ANTIBIOTICS

Antibiotics

Defination-'An antibiotic or an antibiotic substance is a substance produced by the microorganisms, which has the capacity of inhibiting the growth and even of destroying other micro-organisms'

Classification of Antibiotics-

1. **B-*lactamantibiotics:*** *Penicillins, Cephalosporins,* Monobactams, Carbapenems.
2. **β-lactamase inhibitors**—Clavulanic acid, Sulbactam, Tazobactum.
3. ***Tetracyclines:*** *Oxytetracycline, Doxycycline, etc.*
4. ***Nitrobenzene derivative:*** *Chloramphenicol.*
5. ***Aminoglycosides:*** *Streptomycin, Gentamicin,* Amikacin, Neomycin, etc.
6. ***Macrolide antibiotics****: Erythromycin, Roxithromycin,* Clarithromycin, Azithromycin.
7. ***Lincosamide antibiotics****: Lincomycin, Clindamycin.*
8. ***Polypeptide antibiotics:*** *Polymyxin-B, Colistin,* Bacitracin, Tyrothricin.

Classification of Penicillin

1. **Acid-resistant alternative to penicillin G**-Phenoxymethyl penicillin (Penicillin V).
2. **Penicillinase-resistant penicillins** -Methicillin,Cloxacillin.
3. **Extended spectrum penicillins**

(**a) Aminopenicillins**:-Ampicillin, Bacampicillin,Amoxicillin.

(b) Carboxypenicillins: Carbenicillin, Ticarcillin.

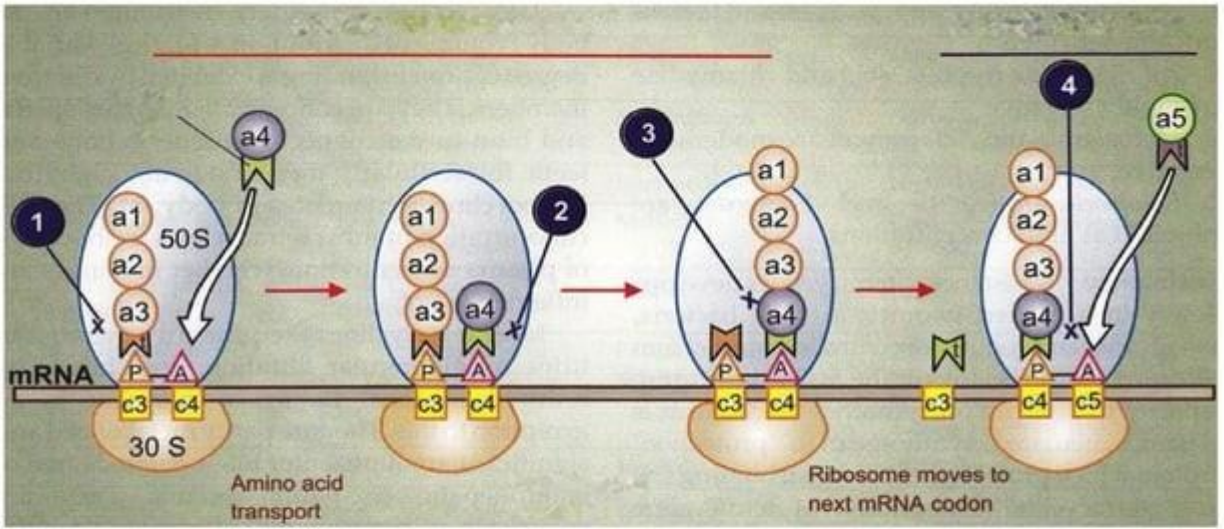
(c) Ureidopenicillins: Piperacillin, Mezlocillin.

4.B-lactamase inhibitors -Clavulanic acid,Sulbactam, Tazobactam

Classification of Cephalosporins

First generation cephalosporins	
Parenteral	*Oral*
Cephalothin*	Cephalexin
Cefazolin	Cephradine
	Cefadroxil
Second generation cephalosporins	
Parenteral	*Oral*
Cefuroxime	Cefaclor
Cefoxitin*	Cefuroxime axetil
Third generation cephalosporins	
Parenteral	*Oral*
Cefotaxime	Cefixime
Ceftizoxime	Cefpodoxime proxetil
Ceftriaxone	Cefdinir
Ceftazidime	Ceftibuten
Cefoperazone	Ceftamet pivoxil
Fourth generation cephalosporins	
Parenteral	
Cefepime	
Cefpirome	

MOA of Antibiotics



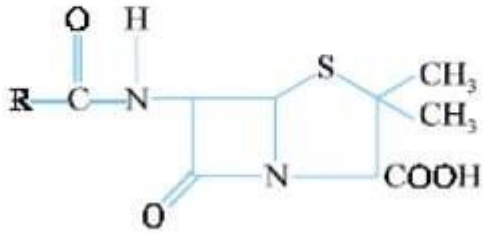
(1) Aminoglycosides bind to several sites at 30S and 50S subunits as well as to their interface-freeze initiation, interfere with polysome formation and cause misreading of mANA code.

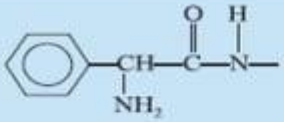
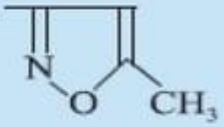
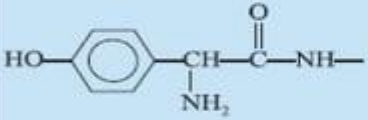
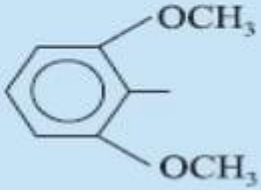
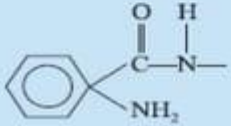
(2) Tetracyclines bind to 30S ribosome and inhibit aminoacyl tRNA attachment to the 'A' site.

(3) Chloramphenicol binds to 50S subunit-interferes with peptide bond formation and transfer of peptide chain from 'P' site.

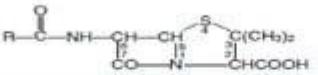
(4) Erythromycin and clindamycin also bind to 50S ribosome and hinder translocation of the elongated peptide chain back from 'A' site to 'P' site and the ribosome does not move along the mANA to expose the next codon. Peptide synthesis may be prematurely terminated.

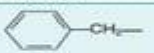
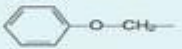
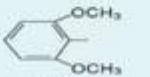
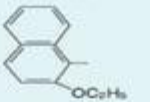
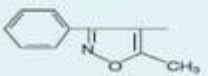
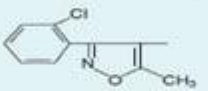
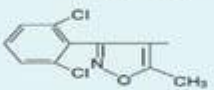
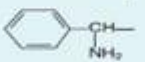
Penicillins

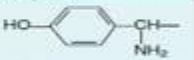
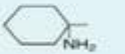
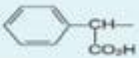
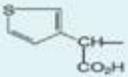
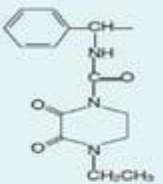
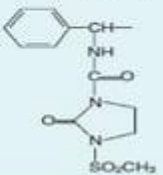


Name	R	Name	R
Ampicillin		Cloxaxillin Sodium	
Amoxicillin Trihydrate		Methicillin Sodium	
Ciclacillin			

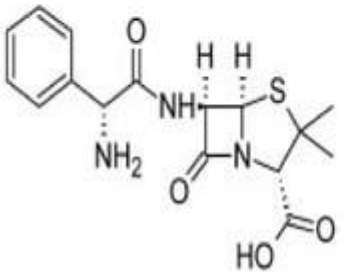
Structure of peniciilin and its derivatives



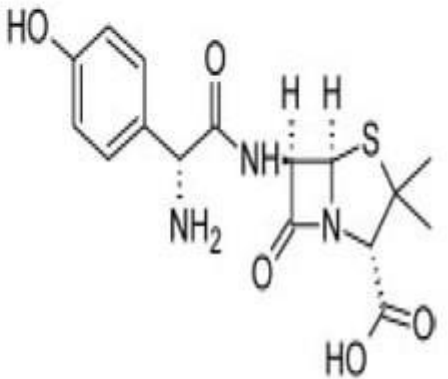
Generic Name	Chemical Name	R Group
Penicillin G	Benzylpenicillin	
Penicillin V	Phenoxymethylpenicillin	
Methicillin	2,6-Dimethoxyphenyl-penicillin	
Nafcillin	2-Ethoxy-1-naphthyl-penicillin	
Oxacillin	5-Methyl-3-phenyl-4-isoxazolylpenicillin	
Cloxacillin	5-Methyl-3-(2-chlorophenyl)-4-isoxazolylpenicillin	
Dicloxacillin	5-Methyl-3-(2,6-dichlorophenyl)-4-isoxazolylpenicillin	
Ampicillin	D-α-Aminobenzyl-penicillin	

Generic Name	Chemical Name	R Group
Amoxicillin	D-α-Amino-*p*-hydroxybenzylpenicillin	
Cyclacillin	1-Aminocyclohexyl-penicillin	
Carbenicillin	α-Carboxybenzyl-penicillin	
Ticarcillin	α-Carboxy-3-thienyl-penicillin	
Piperacillin	α-(4-Ethyl-2,3-dioxo-1-piperazinylcarbonyl-amino)benzylpenicillin	
Mezlocillin	α-(1-Methanesulfonyl-2-oxoimidazolidino-carbonylamino)benzyl-penicillin	

Structure of penicillin

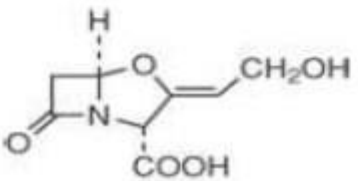


Ampicillin

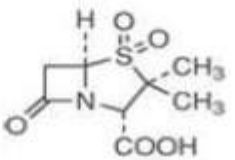


amoxicillin

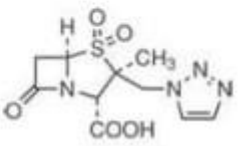
B-lactamase inhibitor



Clavulanic acid



Sulbactam

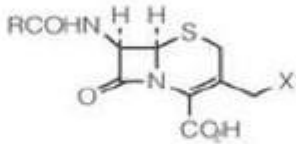


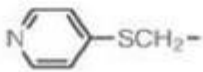
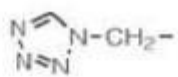
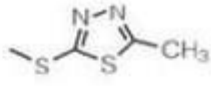
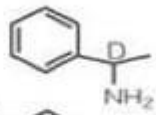
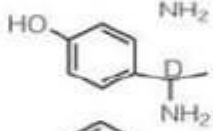
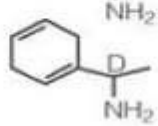
Tazobactam

Therapeutic classification of Cephalosporin

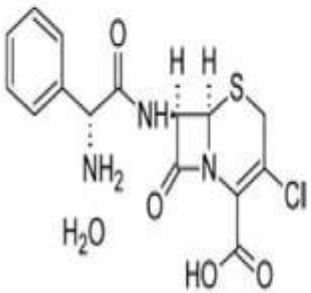
Generation	Characteristics
First generation	Poor serum and tissue concentrat ion Not valuable for systemic infections Lack activity against *Pseudomonas aeruginosa,* Gram-positive organisms, and anaerobes
Second generation	Adequate serum and tissue concentration Good for systemic infections Active against Gram-negative organisms, including *P. aeruginosa; weak activity against Streptococcus pneumoniae; no activity against anaerobes*
Third generation	Once-daily dosing Active against *S. pneumoniae and atypical bacteria; less* active against *P. aeruginosa*
Fourth generation	Active against anaerobes and aerobic Gram-positive and Gram-negative organisms

First Generation of Cephalosporins

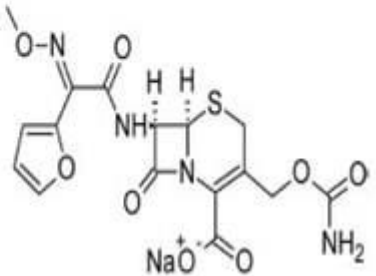


Generic names	Trade names	R	X	Salt
Parenteral Agents:				
Cephapirin	Cefadyl		OAc	Na
Cefazolin	Ancef, Kefzol, Zolicef			Na
Oral Agents:				
Cephalexin	Keflex, Biocef Keftab		H	HCl
Cefadroxil	Duricef		H	-
Oral and Parenteral Agent:				
Cephradine	Velosef		H	-

