Topic 8: Anti-infective agents

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❖ INTRODUCTION

- The history of work on the prevention of bacterial infection can be traced back to the 19th century when Joseph Lister (in 1867) introduced antiseptic principles for use in surgery and posttraumatic injury.

- He used phenol (carbolic acid) in the surgery. Lister’s principles caused a dramatic decrease in the incidence of postsurgical infections.

- Around 1881 and continuing to 1900, microbiologist Paul Ehrlich & Robert Koch, began work with a set of antibacterial dyes and antiparasitic organic arsenicals. “**magic bullets**”

- Anti-infective agents that are used locally are called ***germicides***.

- ***Antiseptics*** are compounds that kill (-***cidal***) or prevent the growth of (-***static***) microorganisms when applied to living tissue.

- **The ideal antiseptic must have**:

- Low-enough toxicity that it can be used directly on skin or wounds.
- It will exert a rapid and sustained lethal action against microorganisms.
- The agent should have a low surface tension so that it will spread into the wound;
- Be nonirritating to tissues
- be non-allergenic, lack systemic toxicity when applied to skin or mucous membranes.

- No antiseptic available today meets all of these criteria. But they have most of them

- A ***disinfectant*** is an agent that prevents transmission of infection by the destruction of pathogenic microorganisms when applied to *inanimate objects*.

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Topic 8A: Disinfectants & antiseptics

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1) ALCOHOLS

- Two of the most commonly used antiseptics and disinfectants are **ethyl and isopropyl alcohol**.

- The antibacterial potencies of the primary alcohols increase with molecular weight until the 8-carbon atom octanol is reached. After 8-carbon water solubility decreases & antimicrobial potency diminishes.

- Branching of the alcohol chain decreases antibacterial potency; branching do not penetrate bacterial cell membranes as efficiently.

- Despite this fact, 2-propanol (isopropyl alcohol) is used commercially instead of n-propyl alcohol, because it is less expensive.

- The activity of alcohols against microorganisms is the result of their ability to **denature important proteins and carbohydrates**.

❑ ETHANOL

- The primary medicinal use of alcohol is external, as an antiseptic, preservative, mild counterirritant, or solvent.

- Alcohol is metabolized in the human body by alcohol dehydrogenase and aldehyde dehydrogenase.

- Acetaldehyde causes nausea, vomiting, and vasodilatory flushing. This fact has been used to treat **alcoholism** with the drug disulfiram, which blocks aldehyde dehydrogenase, allowing acetaldehyde to accumulate.

- The accepted bactericidal concentration is 70% alcohol but there is no study support that conc. between 60% & 95% are less.

- Concentrations below 60% are also effective, but longer contact times are necessary. Concentrations above 70% can be used safely too.

2) PHENOLS & THEIR DERIVATIVES

- Phenol, remains the standard to which the activity of most germicidal substances is compared.

- The ***phenol coefficient*** is defined as the ratio of a solution of a given test disinfectant to the solution of phenol that is required to kill a strain of *Salmonella typhi* under controlled time and temperature conditions.

- As an example, if the solution of a test disinfectant is 10-fold greater than the dilution of phenol, the phenol coefficient is 10.

- Substitution with alkyl, aryl, and halogen (especially in the *para* position) groups on phenol increases bactericidal activity.

- Straight-chain alkyl groups enhance bactericidal activity more than branched groups.

- Alkylated phenols & resorcinol are less toxic than the parent compounds while retaining bactericidal properties.

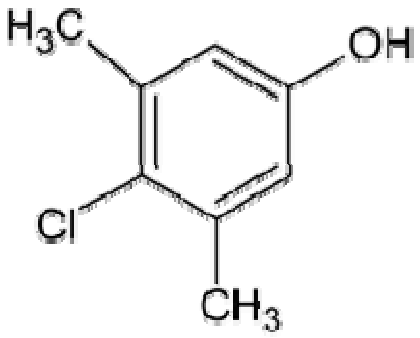
- **Phenols denature bacterial proteins at low concentrations, whereas lysis of bacterial cell membranes occurs at higher concentrations.**

❑ *p*-Chloro-*m*-xylenol

- it is a nonirritating antiseptic agent with broad-spectrum antibacterial and antifungal properties.

- It is marketed in a 2% concentration as a shampoo.

- Used to treat tinea (ringworm) infections such as athlete's foot.

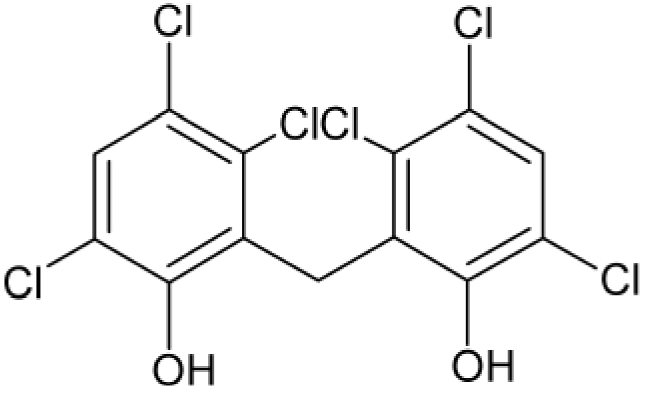


❑ Hexachlorphene

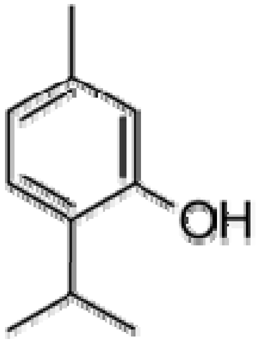
- ➢ increased degree of chlorination of hexachlorophene increases its antiseptic potency.

- ➢ Hexachlorophene is easily adsorbed onto the skin and enters the sebaceous glands. Because of this, topical application elicits a prolonged antiseptic effect, even in low concentrations.

- ➢ Hexachlorophene is used in concentrations of 2% to 3% in soaps, detergent creams, lotions, and shampoos for various antiseptic uses.



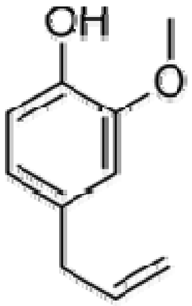
❑ Thymol



- it is extracted from oil of *Thymus vulgaris* (thyme, of the mint family)

- Thymol has mild fungicidal properties and is used in alcohol solutions and in dusting powders for the treatment of tinea (ringworm) infections.

❑ Eugenol



- it is obtained primarily from clove oil.

- Eugenol possesses both local anesthetic and antiseptic activity and can be directly applied on a piece of cotton to relieve toothaches.

- Eugenol is also used in mouthwashes because of its antiseptic property and pleasant taste.

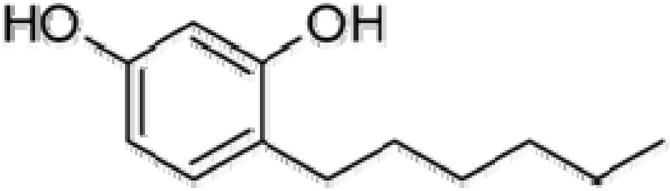
- The phenol coefficient of eugenol is 14.4.

❑ Hexylresorcinol

- Hexylresorcinol is an effective antiseptic, possessing both bactericidal and fungicidal properties.

- The phenol coefficient of hexylresorcinol against *S. aureus* is 98.

- The compound also has local anesthetic activity. Hexylresorcinol is formulated into throat lozenges because of its local anesthetic and antiseptic properties.



3) HALOGEN-CONTAINING COMPOUNDS

❑ Iodophors

- Elemental iodine (I_2) is probably the oldest germicide still in use today.

- Iodine tincture (2% iodine in 50% alcohol with sodium iodide), strong iodine solution (Lugol’s solution, 5% iodine in water with potassium iodide), and iodine solution (2% iodine in water with sodium iodide) are currently official preparations in the USP.

- The iodide salt is mixed to increase the solubility of the iodine and to reduce its volatility.
- Iodine is one of the most effective and useful of the germicides “bactericidal & fungicidal”.

- It acts to inactivate proteins by iodination of aromatic residues (phenylalanyl and tyrosyl) and oxidation (sulfhydryl groups).

- **Povidone–Iodine**

- It is a charge-transfer complex of iodine with the nonionic surfactant PVP “polyvinylpyrrolidone”.

- The complex is extremely water soluble and releases iodine very slowly.

- the preparation provides a nontoxic, nonvolatile, and nonstaining form of iodine that is not irritating to the skin or to wounds.

- It is used as an aqueous solution for presurgical disinfection of the incision site. It can also be used to treat infected wounds and damage to the skin, and it is effective for local bacterial and fungal infections.

4) CATIONIC SURFACTANTS

- All of the cationic surfactants are quaternary ammonium compounds. The compounds, with a polar head group and nonpolar hydrocarbon chain, form micelles by concentrating at the interface of immiscible solvents.

- The cationic surfactants exert a bactericidal action against a broad spectrum of Gram-positive and Gram-negative bacteria. They are also active against several pathogenic species of fungi and protozoa. All spores resist these agents.

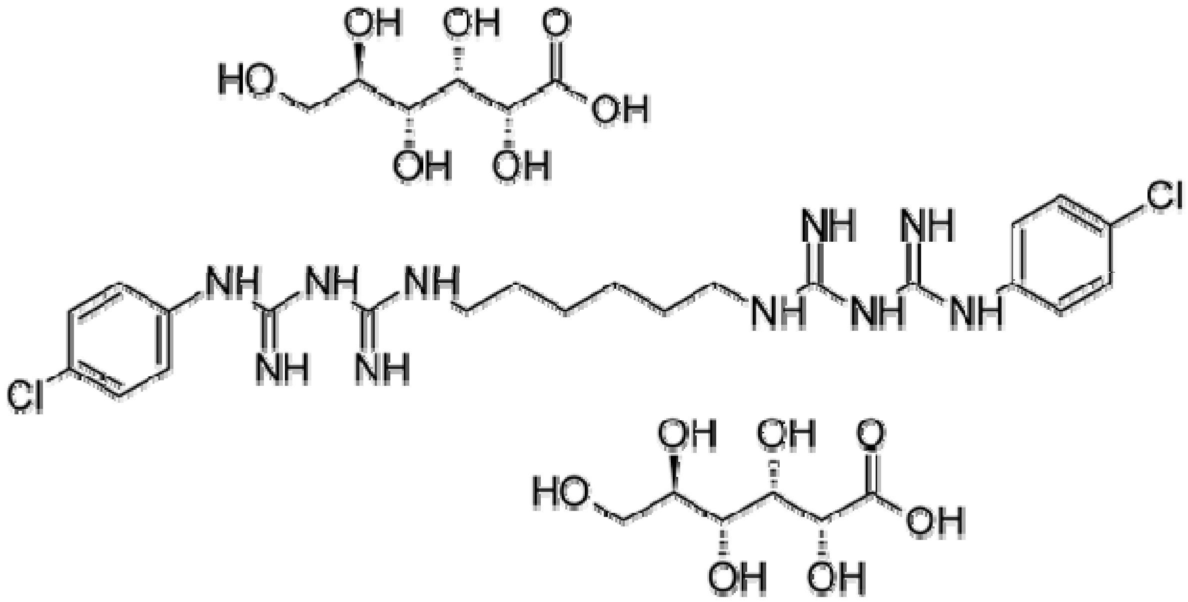
- The mechanism of action probably involves dissolution of the surfactant into the microbial cell membrane, destabilization, and subsequent lysis. The surfactants may also interfere with enzymes associated with the cell membrane.

❑ Chlorhexidine Gluconate

- ➢ it is the most effective of a series of antibacterial biguanides originally developed in Great Britain.

- ➢ Chlorhexidine has broad-spectrum antibacterial activity but is not active against acid-fast bacteria, spores, or viruses.

- ➢ Chlorhexidine is not absorbed through skin or mucous membranes and does not cause systemic toxicity.



Topic 8B: Antibacterial agents

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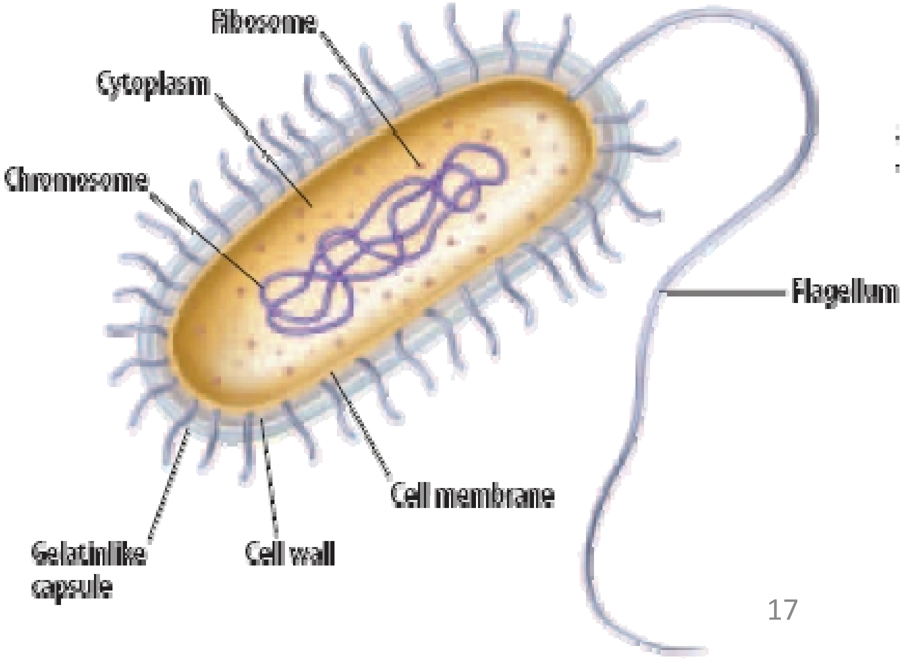
❖The bacterial cell

- The success of antibacterial agents owes much to the fact that they can act selectively against bacterial cells rather than animal cells. This selectivity comes from the difference between bacterial & animal cells.

- Differences between bacterial cell "prokaryotic" & animal cell "eukaryotic":

- The bacterial cell does not have a defined nucleus, whereas animal cell does.
- Animal cells contain a variety of structures called organelles "mitochondria, endoplasmic reticulum, etc." whereas the bacterial cell is relatively simple.



- The biochemistry of a bacterial cell differs significantly from that of an animal cell.

  -E.g., bacteria may have to synthesize essential vitamins which animal cells can acquire intact from food. The bacterial cells must have the enzymes to catalyze these reactions. Animal cells do not, because the reactions are not required.

- the bacterial cell has a cell membrane and a cell wall, whereas the animal cell has only a cell membrane. The cell wall is crucial to the bacterial cell´s survival.

  - if bacterial cell lacking a cell wall was placed in aq. environment containing a low concentration of salts, water would enter the cell due to osmotic pressure. This would cause the cell to swell and burst. This called **lysis**.

❖Mechanisms of antibacterial action

1. Inhibition of cell metabolism.

- They are called antimetabolites. These compounds inhibit the metabolism of a microorganism, but not the metabolism of the host.

- The best known examples of antibacterial agents acting in this way are the sulfonamides.

2. Inhibition of bacterial cell wall synthesis.

- Inhibition of cell wall synthesis leads to bacterial cell lysis (bursting) and death.
- Agents operating in this way include penicillins, cephalosporins & vancomycin.

- Human cells do not have a cell wall, they are unaffected by such agents.

3. Interactions with the plasma membrane.

- They interact with the plasma membrane of bacterial cells to affect membrane permeability. This has fatal results for the cell.
- Polymyxins operate in this way.

4. Disruption of protein synthesis.

- The essential enzymes required for the cell's survival can no longer be made.
- E.g rifamycins, aminoglycosides, tetracyclines, & chloramphenicol.

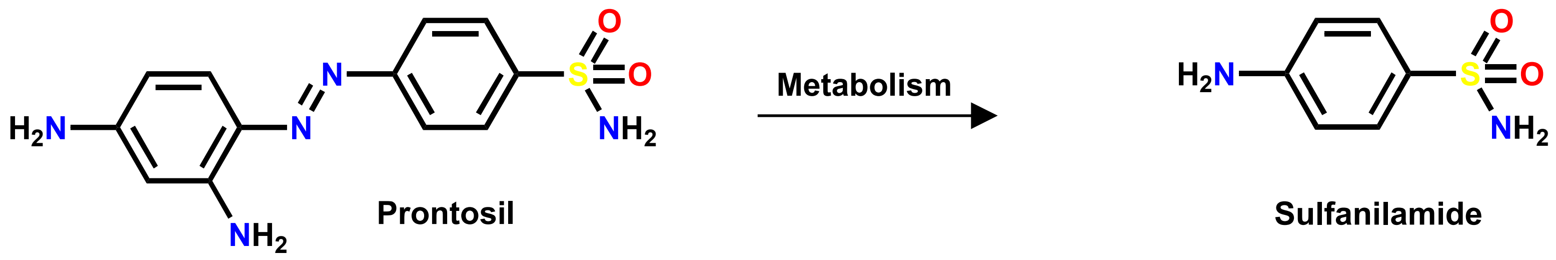
5. Inhibition of nucleic acid transcription and replication.

- Inhibition of nucleic acid function prevents cell division and/or the synthesis of essential enzymes.
- Agents acting in this way include nalidixic acid

1) Antibacterial agents which act against cell metabolism

1) Sulfonamides:

a. Lead Compound



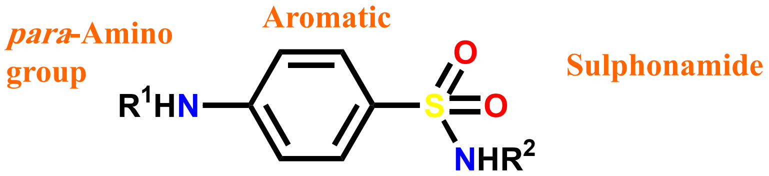
Prontosil

Sulfanilamide

Notes

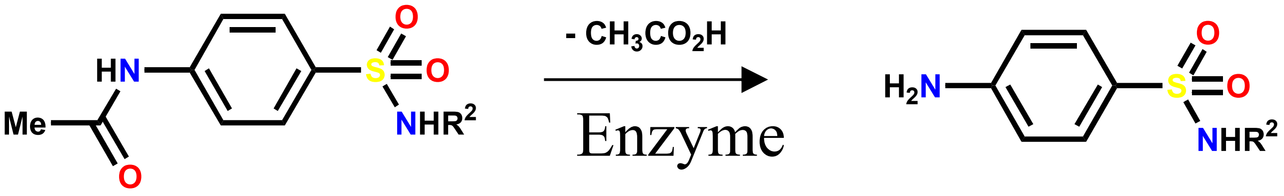
- Prontosil - red dye had antibacterial activity *in vivo* (1935)
- Inactive *in vitro*
- Metabolised to active sulphonamide
- Acts as a prodrug
- Sulphanilamide - first synthetic antibacterial agent acting on a wide range of infections

b. Structure-Activity Relationships



- Primary amino group is essential (R^1=H)
- Amide groups (R^1=acyl) are allowed
  - inactive *in vitro*, but active *in vivo*
  - act as prodrugs
- Aromatic ring is essential
- *para*-Substitution is essential
- Sulphonamide group is essential
- Sulphonamide nitrogen must be primary or secondary
- R^2 can be varied

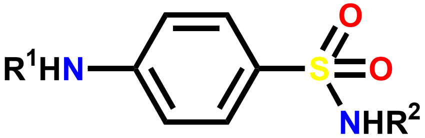
c. Prodrugs of sulfonamides



Notes

- Amide group lowers the polarity of the sulphonamide
- Amide cannot ionise
- Alkyl group increases the hydrophobic character
- Crosses the gut wall more easily
- Metabolised by enzymes (e.g. peptidases) *in vivo*
- Metabolism generates the primary amine
- Primary amine ionises and can form ionic interactions
- Ionised primary amine also acts as a strong HBD

d. Sulphanilamide analogues



Notes

- R^2 is variable
- Different aromatic and heteroaromatic rings are allowed
- Affects plasma protein binding
- Determines blood levels and lifetime of the drug
- Affects solubility
- Affects pharmacokinetics rather than pharmacodynamices

e. Sulphanilamides - applications

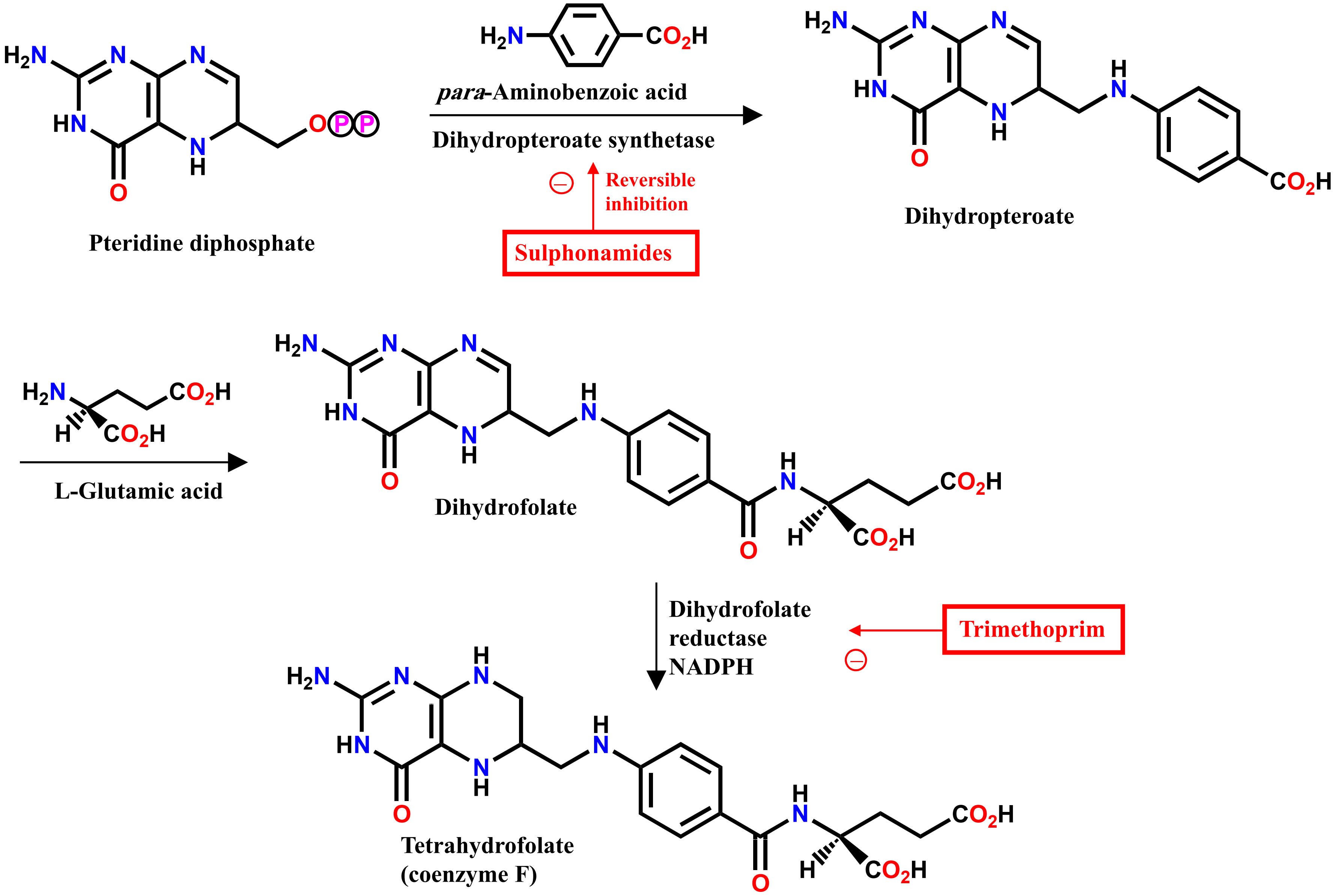
Notes

- Antibacterial drugs of choice prior to penicillins (1930s)
- Superseded by penicillins

