sensitive strains of influenza virus into the cell or by inhibiting the uncoating of the virus particle.

The recently described homo-iso-twistane derivatives, appear to have good potential as antiviral agents.



$R_1 = H; R_2 = H$

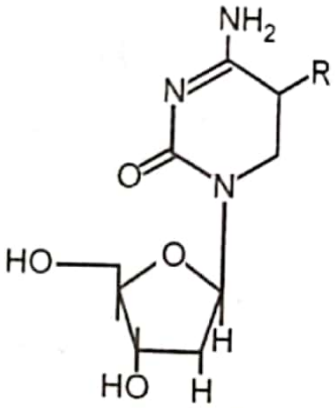
Amantadine

(6) Pyrimidine Nucleosides and Nucleotides:

(i) Halogenated 2'-deoxy pyrimidine nucleoside:

IDU and BDU are sterically very similar to thymidine. These nucleosides enter into and affect a number of *biochemical reactions*. In both, virus infected and non-infected cells they are phosphorylated to their mono, di and triphosphate forms, and they interact with the thymidine kinase, thymidylate kinase and *DNA-polymerase*.

The principal basis of antiviral activity of IDU is incorporation into viral DNA. Replacement of DNA thymidine by IDU results in the formation of either no protein or an inadequate one.

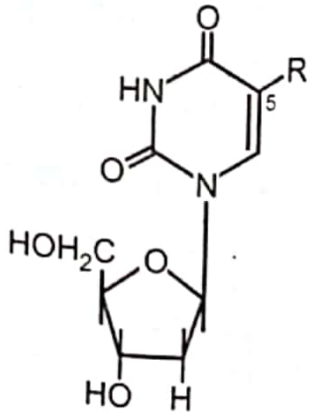


R = I; (IDU)

R = Br; (BDU)

(ii) 5-Alkyl and Other-5-Substituted-2-deoxy-pyrimidine Nucleosides:

The compound is an inhibitor of DNA-synthesis.



R = — CH_2 CH_3

5 - ethyl - 2' - deoxyuridine

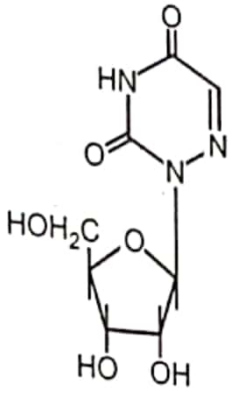
(iii) Pyrimidine Arabino-nucleosides:

The anticancer drug ara-C has a strong inhibitory effect on a number of DNA virus. Ara-C is involved in several biochemical processes.

The nucleoside is a *strong inhibitor of the synthesis of both DNA and RNA* and the triphosphate has been shown to inhibit both *DNA and RNA polymerase*. Incorporation of *ara*-C into both DNA and RNA has also been observed.

(iv) Azapyrimidine Nucleoside:

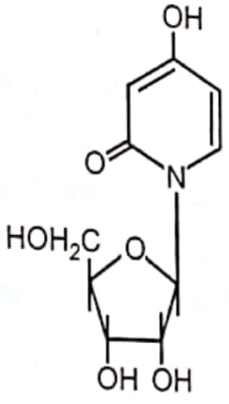
6-Azuridine, which inhibits several DNA and RNA viruses.



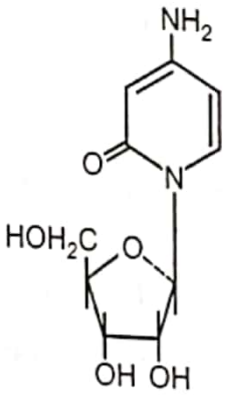
6-azaridine

Conversion of this nucleoside to the 5-monophosphate, which inhibits the orotidylic acid decarboxylase. Inhibition of this route of biosynthesis of uridine-5-monophosphate' Presumably interferes with *formation of viral RNA* during the replicative cycle of sensitive virus.

(v) Deaza Pyrimidine Nucleoside:



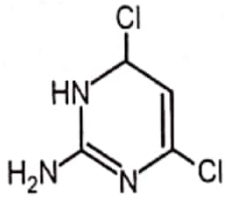
3-Deazauridine



3-Deazacytidine

They inhibit the *replication of RNA* of several viruses in-vitro.

(7) Pyrimidine Heterocycles:



2 - amino - 4, 6 - dichloro pyrimidine

They inhibit assembly of polyvirus proteins in procapsid, subsequently preventing coating of available infectious viral nucleic acid to form mature virus particles.

(8) Purine Nucleosides and Nucleotides:

(i) Purinearabino nucleoside and arabinonucleotides: The clinically active drug is Ara-A. The compound is being deaminated to its hypoxanthin form (9-β-D Arabinofuranosyl- hypoxanthin).

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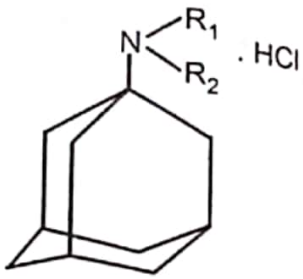

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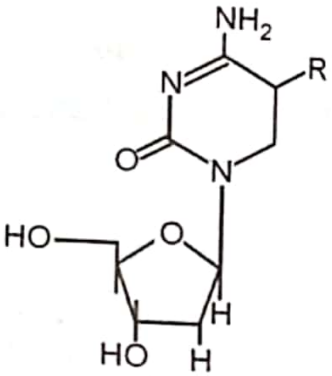

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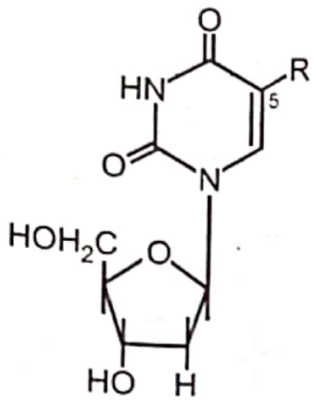

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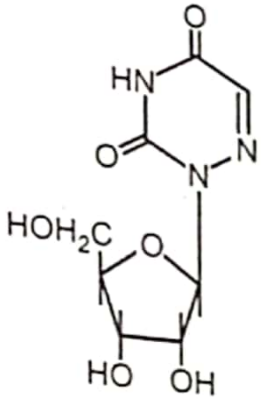

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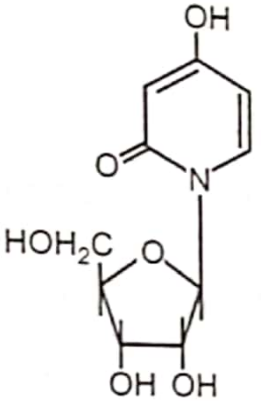
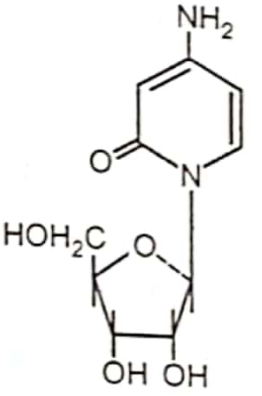


3-Deazauridine



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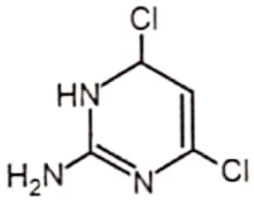

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The hypoxanthine arabino nucleoside may exert antiviral action in that form, or as the monophosphate (9-β-D arabinofuranosyl hypoxanthine-5-monophosphate, Ara HxMP), diphosphate or triphosphate. Ara-Hx may itself be broken down to the inactive hypoxanthine. Ara-Hx does apparently retain a degree of antiviral activity.

(ii) Dezapurine nucleoside: These compounds are *inhibitory to the hypoxanthine guanine phosphoribosyl transferase and inosin monophosphate dehydrogenase.* The antiviral activity is thought to be by inhibition of latter enzyme that result *in inhibition of viral nucleic acid.*

(9) Azole nucleosides and Related Compounds:

Most significant compound is *ribovirin.* It may interfere with *conversion of inosin-5-monophosphate on the pathway to nucleic acid.*

It inhibits the viron and complementary *RNA synthesis of an influenza-A virus.*

Ribovirin also *inhibits* virus induced *polypeptide synthesis.*

(10) Substituted Polycyclic Aromatic Compounds:

2,7-bis (2-dimethyl aminomethoxy) 9-fluorenone dihydrochloride (tilorone HCl) is an oral inducer of interferon production and effective against DNA and RNA virus.

This compound changes the physicochemical properties *of DNA.* The interaction of tilorone with DNA stabilizes the double helical structure indicating the possibility of intercalation as the mode of binding of tilorone. The complex of tilorone with double stranded DNA may result in modified nucleic acid that act as an *interferon inducer.* In addition, it inhibits DNA template function in DNA and RNA polymerase reactions.

(11) Polynucleotides and Other Polymers:

A number of polynucleotides have demonstrated considerable antiviral activity against wide variety of virus in cell culture and in animals which usually is correlated with *stimulation of interferon production.* The observation was that a double stranded RNA was effective as an interferon inducer, promoted the investigation of synthetic polynucleotides as possible inducers of interferon and it was found that certain double stranded nucleotides were especially effective.

Effective double stranded homopolymers include polyinosinic acid, polycytidylic acid (Poly I Poly C) and polyguanalic acid and polycytidylic acid (Poly G Poly C). Alternating copolymers such as Poly (inosinic acid cytidylic acid) and poly (adenylic acid uridyclic acid) also induce interferon formation.

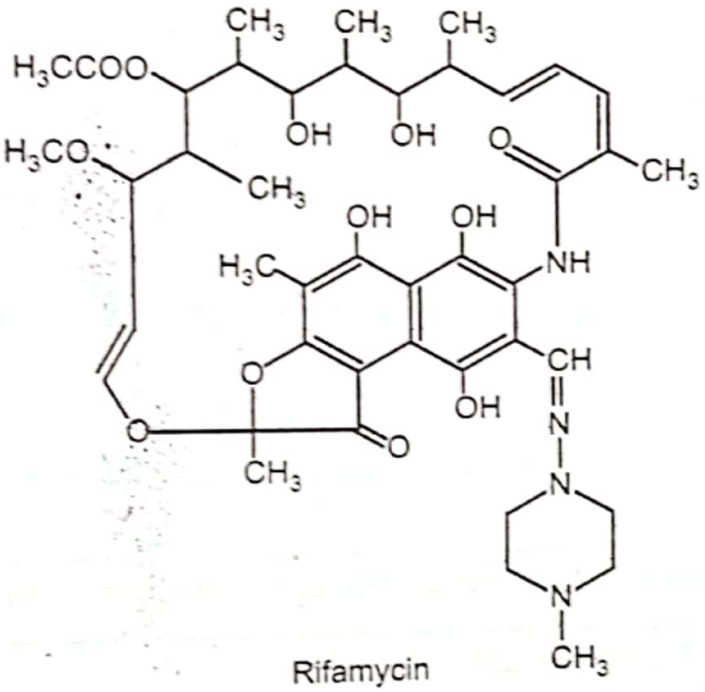
Other significant interferon inducers include polycrylic acid, polymethacrylic acid, malic anhydride-divinyl ether (pyran) copolymer and chlorite oxidised oxyamylose which have had demonstrable efficacy when used prophylactically against both DNA and RNA virus infections.

(12) Antibiotics:

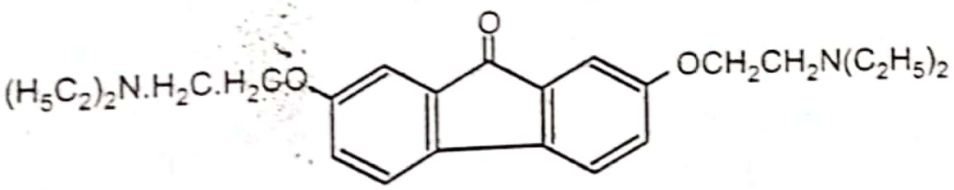
The rifamycins, the streptovaricins and the dissamycin.

(i) Rifamycins: It is isolated from the fermentation broth of *Streptomyces mediterranei.*

It binds to the polymerase molecule in the RNA leukemia virus as well as to the nucleic acid templet, inhibiting DNA synthesis of DNA tumor virus. The non-polar side-chain of antibiotic may impart a detergent quality. Rifampin appears to interfere with assembly of viral components resulting in formation of abnormal viral particles.



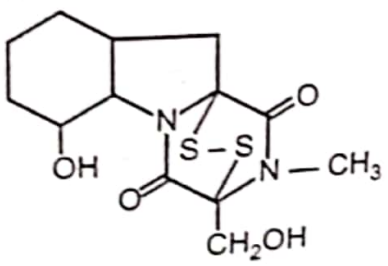
Rifamycin



Tilorone

(ii) Streptovaricins: The streptovaricins are other members of ansamycin antibiotics, obtained from the fermentation strains of Streptomyces, Streptovaricins spectabills. Streptovaricins inhibit the DNA - dependent RNA polymerase but because of the formation of unstable complex with RNA polymerase, it has lower efficacy than rifamycin.

(iii) Gliotoxin: It is a fungal metabolic product. The compound is thought to inhibit viral RNA-dependent RNA polymerase and particularly it inhibits the synthesis of viral RNA.



Gliotoxin

(iv) Distamycins: The mode of action of Distamycin A involves its interaction with viral DNA. This antibiotic also inhibits the RNA-dependent DNA polymerase of RNA-Tumor viruses.

(v) 9-Methyl streptimidone: 9-methylstreptimidone is an isolate from the culture filtrate of a streptomyces species. It acts as an inducer of interferon.

(vi) Bleomycin: Bleomycin A_2 is one of the members of the group of glycopeptide antibiotic. It inhibits the synthesis of the viral nucleic acid. Thus, it causes breaks in cellular DNA.

(13) Miscellaneous Compounds:

(i) Phosphonoacetic Acid: It is active against several viruses. It selectively inhibits the viral DNA-synthesis and it is a specific inhibitor of DNA polymerase.

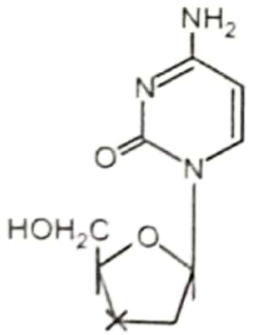
(ii) Kethoxal: 3-Ethoxy 2-oxobutyral-dehyde hydrate (kethoxal) has antiviral activity against several RNA and DNA viruses in-vitro.

The compound is potent inactivator of extracellular virus but also exhibits an inhibition of intracellular virus multiplication.

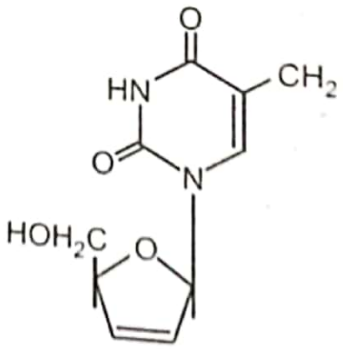
(iii) Decalin derivatives: Several compounds having structure based on the transdecalin nucleus are inhibitory to certain selected influenza viruses. They act by blocking penetration of virus into cell.

Table 8.2: Reverse transcriptase inhibitors

(A) Nucleoside Reverse Transcriptase Inhibitors:

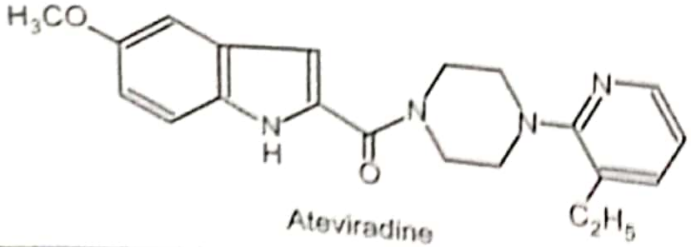


Zalcitabine : X = CH_2
Lamivudine : X = S

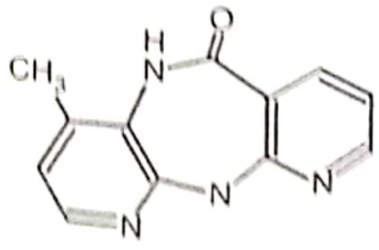


Stavudine

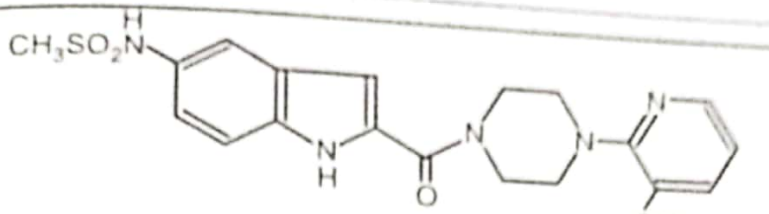
(B) Non-nucleoside Reverse Transcriptase Inhibitors:



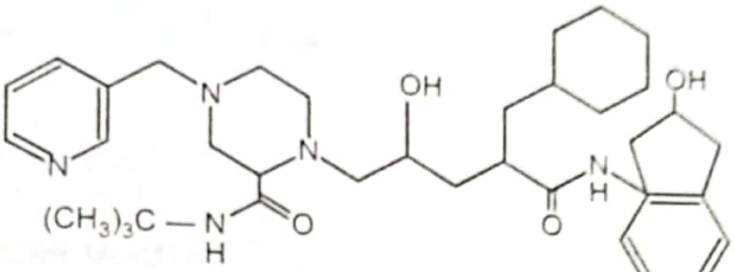
Ateviradine



Nevirapine



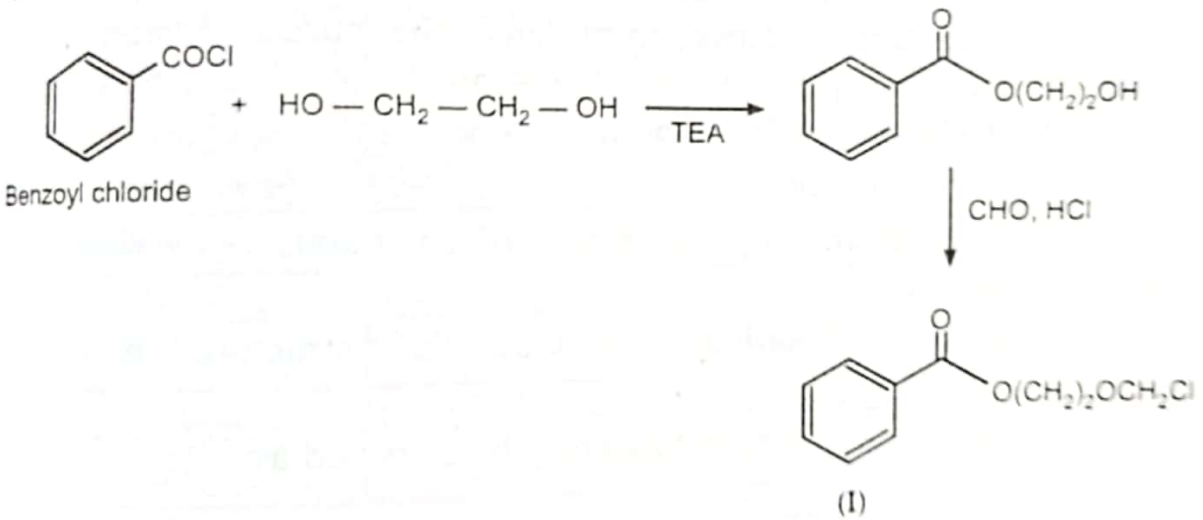
Delavirdine



Indinavir

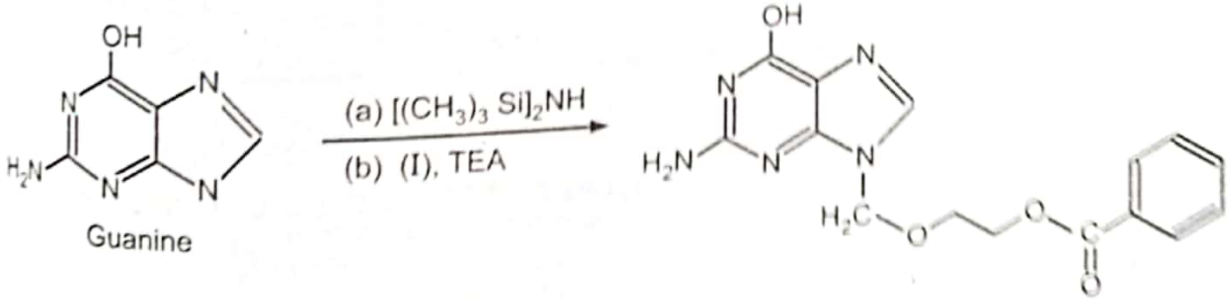
Synthesis

Acyclovir:

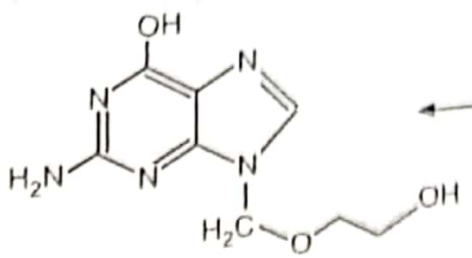


Benzoyl chloride

(I)



Guanine



Acyclovir

UNIT IV

Chapter...9

ANTIFUNGAL AGENTS

♦ SYNOPSIS ♦

9.1 INTRODUCTION

Fungus is a parasite. The human fungi parasitic relationship results in mycotic illnesse the majority of which involve superficial invasion of skin or the mucous membranes of bo orifices. Fungi have different shapes and sizes. Some are large while others are minu parasitic and saprophytic cells. They differ from

- algae by lack of photosynthetic ability.
- protozoa by the lack of motility, possession of chitinuous cell-wall and ease culture on simple media.
- bacteria by greater size and having certain intracellular structure like mitochondri and nuclear membrane.

Depending upon some basic differences, fungi may be classified as:

(a) Phycomycetes (algae like)
(b) Ascomycetes (sac like)
(c) Basidiomycetes (mushrooms), and
(d) Dueteromycetes.

In addition, the 'higher bacteria' like Actinomyces and Nocardia are sometimes groupe with the fungi.

Fungal diseases are generally called as 'mycoses'. Fungal infections fall into two wel defined groups: the superficial and the deep seated mycoses. These mycotic infections ma be categorised broadly as:

(i) Dermatophytoses (skin infections) of contagious nature, caused by various Tricho phyton, Microsporum and Epidermophyton species. These include superficial infections o keratinized tissues like, stratum corneum, hair, nails etc. *Tinea capitis, Tinea corporis, Tinea cruris, Tinea unguium, Tinea versicolor, Tinea nigra* and candidiasis are all grouped under superficial fungal infections. As a rule, these lesions are mild, superficial and restricted. The

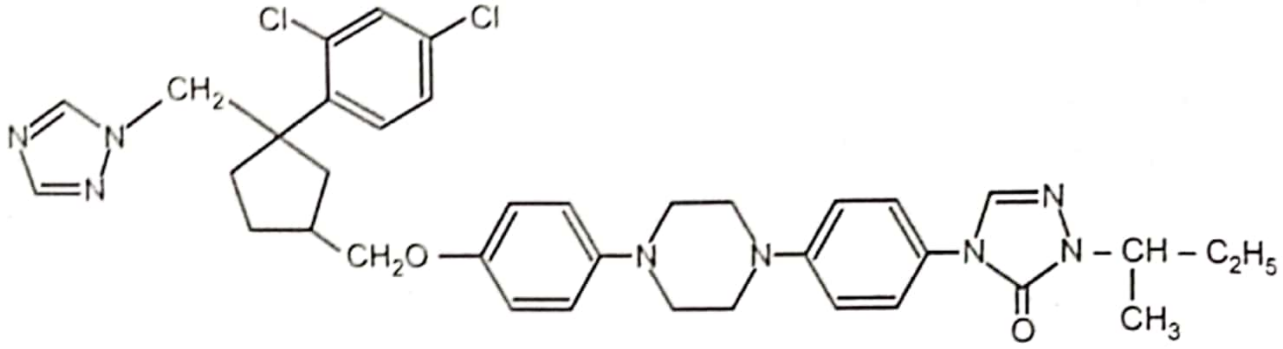
causative microbes are specialized saprophytes with the unusual ability to digest keratin. They have ultimate reservoir in the soil. Topical antifungal agents are effective here. However, to treat deeper infections systemic antifungal (e.g., griseofulvin) may also be given alongwith topical antifungal agents. Because of the keratolytic action of salicylate, it may often be used along with the topical antifungal agent to improve the drug penetration. Salicylic acid helps the drug to reach the site deep within the hyperkeratotic epidermis.

(ii) Candidiasis affects mainly the skin and mucous membrane. It is caused by Candida albicans. These infections mainly develop in the mouth, bowel or vagina and are called as local infections. They may sometimes become systemic and contagious.

Table 9.1: Drugs of choice in the treatment of systemic fungal infections

Sr. No.	Disease	Fungus	Effective antifungal agents
1.	Actinomycosis	*Actinomyces israelii*	Penicillin G, cephalosporin, tetracycline
2.	Aspergillosis	*Aspergillus fumigatus* *Aspergillus niger*	Amphotericin B, rifampin
3.	Blastomycosis (North American type)	*Blastomyces dermatidis*	Amphotericin B, rifampin and hydroxystibamidine, itraconazole
4.	Blastomycosis (South American type)	*Blastomyces brasiliensis*	Amphotericin B, miconazole
5.	Candidiasis	*Candida albicans*	Amphotericin B, nystatin, flueonazole
6.	Chromoblastomycosis	*Cladosporium*	Flucytosine, amphotericin B, potassium iodide
7.	Coccidioidomycosis	*Coccidioides immitis*	Amphotericin B, fluconazole miconazole, ketoconazole
8.	Cryptococcosis	*Cryptococcus neoformans*	Amphotericin B, flucytosine, fluconazole
9.	Histoplasmosis	*Histoplasma capsulatum*	Amphotericin B, hydroxystilbamidine, rifampin
10.	Phycomycosis (Mucormycosis)	*Mucor species*	Amphotericin B, hydroxystilbamidine
11.	Nocardiosis	*Nocardia asteroides*	Amoxicillin, cotrimoxazole, minocycline
12.	Paracoccidioidomycosis	-	Ketoconazole, amphotericin B
13.	Sporotrichosis	*Sporothrix schenckii*	Potassium iodide, amphotericin B, itraconazole
14.	Fusariosis	-	Amphotericin B
15.	Pseudoallescheriosis	-	Ketaconazole, itraconazole
16.	Zygomycosis	-	Amphotericin B

Itraconazole is a 1: 1: 1: 1 racemic mixture of 04 diastereomers, each possessing three chiral centers.



Itraconazole

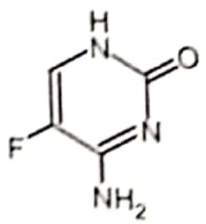
(Janseen, 1992)

(iii) Systemic fungal infection (see Table 9.1), is the third major category that involves fungal infections of bones, viscera, lungs and meninges. Many fungal infections occur either on skin (a vascular region) or in poorly vascularized area (e.g., nails and hair). At such places, the drug cannot build up its therapeutic concentration necessary to exhibit antifungal activity. Besides this, clinical utility of many drugs is hampered mainly because of poor solubility and poor penetration ability. Currently there exist neither clinically available vaccines nor effective antisera for mycotic diseases. Due to various reasons (e.g., differences in solubility, diffusibility or inactivation by serum components), the agents showing excellent antifungal activity *in-vitro* studies, disappointed us when tested *in-vivo*. For example, miconazole and clotrimazole are inactivated by phospholipids and triglycerides. To cover such a broad range of systemic fungal infections, very few antifungal agents are available. These include polyene antibiotics (e.g., amphotericin B, nystatin), antimetabolites (e.g., flucytosine), griseofulvin and imidazoles (e.g., ketoconazole, miconazole and clotrimazole).

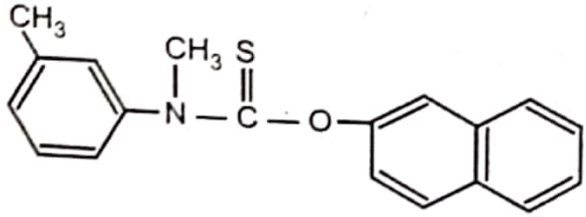
Regardless of the type of fungus that is causing an infection, treatment is extremely difficult because fungi, like mammalians, are eukaryotes. Many biochemical structures, especially the cell membranes, are nearly identical, as are many biochemical reactions.

In human cells, the sterol in the membrane is cholesterol. In fungi, the sterol is ergosterol. This difference amounts to the only source of selectivity that we have in treating fungal infections.

Table 9.2: Some clinically used antifungal agents

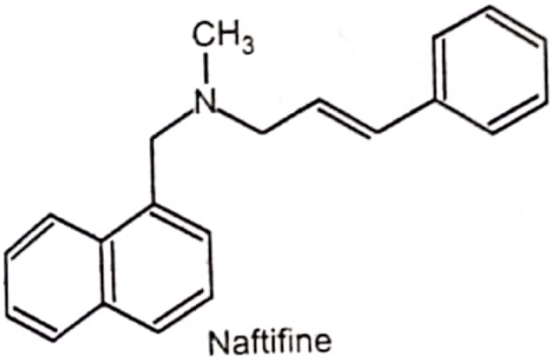


5-Fluorocytosine

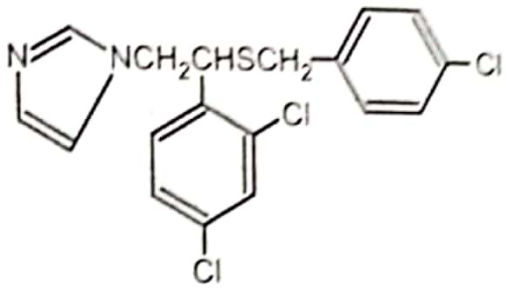


Tolnaftate

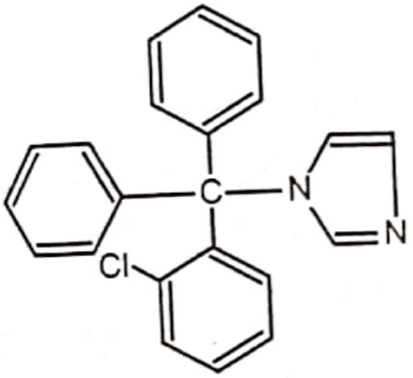
Contd..



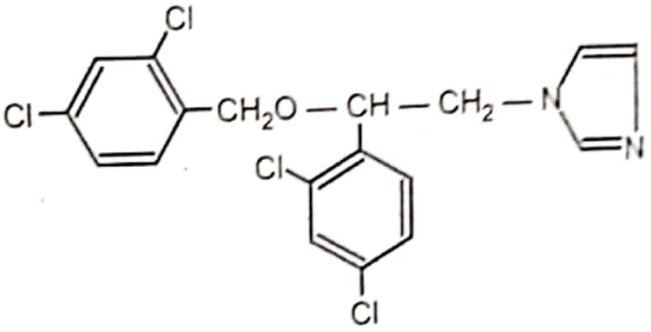
Naftifine



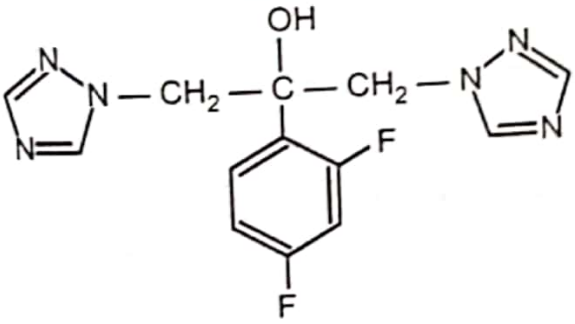
Sulconazole



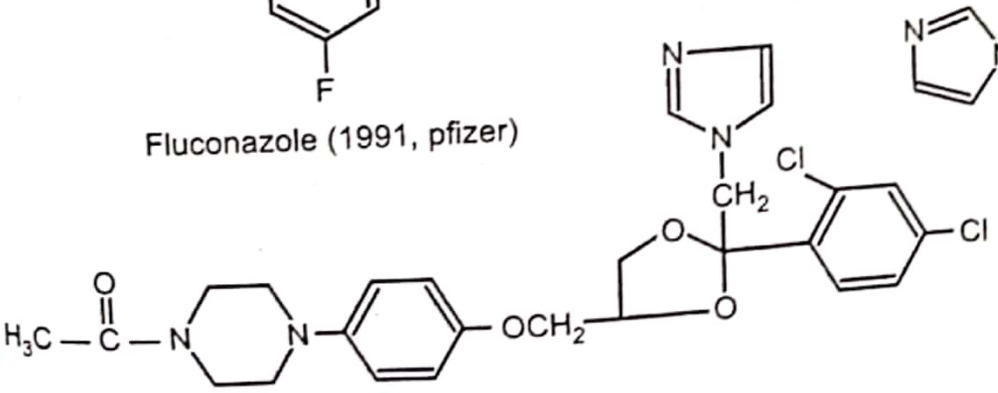
Clotrimazole



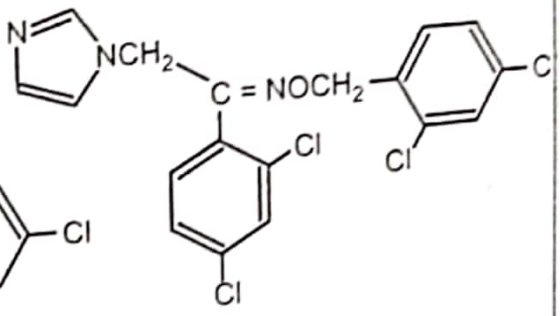
Miconazole



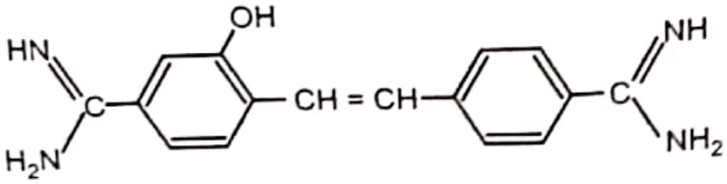
Fluconazole (1991, pfizer)



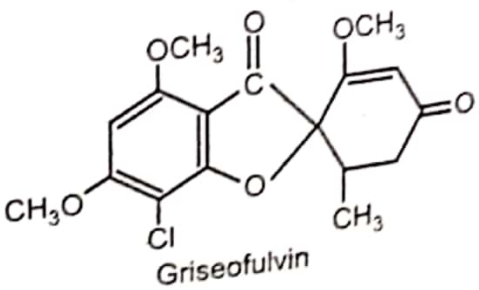
Ketoconazole (1981, Janssen)



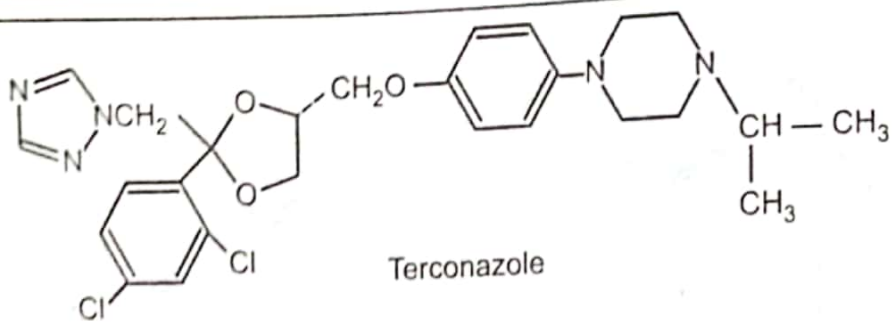
Oxiconazole



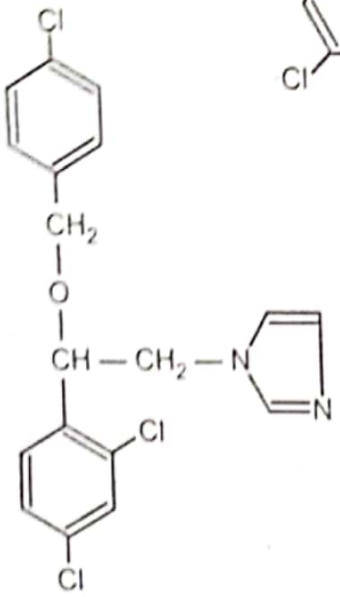
Hydroxystilbamidine



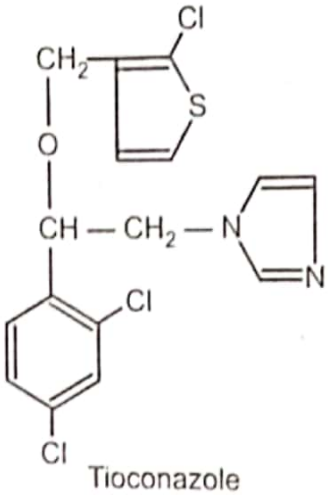
Griseofulvin



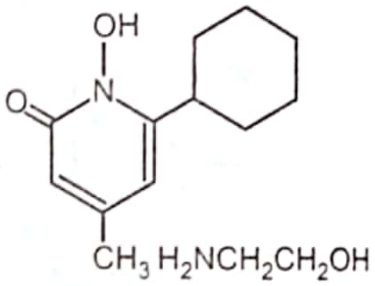
Terconazole



Econazole



Tioconazole



Ciclopirox olamine

9.2 CLASSIFICATION

On the chemical basis, currently used antifungal agents can be categorised as:

(a) Fatty acids
(b) Pyrimidine derivatives
(c) Imidazoles
(d) Allylamines
(e) Amidines
(f) Antifungal antibiotics
(g) Miscellaneous agents.