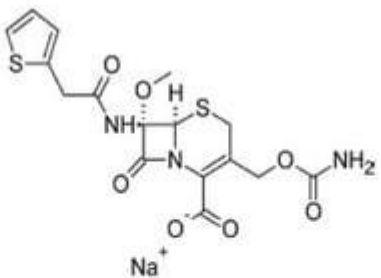
Second generation cephalosporin



cefaclor

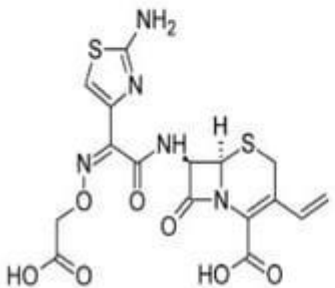


Cefuroxime sodium

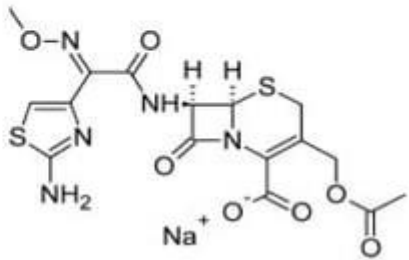


Cefoxitin sodium

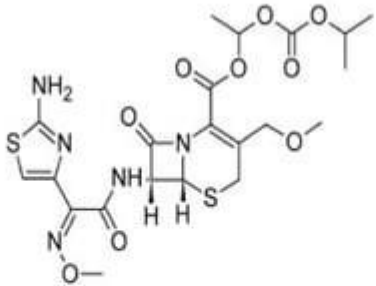
Third generation cephalosporin



cefixime

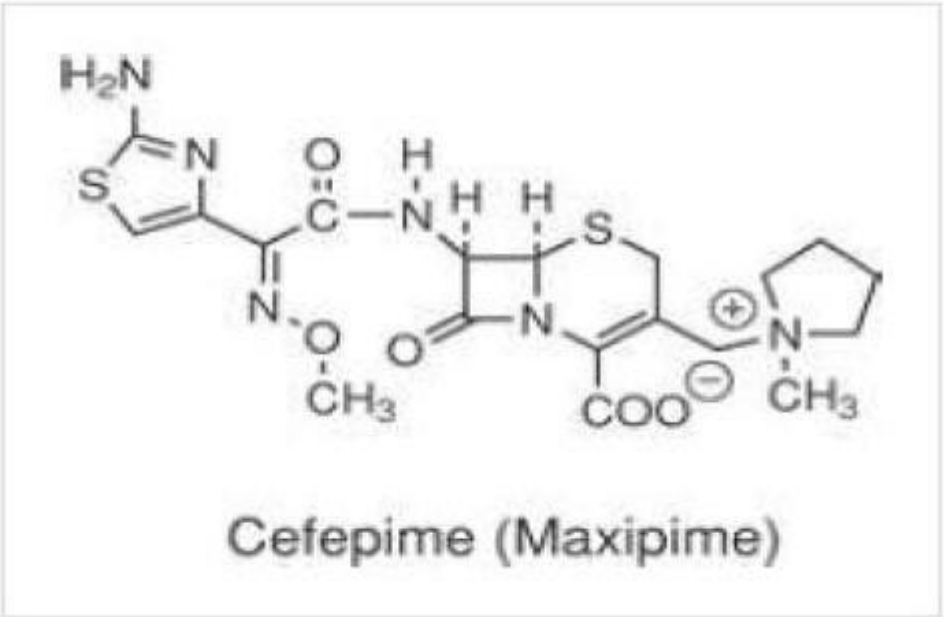


cefotaxime



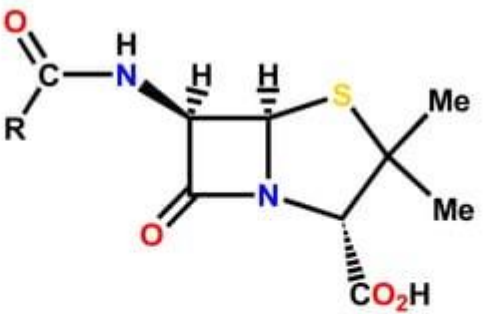
Cefpodoxime proxetil

Fourth Generation Cephalosporins



Cefepime (Maxipime)

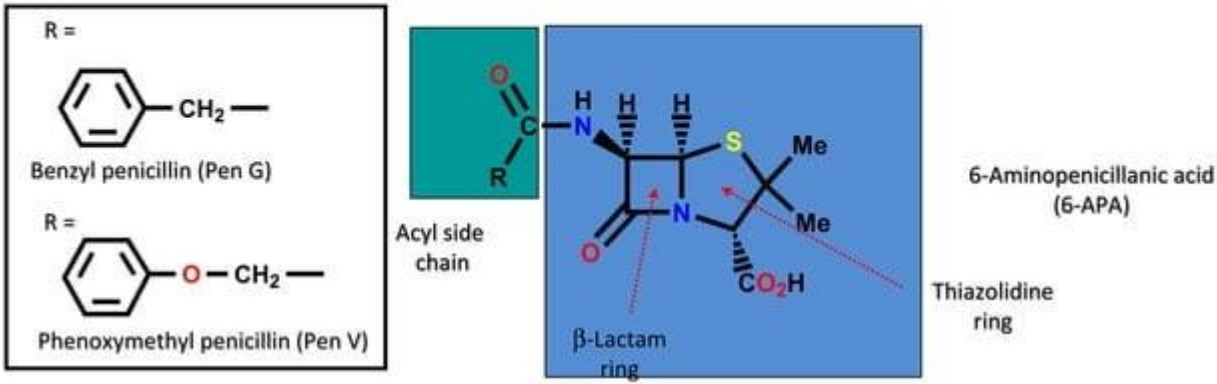
PENICILLINS



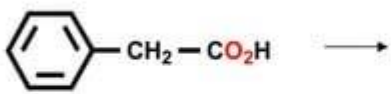
INTRODUCTION TO PENICILLINS

- Antibacterial agents which inhibit bacterial cell wall synthesis
- Discovered by Fleming from a fungal colony (1928)
- Shown to be non toxic and antibacterial
- Isolated and purified by Florey and Chain (1938)
- First successful clinical trial (1941)
- Produced by large scale fermentation (1944)
- Structure established by X-ray crystallography (1945)
- Full synthesis developed by Sheehan (1957)
- Isolation of 6-APA by Beechams (1958-60)
  - development of semi-synthetic penicillins
- Discovery of clavulanic acid and β-lactamase inhibitors

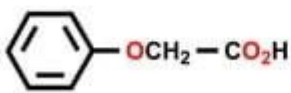
STRUCTURE



Side chain varies depending on carboxylic acid present in fermentation medi

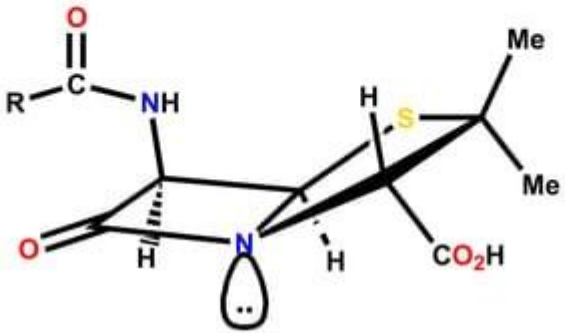


present in corn steep liquor → Penicillin G



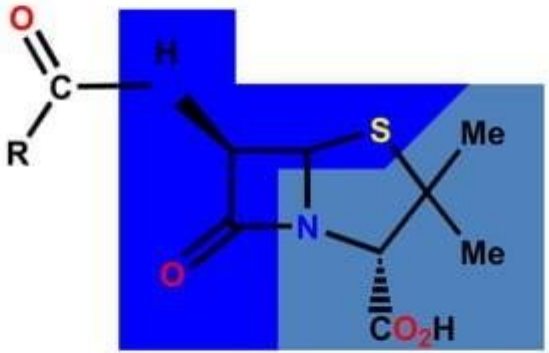
→ Penicillin V
(first orally active penicillin)

Shape of Penicillin G



Folded ‘envelope’ shape

Biosynthesis of Penicillins



Properties of Penicillin G

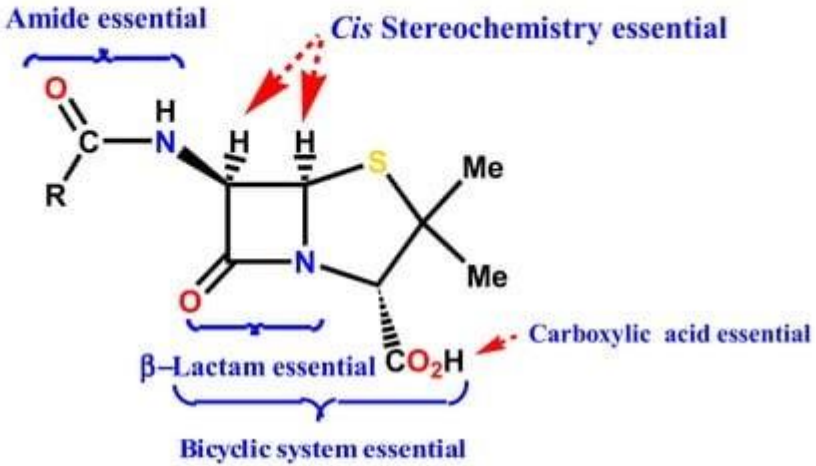
- Active vs. Gram +ve bacilli and some Gram -ve cocci
- Non toxic
- Limited range of activity
- Not orally active - must be injected
- Sensitive to β-lactamases
  (enzymes which hydrolyse the β-lactam ring)
- Some patients are allergic
- Inactive vs. *Staphylococci*

Drug Development

Aims

- To increase chemical stability for oral administration
- To increase resistance to β-lactamases
- To increase the range of activity

SAR

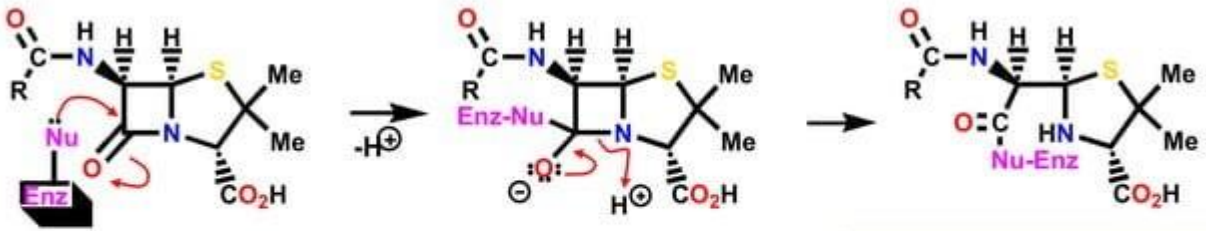


Conclusions

- Amide and carboxylic acid are involved in binding
- Carboxylic acid binds as the carboxylate ion
- Mechanism of action involves the β-lactam ring
- Activity related to β-lactam ring strain (subject to stability factors)
- Bicyclic system increases β-lactam ring strain
- Not much variation in structure is possible
- Variations are limited to the side chain (R)

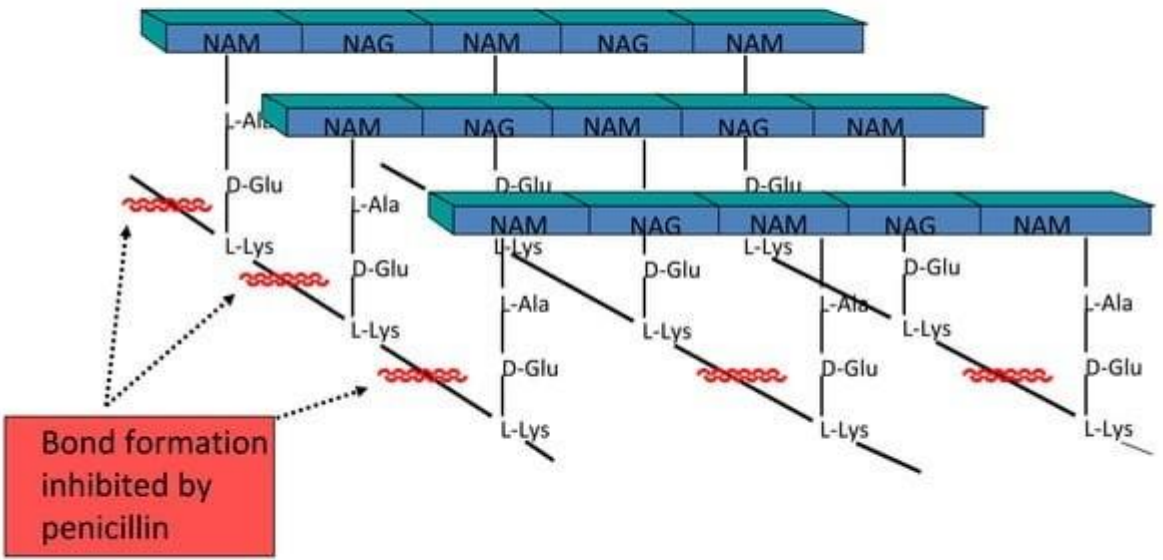
Mechanism of action

- Penicillins inhibit a bacterial enzyme called the transpeptidase enzyme which is involved in the synthesis of the bacterial cell wall
- The β-lactam ring is involved in the mechanism of inhibition
- Penicillin becomes covalently linked to the enzyme's active site leading to irreversible inhibition