Current uses

- Treatment of urinary tract infections
- Eye lotions
- Treatment of gut infections
- Treatment of mucous membrane infections

f. Mechanism of action


Pteridine diphosphate

para-Aminobenzoic acid

Dihydropteroate synthetase

⊖ Reversible inhibition

Sulphonamides

Dihydropteroate

L-Glutamic acid

Dihydrofolate

Dihydrofolate reductase NADPH

⊖

Trimethoprim

Tetrahydrofolate (coenzyme F)

f. Mechanism of action

Target enzyme

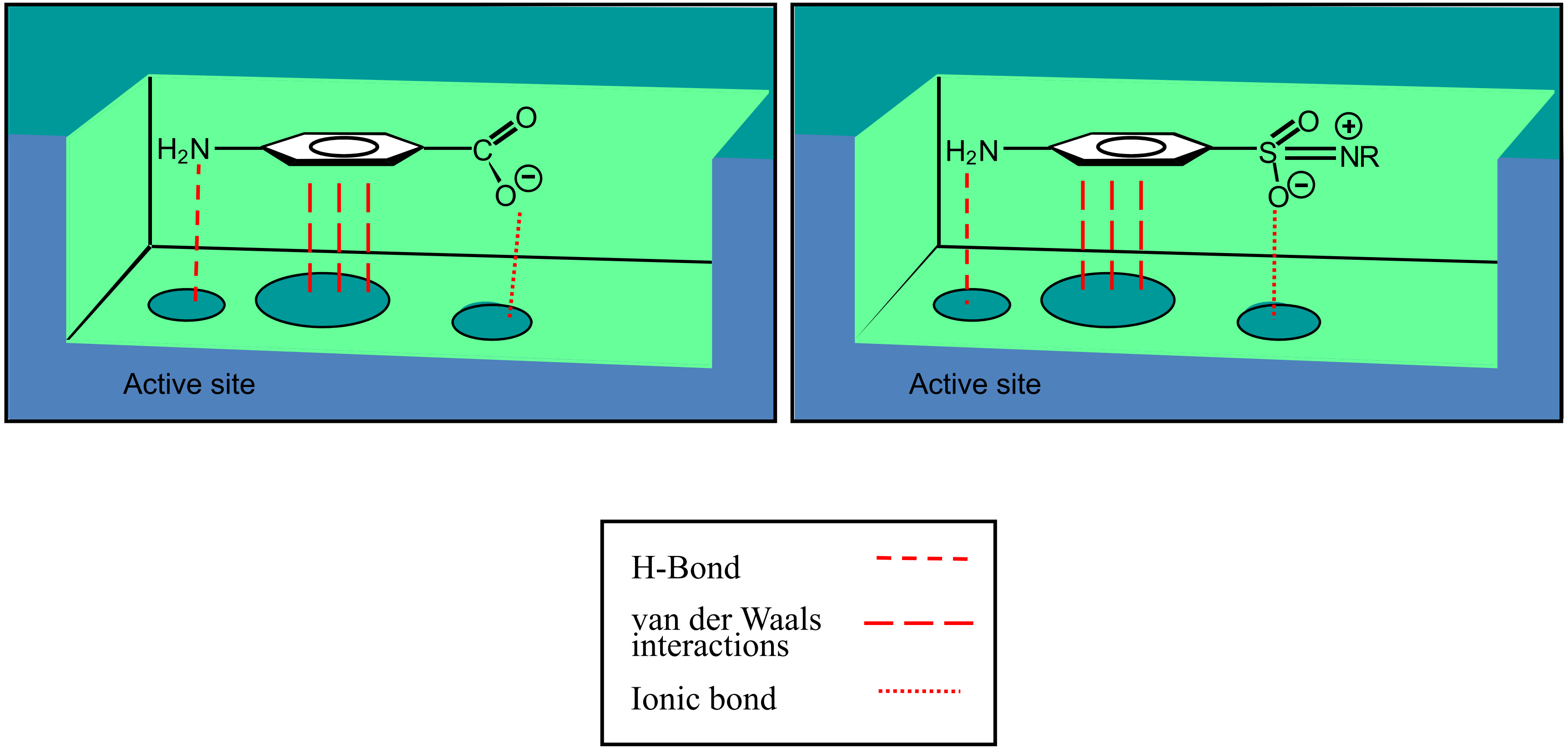
- Dihydropteroate synthetase - bacterial enzyme
- Not present in human cells
- Important in the biosynthesis of the tetrahydrofolate cofactor
- Cofactor is crucial to pyrimidine and DNA biosynthesis
- Crucial to cell growth and division

Sulphonamides

- Competitive enzyme inhibitors
- Bacteriostatic agents “agents that inhibit cell growth”
- Mimic the enzyme substrate - *para*-aminobenzoic acid (PABA)
- Bind to the active site and block access to PABA
- Reversible inhibition
- Resistant strains produce more PABA

f. Mechanism of action

Binding interactions



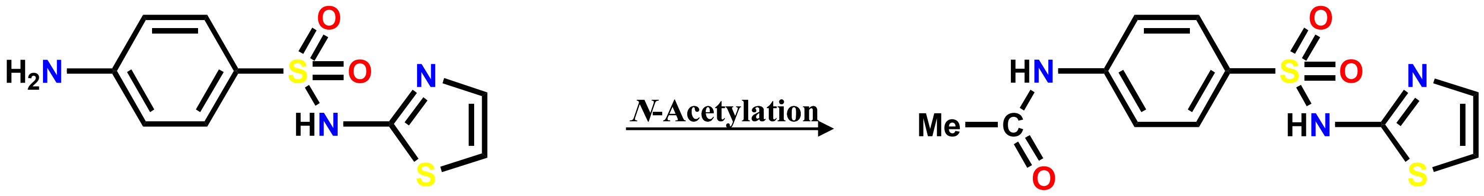
- **The Big Question**: tetrahydrofolate is necessary for the survival of bacterial cells but it is also vital for the survival of human cells so why are the sulfa drugs not toxic to human?
- In human cells, tetrahydrofolate is synthesized from folic acid which is obtained from the diet as a vitamin. Folic acid is brought across cell membranes by a **transport protein**.
- Bacteria lacks the transport protein necessary to transport folic acid.

Metabolic differences between bacterial and mammalian cells

Dihydropteroate synthetase is present only in bacterial cells

Transport protein for folic acid is only present in mammalian cells

g. Sulphonamides - Drug Metabolism



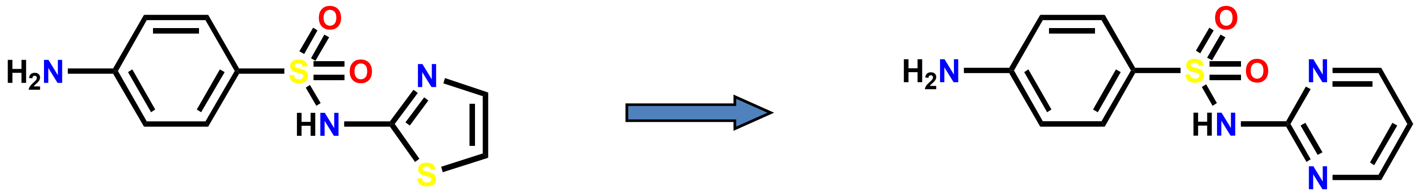
Sulphathiazole

Insoluble metabolite

Notes

- Sulphonamides are metabolised by *N*-acetylation
- *N*-Acetylation increases hydrophobic character
- Reduces aqueous solubility
- May lead to toxic side effects

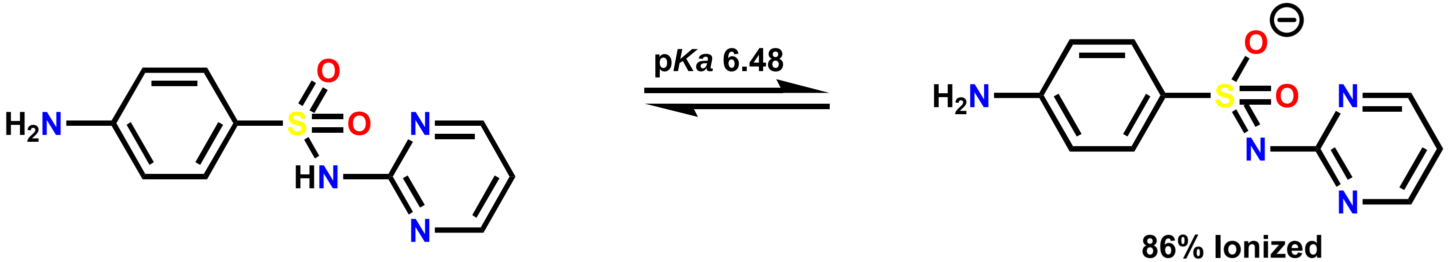
h. Sulfonamides with reduced toxicity



Sulphathiazole **Sulphadiazine**

Notes

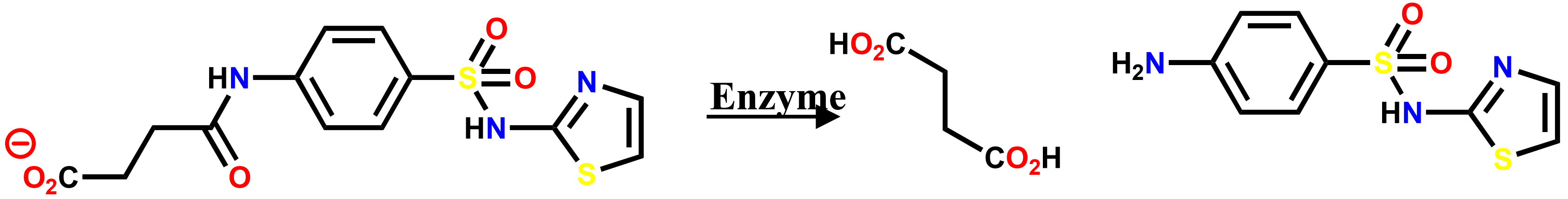
- Thiazole ring is replaced with a pyrimidine ring
- Pyrimidine ring is more electron withdrawing
- Sulphonamide NH proton is more acidic and ionisable
- Sulphadiazine and its metabolite are more water soluble
- Reduced toxicity
- Silver sulphadiazine is used topically to prevent infection of burns



86% Ionized

i. Examples of Sulphonamides

Succinyl sulphathiazole

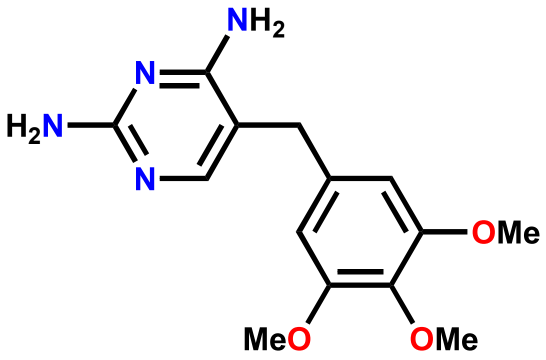


Succinyl sulphathiazole **Succinic acid** **Sulphathiazole**

Notes

- Acts as a prodrug of sulphathiazole
- Ionised in the slightly acidic conditions of the intestine
- Too polar to cross the gut wall
- Concentrated in the gut
- Slowly hydrolysed by enzymes in the gut
- Used versus gut infections

2) Trimethoprim:



- Trimethoprim is a diaminopyrimidine structure.

- It acts against dihydrofolate reductase.

-The enzyme which carries out the conversion of folic acid to tetrahydrofolate.

- Thus the effect is the same as with sulphonamides, inhibition of DNA synthesis and cell growth.

- Dihydrofolate reductase is present in mammalian cells as well as bacterial cells, but trimethoprim can distinguish between the enzymes in either cell because of difference in structure between the two enzymes

- So trimethoprim inhibits the bacterial enzyme but not the mammalian enzyme.

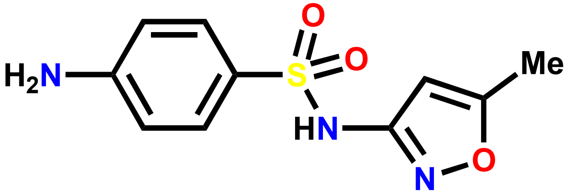
- Trimethoprim is given: in conjunction with the sulfonamide (sulfamethoxazole). The combination is called Cotrimoxazole

- Sulfamethoxazole inhibits the incorporation of PABA, while Trimethoprim inhibits dihydrofolate reductase.

- Thus two enzymes are inhibited and the advantage is that the doses of both drugs can be kept down to safe levels.

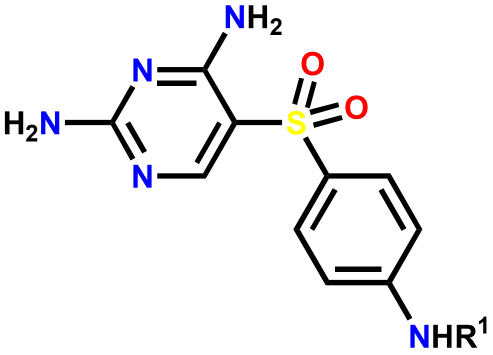
- This approach is called **'sequential blocking**‘

- This combination is less likely to induce bacterial resistance than either agent alone.



Sulphamethoxazole

3. Sulphones



- **Thought to inhibit dihydropteroate synthetase**
- **Used in the treatment of leprosy**

2) Antibacterial agents which inhibit cell wall synthesis

1) PENICILLINS:

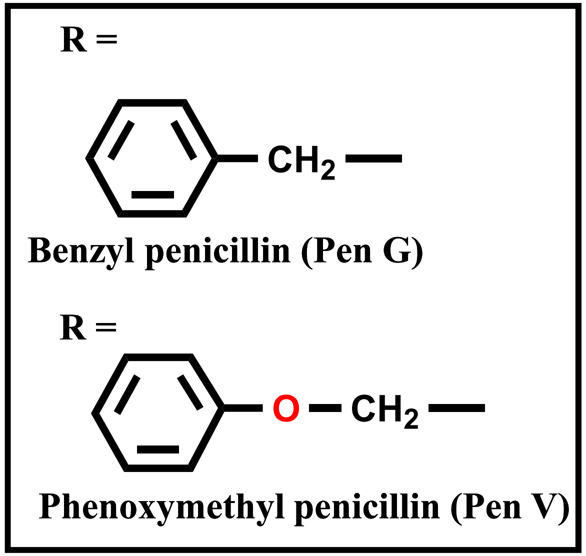
Introduction to penicillins

- Discovered by Fleming from a fungal colony (1928)

-There was an area surrounding the fungal colony where the bacterial colonies were dying. fungus belongs to species of Penicillium

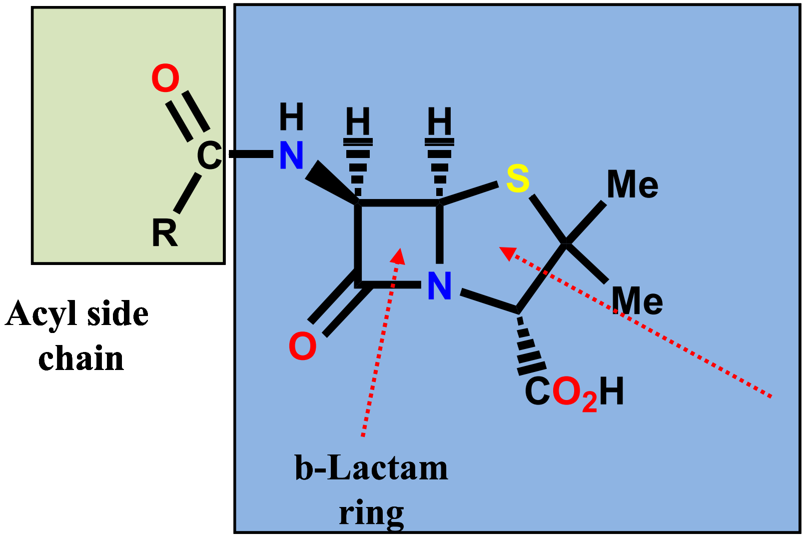
- Shown to be non toxic and antibacterial
- Isolated and purified by Florey and Chain (1938) & the first successful clinical trial conducted by them was in (1941)
- Produced by large scale fermentation (1944)
- Structure established by X-ray crystallography (1945)
- Full synthesis developed by Sheehan (1957)
- Beechams company: Isolated a biosynthetic intermediate of penicillin called 6-aminopenicillanic acid (6-APA).(1958-60)
- This provided the starting material for a huge range of semisynthetic penicillins.

STRUCTURE



Benzyl penicillin (Pen G)

Phenoxymethyl penicillin (Pen V)



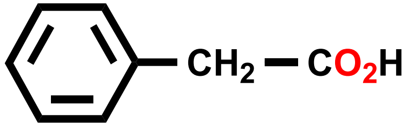
Acyl side chain

b-Lactam ring

Thiazolidine ring

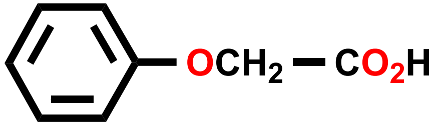
6-Aminopenicillanic acid (6-APA)

Side chain varies depending on carboxylic acid present in fermentation medium



→ Penicillin G

present in corn steep liquor



→ Penicillin V
(first orally active penicillin)

Properties of Penicillin G

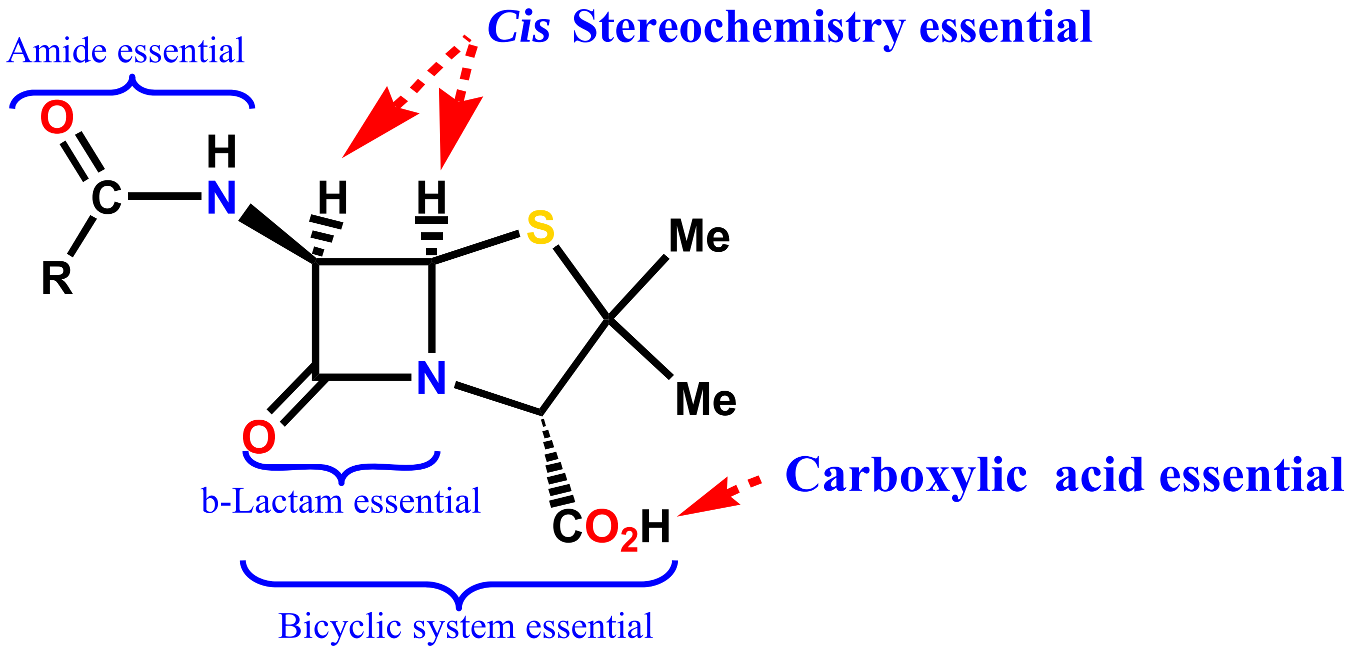
- Active vs. Gram +ve bacilli and some Gram -ve cocci
- Non toxic
- Limited range of activity
- Not orally active "is broken down in the stomach acid" - must be injected
- Sensitive to β-lactamases
  (enzymes which hydrolyse the β-lactam ring)
- Some patients are allergic
- Inactive vs. *Staphylococci*

Drug Development

Aims

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

Structure activity relationship



Amide essential

Cis **Stereochemistry essential**

Carboxylic acid essential

b-Lactam essential

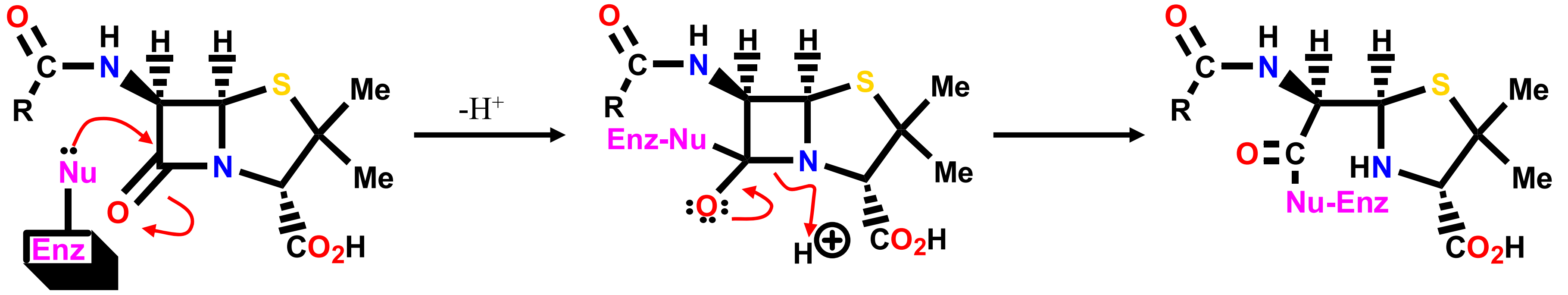
Bicyclic system essential

Conclusions

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

Mechanism of action

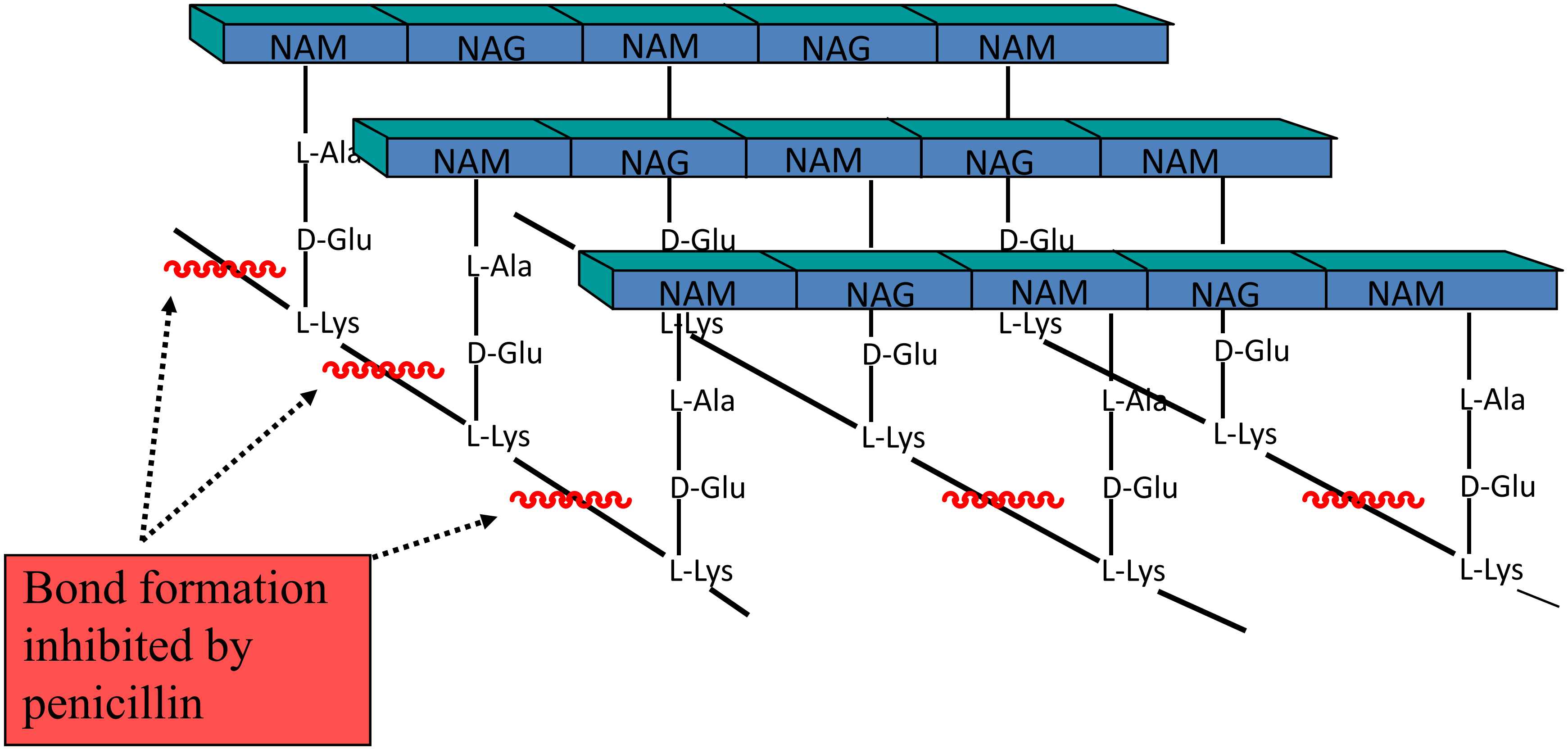
- Penicillins inhibit a bacterial enzyme called the transpeptidase enzyme which is involved in the synthesis of the bacterial cell wall
- The β-lactam ring is involved in the mechanism of inhibition
- Penicillin becomes covalently linked to the enzyme's active site by means of an ester link to a serine residue
- Penicillin is not split in two and acts as a steric shield to prevent access of substrate or water to the active site
- Results in irreversible inhibition