Due to diversified structures of various antifungal agents, attempts to define SAR also failed. In such cases, interpretation of the activity in terms of drugs physicochemical parameters projects better understanding of SAR studies.

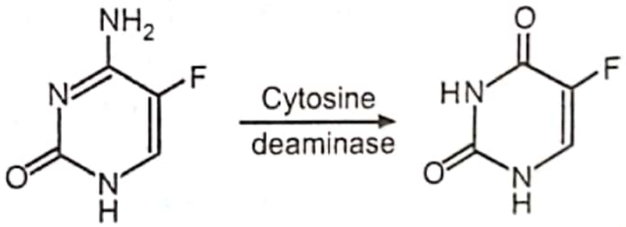
(a) Fatty Acids:

In 1939, Peck reported that sweat has antifungal properties. The antifungal activity of ingredients of perspiration has nothing to do with the pH of the perspiration and the activity is attributed to the presence of fatty acids and their salts. Propionic acid, undecylenic acid and sodium caprylate are effective against infections due to trichophytons, microsporons and *Candida albicans*. The recognition of their antifungal activity led to their clinical use mainly in the form of sodium and zinc salts. For example, the mixture of sodium propionate and propionic acid may be used in the treatment of ringworm while undecylenic acid and zinc undecylenate, in the form of mixture, are effective against ringworm and monilial

vaginitis. Effectiveness increases when zinc salts of these acids are used. They are applied in the form of an ointment, lotion or dusting powder. However, their application to eyes, ears, nose or other areas of mucous membrane should be avoided. Their antifungal activity is related to their ability to precipitate the fungal proteins. Heavy metal ions like Ag^{+}, Hg^{++}, Cu^{++} and Zn^{++} combine with the functional groups present on the surface of enzymes. Hence, when combined with an organic molecule, they potentiate the antifungal activity of the latter.

(b) Pyrimidine Derivatives:

5-Fluorocytosine is a fluorinated pyrimidine and is related in structure to fluorouracil and flouridine. First introduced in 1957, it failed to build up its career as an effective antineoplastic drug. Ten years thereafter when tested for antifungal activity, it proved its potential against Candida species and Cryptococcus neoformans infections.



5-Flucytosine 5-Fluorouracil (An active metabolite)

The antifungal activity of 5-fluorocytosine is attributed to the formation of 5-fluorouracil (an active metabolite) from the drug by fungal cytosine deaminases.

The active metabolite is further converted to 5 - fluro - 2' - deoxyuridylic acid which interrupts the fungal DNA synthesis by inhibiting thymidylate synthetase enzymes. Since mammalian cells do not contain cytosine deaminase, their function is not affected by flucytosine. Besides this, it is also suspected to interfere in protein synthesis.

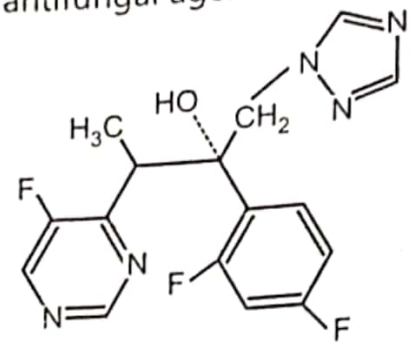
It is not used topically. When used orally, about 4% of administered dose is bound to the plasma-proteins. It has a plasma half-life of 4.2 - 4.5 hours. It is rapidly deaminated in fungal cells to the antimetabolite, 5-fluorouracil. About 80% dose appears in the urine in unchanged form.

Adverse effects include nausea, vomiting, diarrhoea, enterocolitis, headache, skin rashes, vertigo, anemia, sedation, hepatomegaly, hepatic necrosis, leukopenia, agranulocytosis and thrombocytopenia.

Flucytosine is effective against infections caused by *C. neoformans*, *C. albicans*, *T. glabrata* and *S. schenckii*. It is also effective against chromomycosis caused by cladosporium species and phialophora species. It is used in combination with amphotericin B for the treatment of infections due to yeasts and yeast like fungi. Amphotericin renders the yeast cell-membrane more permeable to flucytosine. Hence, both drugs are used in the treatment of cryptococcal meningitis, Candida endocarditis, pulmonary and urinary tract infection.

It may also be used in the treatment of chromoblastomycosis.

Voriconazole: It is a triazole antifungal agent.



(c) Imidazoles:

Imidazole derivatives are associated with many therapeutic fields. Some have been employed as anthelmintics. Antibacterial and antiprotozoal activities are also observed in some analogous. The first azole to become available for clinical use (as a topical agent) was chlormidazole, introduced by Chemie Gruenenthal in 1958. It was followed in 1969 by Janssen's miconazole and Bayer's clotrimazole, and econazole was launched by Janssen in 1974. Even today, the latter three agents remain the mainstay of topical therapy for many dermatophytoses. Miconazole, clotrimazole, ketoconazole, econazole, itraconazole, fluconazole, tioconazole, bifonazole and terconazole are some currently used antifungal imidazole derivatives. They all have activity against a broad range of microorganisms including both fungi and bacteria. Clotrimazole, econazole and tioconazole are effective against superficial fungal infections while bifonazole and terconazole are effective in vulvovaginal candidiasis. Other imidazoles like, ketoconazole and miconazole are effective against both, superficial and systemic infections.

Imidazole derivatives act by damaging the fungal cell-membrane. They enhance the membrane permeability by inhibiting the synthesis of ergosterol which is the primary cellular sterol of fungi.

Clotrimazole shows poor oral absorption. Whatever amount absorbed, gets rapidly inactivated by cytochrome P-450 enzymes in the liver. It is a broad spectrum antifungal agent having fungistatic activity against dermatophytes, *C. albicans*, *C. neoformans*, *S. schenckii* and *B. dermatitidis*. It is used topically in 1-2% concentration as cream, lotion and vaginal preparation to treat cutaneous candidiasis, vulvovaginal candidiasis and dermatophyte infections. It may sometimes be combined with an antibacterial agent in some regimen. Miconazole is a potent antifungal imidazole derivative that may be used topically, intravenously or intrathecally. About 90% of administered dose is bound to the plasma proteins. It has a plasma half-life of 24 hours. It is extensively metabolized in the liver.

The azole class of antifungals act by damaging the fungal cell-membrane. These drugs selectively inhibit the biosynthesis of ergosterol by inhibiting fungal cytochrome P-450 sterol 14α-demethylase. Azoles thus inhibit demethylation at C_{14} of lanosterol thereby causing accumulation of 14 α-methylated sterols that disrupt the various sterol functions in the cell. This mode of action, while leading to inhibition of fungal cell growth, does not result in fungal cell death. Hence, the azoles are fungistatic.

Adverse effects include nausea, vomiting, headache, blurred vision, skin rash, burning, itching, irritation, weakness, arthralgia, seizures, confusion, anemia and thrombocytopenia.

It is effectively used topically in the treatment of tinea pedis, tinea cruris, tinea versicolor, onychomycosis, cutaneous candidiasis, pruritus and other superficial dermatomycoses (2% cream, spray, powder or lotion may be applied topically twice a day for 1-2 weeks). To treat vulvovaginal candidiasis and vaginal infections caused by *T. glabratus* it may be used in the form of a 2% vaginal cream or 100 mg suppositories. The latter may be applied deep in vagina at bed time for 7 days and in the case of 200 mg vaginal suppositories, a three day treatment is usually advised.

Parenterally, it may be used to control systemic fungal infections like, coccidioidomycosis, paracoccidioidomycosis, cryptococcosis, systemic candidiasis and mucocutaneous candidiasis. In patients with coccidioidal meningitis and urinary bladder infections, the I.V. route must be supplemented by intrathecal and intrabladder irrigation routes respectively. The free base may also be used topically to treat ophthalmic mycoses. Ketoconazole is an orally active, broad spectrum antifungal agent that is chemically related to miconazole. About 99% of administered dose is bound to the plasma proteins. It has a plasma half-life of 2 - 4 hours. It is extensively metabolized in the liver primarily by oxidative 0-dealkylation and aromatic hydroxylation to various inactive metabolites that are excreted (85 - 90%) in the bile. It is effective antifungal agent in the treatment of mucocutaneous candidiasis, vaginitis, oral thrush, blastomycosis, coccidioidomycosis, non-meningeal cryptococcal disease, histoplasmosis and some dermatomycoses. It may also be concomittantly administered with flucytosine in the treatment of cryptococcal meningitis.

Econazole nitrate is used topically for the treatment of superficial fungal infections of the skin. While tioconazole is used in the treatment of dermatophyte infections and candidiasis.

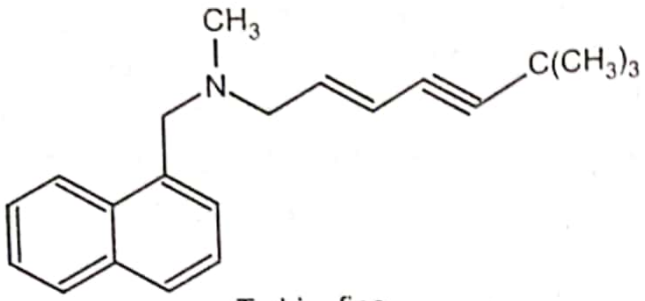
From the many series of azoles that have been reported by diverse groups, several common structural features emerge: an imidazole or triazole heme-coordinating group, a halosubstituted aromatic ring separated from the azole moiety by two atoms, and a side chain. The latter represents the feature of greatest diversity across the family.

Variable length of the side chain explored by various groups suggests that this part of the pharmacophore may extend beyond the substrate binding site, perhaps into the substrate access channel.

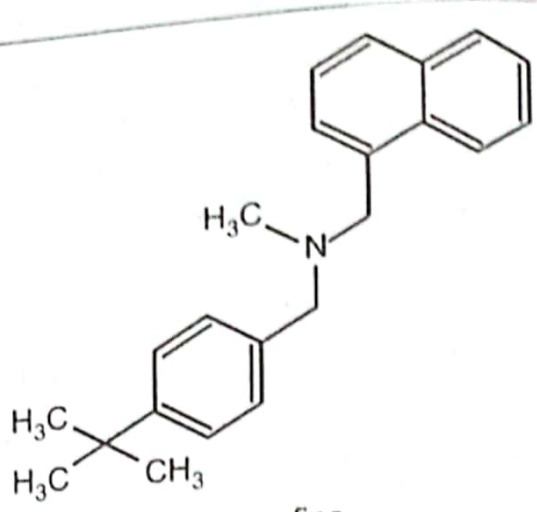
(d) Allylamines:

The allylamines are the most prominent of a number of antifungal classes that exert their activity by inhibition of squalene epoxidase; the intracellular accumulation of squalene that results is thought to be the primary cause of the fungicidal consequences of exposure to the drug. The predominant example of this class of antifungal agents is terbinafine, which is one of the main drugs for the treatment of dermatophytosis.

The inhibition of squalene epoxidase by the allylamines is reversible and non-competitive with respect to squalene, NADPH, and FAD, and the agents have no effects on other enzymes in the ergosterol biosynthetic pathway.



Terbinafine



Butenafine

The structural requirements for potent activity are represented in the broadest sense by two lipophilic domains linked to a central polar moiety by spacer of appropriate length; for good activity, the polar moiety is a tertiary amine, and one of the lipophilic domains consists of a bicyclic aromatic ring system such as naphthalene or benzo [b] thiophene.

(e) Amidines:

In this category, hydroxystilbamidine and stilbamidine represent the examples of effective antifungal agents. These agents are active against fungi and protozoa. Generally, they are used in the treatment of cutaneous blastomycosis, actinomycosis and cryptococcosis.

Hydroxystilbamidine disethionate is administered only by I.V. route. Nothing is known about its bio-transformation and excretion. Adverse effects include nausea, vomiting, diarrhoea, anorexia, rash, fever, chills, anaphylaxis, headache, malaise, fainting, hypotension, dizziness, pancreatitis, hypoglycemia and hepatotoxicity. It is used in the treatment of cutaneous and pulmonary blastomycosis and visceral leishmaniasis.

A solution of 225 mg in 200 ml of 5% dextrose water is freshly prepared and is given by I.V. infusion over a period of 2-3 hours every 24 hours.

(f) Antifungal Antibiotics:

Griseofulvin:

It is isolated in 1939 by Oxford, Raistrick and Simonart. Since it was ineffective against bacteria, its appearance on the clinical screen was delayed by almost about 20 years merely due to an ignorance about its antifungal activity. It is obtained from the yeast, *Penicillium griseofulvum*. Gentles in 1958, first reported its antifungal activity.

Griseofulvin affects only fungi with chitinious cell-wall. The drug is fungistatic rather than fungicidal. It is ineffective in the treatment of systemic mycoses. It may be used orally or topically in the treatment of superficial mycoses of skin, hair and nails caused by most strains of Microsporum, Trichophyton and Epidermophyton. It does not have effect on bacteria, yeasts or other fungi. Its antifungal activity is mainly due to its interaction with the polymerized microtubules. Microtubules are the protein structures found in eukaryotic cells that are responsible for the formation of mitotic spindles. The drug-induced disruption of mitotic spindles slows down the oxidative phosphorylation and nucleic acid synthesis in the fungal cells.

Major metabolite is 6-methyl-griseofulvin that is excreted in the urine. Some fraction also appears unchanged in the faeces. Besides its effectiveness in mycotic infections, it also showed promising results in lichen planus, anginal attacks and Raynaud's syndrome.

Polyene antibiotics:

In the early 1950s, polyene antibiotics were first identified. As the name implies, these compounds contain, unsaturated carbon rings or chains. About 60 polyene antibiotics (all produced by actinomycetes) have been reported in the literature.

They all are characterized by the presence of a large ring containing a lactone group (i.e., macrocyclic lactone) and a hydrophobic region coupled with conjugated polyene system of four to seven double bonds. Many of them, contain a glycosidically linked amino sugars. For example, an aminodesoxy hexose (i.e. mycosamine) is present in amphotericin B and nystatin. The polyene antibiotics are poorly soluble in water. The number of double bonds present in the skeleton serves as a basis of classification of polyenes. For example, they may be categorised as tetraenes (nystatin), pentaenes (filipin), hexaenes (endomycin) and heptaenes (amphotericin B). The most important polyenes are amphotericin B and nystatin. The former being an important therapeutic agent against most of the systemic antifungal diseases. Depending upon the concentration employed, polyene antibiotics exert either fungistatic or fungicidal effects.

These polyene macrolides preferentially bind ergosterol, the predominant fungal sterol, resulting in a permeable plasma membrane and rapid cell death. However, amphotericin also binds to cholesterol of mammalian cytoplasmic membrane. This results in alteration of mammalian cytoplasmic membrane and may explain the severe nephrotoxicity accompanying the use of amphotericin B.

Amphotericin B:

Amphotericin is a mixture of two compounds A and B, obtained from Streptomyces nodosus, a soil actinomycete reported in 1956. As the name suggests, amphotericins are amphoteric in nature. Amphotericin B is more potent and it possesses a broad spectrum of activity. It is effective against *Aspergillus fumigatus, B. dermatitidis, Candida species, C. neoformans, H. capsulatum, Coccidioides immitis, M. audouinii, Paracoccidiodes brasiliensis, Rhizopus species, Rhodotorula species, Sporothrix schenckii, Torulopsis glabrata, Trichophyton* species and Mycobacterium leprae. It exerts maximum antifungal effect between the pH range of 6.0 - 7.5.

It is available in the form of mixture, lozenges and ointment. It has poor oral absorption pattern. It is effective against a number of fungal infections including aspergillosis, blastomycosis, candida infections, leishmaniasis, histoplasmosis, sporotrichosis and coccidioidomycosis. It is used topically to treat external ocular infections (i.e. mycotic conjunctivities). For topical use, amphotericin B is available in a 3% concentration as a cream, lotion and ointment. It may be used intravenously or subconjunctivally in the treatment of fungal corneal ulcers or endophthalmitis. In cryptococcal meningitis, it is usually combined with 5-fluorocytosine to get a synergistic action. It may also be given intra-articularly especially in sporotrichosis and coccidioidomycosis.

In many occasions, flucytosine, minocycline or rifampin in fact may be added to the regimen in order to reduce the minimum antifungal concentration of amphotericin B. Since the latter is one of the most toxic antimicrobial agents which is in use today, the reduction in the dose of amphotericin B, helps to improve the patient comfort. Amphotericin B methyl ester has equipotent antifungal activity but has better pharmacokinetic features. However, it could not reach the market because of its ability to cause leukoencephalopathy.

Nystatin:

It is first isolated from Streptomyces noursei in 1951 by Hazen and Brown. The name of this antibiotic was derived from New York state from where it was discovered. It is only slightly soluble in water and is unstable to moisture, heat, light and air. It exerts no effect on bacteria, protozoa or viruses. When used topically, nystatin may sometimes be combined with iodochlorhydroxyquin.

It is effective specifically against Candida, Microsporum, Trichophyton, Leishmania, *B. dermatitidis, C. neoformans, H. capsulatum, T. vaginalis* and dermatophytes. It is often combined with gentian violet, procaine hydrochloride, antibiotics or hydrocortisone and is used topically in the treatment of candidiasis of skin, mouth, intestine, conjunctival sac, nails and vagina. It may be given as aerosol or instilled into conjunctival sac. However, it should not be used parenterally due to severe systemic toxicities.

Natamycin is yet another tetraene antifungal antibiotic obtained from Streptomyces natalensis in 1958. It is a broad spectrum antifungal agent specifically effective against occular infections caused by Fusarium solani and Myceliating fungi. It is used to treat fungal keratitis, fungal blepharitis and fungal conjunctivitis. It may also be inhaled into the respiratory tract to cure broncho-pulmonary aspergillosis and candidiasis.

Hamycin and candicidin are the examples of other antifungal polyene antibiotics. Candicidin was isolated in 1953 from Streptomyces griseus and is used topically in the treatment of vaginal candidiasis. Hamycin is isolated from *Streptomyces pimprima* and is effective against *Blastomyces dermatitidis, Histoplasma capsulatum, Aspergillus fumigatus, Cryptococcus neoformans* and *Candida albicans*. It may be used topically to control vaginal candidiasis.

(g) Miscellaneous Agents:

Tolnaftate:

It is a topical antifungal agent available as cream, solution and powder and aerosol in 1% concentration. It is a thiocarbamate derivative and is used in the treatment of cutaneous mycoses or ringworm infections. When used against other fungal or bacterial infections, it remains ineffective.

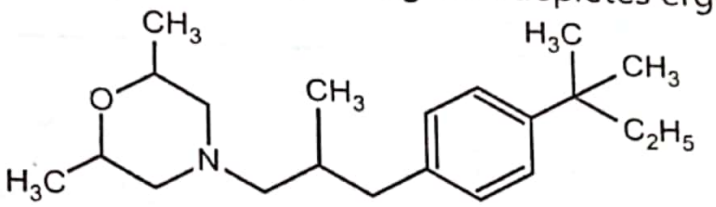
Like the allylamines, it is non-competitive inhibitor of squalene epoxidase, with inherent selectivity for the fungal enzymes over mammalian.

It is topically applied to the affected area twice a day. However, relapses may occur after cessation of therapy.

Cyclopirox olamine:

It is a topically used broad spectrum fungicidal agent effective against dermatophytes. Its antifungal activity may be related to its ability to interfere in uptake of precursors needed for the synthesis of proteins and nucleic acid core. Adverse effects are few and include pruritis and burning at the site of application.

Amorolfine: It is a morpholine antifungal drug that depletes ergosterol.



Potassium iodide:

In the form of saturated solution of 1 g/ml, it may be used orally as an effective antifungal agent in the treatment of cutaneous and lymphatic forms of sporotrichosis, caused by *S. schenckii*. It is excreted in the urine. It probably acts by iodination of proteins in fungus cell membrane. Adverse effects include nausea, vomiting, diarrhoea, heart-burn, sneezing, shedding tears, metallic taste, acneiform skin lesions and swelling of parotid gland. Iodism is frequently encountered during the therapy.

Table 9.3: Clinically used antifungal agents

	Drug	Uses	Possible mechanism of action
1.	Polyene antibiotics	Candidiasis, Histoplasmosis, Blastomycosis, Sporotrichosis	interacts with fungal membrane sterols and change the selective permeability of fungal membrane.
2.	Griseofulvin	*T. capitis, T. circinata, T. pedis*, Onochomycosis	interacts with fungal micro-tubules and prevents cell division.
3.	Imidazole derivatives	Cryptococcosis, Histoplasmosis, Mucormycosis	inhibits ergosterol synthesis in fungal cell membrane resulting into leakage of cell constituents.
4.	Flucytosine	Candidiasis Aspergillosis	inhibits the formation of fungal nucleic acids.
5.	Tolnaftate	Cutaneous mycoses	inhibits transport of precursors for proteins and nucleic acid in fungi.
6.	Cyclopirox	Cutaneous mycoses	inhibits transport of precursors needed for the synthesis of proteins and nucleic acid in fungi.
7.	Potassium iodide	Sporotrichosis	iodination of proteins in fungal cell-membrane.

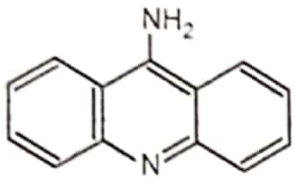
Whitfield's ointment:

It contains a mixture of benzoic acid (fungistatic agent) and salicylic acid (keratolytic agent). The keratolytic action of salicylic acid helps for desquamation of stratum corneum. This promotes the removal of offending fungus resulting into better penetration of antifungal agent.

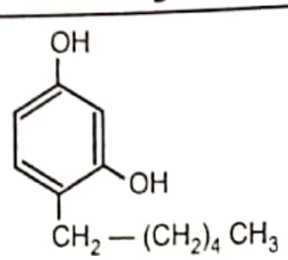
The cell-wall is one of the most attractive targets in the fungal cell. It morphologically defines and protects the cell from lysis and its continued biosynthesis is essential for growth and survival. It is composed primarily of three polysaccharides; β-1, 3-glucan, chitin (β-1, 4-N-acetylglucosamine) and mannoprotein (largely α-1, 6-mannose).

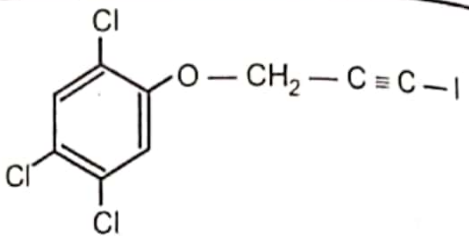
None of the major components of the cell-wall or enzymes involved in their biosynthesis occur in mammalian cells.

Table 9.4: Some Organic Antifungal Agents

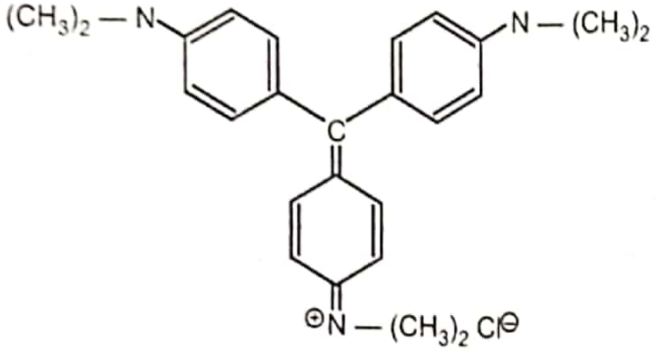


Acrisorcin

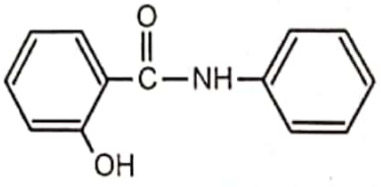




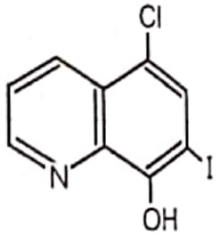
Haloprogin



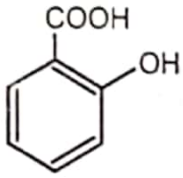
Gentian violet



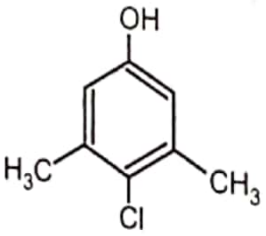
Salicylanilide



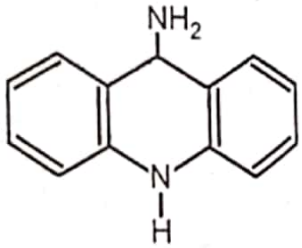
Iodochlorhydroxyquin (Clioquinol)



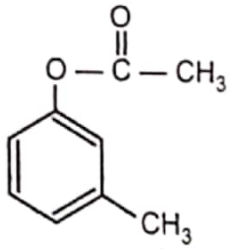
Salicylic acid



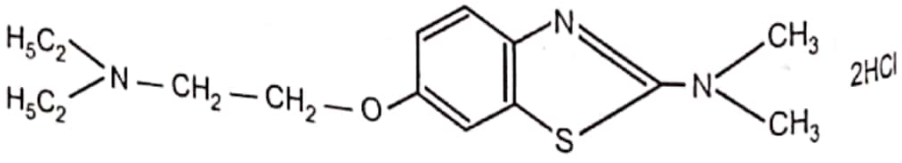
p-Chlorometaxylenol



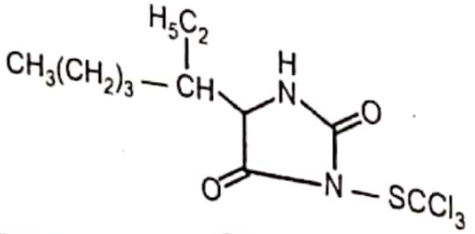
Aminacrin



m-Cresyl acetate



Diamethazole dihydrochloride



Chlordantoin

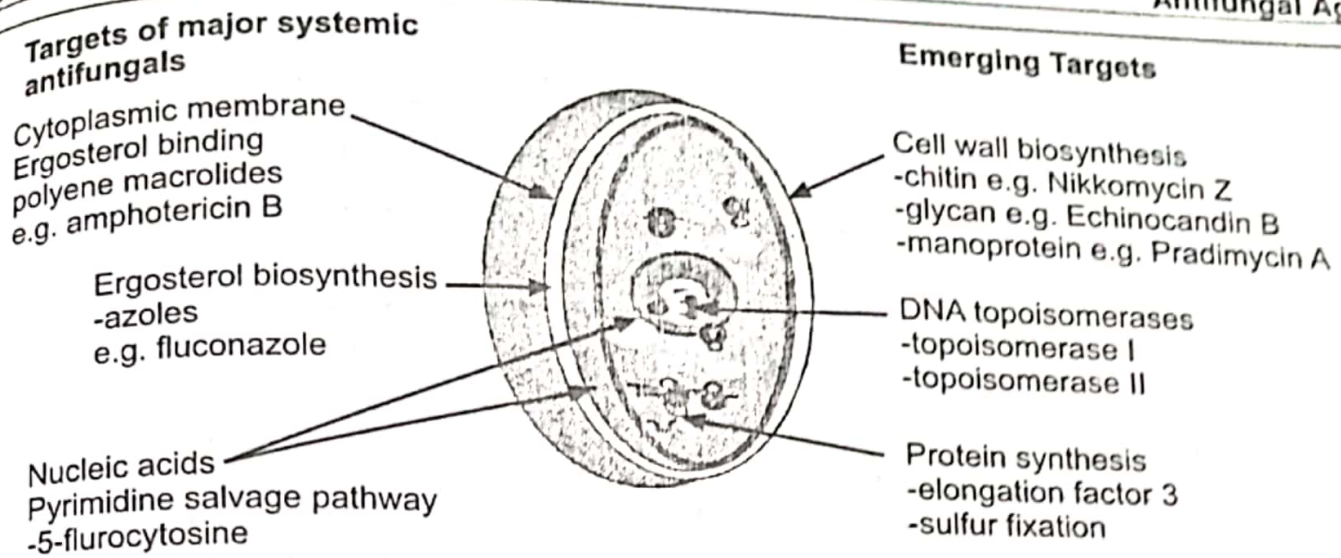


Fig. 9.1: Targets of known and emerging antifungal drugs

Antibiotics:

Many antibiotics like, minocycline, tetracyclines, penicillines and rifampicin may be administered concomittantly with antifungal agents. They do not have antifungal potential. However, they may enhance the activity of antifungal agent (e.g. amphotericin B) when used in combination. Beside them, pyrrolnitrin, variotin and siccanin are other examples of antibiotics used in mycotic diseases.

Plant Products:

Many workers have described the plant products as effective antifungal agents in the treatment of skin disorders. However, the reports over antifungal properties of essential oils are quite meagre and fragmentary.

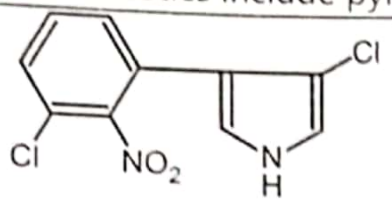
Other Organic Compounds:

Salicylic acid, aminacrine, acrisorcin, halopragin, p-chloro-metaxylenol, salicylamide, salicylanilide, iodochlorhydroxyquin, m-cresyl acetate, diamethazole, chlordantoin, gention violet, pecilocin, di-iodohydroxy quinoline, iodine, phenyl mercuric nitrate, thymol and zinc pyrithione possess significant antifungal activity.

Most of them may be used in the treatment of ringworm infections of scalp, feet and groin. Haloprogin is a synthetic iodinated trichlorophenol available as a 1% cream. It is effective against various candidial and ringworm infections of the skin. Thymol in 1 - 2% concentration may be added to Whitfield's ointment to enhance its antifungal potential. Zinc pyrithione may be used as 1% solution to control infections due to the Pityrosporum ovale and Tinea versicolor. While salicylanilide may be used alongwith undecylenic acid in the form of 5% ointment to exhibit antifungal activity.

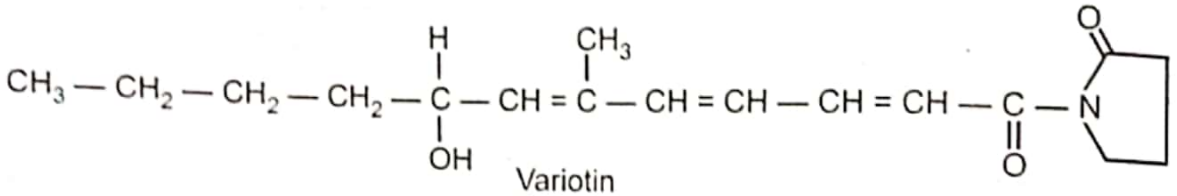
9.3 NATURAL PRODUCTS

The various fungal organisms produce antibacterial substances along with such antifungal agents which do not exhibit significant antibacterial properties. Such types of anti-fungal antibiotics include pyrrolnitrin, variotin, siccanin and natamycin.

i) 

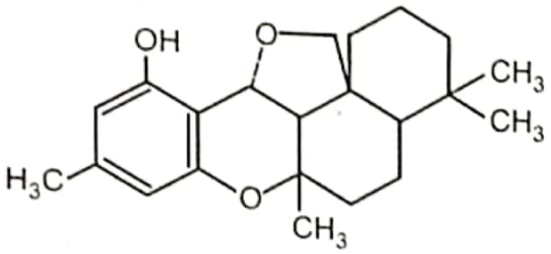
Pyrrolnitrin

Source : Pseudomonas fluoroscence

ii) 

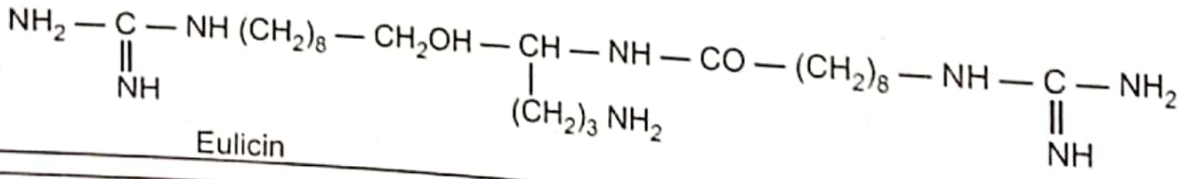
Variotin

Source : Paecilomyces variotii

iii) 

Siccanin

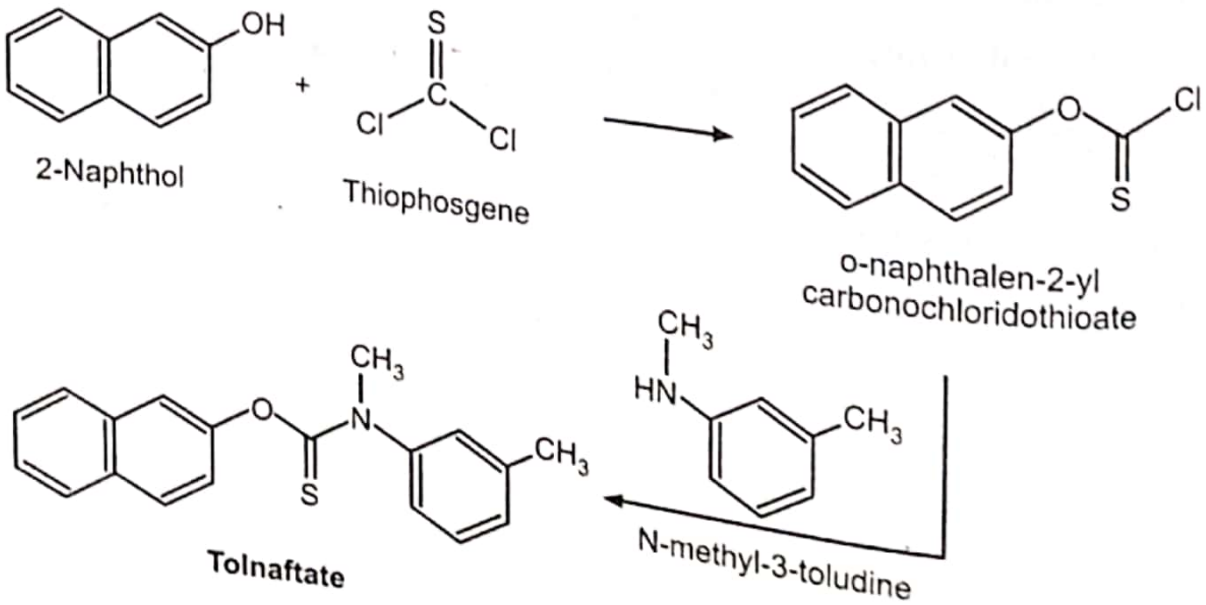
Source : Helminthosporium siccans

iv) 

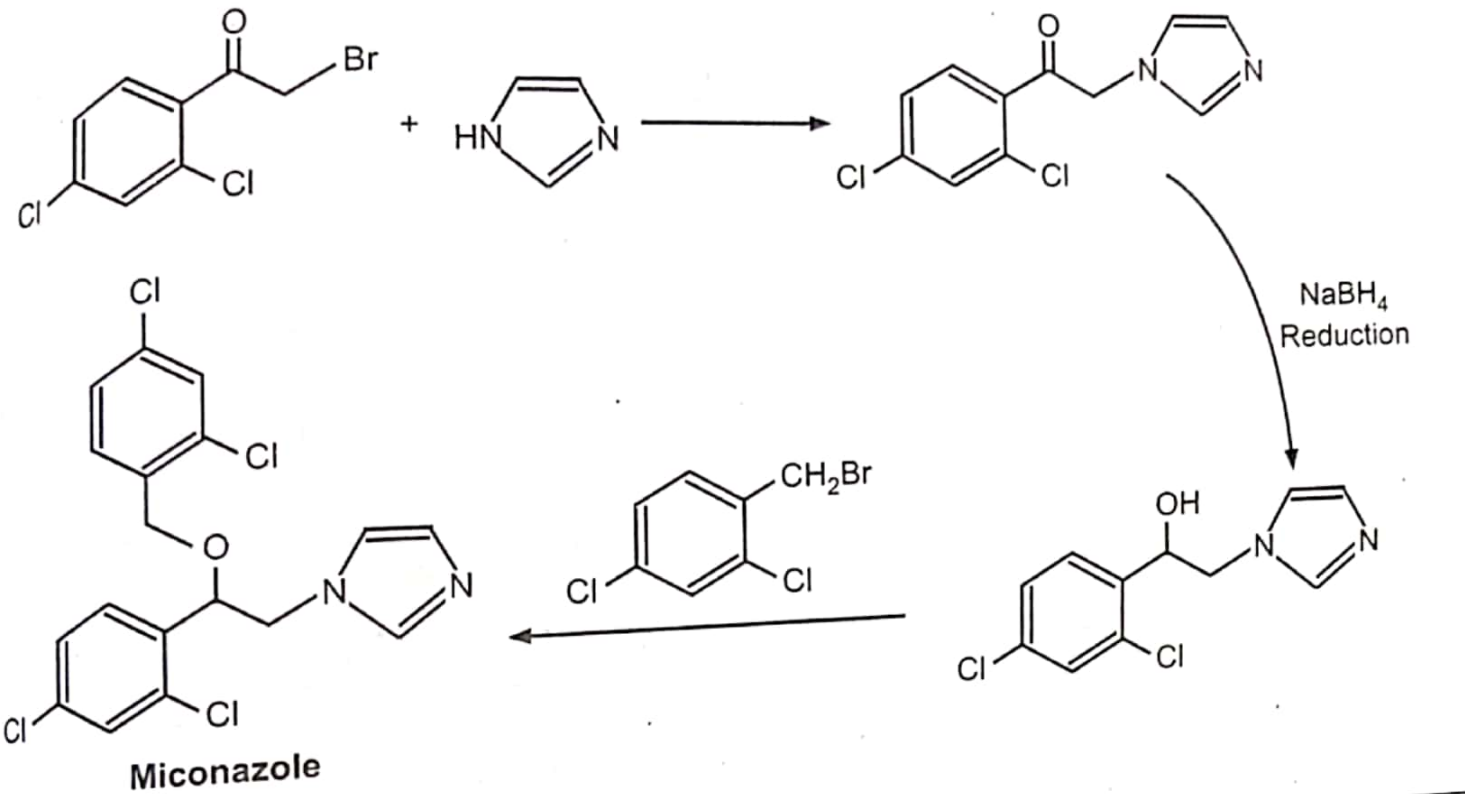
Eulicin

Synthesis

(i) Tolnaftate:



(ii) Miconazole:



Miconazole

Chapter...11

ANTHELMINTICS

♦ SYNOPSIS ♦

11.1 INTRODUCTION

Infestation with the parasitic worms is the most common disease in many tropical and subtropical countries. These parasitic worms firmly hold the intestinal mucosa and continue their reproduction by egg production. Worms parasitic to man can be categorised as cestodes (tapeworm), nematodes (round worms) and trematodes (flukes). Anthelmintics are the agents which are used to destroy or eliminate these parasitic worms from the gastrointestinal tract. They act by killing or paralyzing the worms so that such worms could be easily expelled out of gut. Some anthelmintic agents also impair the egg production process in worms. Depending upon the action, anthelmintic agents are categorised into:

(i) vermifuges or drugs that expel the worms from the body, and

(ii) vermicides or drugs that kill the worms in the body.