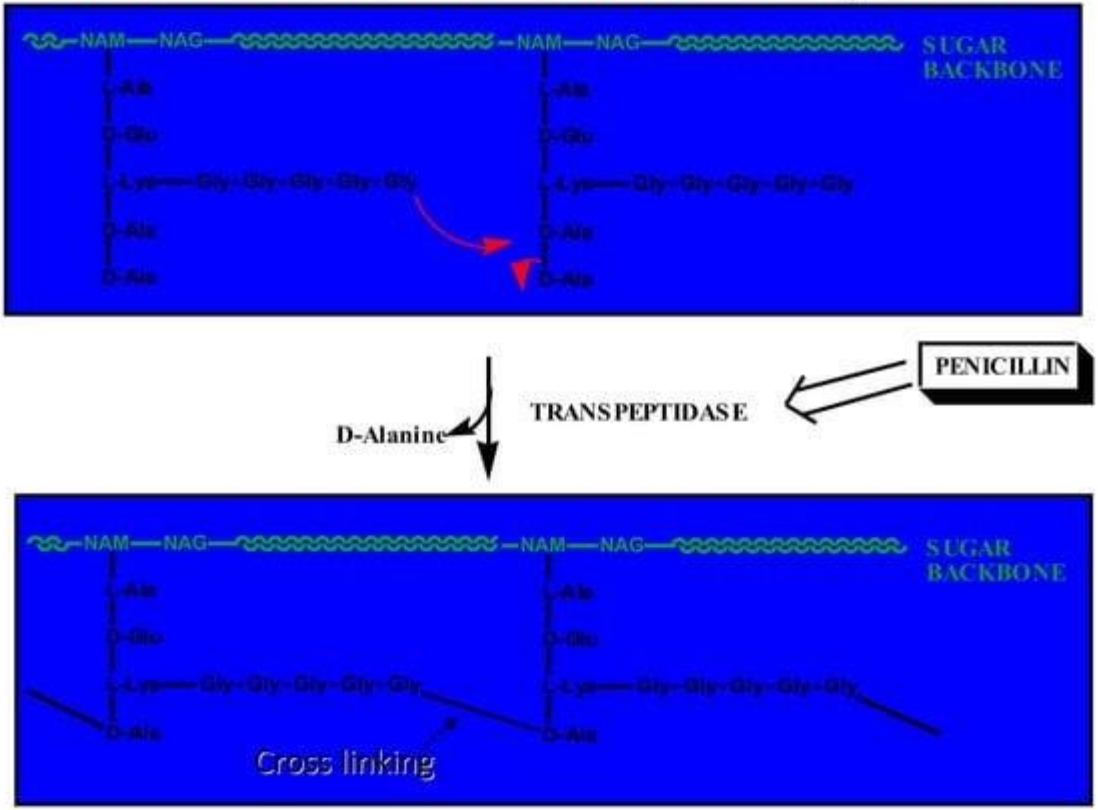


- Covalent bond formed to transpeptidase enzyme
- Irreversible inhibition

Mechanism of action - bacterial cell wall synthesis



Mechanism of action - bacterial cell wall synthesis



Mechanism of action - bacterial cell wall synthesis

- Penicillin inhibits final crosslinking stage of cell wall synthesis
- It reacts with the transpeptidase enzyme to form an irreversible covalent bond
- Inhibition of transpeptidase leads to a weakened cell wall
- Cells swell due to water entering the cell, then burst (lysis)
- Penicillin possibly acts as an analogue of the L-Ala-γ-D-Glu portion of the pentapeptide chain. However, the carboxylate group that is essential to penicillin activity is not present in this portion

Gram +ve and Gram -ve Cell Walls

- Penicillins have to cross the bacterial cell wall in order to reach their target enzyme
- Cell walls are porous and are not a barrier
- The cell walls of Gram +ve bacteria are thicker than Gram -ve cell walls, but the former are more susceptible to penicillins

Resistance to Penicillins

Factors

- Gram -ve bacteria have a lipopolysaccharide outer membrane preventing access to the cell wall
- Penicillins can only cross via porins in the outer membrane
- Porins allow small hydrophilic molecules such as zwitterions to cross

- High levels of transpeptidase enzyme may be present
- The transpeptidase enzyme may have a low affinity for penicillins
  (e.g. PBP 2a for *S. aureus*)

- Presence of β-lactamases
- Concentration of β-lactamases in periplasmic space

- Mutations
- Transfer of β-lactamases between strains
- Efflux mechanisms pumping penicillin out of periplasmic space

Penicillin Analogues - Preparation

1) By fermentation

- Vary the carboxylic acid in the fermentation medium
- Limited to unbranched acids at the α-position i.e. RCH_2CO_2H
- Tedious and slow

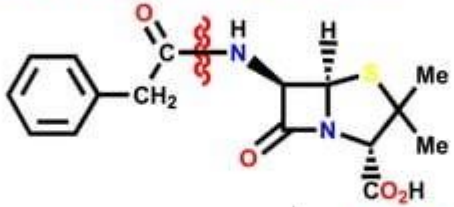
2) By total synthesis

- Only 1% overall yield
- Impractical

3) By semi-synthetic procedures

- Use a naturally occurring structure as the starting material for analogue synthesis

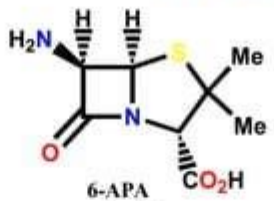
Penicillin Analogues - Preparation



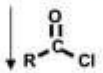
Penicillin G

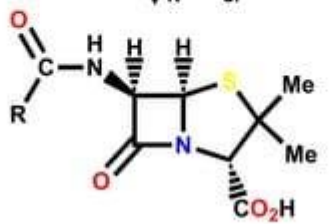
Penicillin acylase
or chemical hydrolysis

Fermentation



6-APA



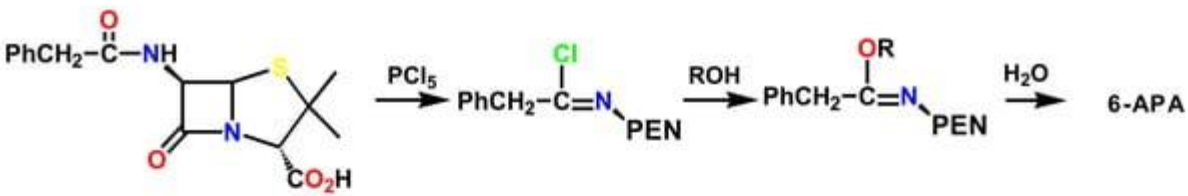


Semi-synthetic penicillins

Penicillin Analogues - Preparation

Problem - How does one hydrolyse the side chain by chemical means in presence of a labile β-lactam ring?

Answer - Activate the side chain first to make it more reactive



Note - Reaction with PCl_5 requires the involvement of a lone pair of electrons from nitrogen. Not possible for the β-lactam nitrogen.

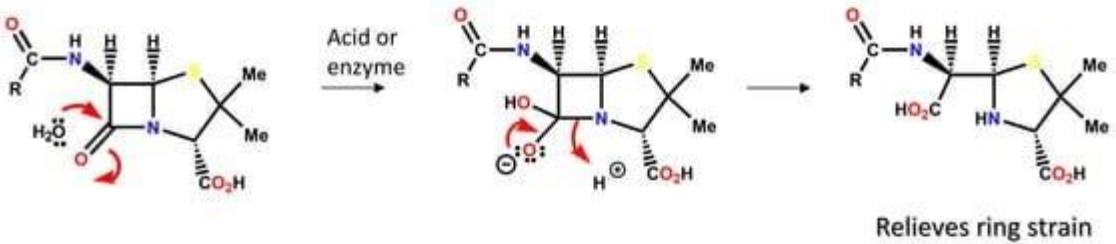
Problems with Penicillin G

- It is sensitive to stomach acids
- It is sensitive to β-lactamases - enzymes which hydrolyse the β-lactam ring
- It has a limited range of activity

Problem 1 - Acid Sensitivity

Reasons for sensitivity

1) Ring strain



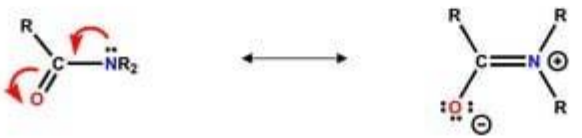
Problem 1 - Sensitivity

Reasons for sensitivity

2) Reactive β-lactam carbonyl group

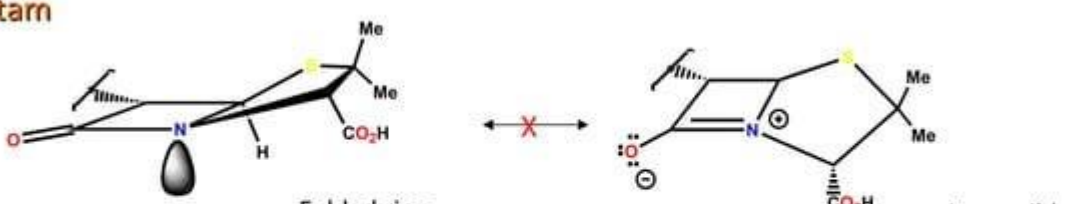
Does not behave like a tertiary amide

Tertiary amide



Unreactive

β-Lactam



Folded ring system

Impossibly strained

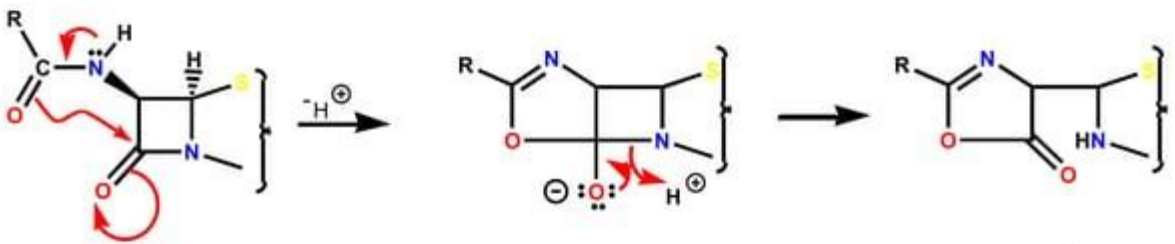
- Interaction of nitrogen's lone pair with the carbonyl group is not possible
- Results in a reactive carbonyl group

Problem 1 - Sensitivity

Reasons for sensitivity

Acyl side chain

Neighboring group participation in the hydrolysis mechanism



Further reactions

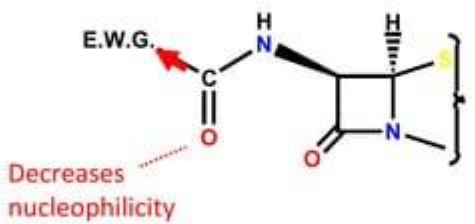
Problem 1 - Sensitivity

Conclusions

- The β-lactam ring is essential for activity and must be retained
- Cannot tackle factors 1 and 2
- Can only tackle factor 3

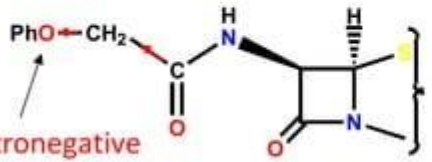
Strategy

Vary the acyl side group (R) to make it electron-withdrawing to decrease the nucleophilicity of the carbonyl oxygen

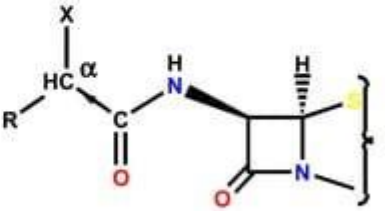


Problem 1 - Sensitivity

Examples



Penicillin V
(orally active)



X = NH_2, Cl, PhOCONH, Heterocycles, CO_2H

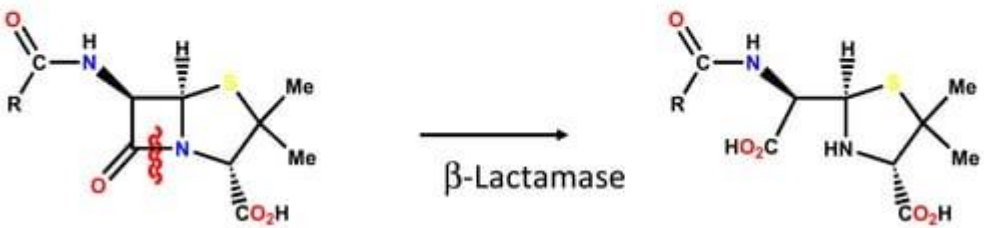
- Better acid stability and orally active
- But sensitive to β-lactamases
- Slightly less active than penicillin G
- Allergy problems with some patients

Very successful semi-synthetic penicillins
e.g. ampicillin, oxacillin

Problem 2 - Sensitivity to β-Lactamases

β-Lactamases

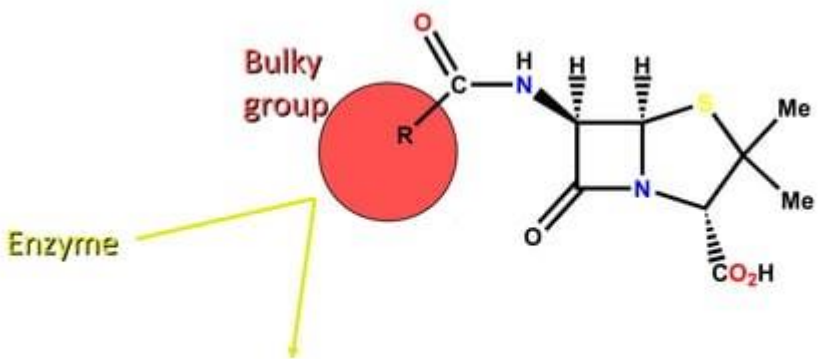
- Enzymes that inactivate penicillins by opening β-lactam rings
- Allow bacteria to be resistant to penicillin
- Transferable between bacterial strains (i.e. bacteria can acquire resistance)
- Important with respect to *Staphylococcus aureus* infections in hospitals
- 80% *Staph*. infections in hospitals were resistant to penicillin and other antibacterial agents by 1960
- Mechanism of action for lactamases is identical to the mechanism of inhibition for the target enzyme
- But product is removed efficiently from the lactamase active site