- **Covalent bond formed to transpeptidase enzyme**
- **Irreversible inhibition**

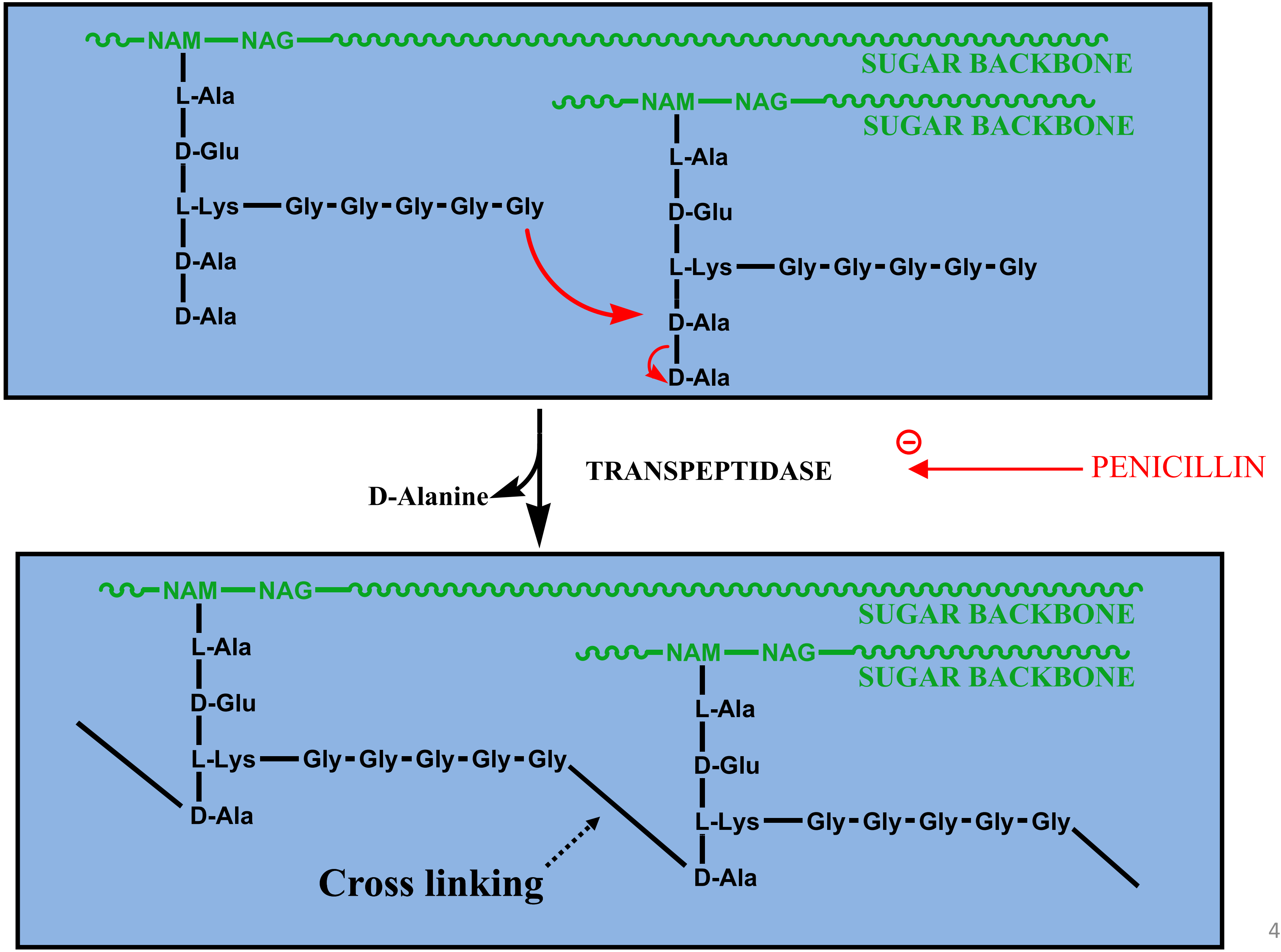
Mechanism of action - bacterial cell wall synthesis

- the wall is peptidoglycan structure. 2 types of sugars “N-acetyl muramic acid & N-acetylglucosamine”.



Bond formation inhibited by penicillin

Mechanism of action - bacterial cell wall synthesis



NAM—NAG

SUGAR BACKBONE

L-Ala

D-Glu

L-Lys—Gly-Gly-Gly-Gly-Gly

D-Ala

D-Ala

NAM—NAG

SUGAR BACKBONE

L-Ala

D-Glu

L-Lys—Gly-Gly-Gly-Gly-Gly

D-Ala

D-Ala

D-Alanine

TRANSPEPTIDASE

⊖

PENICILLIN

NAM—NAG

SUGAR BACKBONE

L-Ala

D-Glu

L-Lys—Gly-Gly-Gly-Gly-Gly

D-Ala

NAM—NAG

SUGAR BACKBONE

L-Ala

D-Glu

L-Lys—Gly-Gly-Gly-Gly-Gly

D-Ala

Cross linking

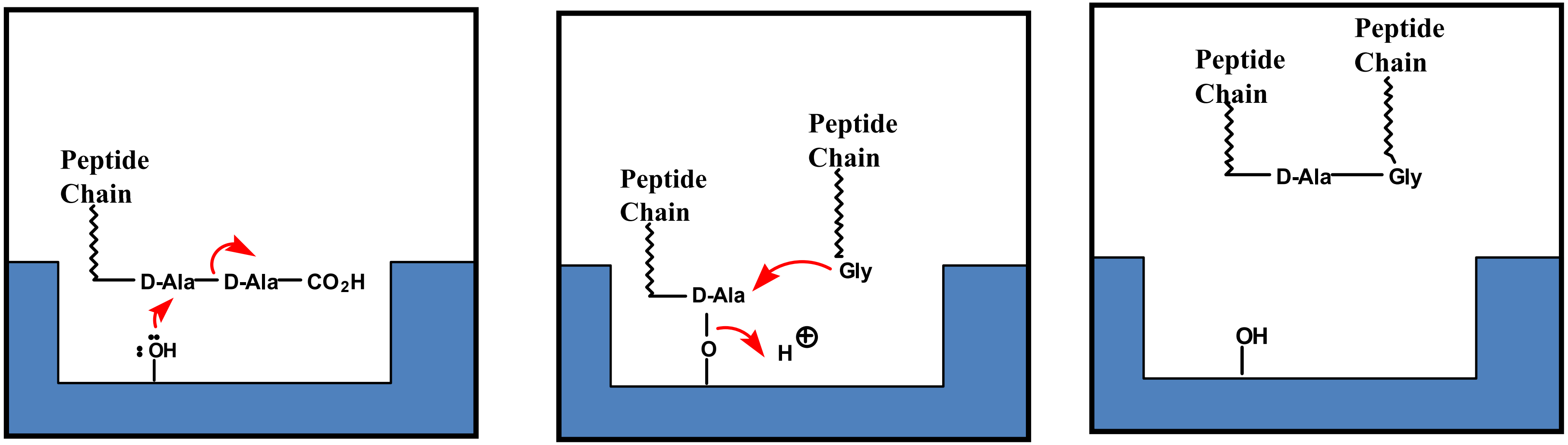
Mechanism of action - bacterial cell wall synthesis

- Penicillin inhibits final crosslinking stage of cell wall synthesis
- It reacts with the transpeptidase enzyme to form an ester linkage with a serine residue
- The ring-opened penicillin acts as a steric shield
- Neither substrate nor water is capable of reaching the ester link
- Results in irreversible inhibition
- Inhibition of transpeptidase leads to a weakened cell wall
- Cells swell due to water entering the cell, then burst (lysis)
- Penicillin thought to mimic *D*-Ala-*D*-Ala

Mechanism of action - bacterial cell wall synthesis

Alternative theory- Pencillin mimics D-Ala-D-Ala.

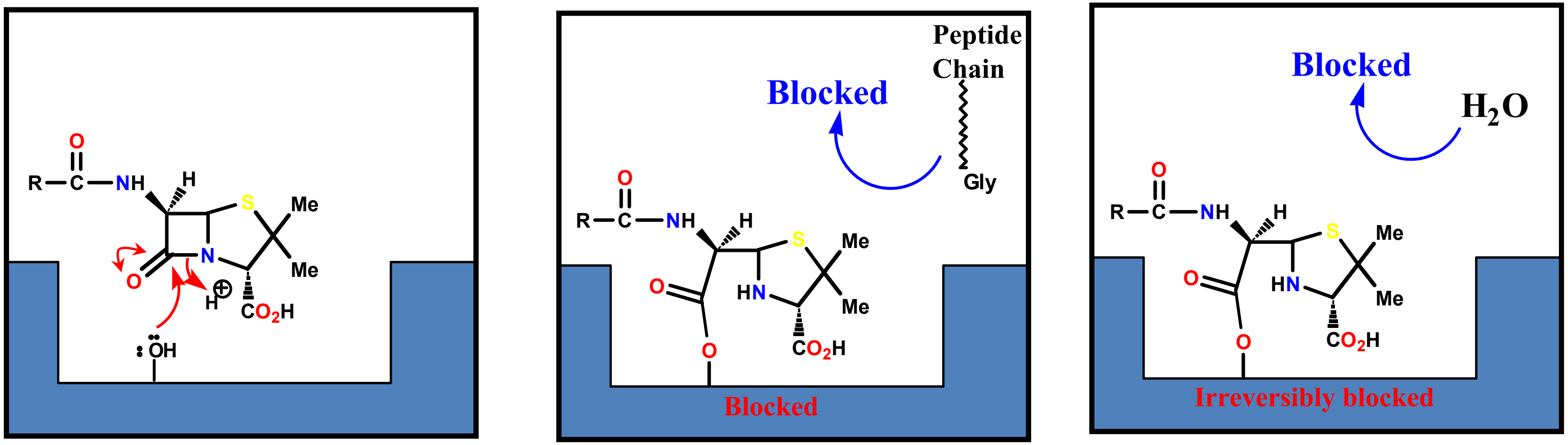
Normal Mechanism



Mechanism of action - bacterial cell wall synthesis

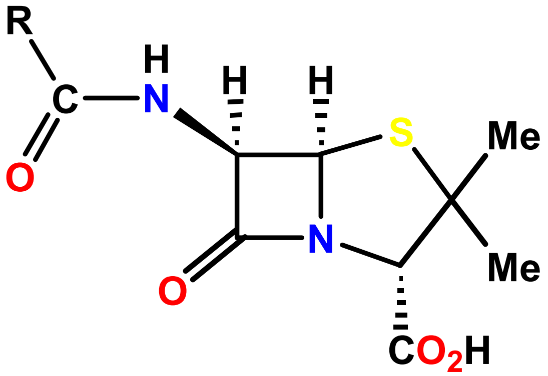
Alternative theory- Pencillin mimics D-Ala-D-Ala.

Mechanism inhibited by penicillin

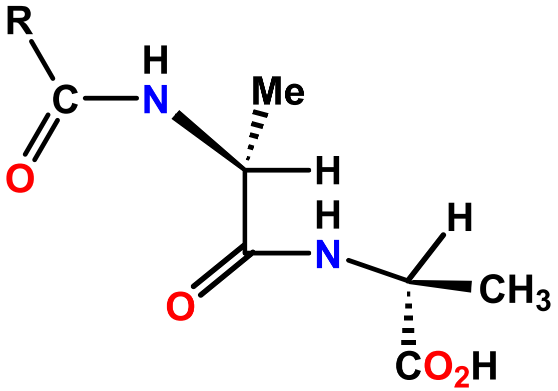


Mechanism of action - bacterial cell wall synthesis

Penicillin can be seen to mimic acyl-D-Ala-D-Ala



Penicillin



Acyl-D-Ala-D-Ala

Gram +ve and Gram -ve Cell Walls

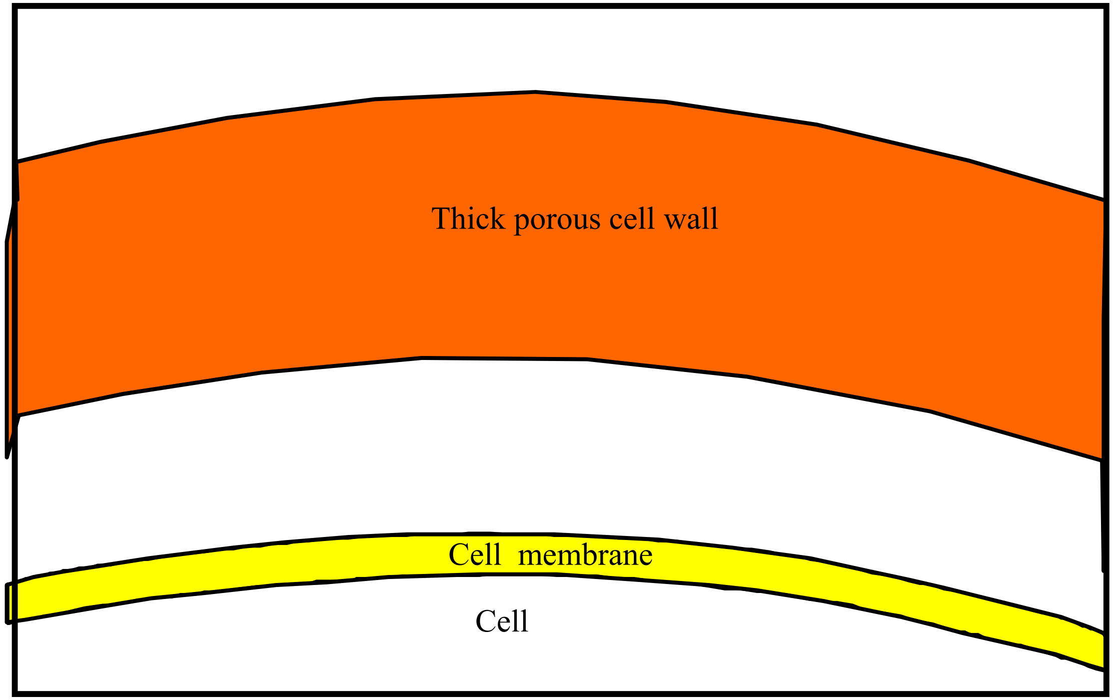
•Penicillins have to cross peptidoglycan layers in order to reach their target enzyme

•Peptidoglycan layers are porous and are not a barrier

•The peptidoglycan layers of Gram +ve bacteria are thicker than Gram -ve cell walls, but the former are more susceptible to penicillins

Gram +ve and Gram -ve Cell Walls

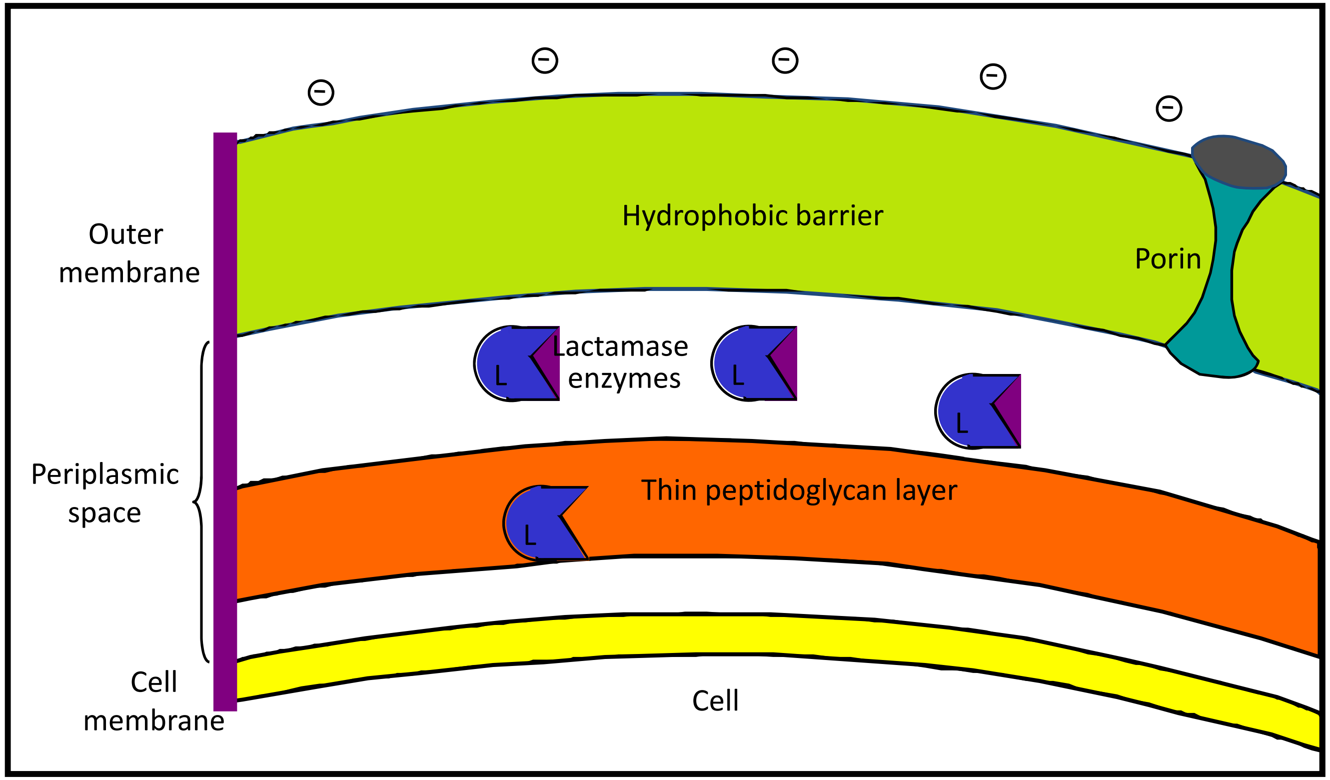
Gram +ve bacteria



- Thick porous peptidoglycan cell wall
- No outer membrane
- Penicillins cross cell wall easily
- Gram +ve more susceptible to penicillins

Gram +ve and Gram -ve Cell Walls

Gram -ve bacteria



- **Thin peptidoglycan layer**
- **Hydrophobic outer membrane**

- acts as a barrier to penicillins

- **Gram –ve more resistant to penicillins**

Resistance to Penicillins

1) Gram -ve bacteria have a lipopolysaccharide outer membrane preventing access to the periplasmic space.

- Penicillins can only cross via porins in the outer membrane. Porins allow small hydrophilic molecules such as zwitterions to cross.

2) High levels of transpeptidase enzyme may be present.

3) The transpeptidase enzyme may have a low affinity for penicillins (e.g. Pencillin binding protein “PBP” 2a for *S. aureus*).

4) Efflux mechanisms pumping penicillins out of the periplasmic space.

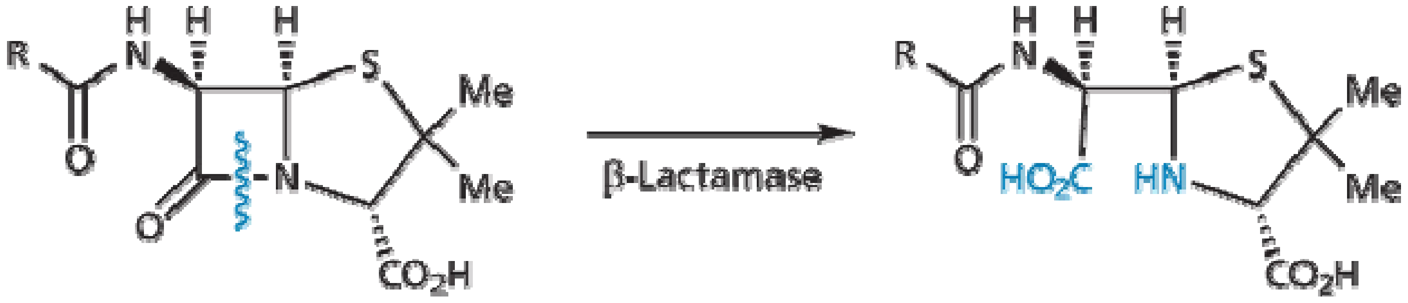
5) Mutations can occur which will affect any of the above mechanisms such that they are more effective in resistance. Transfer of b-lactamases between strains.

6) Presence of β-lactamase enzymes is the most important mechanism by which bacteria gain resistance to pencillin.

- β-lactamase are enzymes which have mutated from transpeptidases & so they are quite similar in nature.

- Unlike transpeptidase enzyme, β-lactamases are able to hydrolyse the ester link resulting the ring-opened penicillin. They hydrolyze also the β-lactam ring.