Table 11.1: Clinically used anthelmintic agents

Sr. No.	Worm infection	Drugs commonly used
1.	Ascariasis	Mebendazole, piperazine, pyrantel, bephenium
2.	Cestode infection	Paramomycin
3.	Cutaneous larva migrans	Thiabendazole
4.	Dracunculiasis	Niridazole
5.	Enterobiasis	Piperazine, pyrantel, pyrvinium
6.	Filariasis	Diethylcarbamazine
7.	Fascioliasis	Bithionol, hexylresorcinol
8.	Nematode (round worm) infection	Bephenium, mebendazole, pyrantel, hexyl resorcinol
9.	Onchocerciasis	Diethylcarbamazine, suramin

Contd.

10.	Paragonimiasis	Bithionol
11.	Schistosomiasis	
	S. haematobium	Lucanthrone, niridazole, metrifonate, praziquantel
	S. mansoni	Hycanthone, niridazole, oxamniquine, praziquantel
	S. Japonicum	Praziquantel, niridazole, tartar emetic
12.	Strongyloidiasis	Pyrvinium, thiabendazole
13.	Trematode (fluke) infections	Bithionol
14.	Trichuriasis	Mebendazole
15.	Trichiniasis	Mebendazole, corticosteroids

11.2 CLASSIFICATION

Presently available anthelmintic agents are structurally diversified. On the chemical basis, they can be classified as:

(a) Phenols
(b) Chlorinated hydrocarbons
(c) Antimonial compounds
(d) Dyes
(e) Piperazine analogs
(f) Heterocyclic compounds
(g) Alkaloids and plant extracts, and
(h) Miscellaneous agents

(a) Phenolic Anthelmintic Agents:

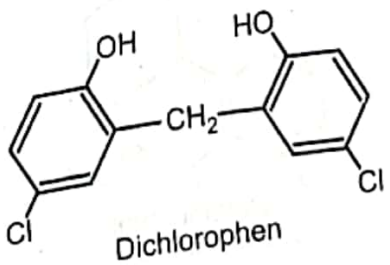
(i) Hexylresorcinol:

It is an orally active broad spectrum anthelmintic agent, originally introduced as an urinary antiseptic. It is particularly effective in the treatment of trichuriasis. It causes tissue irritation and is not used in patients with peptic ulcer. Adult oral dose is 1000 mg once a day.

(ii) Dichlorophen:

It is an anthelmintic agent effective in the treatment of tapeworm infections. It detaches worm from the bowel wall and promotes its digestion in the gut.

Adult oral dose is 75 mg / kg body weight, taken on an empty stomach in the morning. A saline cathartic should be given 2 - 4 hours after the drug to minimize the danger of cysticercosis.



Dichlorophen

(b) Chlorinated Hydrocarbons:

(i) Tetrachloroethylene:

It is an unsaturated chlorinated hydrocarbon. It is not used as an anthelmintic agent presently and is now of historical importance only. Presently, it is used only in veterinary practice and in the treatment of fluke infections in humans.

(c) Antimonial Compounds:

These agents are now less favoured as anthelmintics because of their high toxicity and difficulty of administration. They exert anthelmintic activity by selectively inhibiting schistosomal phosphofructokinase. This enzyme is necessary to catalyze the conversion of fructose - 6 - phosphate to fructose-1, 6-diphosphate. Examples include potassium and sodium antimony tartarate. Stibophen is a trivalent effective antimonial leishmanicidal agent with less intensity of adverse effects.

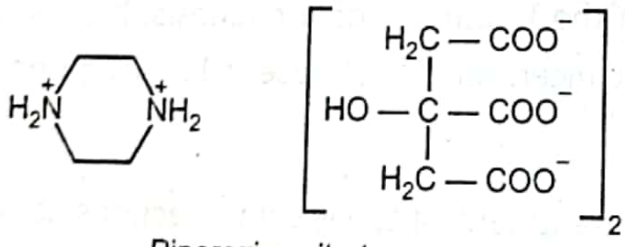
Adult intramuscular dose is 100 mg every second day for 1 - 3 weeks.

(d) Individual Anthelmintic Agents:

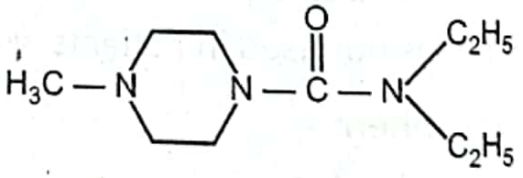
(i) Thiabendazole:

It is an orally active, broad spectrum anthelmintic agent, effective against a wide range of nematodes. It exerts vermicidal activity specifically against Strongyloides stercoralis, Trichinella spiralis and Trichiuris trichiura. It acts by deforming the worm-eggs. However, the egg count returns to normal if therapy is discontinued. It has no effect on filariasis. It has a plasma half-life of 1.2 hours. It is excreted in urine mainly as 5-hydroxy thiabendazole either in the form of glucuronide or sulfate. Adverse effects include nausea, vomiting, epigastric distress, anorexia, diarrhoea, numbness, skin rash, hyperglycemia, crystalluria and transient leukopenia.

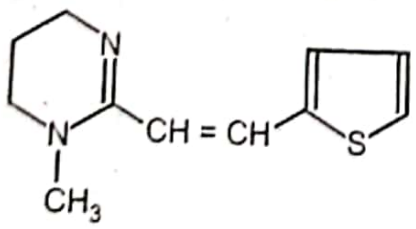
Table 11.2: Some clinically used anthelmintic agents



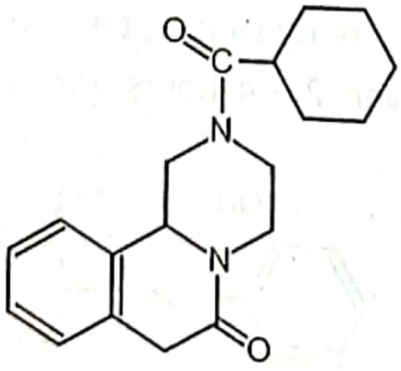
Piperazine citrate



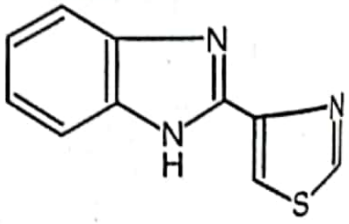
Diethylcarbamazine



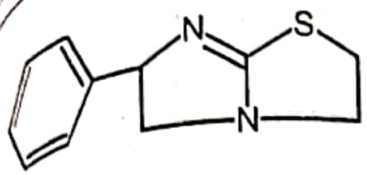
Pyrantel



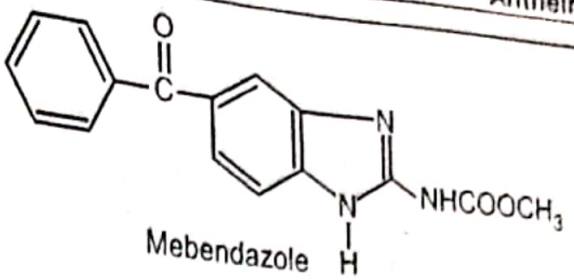
Praziquantel



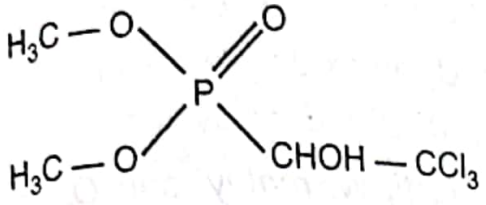
Thiabendazole



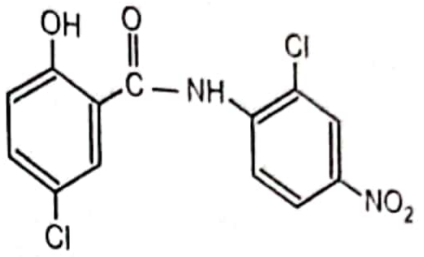
Levamisole



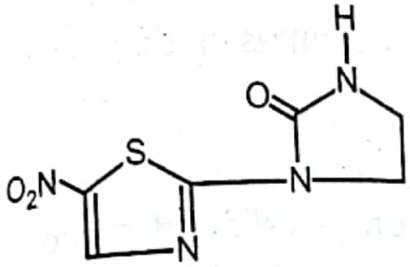
Mebendazole



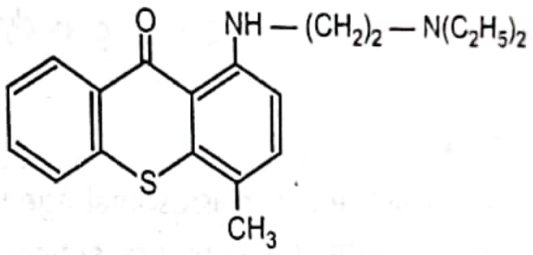
Metrifonate



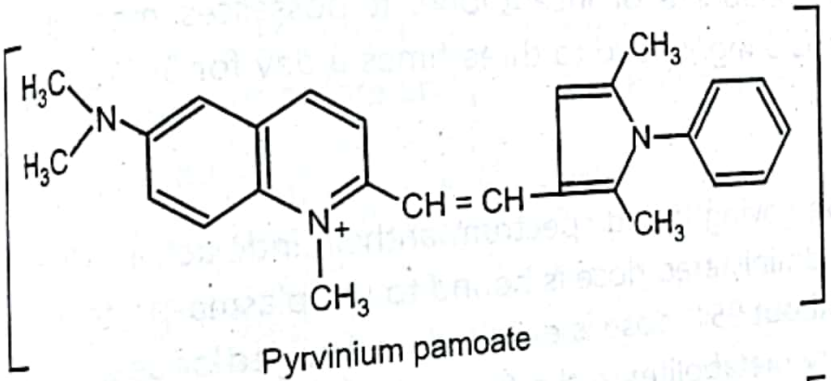
Niclosamide



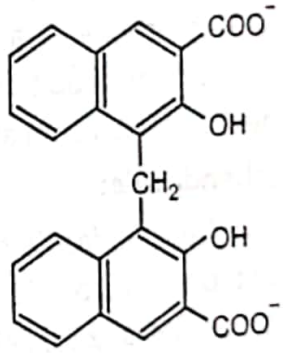
Niridazole

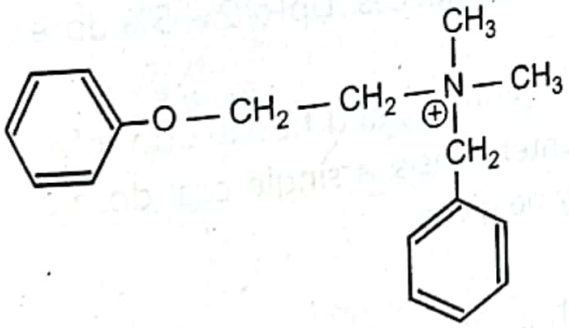


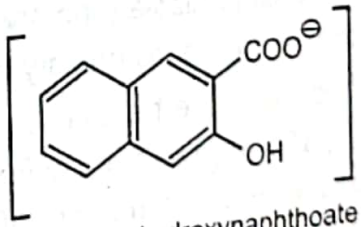
Lucanthone



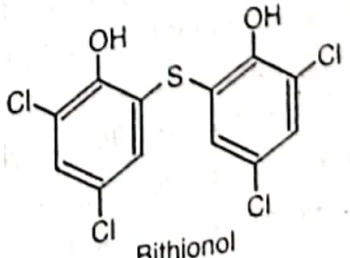
Pyrvinium pamoate



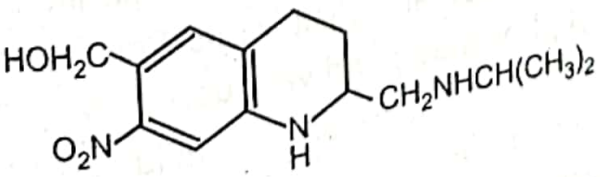




Bephenium hydroxynaphthoate



Bithionol



Oxamniquine

Thiabendazole is effective in the treatment of A. duodenal (common hookworm), *E. vermicularis* (pin worm), *A. lumbricoides* (round worm) and *S. stercoralis* infections. Adult oral dose is 3 g per day. In early trichinosis, treatment may be continued for 2-3 additional days while in disseminated Strongyloidiasis, treatment may be continued for atleast 5 days.

(ii) Diethylcarbamazine:

It is an orally active agent belonging to piperazine category. It acts by sensitizing the microfilaria so that they become plagocytozed by fixed tissue macrophages. It is orally well absorbed and has a plasma half-life of 8 - 12 hours. Upon extensive metabolism, a variety of inactive metabolites are excreted in the urine alongwith 10% dose as unchanged drug.

It is used to treat infections caused by *W. bancrofti*, *W. malayi* and *O. volvulus*. It is a drug of choice for the treatment of filariasis due to Tetrapetalonema perstans or Tetrapetalonema streptocerca. It may also be used in tropical eosinophilia and Ascaris infections. Adult oral dose is 2 mg/kg body weight three times a day after meals for 1-3 weeks.

(iii) Lucanthone:

It is an orally active antischistosomal agent derived from a yellow dye and is effective against *S. haematobium*. It acts by preventing helmintic ova production or its release. This results in the destruction of the parasite. It is less favoured clinically because of its adverse effects. Hycanthone is a hydroxymetabolite of lucanthone. It possesses marked schistosomicidal activity. Adult oral dose is 5 mg/kg two to three times a day for 5 - 10 days in the treatment of schistosomiasis.

(iv) Mebendazole:

It is a benzimidazole derivative having broad spectrum anthelmintic activity. It is poorly absorbed orally. About 78 - 80% administered dose is bound to the plasma-proteins. It has a plasma half-life of 2.5-5.5 hours. About 95% dose is excreted unchanged or as 2 - amino - 5 - benzoyl - benzimidazole (primary metabolite) in the faeces. Upto 2 - 5% dose appears in the urine unchanged or as primary metabolite.

It is effective in the treatment of ascariasis, trichuriasis, and hookworm infections in the oral dose of 100 mg twice a day for 3 days. In enterobiasis, a single oral dose of 100 mg is given. After 2 weeks, second dose of 100 mg may be given.

(v) Metrifonate:

It is an organophosphorus compound which is an inhibitor of cholinesterase enzyme, and is used as anthelmintic and agricultural insecticide. It has a plasma half-life of 1.5 hours. It is extensively metabolized by plasma and schistosomal arylesterases. Principal metabolites include dichloro- (2, 2 - dichlorovinyl dimethyl phosphate) and various inactive products.

Adverse effects include nausea, vomiting, colic, abdominal pain, mild vertigo, lassitude, decreased sperm count and some intestinal nematodes.

It is used for the treatment of urinary schistosomiasis and of *S. haematobium* infections.

Adult oral dose is 5-15 mg/kg body weight 3 times after every 2 weeks.

(vi) Niridazole:

It is an orally active nitrothiazole derivative having anthetmintic and antibacterial activities. It also has amoebicidal and schistosomicidal activities. It is also effective against guinea worm (*D. medinensis*) infestation.

It undergoes an extensive first pass hepatic metabolism. 1-Thiocarbamoyl-2-imidazolidinone is the active metabolite. The metabolites appear both, in urine and faeces.

It is used in the treatment of *D. medinensis*. It may also be used for the therapy of schistosomiasis due to *S. japonicum*.

(vii) Niclosamide:

It is a salicylanilide derivative having anthelmintic activity. It is effective only in the treatment of intestinal cestodes. Its oral absorption is poor. Major dose is excreted through

It is used in the treatment of diphyllobothriasis (*Diphyllobothrium latum*), Hymenolepiasis (*H. nana*), Taeniasis (*T. saginata*) and Dipylidiasis (*Dipylidium conium*). *Enterobium vermicularis* is also susceptible. For destruction of tapeworm, niclosamide is the most effective and safe drug.

Adult oral dose is 500 mg usually in the fasting condition 3 - 4 times a day. Antiemetic may be given one hour before and purgative about 2 hours, after the treatment.

(viii) Oxamniquine:

It is an orally active yellow dye having anthelmintic activity. Chemically, it is 2-amino methyl tetra-hydroquinoline derivative. It is especially effective against *Schistosoma mansoni* and has a plasma half-life of 1 - 2.5 hours. Principal metabolites include 6-carboxyl and 2-carboxylic acid derivatives which are excreted in the urine alongwith traces (0.4 - 1.9% dose) of parent drug.

It is an effective schistosomicidal agent used against *Schistosoma mansoni* and is also used in combination with metrifonate in the treatment of mixed mansoni and haematobium infections.

Adult oral dose is 15 - 60 mg/kg of body weight as a single dose given at bedtime.

(ix) Pyrvinium Pamoate:

It is an organic dye used as anthelmintic agent. It is not orally absorbed. Major portion of administered dose appears unchanged in the faeces. Adverse effects are few and include gastric irritation, photo-sensitization, nausea, vomiting, diarrhoea, cramps and skin rash. It is used in the treatment of enterobiasis caused by pin worm (*E. vermicularis*). Adult oral dose is 5 mg (base) / kg body weight per day.

(x) Suramin:

It is a dye derivative having trypanocidal activity. Freshly prepared solution should always be used for I.V. administration. About 99% administered dose is bound to the plasma-proteins. It has a plasma half-life of 48 - 49 days. It does not undergo significant metabolism. About 60% dose appears unchanged in the urine.

It is used in the treatment of African trypanosomiasis and for onchocerciasis either alone or with arsenical therapy. It is effective in the prophylaxis of Rhodesian and Gambian trypanosomiasis.

Adult I.V. dose is 1 g on days 1, 3, 7, 14 and 21 that is slowly injected in the form of 10% solution. The therapy may be continued for additional 5 weeks.

(xi) Piperazine citrate:

It is an orally active anthelmintic agent available as citrate, phosphate or adipate salt form. It is effective against *Ascaris lumbricoides* and *Enterobium vermicularis* (oxyuriasis). Major dose fraction appears unchanged in the urine.

It is used in the treatment of oxyuriasis, a single daily dose of 65 mg/kg of body weight may be given for a week. The dose-schedule may be repeated after 15 days. It may also be used in the treatment of combined ascariasis and oxyuriasis.

(xii) Bephenium hydroxynaphthoate:

It is a quaternary ammonium salt previously designed to treat hookworm infections. It is poorly absorbed from GIT. It is used to treat infections caused by *Ascaris lumbricoides*, *Trichostrongylus orientalis* and ternideniasis. It is excreted in urine only in trace amounts.

Adult oral dose is 5 g twice a day with empty stomach for 3 - 7 days.

(xiii) Bithionol:

It is phenolic anthelmintic agent used for the treatment of paragonimiasis and fascioliasis. Adverse effects include nausea, vomiting, diarrhoea, colic, urticaria and photodermatitis.

Adult oral dose is 50 mg/kg of body weight every second day for ten doses.

(xiv) Chloroquine:

It is an orally effective 4-aminoquinoline antimalarial agent which may be used in the treatment of clonorchiasis. It acts by depressing ova output. It does not exert vermicidal effect.

(xv) Pyrantel palmitate:

It is a poor orally absorbed broad-spectrum anthelmintic and neuromuscular blocking agent. It is effective against infections with hook-worm, pinworm, and round-worm. About 60 - 70% dose appears in the faeces in unchanged form. Inactive metabolites appear in the

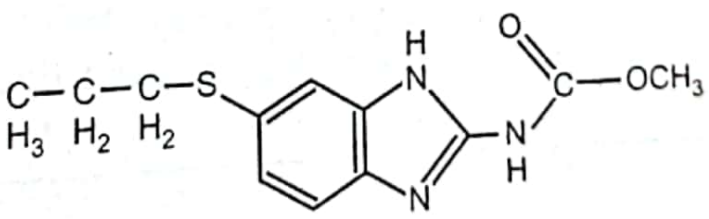
urine alongwith 10 - 20% dose in unchanged form. It is contraindicated during pregnancy and in children less than 2 years of age.

It is effective in the treatment of ascariasis, enterobiasis, ancylostoma, *Necator americanus* and Trichostrongylus. It may be used alongwith oxantel to treat mixed infections with Trichuris trichiura. Adult oral dose is 11 mg/kg to a maximum of 1 g per day. Treatment may be repeated, if necessary after an interval of two weeks.

In general, various anthelmintic agents exert their activity by producing toxic effects on both, gastrointestinal tract and the parasitic worms. They induce contraction of the worms which is followed by their tonic paralysis. Thereafter the worms are removed by the peristaltic movement of smooth muscles of GIT that helps to excrete out these parasites in the faeces. Metrifonate, an organophosphorus inhibitor of cholinesterase enzymes, produces rapid and almost complete inhibition of cholinesterases in the worms. Of course, the drug, to a certain extent, also affects the host due to its cholinesterase-inhibitory activity.

Depending upon the type of worm involved, anthelmintic agents are used alone or in combination with each other. For example, in tapeworm infections, niclosamide is a drug of choice. In nematode infections, piperazine, mebendazole and pyrantel palmitate are used because of their broad-spectrum of anthelmintic activity. For filaria, diethylcarbamazine is generally used, while in trematode infections, niridazole, bithionol and oxamniquine are effective agents.

(xvi) Albendazole: Developed in 1975, it is used for the treatment of a variety of parasitic worm infestations. The most common side-effects include headache and abnormal liver function. Albendazole induces degenerative alterations in the intestinal cells of the worm by binding to the colchicine-sensitive site of β-tubulin, thus inhibiting its polymerization or assembly into microtubules. At higher concentrations, albendazole inhibits metabolic enzyme, such as, malate dehydrogenase and fumarate reductase. This leads to production of less energy (ATPs) by Krebs cycle resulting into immobilization and death of the parasites.

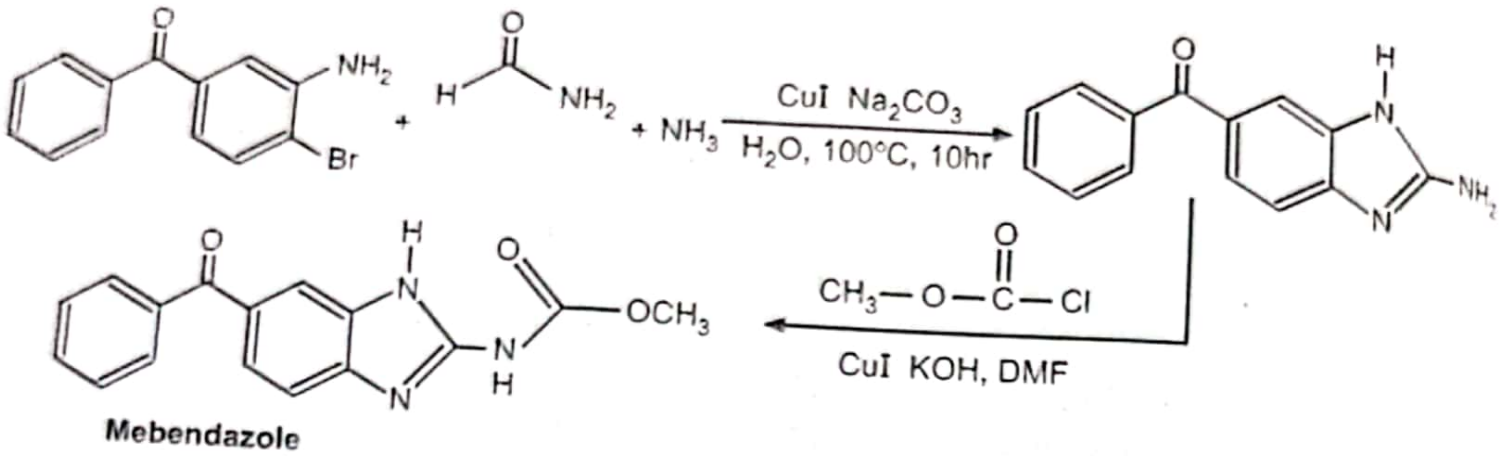


(xvii) Praziquantel: Approved for medical use in 1982, praziquantel is used in the treatment of schistosomiasis, clonorchiasis, tapeworm infections, hydatid diseases and other fluke infections. The drug induces contraction of parasites by increasing the permeability of the membranes of schistosome cells towards calcium ions.

(xviii) Ivermectin: Approved for medical used in 1981, ivermectin is used to treat head lice, scabies, trichuriasis and lymphatic filariasis. Common side-effects include red eyes, dry skin and burning skin. Ivermectin is also used as antinematode drug against pinworm infection.

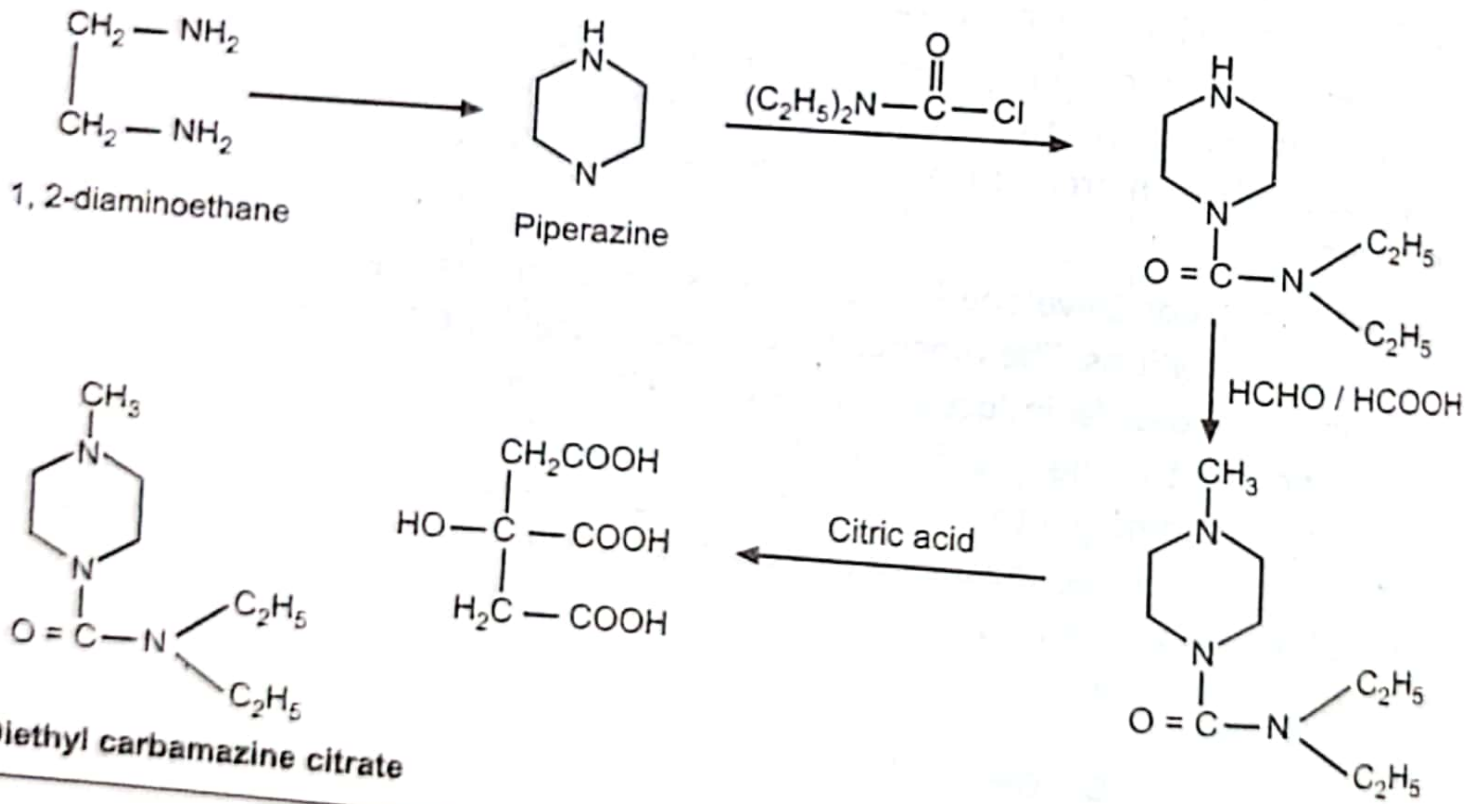
Synthesis

(i) Mebendazole:



Mebendazole

(ii) Diethyl Carbamazine Citrate:



Diethyl carbamazine citrate

Chapter...10

ANTIPROTOZOAL AGENTS

♦ SYNOPSIS ♦

10.1 AMOEBIASIS

Amoeba is a shapeless moving mass of protoplasm which usually measures 20 - 40 µm. It contains a nucleus and food vacuoles in a granular endoplasm, surrounded by a clear ectoplasm. The food vacuoles may contain red cells.

Amoebiasis is an infection of the mucous membrane of the large intestine where Entamoeba histolytica is the causative organism. This organism occurs in the intestine in the form of trophozoites and cysts. In most infections, trophozoites (vegetative form) appear to feed on intestinal bacterial flora and multiply in the colonic lumen without causing any symptoms. But under certain circumstances, trophozoites may get activated and invade the intestinal mucosa, causing tissue lysis and producing dysentery or diarrhoea. Under unfavourable atmosphere however, trophozoites are encysted and the cyst form is excreted in the faeces. In majority of cases, the infected persons do not show any symptoms and excrete both cysts and trophozoites in the faeces. The cyst form of the organism is ineffective and the infection is acquired by ingestion of amebic cysts in food or water contaminated due to handling by such asymptomatic infected person. Ingested cysts liberate trophozoites in the intestine and continue the process in the new host. The infection usually prevails in the areas of poor hygienic conditions and inadequate sanitation.

Amoebic dysentery: *Amoeba can cause attacks of acute dysentery which is characterized by presence of blood and mucous in the stools and severe abdominal pain. This occurs when the amoebae invade the wall of intestine, multiply and cause tissue damage, often forming layer, ulcers. Later amoebae may invade the blood vessels and be carried to the liver and even to brain. Death can occur from liver abscess which can perforate into the lung and elsewhere. Of the several species of amoebae that occur in the human intestinal tract, only Entamoeba histolytica is pathogenic.*

Invasive intestinal amoebiasis may vary in its symptoms from a mild illness to severe amoebic dysentery with blood and mucuos in the stool. Trophozoites may spread to the liver through portal vein and produce either an acute amoebic hepatitis or may encyst and produce amoebic liver abscess. The latter may be complicated by rupture or extension of infection to adjacent organs. It may be associated with chronic colonic dysfunction, acute colonic perforation, insidious peritonitis and amoebic granuloma.

Amoebic abscess of the liver is the most common extra-intestinal manifestation of invasive amoebiasis. It may be characterized by abdominal pain, anorexia, fever, weight loss and hepatomegaly. In the mild amoebiasis, the symptoms are mainly of intestinal origin which appear due to invasion of the intestinal wall by the multiplying trophozoites. In more severe form other vital organs like liver, lungs, brain, and genitourinary tract may get affected. This constitutes the cause of extra-intestinal symptoms.

10.2 CHEMOTHERAPY

Various drugs used in the treatment of amoebiasis can be categorised on the basis of their sites of actions. For example,

(i) Luminal amoebicides:

These drugs are effective against the organism present in the bowel lumen. Most of them owe their effectiveness due to their poor oral absorption. This helps the drug to stay more time in the intestine. They are highly effective in eliminating cysts in asymptomatic carriers. Examples include, diloxanide furoate, iodoquinol, clioquinol etc.

Pentamidine Isethionate: The term isethionates refers to a group of esters of long chain aliphatic carboxylic acids ($C_8 - C_{18}$) with isethionic acid (2-hydroxyethane sulfonic acid) or salts there of such as ammonium or sodium isethionate. They have good detergent activity.

Pentamidine isethionate, an aromatic diamidine, is an antiprotozoal agent effective against Pneumocystis carinii pneumonia. It may be given in combination with co-trimoxazole.

(ii) Luminal trophozoitocidal agents:

These agents mainly attack intestinal trophozoites and are effectively used to treat invasive intestinal amoebiasis. Examples include metronidazole, tinidazole, paromomycin, tetracycline and erythromycin.

(iii) Systemic amoebicides:

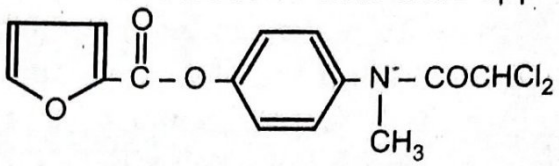
As the name indicates, these drugs are not acting locally in the intestine. When trophozoites spread into liver, brain or lungs, these drugs may be used to treat extra-intestinal manifestations of invasive amoebiasis. Examples include metronidazole, tinidazole, chloroquine and dehydroemetine. Metronidazole and tinidazole thus not only eliminate trophozoites present in the intestine but are also effective at extra-intestinal sites.

In addition to the chemotherapeutic measures, the large liver abscesses may be aspirated percutaneously for better results.

(a) Diloxanide furoate:

It is a cheap, relatively non-toxic dichloroacetamide derivative, mainly used in th treatment of chronic amoebiasis. It is less effective in the treatment of acute intestin

amoebiasis. Studies of diloxanide ester showed that, in the form of furoate, the drug is less soluble and hence more effective locally. This ester undergoes hydrolysis in upper intestine to release diloxanide which is then absorbed. Major amount of drug is excreted in the urine in the form of its inactive glucuronide. About 4 - 10% dose appears unchanged in the faeces.



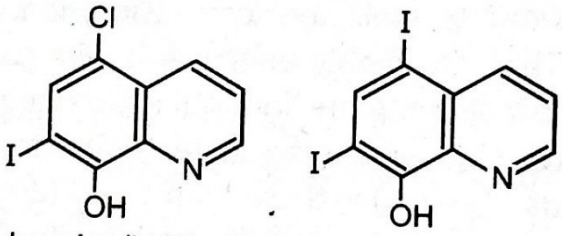
Diloxamine furoate

Adverse effects include vomiting, abdominal cramps, flatulence, diarrhoea, urticaria, and pruritus. It is contraindicated during pregnancy and in children under 2 years of age.

Little is known about its mechanism of action. It appears to interfere with protein synthesis and with the activity of some essential enzymes of protozoa. It is used in the treatment of cyst-passing patients. In the treatment of extraintestinal amoebiasis, it may be used along with a systemic amoebicidal agent.

Teclozan and etofamide are other effective amoebicidal members of dichloroacetamide series.

(b) 8-Hydroxyquinolines:



Diiodohydroxyquin (iodoquinol), iodochlorhydroxyquin (clioquinol), dibromoquin, chiniofon and chloroquinaldol are examples of halogenated derivatives of 8-hydroxyquinoline having luminal amoebicidal activity.

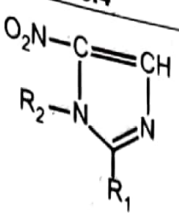
They are used alone in the treatment of chronic intestinal amoebiasis. In acute amoebiasis they are generally used in combination with either emetine, metronidazole or carbarsone. Since the drug therapy increases plasma iodine levels, these agents must be used with caution in patients hypersensitive to iodine or with thyroid dysfunction. In long term therapy, these agents are reported to cause optic neuritis and may prove to be dangerous.

Iodoquinol is excreted mainly through the faeces. Adverse effects include nausea, vomiting, diarrhoea, stomach pain, blurred vision, optic atrophy, skin rashes, chills, muscle pain, weakness, headache and peripheral neuropathy.

It kills trophozoites and cysts in intestinal tract by chelating ferrous ions which are essential for the protozoal metabolism. As a luminal amoebicide, it is used in the dose of 2 g daily for 20 days.

(c) Nitroimidazole derivatives:

First report of study on nitroimidazole derivatives appeared in 1955 by Nakamura and coworkers. Four such active members of the series include, metronidazole, tinidazole, timorazole and ornidazole.