Problem 2 - Sensitivity to β-Lactamases

Strategy

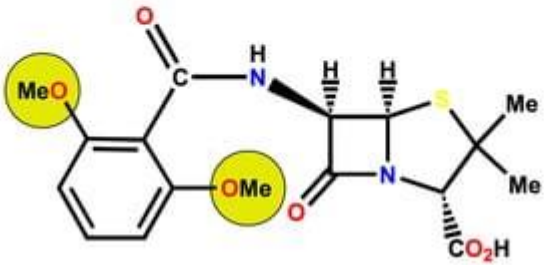
- Use of steric shields
- Block access of penicillin to the active site of the enzyme by introducing bulky groups to the side chain
- Size of shield is crucial to inhibit reaction of penicillins with β-lactamases, but not with the target transpeptidase enzyme



Problem 2 - Sensitivity to β-Lactamases

Examples - Methicillin (Beechams - 1960)

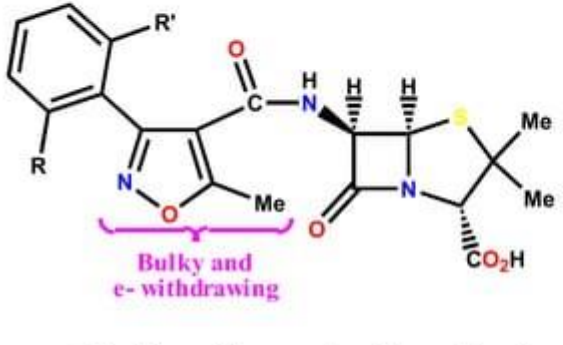
ortho groups important



- Methoxy groups block access to β-lactamases but not to transpeptidases
- Binds less readily to transpeptidases compared to penicillin G
- Lower activity compared to Pen G against Pen G sensitive bacteria
- Poor activity vs. some *streptococci*
- Inactive vs. Gram -ve bacteria
- Poor range of activity
- Active against some penicillin G resistant strains (e.g. *Staphylococcus*)
- Acid sensitive since there is no electron-withdrawing group
- Orally inactive and must be injected

Problem 2 - Sensitivity to β-Lactamases

Examples - Oxacillin



Oxacillin R = R' = H
Cloxacillin R = Cl, R' = H
Flucloxacillin R = Cl, R' = F

- Orally active and acid resistant
- Resistant to β-lactamases
- Active vs. *Staphylococcus aureus*
- Less active than other penicillins
- Inactive vs. Gram -ve bacteria
- Nature of R & R' influences absorption and plasma protein binding
- Cloxacillin better absorbed than oxacillin
- Flucloxacillin less bound to plasma protein, leading to higher levels of free

Problem 3 - Range of Activity

Factors

1) Cell wall may have a coat preventing access to the cell
2) Excess transpeptidase enzyme may be present
3) Resistant transpeptidase enzyme (modified structure)
4) Presence of β-lactamases
5) Transfer of β-lactamases between strains
6) Efflux mechanisms

Strategy

- The number of factors involved make a single strategy impossible
- Use trial and error by varying R groups on the side chain
- Successful in producing broad spectrum antibiotics
- Results demonstrate general rules for broad spectrum activity.

Problem 3 - Range of Activity

Results of varying R in Pen G

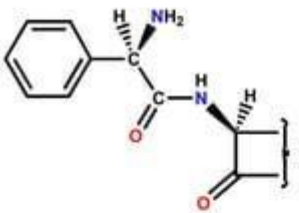
1) Hydrophobic side chains result in high activity vs. Gram +ve bacteria and poor activity vs. Gram -ve bacteria
2) Increasing hydrophobicity has little effect on Gram +ve activity
   but lowers Gram -ve activity
3) Increasing hydrophilic character has little effect on Gram +ve activity
   but increases Gram -ve activity
4) Hydrophilic groups at the α-position (e.g. NH_2, OH, CO_2H) increase activity vs Gram -ve bacteria

Problem 3 - Range of Activity

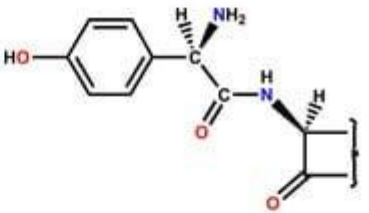
Examples of Broad Spectrum Penicillins

Class 1 - NH_2 at the α-position

Ampicillin and amoxicillin (Beechams, 1964)



Ampicillin (Penbritin)
2nd most used penicillin



Amoxicillin (Amoxil)

Problem 3 - Range of Activity

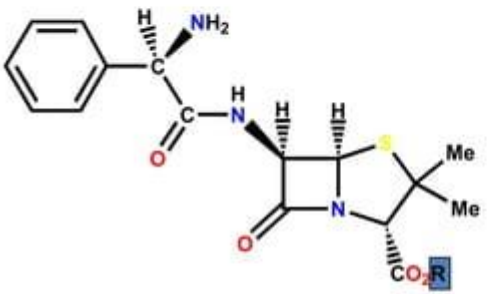
Examples of Broad Spectrum Penicillins

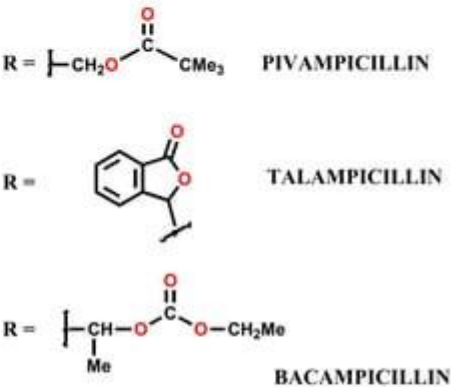
Properties

- Active vs Gram +ve bacteria and Gram -ve bacteria which do not produce β-lactamases
- Acid resistant and orally active
- Non toxic
- Sensitive to β-lactamases
- Increased polarity due to extra amino group
- Poor absorption through the gut wall
- Disruption of gut flora leading to diarrhea
- Inactive vs. *Pseudomonas aeruginosa*

Problem 3 - Range of Activity

Prodrugs of Ampicillin (Leo Pharmaceuticals - 1969)



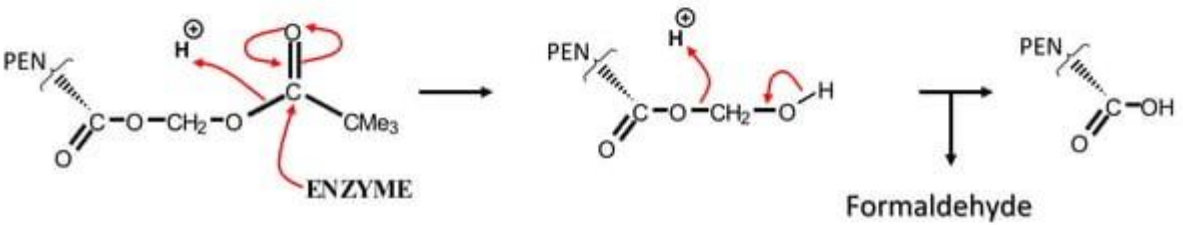


Properties

- Increased cell membrane permeability
- Polar carboxylic acid group is masked by the ester
- Ester is metabolised in the body by esterases to give the free drug

Problem 3 - Range of Activity

Mechanism of prodrug activation



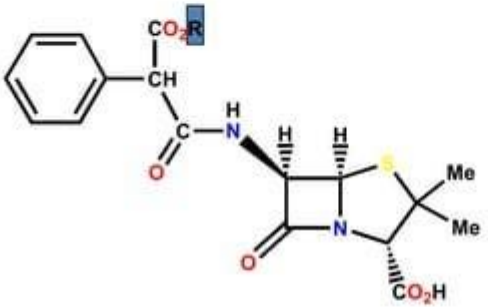
- Extended ester is less shielded by the penicillin nucleus
- Hydrolysed product is chemically unstable and degrades
- Methyl ester of ampicillin is not hydrolysed in the body
- Bulky penicillin nucleus acts as a steric shield for methyl ester

Problem 3 - Range of Activity

Examples of broad spectrum penicillins

Class 2 - CO_2H at the α-position (carboxypenicillins)

Examples



R = H Carbenicillin
R = Ph Carfecillin

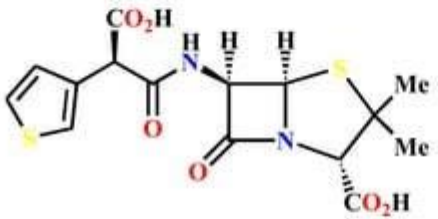
- Carfecillin = prodrug for carbenicillin
- Active over a wider range of Gram -ve bacteria than ampicillin
- Active vs. *Pseudomonas aeruginosa*
- Resistant to most β-lactamases
- Less active vs Gram +ve bacteria (note the hydrophilic group)
- Acid sensitive and must be injected
- Stereochemistry at the α-position is important
- CO_2H at the α-position is ionised at blood pH

Problem 3 - Range of Activity

Examples of broad spectrum penicillins

Class 2 - CO_2H at the α-position (carboxypenicillins)

Examples



TICARCILLIN

- Administered by injection
- Identical antibacterial spectrum to carbenicillin
- Smaller doses required compared to carbenicillin
- More effective against *P. aeruginosa*
- Fewer side effects
- Can be administered with clavulanic acid

Problem 3 - Range of Activity

Examples of broad spectrum penicillins

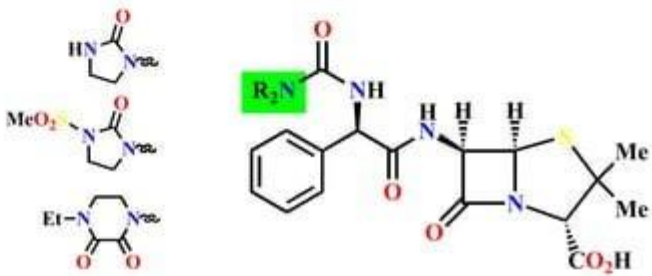
Class 3 - Urea group at the α-position (ureidopenicillins)

Examples

Azlocillin

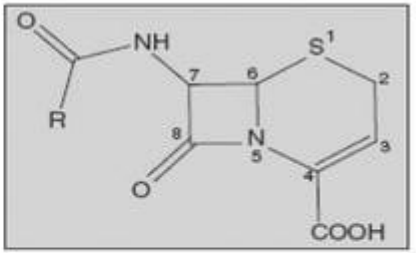
Mezlocillin

Piperacillin



- Administered by injection
- Generally more active than carboxypenicillins vs. streptococci and *Haemophilus* species
- Generally have similar activity vs Gram -ve aerobic rods
- Generally more active vs other Gram -ve bacteria
- Azlocillin is effective vs *P. aeruginosa*
- Piperacillin can be administered alongside tazobactam

SAR of cephalosporins



1. 7-Acylamino substituents:

a) Acylation of amino group generally increases the potency against gram-positive bacteria, but it is accompanied by a decrease in gram-negative potency.

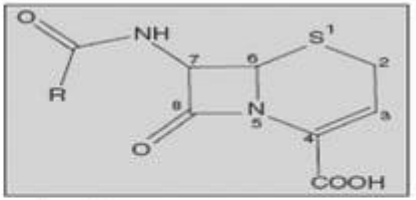
b) High antibacterial activity is observed only when the new acyl groups are derived from carboxylic acids for gram-positive bacteria.

c) Substituents on the aromatic ring that increases lipophilicity provide higher gram-positive activity and generally lower gram-negative activity.

d) The phenyl ring in the side-chain can be replaced with other heterocycles with improved spectrum of activity and pharmacokinetic properties, and these include thiophene, tetrazole, furan, pyridine, and aminothiazoles

2. C-3 substituents: The nature of C-3 substituents influences pharmacokinetic and pharmacological properties as well as antibacterial activity. Modification at C-3 position has been made to reduce the degradation (lactone of desacetyl cephalosporin) of cephalosporins.