- Presence and concentration of b-lactamases in the periplasmic space (gram –ve bacteria)



Penicillin Analogues - Preparation

1) By fermentation

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

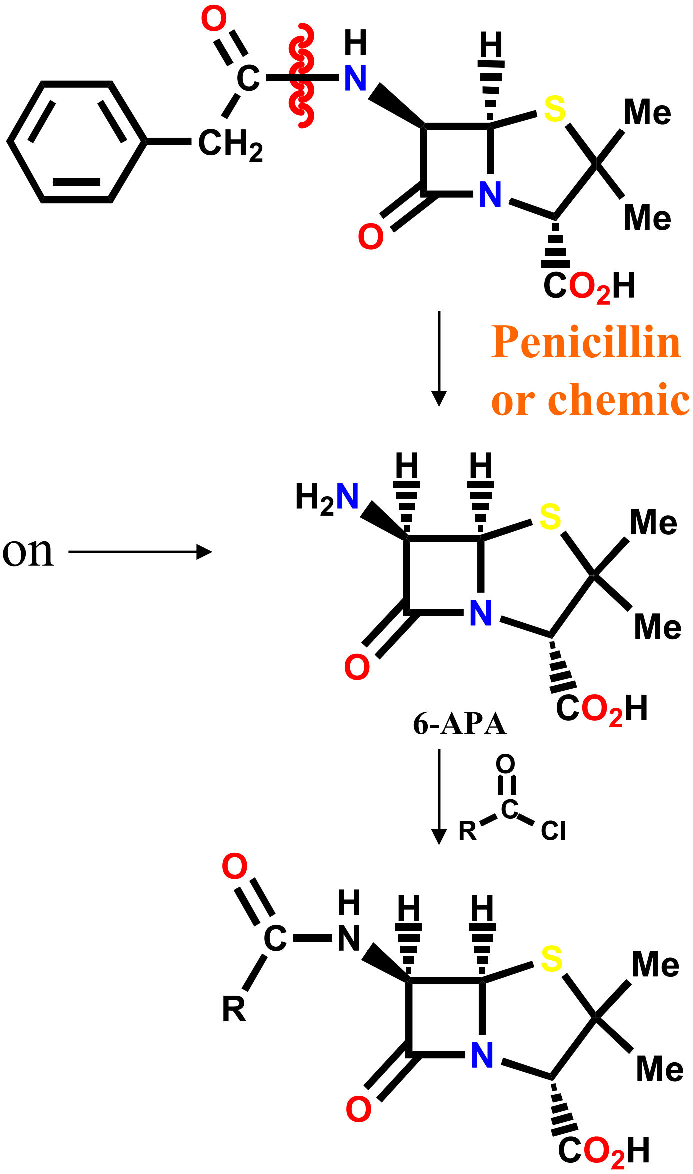
2) By total synthesis

- 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

Problems with Penicillin G

- **It is sensitive to stomach acids**

- **It is sensitive to b-lactamases - enzymes which hydrolyse the b-lactam ring**

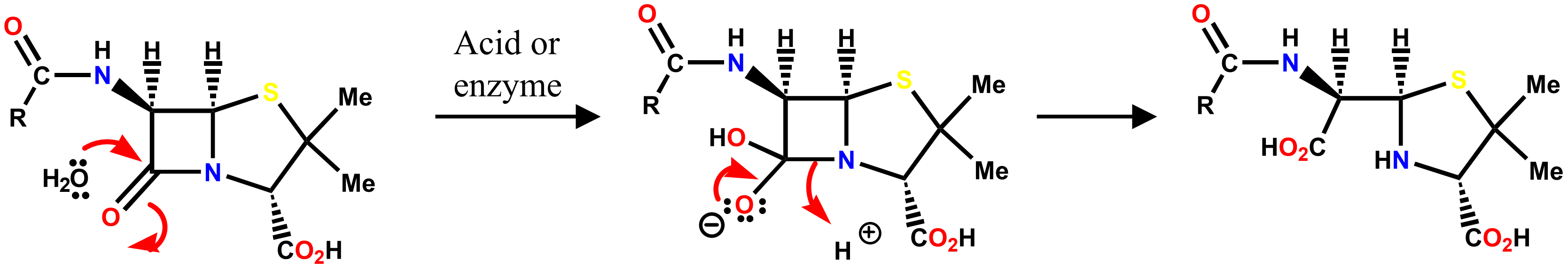
- **It has a limited range of activity**

Problem 1 - Acid Sensitivity

Reasons for sensitivity

1) Ring strain

- It suffers large angle and torsional strains. Acid catalyzed ring opening relieves these strains by breaking open the more highly strained β-lactam ring.



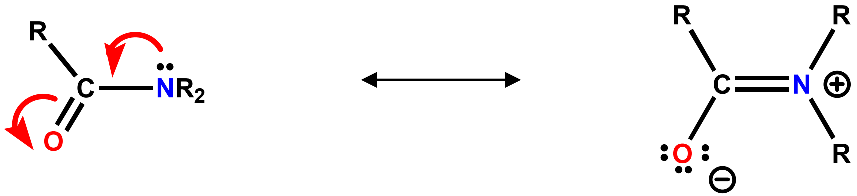
Relieves ring strain

2) Reactive b-lactam carbonyl group

Does not behave like a tertiary amide

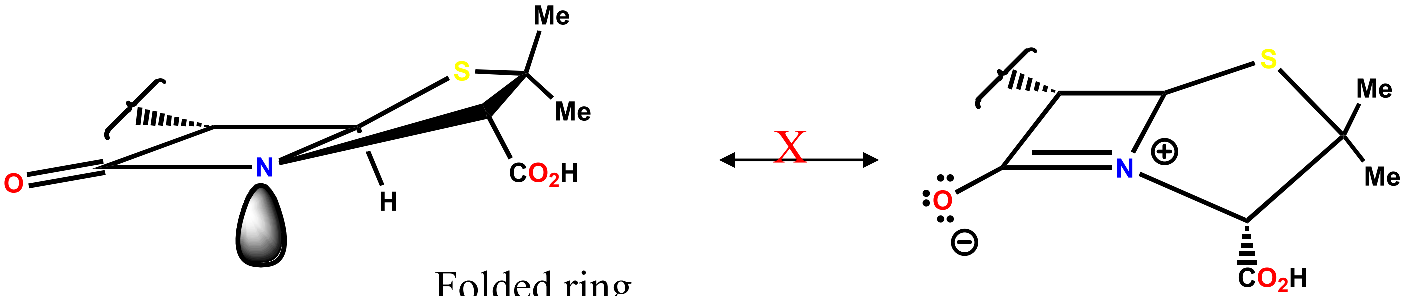
- In 3° amide is resistance to nucleophilic attack because the carbonyl group is stabilized by the neighbouring nitrogen atom. N can feed its Lone pair into carbonyl gp to form a resonance.

Tertiary amide



Unreactive

β-Lactam



Folded ring system

Impossibly strained

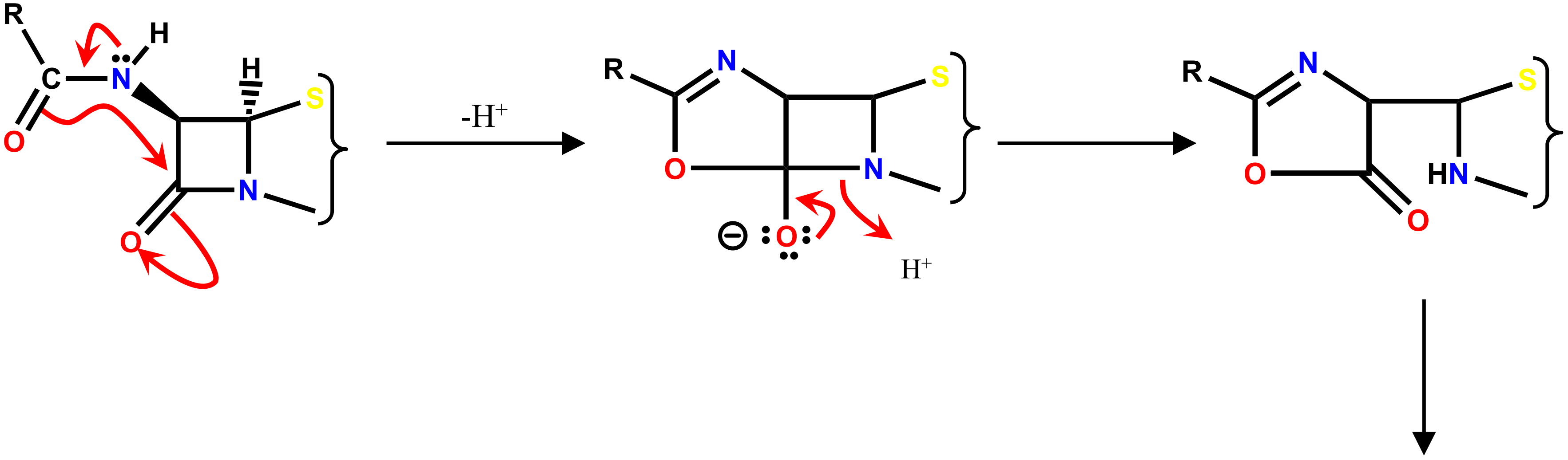
- Interaction of nitrogen’s lone pair with the carbonyl group is not possible
- Results in a reactive carbonyl group

Problem 1 - Acid Sensitivity

Reasons for sensitivity

3) Acyl side chain

Neighbouring group participation in the hydrolysis mechanism



Further reactions

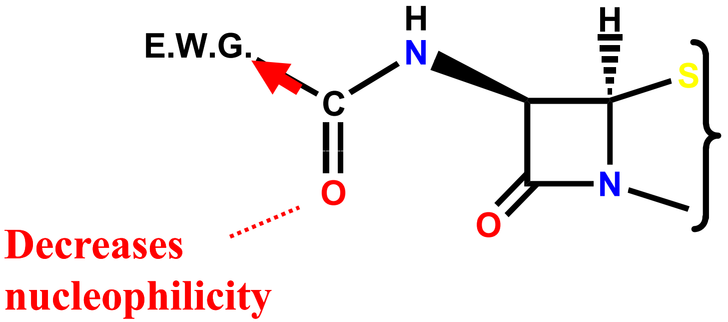
Problem 1 - Acid Sensitivity

Conclusions

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

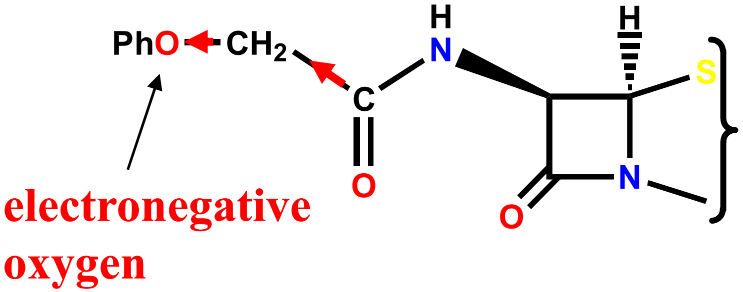
Strategy

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



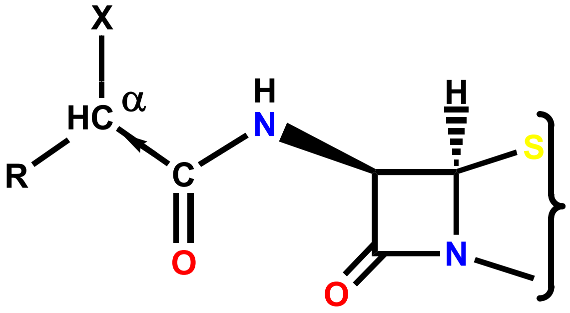
Problem 1 - Acid Sensitivity

Examples



electronegative oxygen

Penicillin V
(orally active)



X= NH_2, Cl, PhOCONH, heterocycles. CO_2H

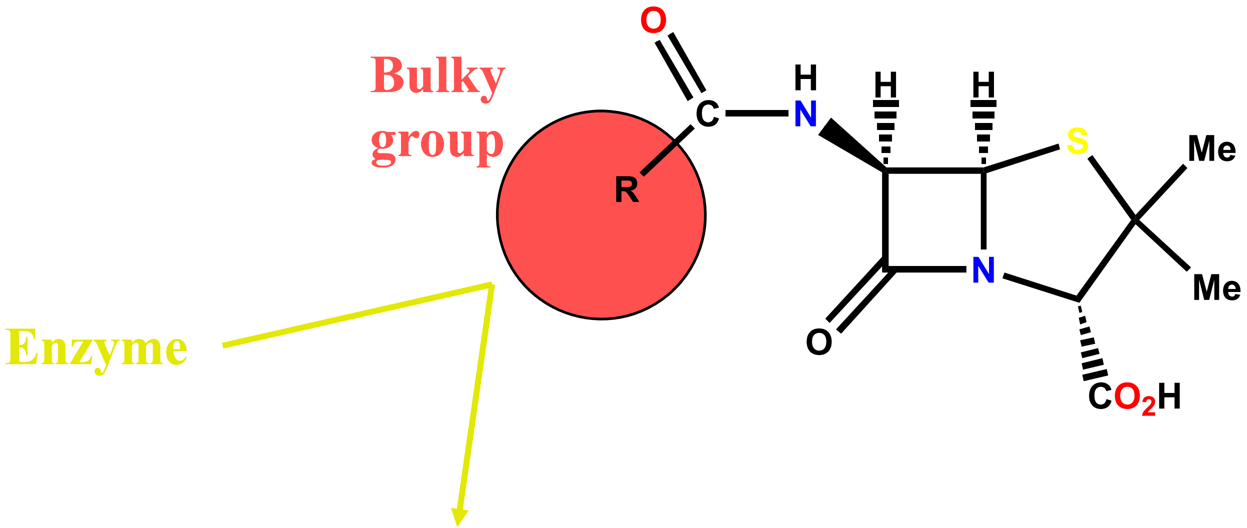
- •Better acid stability and orally active
- •But sensitive to b-lactamases
- •Slightly less active than penicillin G
- •Allergy problems with some patients

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

Problem 2 - Sensitivity to β-Lactamases "penicillinase"

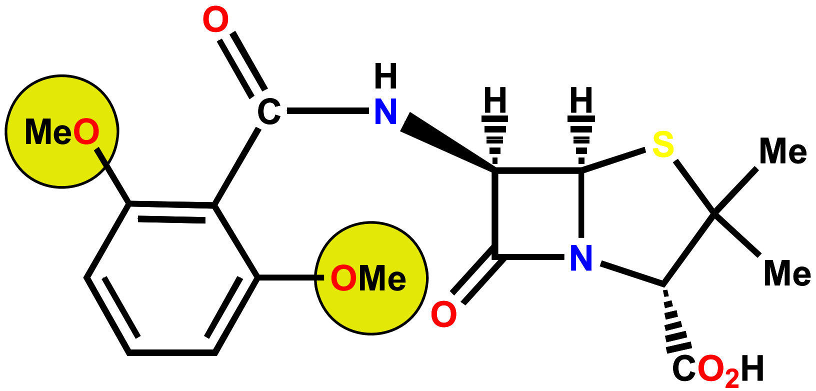
Strategy

- 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 "penicillinases"

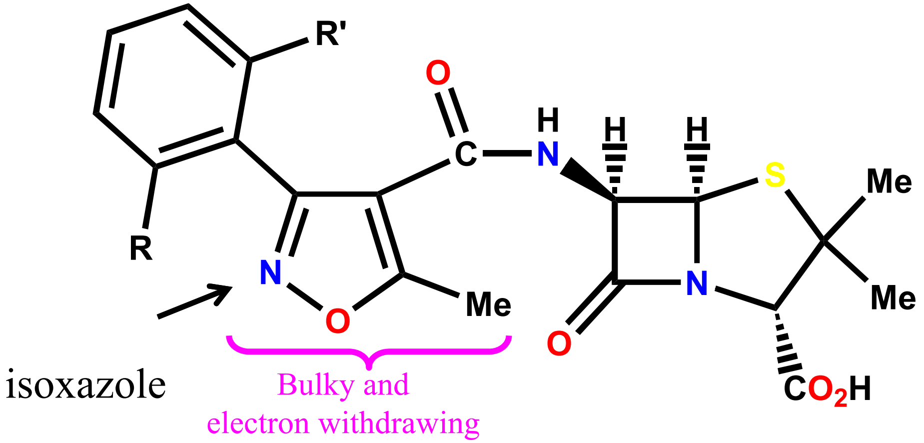
Examples - Methicillin (Beechams - 1960)



- 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
- Poorer 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 “penicillinase”

Examples



isoxazole

Bulky and electron withdrawing

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 drug**

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 b-lactamases
5) Transfer of b-lactamases between strains
6) Efflux mechanisms

Strategy

- The number of factors involved make a single strategy impossible
- Use of trial and error to vary 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 groups 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

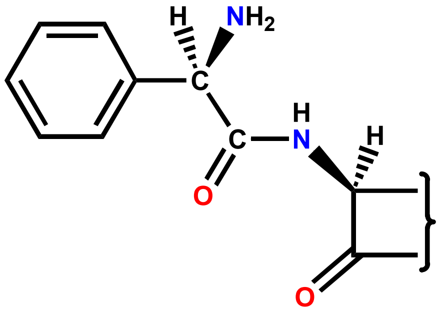
4) Hydrophilic groups at the α-position (e.g. NH_2, OH, CO_2H) increase activity vs Gram -ve bacteria

Problem 3 - Range of Activity

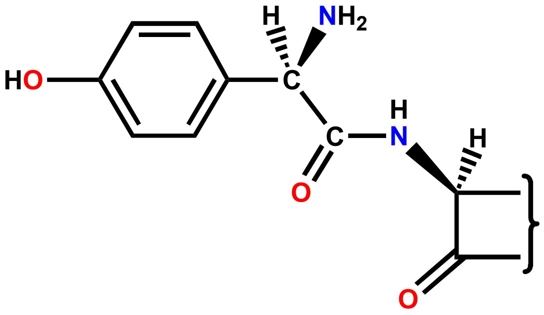
Examples of Broad Spectrum Penicillins

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

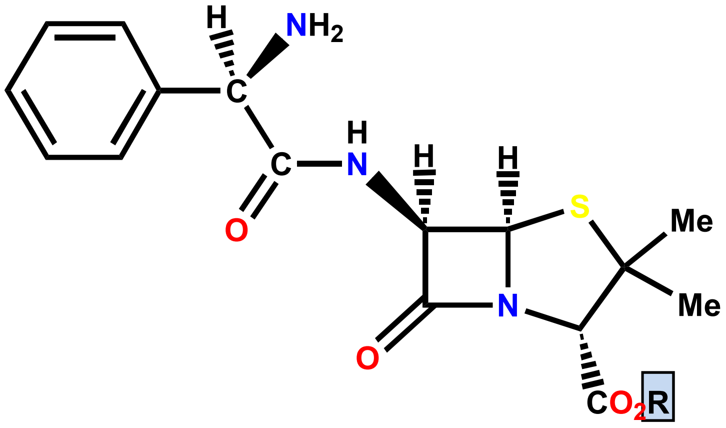
Examples of Broad Spectrum Penicillins

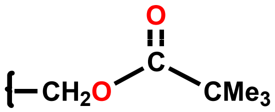
Properties

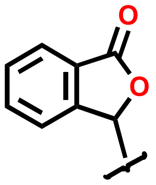
- **Active vs Gram +ve bacteria and Gram -ve bacteria which do not produce b-lactamases**
- **Acid resistant and orally active**
- **Non toxic**
- **Sensitive to β-lactamases "penicillinase"**
- **Increased polarity due to extra amino group**
- **Poor absorption through the gut wall**
- **Disruption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

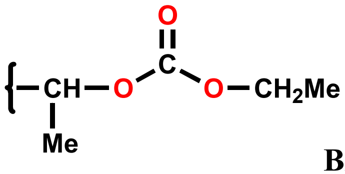
Problem 3 - Range of Activity

Prodrugs of Ampicillin (Leo Pharmaceuticals - 1969)



R =  $-CH_2O-C(=O)-CMe_3$ **PIVAMPICILLIN**

R =  **TALAMPICILLIN**

R =  $-CH(Me)-O-C(=O)-O-CH_2Me$ **BACAMPICILLIN**

Properties

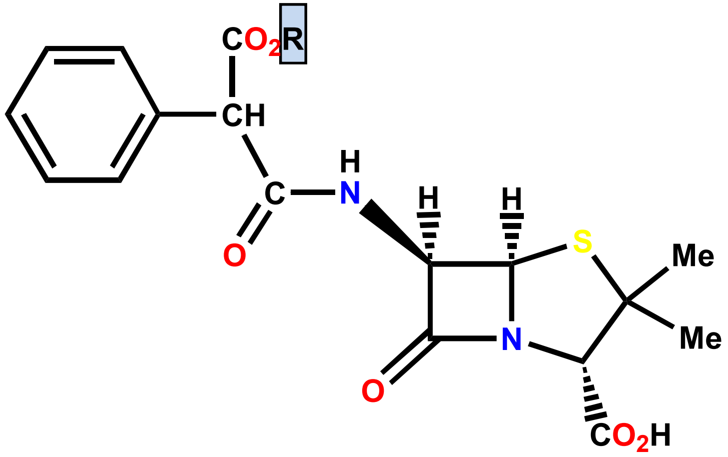
- Increased cell membrane permeability
- Polar carboxylic acid group is masked by the ester
- Ester is metabolised in the body by esterases to give the free drug

Problem 3 - Range of Activity

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***
- **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**

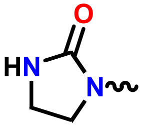
Problem 3 - Range of Activity

Examples of broad spectrum penicillins

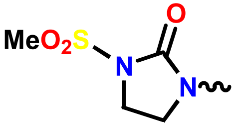
Class 3 - Urea group at the a-position (ureidopenicillins)

Examples

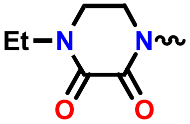
Azlocillin

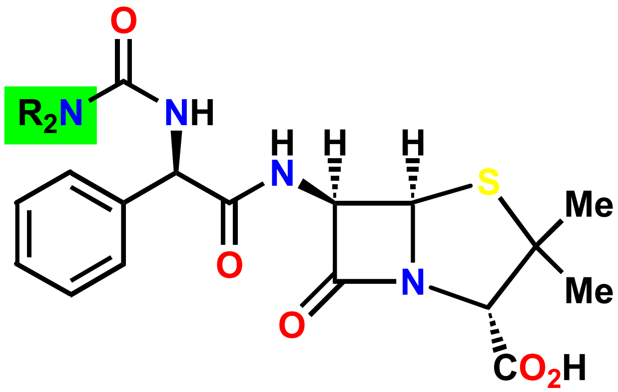


Mezlocillin



Piperacillin





- •Administered by injection
- •Generally active 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 *Pseudomonas aeruginosa*

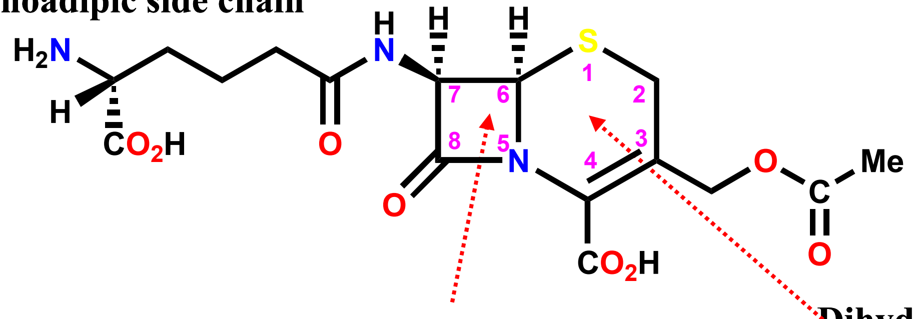
2) CEPHALOSPORINs:

a. Introduction

- **Antibacterial agents which inhibit bacterial cell wall synthesis**
- **Discovered from a fungal colony in Sardinian sewer water (1948)**
- **Cephalosporin C identified in 1961: the first cephalosporin**

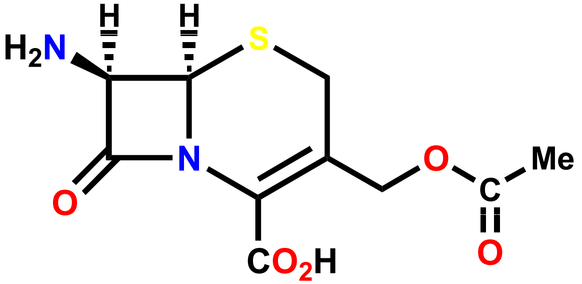
b. Structure of Cephalosporin C

7-Aminoadipic side chain



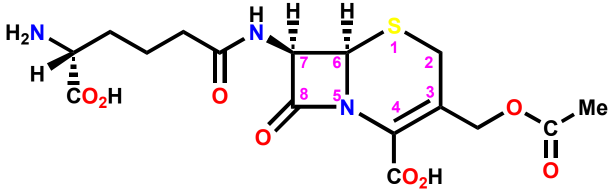
β-Lactam ring

Dihydrothiazine ring



7-Aminocephalosporinic acid (7-ACA)

c. Properties of Cephalosporin C



Disadvantages

- Polar due to the side chain - difficult to isolate and purify
- Low potency - limited to the treatment of urinary tract infections where it is concentrated in the urine
- Not absorbed orally