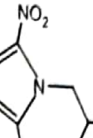
(i) Metronidazole

$R_1 = -CH_3$; $R_2 = -CH_2CH_2OH$

(ii) Tinidazole

$R_1 = -CH_3$; $R_2 = -CH_2CH_2SO_2C_2H_5$

(iii) Timorazole

$R_1 = H$; $R_2 = -CH_2CH_2N$ 

Ornidazole

Chemically ornidazole is a nitroimidazole derivative. It is an antibiotic highly effective against anaerobic micro organisms and parasitic infections. It is used to treat protozoan infections. It is an effective drug to treat both, intestinal and hepatic amoebiosis.

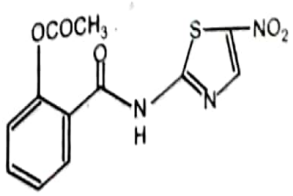
Metronidazole was introduced in 1959 for the systemic treatment of trichomonal infections of urinogenital tract. It has exhibited a high degree of activity against amoebiasis, trichomoniasis, giardiasis, balantidiasis and dracunculiasis. It has been found to possess extremely broad spectrum of both, antibacterial and antiprotozoal activities.

Its effectiveness is due to the presence of nitro group which participates in the endogenous reduction reaction as an electron acceptor. Since it has a redox potential lower than the protein, ferredoxin, which is found in anaerobic organisms, its nitro group is reduced. This reduced form of metronidazole then causes interference in the carbohydrate metabolism and nucleic acid synthesis. It also binds with the cytoplasmic proteins of susceptible cells.

Metronidazole is an orally active drug. About 10% of administered dose is bound to the plasma proteins. It has a plasma half-life of 8.5 to 10.0 hours. The 2-hydroxy methyl metabolite and several inactive metabolites appear in the urine in the form of their glucoronides. Adverse effects include nausea, vomiting, anorexia, metallic taste, stomatitis, glossitis, vertigo, dizziness, flushing, neutropenia and thromboplebitis.

It is effectively used in the treatment of amoebiasis, giardiasis and trichomoniasis. However metronidazole alone, may not always eradicate the intestinal phase. Hence, it should be given along with diloxanide (a luminal amoebicide) in order to prevent recurrence. It is also effective against a number of anaerobic bacteria (e.g., Bacteroides, Eubacterium, Clostridium, Fusobacterium, Peptococcus etc.). It may sometimes be used with other antibiotics to control the infections caused by aerobic microorganisms. It is contraindicated in patients with blood dyscrasias and with alcoholic drinks (because of its disulfiram like effects). In children, a tasteless oral suspension of benzoyl metronidazole is usually preferred.

Nitazoxanide: It is a synthetic nitrothiazolyl salicylamide derivative used as antiprotozoal agent.



(d) Antibiotics:

Tetracycline, chlortetracycline, oxytetracycline, erythromycin and paromomycin are the examples of antibiotics which can be used in the treatment of amoebiasis. Since amoebicidal action is not their main activity, they are usually used in combination with other principal amoebicidal agents in the treatment of mild to severe stages of intestinal amoebiasis.

Most of the trophozoites present in the lumen, feed on the intestinal bacterial flora and then multiply. Except paromomycin, rest of the antibiotics exert an indirect trophozoitocidal action by destroying the enteric bacteria necessary for amoebic proliferation. Paromomycin is an aminoglycoside antibiotic obtained from *Streptomyces rimosus*. It has a direct effect on the amoebae present in the lumen. It interferes in the protozoal DNA and RNA synthesis and it is generally used as a supplementary therapy in amoebiasis and in the treatment of various tapeworm infections. Adverse effects include diarrhoea and anorexia.

(e) Carbarsone:

It is an example of organic arsenicals. It may be used in combination with other amoebicides in the treatment of both acute and chronic intestinal amoebiasis. They are of no value in the treatment of systemic amoebiasis. Examples of drugs include carbarsone, glycobiarsol (a bismuth salt of phenylarsonic acid) and diphetarsone. They are effective antiamoebic agents due to the presence of arsenic in their structures. Arsenic exerts amoebicidal effect by non-specifically inactivating the enzymes containing sulfhydryl groups. They are less favoured due to severe toxicity associated with their use.

(f) Chloroquine:

It is an effective agent in the treatment of systemic amoebiasis. It is used only in the cases where other drugs either fail or are contraindicated. It is highly effective drug when used alongwith quinine, in the treatment of hepatic amoebic abscesses or amoebic hepatitis because of its preferential localization in the liver. To achieve complete cure it is given along with intestinal amoebicide.

It is not much effective in the treatment of colonic amoebiasis in which its only function is to prevent development of liver abscesses.

(g) Ipecacuanha alkaloids:

These include emetine and dehydroemetine. The latter is a synthetic analog of emetine, an alkaloid obtained from the roots of ipecac plant (Cephaelis ipecacuanha). Emetine is highly effective agent in systemic amoebiasis but it fails to act as luminal amoebicide because of poor concentration of the drug in that area. However it may be used as luminal amoebicide in the form of emetine bismuth iodide which contains about 25% anhydrous emetine and 20% bismuth. It releases emetine slowly in the intestinal lumen.

Dehydroemetine is a better chemotherapeutic agent than emetine. Both are more effective against motile trophozoites than cysts. They affect protein synthesis by inhibiting the translocation of peptidyl-t RNA on ribosomes resulting in the inhibition of polypeptide chain elongation.

In severe cases of amoebiasis, emetine is used in combination with either chloroquine, tetracycline or paromomycine, while dehydroemetine may be used for rapid relief of symptoms in patients with severe amoebic dysentery. Emetine is not a safe drug. Administration of either drugs need close medical supervision. A number of adverse effects affecting gastrointestinal, neuromuscular, cardiovascular systems are reported with their use. Dehydroemetine is less toxic than emetine.

(h) Miscellaneous agents:

Many compounds exhibit amoebicidal activity when used orally. Important examples of such clinically used agents include chlorbetamide, chlorphenoxamine, chlorphenoxamine ethyl ether, phanquone and teclozan. These agents are used as luminal amoebicides.

10.3 LEISHMANIASIS

This infection is transmitted to the humans by the bites of infected female flies which are pre-infected by biting the cats, dogs or rodents, the non-human mammalian reservoirs. Depending upon the protozoa involved and the organ affected, leishmaniasis may be of three different types.

(a) *Visceral leishmaniasis*:

It is caused by *Leishmania donovani*. This protozoa parasitizes the reticuloendothelial cells that results into an enlargement of lymph nodes, liver and spleen. The main symptoms of the disease include fever, dysentery, severe anemia and the spleen becomes massive.

(b) *Cutaneous leishmaniasis*:

It is caused by *Leishmania braziliensis* and involves ulceration of skin and formation of skin lesions. It is a form of localized and non-systemic infection.

(c) *American leishmaniasis*:

It is caused by *Leishmania tropica*. The infection is characterized by the ulceration of mucous membranes of nose, mouth and pharynx. Skin lesions may also appear.

Of all the types, visceral leishmaniasis (kala azar) is the commonly occurring form of infection while other two forms are rare. The occurrence of localized or systemic (kala azar) form of disease depends on the type of the infecting protozoa and the host immunological system.

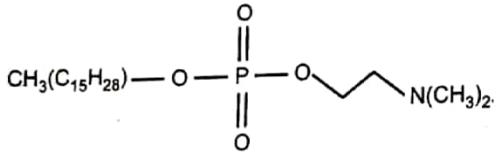
Leishmania species occur intracellularly in the amastigate form in MPS (mononuclear phagocytic system) cells of the host, mainly in those of spleen, liver, bone marrow or lymph nodes in the visceral disease (kala-azar) and mainly in the skin and/or mucous membranes in the cutaneous diseases (tropical sore chiclero' ulcer, etc.).

One cell may contain 10 or more organisms. The cytoplasm of organism may be vacuolated and the organism may occur in vacuoles in cytoplasm of host cell or simply in the continuity of cytoplasm.

Most of the drugs used in the treatment of leishmaniasis belong to pentavalent antimonial category. These include sodium stibogluconate, urea stibogluconate, ethyl stibamine, dihydroxy stibamidine isethionate and meglumine antimonate (i.e., N-methyl glucamine antimonate). These pentavalent antimonials get *in-vivo* converted to trivalent antimonials. The latter then inhibits phosphofructokinase, an enzyme that catalyses a rate limiting step in glycolysis. Hence, the organisms whose growth is dependent upon the anaerobic metabolism of glucose, can not survive with lack of energy source. Besides this, these agents were also reported to inhibit several enzyme systems of the protozoa. The effectiveness of sodium stibogluconate in the treatment of visceral leishmaniasis is due to its ability to concentrate in liver and spleen.

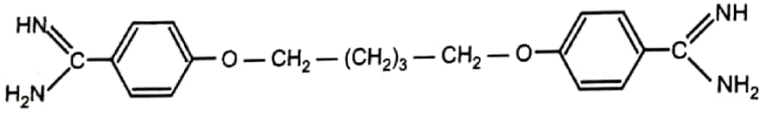
Orginally developed as an antimalarial agent, atovaquone is mainly used against Pneumocystosis infections as an alternative to trimethoprim/ sulfamethoxazole therapy. Due to structural similarity, it acts as an antimetabolite to ubiquinone 6, an essential component of the mitochondrial electron transport chain in microorganisms.

Miltefosine: Originally developed as antineoplastic, it is used now also as an antiprotozoal drug. It is currently the only effective oral treatment for leishmaniasis.

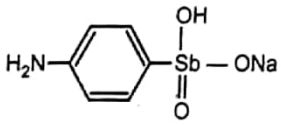


Miltefosine

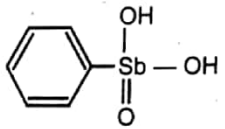
Table 10.1: Clinically used agents in the treatment of leishmaniasis



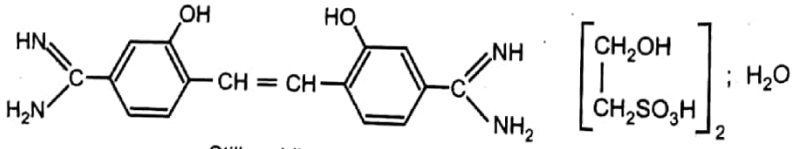
Pentamidine



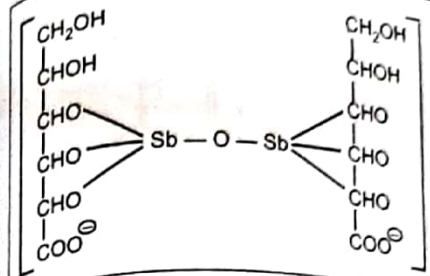
Stibamine



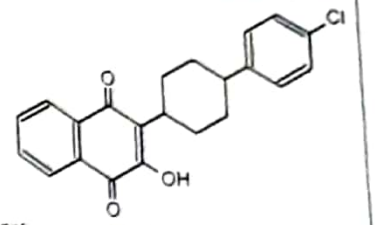
Phenyl stibonic acid



Stilbamidine isethionate



Sodium stibogluconate



Atovaquone

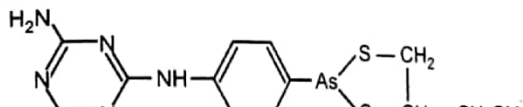
Pentamidine isethionate is a poor orally absorbed aromatic diamidine having antiprotozoal and fungicidal activities. About 80% of administered dose is bound to the plasma proteins. It is excreted unchanged in the urine at much slow rate.

Adverse effects include nausea, vomiting, anorexia, headache, fever, rash, pancreatitis, leukopenia, thrombocytopenia, dizziness, confusion, hypotension and hypoglycemia.

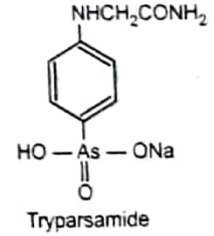
It is used as a prophylactic agent in systemic blastomycosis, trypanosomiasis, cutaneous leishmaniasis and in pneumonia due to *P. carinii*. The latter infection may be associated with acquired immuno deficiency syndrome (AIDS).

Amphotericin B is also reported to be effective in the cases of leishmaniasis, not responding to other drugs.

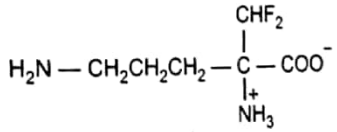
Table 10.2: Clinically used agents in the treatment of Trypanosomiasis



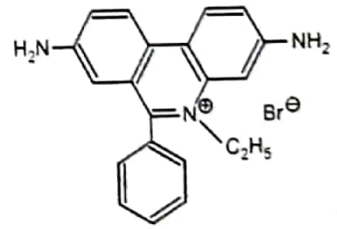
Melarsoprol



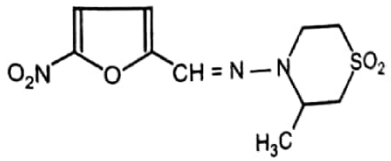
Tryparsamide



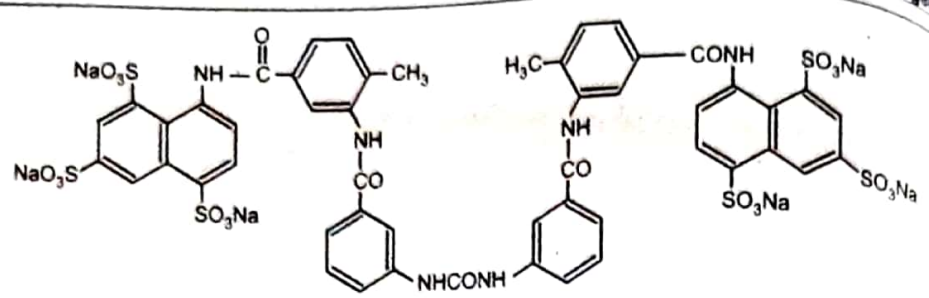
Eflornithine



Ethidium bromide



Nifurtimox



Suramin sodium

10.4 TRYPANOSOMIASIS

Trypanosomes are mobile protozoan parasites that require two hosts including man (vertebrate) and insect (invertebrate). The parasites may be found in the blood and spinal fluid of the infected person. Some trypanosomes are non-pathogenic and live silently in the body of the host. Trypanosomiasis is of two types based upon the species of trypanosomes involved.

(a) African trypanosomiasis:

It is caused by the bite of the fly belonging to Glossina species. The infective protozoa is either *T. gambiense* or *T. rhodesiense*. The disease caused is known as sleeping sickness which is characterised by fever, headache, lymph node enlargement, drowsiness, lethargy, weakness and mental disturbances. Since, the protozoan enter into the cerebrospinal fluid and CNS, the signs of mental disturbances are seen.

(b) American trypanosomiasis (Chagas disease):

It is caused by *T. cruzi* and is transmitted by kissing bugs.

(i) Suramin sodium:

It is a non-metallic dye derivative having trypanocidal activity. Freshly prepared solution should always be used for I.V. administration. About 99% of administered dose is bound to the plasma-proteins. It has a plasma half life of 48 - 49 days. It does not undergo metabolism and is excreted slowly in urine in unchanged form over a period of months. Since, a single injection provides adequate blood concentration for several months, it can be used in both, prophylaxis and treatment of *African trypanosomiasis*.

Because of its anionic nature, it binds with the cationic sites present in proteins and enzymes in glycolytic pathways and inhibits their functioning. Protozoa may die due to the lack of energy source. It does not readily penetrate mammalian cells. This explains its selectivity of action. It is effective in treatment of onchocerciasis either alone or along with arsenicals and is also effective in the prophylaxis of Rhodesian and Gambian trypanosomiasis.

(ii) Melarsoprol:

It is a dimercaprol derivative of melarsen oxide used as antiprotozoal agent. Trimelarsan is a water soluble derivative of melarsoprol which may be used intra-muscularly. The activity is due to the arsenic content which inhibits the essential sulfhydryl group containing enzymes. This inhibitory action of arsenic is of non-specific and non-selective nature which explains the severe adverse effects associated with the use of this drug. Drug-resistant strain of protozoa are less permeable to the drug.

It is marketed as a 3.6% w/v sterile-solution in propylene glycol. It is used to treat *African trypanosomiasis* involving CNS symptoms.

(iii) Tryparsamide:

It is a pentavalent arsenical, once used in the treatment of advance cases of trypanosomiasis. It contains about 25% antimony and can be used along with suramin in the treatment of West African trypanosomiasis. Because of drug-induced optic nerve damage, it is a less preferred agent.

(iv) Nifurtimox:

It is a nitrofuran derivative, effective specifically against *T. cruzi* infections and is an orally effective drug. It has a plasma half life of about 8 hours. Inactive metabolites appear in the urine along with 5% dose in unchanged form.

Adverse effects include nausea, vomiting, headache, weight loss, euphoria, tremors, insomnia, drowsiness, psychic disturbances and peripheral neuropathy.

The nitro group of nifurtimox is converted to nitro anion radical in the presence of pyridine nucleotides. This anion produces superoxide by reaction with molecular oxygen, resulting into regeneration of nifurtimox. This superoxide then may interfere with the synthesis of proteins and in the functioning of protozoal enzymes.

Nitrofurazone is a topical antibacterial agent effective against many gram-positive and gram-negative bacteria and some protozoa. It is used to treat special cases of American trypanosomiasis which are resistant to other drugs. The adult oral dose in the treatment of trypanosomiasis is 500 mg daily for 3 days and then 500 mg every 8 hours for a week. This dose schedule may be repeated thrice with a week's interval each time.

Eflornithine acts by inhibiting ornithine decarboxylase, an enzyme responsible for biosynthesis of polyamines essential for the regulation of DNA synthesis and cell proliferation in microorganisms. It is used to treat African trypanosomiasis.

10.5 TRICHOMONIASIS

Trichomonas are unicellular, flagellated protozoal parasites. Most of them are non-pathogenic in nature. In humans, the pathogenic parasites reside in the urinogenital tract. The pathogenic species include *Trichomonas vaginalis* in man and *T. foetus* in cattles.

Males are asymptomatic carriers but female often develop severe vaginitis and cervicitis. *T. vaginalis* infection in male appears as a symptom-free urethritis while in female, it occurs as vaginitis which is characterised by a frothy pale yellow discharge. Age of the female, pH at vaginal region and period of her menstrual cycle are some of the factors that affect her susceptibility to the infection. Since the transmission of disease is effected due to sexual contacts, the male sexual partner should be treated simultaneously to prevent occurrence of relapses in the female.

Aminitrozole, niridazole, acetarsol, furazolidone and nifurtimox are the agents which can be used in the treatment of vaginitis. Alongwith these agents, metronidazole, nimorazole, tinidazole, arsenicals, 8-hydroxyquinolines and certain polyene antibiotics (e.g, pimaricin and hachimycin) can also be used. A single dose of 2 g of either metronidazole or tinidazole has been shown to be effective, if the patient is not reinfected.

10.6 GIARDIASIS

Giardiasis is an intestinal protozoal infection caused by Giardia lamblia. Unhygienic conditions, low socio-economic status and homosexuality are some factors that contribute into the spread of giardiasis. The asymptomatic patients may release protozoal parasites in the form of cysts in the faeces. Transmission then occurs after ingestion of cysts in contaminated food or water. The main symptoms include diarrhoea, anorexia, bloating, flatulence and weight loss.

Though all amoebicidal drugs can be used in the treatment of giardiasis, metronidazole, tinidazole and quinacrine are more preferred agents.

(a) Quinacrine:

Though it is no longer used in the prophylaxis and treatment of malaria still it is used as an important agent in the treatment of giardiasis. It is an orally active, long acting acridine derivative which is very slowly excreted in the urine. The metabolic pathways for quinacrine are poorly understood.

Adverse effects include nausea, vomiting, anorexia, diarrhoea, headache, ocular toxicity, discoloration of nails, dizziness, anxiety, restlessness, blood dyscrasias and psychosis. It is contraindicated during pregnancy and in patients receiving antimalarial therapy with primaquine.

It binds to DNA through an intercalation mechanism, and strongly inhibits DNA replication and RNA transcription process. It also interferes with protein synthesis and functioning of various enzyme systems. It is used as anticonvulsant agent in certain cases. It exerts quinidine like effects on the heart.

It is a drug of choice in the treatment of giardiasis and is used orally in dose of 100 mg three times a day for 5 - 7 days. The dose-schedule can be repeated, if necessary about 2 weeks later. Besides this furazolidone and paromomycin can also be used in the treatment of giardiasis.

10.7 TOXOPLASMOSIS

It is caused by the ingestion of oocysts of *T. plasma gondii* from the faeces of infected cats or ingestion of cysts affected raw meat. Active ocular toxoplasmosis, systemic toxoplasmosis and congenital toxoplasmosis (in newborns) are the important types of this infection.

Drugs like pyrimethamine, sulfadiazine, and spiramycin (a macrolide antibiotic) can be used alone or in combination with corticosteroids in the treatment of infection. Folinic acid (Leukovorin calcium) may be concomittantly administered alongwith pyrimethamine to minimize the adverse effects of pyrimethamine on folic acid metabolism.

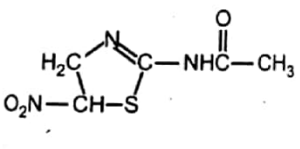
10.8 BALANTIDIASIS

This intestinal infection is caused by *Balantidium coli*, which is normally a parasite of pigs. Trophozoites may induce either a superficial necrosis or a deep ulceration in mucosa and submucosa of large intestine. This results into a variety of symptoms including nausea, vomiting, abdominal pain, diarrhoea or severe dysentery.

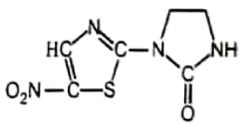
The infection can be treated by giving tetracycline, 500 mg four times daily for 10 days. Iodoquinol, 650 mg 3 times a day is also effective in the treatment of balantidiasis.

Other pathogenic intestinal protozoan infections include isosporiasis (*Isospora belli* and *I. hominis*) and *Pneumocystis carinii* infection. Both are treated with either pyrimethamine-sulphonamide or co-trimoxazole combination preparations. Pentamidine can be given intramuscularly to control pneumocystosis.

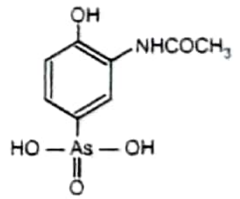
ANTITRYPANOSOMAL DRUGS



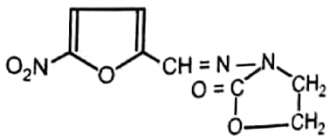
Aminitrozole



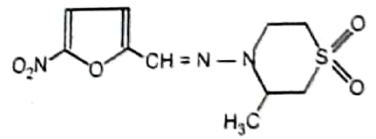
Niridazole



Acetarsol

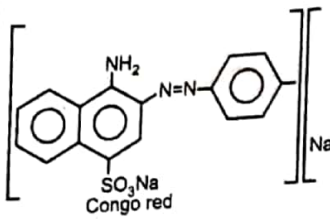


Furazolidone

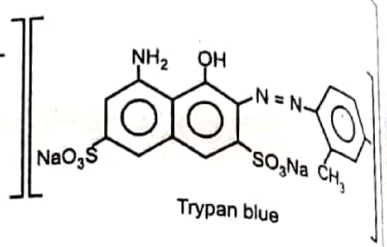


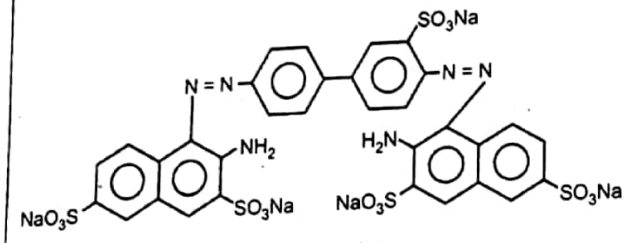
Nifurtimox

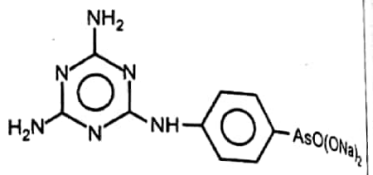
ANTITRYPANOSOMAL DRUGS

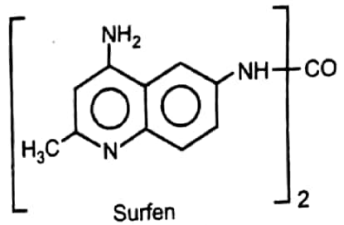

Congo red

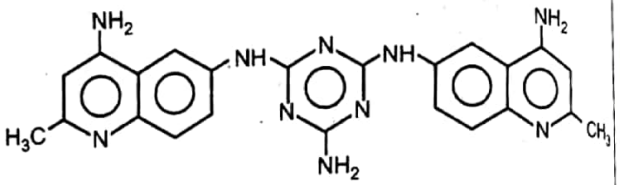

Benzopurpurin

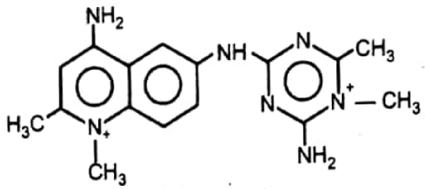

Trypan blue

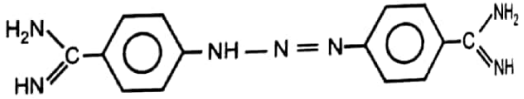

Trypan red

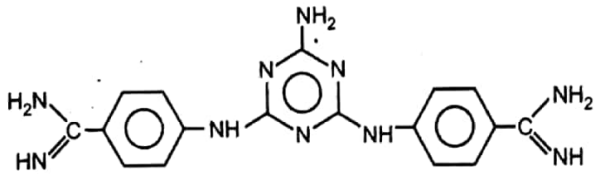

Melarsen sodium

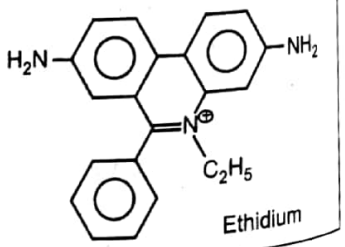

Surfen

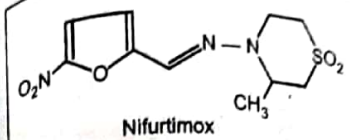

Surfen C

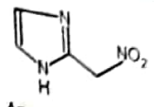

Quinapyramine

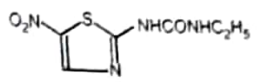

Diminazene

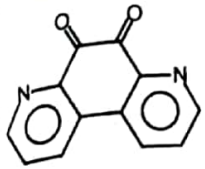

Diamidine-type antitrypanosomal drug

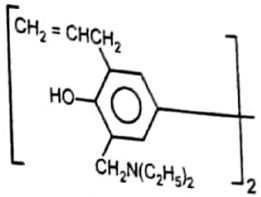

Ethidium

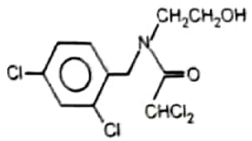

Nifurtimox


Azomycine


Nithiazide

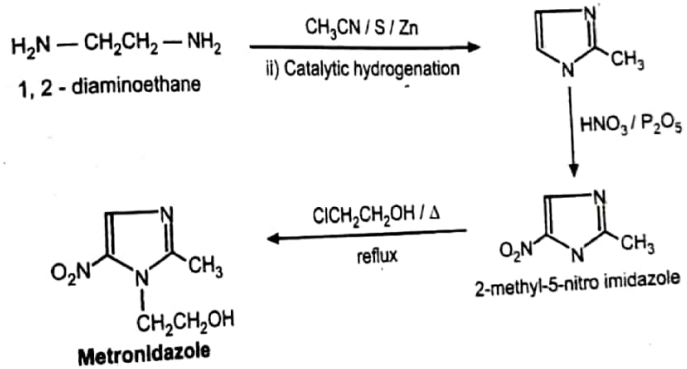

Phanquone


Bialamicol


Chlorbetamide

Synthesis

(i) Metronidazole:



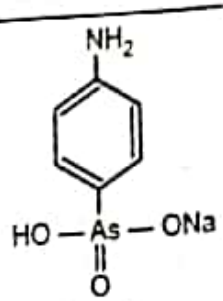
Chapter...12

SULPHONAMIDES AND SULFONES

♦ SYNOPSIS ♦

12.1 INTRODUCTION

The modern chemotherapy and the concept of prodrug was successfully utilized with the introduction of sulphonamides. They were the first effective chemotherapeutic agents to be employed systemically.



Atoxyl

Paul Ehrlich (1854 - 1915) is regarded as the father of modern chemotherapy due to his pioneering work in this field. He synthesized and successfully used Atoxyl in the treatment of sleeping sickness.

The next major achievement in the field of chemotherapy is credited to Gerhard Domagk, a research director of Bayer laboratories, who in 1932, recognized the antibacterial activity of an azodye, Prontosil Red. It was found to be effective in the treatment of hemolytic streptococcal infections in the mice. For this work, he was awarded Nobel prize in Medicine in 1938. Though synthesized first in 1908, sulphonamides did not receive much attention till 1937 when it was proved by some workers at Pasteur Institute in France that prontosil is a prodrug and the active drug, sulfanilamide gets released into the body after *in-vivo* cleavage of the azo linkage.

This discovery led to the synthesis of atleast 5500 congeneric substances which are known as 'sulphonamides'. However, only few of them have retained the place in therapeutics. Sulphonamides are all white crystalline powders, mostly poorly soluble in water. Their sodium salts are usually used because of aqueous solubility. The solubility parameter is greatly influenced upon by the nature of the substituents on – SO_2NH_2 group. These substituents modify the chemical features of the molecule. Hence, they play an important role in governing the rates of absorption and excretion of sulphonamides.

The term sulphonamide is usually employed as a generic name for the derivatives of para-amino benzene sulphonamides. Sulphonamides are bacteriostatic agents but in the rare circumstances where bacteria are exposed to thymineless medium, they may act as bactericidal. Sulfanilamide is the basic skeleton of this category. The successful exploitation of this lead nucleus opened up new avenues in the field of chemotherapy. Presence of free amino group ($-NH_2$) is essential for antibacterial activity. However, it can be replaced only by groups which can be reconverted in body to free amino group. Examples include acetamido ($-NHCOCH_3$); succinylamido and phthalylamido groups. The compounds in which these groups are present, may undergo metabolism to regenerate free amino functional group.

Pharmacologically, all sulphonamides exert similar actions. However, they differ from one another in solubility, rates of absorption, distribution, metabolism and excretion and in protein binding behaviour. These differences served as the basis of their clinical classification. For example, relatively insoluble sulphonamides largely remain unabsorbed in GIT after oral administration. Hence, such agents may be of value in the treatment of GIT-infections, while the sulphonamides with rapid excretion feature may be used in the treatment of urinary tract infections.

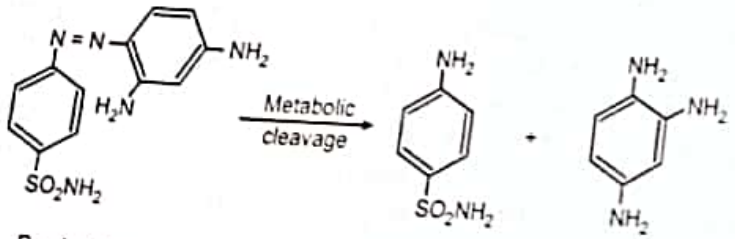
Sulphonamides are less potent antibacterials than most antibiotics. Their antibacterial potential gradually drops by the presence of pus, tissue fluids and such drugs which contain para amino benzoic acid (PABA) as a basic skeleton (e.g., local anaesthetics). Moreover many staphylococci, enterococci, clostridia and pseudomonas species remain highly resistant to the sulphonamide action. The popularity of sulfonamides as antibacterial agents declined after 1945 because of

(a) Publications of reports regarding sulphonamide toxicity in some patients.

(b) Development of sulphonamide-resistant bacterial strains, and

(c) Introduction of clinically more effective antibiotics.

However, the impression as the 'wonder drugs' created by penicillins in their early days unfortunately could not be maintained. Many factors contributed for their clinical devaluation. Acid-instability and microbial resistance were important amongst such factors. Attempts to synthesize new sulphonamides with improved qualities thus began in 1957 after realising clinical deficiencies associated with the use of antibiotics. The high clinical merits ssociated with the combination of trimethoprim and sulphamethoxazole reawakened the terest in sulphonamides. Many are still employed in the treatment of various bacterial, otozoal and viral infections.

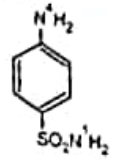


Prontosil red

Sulphanilamide

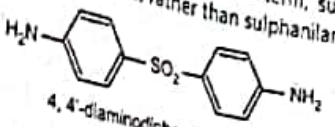
12.2 NOMENCLATURE AND CLASSIFICATION

Sulphonamides can be considered as the derivatives of para-amino benzene sulphonamide (i.e., sulphanilamide) skeleton. Since the sulphonamide (SO_2NH_2) group is the most important moiety for the antibacterial activity of sulphonamides, the amide nitrogen is designated as N^1 while nitrogen of para amino functional group is designated as N^4. Most of the clinically used sulphonamides belong to the class of N^1-substituted sulphonamides.



Sulphanilamide

A third but separate category is described by the term, sulphones where the basic nucleus is 4, 4' - diaminodiphenyl sulphone, rather than sulphanilamide.



4, 4'-diaminodiphenyl sulphone

Sulphonamides can be classified in various ways. Many criteria may be utilized as the basis of their classification. These include:

(a) On the basis of pharmacokinetic properties, they may be classified as:

(i) Agents which are rapidly absorbed and are rapidly excreted: They are also known as systemic sulphonamides. Examples include: sulphamethoxazole, sulfisoxazole, sulfapyridine, sulphadiazine.

(ii) Agents which are poorly absorbed in GIT: They are known as locally acting drugs. Their use is intended to exert local antibacterial effect in bowel lumen either to treat specific intestinal disease or to reduce luminal bacterial population prior to bowel surgery. This helps to reduce the changes of postoperative wound infection after colon surgery. Examples include sulphasalazine, phthalylsulfathiazole etc.

(iii) Agents which are employed topically: They are applied only in conjunctival sac, otic canal and vagina to treat bacterial infections. They may also be used topically to abolish bacterial colonization of burns. Examples include mafenide, sodium sulphacetamide and silver sulphadiazine.

(b) On the basis of chemical nature, they may be classified as:

(i) Agents carrying substituents on amino group: These are N^4-substituted sulphonamides which undergo metabolism in body to release free amino group. Hence, they may be considered as prodrugs. Examples include, prontosil, solucopticin.

(ii) Agents having substituents on amido group: These are N^1-substituted sulphonamides. Most of the clinically used sulphonamides belong to this category. Examples include, sulphadiazine, sulphacetamide, sulphadimidine etc.

(iii) Agents having substituents at both, amino and amido nitrogens: They are also known as N^1, N^4-disubstituted sulphonamides. Examples include succinyl sulphathiazole, phthalyl sulphathiazole, etc.

(iv) Agents missing the amino functional group from the benzene nucleus: They are also known as non-anilino sulphonamides. Example include - mafenide.

(c) On the basis of pharmacological activity, sulphonamides can be categorized as:

(i) Antibacterial agents: *Examples include sulphacetamide, sulphadiazine, sulphisoxazole etc.*

(ii) Oral hypoglycemic agents: *Example include, tolbutamide.*

(iii) Diuretics: *Examples include, furosemide chlorthalidone, bumetanide etc.*

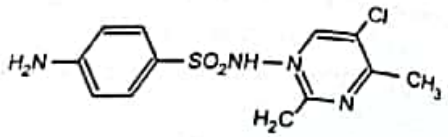
(d) On the basis of duration of action sulphonamides can be classified as:

(i) Long-acting sulphonamides: *They have plasma half-life greater than 24 hours. They have a greater ability to cause hyper-sensitivity reactions. Examples include, sulfamethoxypyridazine, sulphamethoxy diazine, sulfadimethoxine.*

(ii) Intermediate - acting sulphonamides *They have plasma half-life between 10 - 24 hours. Examples include sulphasomizole, sulphamethoxazole.*

(iii) Short-acting sulphonamides: *They have plasma half-life less than 10 hours. Examples include sulphamethizole, sulphasomidine, sulfisoxazole etc.*

(iv) Ultra-long-acting sulphonamides: *These agents have plasma half-life greater than 50 hours. Examples include sulfalene, sulpho-rmethoxine, sulphasalazine, sulfa-methopyrazine, sulfadoxine. (plasma half-life = 150 hours). These agents should never be used in patients with renal insufficiency.*



Sulphaclomide

Recently, a new broad spectrum sulpha drug, sulphaclomide has been introduced. It is found to achieve higher serum level than all presently available sulpha drugs.