SAR of cephalosporins

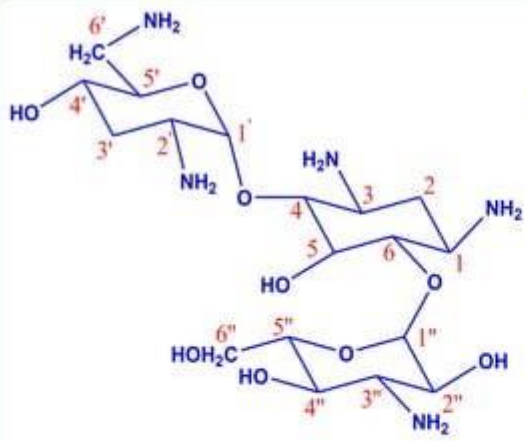


3. Pyridine and imidazole-replaced acetoxy groups show improved activity against *P. aeruginosa*. Displacement of acetoxy group by azide ion yields derivatives with relatively low gram-negative activity.
4. Displacement with aromatic thiols of 3-acetoxy group results in an enhancement of activity against gram-negative bacteria with improved pharmacokinetic properties.
5. Replacement of acetoxy group at C-3 position with $-CH_3$, Cl has resulted in orally active compounds.
6. Oxidation of ring sulphur to sulphoxide or sulphone greatly diminishes or destroys the antibacterial activity.
7. Replacement of sulphur with oxygen leads to oxacepam (latamoxef) with increased antibacterial activity, because of its enhanced acylating power.
8. Similarly, replacement of sulphur with methylene group (loracarbef) has greater chemical stability and a longer half-life.
9. The carboxyl group of position-4 has been converted into ester prodrugs to increase bioavailability of cephalosporins, and these can be given orally as well. Examples include cefuroxime axetil and cefodoxime proxetil
10. Olefinic linkage at C 3-4 is essential for antibacterial activity. Isomerization of the double bond to 2-3 position leads to great losses in antibacterial activity.

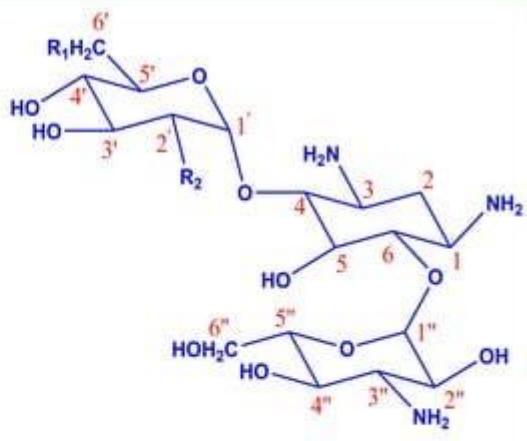
Medicinal Chemistry of Aminoglycoside Antibiotics

Introduction

- ✓ Antibiotics contain an aminocyclitol moiety to which aminosugars are glycosidically linked.
- ✓ They may be more correctly called aminocyclitol antibiotics.



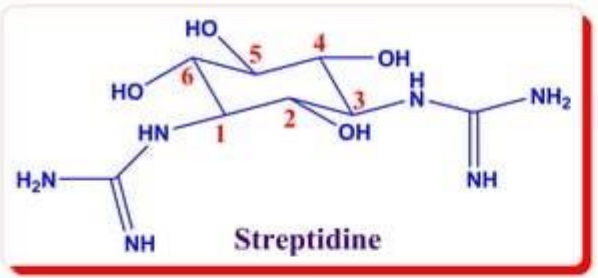
Tobramycin

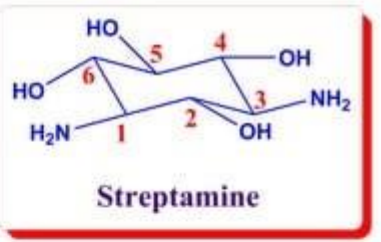


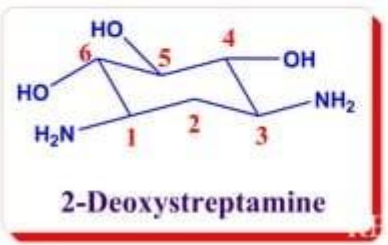
Kanamycins

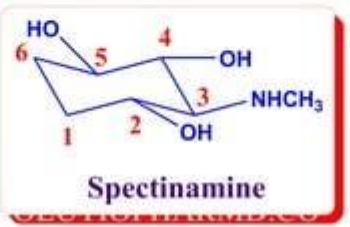
Aminocyclitols???

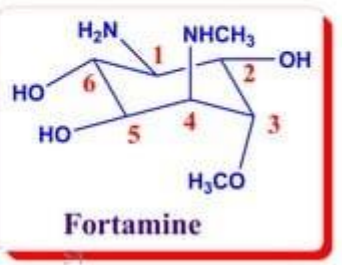
- ✓ Cyclohexanes with several substituted or unsubstituted amino and hydroxyl groups which bring them high water solubility.
- ✓ Streptidine and Streptamine can be called 1,3-diguanidino and 1,3-diamino inositol, respectively.



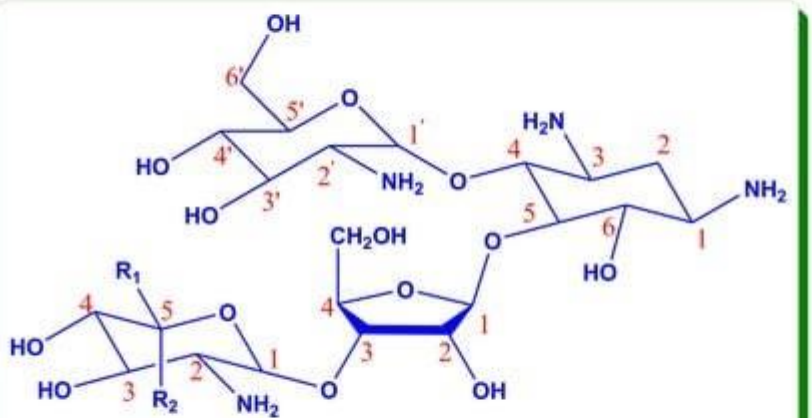








- ✓ All have an aminohexose as the amino sugar and some have a pentose as an extra sugar.



Paromomycin I: R_1= H; R_2= CH_2NH_2
Paromomycin II: R_1= CH_2NH_2; R_2= H

Spectrum of Antimicrobial Activity

- ✓ Aminoglycosides are broad-spectrum antibiotics effective in:

1. Systemic Infections caused by aerobic G(-) bacillus (klebsiella, proteus, enterobacters).
2. Tuberculosis, Brucellusis, Tularaemia and yersinia infections.
3. Amoebic dysentery, shigellosis and salmonellosis.
4. Pneumonia and urinary infections caused by *Pseudomona aeroginosa.*

- ✓ G(+) and G(-) aerobic cocci except staphylococci and anaerobic bacteria are less susceptible.

Microbial Resistance against Aminoglycosides

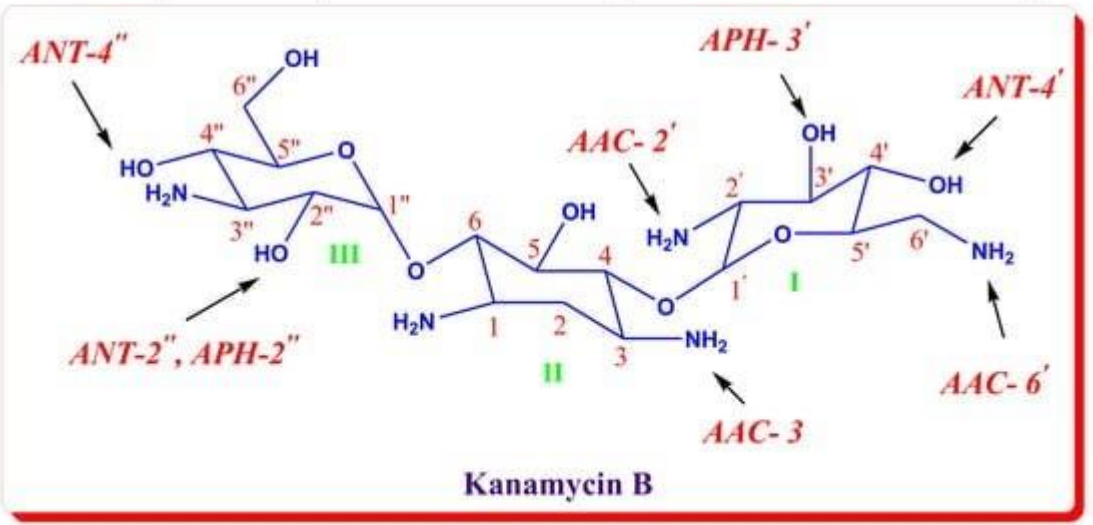
- ✓ Resistant strains have emerged against streptomycin, kanamycin and gentamycin in clinic.
- ✓ R factor is resposible for the production of aminoglycoside deactivating enzymes:

1) Acetyl transferases (AAC)
2) Phosphotransferases (APH),
3) Nucleotidyl transferases (ANT)

- ✓ These enzymes transfer to hydroxyl and amino groups of the drug.

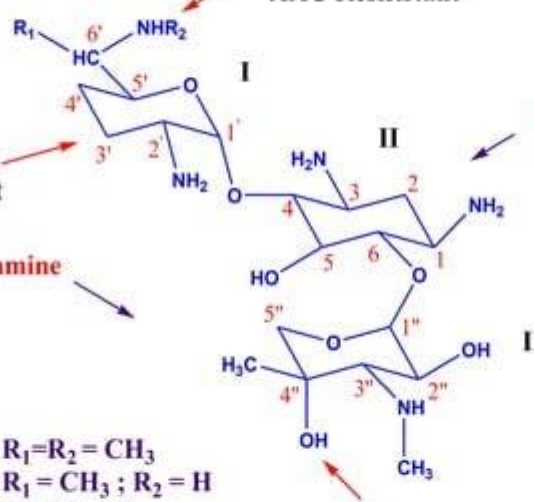
Aminoglycoside Deactivating Enzymes

- AAC acetylates 3-NH_2 of the ring II, and 2`, 6`- NH_2 of the ring I.
- APH phosphorylates 3`-OH of the ring I and 2``-OH of the ring III.
- ANT adenylates 2``,4``-OH of the ring III and 4`-OH of the ring I.



Gentamycin and Deactivating Enzymes

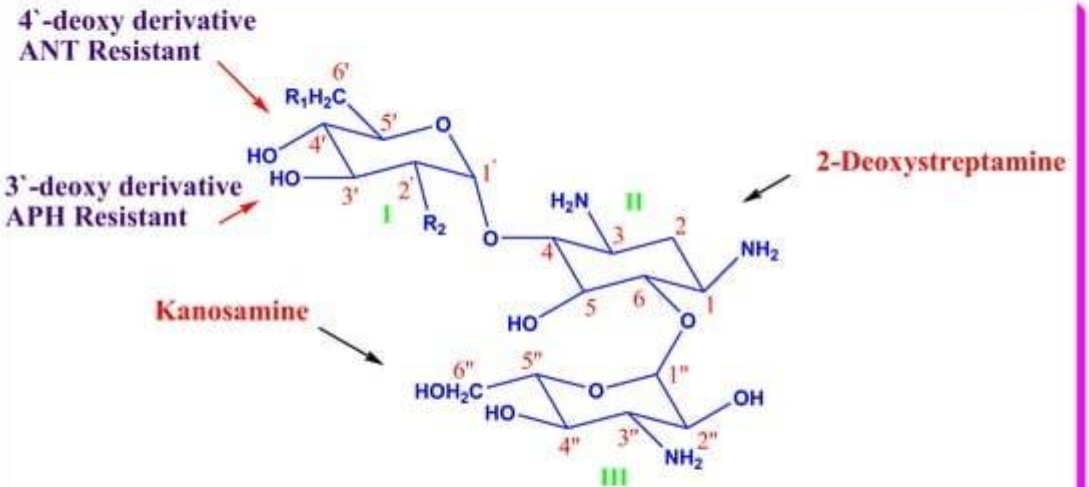
Secondary amino group at 6`-NH_2 in Gentamycin C1, spacial hynderance
AAC Resisistant



Gentamicin C_1: R_1=R_2 = CH_3
Gentamicin C_2: R_1 = CH_3 ; R_2 = H
Gentamicin C_{1a}: R_1=R_2 = H

Axial and tertiary 4``-OH instead of equatorial secondary 4``-OH in Kanamycin
ANT Resistant