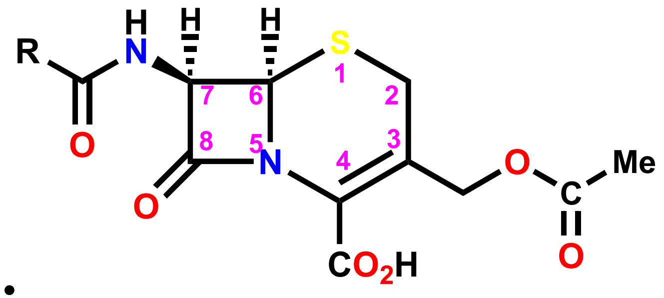
Advantages

- Non toxic
- Lower risk of allergic reactions compared to penicillins
- More stable to acid conditions
- More stable to β-lactamases
- Ratio of activity vs Gram -ve and Gram +ve bacteria is better

Conclusion

- Useful as a lead compound

d. SAR of Cephalosporins

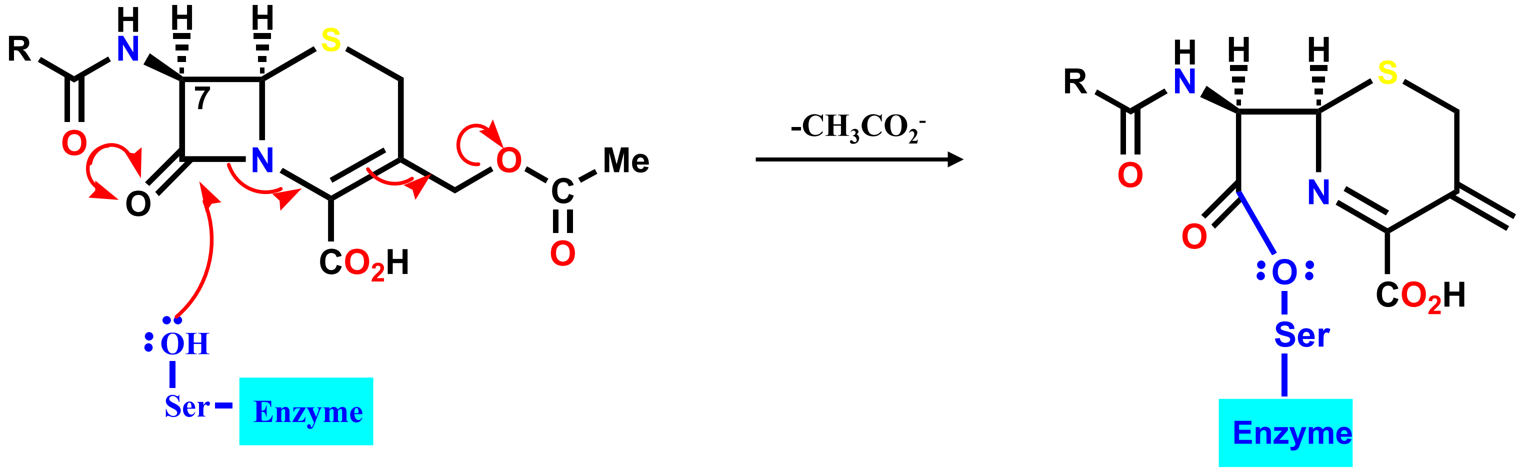


- Similar to penicillins
- The β-lactam ring is crucial to the mechanism
- The carboxylic acid at position 4 is important to binding
- The bicyclic system is important in increasing ring strain
- Stereochemistry is important
- The acetoxy substituent is important to the mechanism

Possible modifications

- 7-Acylamino side chain
- 3-Acetoxymethylene side chain
- Substitution at C-7

e. Mechanism of Action

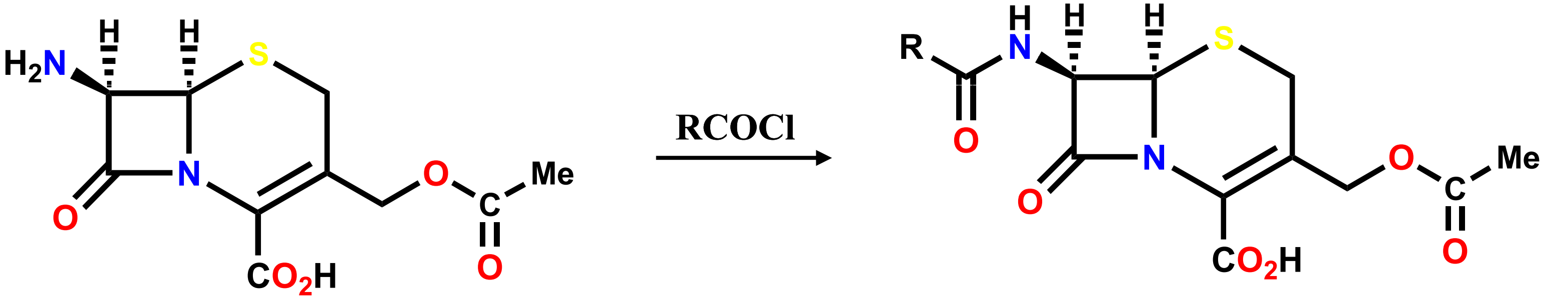


Note

The acetoxy group acts as a good leaving group and aids the mechanism

f. Variation of the 7-Acylamino Side Chain

- Not possible to generate analogues by fermentation
- Not possible to generate analogues by a full synthesis
- Restricted to semi-synthetic procedure



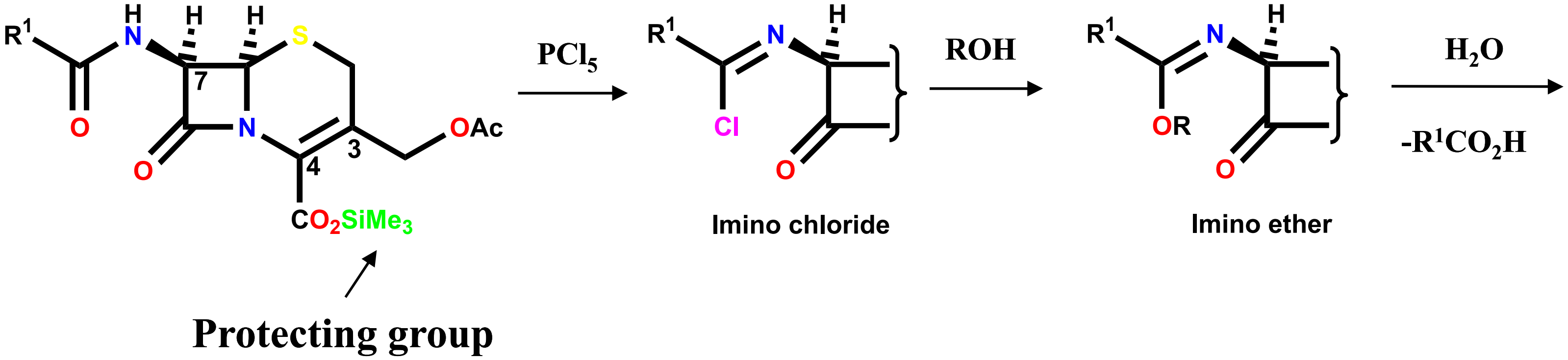
7-ACA

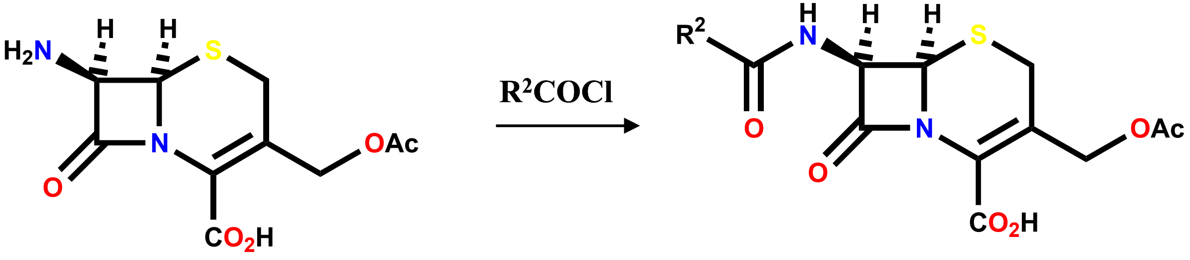
- 7-ACA not available by fermentation
- 7-ACA not available by enzymatic hydrolysis of cephalosporin C
- Generated by a chemical hydrolysis

f. Variation of the 7-Acylamino Side Chain

Generation of 7-ACA

- Need to hydrolyse a relatively unreactive secondary amide in the presence of a labile β-lactam ring



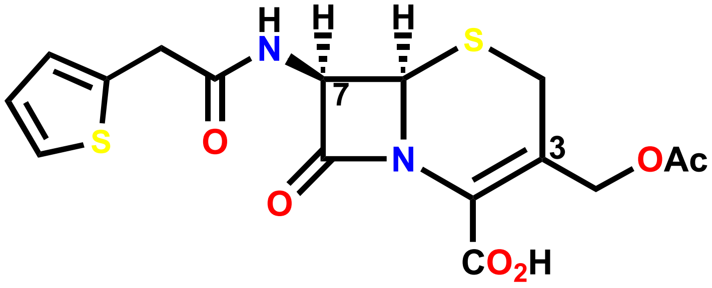


7-ACA

Range of cephalosporins

g. First Generation Cephalosporins

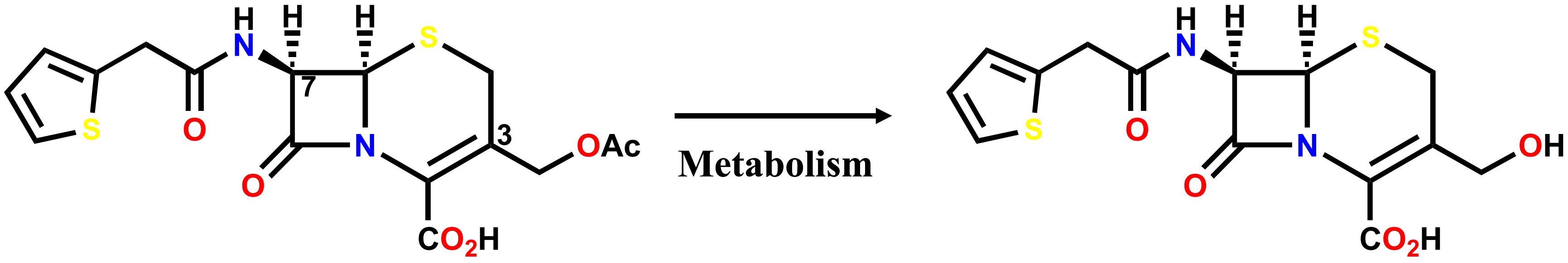
Cephalothin



- •More active than penicillin G vs. some Gram -ve bacteria
- •Less likely to cause allergic reactions
- •Useful vs. penicillinase producing strains of *S. aureus*
- •Not active vs. *Pseudonomas aeruginosa*
- •Poorly absorbed from GIT
- •Administered by injection
- •Metabolised to give a free 3-hydroxymethylene group (deacetylation)
- •Metabolite is less active

g. First-generation Cephalosporins

Cephalothin - drug metabolism



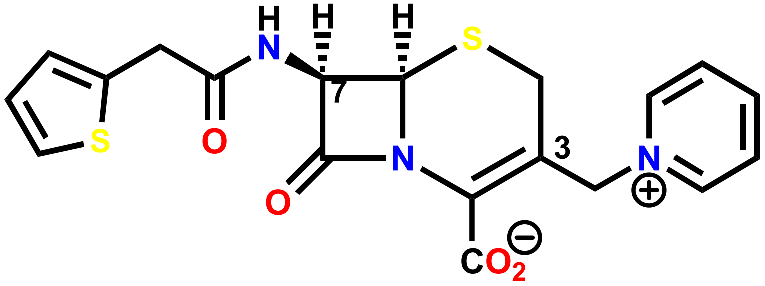
- Less active
- OH is a poorer leaving group

Strategy

Replace the acetoxy group with a metabolically stable leaving group

g. First-generation Cephalosporins

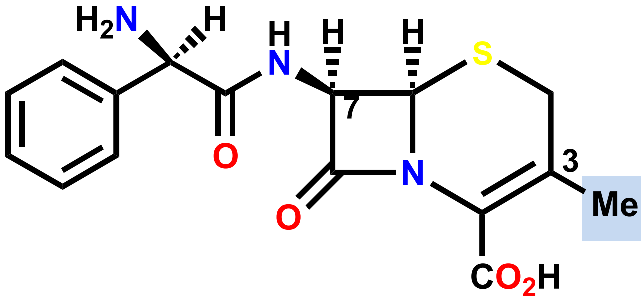
Cephaloridine



- The pyridine ring is stable to metabolism
- The pyridine ring is a good leaving group (neutralisation of charge)
- Cephaloridine exists as a zwitterion and is soluble in water
- Poorly absorbed through the gut wall
- Administered by injection

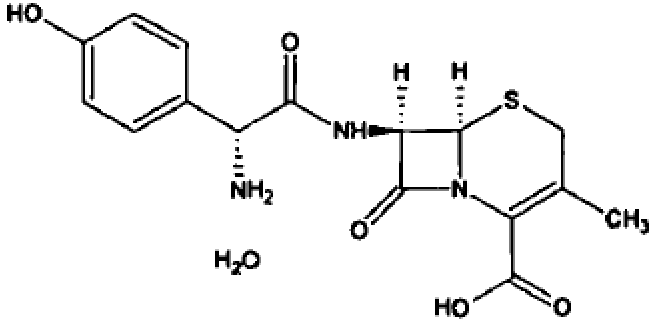
g. First-generation Cephalosporins

Cefalexin

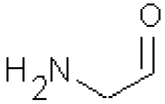


- The 3-methyl group is a poor leaving group
- Methyl group is bad for activity but aids oral absorption
- Can be administered orally
- A hydrophilic amino group at the a-carbon of the side chain helps to compensate for the loss of activity due to the methyl group

Cefadroxil (Duracef®, Biodroxil®, Cefadrox®)



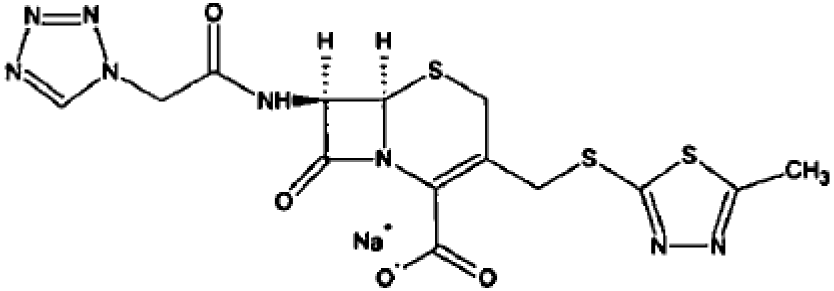
- Semisynthetic derivative of 7-ACA in which the 7 acyl group is the *p*-hydroxylphenylglycyl moiety.



→ glycyl moiety

- The main advantage claimed for cefadroxil:
  - Prolonged duration of action is related to a slow urinary excretion of the drug compared with other cephalosporins.
- The D-*p*- hydroxylphenylglycyl isomer is much more active than the L- isomer.

Cefazoline sodium, (Cefazin®-BPC)



- First generation cephalosporin
- It is semisynthetic cephalosporins
- Cefazoline structure:
  - The C-3 acetoxy has been replaced by a thiol-containing heterocycle (5-methyl-2-thio- 1,3,4-thiadiazole).
  - It also contains tetrazolylacetyl group.
- Cefazolin was released in 1973 as a water-soluble sodium salt.
- It is active only by parenteral administration.

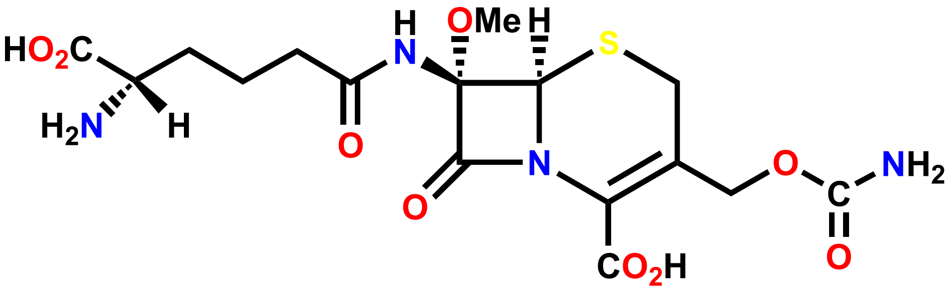
g. First-generation Cephalosporins

Summary

- Generally lower activity than comparable penicillins
- Better range of activity than comparable penicillins
- Best activity is against Gram positive cocci
- Useful against some Gram negative infections
- Useful against *S. aureus* and streptococcal infections when penicillins have to be avoided
- Poorly absorbed across the gut wall (except for 3-methyl substituted cephalosporins)
- Most are administered by injection
- Resistance has appeared amongst Gram negative bacteria (presence of more effective β-lactamases)

h. Second-generation Cephalosporins

h.1 Cephamycins

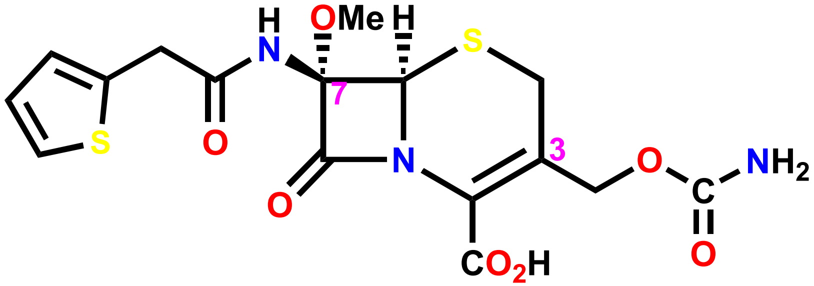


Cephamycin C

- **Isolated from a culture of *Streptomyces clavuligerus***
- **First β-lactam to be isolated from a bacterial source**
- **Contains a 7-methoxy group**
- **Modifications carried out on the 7-acylamino side chain**

h. Second-generation Cephalosporins

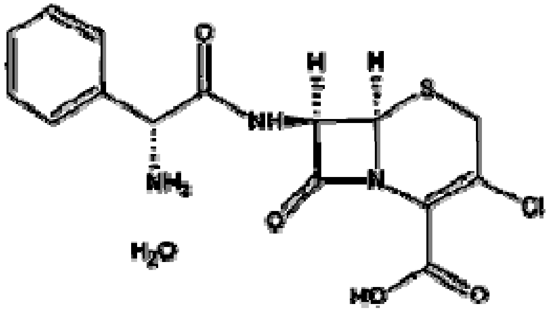
h.1 Cephamycins



Cefoxitin

- **Broader spectrum of activity than most first-generation cephalosporins**
- **Greater resistance to β-lactamase enzymes**
- **The 7-methoxy group may act as a steric shield**
- **The urethane group at position 3 is stable to metabolism**

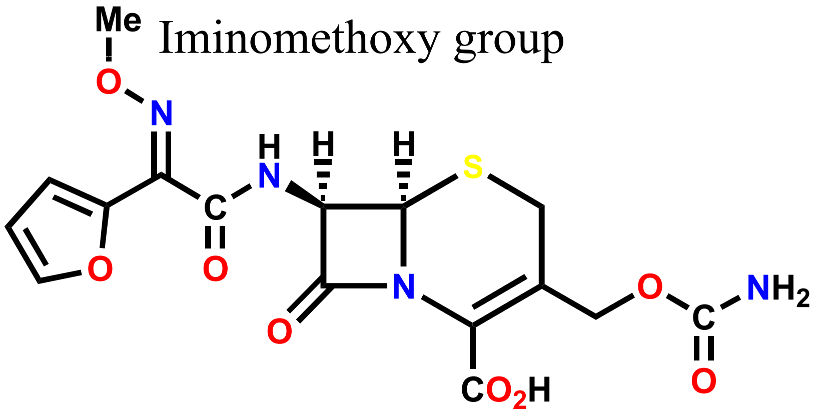
Cefaclor (Ceclor ®)



- Second generation cephalosporin
- It is an orally active
- It differs structurally from cephalexin in that
  - The 3-methyl group has been replaced by a chlorine atom.
- Cefaclor is moderately stable in acid and achieves enough oral absorption.

h. Second-generation Cephalosporins

h.2 Oximinocephalosporins

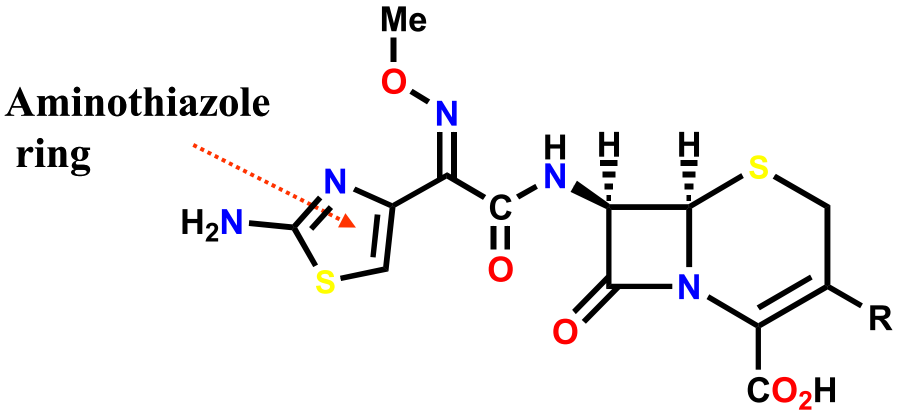

Iminomethoxy group

Cefuroxime

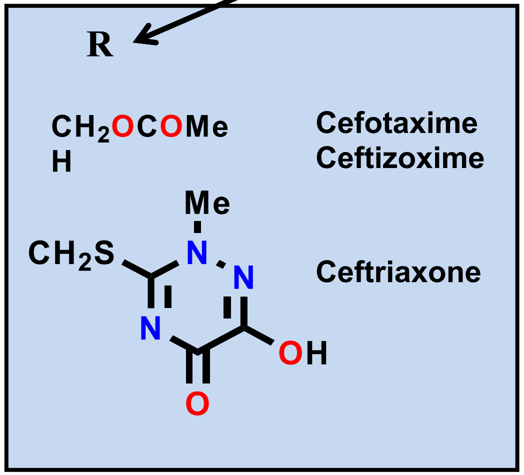
- Much greater stability against some β-lactamases due to the iminomethoxy group at α-position of the acyl side chain.
- Resistant to esterases due to the urethane group
- Wide spectrum of activity
- Useful against organisms that have gained resistance to penicillin
- Not active against *Pseudomonas aeruginosa*
- Used clinically against respiratory infections

i. Third-generation Cephalosporins

Oximinocephalosporins



vary the pharmacokientic properties

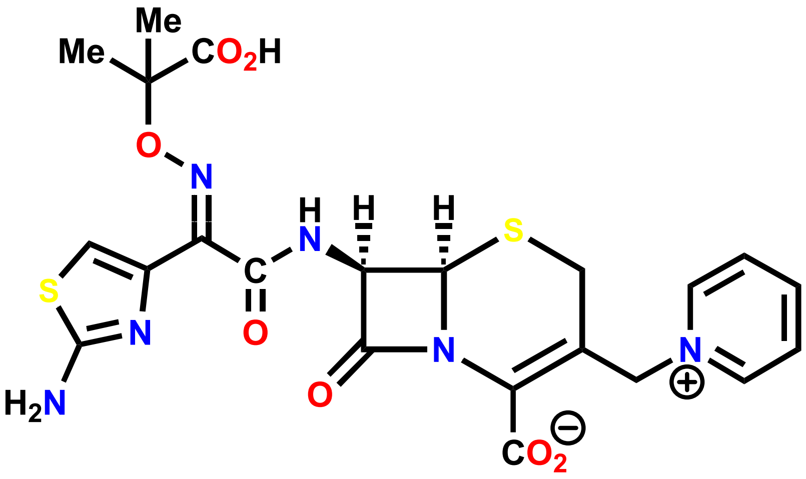


R	
CH_2OCOMe	Cefotaxime
H	Ceftizoxime
	Ceftriaxone

- Replacing the furan ring with an aminothiazole ring enhances penetration of cephalosporins across the outer membrane of Gram -ve bacteria
- May also increase affinity for the transpeptidase enzyme
- Good activity against Gram -ve bacteria
- Variable activity against Gram +ve cocci
- Variable activity vs. *Pseudomonas aeruginosa*
- Generally reserved for troublesome infections