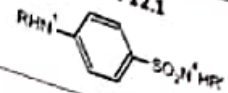
12.3 PHARMACOKINETIC FEATURES OF SULPHONAMIDES

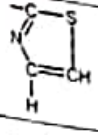
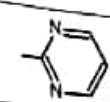
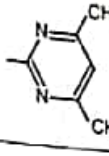
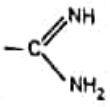
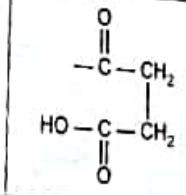
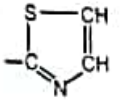
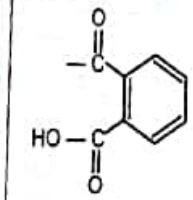
All sulphonamides in systemic use are well absorbed primarily in small intestine. Upon absorption, they are widely distributed to all organs and to pleural, peritoneal and articular body fluids. They can also cross placental barrier. They are also found to appear in erebrospinal fluid. These agents vary in their ability to bind with plasma-proteins. or example, sulphadiazine is poorly bound (20%) while sulphamerazine is 85% bound to the sma-proteins. Usually acetylated derivatives are extensively bound to plasma-proteins. tein binding of sulphonamides is directly proportional to plasma albumin concentration. ce, renal adverse effects are more pronounced in patients having hypoalbuminea due to er concentration of free drug in the plasma.

ne main metabolic pathways for sulphonamides include acetylation and oxidation. ated metabolites do not retain antibacterial activity. They are usually more toxic and ter-soluble than the parent drugs. The extent of acetylation for any sulphonamide is onal to the duration of stay of that agent in the body.

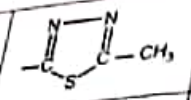
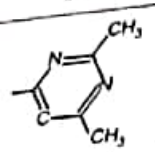
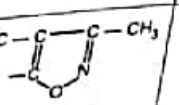
of the metabolites and active drugs are excreted in the urine in free or ated form. Some loss may also occur in sweat, tears, saliva, milk and faeces. des accumulate in the patients suffering renal failure.

Table 12.1

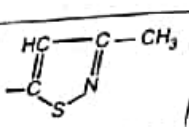
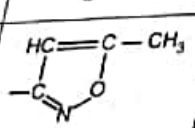


Name	R	R'	Features
1. Sulphapyridine	H		Toxic, not used
2. Sulphathiazole	H		Toxic, not used
3. Sulphacetamide	H	$-COCH_3$	Ophthalmological use
4. Sulphadiazine	H		Used in meningitis
5. Sulphadimidine	H		
(A) COMPOUNDS USED IN GIT INFECTIONS			
1. Sulphaguanidine	H		
2. Succinylsulphathiazole			
3. Phthalylsulphathiazole		same as above	

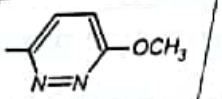
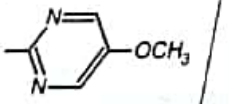
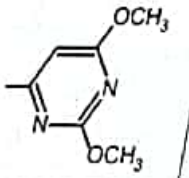
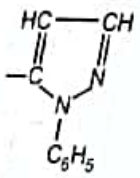
(B) SHORT ACTING SULPHA DRUGS

1. Sulphamethizole	H		Used in Urinary tract infections
2. Sulphasomidine	H		
3. Sulfisoxazole	H		Used in Urinary tract infections

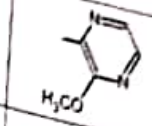
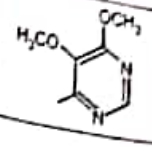
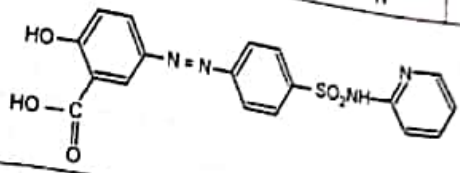
(C) INTERMEDIATE ACTING SULPHA DRUGS

1. Sulphasomizole	H		
2. Sulphamethoxazole	H		

(D) LONG ACTING SULPHA DRUGS

1. Sulphamethoxy pyridazine	H		
2. Sulphamethoxydiazine	H		
3. Sulphadimethoxine	H		
Sulphaphenazole	H		

(E) EXTRA LONG ACTING SULPHA DRUGS

1. Sulfalene	H		
2. Sulfadimethoxine	H		
3. Sulfasalazine			

12.4 SAR STUDIES OF SULPHONAMIDES

Sulphonamide being an important chemical class, several thousand sulphonamides had been investigated for their activity on infective organisms. In antibacterial therapy, they are placed next to antibiotics, sometimes even preferred over the latter. The major features of SAR of sulphonamides include:

(a) Sulphanilamide skeleton is the minimum structural requirement for antibacterial activity.

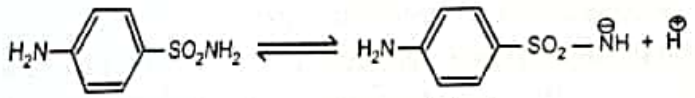
(b) Sulfur atom should be directly linked to the benzene ring.

(c) In N^1-substituted sulphonamides, activity varies with the nature of the substituent at amido group. With substituents, imparting electron rich character to SO_2 group, bacteriostatic activity increases. Heterocyclic substituents lead to highly potent derivatives. Sulphonamides that contain a single benzene ring at N^1-position, are considerably more toxic than heterocyclic ring analogs.

(d) The free aromatic amino group should reside para to the sulphonamido group. Its substitution at ortho or meta position results in compounds devoid of antibacterial activity.

(e) The presence of free amino group is very essential for the activity. Any substitution of amino group either results in prodrug nature or in the loss of activity.

(f) The active form of sulphonamides is the ionized form. Maximum activity is observed between the pKa values 6.6 to 7.4.



(g) Substitutions in the benzene ring of sulphonamides have also been tried. All attempts ended up in the formation of inactive compounds.

(h) Substitution of free sulfonic acid (– SO_3H) group for sulphonamido function, destroys the activity but replacement by a sulfinic acid group (– SO_2H) and acetylation of N_4-position retains the activity.

12.5 ADVERSE EFFECTS

The toxicities of sulphonamides vary considerably and may have little relationship with the dose of the drug. Adverse effects of sulphonamides may be studied as per the organ involved. For example,

(a) Gastro-intestinal tract: Adverse effects include nausea, vomiting, anorexia, diarrhoea, hepatitis etc.

(b) Urinary tract: More effective sulphonamides are usually less soluble in acidic urine. Moreover, acetylated metabolites of most of the sulphonamides are sparingly water-soluble. This leads to the deposition of crystalline aggregates of parent drug and/or its metabolites in the kidney, ureters or bladders. Oliguria, crystalluria and other renal complications may thus result. Such damage can result in epithelial irritability, bleeding and/or complete obstruction of kidneys.

The smooth excretion of drug is possible only when it is water soluble (ionized form). The water solubility of drug can be ensured by adjusting its pKa. Because urine pH is typically about 6, many sulpha drugs are designed to have pKa, closer to the pH of urine, for example, sulfamethoxazole (pKa = 6.1), sulfadiazine (pKa = 6.5) etc.

Crystal formation is dependent upon the solubility of sulphonamide, pH and volume of urine of the patient. Hence, patient undergoing sulphonamide therapy should consume adequate fluid so as to produce atleast 1.5 litres of urine per day. Chances of development of crystalluria may be minimized by

(i) Increasing the fluid intake.

(ii) Keeping the pH of the urine in alkaline range by using alkalinizing agents (e.g., sodium salts of bicarbonate, lactate, acetate or citrate). However, alkalinizing agents also lower down plasma sulphonamide concentration by enhancing its renal excretion.

(c) Nervous system: Effects on nervous system include headache, dizziness, confusion, mental depression, peripheral neuritis (motor and sensory neuropathy) and optic neuritis.

(d) Hematopoietic system: Effects include leukopenia, thrombocytopenia, agranulocytosis and marked decrease in erythrocytes and haemoglobin contents. Sulphonamides cause acute hemolytic anemia in patients with glucose-6 phosphate dehydrogenase enzyme deficiency in their erythrocytes.

(e) Hypersensitivity reactions: These include, skin and mucous membrane eruptions, fever, headache, vascular lesions and serum sickness. Jaundice, hamaturia or sore throat are indications to withdraw the drug immediately.

(f) Effects on fetus and neonate: Sulphonamides compete with bilirubin for the binding sites on plasma proteins (specifically on albumin). As a result, the unbound bilirubin concentration increases in patients under sulphonamide therapy. If sulphonamide is given to pregnant woman, the unbound bilirubin may get deposited in basal ganglia and subthalamic nuclei in CNS of the fetus or newborn, causing kernicterus, a toxic encephalopathy. Hence, it should not be given to newborns or pregnant women.

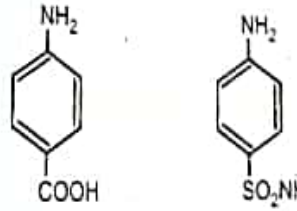
(g) Miscellaneous effects: These include conjunctivitis, porphyria, arthralgia and pulmonary eosinophilia. Sulphonamides also compete for binding sites on plasma albumin with many drugs like aspirin, phenylbutazone, coumarins and methotrexate. Hence, sulphonamides may cause sudden and unexpected rise in the plasma concentration of these drugs by displacing them from plasma-proteins. This results into the appearance of adverse effects characteristic of displaced drug.

12.6 MECHANISM OF ACTION

The therapeutic effect of sulphonamides is achieved by arresting the growth and multiplication of the infectious organism and thus allowing the host to eradicate the infection by its cellular and humoral defense mechanisms. Various folate derivatives like, folinic acid; N_5,N_{10}-methylenetetrahydrofolic acid and N_{10}-formyltetrahydrofolic acid act as coenzymes in transport of one-carbon units in several biochemical reactions in human and microorganisms. These one carbon transfer reactions are necessary for the synthesis of purines, thymidine and some amino acids.

One of the products of these folate dependent biochemical reactions is deoxythymidine monophosphate which is involved in nucleic acid synthesis. Sulphonamides block the biosynthesis of these folate coenzymes resulting into the arrest of bacterial growth and cell division.

Fildes and Woods independently suggested in 1940, that the para-amino benzoic acid (PABA) is essential in the biosynthesis of various folate enzymes and cofactors. The structural similarity with PABA results into competitive inhibition of take-up of PABA in microorganisms by sulphonamides. Sulphonamides inhibit the incorporation of PABA in dihydropteroic acid which is a precursor of folic acid needed for the synthesis of DNA and 1-carbon fragments. Thus, by acting as antimetabolite, sulphonamides prevent the formation of pteroylglutamic acid (PGA) in microorganisms. Mammalian cells can utilize the preformed PGA present in the diet while bacterial cells can not utilise preformed PGA and they have to synthesize PGA from PABA. Hence, sulphonamides do not affect mammalian cells.



PABA Sulphanilamide

The antibacterial action of sulphonamides depends upon:

(i) The form (ionized/unionized) in which they are circulated in the body and

(ii) The dose of sulphonamide. Greater the concentration of drug in the plasma, greater will be the activity.

PABA has much greater affinity for the bacterial enzyme system. Since, sulphonamide activity is based upon the principle of competitive antagonism, it is necessary to maintain always a high concentration of sulpha drug in the tissue to achieve the desired effect. Hence, certain drugs having PABA as the basic skeleton (e.g., procaine) will antagonize the action of sulphonamides *in-vivo*.

Sulphonamides exert only bacteriostatic effect in the body. They possess a wide range of antimicrobial activity against both, gram-positive and gram-negative bacteria. These include, *Mycobacterium tuberculosis, Strep. pneumoniae, Haemophilus influenzae, Corynebacterium diphtheria*, Nocardia, Actinomyces, *E. Coli*, Meningococci, etc. Sulphonamides therefore have applications in the treatment of tonsillitis, septicaemia, pneumonia, meningococcal meningitis, bacillary dysentery and a number of infections of urinary tract.

The conversion of dihydrofolic acid to tetrahydrofolic acid is catalyzed by the enzyme, dihydrofolate reductase. Trimethoprim is a potent and selective competitive inhibitor of this enzyme. It appears to be the most active agent that exerts a synergistic antibacterial activity when combined with a sulpha drug. Combination of sulpha drug with other bacteriostatic agent (e.g., tetracycline) may also give synergistic effect, while in antimalarial therapy, pyrimethamine and sulpha combination yields synergistic effect.

12.7 BACTERIAL RESISTANCE

Wide and non-selective use of sulpha drugs lead to the development of drug-resistant bacterial strains. First seen in *N. gonorrhoeae*, resistance to sulpha drugs then rapidly developed in the variety of staphylococci, hemolytic streptococci, meningococci, pneumococci and shigellae.

The bacterial resistance may be:

(1) Natural (intrinsic) resistance or

(2) Acquired resistance.

Bacterial resistance develops mainly due to mutation process. Bacterial plasmids can cause production of altered enzyme that can bypass the due affinity for sulphonamides. Other possible mechanisms of bacterial resistance include:

(a) an increased production of PABA in the bacterial cell,

(b) an increased ability of bacterial cell to destroy or inactivate the sulpha drug,

(c) production of sulpha drug antagonist by the bacterial cell,

(d) decreased bacterial permeability to sulpha drug,

(e) a production of an altered dihydrofolate reductase.

The development of drug resistance severely limits the therapeutic efficacy of the drug. A microorganism is resistant to one sulphonamide, it develops resistance against all sulphonamides. Similarly, the sulphonamide-sensitive species may develop drug resistance when it comes in contact with a resistant bacterial species. The use of trimethoprim with sulfamethoxazole may reduce development of resistance to sulpha drugs.

12.8 THERAPEUTIC USES

Depending upon the solubility, sulphonamides may be used systemically, topically or may be used orally for local effects. Following are some principal uses of sulphonamides:

1. Uncomplicated urinary tract infections
2. Intestinal infections: e.g., sulphaguanidine
3. Ophthalmic infections: e.g., sulphacetamide
4. Ulcerative colitis: e.g., salicylazosulfapyridine
5. Rheumatic fever: e.g., sulfadiazin, sulfisoxazole.
6. Nocardiosis: e.g., sulfadiazine, sulfisoxazole
7. Chancroid (a veneral infection caused by Haemophilus ducreyi): sulfasalazine.
8. Toxoplasmosis: e.g., sulpha drug with pyrimethamine.
9. Respiratory tract infections: e.g. Cotrimoxazole.
10. Otitis media: sulpha drug is used in combination with erythromycin.
11. Dermatitis herpetiformis: e.g., sulfapyridine
12. Vaginal infections: sulfisoxazole diethanol amine.
13. Infected burns: e.g., silver sulfadiazine.
14. Meningococcal meningitis: e.g., sulfadiazine.
15. Trachoma and inclusion conjunctivitis: sulfisoxazole, sulfadiazine, sulfacetamide etc.

12.9 SYSTEMIC SULPHONAMIDES

These agents are rapidly absorbed into the circulation when given orally. They can also be given parenterally. They are readily excreted from the body by the efficient excretion process. Depending upon their duration of action, they can be further sub-divided into:

(i) short-acting sulphonamides,

(ii) intermediate-acting sulphonamides, and

(iii) long-acting sulphonamides.

Along with the therapy of systemic sulphonamides, an adequate fluid intake is necessary to minimize the risk of crystalluria. In certain cases, urinary alkalinizer may also be tried to help rapid excretion of the drug and/or its metabolites.

(a) Sulfisoxazole:

It is an example of short-acting sulphonamide. Other examples of this group include, sulfamethizole, sulfacetamide, sulfamerazine, sulfamethazine, sulfisomidine and sulfadiazine. All have an ability to cause crystalluria. A mixture of sulfamerazine, sulfamethazine and sulphacetamide is marketed under the name of trisulfapyrimidine. It has an advantage of low potential to cause crystalluria.

Sulfisoxazole is an orally effective agent. About 91 - 93% of administered dose is bound to the plasma proteins. It has a plasma half-life of 7.0 hours. About 40% dose appears unchanged in the urine along with 20-30% dose in acetylated form.

Adverse effects include nausea, vomiting, anorexia, diarrhoea, dizziness, hypersensitivity reactions, crystalluria and blood dyscrasias.

It is available in a fixed dose combination (sulfisoxazole, 500 mg and phenazopyridine, 50 mg) form which is used in the treatment of urinary tract infections caused by the susceptible strains of *E. coli, Klebsiella, S. aureus, P. mirabilis* and *Proteus vulgaris*. It may also be used alongwith erythromycin ethyl succinate in the treatment of otitis media, specifically in children. Sulfisoxazole and sulfadiazine may be used prophylactically to control streptococcal infections in rheumatic fever patients who are hypersensitive to penicillins. It may be used as alternative drug for the treatment of meliodiosis caused by *Pseudomonas pseudomallei* and for the infections caused by nocardiae. It may also be used topically as a 10 % cream in the treatment of infections of eye, ear, nose or vagina.

(b) Sulphadiazine:

It is an orally active sulphonamide used in the treatment of nocardiosis and other infections caused by Chlamydia and Toxoplasma gondii. Optimal antibacterial activity was probably achieved in 1908 with the introduction of this agent. About 54 - 58% of administered dose is bound to the plasma proteins. It has a plasma half-life of 7 - 10 hours. About 30 - 40% dose is acetylated during metabolism. It is excreted in the urine along with about 55 - 60% dose in unchanged form. Its relative insolubility in acidic urine exposes the patient to the high risk of crystalluria. Hence, adequate sodium bicarbonate (a urine alkalinizer) may be given.

It may be used intravenously in the therapy of meningitis and for prophylaxis against meningococcal meningitis if Neisseria are the basic cause. It is also used as an antimalarial agent when given in combination with pyrimethamine.

12.10 LOCALLY ACTING SULPHONAMIDES

These agents are poorly absorbed from GIT when they are given orally. Hence they are intended to be used for exerting local sterilizing effect on the bowel. Examples of such agents include, phthalylsulfathiazole, succinyl sulfathiazole, phthalylsulfacetamide and salicylazosulfapyridine. All these are the examples of N^1, N^4-disubstituted sulphonamides in which an organic acid is conjugated at N^4-position.

(a) Salicylazosulfapyridine (sulfasalazine): It is a poor orally absorbed sulphonamide and does not have antibacterial activity. It splits in the gut into sulfapyridine and 5-aminosalicylic acid moieties. The former is absorbed systemically and appears in the urine while the latter is excreted in the faeces. The enzyme responsible for splitting of sulfasalazine is azoreductases, amidases, and glycosidases.

Hence, it is effective in inflammatory bowel disease. Part of its effectiveness is attributed to anti-inflammatory effects of 5-aminosalicylate. It inhibits prostaglandin synthesis.

Adverse effects include nausea, vomiting, anorexia, gastric distress, pancreatitis, headache, rashes, fever, arthralgia, anaemia, agranulocytosis and thrombocytopenia.

It is effective in the long-term treatment of ulcerative colitis, granulamatous colitis and regional enteritis. It may also be used in the therapy of malaria, conjunctivitis, meningococcal meningitis, nocardiosis, otitis media, toxoplasmosis and chancroid (a venereal infection caused by *Haemophilus ducreyi*).

12.11 TOPICALLY USED SULPHONAMIDES

These agents are extremely useful in decreasing the bacterial colonization of burnt skin and thereby preventing burn-wound sepsis. For antibacterial effect, they may also be applied topically to eye, ear, nose, and vagina. Examples include, mafenide, silver sulfadiazine, sulfapyridine, sulfisoxazole diethanolamine and sodium sulfacetamide.

(a) Mafenide acetate: It is a sulphonamide antibacterial agent effective against Pseudomonas aeruginosa, an organism that colonizes the burns. It is effective in the presence of necrotic tissue and also inhibits other gram-positive and gram-negative bacteria. It is partly absorbed systemically upon topical application and is converted to p-carboxylbenzene sulphonamide. In the form of acetate, it may act as carbonic anhydrase inhibitor and cause either alkalosis or acidosis.

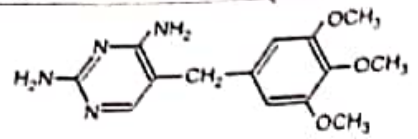
Adverse effects include skin rashes, eczema, urticaria, exfoliative dermatitis, metabolic acidosis, intense pain at the site of application and chances of superinfection with Candida.

It is available as a cream containing 85 mg/g of mafenide and is applied once or twice a day over burnt skin till desired response is obtained. Occasional cleansing of wound and removal of debris is necessary.

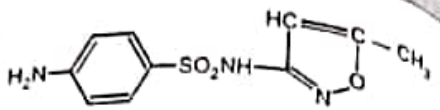
(b) Silver sulphadiazine: Silver ions are especially effective against Gonococci and Pseudomonas species. Silver salts are highly germicidal. Silver sulphadiazine is available in microionized form. It may be used topically in the form of cream (10 mg/g) to inhibit the growth of most bacteria, yeast form of some fungi and herpes simplex. It is effectively used to treat extensive burns and burn infections.

Sulfapyridine is relatively toxic and less effective antibacterial agent. It may be of some value in the treatment of dermatitis herpetiformis. While in the form of eye-drops, sodium sulphacetamide may be used to treat blepharitis and conjuctivitis, and may also be used in prophylaxis against trachoma and inclusion conjuctivitis. It penetrates into the ocular tissues in high concentration. Hence, it is suitable for local management of ophthalmic infections.

12.12 FOLATE REDUCATSE INHIBITORS



Trimethoprim



Sulphamethoxazole

The synergistic effect achieved by the combination of trimethoprim and sulphamethoxazole is recognized as the major advance in the field of chemotherapy.

Bacteriostatic activity is observed due to the inhibition of two prominent steps in bacterial enzymatic pathway involved in folate synthesis. Sulphamethoxazole inhibits utilization of PABA in the formation of dihydrofolate, while trimethoprim is a potent and selective inhibitor of the enzyme that catalyzes the conversion of dihydrofolate to tetrahydrofolate. Thus, a synergistic antimicrobial effect is observed due to the double sequential effects on the bacterial metabolism.

Originally, introduced as antimalarial agent, trimethoprim has also shown significant bacteriostatic activity. It is effective against most of the gram-positive and gram-negative organisms with exceptions of *P. aeruginosa* and *S. faecalis*.

Sulphamethoxazole is selected from systemic sulphonamide class on the basis that it has similar pharmacokinetic features (i.e., rates of absorption and elimination) to that of trimethoprim. It is hence coadministered with trimethoprim in a fixed dose ratio of 5 : 1. This dose ratio yields a fairly constant plasma concentration of sulphamethoxazole - trimethoprim as 20: 1 ratio which is found to be the most effective concentration range to exhibit a synergistic effect against most of the pathogenic microorganisms.

Co-trimoxazole is thus effective against most gram-positive cocci and gram-negative bacteria. Neisseria meningitidis and gonococci are also susceptible. It is used in the treatment of infections of urinary, intestinal and lower respiratory tracts. It is also effective in the treatment of acute otitis media, chronic bacterial prostatis, meningococcal infections, gonorrhea, nocardiosis and antibiotic resistant salmonellae and shigellae infections.

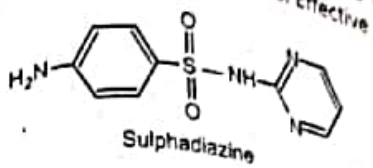
This combination preparation is preferably used in the treatment of acute and recurrent urinary tract infections, typhoid fever, brucellosis, endocarditis, salmonella sepsis, acute bacterial exacerbations of chronic bronchitis and pneumocystis.

Adverse effects of this combination arise as the summation of adverse effects of individual components. However, most of the adverse effects of cotrimoxazole are mainly due to sulphamethoxazole moiety. Trimethoprim just helps to intensify some of these (e.g., hematologic adverse effects) toxicities.

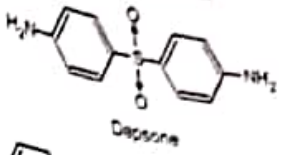
12.13 SULPHONES

Though the antimalarial potency of sulphonamides was proved long back in 1943, they were neglected because of their low therapeutic index. Later on, due to the development of chloroquine resistant strains of *P. falciparum*, long acting sulphonamides were tried in

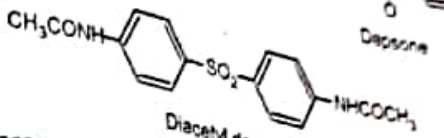
combination with pyrimethamine or trimethoprim. Dapsone, in combination with pyrimethamine can also be effectively used as a chemoprophylactic agent against drug-resistant strains of malarial parasites. Effective members of this class include.



Sulphadiazine



Dapsone

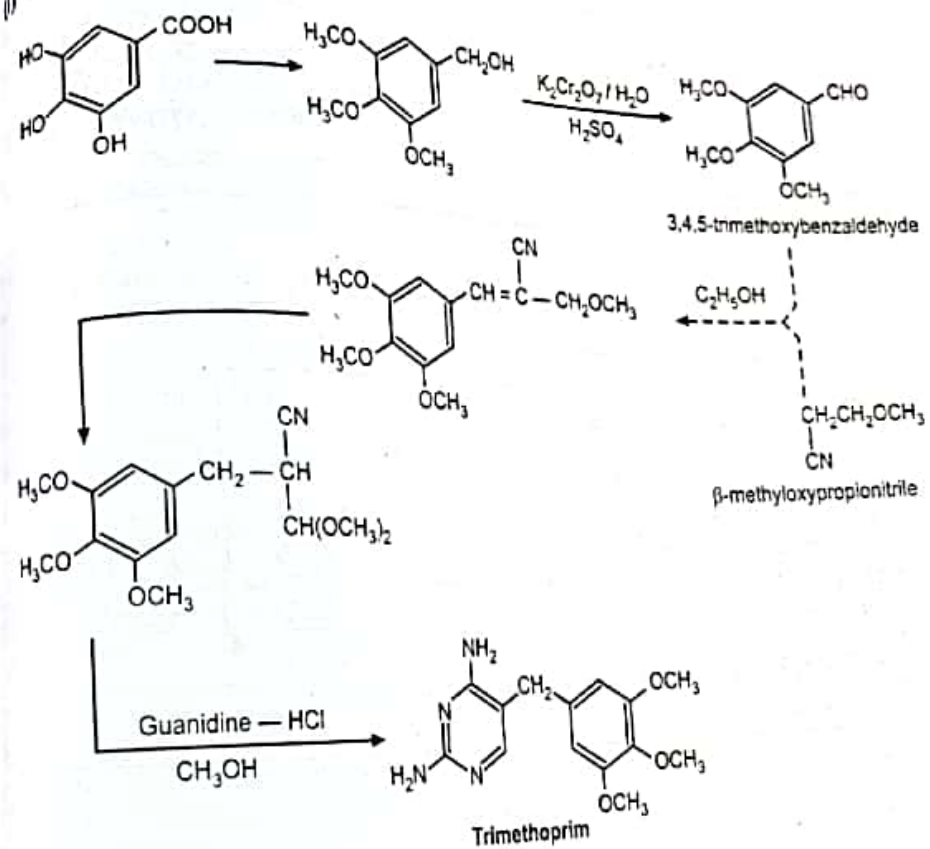


Diacetyl dapsone

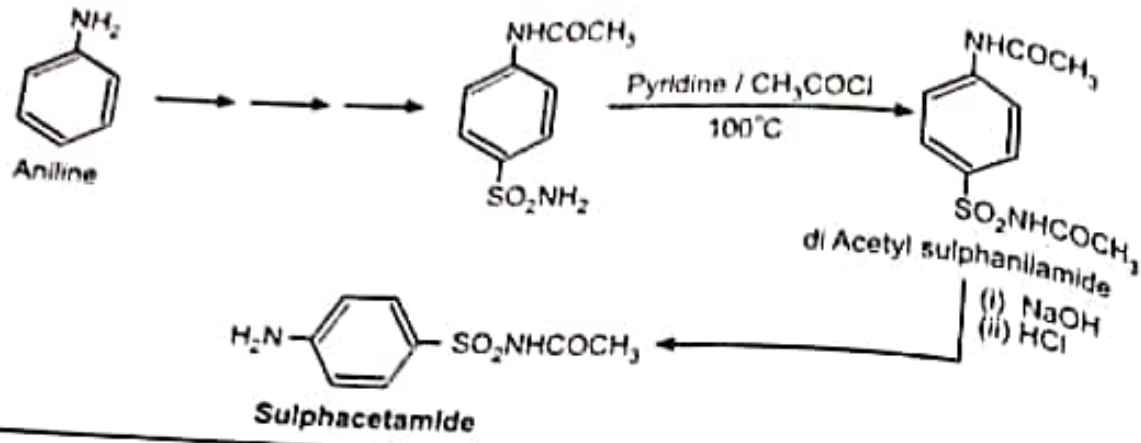
Dapsone is found to possess mild toxicity and prolonged duration of action. Both, sulphonamides and sulphones are active only against erythrocytic stages of malarial parasite. They are ineffective in the treatment of *P. vivax* infections.

Synthesis

(I) Trimethoprim:

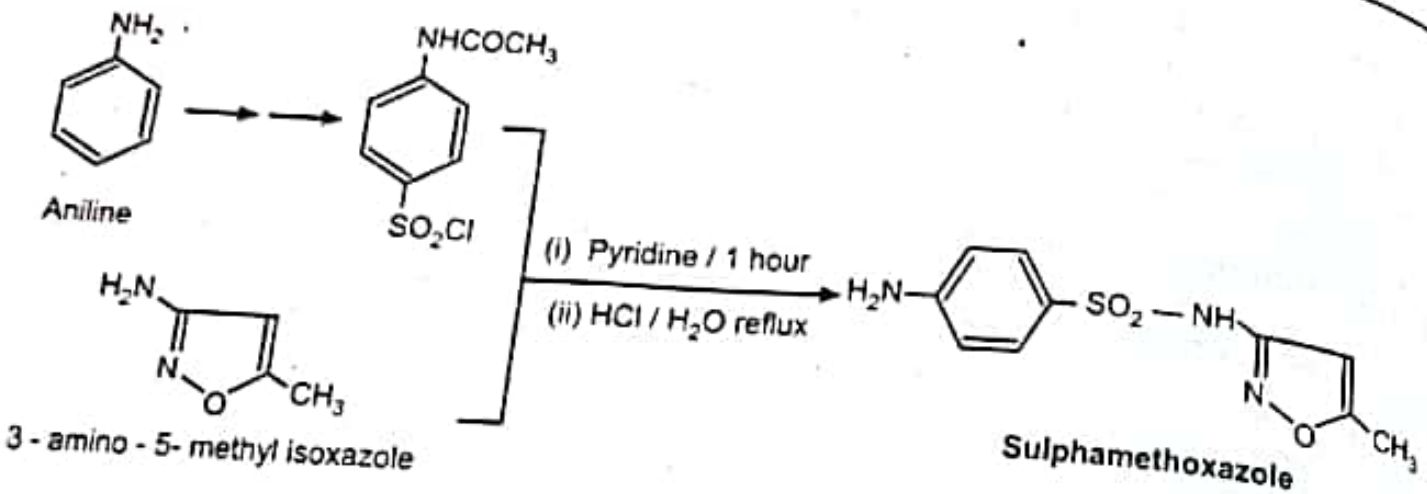


(ii) Sulphacetamide:



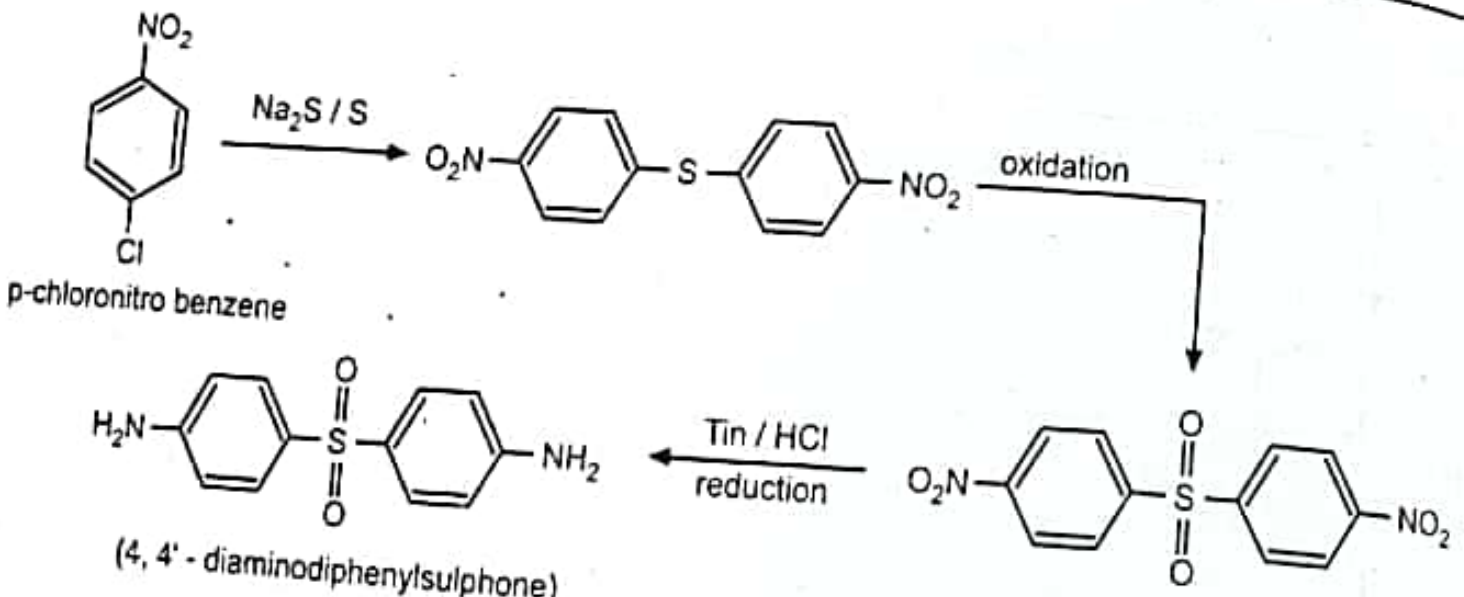
Sulphacetamide

(iii) Sulphamethoxazole:



Sulphamethoxazole

(iv) Dapsone:



Dapsone

UNIT V

Chapter...13

INTRODUCTION TO DRUG DESIGN

♦ SYNOPSIS ♦

13.1 METHODS OF LEAD DISCOVERY

There are several approaches which can be employed for lead identification. In order to identify a lead nucleus in a given series, the whole series should be analysed for a particular biological activity. Once the lead is identified, it can be structurally modified to improve the potency. There is a difference between the terms, activity and potency. Activity is the particular pharmacological activity while potency is the strength of that effect. Following are some of the important methods which can be used for lead identification.

a) Random screening:

In this method, all compounds (including synthetic chemicals and natural products of plant, marine and microbial origin) from a given series are tested. Inspite of budgetary and manpower overuse, this method may be used to discover drugs or leads that have unexpected activities. Antibiotics like, streptomycin and tetracyclines were found out by this method.

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UNIT V

Chapter...13

INTRODUCTION TO DRUG DESIGN

• SYNOPSIS •

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which resulted in the discovery of many novel structures and some spectacularly useful groups of antibiotics, notably the tetracyclines.

Recently, the large scale automated testing of microbial mutants has been done in combination with recombinant DNA techniques to speed up the efficient discovery and production of new antibiotics.

(b) Non-random screening:

It is a modified form of random screening which was developed because of budgetary and manpower restrictions. In this method, only such compounds having similar structural skeletons with that of lead, are tested.

(c) Drug metabolism studies:

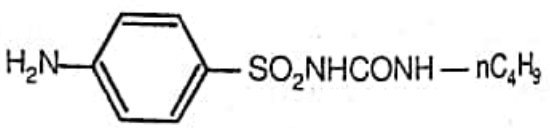
Metabolism of drug occurs as an attempt by metabolizing enzymes to cut short period of stay of the drug in the body. Structural modifications (i.e. metabolic biotransformation) are done in drug molecule by the enzymes to increase its polarity. It is brought about regardless of whether the resulting drug metabolite possesses more activity or toxicity. The discovery of sulfanilamide is reported through the metabolic studies of prontosil.

The antipyretic action of acetanilide was discovered by chance when a nurse by mistake dispensed acetanilide to a patient. Due to its toxicities, acetanilide could not stand in the market. Metabolic studies showed that the toxicities are due to its *in-vivo* metabolite, p-aminophenol. These observations led to development of phenacetin and paracetamol.

(d) Clinical observations:

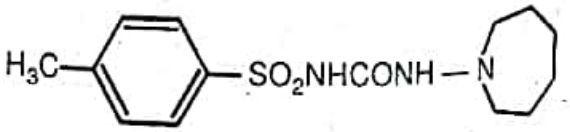
Many times the drug possesses more than one pharmacological activities. The main activity is called as therapeutic effect while rest of the actions are known as side-effects of the drug. Such drug may be used as lead compound for structural modifications to improve the potency of secondary effects.

Sulphonamide oral hypoglycemics arose directly from the clinical observation, in 1942, that a sulphathiazole derivative, which was being used specifically for treating typhoid, lowered the blood sugar drastically. The pronounced hypoglycemia exerted by 5 - isopropyl - 2 sulphanilamido - 1, 3, 4-thiadiazole indicated that an arylsulponyl thiourea moiety ($ArSO_2 - NH - C (= N) - S$) present in thiadiazoles is responsible for their blood glucose lowering effect. This observation led to the development of carbutamide by Franke and Fuchs through opening of thiazole ring to give a thiourea moiety in which = S was then replaced by = O.

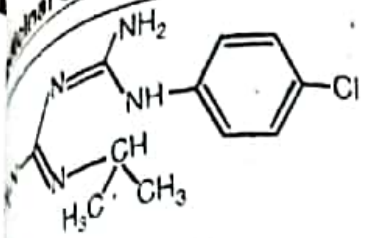


Tolbutamide

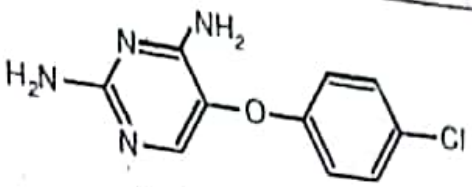
In order to nullify the toxicity and antibacterial activity of the 4-amino group, it was replaced by other substituents resulting into tolbutamide, chlorpropamide and tolazamide.