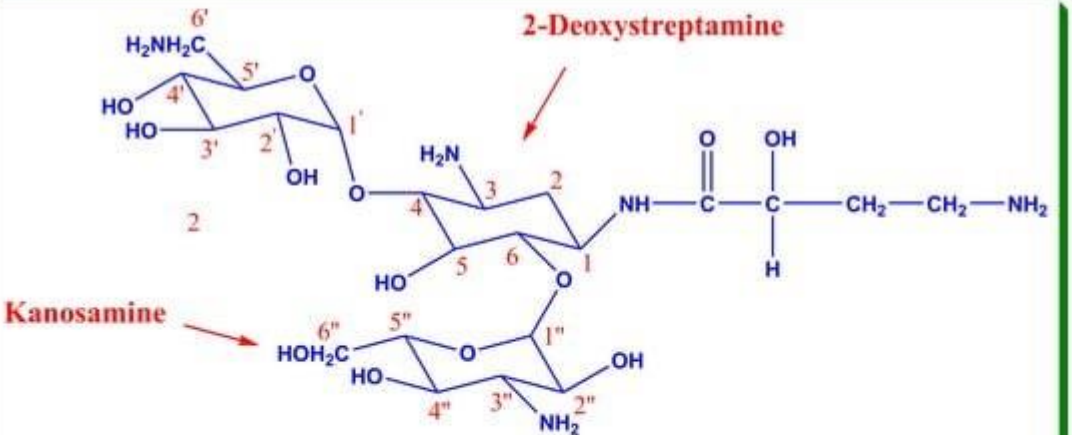
Kanamycin and Deactivatig Enzymes



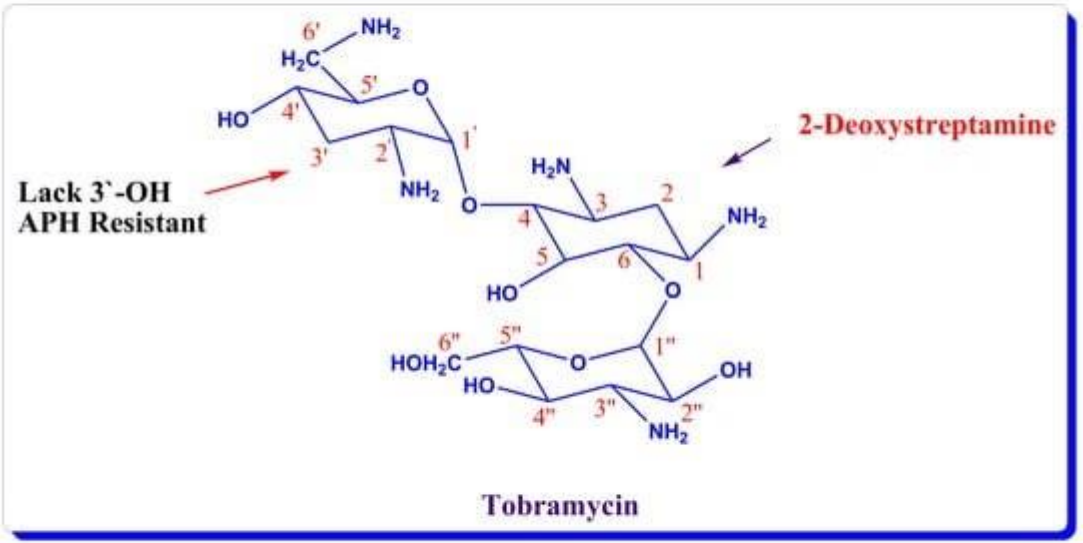
Kanamycin A: $R_1 = NH_2$; $R_2 = OH$
Kanamycin B: $R_1 = NH_2$; $R_2 = NH_2$
Kanamycin C: $R_1 = OH$; $R_2 = NH_2$

Amikacin and Dactivating Enzymes

- 1-*N*-L-(-)-amino-α-hydroxybutyric acid derivative of kanamycin A. Susceptible only against the action of AAC 6`-amino and ANT 4`-OH, resistant against all other deactivating enzymes.



Tobramycin and Deactivating Enzymes



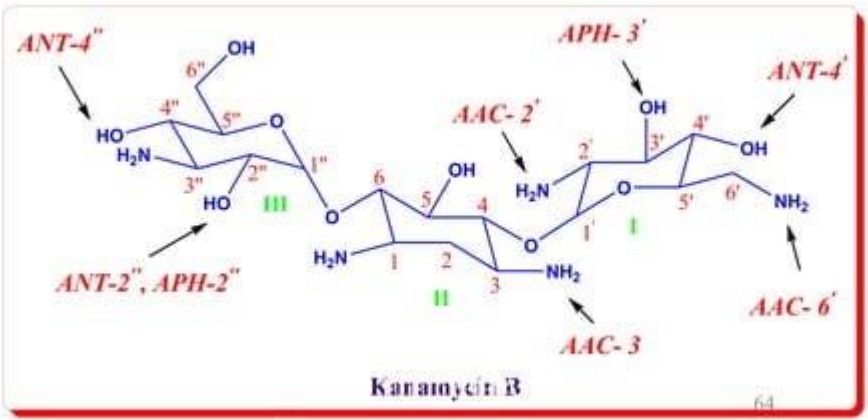
The Minor Mechanism for Microbial Resistance

- ✓ Decreased uptake of the drug in some strains of *p. aeroginosa* in hospital infections because of blockade in the active transport of aminoglycosides.
- ✓ Aminoglycoside molecules attach through their cationic groups to anionic portions of membrane phospholipids of bacteria. Upon this attachment the the ATP-dependent uptake occurs.
- ✓ Bivalent cations such as Ca^{2+} and Mg^{2+} compete with the drug in this process and antagonise them.
- ✓ Anaerobic bacteria lack the ATP-dependent uptake process, so they are resistant to aminoglycosides.

SAR of Aminoglycosides

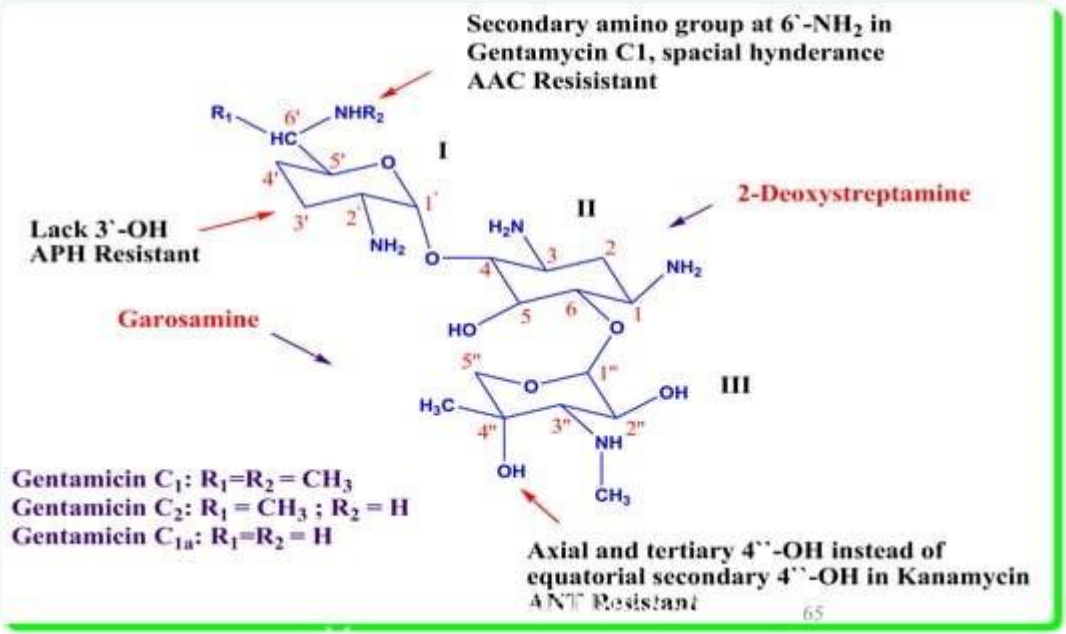
- ✓ Ring I is very necessary for broad-spectrum antibacterial activity.
- ✓ 2` and 6`-NH_2 groups are specially important.

Exchanging of one of them in kanamycin B with hydroxyl group decreases the activity (kanamycin A, C)



SAR of ring I *continued*

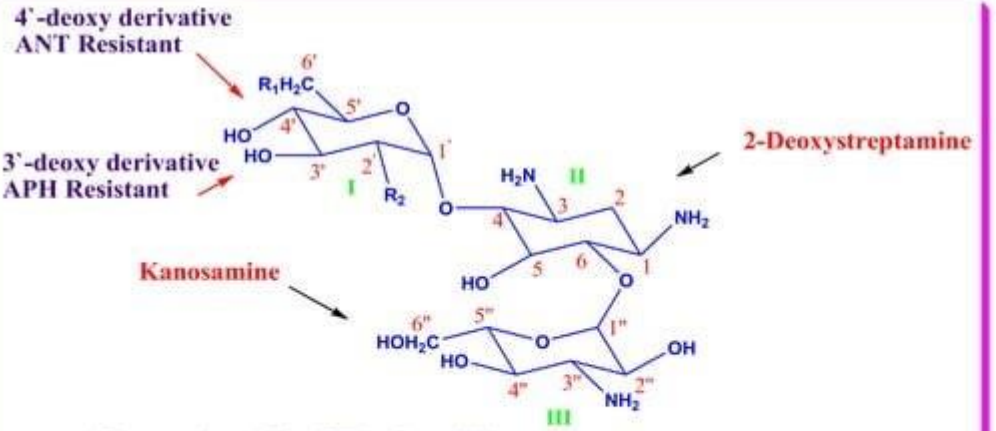
- ✓ Methylation of C-6` or 6`- NH_2 doesn't alter the antibacterial activity, but increases the resistance against AAC.



SAR of ring I *continued*

- ✓ Omitting the 3`-OH and/or 4`-OH in kanamycin doesn't decrease the antibacterial activity but increases the resistance against AAC: 3`,4`-dideoxykanamycin B: Dibekacin.

  The same is true for gentamicin.

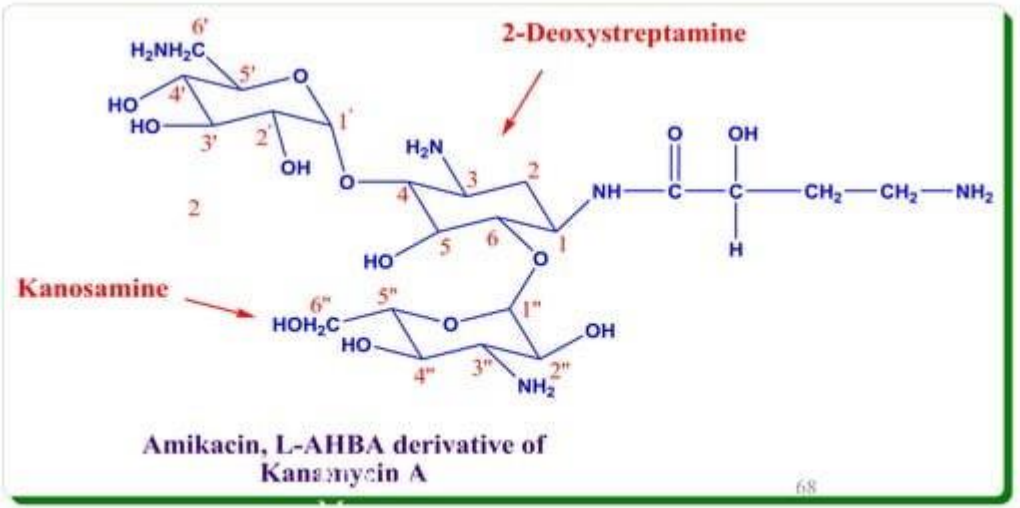


SAR of ring I *continued*

- ✓ Omitting the 3`-OH and 4`-OH and the addition of a double bond between C-4` and C-5`has the same effect.

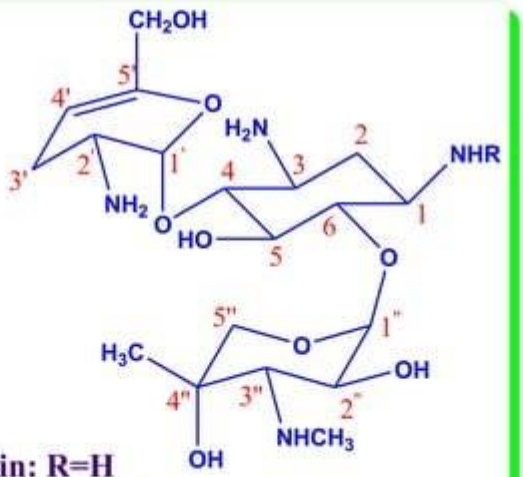
SAR of Aminoglycosides *continued*

- ✓ Ring II is flexible toward changes. 1-NH_2 in kanamycin can be acylated and the antibacterial activity remains almost unchanged , but resistance against deactivating enzymes increases: Amikacin



SAR of ring II *continued*

- ✓ 1-NH_2ethylation of sisomycin saves the antibacterial activity and increases the enzymatic resistance: Netilmycin

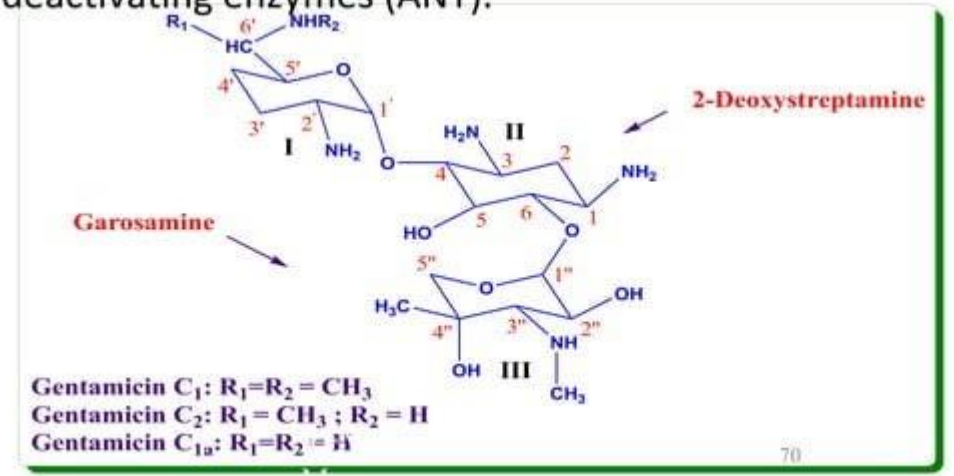


Sisomicin: R=H

Netilmicin: R=C_2H_5

SAR of Aminoglycosides *continued*

- ✓ Ring III functional groups are less sensitive to modifications:
- ✓ 2``-deoxy gentamicins are less active than 2``-OH ones, but 2``-NH_2 derivative (seldomycin) are very active.
- ✓ 3``- NH_2 can be primary or secondary.
- ✓ 4``-OH can be axial or equatorial, the former is resistant against the deactivating enzymes (ANT).