i. Third-generation Cephalosporins

Oximinocephalosporins

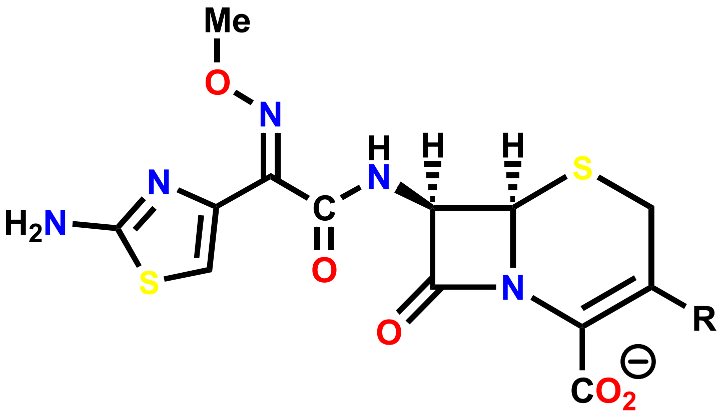


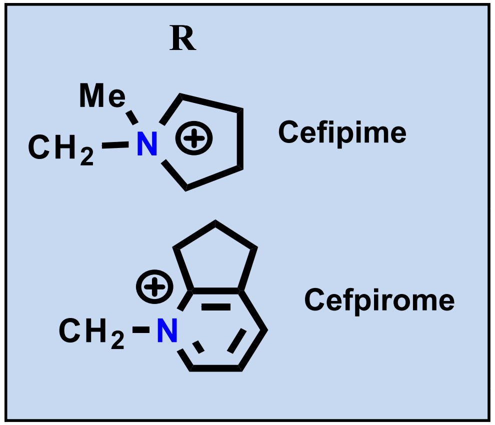
Ceftazidime

- **Injectable cephalosporin**
- **Excellent activity vs. *P. aeruginosa* and other Gram -ve bacteria**
- **Can cross the blood brain barrier**
- **Used to treat meningitis**

j. Fourth-generation Cephalosporins

Oximinocephalosporins



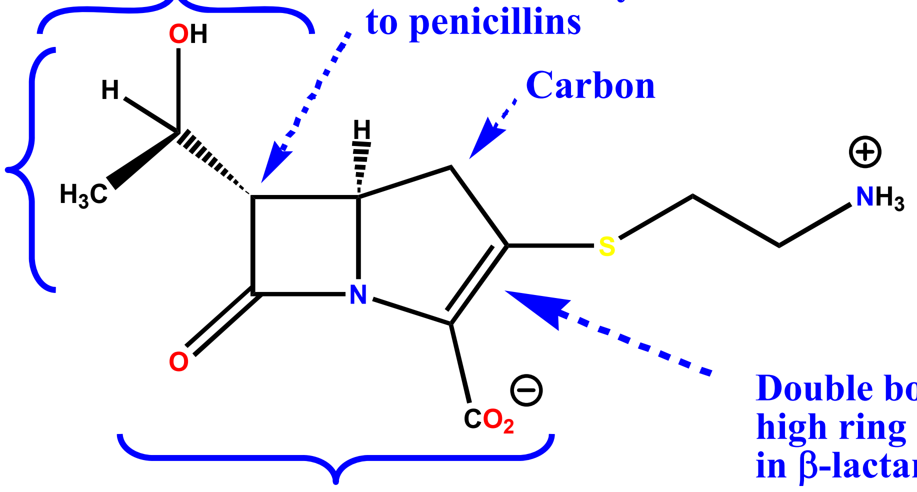


- Zwitterionic compounds
- Enhanced ability to cross the outer membrane of Gram negative bacteria
- Good affinity for the transpeptidase enzyme
- Low affinity for some b-lactamases
- Active vs. Gram +ve cocci and a broad array of Gram -ve bacteria
- Active vs. *Pseudomonas aeruginosa*

3) OTHER β-LACTAM ANTIBIOTICS

3.1) Thienamycin

Merck 1976



Acylamino side chain absent

Opposite stereochemistry to penicillins

Carbon

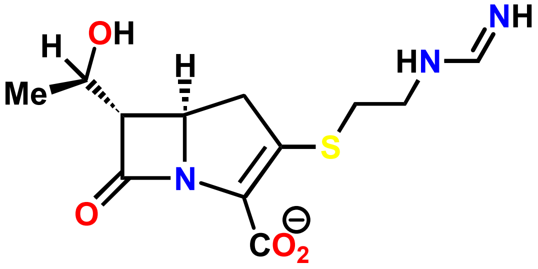
Plays a role in b-lactamase resistance

Double bond leading to high ring strain and an increase in β-lactam ring reactivity

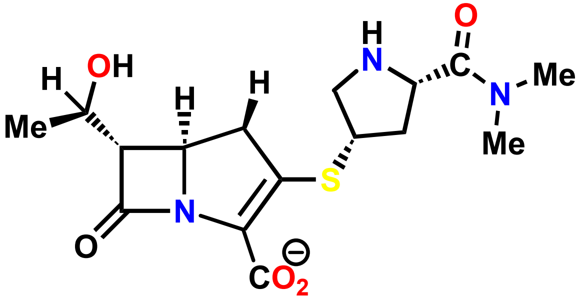
Carbapenam nucleus

- Isolated from *Streptomyces cattleya*
- Potent and wide range of activity vs Gram +ve and Gram -ve bacteria
- Active vs. *Pseudomonas aeruginosa*
- Low toxicity
- High resistance to β-lactamases
- Poor stability in solution (ten times less stable than Pen G)

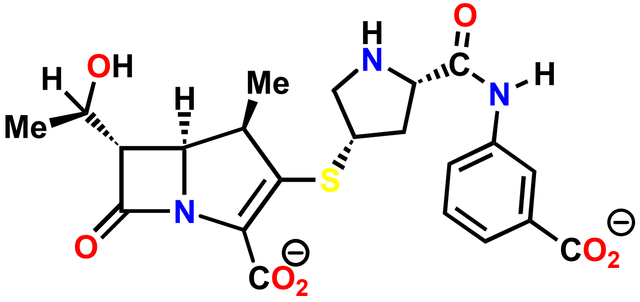
Thienamycin analogues used in the clinic



Imipenem



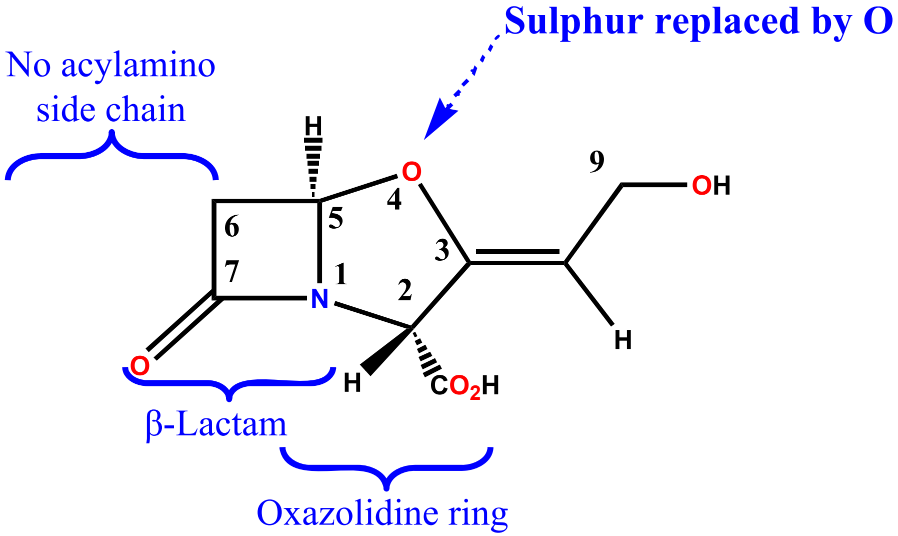
Meropenem



Ertapenem(2002)

3.2. β-Lactamase Inhibitors

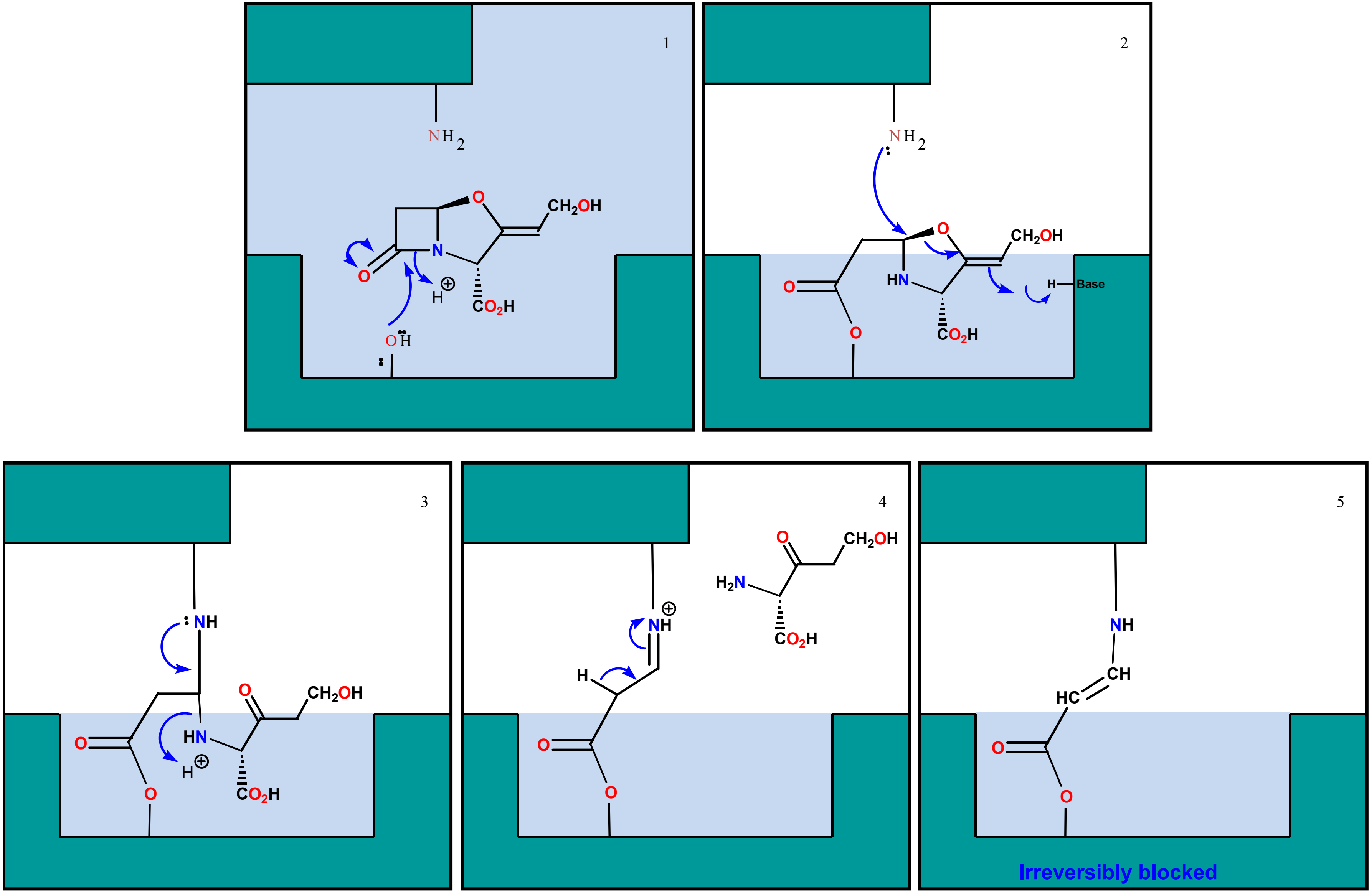
Clavulanic acid (Beechams 1976)



- Isolated from *Streptomyces clavuligerus*
- Weak, unimportant antibacterial activity
- Powerful irreversible inhibitor of β-lactamases - suicide substrate
- Used as a sentry drug for ampicillin
- Augmentin = amoxicillin + clavulanic acid
- Allows less ampicillin per dose and an increased activity spectrum

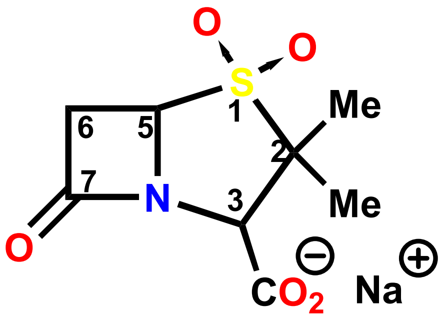
3.2. β-Lactamase Inhibitors

Clavulanic acid - mechanism of action

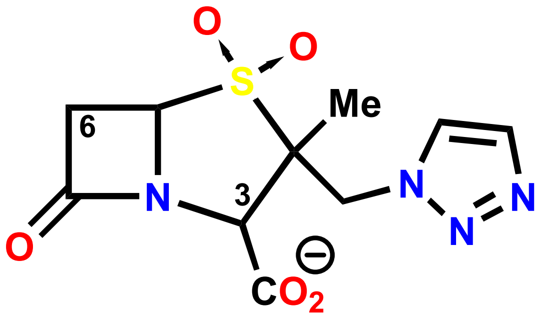


3.3. β-Lactamase Inhibitors

Penicillanic acid sulfone derivatives



Sulbactam



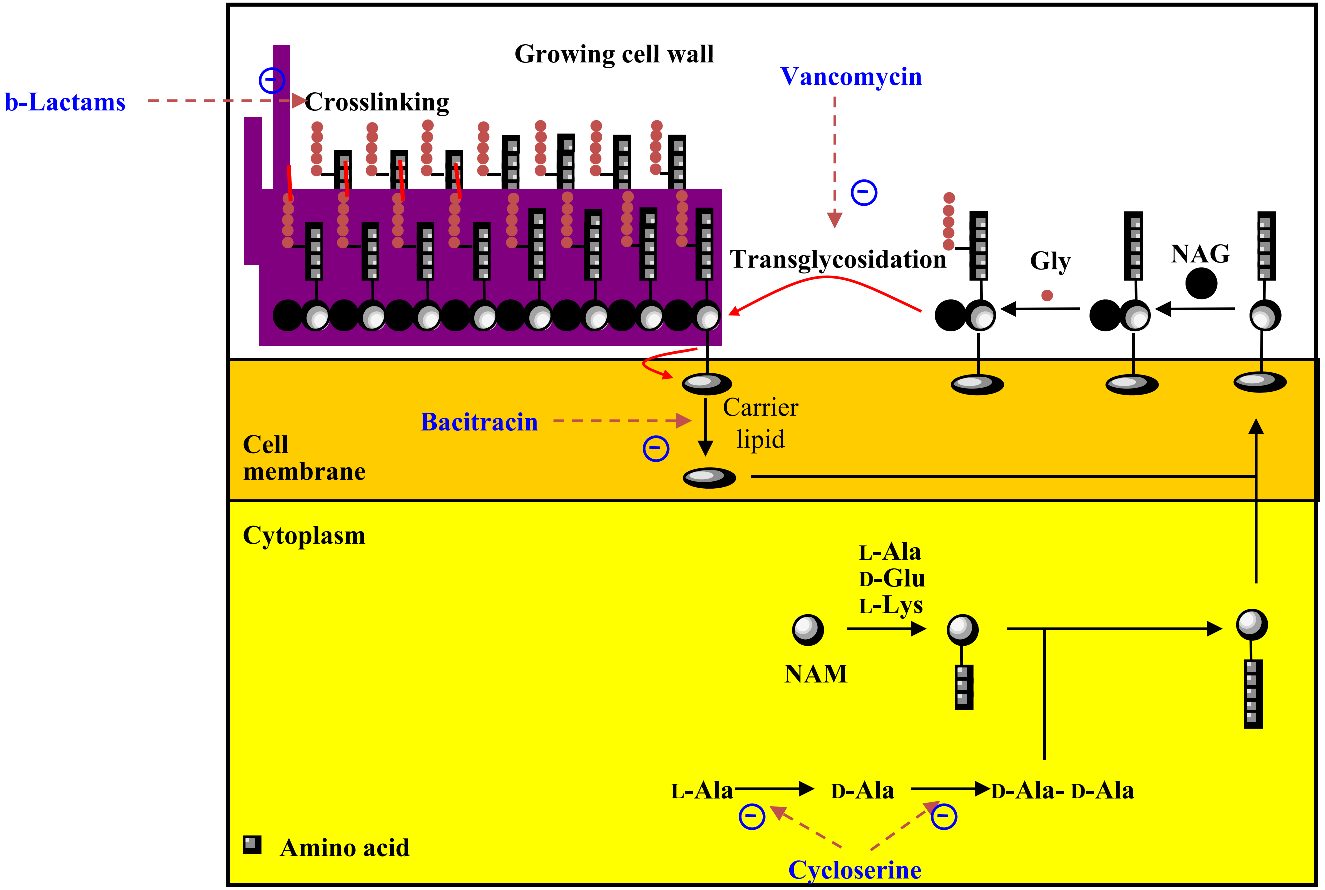
Tazobactam

- **Suicide substrates for β-lactamase enzymes**
- **Sulbactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, but is less potent**
- **Unasyn = ampicillin + sulbactam**
- **Tazobactam has a broader spectrum of activity vs β-lactamases than clavulanic acid, and has similar potency**
- **Tazocin or Zosyn = piperacillin + tazobactam**

4) OTHER DRUGS THAT ACT ON BACTERIAL CELL WALL BIOSYNTHESIS

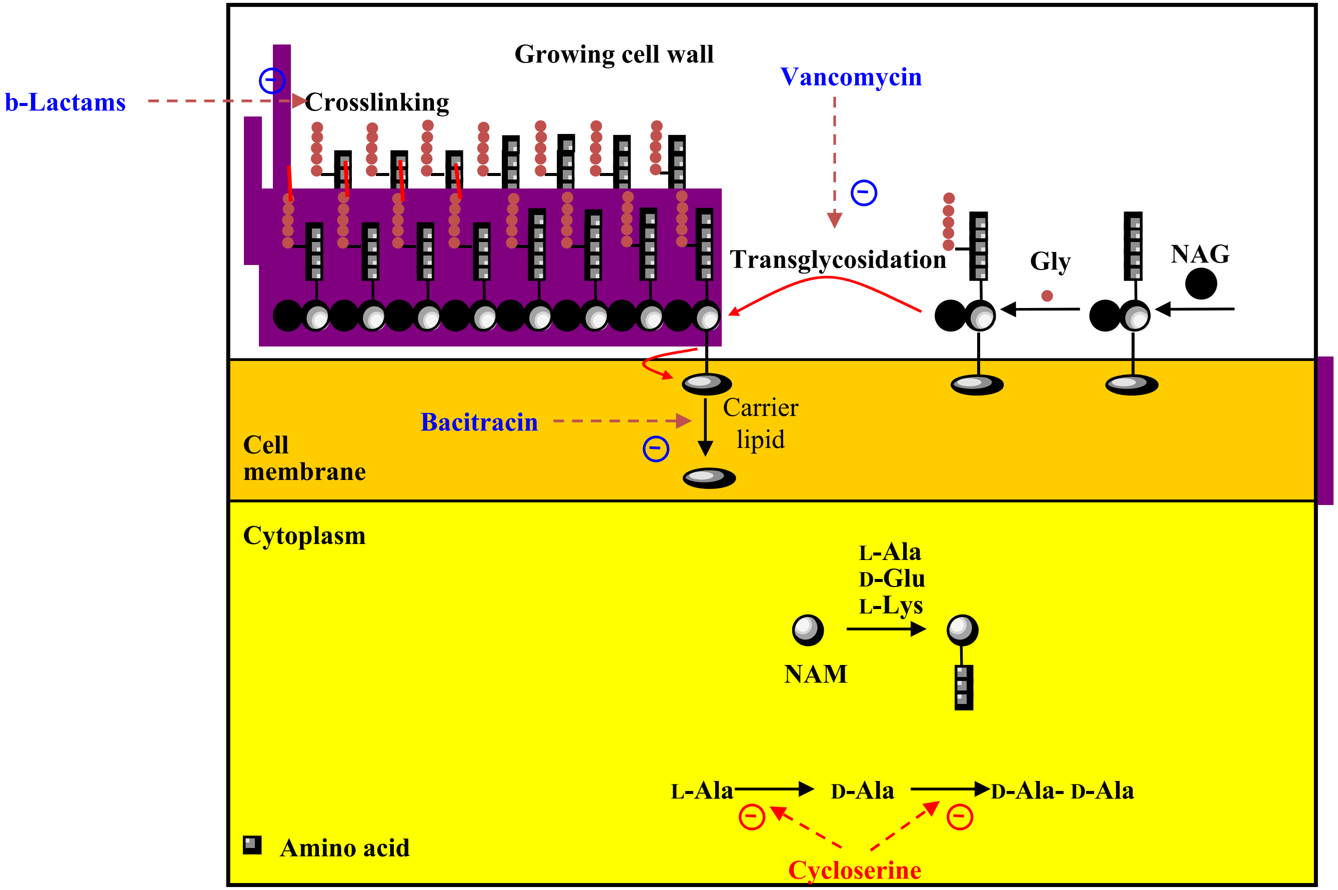
CYCLOSERINE
BACITRACIN
VANCOMYCIN
VANCOMYCIN ANALOGUES

1. Cell Wall Biosynthesis



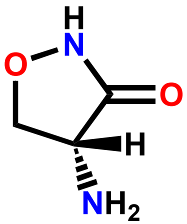
- Building block partially constructed in cytoplasm
- Transported across cell membrane and completed
- Constructed from 2 sugars (NAM, NAG) and a peptide chain
- Linked to growing cell wall by enzyme (transglycosidation)
- Final crosslinking reaction catalysed by transpeptidase enzymes

2. D-Cycloserine

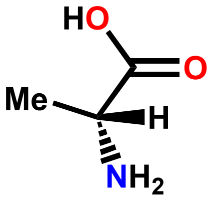


- **Natural product produced by *Streptomyces garyphalus***
- **Inhibits L-alanine racemase and D-Ala-D-Ala ligase**
- **Blocks biosynthesis of D-Ala-D-Ala**
- **Mimics the structure of D-Ala**