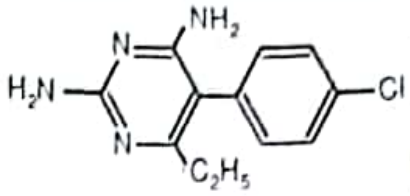
Tolazamide



Chlorguanide



Diaminopyrimidine



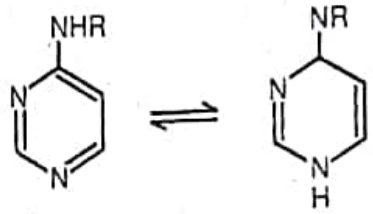
Pyrimethamine

Using 4-methylhistamine as a lead, Ganellin and his colleagues developed H_2 - receptor antagonists with a side-chain terminating in a thiourea group. Because of severe side-effects seen in these thiourea derivatives, thiourea group was bioisosterically replaced by guanidine. Cyanoguanidine when introduced into the side-chain, resulted into cimetidine.

A series of aminoalkyl derivatives of iminodibenzyl was synthesized as analgesics, sedative and anti-histaminics by Hafliger and Schindler in 1951. Imipramine, one of the compounds, appeared to be potential anti-depressant during clinical studies by Kuhn in 1957. Many tricyclic anti-depressants, therefore were synthesized.

Similarly, due to the antifolate activity shown by chlorguanide, various diaminopyrimidines were synthesized. Pyrimethamine was designed by deleting the bridging between two rings.

With the knowledge of antimalarial activity of sulfapyrimidines at hand, British medicinal chemists F.L. Rose and F.H.S. Curd spotted a tautomeric proton shift in aminopyrimidines which was supposed to be an essential prerequisite for potent antimalarial activity as per Schonhofer's hypothesis proposed for aminoquinolines. Less toxicity may be expected from pyrimidine series when compared with quinolines/acridines, as the former are components of nucleotides.



Proton tautomerism in aminopyrimidines

(e) Rational approaches to lead discovery:

The knowledge about the receptors and their mode of interaction with drug molecules play an important role in drug design. This knowledge may be used to develop conformationally bioactive skeletons having exact three-dimensional complementarity to a receptor. Greater potency, higher selectivity and less adverse effects are expected by reducing the flexibility of the drug structure. For example, replacement of a terminal N, N-diethylamino group by piperidino exploits the decreasing valency angle at the tertiary nitrogen of the latter so that access of the basic group to anionic sites might be improved. This modification leads to the development of major tranquilizers, local anaesthetics, antihistaminics and spasmolytics. Incorporating a rigid ring leads to altered pharmacokinetic and pharmacodynamic features due to altered pKa of the amine and lipophilicity of the molecule.

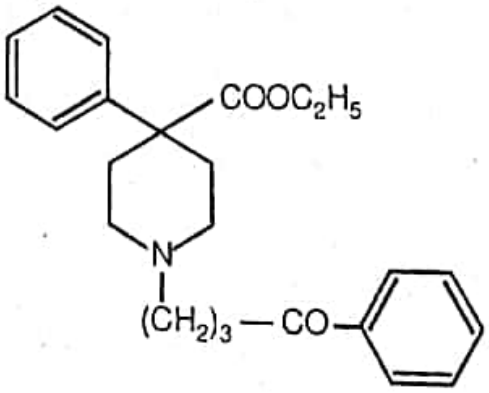
This approach is of greater importance in identification of lead nucleus. It involves the use of signs and symptoms of the disease. Most diseases, atleast in part, arise from an

imbalance of particular endogenous bioactive substances in the body. These imbalances may be corrected by agonism or antagonism of a receptor or by inhibition of a particular enzyme. Once the real site of such imbalance is identified, the natural enzyme substrate or endogenous substance may be used as a lead nucleus. For example, endogenous hormones, progesterone and 17 β-estradiol were used for developing oral contraceptives. The development of an anti-inflammatory drug, indomethacin from the lead nucleus, serotonin resulted at Merck with a belief that serotonin is a possible mediator of inflammation.

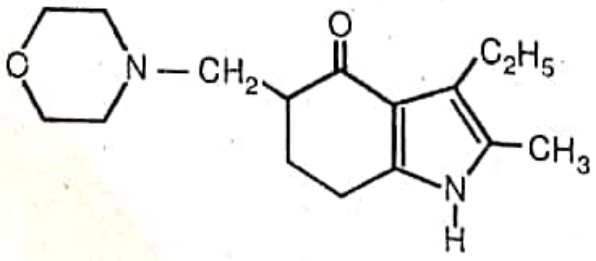
Medicinal chemistry has many examples of the development of successful therapeutics based on an exploration of endogenous compounds. The treatment of diabetes mellitus, for example, is based upon the administration of insulin, the hormone that is functionally deficient in this disease. The current treatment of Parkinson's disease is based upon the observation that the symptoms of Parkinson's disease arise from a deficiency of dopamine an endogenous molecule within the human brain. Since, dopamine cannot be given as a drug, since it fails to cross the blood-brain barrier and enter the brain, its biosynthetic precursor, L-DOPA, has been successfully developed as an anti-Parkinson's drug.

Analogously, the symptoms of Alzheimer's disease arise from a relative deficiency of acetylcholine within the brain. Current therapies for Alzheimer's-type dementia are based upon the administration of cholinesterase enzyme inhibitors that prolong the effective half life of remaining acetylcholine molecules within the brain.

Paul A. J. Janseen developed meperidine derivatives by replacing methyl group of piperidine nitrogen by alkyl aryl keto groups. While searching for a better substituent to replace carbethoxy group, tertiary alcohol group was finally selected.



Substitution of the aryl nucleus by halogens and pseudohalogens (F_3C) demonstrated that fluorine para to the keto group was optimal for neuroleptic potency. Out of several hundreds of analogs, haloperidol was selected in 1958 finally for clinical trial. Haloperidol was subjected to various molecular modifications to enhance neuroleptic activity at the expense of analgetic properties. For example, tetrahydropyridyl and piperazinyl rings were used to replace piperidine ring.



Since, the aminobutyrophenones are δ-aminoketones, homologs were synthesized. Molindone, a Mannich base of pyrrole ketone, is used as an antipsychotic. In rational drug design, this cycle of "design-test-redesign-retest" can go on for several iterations until the optimized molecule is achieved.

In a chance test, numbing on the tongue was exerted by 2-dimethylamino-2-aceto-toluidine, an intermediate in the synthesis of gramine. This led to the synthesis of various anilides to get local anaesthetics. The presence of two sterically hindering ortho methyl groups protect the anilide linkage from hydrolysis and increase the duration of action of lidocaine. This principle was extended further to develop mepivacaine and dimethisoquine.

In Postwar France, the Berthier Pharmaceutical Company in Grenoble began to pursue a sideline project of producing soothing liquid bismuth preparations for acute tonsillitis. Being dissatisfied with the commonly used oils, they elected to use the physiologically inert valproic acid as a solvent for their bismuth compounds. Valproic acid is now used in the treatment of epilepsy and (migraine).

In 1962, Pierre Eymard, a graduate student at the University of Lyon, synthesized a series of Khellin. Khellin is a biologically active substance that occurs in the fruit of the wild Arabian Khell plant and which has been used for centuries for the treatment of kidney stones. When attempts to produce a solution of these Khellin compounds failed, advice was sought from H. Meunier of the nearby Laboratory, Berthier. In the view of Berthier's recent interest in valproic acid as a non-toxic inert solvent, Eymard's Khellin derivatives were dissolved in valproic acid and they were studied for anticonvulsant activity. These preliminary studies revealed profound anticonvulsant activity. The antiepileptic action of valproic acid was thus discovered completely by accident, with the first successful clinical trial occuring in 1963.

Bromine was discovered in seawater in 1826. Recognizing its chemical similarity to iodine, French physicians immediately exploited it as an iodine alternative for the treatment of numerous conditions, including syphilis and thyroid goiter. Although no beneficial effects were reported for either bromine or its potassium salt, their widespread use eventually helped to recognize the depressant effect of potassium bromide on the nervous system.

In 1857, Sir Charles Locock, the physician, with the view that epilepsy arose from excessive sexuality, introduced bromide to suppress the supposed hypersexuality of epileptics. The bromide salts (e.g., potassium bromide, sodium bromide) were administered in substantial doses ranging from 0.3 g/day in children to a staggering 14 g/day in adults. Although side effects had been considerable (and included psychoses and serious skin rashes), bromides were successful in 13 of the 14 patients treated. On 11 may 1857, at a meeting of the Royal Medical Society, Lucock proudly reported his success in treating "hypersexual" epilepsies with bromides.

Bromides were a major step forward in the treatment of epilepsy and their use persisted until the introduction of Phenobarbital in 1912.

13.2 OPTIMIZATION OF THE LEAD

Once the lead nucleus is identified, it is easy to exploit. This process is rather straight forward. Various approaches are employed in order to improve the desired pharmacological properties of the lead nucleus. Important amongst them are,

(a) Identification of the active part (the pharmacophore):

Any drug molecule consists of both, essential and non-essential parts. Essential part is important in governing pharmacodynamic (drug-receptor interactions) property while non-essential part influences pharmacokinetic features. The relevant groups on a molecule that interact with a receptor are known as bioactive functional groups. They are responsible for the activity. The schematic representation of nature of such bioactive functional groups along with their interatomic distances is known as pharmacophore.

A pharmacophore was first defined by Paul Ehrlish in 1909, as "a molecular framework that carries the essential features responsible for a drug's biological activity". In 1977, this definition was updated by Peter Gund to "a set of structural features in a molecule that is recognized at a receptor site and is responsible for that molecule's biological activity". The IUPAC definition of pharmacophore is "an ensemble of steric and electronic features that is necessary to ensure the optimal supra molecular interactions with a specific biological target and to trigger (or block) its biological response".

In 1958, Daniel Koshland suggested a modification to the lock and key model. Since, enzymes are rather flexible structures the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme. The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined.

The active site geometry of a protein complex depends heavily upon conformational changes induced by the bound ligand.

Once such pharmacophore is identified, structural modifications can be done to improve pharmacokinetic properties of the drug. For example, the presence of a phenyl ring, asymmetric carbon, ethylene bridge and tertiary nitrogen are found to be minimum structural requirement for a narcotic analgesic to become active. Similarly, the presence of two anionic sites and one cationic site must be present in cholinergic agent. Morphine, the prototype narcotic agent has a pentacyclic structure. The complexity of structure leads to appearance of several adverse effects. Hence, the pharmacophore of morphine has been recognized through molecular dissection and was used to develop still simpler and even acyclic analogs. For example, methadone is as potent as analgesic as morphine.

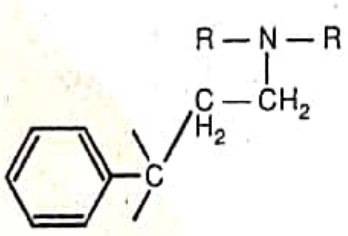


Fig. 13.1: Pharmacophore for Narcotic Drug

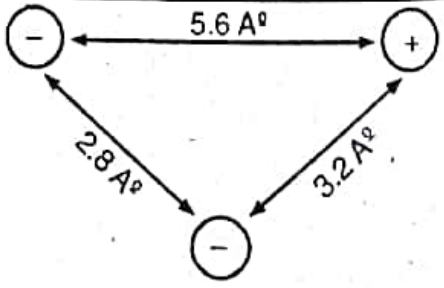


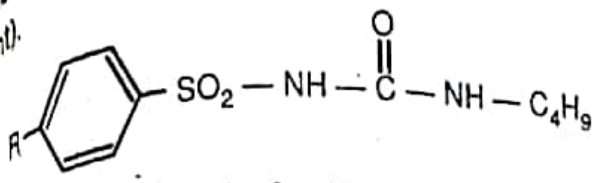
Fig. 13.2: Pharmacophore for Cholinergic Drug

Similarly, in estrogenic compounds two bioactive sites, having ability to undergo bonding, should be separated by a minimum distance of 8.5 A°.

In 17-β-estradiol, the distance is 10.9 A° while in diethyl stilbestrol it is 12.1 A°.

b) Functional group optimization:

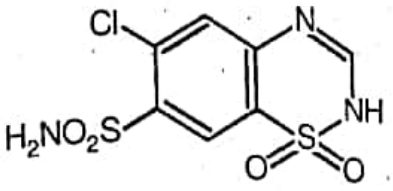
The activity of a drug can be correlated to its structure in terms of the contribution of its functional groups to the lipophilicity, electronic and steric features of the drug skeleton. Hence, by selecting proper functional group, one can govern the drug distribution pattern and can avoid the occurrence of side-effects. For example, the amino group of carbutamide (antibacterial agent) was replaced by a methyl group to give tolbutamide (antidiabetic agent).



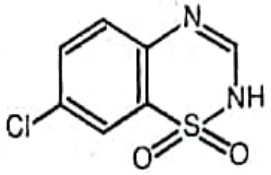
Carbutamide : $R = NH_2$

Tolbutamide : $R = CH_3$

Similarly, removal of sulfonamide side-chain of chlorothiazide (an antihypertensive drug with diuretic activity) helped to design diazoxide (an antihypertensive drug without diuretic activity).

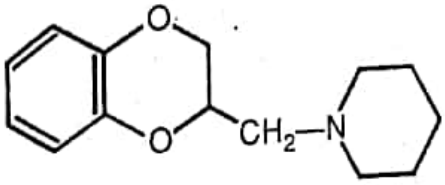


Chlorothiazide

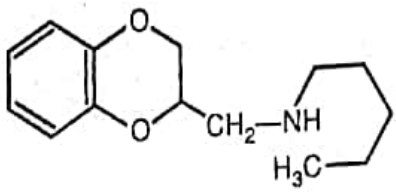


Diazoxide

Since a neuroleptic activity runs parallel with the α-adrenoceptor blocking activity, piperoxan, an alpha adrenoceptor blocking agent was chosen as a lead to get pentamoxane which showed high neuroleptic activity in animal studies.



Piperoxan



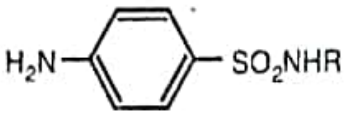
Pentamoxane

The replacement of –Cl by CF_3 in ring position 2 and the modification of the basic side-chain to include a piperazine moiety in chlorpromazine is thought to enhance neuroleptic potency by increasing lipophilicity so that CNS-entry of the drug is facilitated.

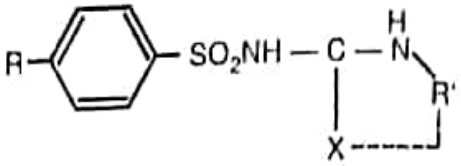
c) Structure-activity relationship studies:

The physiological action of a molecule is a function of its chemical constitution. This observation is the basis of SAR studies. SAR studies usually involve the interpretation of activity in terms of the structural features of a drug molecule. Generalised conclusions then can be made after examining a sufficient number of drug analogs. For example, sulphonamides are found to be associated with diuretic and antidiabetic activities in

addition to their antibacterial activity. The generalised structures needed for individual activity are represented below.

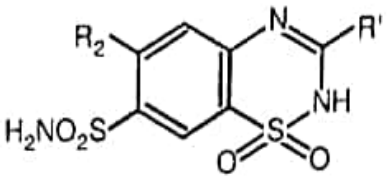


Antibacterial sulphonamide



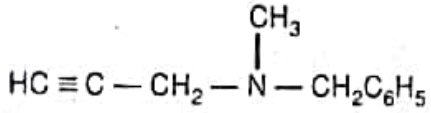
Antidiabetic sulphonamide

(X = O, S or N)

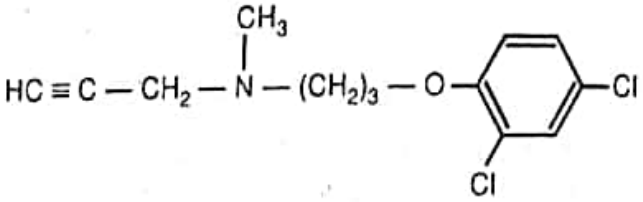


Diuretic sulphonamide

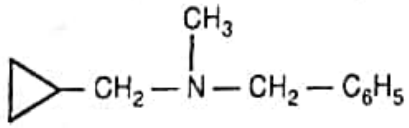
Because of hepatotoxic side-effects of hydrazines and hydrazides, structurally diversified compounds were synthesized resulting into the introduction of pargyline and tranylcypromine. Tranylcypromine was developed as a structural analog of amphetamine and is used as an anti-depressant agent. Due to pronounced effect on blood pressure, the former was used as an antihypertensive agent. Further structural modification of pargyline skeleton resulted into cyclogyline.



Pargyline



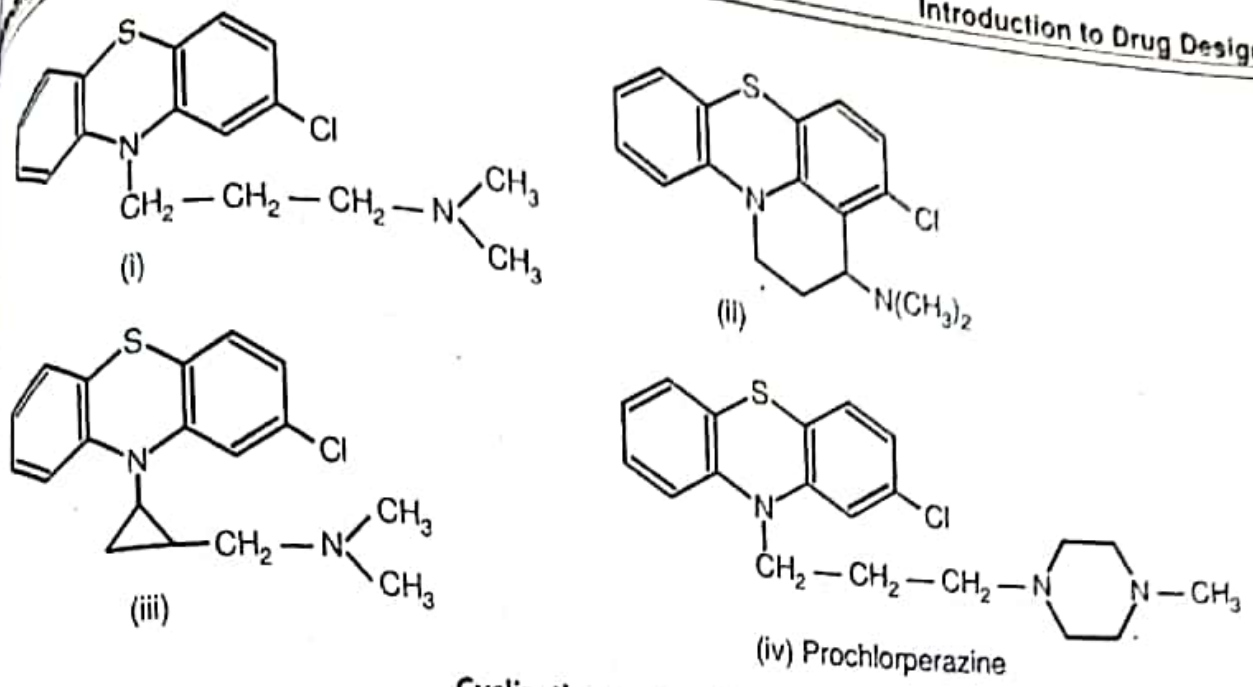
Clorgyline



Cyclogyline

(d) Homologation:

The variation in the substituent can be used to increase or decrease the polarity, alter the pKa, and change the electronic properties of a molecule. Exploration of homologous series is one of the most often used method to induce these changes in a very gradual manner. A homologous series is a group of compounds that differ by a constant unit, generally a CH_2 group. Usually, increasing the length of a saturated carbon side-chain from one (CH_3) to 5 to 9 atoms (pentyl to nonyl) produces an increase in pharmacological effects. Further increase results in a decrease in the activity. This is probably either due to increase in lipophilicity beyond optimum value (hence decreased absorption and distribution) or decrease in concentration of free drug (i.e., micelle formation). For example, maximum hypnotic activity is seen from 1-hexanol to 1-octanol. Thereafter activity decreases for higher



Cyclization of the side chain

homologs. Similarly, in a series of 4-alkyl substituted resorcinol derivatives, 4-n-hexyl - resorcinol (clinically used topical anaesthetic in throat lozenges) was found to possess maximum antibacterial activity. While in a series of mandelate esters, n-nonyl ester has maximum antispasmodic activity. In the same series, branching leads to decrease in the activity, probably due to interference with receptor binding. For example, primaquine (an antimalarial agent) is much more potent than its secondary or tertiary homologs.

(e) Cyclization of the side-chain:

Change in the potency or change in the activity spectra can be brought about by transformation of alkyl side-chain into cyclic analogs. For example, chlorpromazine (i) has more neuroleptic activity than its cyclic analog (ii). Similarly the compound (iii) has anti-depressant (imipramine like) activity than neuroleptic activity. While in compound (iv) the antiemetic activity is greatly enhanced.

Sometimes bridging of two carbon atoms (secondary cyclization) also leads to an increase in potency or change in activity spectrum. Examples include thebaine (oripavine) derivatives, atropine, bridged piperazine derivatives of phenothiazines etc.

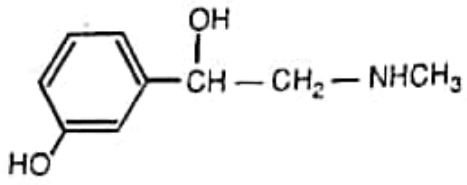
(f) Bioisosterism:

The purpose of molecular modification is usually to improve potency, selectivity, duration of action and reduce toxicity. The physicist Langmuir introduced the term, isosterism in 1919. The term, bioisosterism, introduced by Friedmen in 1951. Bioisosters are substituents or groups that have similar physical or chemical properties and hence similar biological activity pattern. Isosteric groups, according to Erlenmeyer's definition are isoelectronic in their outermost electron shell. Bioisosteric replacement may help to decrease toxicity or to change activity spectra. It may also alter the metabolic pattern of the drug. The parameters being changed are molecular size, steric shape (bond angles, hybridization), electron distribution, lipid solubility, water solubility, the pKa, the chemical reactivity to cell

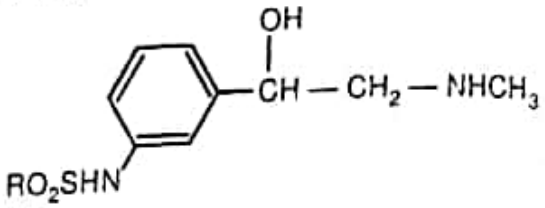
components, and the capacity to undergo H-bonding (receptor interaction). Even if the bioisosteric replacement is relatively minor (Cl for CH_3 or vice versa). Cl may block metabolic hydroxylation, whereas CH_3 may be bio-oxidized and the compound may have shorter half-life. For example, tolbutamide ($R=CH_3$) has shorter half-life than chlorpropamide ($R=Cl$). Erlenmeyer defined isosters as atoms, ions or functional groups in which the peripheral layers of electrons can be considered to be identical. These are known as classical bioisosters. While non-classical bioisosters do not have the same number of atoms and do not fit the steric and electronic rules of classical isosters, but they produce a similarity in biological activity.

13.3 APPLICATIONS OF BIOISOSTERISM IN DRUG-DESIGN

(a) An important compound from catecholamine series is phenylephrine in which phenolic hydroxyl group takes part in H-bonding with bioactive site on the receptor. The hydroxyl group can be replaced by other group having ability to undergo H-bonding. Hence, alkylsulphonamido derivative of phenylephrine was found to retain activity.

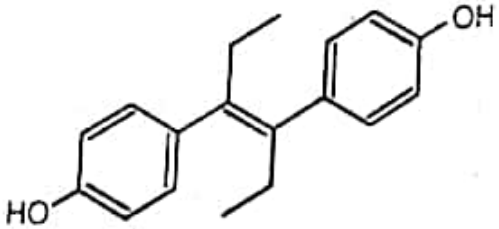


Phenylephrine

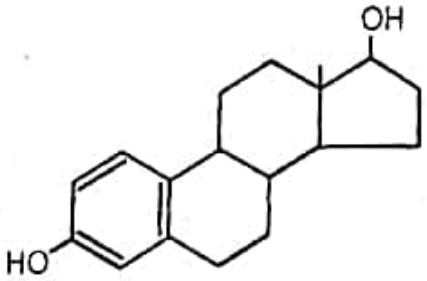


Alkylsulphonamido derivative

(b) A classic example of ring versus non-cyclic structure is diethylstilbestrol and 17 β-estradiol.



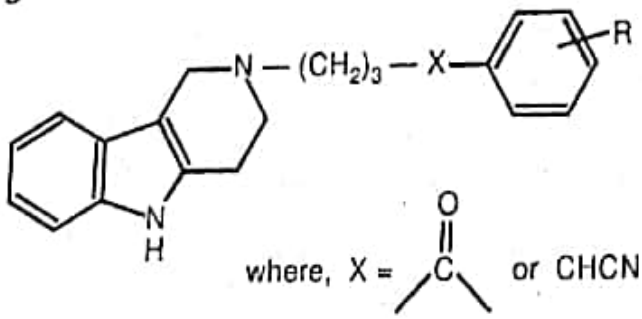
Trans-Diethyl stilbestrol



17-β-estradiol

Diethylstilbestrol has about the same potency as that of naturally occurring estradiol. The central double bond of diethyl stilbestrol is highly important for the correct orientation of the phenolic and ethyl groups (trans) at the receptor site.

(c) Bioisosteric analogs in neuroleptic category include



The term 'drug design' represents mainly the efforts to develop new drugs on rational basis. The various approaches used in drug design include,

1. Random screening of synthetic compounds or chemicals and natural products by bioassay procedures.
2. Novel compound preparation based on the known structures of biologically active, natural substances of plant and animal origin, i.e., lead skeleton.
3. Preparation of structural analogues of lead with increasing biological activity and
4. Application of the bioisosteric principle.

Of a number of procedures involved in drug design, the first step is the detection of some biological action in a group of compounds so as to serve as a lead. This is followed by molecular manipulations to increase or modify the activity. Identification of a lead nucleus depends upon the consideration of the following points:

(i) molecular structure of the drug,

(ii) behaviour of the drug in the biophase,

(iii) geometry of the receptor,

(iv) drug-receptor interaction,

(v) changes in the structure on binding, and

(vi) the observed biological response.

After following such a tedious process, only fewer drugs can reach to the level of clinical applicability. Such compounds have to be given extensive trials before they are tried on humans. This adds to the cost of research for new drugs. Broadly, this means that if the development of new drugs is to remain economically feasible, the ratio of output to input must be increased.

The lead is a prototype compound that has the desired biological or pharmacological activity, but may have many undesirable characteristics, e.g., high toxicity, other biological activities, insolubility or metabolic problems. Early SAR studies (prior to 1960s), simply involved the synthesis of as many analogues as possible of the lead and their testing to determine the effects of structure on activity. Attempts were made to interpret chemical structure in terms of physical and chemical properties, transport and distribution of a drug in a biological multicompartment system, the affinity of the drug to a complementary structurally unknown-receptor and the interaction of the drug with its receptor. Corwin Hansch's classic work which appeared in 1964 can be taken as turning point in the study of chemical SARs.

The understanding of structure-activity relationships has developed extensively over the past decades, with very powerful statistical techniques available to predict activities of designed, but yet unsynthesized compounds.

Between 1858 and 1861, three chemists: Archibald, Freidrich August Kekule von Strandonitz and Aleksandr Mikhailovich Butleroy independently introduced the general rules of valence for organic chemistry and the first written structure involving chains of carbon atoms drawn as 'Bonds' to substituent atoms and groups. The term 'Chemical structure' was first used at this time.

The first recorded use of physical molecular model in organic chemistry was done by August Wilhelm Hoffmann in 1865. He used the metaphor of croquet balls joined by sticks to describe methane, chloroform and other compounds. Hoffmann established colour scheme which is still widely used today: white for hydrogen, black for carbon, red for oxygen, blue for nitrogen and green for chlorine.

The introduction of the Hansch model in 1964 enabled medicinal chemists to formulate their hypotheses of structure-activity relationships in quantitative terms and to check these hypotheses by means of statistical methods. From such Quanitative Structure Activity Relationships (QSAR), it is possible to elucidate the influence of various physiological properties on drug potency and to predict activity values for new compounds within certain limits.

QSAR techniques employ powerful computers, molecular graphics and sophisticated softwares; they may be of enormous assistance to those trying to generate the large data bases resulting from the massive efforts in drug research.

The goals of Quantitative Structure Activity Relationship studies were first proposed about 1865 to 1870 by Crum-Brown and Fraser who showed that the gradual chemical modification in the molecular structure of a series of poisons produced some important differences in their action.

They observed that a series of quaternized strychnine derivatives could be prepared which, to a varying degree depend on the quaternary substituent, possessed activity similar to curare in paralyzing muscle. In their paper (1868), they proposed the equation shown below, in which f is a measure of biological activity (Physiological action) and C is a measure of chemical structure (Chemical constitution).

$$F = f(C)$$

Overton related tadpole narcosis (induced by a series of non-ionized compounds added to the water in which the tadpoles were swimming) to the ability of the compounds to partition between oil and water.

Testing of 51 compounds including alcohols, ethers and amides as narcotics on tadpoles yielded the following equation.

$$\text{Log } (1/C) = 0.94 \log P + 0.87$$

$$r = 0.97 \text{ and } n = 51$$

Thus, the strong correlation between the biological activity and partition coefficient supports the proposed mechanism.

Traube found a linear relation between narcosis and surface tension in 1912.

The depressant action of structurally non-specific drugs were rationalized by Fergusson, in 1939, working in ICI laboratories, who formulated a concept linking narcotic activity, partition coefficient and thermodynamics. Relative saturation was termed as thermodynamic activity by Fergusson.

Shortly thereafter, Richardson noted that the hypnotic activity of aliphatic alcohols was a function of their molecular weight. These observations are the basis of QSAR.

QSAR is essentially a computerised statistical method which tries to explain the observed variance in the biological effect of certain classes of compounds as a function of molecular changes caused by the substituents. It assumes that the potency of a certain biological activity exerted by a series of congeneric compounds is a function of various physicochemical parameters of the compounds. Once statistical analysis shows that certain physico-chemical properties are favourable to the concerned activity, the latter can be optimised by choosing such substituents which would enhance such physico-chemical properties.

It involves the mathematical and statistical analysis of SAR-data which helps to reduce the number of educated guesses in molecular modification. The ultimate objective of such studies is to understand the forces governing the activity of a particular compound or a class of compounds. QSAR is thus a scientific achievement and an economic necessity to reduce an empiricism in drug design to ensure that every drug synthesized and pharmacologically tested should be as meaningful as possible.

13.5 QSAR-PARAMETERS

Physical organic chemistry deals with characterization of the structure and prediction of the properties, the descriptors for which, are usually found experimentally. If some property depends on the set of selected descriptors, the ordering of the structure will parallel the ordering of the properties. In other words, the structural information is coded in these properties. Therefore, good correlation of physico-chemical properties with a particular set of indices may help in understanding the contribution of these invariants in determining the property.

Studies of Meyer and Overton suggested, inter alia, correlations between insecticidal activity and boiling points and narcotic activity with surface tension. This revealed that the biological activity of a drug is a function of chemical features (i.e., lipophilicity, electronic and steric properties) of the substituents and the skeleton of a drug molecule. For example, lipophilicity is the main factor governing transport, distribution and metabolism of drugs in biological systems. Similarly, electronic and steric features influence the metabolism and pharmacodynamic processes of a durg. For example, steric crowding of substituents leads to lower the predicted activity and co-operative binding to receptor leads to increase in the predicted activity. An optimum was observed for antibacterial activity and dissociation constant (pKa) in sulphonamide series. Thus, a substituent may affect activity by altering the physical as well as the chemical properties of a skeleton.

Biological activity reflects the fundamental physico-chemical properties of the bioactive compounds. Enantiomers are related physico-chemically very closely. They only differ sterically but are usually identical with respect to lipophilicity, polarity, charge distribution etc. A major problem in QSAR studies arises because the hydrophobic, electronic and steric effects overlap and can not be neatly separated. The parameters which are used to obtain such correlations can be divided into:

(i) Those which describe mainly the physical properties of a skeleton, such as water solubility, partition coefficient, chromatographic R_f values, molecular weight, surface tension etc.

(ii) Those which describe the chemical properties such as dipole moment, charge densities, electron donar-acceptor properties, Hammett's electronic constants, Taft's steric constants etc. (see Table 13.1).

Various QSAR methods are developed with an assumption that the biological properties of organic compounds are a direct consequence of their chemical and physical properties.

Table 13.1: Physico-chemical Parameters used in QSAR

	Physico-chemical parameters	Symbol
(I)	**Hydrophobic parameters:**	
	(i) Partition coefficients	log P, $(\log P)^2$
	(ii) Pi substituent constants	π, π^2
	(iii) R_M - chromatographic parameter	log R_M
	(iv) Solubility	δ
	(v) Elution time in HPLC	log K'
	(vi) Parachor	[P]
(II)	**Electronic parameters:**	
(a)	**Experimental parameters:**	
	(i) Ionization constants	pKa, Δ pKa
	(ii) Sigma substituent constants	σ, σ^2, σ^-, σ^+, σ I, σ^*
	(iii) Spectroscopic chemical shift	Δ Fr, ppm
	(iv) Resonance effect	R
	(v) Field effect	F
	(vi) Ionization potential	I
(b)	**Theoretical quantum mechanical indices (MO-indices):**	
	(i) Atomic charge densities	ε
	(ii) Atomic net charge	q, QT, q^σ, Q^σ, q^π, Q^π
	(iii) Super delocalizability	S_r^N, S_r^E, S_r^R
	(iv) Energy of molecular orbit	E_{LEMO}, E_{HOMO}
	(v) Others	$\pi'N$, N, $\pi'N$, NH, F[A], F[A]

Steric parameters:	
(i) Taft's steric substituent constant	Es
(ii) Van der Waals radii	γ
(iii) Inter atomic distances	B, L
(iv) Molar refractivity	MR
(v) Molar volume	MV

13.6 QSAR-METHODS

The introduction of Hansch method in 1964 enabled chemists to describe SAR-studies in quantitative terms. During past decades, QSAR started to develop from a merely intuitive and empirical discipline to more and more advanced state. In a given series of compounds, to get a quantitative information about SAR, either of following approaches can be employed:

(a) One may use QSAR methods based on linear free-energy relationships which relate the biological activity of a molecule with contributions from various free-energy related physico-chemical parameters of the substituents, the constants associated with each physico-chemical parameter being generated by regression analysis for the biologically tested compounds.

(b) In statistical approach mathematical models rather than linear free-energy relationships are used to express the dependence of biological activity on the nature and location of the substituents.

Various methods used in QSAR analysis can be summarised as follows:

Table 13.2: QSAR Methods

(I) Free Energy Models:
(a) Hansch Method: linear free energy relationship
(b) Martin and Kubinyi: non-linear free-energy relationship
(c) Free-Wilson Mathematical Model:
(II) Other Statistical Methods:
(a) Discriminant analysis
(b) Principal component analysis
(c) Factor analysis
(d) Cluster analysis
(e) Combined multivariate analysis
(III) Pattern Recognition
(IV) Topological Methods
(V) Quantum Mechanical Methods
(VI) Molecular Modelling

13.7 SUBSTITUENT CONSTANTS

During its journey from the site of administration to the site of action, the drug molecule undergoes continuous changes in conformation. Each conformation has a specific free energy as per its specific environment. Thermodynamically, the extent to which each of the binding, transport and metabolism of the drug occur, is directly thus dependent on the associated change in free energy. The change in free energy is determined from the relative probability of finding the system in a given state.

The pharmacokinetic and pharmacodynamic properties are governed by lipophilic (log P), electronic (σ) and steric feature (Es) of the drug molecule. The Biological Activity (BA) in a given series is influenced by the lipophilicity, electronic and steric properties of the substituent at given position

$$\log (BA) = a \log P + b\sigma + cEs + d$$

where a, b, c and d are the numerical values.

A non-polar drug and a hydrophobic region of a receptor are surrounded by a layer of water molecules which are more or less ordered and therefore in a lower state of energy than in free solution. When such a drug molecule contacts receptor, it results in the displacement of water layers and an increase in entropy. This gain in the free energy is proportional to the number of water molecules changed from an ordered to a disordered state, i.e., proportional to the surface area of the non-polar part of the drug and receptor.

Lipophilicity (hydrophobicity) of a drug can be measured readily by disribution of the compound between an aqueous and non-aqueous, water immisible solvent. The non-aqueous solvent usually chosen is 1-octanol. The octanol-water partition coefficient is designated as P and the Hansch value π is the effect of a given substituent on log P of the basic skeleton.

Largely with the initiative of Hansch, n-octanol now seems to be the organic solvent of choice. n-octanol has, a long saturated fatty alkyl chain, hydroxyl group for H-bonding and dissolves water to the extent that saturated octanol contains 1.7 M water. This combination of lipophilic chains, hydrophilic hydroxyl group and water molecules appears to give n-octanol, properties very close to those of natural membranes and macro-molecules.