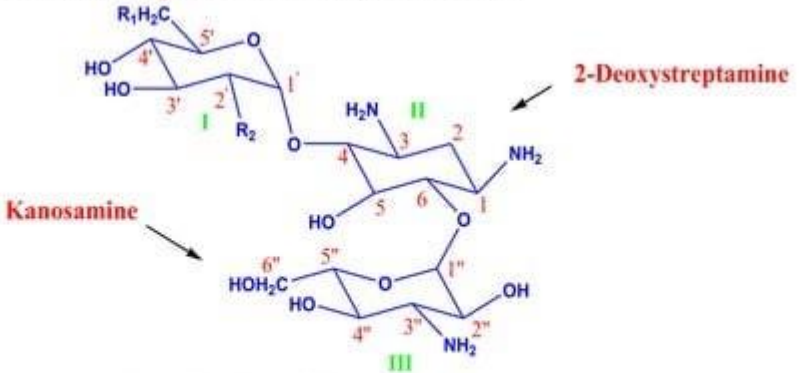


Mechanism of Action of Aminoglycosides

- ✓ Inhibition of protein biosynthesis initiation upon attachment to 30s portion of ribosomes.
- ✓ Misreading mutation of the genetic code and the synthesis of nonesense proteins which are not normal proteins so they cannot take part in cellular activities.
- ✓ Nonesense proteins disturb the semipermeability of the bacterial cell and aminoglycoside molecules enter the cell easily and kill it.

Therapeutic Agents
Kanamycin

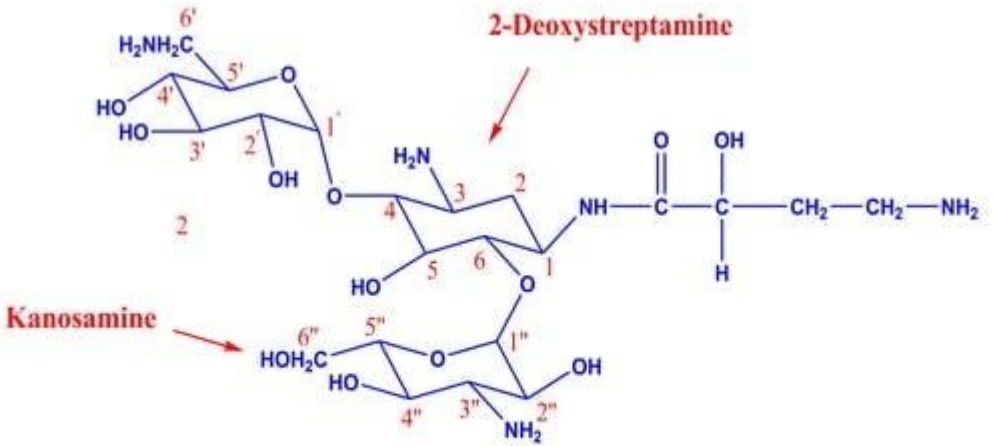
- ✓ Isolated from cultures of *Streptomyces kanamyceticus*. The least toxic member in the market is kanamycin A.
- ✓ It is used for the treatment of GI infections, such as dysentery and systemic G(-) bacillus infections caused by klebsiella, proteus, enterobacters.
- ✓ For disinfection of GI before an operation.



Kanamycin A: $R_1 = NH_2$; $R_2 = OH$
Kanamycin B: $R_1 = NH_2$; $R_2 = NH_2$
Kanamycin C: $R_1 = OH$; $R_2 = NH_2$

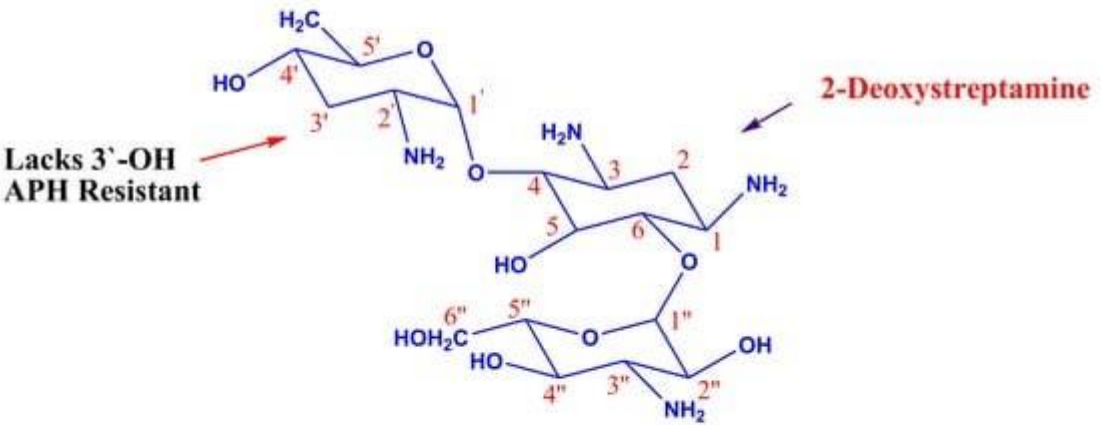
Amikacin

- ✓ A semisynthetic derivative of kanamycin A.
- ✓ It is used in the treatment of infections caused by *Mycobacterium tuberculosis, Yersinia tularensis, Pseudomona aeroginosa.*
- ✓ The suffix "micin" denotes its origin.



Tobramycin

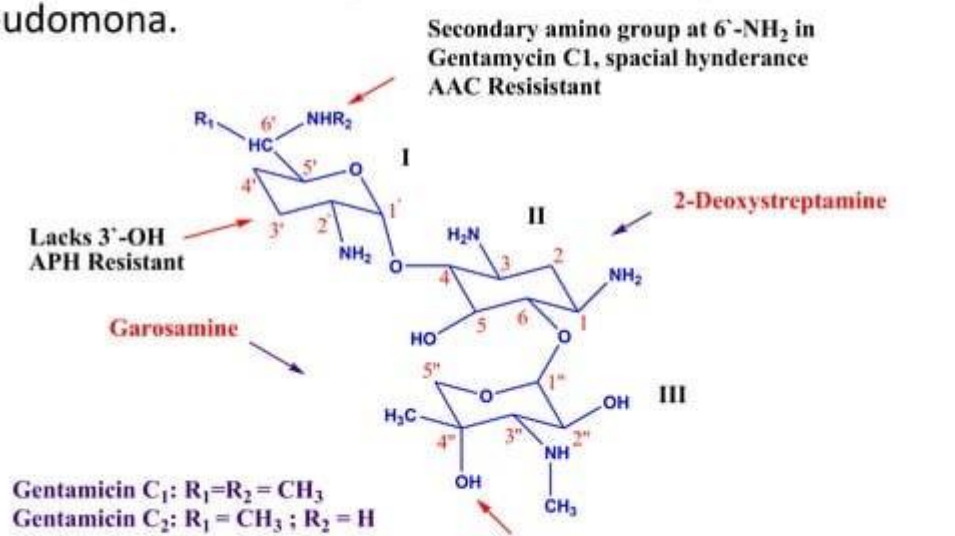
- Isolated from cultures of *Streptomyces tenebrarius.*
- Antimicrobial activity against resistance *P.aeroginosa.*



Tobramycin

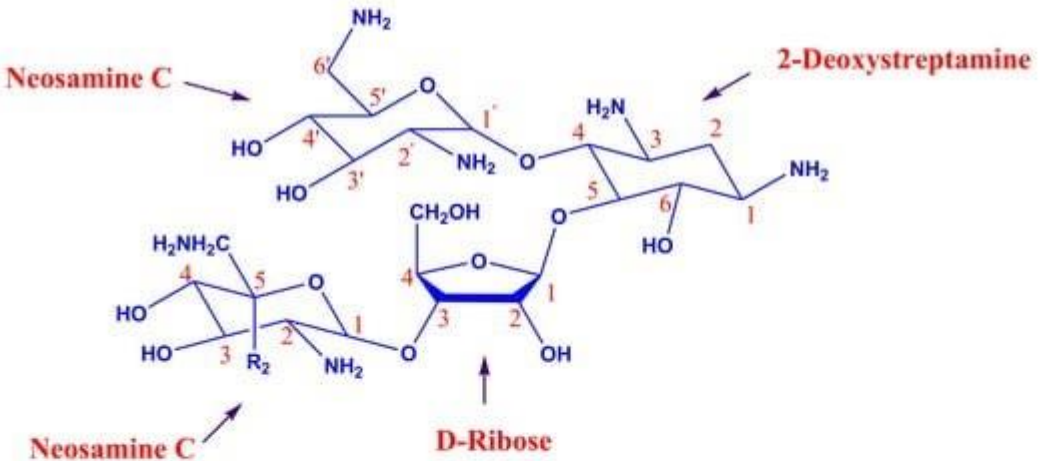
Gentamicin

- ✓ Isolated from cultures of *Micromonospora purpurea.*
- ✓ The suffix "micin" denotes its origin.
- ✓ It is used against urinary infections caused by G(-) and pseudomona.



Neomycin

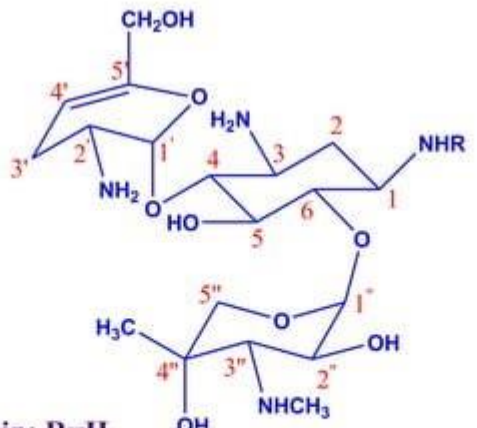
- ✓ Isolated from cultures of *Streptomyces fradia* along with an antifungal subsance: Fradicin.
- ✓ Effective against GI and dermal infections.



Neomycin

Netilmicin

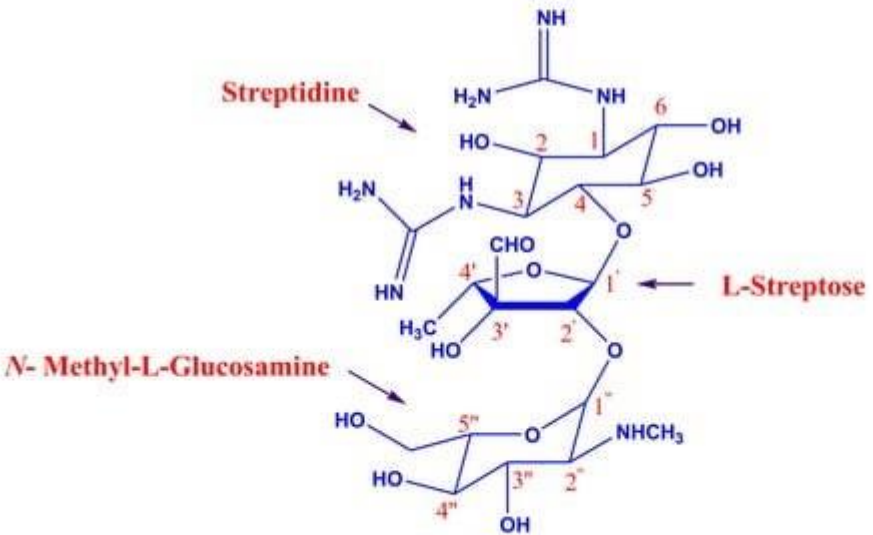
- A semisynthetic ethyl derivative of sisomicin isolated from *Micromonospora inyoensis*.
- Ethylation causes spacial hynderance against APH and ATN enzymes.
- Against gentamicin resistant pseudomona and proteus.



Sisomicin: R=H
Netilmicin: R=C_2H_5

Streptomycin

- ✓ Has a different aminocyclito (a 1,3-diguanidinoinositol).



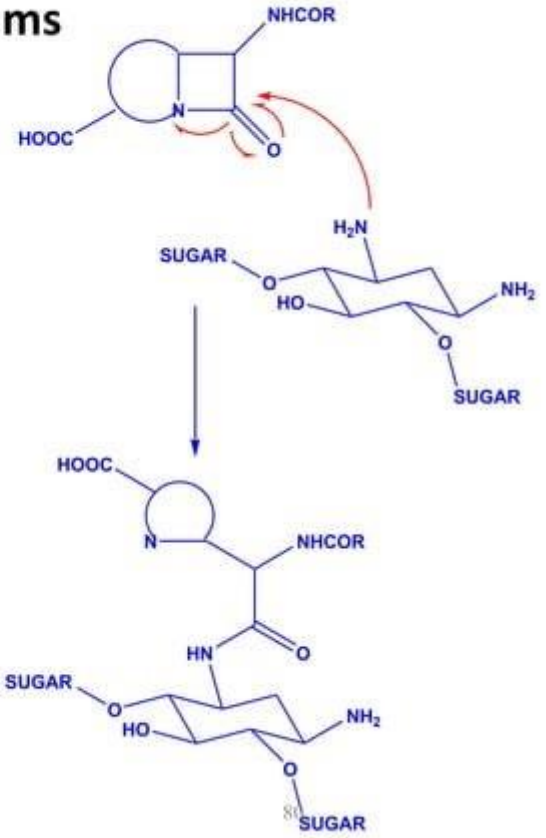
Streptomycin

Streptomycin *continued*

- ✓ Isolated from cultures of *Streptomyces griseus.*
- ✓ It was introduced against tuberculosis in 1943, kanamycin and amikacin are effective against tuberculosis, but not as much as streptomycin.
- ✓ Streptomycin brought Waxman the Noble prize in 1952.