2. D-Cycloserine

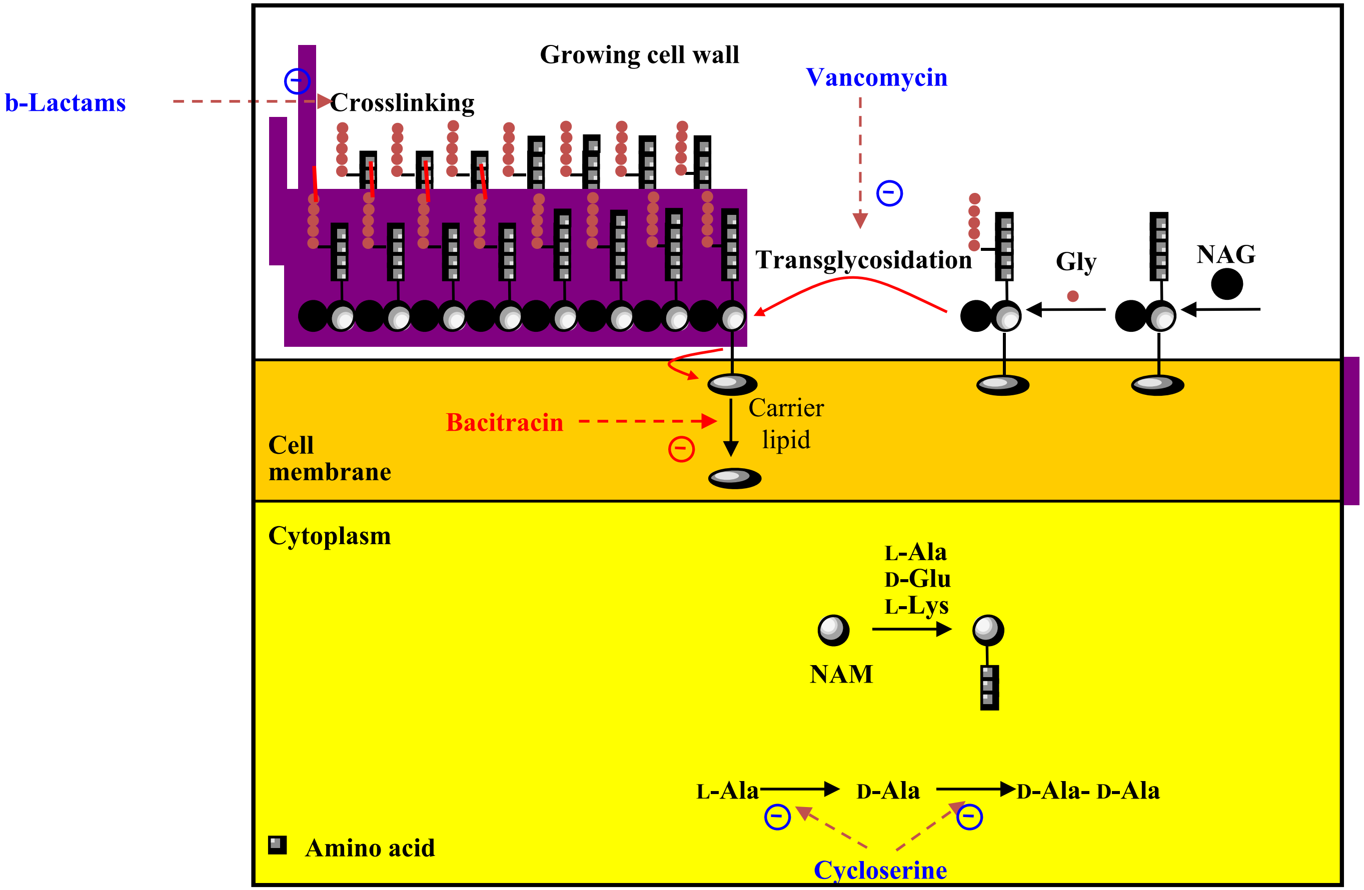


D-Cycloserine



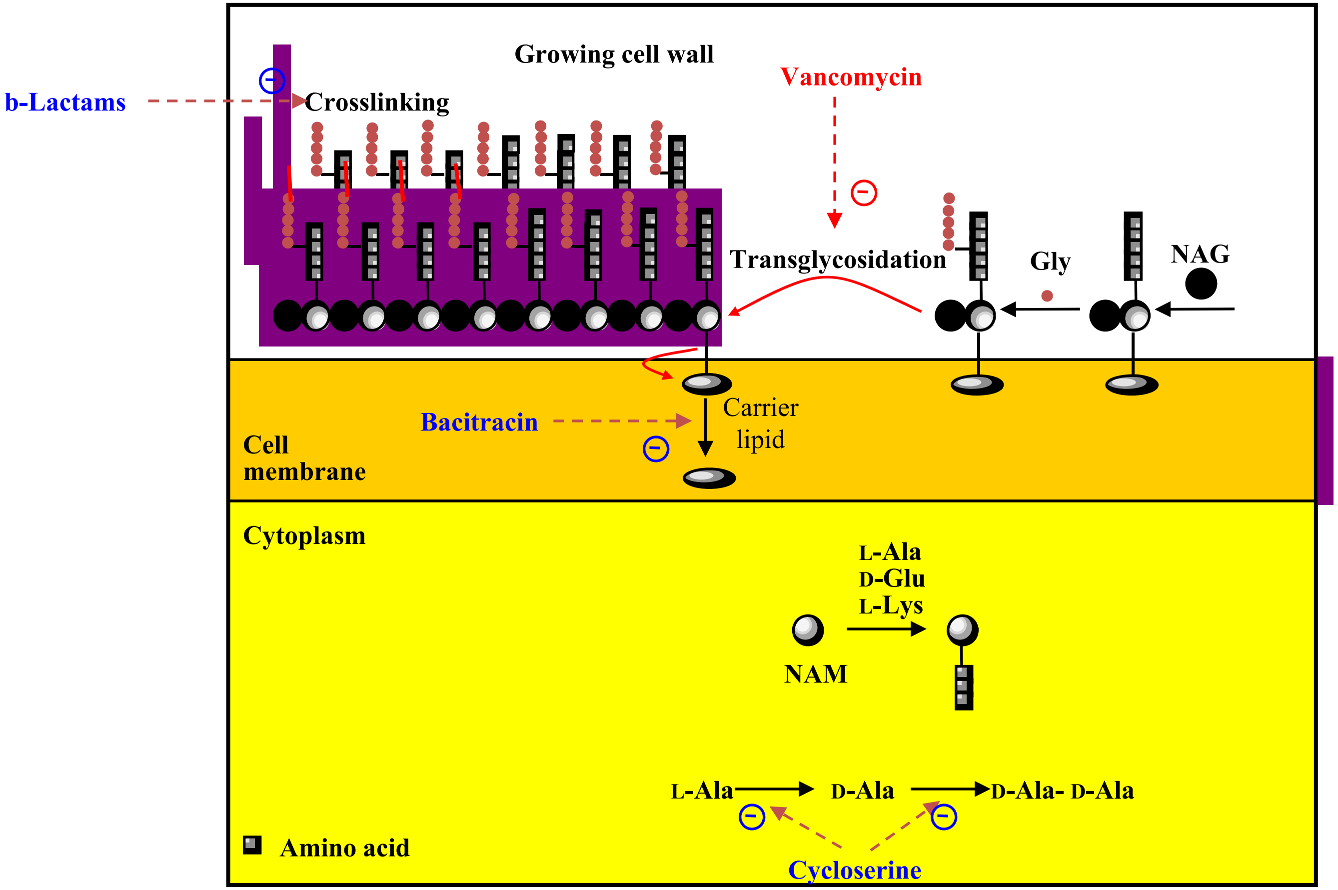
D-Alanine

3. Bacitracin



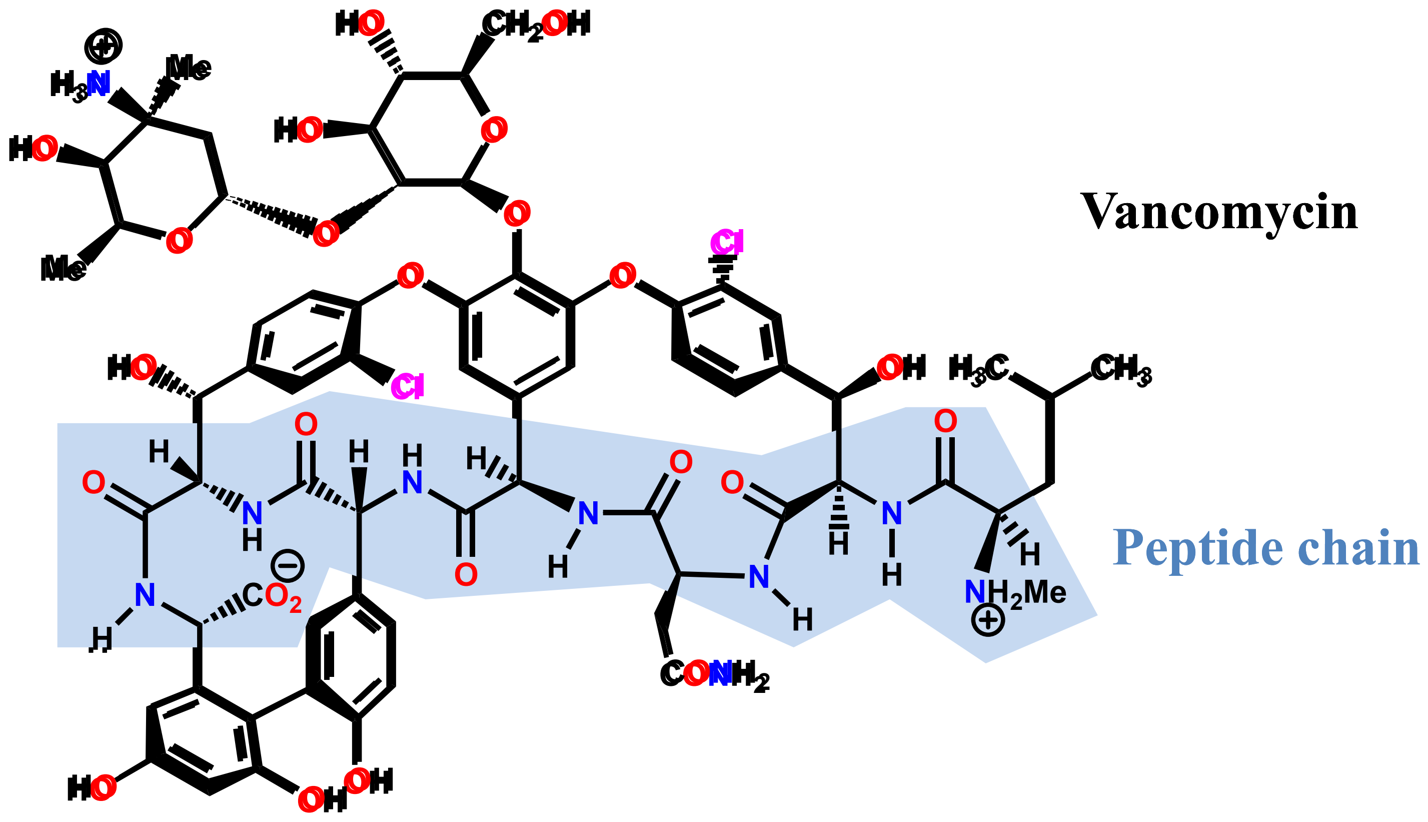
- Polypeptide produced by *Bacillus subtilis*
- Binds to the carrier lipid
- Prevents the carrier lipid from transporting the NAM - pentapeptide building block across the cell membrane

4. Vancomycin and vancomycin analogues



- Narrow spectrum bactericidal glycopeptide
- Produced by *Streptomyces orientalis*
- Blocks transglycosidation

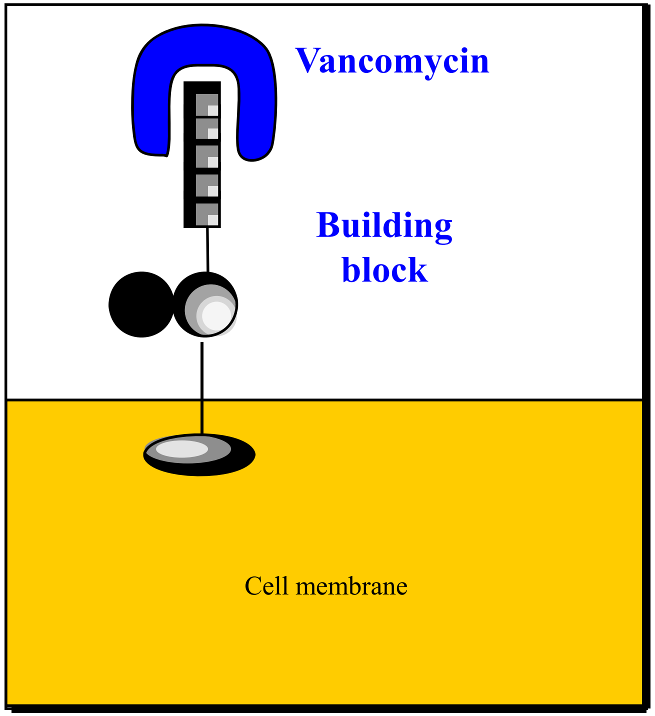
4. Vancomycin and vancomycin analogues



- •Important antibacterial agent
- •Caps the building block used in the synthesis of the bacterial cell wall
- •Contains a peptide chain which forms hydrogen bonds to the target
- •Vancomycin acts as a receptor for the building block

4. Vancomycin and vancomycin analogues

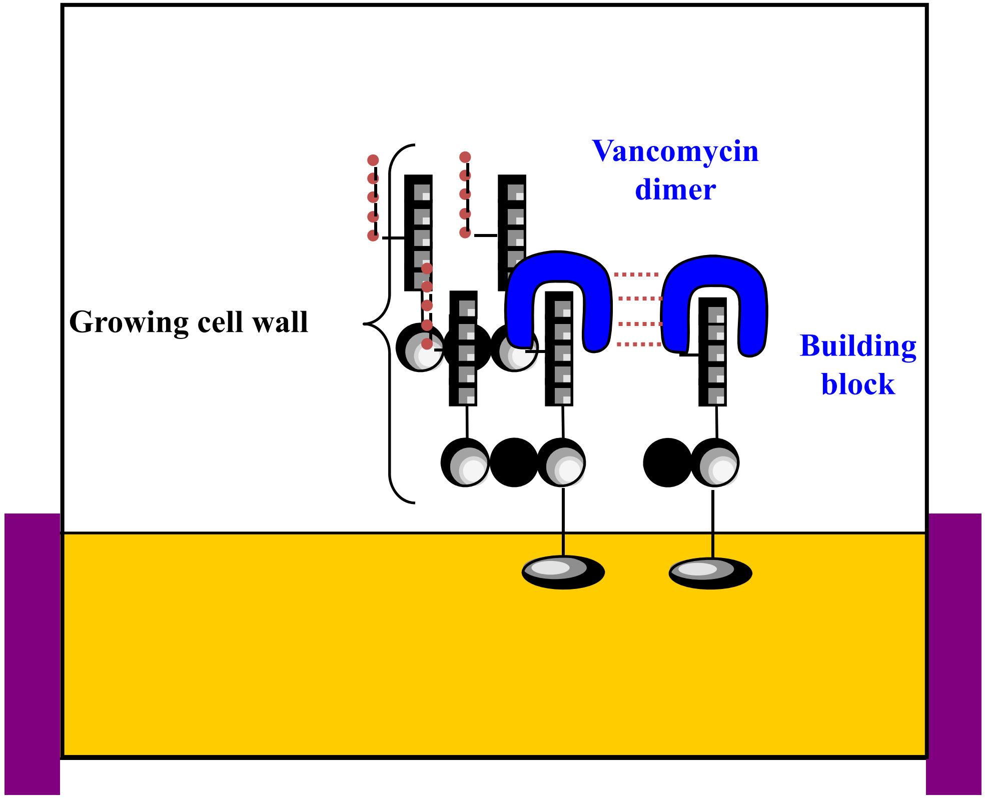
Mechanism of inhibition



Notes

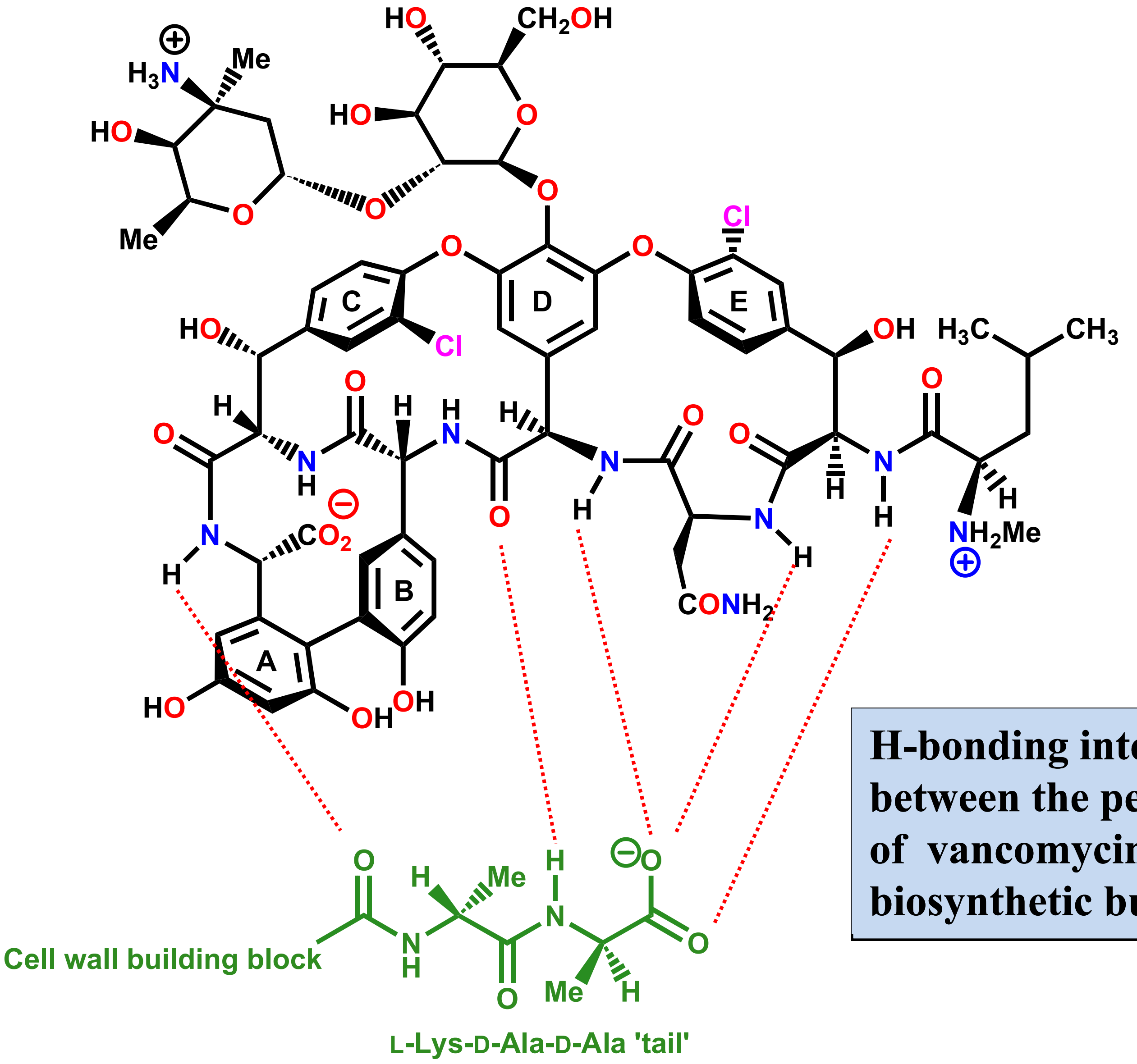
- Vancomycin provides binding pocket for tail of biosynthetic building block
- Vancomycin binds to the tail of the building block's peptide chain
- Caps the building block
- Disguises the building block from the transglycosidation enzyme

4. Vancomycin and vancomycin analogues



- Dimerisation occurs
- Dimer is highly stable
- Large vancomycin molecule acts as a steric shield

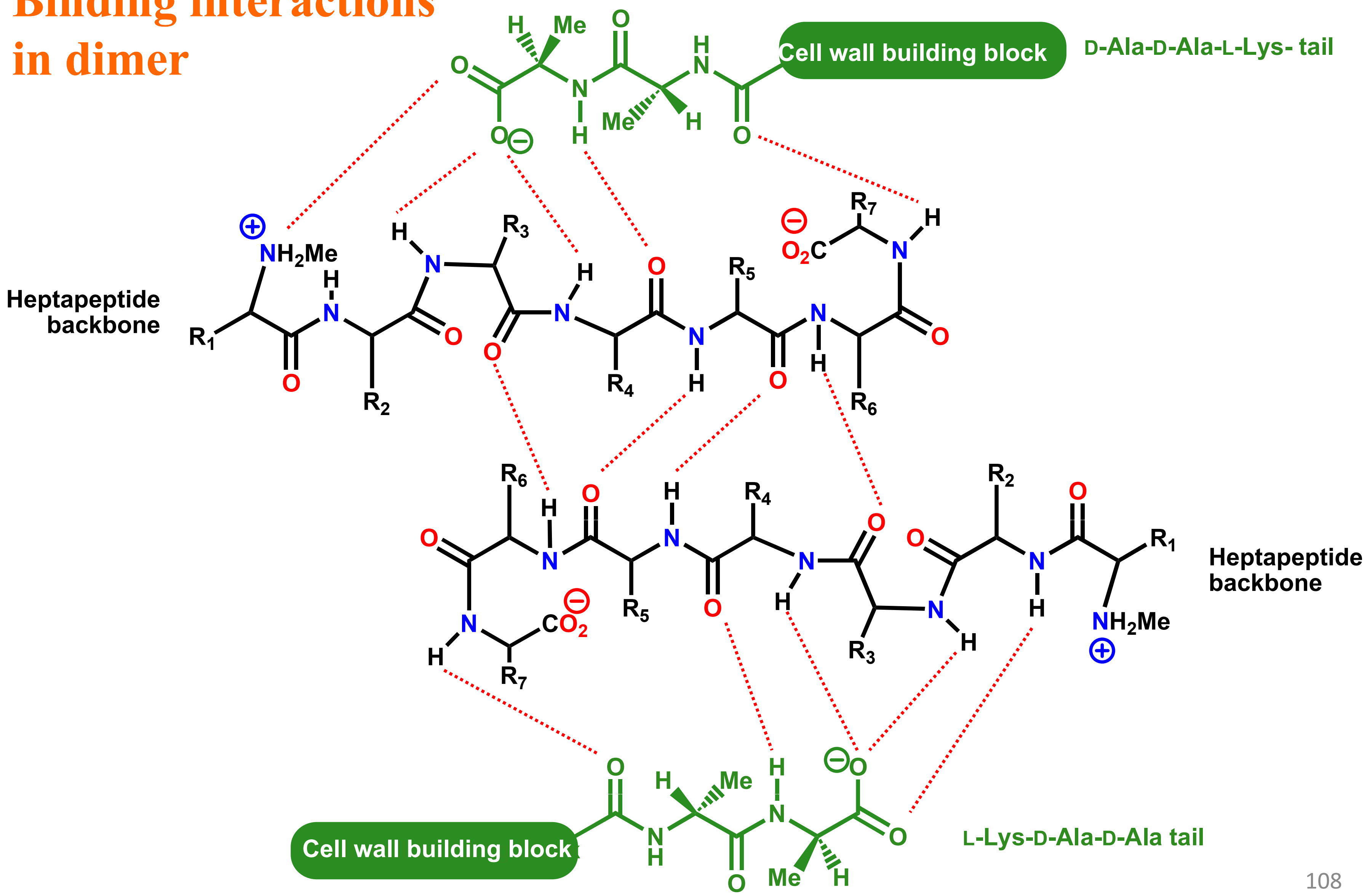
4. Vancomycin and vancomycin analogues



H-bonding interactions between the peptide backbone of vancomycin and the biosynthetic building block

4. Vancomycin and vancomycin analogues

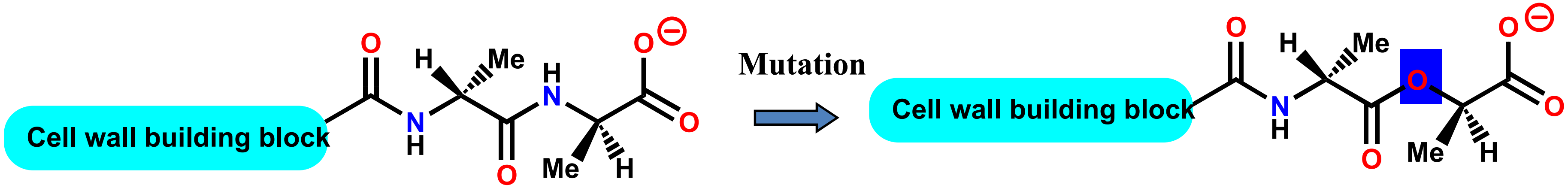
Binding interactions in dimer



4. Vancomycin and vancomycin analogues

Drug resistance

- Vancomycin-resistant *Staphylococcus aureus* (VRSA) (1996)
- Vancomycin-resistant enterococci (VRE) (1989)
- Resistance due to mutation in pentapeptide chain of cell wall building block
- Terminal D-alanine replaced by D-lactate



L-Lys-D-Ala-D-Ala tail

L-Lys-D-Ala-D-Lactate tail

- Peptide link replaced by ester link
- Loss of NH (HBD)
- Weakens binding affinity of vancomycin with ‘tail’
- Lactate acts as a leaving group in cell wall synthesis

3) Antibacterial agents which act on the plasma membrane structure

- The peptides like valinomycin and gramicidin A both act as ion conducting antibiotics
- It allows the uncontrolled movement of ions across the cell membrane

1. Gramicidin A (Decomb ®):

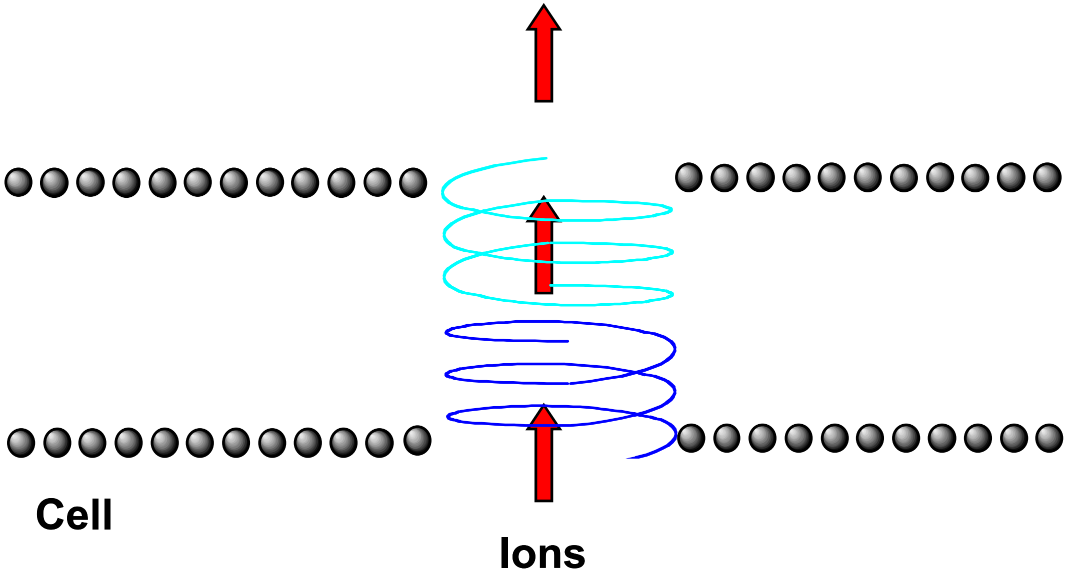
- It is a peptide containing 15 amino acids which is thought to coil into a helix

- The outside of the helix is hydrophobic and interacts with the membrane lipids.