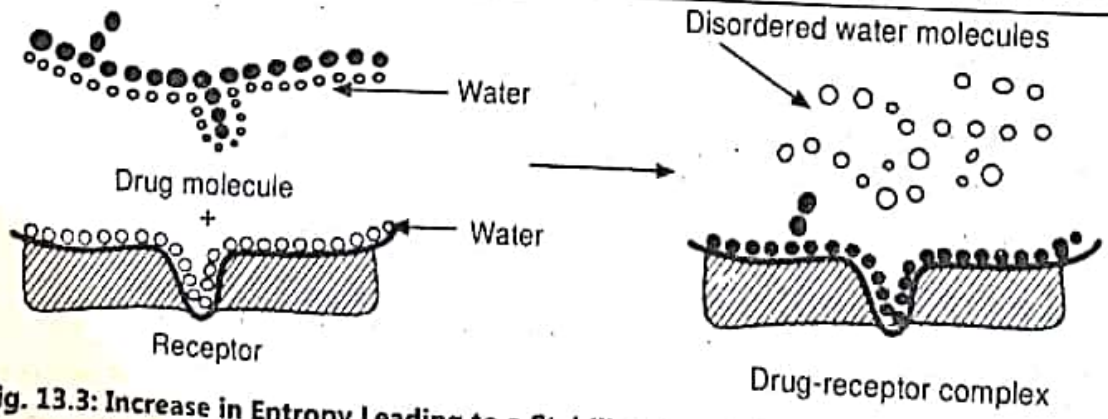


Fig. 13.3: Increase in Entropy Leading to a Stabilization of the Drug-receptor Complex

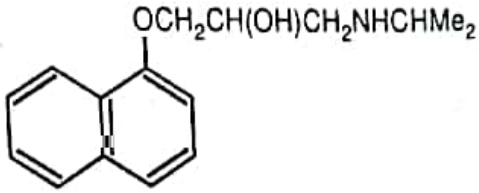
In addition, n-octanol has low vapour pressure at room temperature and it is well suited for direct measurement of concentrations in the ultraviolet region due to its low absorption over a wide range. n-octanol-water partition coefficients are available from the literature and from the Hansch data bank for a large number of drugs.

Partition coefficient is a free energy related parameter which expresses the relative free energy change occurring on moving a substituent from one phase to another. This is an additive property. It means, with the help of π values of the substituents, the log P value of any molecule may be calculated by simple addition.

$$\log P = \sum_{l}^{m} \pi \text{ (additive free energy)}$$

For example, propranolol.

(i)



log P (naphthalene) = 3.37

$\pi - OCH_2 -$ = − 0.02 (from anisole)

$\pi - CH -$ = 0.50

$\pi - OH$ = − 1.39 (from 2-butanol)

$\pi - CH_2NHCH -$ = − 0.17 (from N-methylbutylamine)

$2 \times - \pi\ CH_3$ = 1.00

$\Delta\ \pi$ branch = − 0.20

$\log P_{(propranolol)}$ = 3.09

(log P exp. = 3.33)

(ii) $\log P_{C_6H_5CH_2CH_2C_6H_5}$ = $2 \log P_{C_6H_5} + 2\ \pi\ CH_3$

= 4.26 + 1.00 = 5.26

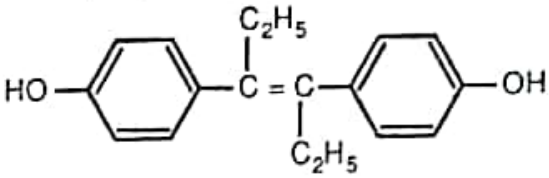
Expt. log P = 4.79

The environment of a substituent has a significant influence on its chemical properties and therefore different activity contributions may be observed for the same substituent in different positions of a molecule. The values of π are highly position–dependent. It means the π value of a given substituent will not be same for ortho, meta or para position.

Examples:

(a) Calculate log P value for an anticancer drug, diethylstilbestrol (DES).

The structure of DES is as follows:



Hence, $\log P\ (DES) = 2\pi\ CH_3 + 2\pi\ CH_2 + \pi\ CH_2 = CH + 2 \log PhOH - 0.40$

$= 2\ (0.50) + 2\ (0.50) + 0.69 + 2\ (1.46) - 0.40$

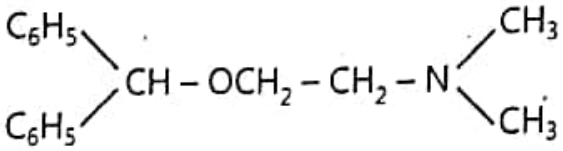
$= 5.21$ (Expt. log P = 5.07)

Here $\pi\ CH = CH = \frac{1}{2}(\pi\ CH = CH - CH = CH)$

To account for two branching points, – 0.40 is added in the equation.

(b) Calculate log P value for anti-histamine, diphenhydramine.

The structure of diphenhydramine is as follows:



Hence,

$\log P = 2\pi Ph + \pi CH + \pi OCH_2 + \pi CH_2 + \pi NMe_2$

$= 2\ (2.13) + 0.30 - 0.73 + 0.50 - 0.95$

$= 3.38$ (Expt. log P = 3.27)

Here 2.13 is log P for benzene; 0.30 is π CH (0.5 – 0.2 for branching); – 0.73 is obtained by subtracting 1.50 ($2\pi CH_3 + \pi CH_2$) from log $P_{CH_3CH_2OCH_2CH_3}$ (= 0.77); and – 0.95 is the value for $\pi\ N(CH_3)_2$.

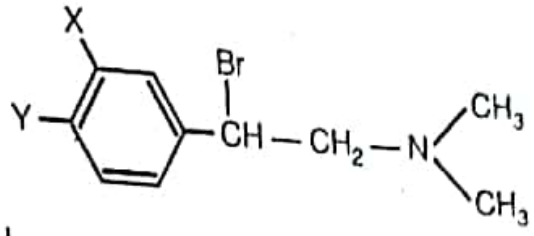
A negative value of π implies that the substituent prefers an aqueous phase while a positive value implies that an organic (lipoidal) phase is favoured by the drug for distribution.

13.8 LINEAR RELATIONSHIP BETWEEN LOG P AND BIOLOGICAL ACTIVITY

The first linear relationship was observed by Meyer and Overton who found that the narcotic activity of various organic compounds paralleled their oil-water partition coefficients. Exactly linear relationship between lipophilicity and biological activity (log 1/c) is frequently observed, especially for the binding of drugs by proteins, for drugs eliciting unspecific toxic, anaesthetic, bactericidal, fungicidal, narcotic or hemolytic properties. The straight line obtained (y = mx + c) when log P and log 1/c are plotted, can be represented by following equation

$$\log 1/c = a \log P + b$$

In such a linear relationship, the biological activity increases as the lipophilicity increases. Examples include, the antiadrenergic activity of substituted phenethylamines which can be represented by the following equation.



X = H, F, Cl, Br, I, CH_3

Y = H, F, Cl, Br, I, CH_3.

$$\log 1/c = 1.15 (\pm 0.19) \pi - 1.47 (\pm 0.38) \sigma + 7.82$$

This is an example of multiparameter linear relationship between activity (log 1/c), lipophilicity (π) and electronic (σ) properties.

Similarly, corrrelation analysis indicated that ascites cell respiration inhibition (pI_{50}) is linearly dependent on π.

$$pI_{50} = 0.46 (\pm 0.11) \pi + 3.22$$

13.9 NON-LINEAR RELATIONSHIP BETWEEN LOG P AND BIOLOGICAL ACTIVITY

Linear relationship between lipophilicity and biological activity only apply to certain range of lipophilicity. If lipophilicity exceeds a definite limit, a more or less sharp decrease of biological activity results for each series of compounds and each type of biological activity. In linear equations, the lipophilicity limits are still beyond the ranges of optimum lipophilicity. If there were no optimum lipophilicity in each series, compounds with infinite biological activity would result if only their lipophilicity were high enough.

In series of compounds, where biological activity is dependent mainly upon lipophilicity, one can not go on increasing the biological activity indefinitely by increasing lipophilicity of the compound. Activity rises to a maximum (log P_o) and then declines.

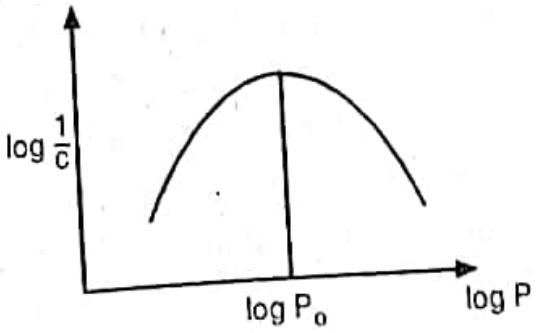


Fig. 13.4: Parabolic Relationship Between Biological Activity (log 1/c) and Partition Coefficient (log P_o)

P_o = The optimum value for the partition coefficient in the congeneric series unde nvestigation. This remains constant for that particular series.

The main reasons for the decrease in the biological activity beyond a certain range of lipophilicity, include:

(i) Because of the high lipophilicity of the drug molecule, the compound becomes so lipid-soluble that it no longer can circulate in the blood stream but merely becomes 'glued' to the first lipid membrane or macromolecule with which it comes in contact. Highly polar compounds are so insoluble in organic phases that they can not cross lipid membranes and will remain in the first aqueous phase. Hence, only compounds of intermediate lipophilicity will be able to cross lipophilic as well as hydrophilic barriers to reach their target.

(ii) Micelle formation at higher concentration of the drug, may be responsible for non-linear lipophilicity-activity relationships. While drug in dilute aqueous solution is dissolved as monomers, an increase in the concentration may lead to formation of micellar aggregates, consisting of some hundreds or thousands of molecules. Hence, the effective concentration of monomers being capable of interacting with the biological system is significantly lowered down, resulting into a decrease in the biological activity.

13.10 ORTHO EFFECT

The log P contribution by the substituent at ortho position is difficult to measure due to the following reasons:

(i) Mutual electronic interaction may occur between the substituents.

(ii) When there are two substituents 'ortho' to each other, in body fluids both such groups compete for the same layer of water molecules which forms an envelope of ordered structure around the receptor. Therefore, when an ortho disubstituted compound moves from an aqueous phase to lipid phase, the total gain in entropy of aqueous phase is less than its isomeric disubstituted compound. There occurs overall reduction in expected log P and π values. This effect is known as ortho effect.

By subtracting the value of π for para substituent from the value of π of ortho substituent, one may get an approximate estimation of the ortho effect. Thus,

$$\text{Ortho effect } (\Delta\pi \text{ ortho}) = \pi \text{ O-substituent} - \pi \text{ P-substituent}$$

Besides this, the partition coefficient of drug is affected by pH and temperature of the system. In addition to the complications arising from dissociation of acids and bases and from temperature dependence, partition coefficients of charged species can be significantly affected by the formation of ion pairs.

Single parameter is satisfactory for the quantitative description of unspecific biological activities or for small sets of congeneric compounds. However, a single parameter can not be used to describe the biological effects of a larger group of structurally diversed compounds. In such cases, electronic or steric parameters also may influence the biological activity.

$$\log (1/c) = a \text{ (lipophilic parameters)} + b \text{ (electronic parameters)} + c \text{ (steric parameters)} + d$$

13.11 ELECTRONIC PARAMETERS

Electronic parameters mainly indicate the influence of polar characters of the drug on its biological activity. They affect (i) metabolism and elimination pattern of the drug, and (ii) the drug receptor interaction. The commonly used electronic parameters are shown in the Table 13.3. In 1940, L.P. Hammett published his book on "Physical Organic Chemistry" in which he introduced - constants as a quantitative measure of the electronic effects of substituents of aromatic rings on reaction rates and equilibria. Hammett postulated that the electronic effect of a set of substituents on different organic reactions should be similar. He selected substituted benzoic acids $X\text{-}C_6H_5\text{-}COOH$ as the standard system to develop the numerical σ constant scale.

The most commonly used electronic parameter is Hammett substituent constant 'σ' which can be obtained from the dissociation constants KX and KH of the benzoic acids X-Ph-COOH and Ph-COOH respectively.

$$\sigma = \log K_X - \log KH = \log(K_X/K_H) = pKa_H - pKa_X$$

The substituent constant σ is linearly dependent on ΔG, the change in the free energy arising due to dissociation of benzoic acids.

The reason for using the logarithm of biological response has thermodynamic origins. Here $\log \frac{K_X}{K_H}$ is used instead of free energy change because equilibrium constants are logarithmically related to free energy (ΔG) change through the Van't Hoff equation and therefore additive.

Hammett proposed that an electron withdrawing group, attached to aromatic ring of benzoic acid would increase the acid strength of the carboxyl group and the greater the electron withdrawing power, the greater will be an increase in the strength. Thus electron withdrawing groups have positive values, electron donating groups have negative values and hydrogen has a zero value.

Table 13.3: Electronic Parameters

Parameters	Comments
σ_m	Hammett constant for meta substituent derived from ionisation of benzoic acid.
σ_p	Hammett constant for para substituent derived from ionisation of benzoic acid.
σ_p^-	Hammett constant used when there is direct conjugation between substituent and reaction centre; derived from anilines and phenols.
σ_p^+	H.C. Brown constant derived from solvolysis of dimethylphenyl-carbinyl chlorides.

Contd...

σ_1	Constant describing solely polar effects.
σ_R	Constant describing solely mesomeric effects.
σ^*	Taft's polar substituent constant derived from hydrolysis of aliphatic esters.
σ	Homolytic constant for substituent interacting with a free radical reaction.
F and R	Field and Resonance components derived from linear combination of σ_m and σ_p values.

Polarizability (α) plays an important role in the interaction of small molecules with proteins. In 1880, Lorenz-Lorenz derived the following equation from the electro magnetic theory of light.

$$R = \alpha = \frac{(n^2 - 1)\, M}{(n^2 + 2)\, d}$$

where,

n = refractive index of visible light,

M = molecular weight,

d = density (at the temperature quoted for n, usually 20°C).

Since linear correlations are found to exist between σ and NMR shifts (^1H, ^{13}C, ^{19}F, ^{15}N etc.), molar IR extinction coefficients or IR frequencies, the latter parameters may also be used as indicators of electronic properties of the substituents instead of σ value. Since, pKa value shows variation with temperature, the standard temperature chosen for determination of pKa value is 37°C. The pKa value is determined by potentiometry.

The pKa values for acids or bases can also be calculated from the σ substituent constant by using the following equation, because of its additive nature.

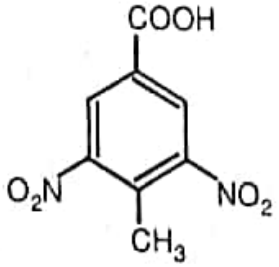
$$pKa = 4.20 - 1.00 \sum \sigma$$

This equation is known as Hammett's equation.

Problems:

(1) Calculate pKa value for 4-methyl -3, 5-dinitrobenzoic acid using Hammett equation. ($\sigma_m NO_2 = 0.71$; $\sigma_p CH_3 = -0.17$)

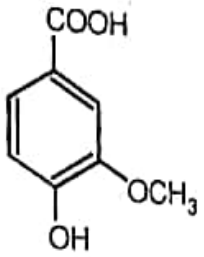
Solution:



$$pKa = 4.20 - 1.00\, \sigma$$
$$= 4.20 - 1.00\, (2 \times 0.71 - 0.17)$$
$$= 2.95 \text{ (predicted); } 2.97 \text{ (exptl.)}$$

(2) Predict the pKa value for 3-methoxy -4-hydroxy benzoic acid using Hammett equation. ($\sigma_m - OCH_3 = 0.14$; $\sigma_p - OH = -0.37$)

Solution:



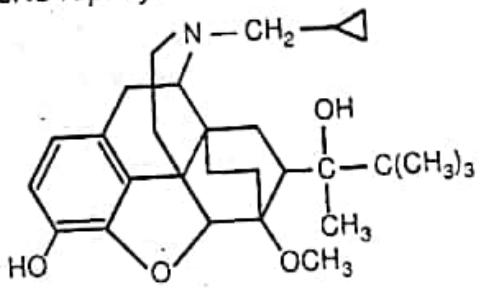
$$pKa = 4.20 - 1.00 \sum \sigma$$
$$= 4.20 - 1.00\ (0.14 - 0.37)$$
$$= 4.43 \text{ (predicted); } 4.50 \text{ (exptl.)}.$$

The negative σ value indicates, the electron releasing nature of the substituent. The F and R values indicate the sign of the charge which the substituent places on the ring. For example, $-NH_2$ by both resonance and field effects makes the ring more negative and hence both its F and R values are negative.

13.12 STERIC SUBSTITUENT CONSTANTS

Steric features of the drug markedly affect the drug receptor interactions reflecting the change in the onset and duration of biological action. For example, buprenorphine, a more lipophilic drug than morphine, is expected to enter the CNS rapidly.

Thus, it is expected to exert rapid onset and shorter duration of action. However, because of bulky substituent at nitrogen, it needs time to get oriented in a favourable conformation. The bulky substituent also delays the detachment of drug from the receptor. This leads to late onset and long duration of action.



Buprenorphine

On the guidelines provided by L.P Hammett, a numerical scale Es for the steric effects of substituents was proposed by Hammett's student, Taft in 1956.

Various parameters are used to describe the steric features of the substituents. The most common is Taft Es constant, which is derived from the acid hydrolysis of aliphatic esters.

$$\log (K/K_0) = Es$$

where,

K = rate of acid hydrolysis of substituted ester

K_0 = rate of hydrolysis of parent ester

This parameter is useful for studying intramolecular steric effects, particularly i reactions where the substituent is near the reaction centre. Other parameters like, mola refractivity (MR), van der Waals radii, molecular weight and molecular connectivity index () can be used to express steric features of the substituents.

Normally, Es is standardised to the methyl group so that Es for CH_3 group is equal to zero. However, it is possible to standardize this parameter to hydrogen i.e. Es (H) = 0.00 and there after adding 1.24 to every additional methyl group. Greater the positive value of Es, greater is the steric effect affecting intramolecular and/ or inter-molecular hindrance to drug-receptor interactions.

Other steric parameter is molar refractivity. Greater the value of the MR, larger is the steric contribution of the substituent. For liquids, the MR value can be calculated in units of volume using the Lorentz-Lorentz equation.

$$MR = \frac{MW\,(n^2 - 1)}{d\,(n^2 + 2)}\ (cm^3/mol)$$

where, MW = molecular weight

n = index of refraction at 20°C

d = density at 20°C

The third steric parameter is molecular connectivity index (χ). It indicates the degree of branching in a given structure. Since, branched isomers of molecule differ in their properties, the arrangement of substructure in the given molecule must be responsible for it. Molecular connectivity describes molecular substructures in topological terms. Correlation of the physical properties with the variation in the structure depends not only on number of atoms in the structure but also upon arrangement of these atoms. Since size and shape of the molecule determines many of the physical parameters that govern the biological activity of drug, molecular connectivity index helps to quantify the effect of size and shape on the biological response.

13.13 ACHIEVEMENTS OF QSAR

QSAR helps to understand the forces that govern the activity in a congeneric series of compounds. It thus helps to reduce the empiricism in drug-design and ensures that every drug synthesized and pharmacologically tested is as meaningful as possible. The main area where QSAR provides insight include:

(a) Forcasting of Biological Activity:

Innumerable applications of QSAR have been reported where successful prediction of biological activity played an important role. Through the regression analysis, parameters or nature and position of substituent which may increase the activity can be guessed. The advanced techniques using computerised programmes even give the structural features of the most possible active compound from the series. However, QSAR is not the final answer to drug discovery. It may be considered as one of the refined tools for drug development.

(b) Selection of Proper Substituents:

Proper selection of substituents to develop a series leads to a decrease in the average number of analogues required to investigate the relationship between substituent parameters and the biological activity. Batch selection and cluster analysis are the examples

of QSAR–techniques that help in proper design of series. Such a planning gives a good chance of finding out what combinations of parameters will optimise the potency. The analogues should vary substantially in each of the properties proposed to be important in the determination of potency. If the minimum number of analogues represent all possible combinations of parameter, decision to terminate synthesis can be taken at early state in a series that does not show promising results.

(c) Bioisosterism:

With the introduction of QSAR, the qualitative concept of bioisosterism has turned to be more quantitative and constitutive. QSAR also helps to decide an isoster which will give better pharmacokinetic and/or pharmacodynamic properties to lead nucleus.

(d) Drug–receptor interactions:

In a congeneric series of compounds, QSAR studies help to predict in quantitative terms, the forces involved in the drug-receptor interactions if the substitutions are made in non-essential part of the drug molecule. Such studies have been reported for the drugs that inhibit mammalian and bacterial dihydrofolate reductase. It is possible to derive a quantitative correlation between the strength of binding and the number and types of bonds possible. If selection of parameters is proper, QSAR may also suggest at which positions of the receptor, increased lipophilicity of drug increases binding, how changes in the strength of potential H-bonds affect binding, etc. The three dimensional feature of receptor and minimum energy active conformational forms of the drug molecules can also be predicted through QSAR-studies.

(e) Pharmacokinetic information:

The correlation between various types of parameters and the pharmacokinetic features of the drug can be done using QSAR. The passive reabsorption of substances from the urinary filtrate to decrease the total amount of drug, excreted in the urine has also been studied by QSAR.

Other recent developments in QSAR include approaches such as HQSAR, Inverse QSAR and Binary QSAR. Improved statistical tools such as Partial Least Square (PLS) can handle situations where the number of variables overwhelms the number of molecules in a data set, which may have collinear X-variables.

Environmental toxicology is also a field in which QSAR has been applied in the study of bio-concentration, toxicity of chemicals and movement through soils. QSAR has provided insight into the structure - activity pattern of taste and olfactory compounds. Drug metabolism and distribution and anesthesiology are also fields for QSAR application. QSAR also finds use in rationalizing the relative lethality of certain classes of drugs in forensic toxicology.

13.14 LIMITATIONS OF QSAR

Even though the applications of QSAR analysis may result in statistically valid equations, it is often difficult to interpret the relationship in biochemical terms. Failure of regression analysis in the prediction of biological activity of analogues results mainly due to:

(a) A poorly designed series or ambiguous regression analysis.

(b) An extrapolation outside the range of the physical properties represented by original substituents.

(c) Improper conditions of the biological testing and

(d) Multiple modes of action.

The most serious problem in QSAR is the lack of fundamental understanding of how to quantitatively describe substituent effects on non-covalent intermolecular (e.g., drug-receptor) interactions. Hence, the knowledge about the sort of interactions and quantification of substituents effect (parameter) on the interactions is essential.

A successful QSAR can provide only indirect (in terms of Es, MV or MR) information about the three dimensional aspects of drug-receptor interactions. However, mutual conformational adaptation of drug and receptor may also occur after interactions. Since, no specific parameter has yet been developed for the description of the variation in conformation, conformational flexibility or three dimensional aspects of the drug, it imposes limitations on the success of QSAR analysis.

Other effects (like electronic or steric) have their own influences on the overall lipophilicity of the molecule. This may result in the wrong correlation and interpretation of activity in a series that mainly depends upon lipophilicity for biological action. Electronic effects of a substituent may change both, the degree of ionization and the charge distribution. The former may affect the amount of active species available to the receptor while the latter may affect the strength of the drug-receptor interaction.

Since, the biological activity is susceptible to considerable experimental variations, a non-linear scatter may be observed during correlation of biological activity with physico-chemical parameters. QSAR fails to explain this built-in scatter mathematically.

Although a variety of linear parameter (about 41) descriptors of electronic features of a substituent are available, several workers have found that quite often in a particular case one electronic parameter has worked while others did not.

Similarly, physiologically active compounds on their way from the site of administration to the target sites, are known to undergo diverse chemical and biochemical transformations. It is likely that they act differently on different bio-targets to exert same kind of activity.

In summary, if the problem is to learn more about the mechanism of action of a congeneric series of compounds or to design a more active drug from the information available, Hansch or Free-Wilson approach may be useful. Best results with the Free-Wilson

approach are obtained in a series with several positions available for substitution and only if, each substituent at any location appears in atleast two compounds of the series.

Alternative statistical techniques can be used in these cases. The methods which have been used for this type of analysis include SIMCA (Soft Independent Modelling of Class Analogy), ADAPT (Automated Data Analysis by Pattern Recognition Techniques), CASE (Computer Automated Structure Evaluation) and CSA (Cluster Significance Analysis).

13.15 MOLECULAR MODELLING IN DRUG DESIGN

Different contributions to molecular interactions can be divided into two types. Bonded interactions include bond stretching, bond angle bending and torsion angle rotation. Non-bonded interactions include London-Van der Waals and Coulomb interactions. Sometimes all of the non-bonded interactions are simply called Van der Waals interactions. This force is very significant when the molecules are close and their contact surface is large.

The formula for this force is $A/(r^6)-B/(r^{12})$, where A and B are constants and r is the distance between them. Note that when the distance is very small there is a significant rejection force driving them apart. The total energy is simply the sum of all of the bonded and non-bonded interactions. These interactions can be used to calculate low energy conformations of a molecule. Energy minimization is routinely used to improve approximate structures obtained from X-ray diffraction of crystals or molecular magnetic resonance data of solutions.

Non-covalent Reactions:

Interactions between a charged ion and a neutral molecule with a dipole moment are called ion-dipole interactions. Ions can also interact with neutral moelcules with zero dipole moment. For example, the permanent dipole moment of CCl_4 molecule is zero because of symmetrical location of all four chlorine atoms at the four corners of a tetrahedron. However, a charge if placed near a CCl_4 molecule, will distort the electronic distribution and the CCl_4 molecule becomes polarized. Interactions between a charged ion and such polarized molecules are called charge-induced dipole interactions.

A molecule with no permanent dipole moment may acquire an instantaneous dipole moment due to fluctuations in the electronic distributions. This instantaneous dipole may induce a dipole in a neighbouring neutral molecule. Interactions between such dipole-induced dipoles are called London interactions. London interaction is always present in all kinds of molecules and is the only attractive force acting between identical rare gas atoms.

Van der Waals interactions are another most common type of interactions. It includes permanent dipole-permanent dipole interactions, permanent dipole-induced dipole attractions and steric repulsions. The London-Van der Waals interactions are usually non-specific forces which contribute to the energies of all reactions.

A hydrogen atom while remaining covalently bonded to one oxygen or nitrogen may form a weak hydrogen bond to another oxygen or nitrogen. The hydrogen bond plays an important role in governing the three-dimensional structures of proteins and nucleic acids.

Hydrophobic (fear-of-water) Interactions:

The molecules of water form a mobile network through hydrogen bonds with four tetrahedrally oriented neighbours. The network is not a rigid one and change of neighbours occurs rapidly because of thermal motions. A hole is created due to insertion of any other molecule into this network. Some hydrogen bonds in the original network are broken.

When two such hydrocarbon groups are inserted into water, each will lead to an unfavourable free energy change. If the two groups cluster together, the disruptive effect on the solvent network will be less than the combined effects of two separate groups. Hence, the clustering of such groups will be thermodynamically favoured. The clustering of the groups is not because they like each other but because they are both disliked by water. The clustered arrangement results in a decrease in the overall free energy of the system in comparison with the separate dispersion of unlike molecules. Hydrophobic interactions are characterised by low enthalpy (energy) changes and are entropy (conformations) driven.

Partial Charges and Dipole Moments:

A molecule is an arrangement of nuclei surrounded by electrons. The electron distribution determines the partial charge on each atom. The electron wave function for all of the electrons in a molecule tells us the electron density at every point in space. The interactions of the assigned bond dipoles produce a net dipole for a molecule. Then, by using the co-ordinates of the nuclei of the atoms, one can estimate the dipole moment.

Thermodynamics deals with interchange among different forms of energy. Interactions between ligand and receptor involve physical contact which if exceeds for a certain time period, results in an attractive force. In general, these interactions mainly depend on the concentration of reactants and salt in the solution.

Bond Stretching and Bond Angle Bending:

Bond stretching and bond angle bending can be treated as if the atoms are connected by springs. The energy of moving the atoms so as to stretch or compress a bond or to change a bond angle depends on the square of the change in bond length or the square of the change in bond angle.

$$u = K_r (r - r_{eq})^2$$

$$u = K_b (\theta - \theta_{eq})^2$$

Here, $r - r_{eq}$ is the difference between the perturbed bond length and the equilibrium bond length, and $\theta - \theta_{eq}$ is the difference between the perturbed bond angle and the equilibrium bond angle. The constants K_r and K_b are positive which means the energy increases when the bonds are perturbed from their equilibrium positions.

Stretching and compressing a bond requires a large amount of energy. For example, changing the bond length of a single bond by 0.1 A° requires about 10 kJ mol^{-1} while a double bond requires about twice as much energy.

Rotation around single bonds can cause large changes in the conformation of a molecule but it does not require much energy. The energy necessary to rotate around a C – C single bond will have maxima and minima corresponding to different orientations of the substitutents on the carbon atoms. It takes a large amount of energy to rotate around a double bond as one has to break the pi bond.

Molecular geometry is a source of information about the molecular architecture, its electronic structure and interaction mechanisms. It helps us to understand many biological or chemical processes at the molecular level. Various spectroscopic techniques like NMR and X-ray diffraction can give valuable insight of molecular geometry. X-ray diffraction is useful when the sample is in crystal state. X-ray scattering is almost entirely due to external electrons and the intensity of the scattered radiation depends upon the electron distribution within the atoms. From electron densities, a density map may be drawn where peaks indicate the location of the atoms. There are about 125000 well resolved experimentally determined crystal structures in the Cambridge structural database. The crystal structure reveals much of the conformational information of the flexible molecule. The success of Cambridge Database or the Brookhaven Protein Data Bank, are the examples of commercial application of geometrical data about organic compounds and protein derived from X-ray diffraction.

NMR derived information directly concerns with conformations of the molecules in solution.

Computer-aided drug design may either ligand-based design or the de novo design relying on the 3D structure of the macromolecular target site. The latter are usually derived by X-ray crystallography. Solved structures are available free of charge on the Brookhaven Protein Data Bank (PDB, http:// www.pdb.bnl.gox) site financed by the US government. This site also contains a number of structures obtained by 2D-NMR spectroscopy. These NMR measurements are useful to derive solution structures or structures of proteins which can not be crystallized for X-ray analysis. As the membrane bound proteins are difficult to crystallize, structural information on these proteins can be obtained using electron diffraction techniques or electron cryomicroscopy. In the absence of experimental data, macromolecular structures can be explored by homology modelling. This method utilizes the knowledge of certain degree of similarity between the primary sequences of unknown protein and the protein whose 3D structure is known. The primary sequence of all the structurally known proteins may be derived from Genbank at ncbi.nlm.nih.gox, the EMBL server at ftp.embl.heidelberg.de and the Swiss Prot server at www.expasy.ch.

Substrate conformations can also be obtained using X-ray diffraction studies and NMR experiments. The Cambridge Structural Database is the most relevant source of 3D structures of small molecules derived from neutron and X-ray diffraction data. The conformational space of the substrate can be explored by the theoretical methods as well.

Site-directed de novo drug design is based on the complementarity between the enerated ligand and its site. It can be achieved by searching for the possible structure from

a database of structure fragments which will maximally fit and satisfy the local constraints of the site. Three-dimensional database searching enables the identification of compounds that match the pharmacophoric distances or shape and electrostatic complementarity. Alignments are generated by random rotations and translations of one structure relative to the other, followed by minimization of the alignment function for each overlay. If we have n fragments and each fragment can be connected to its neighbour by m different ways, there is a possibility of m^n different combinations of fragments. Ranking according to an energy of interaction between the ligand structure and receptor site may then be used as a basis to pick up the final few combinations. Softwares commonly used in De Novo Drug Design methods include DOCK, GRID, CAVEAT, LEGEND, LUDI, SPROUT, NEWLEAD etc.

In general, for larger sites, structure generation is so diverse that detailed inspection of each structure becomes impracticable. In order to reduce the number of combinations and to potentiate the specificity of resulting combinations, larger target sites may be divided into subsets. These subsets include hydrophobic regions, hydrogen-bonding region and dividing the electrostatic potential to regions of maxima and minima. These subsets can also be surveyed energetically by a program such as GRID to identify favourable interaction sites for a large variety of functional polar groups. The software DISCO allows automatic computation of hydrogen bond accepting and bond donating sites. The combinations developed at each subset, then can be connected to generate the drug structure.

Structure-based Lead Generation:

The de novo (computational model) structure-based design is also possible where the structure of the target enzyme or receptor is known. In this case, lead generation may be done both through the application of 3D searches to identify existing compounds and by the de novo design of novel structures including automated structure generation. Various element, substructure or distance range keys (based on single atoms from the functional groups or centroids, lines, planes or excluded volumes derived from the functional groups) are used in 3D searching to screen out rapidly unsuitable structures. These keys act as a very fast filter to eliminate all structures that could not possibly fill the query.

Modelling describes the generation, manipulation, and/or representation of three-dimensional structures of molecules and associated physico-chemical properties. As computers are becoming even more powerful, new methods enabling the modelling of molecular realities have been described.

(a) Molecular structure building:

One of the simplest and most reliable ways is to use libraries of typical organic fragments and the Cambridge X-ray Crystallographic Data Base, which contains about 1,25,000 structures. Several common building functions are involved in these operations, make-bond, break-bond, fuse-rings, delete-atom, add-atom-hydrogens, invert chiral center etc. The molecular structures are generated in a 3-step process. First, molecular connectivities and atom information is entered using either an interactive computer graphics template program or a user written non-graphics program. Second, EMBED, a distance

ometry program is used to obtain three-dimensional co-ordinates. A novel feature of stance geometry is the use of random number generator with a uniform distribution to lect the internal distances so that they lie between the upper and lower bound values. nally, these co-ordinates are refined with molecular programs MM2 or AMBER, CONCORD as been also used to generate three-dimensional structures from two-dimensional structures stored in large industrial databases to provide conformations for newly veloping three-dimensional based techniques.

(b) Molecular mechanics:

With the invent of computers in 1950s, early molecular mechanics programs were developed in 1960s for certain specific compounds. Molecular mechanics deals more with the number of atoms while quantum mechanics is concerned more with the number of orbitals.

Computational chemistry techniques are now used routinely to simulate chemical and physical properties on a computer prior to synthesis. The accuracy of these calculations is highly dependent on the accuracy of the parameters employed, the solvation model used or the completeness of the conformational search.

The traditional force fields used for structural predictions, are formulated on the basis of vast experimental data (i.e. bond lengths, bond angles and other structural/energetic data) available for organic and inorganic molecules in the form of typical small fragments.

Emperical molecular mechanics calculations utilize force fields to reproduce molecular geometries, conformational energies, torsional barriers, inter- and intramolecular interaction energies, vibrational frequencies, heats of formation and other gas-phase and condensed (i.e., solid and liquid) phase properties.

Molecular mechanics consists of a series of mathematical steps used to calculate molecular geometry, energy, vibrational spectra and other chemical (e.g. electronegativity, anomeric and Bohlmann effects) properties.

Molecular mechanics (emperical force field) expresses the potential energy mainly in terms of three main groups: non-bonded energy, electrostatic energy and intramolecular energy. The potential energy reflects the energy necessary to stretch bonds, to distort bond angles and to generate strain in the torsion angles by twisting around the bonds.

Energy terms (Parameters) of molecular mechanics can be classified as follows:

(a) Bonding interactions: Stretching, bending, and torsional.

(b) Non-bonding interactions: Dispersive attractions (Van der Waal's), dipole-dipole and charge-charge interactions.

Bonding parameters can be further subdivided into equilibrium type and force constant type parameters. Equilibrium type parameters (bond length, bond angles, etc.) can be obtained easily from X-ray, neutron or electron diffraction; parameters belonging to the force constant type can be measured by microwave and IR spectroscopy. X-ray structures are suitable for the evaluation of bond lengths and bond angles. Microwave and IR

spectroscopy provide stretching and bending force constants while NMR measurements are helpful to describe torsional profiles. Among non-bonding interactions, atomic radii and E values characteristics of hardness/softness are typical van der Waal's parameters, while electrostatic interactions are usually represented by atomic charges or bond dipoles.

(A) Non-bonded energy describes the energy of interaction between two non-bonded atoms. At long distance the atoms attract each other owing to dispersion forces, whereas at short distances, there is a strong repulsion due to overlap of the atom's electronic clouds. Between the two regions there is a minimum.

Restricted rotations of molecular fragments connected through covalent bonds are qualified by torsional barriers. Dispersive attraction is usually formed between particles which do not have a dipole moment or a charge. Here the induced dipole moments are created by the distortion of electron distribution.

The electrostatic interactions depend mainly on atomic charges, the interatomic distance and a dielectric constant accounting for environmental effects. Atomic charges can be calculated by ab initio or semiempirical calculation. In addition to charges obtained from Mulliken population analysis or natural bond orbital analysis (NBO), ESP charges derived from electrostatic potential are also available. Conformational dependence of atomic charges, however, would make the calculation of electrostatics rather complicated and therefore in most force fields, this conformational effect is neglected.

In the long-range region, even electroneutral molecules exert attractive forces on each other. These forces are function of the intermolecular distances as well as of the elecronic structures. London proposed this theory of attractive forces in 1930. At any given moment, instantaneous dipoles are created because of nuclear and electronic fluctuations. These fluctuating dipoles induce dipoles in other atoms and the interaction of these two dipoles creates a net attraction.

The short-range forces are repulsive forces. When atoms are close, there is considerable overlap of their electronic clouds and these clouds are distorted owing to the Pauli exclusion principle. The net effect is a repulsion between the two atoms.