Mechanism of Chemical incompatility of Aminoglycosides with β-lactams

- ✓ Acylation of aminocyclitol portion by the β-lactam molecule.
- ✓ Begins with nucleophilic addition of the amino group to the carbonyl group of β-lactam ring.

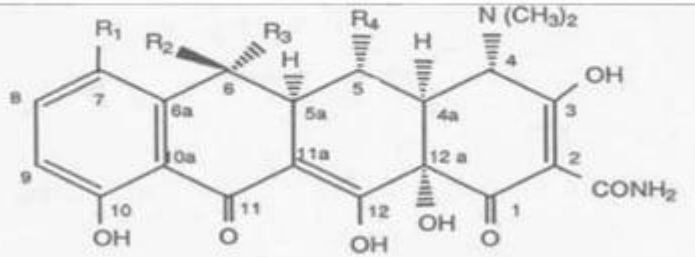


Tetracyclines

TETRACYCLINE

- The tetracyclines are primarily bacteriostatic; inhibit protein synthesis by binding to 305 ribosomes in susceptible organism.
- Subsequent to such binding, attachment of aminoacyl-t-RNA to the mRNA-ribosome complex is interferred.
- As a result,the peptide chain fails to grow.

Classification of tetracycline



S.No.	Name	R_1	R_2	R_3	R_4
1.	Tetracyclines	H	OH	CH_3	H
2.	Chlortetracycline	Cl	OH	CH_3	H
3.	Oxytetracycline	H	OH	CH_3	OH
4.	Demeclocycline	Cl	OH	H	H
5.	Methacycline	H	CH_2	–	OH
6.	Doxycycline	H	CH_3	H	OH
7.	Minocycline	$N(CH_3)_2$	H	H	H
8.	Meclocycline	Cl	CH_2	–	OH

SAR OF TETRACYCLINES

Electron donating (or) electron withdrawing groups at c_7 increased Antibacterial activity

Replacement of $-No_2$ group Gives more potent but carcinogenic compounds.

$(=CH_2$ at c_6 increases the Antibacterial activity Ex: Methacycline).

Elimination of 6-OH group Causes increase lipophilicity And more stable to acids. Ex: Doxycycline.

Presence Of $-N(CH3)2$ group at C_4 Tetracyclines exists Zwitter ion Which can be posible to distribute in The body. Removal of this group loss of activity.

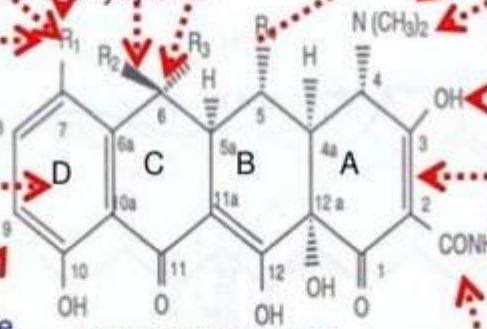
Substitution with –OH Produce water soluble derivatives which can be administered orally.

Substitution of $-N(CH_3)_2$ at 7 increase the activity. Ex: Minocycline.

Little information available.

Epimerization at c_4 and dehydration at 5a results loss of activity.

(any modification at C_3 loss of activity.)



Inviolate zone is essential

The keto-enol tatomerism Between c_2 and c_3 are very important for biological activity.

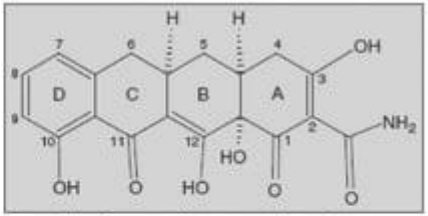
'D' ring should be always aromatic Changes in this ring Leads to biological inactivation of the molecule.

Conversion of corboxamide group to nitriles cause a 20 fold loss of activity.

Additional glycyl amino substitution at the 9th Position leads to the new Class of antibiotics the glycylcyclines. EX: Tigecycline.(Tygacil)

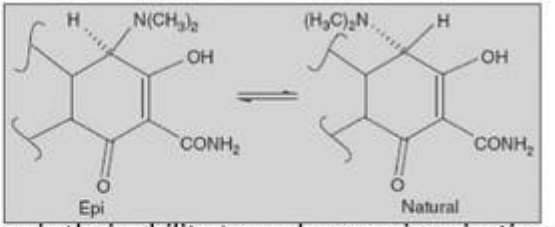
The linearly fused tetracyclic nucleus is most important for the antibiotic activity.

SAR of tetracyclin



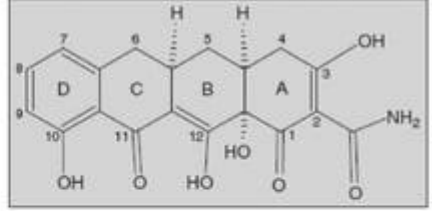
1. C-1 substituents: The keto-enol system of the A-ring is indispensable for antibacterial activity. No variation at the C-1 position has been successful.
2. C-2 substituents: The carboxamide moiety is present in all naturally occurring tetracyclines and this group is crucial for antibacterial activity. The amide is best left unsubstituted, or mono-substitution is acceptable in the form of activated
3. C-3 substituents: In conjugation with the C-1 position, the keto-enol conjugated system is imperative for antibacterial activity.
4. C-4 substituents: The naturally occurring tetracyclines contain α-C-4 dimethylamino substituent that favourably contributes to the keto-enolic character of the A-ring. Replacement of dimethylamino group with a hydrazone, oxime, or hydroxyl group leads to a pronounced loss of activity, probably due to the increase in heteroatom basicity.
5. C-4a substituents: The α-hydrogen at C-4a position of tetracyclines is necessary for useful antibacterial activity.
6. C-5 substituents: Many naturally occurring antibacterial tetracyclines have an unsubstituted methylene moiety at the C-5 position. However, oxytetracycline contains C-5 α-hydroxyl group and was found to be a potent compound, and has been modified chemically to some semi-synthetic tetracyclines. Alkylation of the C-5 hydroxyl group results in a loss of activity.

Effect of pH on tetracyclines



- An interesting property of tetracyclines is their ability to undergo epimerization at C-4 in solutions of intermediate pH range. These isomers are called epitetracyclines. Under the influence of the acidic conditions, an equilibrium is established in about one day consisting of approximately equal amount of isomers. Epitetracyclines exhibit much less activity than natural isomers.
- Strong acids and bases attack the tetracyclines having a hydroxyl group on C-6, causing a loss in activity through modification of C-ring.
- Strong acids produce dehydration through a reaction involving the C-6 hydroxyl group and C-5a hydrogen.
- Bases promote a reaction between the C-6 hydroxyl group and the ketone group at the C-11 position, causing the bond between the C-11 and C-11a atoms to cleave and to form the lactone ring found in the inactive isotetracycline.

SAR of tetracyclin



7. C-5a substituents: The configuration of the naturally occurring tetracyclines places the C-5a hydrogen atom in an α-configuration. Epimerization is detrimental to antibacterial activity.
8. C-6 substituents:

The C-6 position is tolerant of a variety of substituents. The majority of tetracyclines have an α-methyl group and a β-hydroxyl group at this position.

Demeclocyclin is a naturally occurring C-6 demethylated chlortetracycline with an excellent activity. This C-6 methyl group contributes little to the activity of tetracycline.

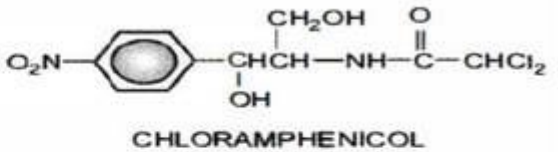
Similarly, the C-6 hydroxyl group also appears to offer little in terms of antibacterial activity; removal of this group affords doxycycline, which is a superb antibacterial.

9. C-7 and C-9 substituents:

The nature of the aromatic D-ring predisposes the C-7 position to electrophilic substitution, and nitro and halogen groups have been introduced.

10. The C-7 acetoxy, azido, and hydroxyl tetracyclines are inferior in terms of antibacterial activity.
11. C-10 substituents: The C-10 phenolic moiety is absolutely necessary for antibacterial activity.
12. C-11 substituents: The C-11 carbonyl moiety is part of one of the conjugated keto-enol systems required for antibacterial activity.
13. C-12a substituents: The C-12a hydroxyl group is needed for antibacterial activity, although this moiety can be esterified to provide tetracycline with increased lipophilicity.

Chloramphenicol



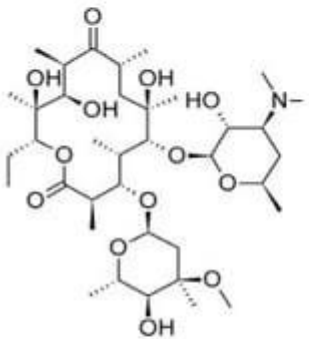
CHLORAMPHENICOL

- MOA of Chloramphenicol-it inhibits bacterial protein synthesis by interferring with 'transfer' of the elongating peptide chain to the newly attached aminoacyl-tRNA at the ribosome-mRNA complex.
- It specifically attaches to the 50S ribosome and thus may hinder the access of aminoacyl-tRNA to the acceptor site for amino acid incorporation
- it prevents formation of peptide bonds

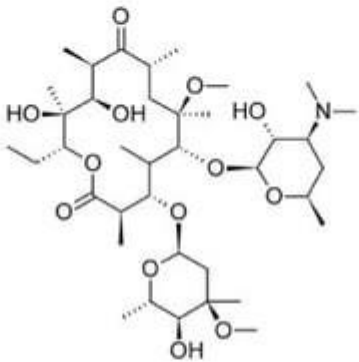
Microlide antibiotics

- MACROLIDE ANTIBIOTICS-The macrolide antibiotics have three common chemical characteristics:
- A large lactone ring
- A ketone group
- A glycosidically linked amino sugar
- These are antibiotics having a macrocyclic lactone ring with attached sugars.
- Among the many antibiotics isolated from the actinomycetes is the group of chemically related compounds called the *macrolides*
- Erythromycin is the first member discovered in the 1950s,Roxithromycin, Clarithromycin and Azithromycin.

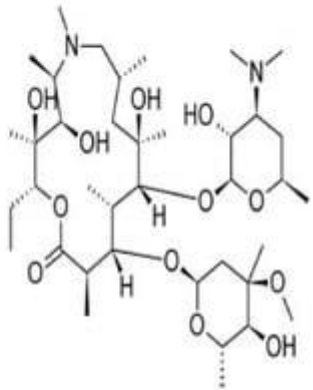
Microlide structures



Erythromycin



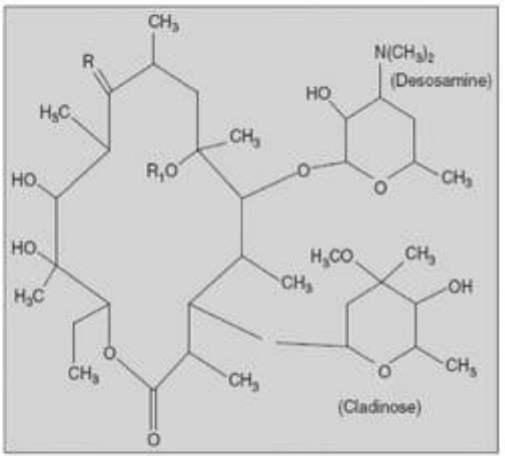
Clarithromycin



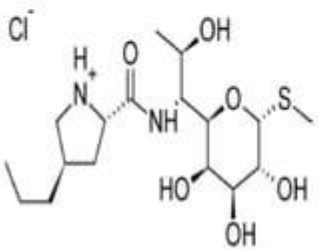
Azithromycin

SAR of Microlide

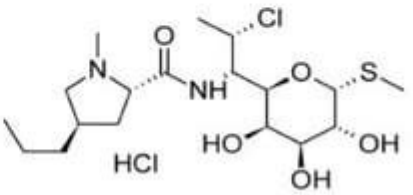
- A number of strategies have been utilized to improve the acid stability of erythromycin.
- The first approach involved the addition of hydroxylamine to the ketone to form oxime— e.g., roxithromycin.
- The second approach involves an alteration of C-6 hydroxyl group, which is the nucleophilic functionality that initiates erythromycin degradation. Modification that removes the nucleophilic nature of this hydroxyl group can retain antibacterial properties if the size of the group is kept small so as not to affect the ribosomal binding —e.g., clarithromycin.
- The azalides (e.g., azithromycin) are semi-synthetic 15-membered congeners in which a nitrogen atom has been introduced to expand a 14-membered precursor, and this leads to an extended spectrum of action.



Lincomycin



Lincomycin Hydrocholride



Clindamycin