(B) Electrostatic energy mainly exists, due to the presence of highly polar groups. In such groups, the Coulomb or dipole-dipole forces are more important. They must be taken into account in conformational calculations where dielectric constant is used to explain the effect of solvent in attenuating the electrostatic interactions of charged groups in an aqueous environment.

The most widely used method for obtaining partial atomic charges is by performing a quantum mechanical calculation and doing a Mulliken population analysis. Partial atomic charges are derived by fitting to quantum mechanically calculated electrostatic potentials.

Consider a molecular force as a collection of atoms held together by elastic or harmonic forces. These forces can be described by potential energy functions of structural feature like bond length, bond angles, non-bonded interaction and so on. The combination of these potential energy function is the 'force field'. Molecular force fields help to calculate

interaction energies. These equations describe both intra and inter-molecular forces including bonding, Van der Waal's interactions and Coulombic interactions. The energy, E, of the molecule in the force field arises from deviations from 'ideal' structural features and can be approximated by a sum of energy contributions.

$$E_{total} = E_s + E_b + E_{(w)} + E_{nb} + ...$$

E is sometimes called as the 'steric energy'. It is the difference in energy between the real molecule and a hypothetical molecule where all the structural values like bond lengths and bond angles are exactly at their 'ideal' or 'natural' values. E_s is the energy of a bond being stretched or compressed from its natural bond length, E_b is the energy of bending bond angles from their natural values, $E_{(w)}$ is the torsional energy due to twisting about bonds, and E_{nb} is the energy of the non-bonded interaction. If there are other intramolecular mechanisms affecting the energy, such as electrostatic (coulombic) repulsions or H-bonding, these too may be added to force field. The most extensively tested force fields are MM2 (hydrocarbons plus a limited selection of simple heteroatom functional groups), AMBER and CHARMM (peptides and nucleic acids) and ECEPP (peptides). MM2 is current standard for small-molecule work, AMBER and CHARMM force fields are similar and are the standard for macromolecules.

Since the development of MM2, the first high-performance molecular mechanics force field in 1977, several general and specialized force fields have been published.

MM4 was obtained by the complete reparameterisation of MM3 to reduce the error that comes from the neglect of inductive and hyperconjugative effect.

General force fields are meant for handling a structurally diverse set of molecules and are therefore of limited accuracy as it would require careful parameterisation based on a very large set of reference molecules. Designing of specialized force fields is promoted for the accurate calculation of a chemical limited class of compounds. The limited diversity of structural units allows precise parameterisation for structural building blocks. This concept has been used in the development of the AMBER force filed by Kollman et al in 1985.

CHARMM is parameterized for high-quality computations of a limited set of molecules on the similar lines of AMBER. Although the first version was only parameterized for amino acids and proteins, CHARMM was finally reparameterized in Hyperchem package to yield the BIO + force field. The other force field include, OPLS, ECEPP and the Merck force field (MMFF 94). The MMFF 94 is one of the most recent molecular mechanics force field developed by Tom Halgren at Merck to handle most types of structures represented in the Merck Index.

The concept of the force field originated in the first half of the twentieth century from vibrational spectroscopy, which considered the forces acting between every pair of atoms in the molecule, or in a lattice in the case of ionic crystals. A formulation which later had a significant effect on molecular modelling was that of Urey and Bradley, in which they wrote quadratic **Hooke's Law** potential equations to describe some of the harmonic vibrations in imple molecules, but found the **Morse potential** give the best fit to empirical data for bond stretching.

Class 2 Force Fields, which contain anharmonic potentials, and utilize explicit off-diagonal terms form the force constant matrix.

The **Class 3 Force Fields** will be able to model the influence of chemical effects, electronegativity, and hyperconjugation on molecular structure and properties.

(c) Molecular dynamics:

Originated in 1957, molecular dynamics simulations were first used for the study of a simple fluid made of two-dimensional hard disks and to evaluate relaxation phenomena and transport properties in liquids.

Molecular dynamics is a method of studying the motions and the configurational space of the molecule in which the time evolution or trajectory of a molecule is described by the classical Newtonian equations of motion.

The molecular dynamics method directly simulates the motions of all the molecules in the system. The system is started in an arbitrary arrangement at a temperature near absolute zero; the atoms are nearly stationary. The velocities of the atoms are then allowed to increase so that the average kinetic energy of the system is increased to correspond to the temperature of interest, such as 300 K. At this temperature, the motion of all the atoms in the system is simulated. A great deal of computer power is needed to simulate the motion even for a few nanoseconds. The simulation studies help us to understand fluctuations in the shape of the molecule and molecule-solvent rearrangements occuring to facilitate binding of a substrate. The analysis of the motions of all the molecules can provide the free energy of the system.

Molecular dynamics aims to reproudce the time-dependent motional behaviour of a molecule. In molecular dynamics simulations, the system is first partially minimized to relieve strain in the system. Then by taking small time steps and integrating the equations of motion, new forces and accelerations are calculated.

The interactions of large molecules may be understood by simulating their motions on a computer. The entropy requires knowledge of how many ways the system can change without affecting the energy; the more rearrangements (conformations) that are possible for the system, the higher the entropy and the lower the free energy. There are two methods for calculating entropy and free energy for a system by simulating the motion of the molecules: Monte Carlo and Molecular dynamics. Both are effective methods used to calculate the energies of different possible conformations of the molecule. Consider two molecules having same minimum energy. If changes in the conformations do not raise the energy much, it is flexible molecule. On the other hand, if any change in the conformation raises the energy greatly, the molecule is said to be rigid. It means the rigid molecule will have always a higher free energy than the flexible molecule.

Two kinds of molecular simulations (behaviour of molecule model as a function of time) may be performed.

(a) Monte Carlo Method.

(b) Molecular dynamics method: It produces trajectories in the configurational space and leading to both static and dynamic properties.

Monte Carlo:

In 1950 with the publication of Barton's short note on how the conformations of steroids affect their chemistry which laid the foundation of the concept, conformational analysis.

From then onward, an appreciation of the 3-D aspect became crucial for understanding the structure, stability, conformation and reactivity. In the year 1953, a group of scientists in Los Alamos published their studies of, "equation of state calculations by fast computing machines". This work carried out on the advanced MANICAC computer, laid the ground work for computer based Monte Carlo methods, established Metropolis algorithm as simulated annealing and was the ancestor of molecular dynamics calculations.

The Monte Carlo method is named after the famous gambling city in Europe. It starts with perturbating slightly the whole conformation or a part of conformation of a large molecule step by step. After each step, calculate the change in energy. If the energy has decreased, you continue the move. If energy is higher, an algorithm is used to determine whether the new configuration is to be accepted. By repeating such moves several times, you will eventually find the lowest free energy for the molecule. The number of perturbations that do not increase the system near the minimum is a measure of the entropy. The Monte Carlo search followed by energy minimization may be used to generate pharmacophore which is further refined by energy minimization of all compounds simultaneously using the Multifit program.

In Monte Carlo technique, several million configurations are generated. Using these configurations, the average of some desired property can be calculated using further equation.

$$x = \frac{\sum_i x_i \exp(-E_i/kT)}{\sum_i \exp(-E_i/kT)}$$

where, x is the desired property, x_i is the value of the property in the configuration i, E_i is the energy of the configuration, k is the Boltzmann constant and T is the temperature.

The knowledge of the accessible structures and insight into accessible spatial relationships is gained through structures of minimum energy, dynamic trajectories of analogues (molecular dynamics) or a series of Monte Carlo configurations. This allows the chemist to judge the importance of a given conformation in interacting with the receptor. New analogues with certain conformation to enhance the stability or potency then may be synthesized.

In principle, molecular dynamics simulations can be used to describe many of the kinds of events involved in drug-receptor interactions, including the solvation and conformational changes required for initial complex formation, and any conformational or covalent rearrangements that may occur subsequent to binding. Molecular dynamics is used simply as a powerful method for generating the samples of thermally accessible molecular configurations that are needed in calculations of entropies, enthalpies and other thermodynamic quantities. The molecular dynamic calculations can be used, for example, to

predict how changes in the chemical structure of a drug will change the equilibrium constant for binding to a receptor if a high resolution structure of the original drug-receptor complex is available.

Molecular dynamics produces a great many molecular conformations. Through docking studies, one can know about the number of unfavourable conformations of the drug which do not fit the receptor. This number may be drastically reduced by inserting a methyl "blocking" group or a ring constraint in the structure to prevent too much conformational freedom. The most favourable drug conformation chosen through the results of a dynamics may be subjected to molecular mechanics.

(d) Quantum mechanics:

Molecules are made of electrons and nuclei. The nuclei attract the electrons but, of course, the electrons repel each other and the nuclei repel each other. The electrons move relative to the nuclei. The balance of the forces determines the structure and the chemical reactivity of each molecule. The nuclei and electrons arrange themselves to obtain the lowest-energy possible. This produces the bonding and the electron distribution of the molecule in its most stable form. Higher energies correspond to excited states of the molecule caused by molecular collisions or by photoexcitation; these states are important in the reactions of molecules. The energies of molecules are quantized.

Quantum mechanics is necessary to describe quantized energy levels and to understand bonding and electronic orbitals of atoms and molecules. Quantum mechanics was introduced during 1930s to describe molecular and subatomic behaviour.

To understand reaction mechanisms and molecular interactions, one should know in detail about bonding, orbitals, electron distribution and charge densities. Quantum mechanics explain molecular interactions in terms of electron distribution and motion.

The nuclei of atoms may be treated as particles with wavelengths that are very much shorter than those of the lighter and faster electrons. The Schrodinger equation may be solved by using widely available ab initio programs and an ever increasing computation power. The Schrodinger equation assigns an amplitude ψ to the electron wave which is known as the wavefunction of the system. It helps to calculate the average position of the electron and its energy in each electronic state. The energy of the electron tells us whether the molecule is stable and what is stable bond distance is. From the wavefunctions, measurable properties can be calculated. These properties are functions of the positions and moments of the electrons and nuclei in the molecule. The electron distribution determines what is the most stable configuration or geometry of the molecule besides bond lengths, force constants and bond angles.

Calculation of electronic properties implicated in physical and chemical reactions of drugs with their biological environment can only be done using quantum mechanical methods. In addition, calculation of energy conformational profiles and intermolecular interactions in a variety of contexts are best done using quantum mechanical methods. In principle, the exact solution of the Schrodinger equation, where H is the Hamilltonian

..., y is the wavefunction and E is the energy of the system, would yield a complete ...on of a molecular system.

$$H_y = E_y$$

...e Schrodinger equation of a given molecular system can be solved either with no ...ximations at all (ab initio) or with the introduction of some approximations (semi-...rical). In most ab initio methods, all electrons are explicitly included. Ab initio method ...e advantage of not requiring any parameterization and therefore can be used for all ...s of systems.

...t is also much easier to identify failings of these methods and improve them in a ...eptually consistent and even-handed way. In semi-empirical method, only valence ...trons are explicitly included, some integrals are neglected and others are approximated ...parameters derived from experiment. The selection of the most appropriate method ...pends not only on the size of molecule but also on the type of molecular property ...g. conformation, electron density, electrostatic potential, frontier orbitals etc.) that is ...rived.

Quantum mechanics defines the behaviour of nuclei and electrons. The quantum ...echanics is based on the Schrodinger equation. The wavefunction derived from ...hrodinger equation, contains all the information needed to describe the properties of ...omic and molecular systems.

Besides the classical Schrodinger equation method, certain semi-empirical methods may ...lso be used to calculate the wavefunctions of valence electrons only. These include,

(a) Extended Huckel Method.
(b) Complete Neglect of Differential Overlap (CNDO) Method.
(c) Intermediate Neglect of Differential Overlap (INDO) Method.
(d) Modified Intermediate Neglect of Differential Overlap (MINDO) Method.
(e) Neglect of Diatomic Differential Overlap (NDDO) Method.
(f) Modified NDDO (MNDO) Method.

Semi-empirical treatments such as AMI, MNDO, CNDO, INDO, EHT, MINDO, PRDDO and PCILO are some of the most popular semi-empirical programs, whereas the GAUSSIAN and HONDO series are typical ab initio programs. AMPAC and MOPAC are QCPE packages that include the AMI, MNDO and MINDO programs. Along with GAUSSIAN series these are among the most popular programs for quantum mechanical calculations. Quantum chemical calculations can provide detail insight into the electronic nature of the molecular structure.

Quantum mechanics offers a much better description of electronic structure than molecular mechanics can ever do. Energy of the highest occupied molecular orbital represents charge distributions in a molecule. Quantum mechanics helps in the electrostatic potential energy evaluation directly using the wavefunction as opposed to using the point charges extracted from the wavefunction. If one computes this energy at the points generated from a molecular surface calculations, the resulting colour-coded surface can be

displayed. The surfaces of drug and receptor protein can be visually compared and evaluated. The optimal docked orientation will be that in which the two surfaces are optimally complementary in shape and charge distribution.

(e) Conformational analysis:

The energy treatment in this approach to QSAR resembles a linear free energy model or Free-Wilson analysis; the added feature is the geometric constraints during the fitting of the data so that the ultimate outcome is a geometric interpretation of the biological activity. In the distance geometry approach, one constructs a geometry of the receptor site from the drug molecular structure and subsequently evaluates the interaction energy matrix so that the given binding mode for each molecule is its optimal binding mode. The method generally focus on the comparison of chemically similar analogues, where it is clear that a substantial subset of the atoms of one drug molecule match corresponding subsets in the other molecule. In reality, however drug molecules bind in whatever orientation and internal conformation will minimize the drug-receptor-solvent system. The distance geometry calculations directly simulate this search for the most favorable binding mode and rather similar compounds may bind quite differently.

Starting from an arbitrary initial conformation, a specified number of attempts, N, are made to generate a random ligand conformation. In each attempt, all rotatable bonds are subjected to a quasi-random change in torsion angle. The possible values for change in torsion angle about a bond are based on the total number of rotating atoms.

The initial docking of the ligand is obtained by alignment of the principal axes of the ligand to the principal axes of the site. There are four possible orientations to be considered. There are two energy terms in the expression for the dock energy, internal energy of the ligand and the interaction energy of the ligand with the protein. The interaction energy is taken as the sum of the Van der Waals energy and elecrostatic energy.

The RMS calculation takes topological symmetry into account and automatically associates atom pairs between the X-ray structure and the docked structure to report the best RMS for all possible topologically equivalent pairings.

(f) Physical properties:

Theoretical calculations can provide a number of indices that may not be directly related to experimental data but that can be very useful since they carry high physical information content. For example, electron densities are useful because they provide a good basis for the stereoelectronic properties of either isolated or interacting molecules. Molecular electrostatic potentials are usually generated from the partial atomic charges derived from a quantum mechanical calculation. Other properties can be calculated by empirical methods; the most popular are the prediction of log P (octanol/water partition coefficient) and molar refractivity.

Distance Geometry:

Distance geometry refers to the study of geometric problems with an emphasis on distance between points. The use of interatomic distances or atomic co-ordinates as representative of molecular shape is common in 3D QSAR. Distance geometry permits both, projection of important interatomic distances in the ligand molecule and postulates the ideal intermolecular binding distances in ligand-receptor interaction.

It has been used extensively to generate molecular structures from NMR data, for NOESY cross-peaks can be translated into distance constraints. Distance geometry is also used to search the conformational space of the ligand by specifying artificial constraints.

13.16 APPROACHES TO MOLECULAR DOCKING

(I) Shape Complementarity Methods:

Geometric matching/shape complementarity methods include molecular surface/complementary surface descriptors. In this case, the receptor's molecular surface is described in terms of its solvent-accessible surface area and the ligand's molecular surface is described in terms of its matching surface description. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Whereas, the shape complementarity based approaches are typically fast and robust, they cannot usually model the movements or dynamic changes in the ligand/protein conformations accurately, although complementarity methods are much more amenable to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.

(II) Simulation Processes:

In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein's active site after a certain number of "moves" in its conformational space. The moves incorporate rigid body transformations such as translations and rotations, as well as internal changes to the ligand's structure including torsion angle rotations. Each of these moves in the conformation space of the ligand induces a total energetic cost of the system, and hence after every move the total energy of the system is calculated. This process is physically closer to what happens in reality, when the protein and ligand approach each other after molecular recognition.

The success of a docking program depends on two components; the search algorithm and the scoring function.

(III) The Search Algorithm:

The search space consists of all possible orientations and conformations of the protein paired with the ligand. This would involve enumerating all possible distortions of each molecule and all possible rotational and translational orientations of the ligand relative to the protein. Each "snapshot" of the pair is referred to as **Pose.**

(IV) The Scoring Function:

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favourable binding interaction.

Most scoring functions are physics based molecular mechanics force fields that estimate the energy of the pose, a low (negative) energy indicates a stable system and thus a likely binding interaction.

Global energy optimization can be accomplished using simulated annealing, the Metropolis algorithm and other Monte Carlo methods or using different deterministic methods of discrete or continuous optimization. The main aim of optimization methods is finding the lowest energy conformation of a molecule or identifying a set of low-energy comformers that are in equilibrium with each other. The force field represents only the enthalpic component of free energy, and only this component is included during energy minimization.

Other applications of MM include potential energy mapping and ligand docking simulations.

MM implements more 'static' energy minimization methods to study the potential energy surfaces of different molecular system. However, MM can also provide important dynamic parameters, such as energy barriers between different conformers or steepness of a potential energy surface around a local minimum. MD and MM are usually based on the same classical force fields. But MD may also be based on quantum chemical methods like DFT.

13.17 MOLECULAR SIMILARITY IN DRUG DESIGN

Drug Design is a multifaceted discipline where molecular similarity is one important tool to dig out new ideas for design. Molecular similarity involves the process of searching the features of similarity in a set of flexible and dissimilar active molecules and use that informtion to design novel drug molecules. Similarity, may be searched for bonding patterns, atomic positions, conformations, electrostatic potential, shape and spatial display of molecule or molecular properties. The result is usually expressed by a similarity coefficient. A least-square fit method is used to find the optimal superimposition for each acceptable combination. Apparently a lower root mean square (rms) deviation between the structures so matched, reveals the better correspondence. The superimpositions so generated by matching programs can be used directly to predict pharmacophore. This pharmacophore with a distance matrix between pharmacophoric points, then can be used to search a 3D structural database for novel structures that fit the query.

The shape of a molecule is represented by its Van der Waals volume. The degree of molecular shape change, that is possible if a molecule is related to the number and position

the rotatable bonds. Rotation and translation of one molecule relative to the other is performed. Many softwares are developed that allow electrostatic potential comparisons of conformationally frozen structures of drugs containing bioisosters.

The atom correspondences or functional group similarities revealed through similarity studies, are usually expressed as points in space with associated properties. A distance matrix is constructed between the points. Alternatively, a novel structure with similar shape may be constructed through a space filling network of bonds with appropriate angles and bond lengths to create molecular skeletons without atomic identities. Rotation and translation of one molecule relative to the other is performed. This matrix we call an envelope in which structurally diverse ligands can be generated. Automated structure generation is also possible if the matrix with complementary shape and electrostatic properties is generated. Atoms may then be incorporated into this network to create molecules with the desired similar properties. The optimization can be achieved by annealing an atom placement procedure from a small library of acyclic and aromatic pieces of combinations of atoms from the set. Not all the molecular surface of the ligand is involved in activation of the receptor site. Hence, optimization of the 'active' surface is critical to yield a novel drug structure.

The properties of a molecule are intimately linked to the 3D structures or conformations that it can adopt. Consideration of the conformational properties (i.e. the energy of a given conformation) of a molecule is therefore essential in any drug design method.

The hypothesis, 'similarity in behaviour implies similarity in structure' highlights the importance of the comparison of structural characteristics in QSAR. Computer graphics allows automated detection of the degree of overlap between given molecular shapes and the common patterns. If molecules to be compared are not closely related, it sometimes becomes difficult to decide how to superimose such ligands to identify common binding interactions with the receptor involved. Molecular similarity studies are also useful to screen large 3D databases in the search of leads containing desired structural features.

Detection and evaluation of the similarity between "structural shapes" (steric complimentarity) and "electronic shapes" (electronic characteristics such as electrostatic potential or electron distribution) are more useful than structural similarity in realistic comparison of chemical or biological activity.

Steric fit plays an important role in recognizing "lock-and-key" models of molecular interactions involved in pharmacophore identification. Encoded or coloured surfaces with reference to the value of property such as electrostatic potential and hydrophobicity may be used to obtain a better insight to judge chemical complementarity. The Van der Waal's contour (or volume) gives a good estimate of the molecular shape for small molecules.

13.18 PHARMACOPHORE MAPPING

The pharmacophore is often described only by a set of distance constraints between atom centers or centroids of a defined environment (normally hydrogen bond acceptor/donar, acidic, basic, aromatic ring / hydrophobic centroid). A pharmacophore highlights the set of "features" a compound must have to elicit a certain biological activity.

The program DOCK, developed at the University of California, San Francisco identifies molecules that are complementary in shape and chemical interactions to a user-supplied receptor structure.

The hardest task to ask of a docking computation is to predict the best binding geometry of a ligand, for this involves assessing the relative free energy of several alternative binding modes.

Many structure based approaches involve the combination of developing a phamacophore hypothesis and then searching 2D or 3D databases. The pharmacophore may be derived from crystallographic information of the binding site or from superimposition of a lead molecule on the NMR structures of peptides in solution. Variety of programs including Chem DBS-3D, ALLADIN UNITY, MACCS-3D, CATALYST, CAVEAT, CSD (Cambridge Structural Database), etc. are used for quick searching of a database of 3D structures.

Both known and novel binding sites can be identified through automated procedures, such as negative imaging approach used with DOCK. The potential pharmacophore points such as hydrogen bond acceptors or donars, positively charged atoms and aromatic ring centroids may be identified. The distances between pairs of these points (distance keys) and data on the number, types and interpoint connectivity (formula keys) may be used as guidelines for pharmacophore mapping. Pharmacophore mapping attempts to find features important for receptor binding. Tracing of pharmacophore pattern in large, more complicated structure can be done by investigating the full structure part by part. Alternatively, the use of structurally rigid molecules (i.e. conformationally restrained analogues) is preferred to probe requirements for receptor binding. Occassionally, there may be several binding modes with similar energy that may be difficult to distinguish.

Pharmacophore mapping may be used for de novo compound design. In the program, NEWLEAD, key fragments from bioactive conformations are joined with spacers to generate new structure to fit the model. While the program SPROUT helps to join the templates, such as five and six-membered rings and acyclic fragments to mimic pharmacophore model.

QSAR analysis is applied to structure-activity data sets only in such cases where molecular geometry of a common receptor is unknown. If the receptor geometry is known, intermolecular locking is usually performed to the exclusion of a QSAR analysis.

through multiple linear regression analysis, QSAR helps to develop functional relations ween biological activity and 2D/3D physico-chemical molecular properties.

The affinity of two molecules that form a non-covalent complex is described by the erall change in the free energy of the system. The system consists of the molecules and lvent (before complex formation) and the complex and solvent. Free energy is the total ergy of the system and includes both enthalpy and entropy.

The types of interaction energies important in drug (ligand)-receptor interactions clude,

(a) Intramolecular ligand conformational energy.

(b) Ligand solvation energy.

(c) Intramolecular receptor conformational energy.

(d) Receptor solvation energy.

(e) Solvent reorganization energy.

(f) Intermolecular ligand-receptor energy.

All above contributions to free energy of binding can be calculated separately and they are additive.

Any 2D/3D QSAR will not be meaningful unless it accommodates or represents all above types of interaction energies.

13.19 COMBINATORIAL CHEMISTRY

Combinatorial Chemistry is a new subfield of Chemistry with the goal of synthesizing very large number of chemical entities by condensing a small number of reagents together in all combinations defined by a given reaction sequence. It is also referred as 'matrix' Chemistry. If a chemical synthesis route consists of three discrete steps, each employing one class of reagent to accomplish the conversion, then employing one type of each reagent class will yield $1 \times 1 \times 1 = 1$ product as the result of $1 + 1 + 1 = 3$ total reactions. While conceptually simple, considerable strategy is required to identify 1,000,000 products worth making and to carry out their synthesis in a manner that minimizes labour and maximizes the value of the resulting organized collection, called a 'chemical library'.

Combinatorial Chemistry or 'molecular diversity' was first popularized by Merrifield in the 1960s who received a Noble prize for his work on solid-phase peptide synthesis (SPPS). The 1970s visualized the emergence of solid phase non-peptide synthesis. It was in the 1980s, primarily peptides or oligonucleotides and recombinant protein or nucleic acid based technologies developed. The need for Combinatorial Chemistry is underscored by the high cost, long time frame and high rate of failure in research and development for new drug. Today, the preferred methods for building libraries are parallel synthesis, split synthesis and

a combination of the two. Parallel synthesis is in essence an automated form of the traditional 'one at a time' approach. It can use either solution or solid phase Chemistry. For split synthesis a range of synthons or reactants are used at each step, in principle, giving a library in which every possible compound from every possible combination of serial step using these synthons, is created.

(i) Solid Phase Synthesis:

In this method, the reacting molecules are covalently bound on a solid support material. The starting material is protected at all reactive functional groups. The product is synthesized step-by-step in a single reaction. The order of reactions can be controlled by the order of deprotection of reactive functional groups. This method is used for the synthesis of pepides. The advantages of solid phase synthesis include the ability to use excess reagents to push reactions to completion and the easy purification of the resin bound product through filtration and washing.

(ii) Solution Phase Synthesis:

Because of the often tedious isolation and purification, the main problem of solution phase combinatorial synthesis is to obtain pure products.

When designing a combinatorial mixture library for lead identification, it is desirable that the compounds within the library can be as diverse as possible, to fully explore the scope of activity against the target. However, the design of a library should take into account many other factors, not least among them is the effort needed to deco volute the mixtures, once hits are obtained. Factors such as cost and availability of reagents or the range of physical properties of library products may also require optimization. Furthermore, in the design of a mixtures, the combinatorial constraint always applies, that is, every subsistent at each position will occur in combination with every subsistent at all other positions. Each of these additional considerations may mean that a certain amount of possible diversity in a library has to be sacrificed.

Library Size, Design and Selection:

Compound libraries need to serve two distinct functions in the drug discovery process, lead identification and lead optimization, which dictate their size and composition. Thus, when a lead has been identified from screening, rapid analog synthesis is performed to provide optimization of both potency and selectivity. Ideal libraries to address this lead optimization phase will invariable be smaller in size than libraries used for lead identification and consists of individual components (≤ 1000 discrete), and can be prepared by parallel synthesis around the lead.

The realization that analog library synthesis impacts the time for the lead optimization phase of drug discovery, is now being extensively exploited by medicinal chemists. Industries such as Pfizer and Lilly have described their capability to optimize lead structure in 6-12

time framed. Pfizer has reported an analog synthesis of an antiatherosclerotic drug 100 fold increase in potency, while Lilly has described the optimization of a 5 HT agonist, for development of a potential new antimigraine drug.

Another function of compound libraries is to provide a range of leads, preferable of different structural types, to initiate the drug discovery process. One simple approach is to focus on structural motifs of molecules of proven efficacy. A good example is, the benzodiazepine libraries with diverse biological activities were discovered and this encouraged many groups to devise libraries around other structural motifs which potentially offer other activity profiles.

Synthetic libraries that have been targeted more specifically at a particular family of receptor or enzyme, by incorporating a key recognition element for binding, are also available. One such an example is the hydroxamate libraries of metalloproteinase inhibitors.

For novel targets, there is the requirement to device libraries of fundamentally distinct structures which offer the best chance of finding novel screening hits. The synthetic hurdles to such libraries are not inconsiderable and it is not surprising, therefore, that much more emphasis has been given over the past two years on applying software design techniques to enhance the content of such libraries, while constraining the size. One of the elements of the design that reflects library composition is molecular diversity.

In a new approach, the concept of libraries for information was proposed, wherein the informatory molecules, used to provide finger print of the structural requirement of the target, have been designed on promiscuity rather than diversity. The overall description of a promiscuous molecule is its proven ability to interact with a wide range of targets. Subsequent libraries are designed and synthesized to allow the separation and identification of the specific binding characteristics that are relevant to a particular target under study and this is achieved by an iterative but convergent approach using experimental design and testing.

Library Diversity: One approach to produce diverse library products has been to ensure that the set of precursors used to construct the library are as diverse as possible. If all the suitable precursors for a given diverse site are grouped on the basis of some desirable features, such as distribution of potential pharmacophoric points, then selecting no more than one from each group should ensure that the set is diverse. To produce a diverse library, it is therefore desirable to assume that every precursor is taken from different group.

A recent study has suggested that maximizing diversity among the precursor set may not necessarily give the most diverse possible set of library products. An alternative is to consider the diversity among the library products themselves. This may be measured by enumerating the library products and either clustering them and attempting to pick as few compounds as possible from each cluster or using a cell-based partitioning method attempting to pick as few compounds as possible from each cell.

13.20 DECONVOLUTION

A number of solutions have been suggested to the problem of deconvolution. These include tagging beads with various types of chemically and spectroscopically readable labels or producing libraries on silicon chips whose identities can later be determined by radiofrequency scannings.

Once this information is known, selective synthesis and testing of all the compounds in the library with that molecular weight will be required. Since, it is desirable to keep this work to a minimum, it is sensible to design a combinatorial library to have the smallest possible number of compounds of any one molecular weight, given the constraints of the required size of the library and the availability of the precursor. For a given number of library products all having the same molecular weight, deconvolution will be simplified further if substituents used at the diversity sites have different molecular weights.

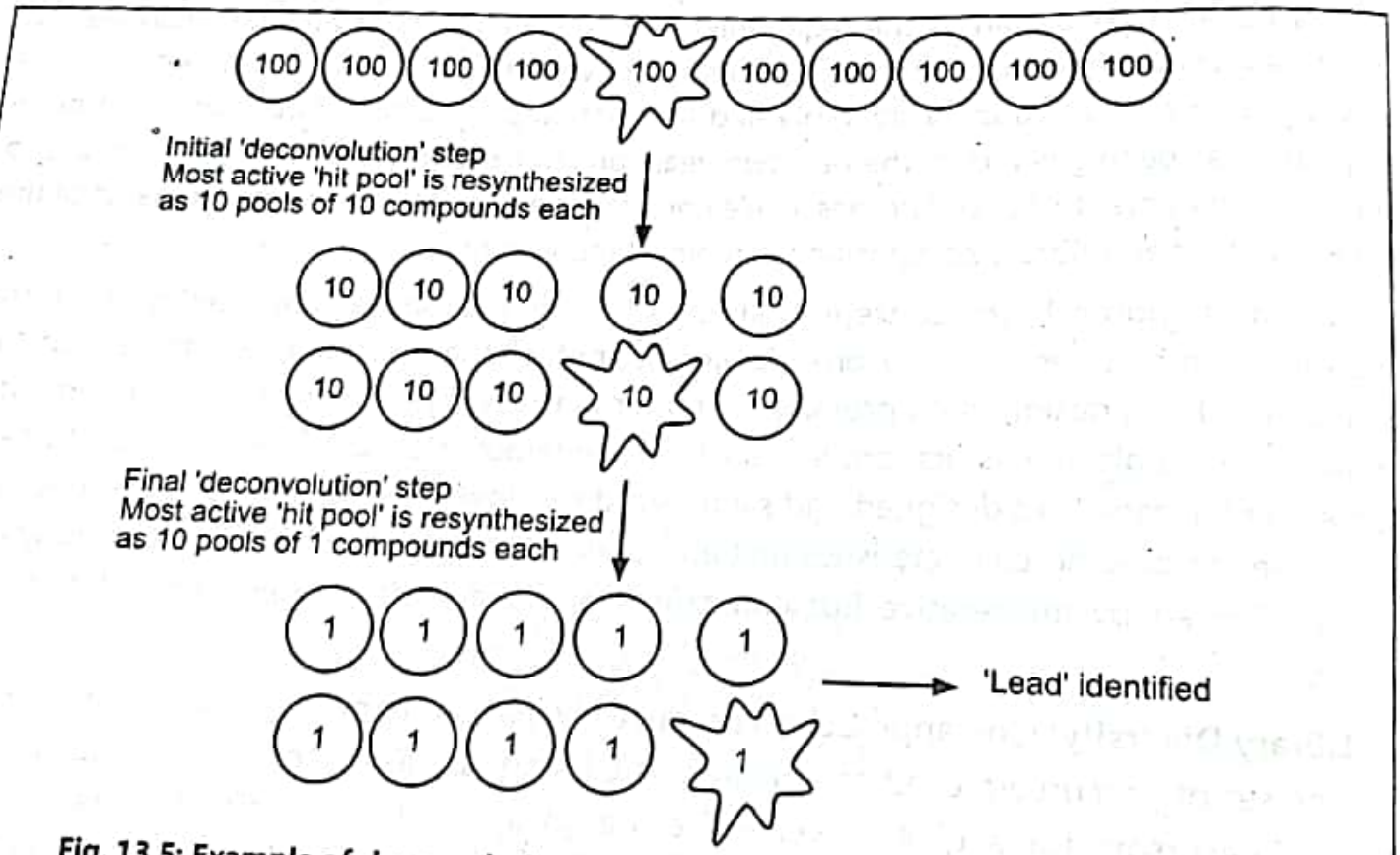


Fig. 13.5: Example of deconvolution process; repeated resynthesis of subsets of hit pools results in the identification of the active compound(s) in a mixture

It is therefore desirable to minimize the substituent molecular weight redundancies for each given product molecular weight. In a 'mix and split' strategy for combinatorial synthesis, the pools of compounds that result from the addition of the final diversity sites are often not mixed. Since, these pools are screened individually the deconvolution problem applies to each subpool separately. A library design strategy must therefore be able to suggest pools within each of which there is the minimum products and substituent molecular weight redundancy.

Problem Size: The number of precursors that are suitable for use in combinatorial mixture library is often larger than the number that can be reasonably be used. Hence, typical mixture library design problems have huge search spaces, the size of which are best illustrated by an example.

In a library, two sites of diversity were available, there were 360 commercially available precursors compatible with the chemistry for R_1 and 259 for R_2. There are therefore, 92, 240 possible library product compounds. The design of this library requires production of 10,000 compounds by combining 100 R_1s with 100 R_2s. The number of ways of selecting k objects from n is:

$$^{n}C_{k} = n!/(n-k)!\,k!$$

And so the number of libraries is

$$^{350}C_{100} \cdot {}^{259}C_{100} = 2.5 \times 10^{164}$$

13.21 ADVANCES IN COMPOUND LIBRARY PRODUCTION

Library design is complex problem, requiring the optimization of a number of often competing factors, over a vast search space. Genetic algorithms have been successfully applied to a wide range of such problems in both chemical and non-chemical domains.

A genetic algorithm is a computational technique that mimics the process of Darwinian evolution. A potential solution to a problem is encoded in a representation termed as chromosome. This is typically a string of bits, integers, real numbers or symbols each of which is termed as gene.

A genetic algorithm operates on a population of these chromosomes that are generated by assigning values to the genes in chromosomes, often at random. A fitness function measures how well adapted each chromosome is to its environment.

There are two pre-requisites to being able to apply a genetic algorithm to a problem. The first is to choose a representation that allows every possible solution to the problem to be encoded in a chromosome. The second is that it must be possible to write a fitness function to decode the chromosome and produce a score that reflects the quality of that solution.

A most promising new approach to drug discovery concerns the synthesis in one-pot reaction, without isolation or purification and the reaction mixture is screened using a competitive binding assay based on pulsed ultrafiltration, electrospray mass spectroscopy (PUF/ESMS) which tentatively identify those derivatives having the highest affinity for the target receptors.

As a model system to test this approach, a synthetic scheme designed to prepare a series of analogs of the adenosine deaminase inhibitor, erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), as diastereomeric mixtures, was carried out. Pulsed ultrafiltration screening of the crude reaction mixtures against controls without protein, detected protonated

molecules corresponding to EHNA-type derivatives and three of its linear, alkyl homologues. It did not show protonated molecules for an isobytyl or benzylic EHNA derivative, suggesting the latter was inactive.

An important feature of combinatorial chemistry is the synthesis of compounds on solid support allowing "split and pool" methodology to be employed for library construction. The method involves the use of an appropriate linker to tether the initial starting substrate to solid support. The linker needs to be stable during the synthesis phase, but capable of facile cleavage to free up the final product.

The ideal linker would be one capable of product release with formation of a carbon-hydrogen bond in place of resin attachment, thus leaving behind no memory of the site of attachment on the solid phase support.

Classes of potential drugs synthesized recently by using solid phase techniques include 1,4-dihydropyridines and polyisoxazolines. A more general separation technique, which introduces the concept of third phase, the so called fluorous phase relies on the preferential partitioning of heavily fluorinated substrates into fluorinated solvents such as FC-72, which can then form a third phase separable from both, the aqueous phase and common organic solvents.

In library synthesis, either reactant or product can constitute the pre-fluorinated substrate which then is separated from organic or inorganic contaminants by liquid-liquid extraction via the fluorinated solvent. Thus spectroscopy and chromatography can be used to both, monitor analytical purity and as a preparative tool to isolate purified product.

In conclusion, the synergy of structure-based design with combinational synthesis is an obvious marriage in enhancing all technologies. As can be seen, there are many design strategies for lead generation libraries. All share certain aspects in common, notably the desire to reduce the physical size of the library while maintaining or enhancing the information content.

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