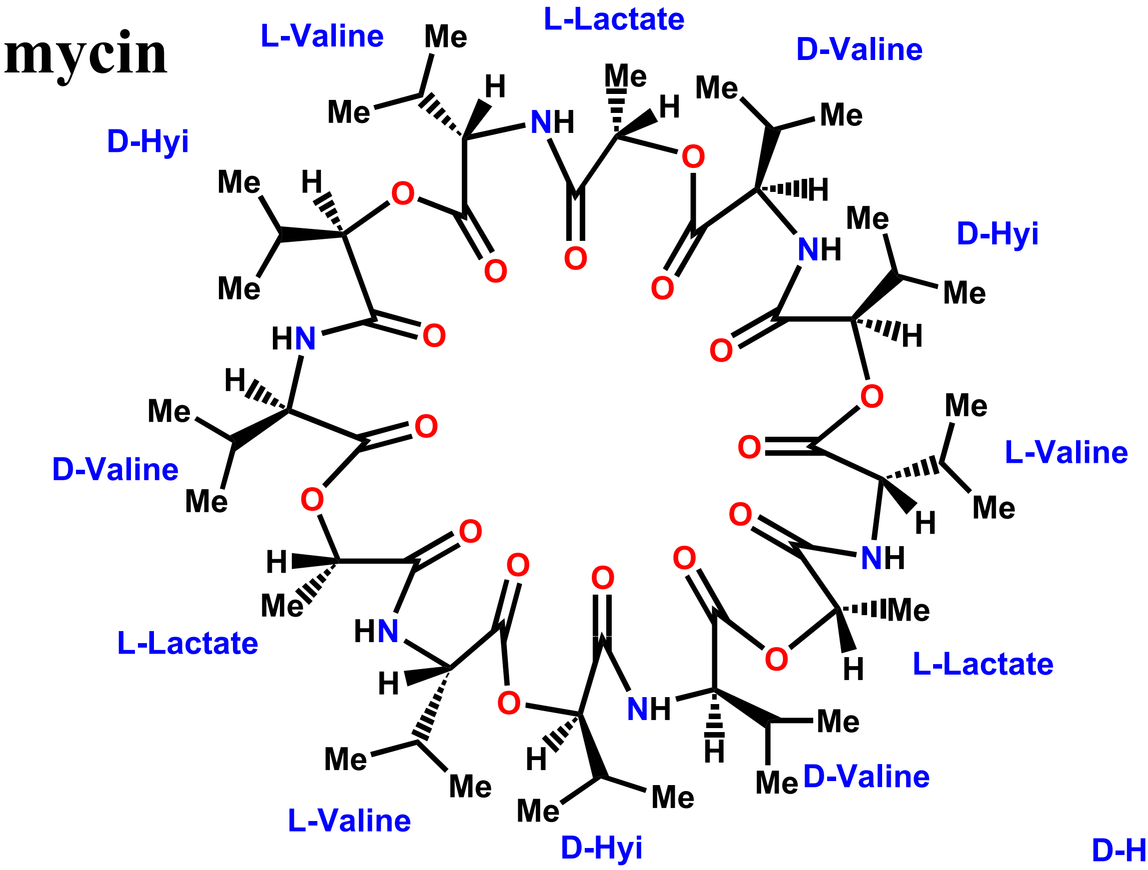
- The inside of the helix contains hydrophilic groups, Thus allowing the passage of ions.

- Gramicidin A could be viewed as an escape tunnel through the cell membrane.

- One molecule of gramicidin would not be long enough to traverse the membrane
- Thus is proposed that two gramicidin helices align themselves end-to-end to achieve the length required



2. Valinomycin



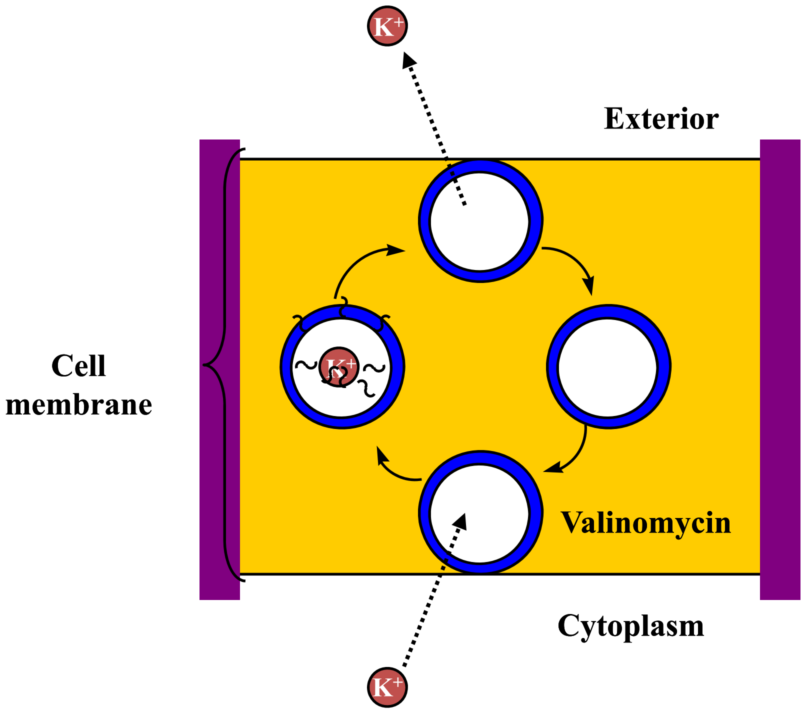
D-Hyi = D-Hydroxyisovaleric acid

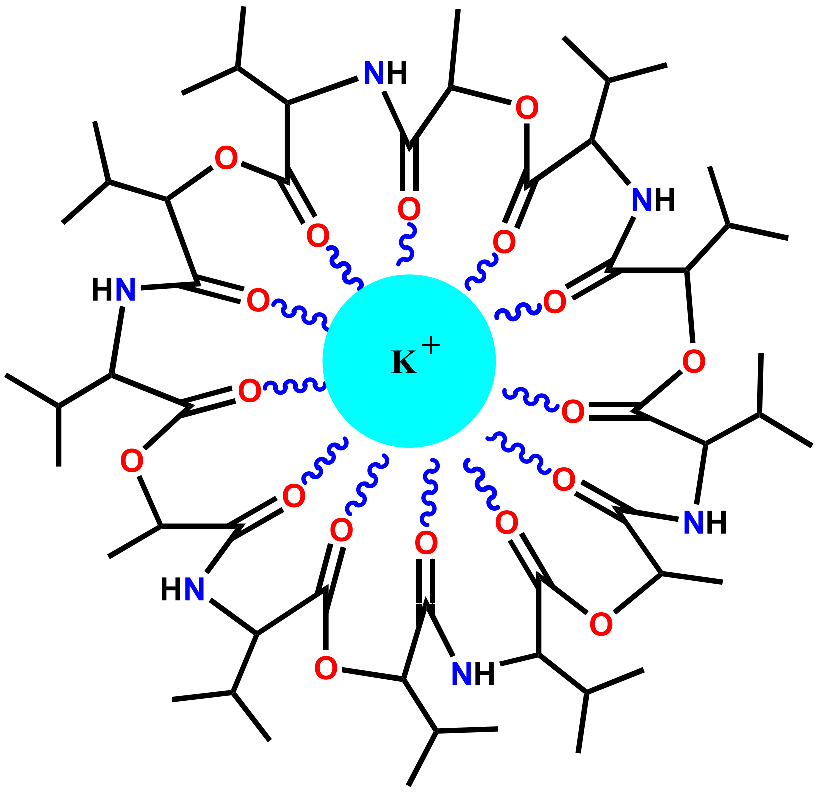
Notes

- Cyclic structure with alternating ester and amide links
- Hydrophobic residues on exterior
- Polar carbonyl groups in interior

Mechanism of action

- Acts as an ion carrier
- Hydrophobic groups on exterior interact with membrane lipids
- Carbonyl groups interact with potassium ion
- Allows uncontrolled escape of potassium ions from cell





3. Polymyxin B (Tribiotic Powder ®-BPC):

- It is a polypeptide antibiotic which operates within the cell membrane.
- It shows selective toxicity for bacterial cells over animal cells
  - This is due to its ability to bind selectively to the different plasma membranes.
  - The mechanism of this selectivity is not fully understood.
- Polymyxin B probably acts like valinomycin
  - but it causes the leakage of small molecules such as nucleosides from the cell.
- The drug is injected intramuscularly and is useful against *Pseudomonas* strains which are resistant to other antibacterial agents.

4) Antibacterial agents which impair protein synthesis: translation

❖ Introduction

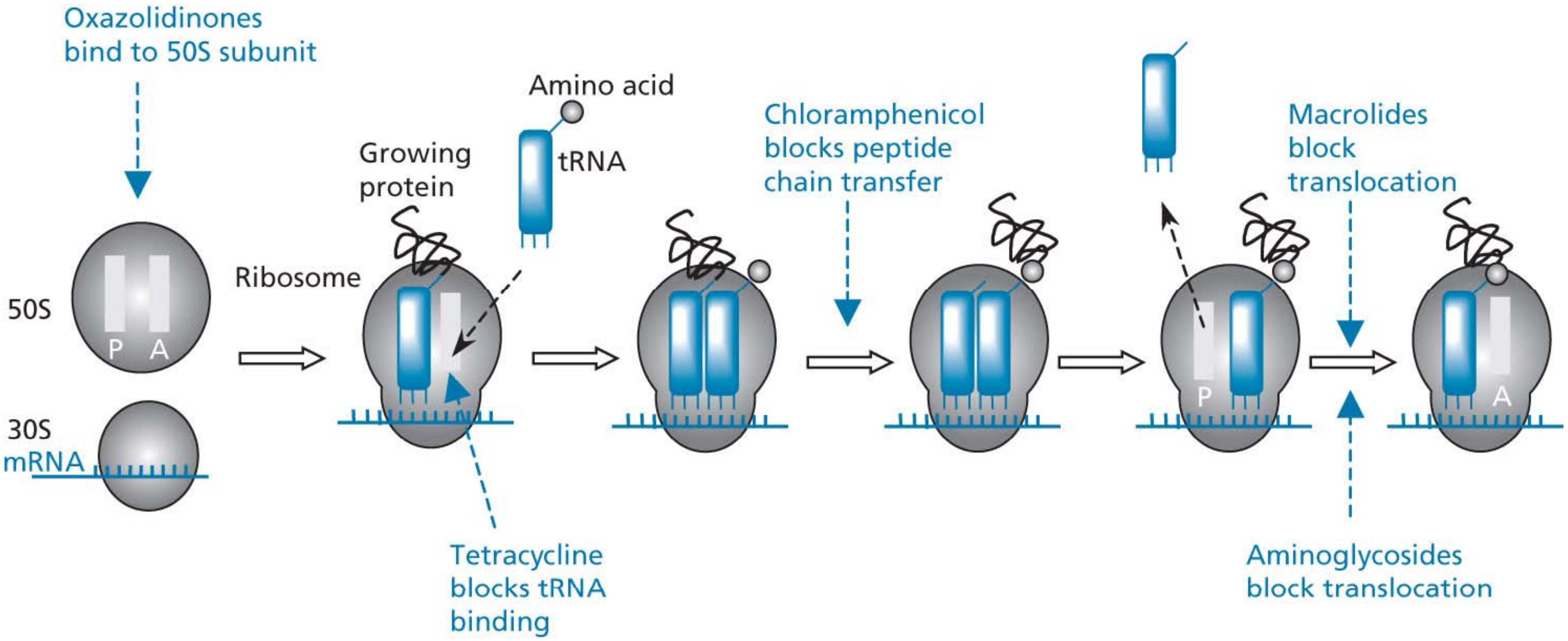
- All inhibit protein synthesis by binding to ribosomes and inhibiting different stages of the translation process.

- the bacterial ribosome is a 70S particle made up of a 30S subunit and a 50S subunit.

- the 30S subunit binds mRNA and initiates protein synthesis.

- The 50S subunit combines with the 30S subunit-mRNA complex to form a ribosome, then binds aminoacyl transfer RNA (tRNA) and catalyses the building of the protein chain.

- the ribosmes of eukaryotic cells are bigger (80S), consisting of a 60S large subunit and a 40S small subuint. This difference give selectivity to this types of antibiotics.


Oxazolidinones bind to 50S subunit
50S
P
A
30S
mRNA
Ribosome
Growing protein
Amino acid
tRNA
Tetracycline blocks tRNA binding
Chloramphenicol blocks peptide chain transfer
P
Macrolides block translocation
A
Aminoglycosides block translocation

1) AMINOGLYCOSIDES

- The drug works by inhibiting protein synthesis.

- they are a carbohydrate structure which includes basic amine groups "aminoglycoside".

- At pH 7.4, they are positively charged which is beneficial to how these agents are absorbed through the outer membrane of gram –ve.

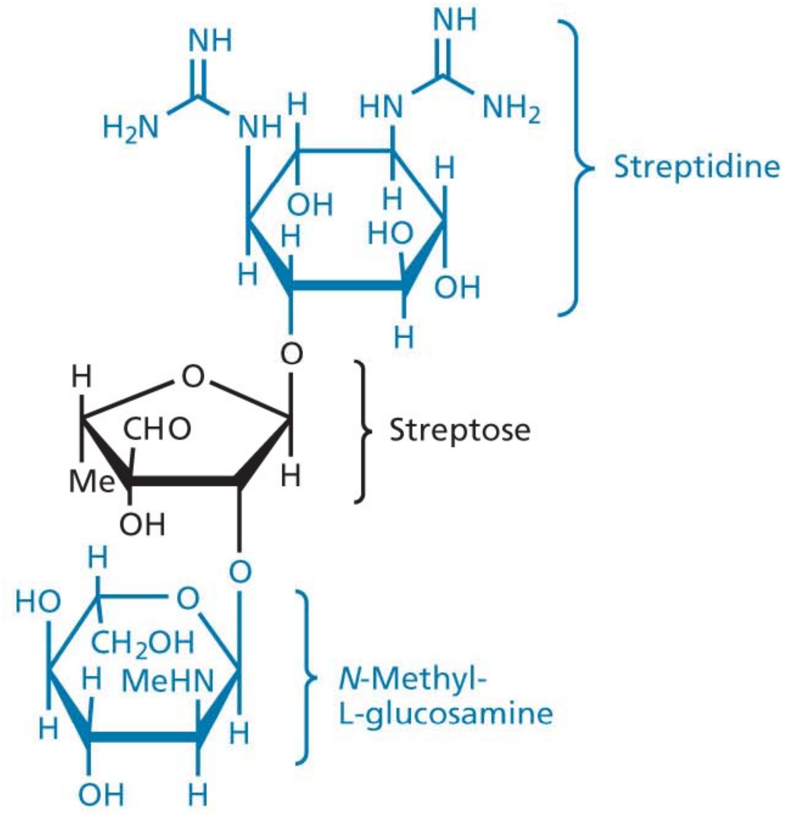
- Then they bind to bacterial ribosmes specifically to 30S ribosome subunit & prevents the movment of the ribosome along mRNA so that the triple code on mRNA no longer be read.

- Aminoglycosides are bactericidal rathar than bacteriostatic.

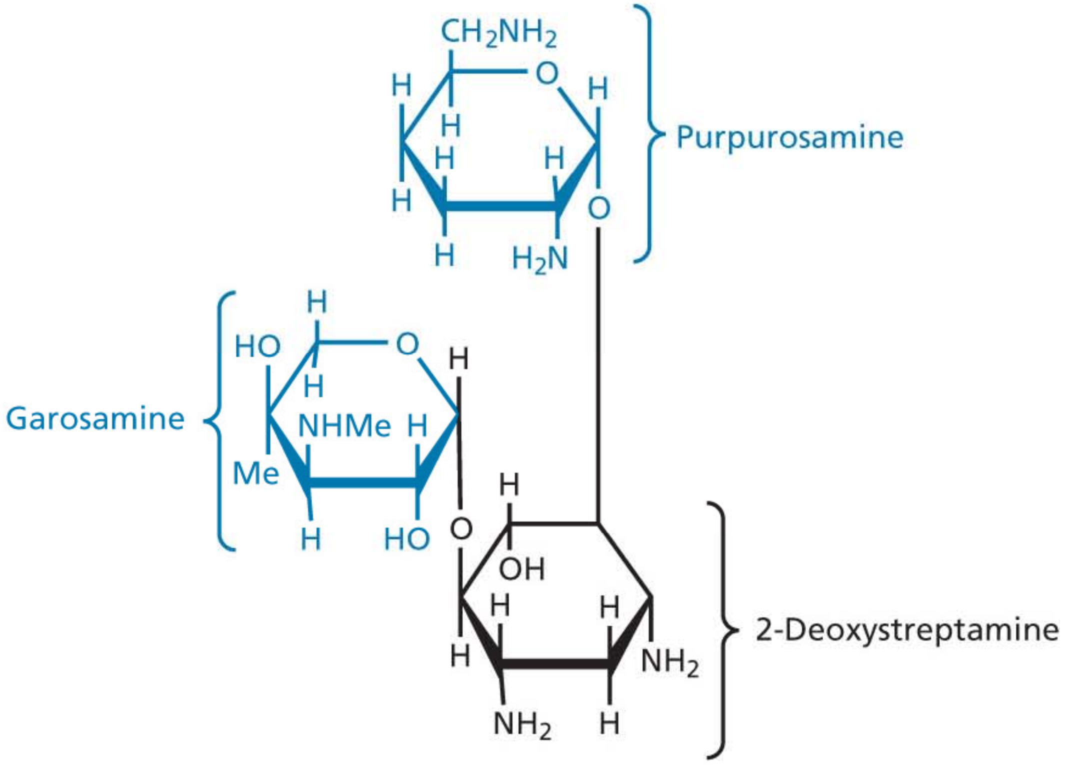
- They are present as inorganic acid salts:
  - They are very soluble in water
  - Usually available in formulation of sulphates salts.
- Their chemical structure:
  - All have at least one aminohexose
  - Some have a pentose without an amino group.
    - Eg: Streptomycine, Neomycin
  - Some aminoglycosides contain a substituted 1,3-diaminocyclohexane (deoxystreptamine) .
    - E.g: Kanamycin,Neomycin, gentamicin and tobramycin
  - Streptomycin contains streptidine.
    - From which the name streptomycine comes

➢ Aminoglycosides include compounds closely related in structure to streptomycin like:

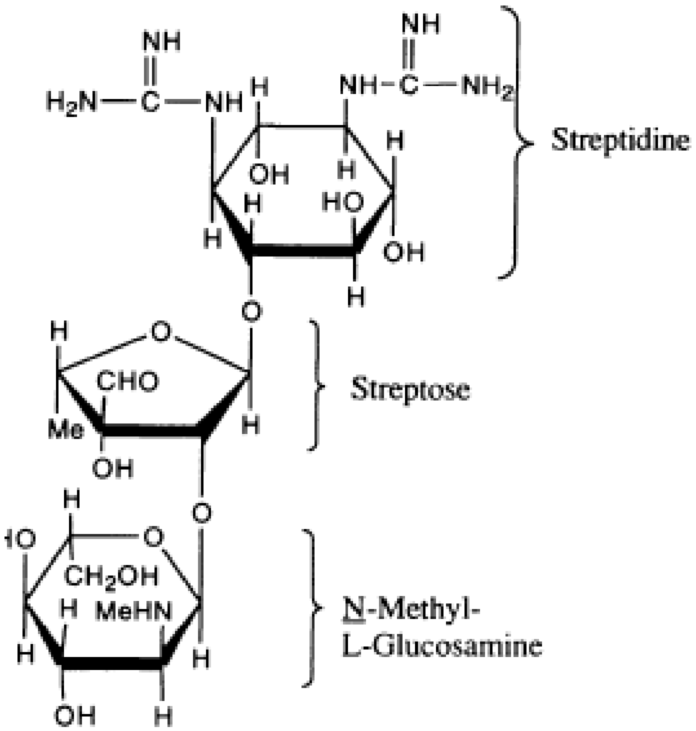
- kanamycin, neomycin, gentamicin, tobramycin and netilmicin.



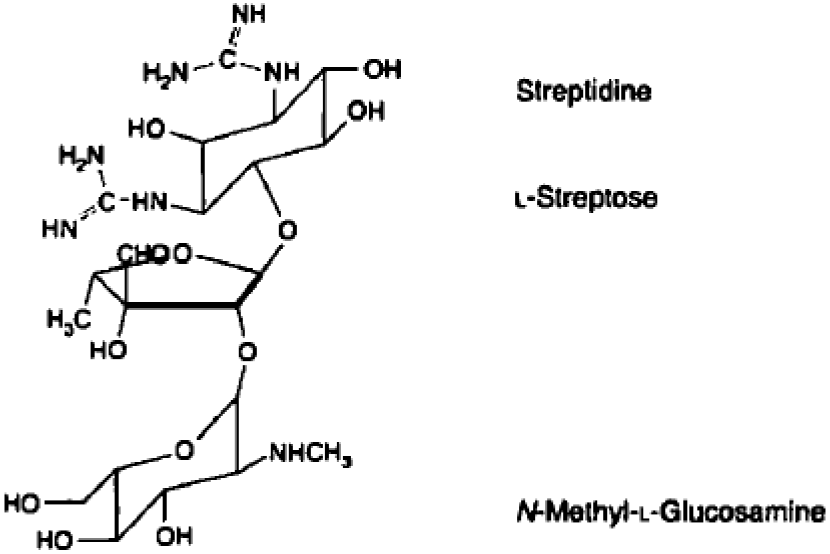
Streptomycin (from *Streptomyces griseus*)



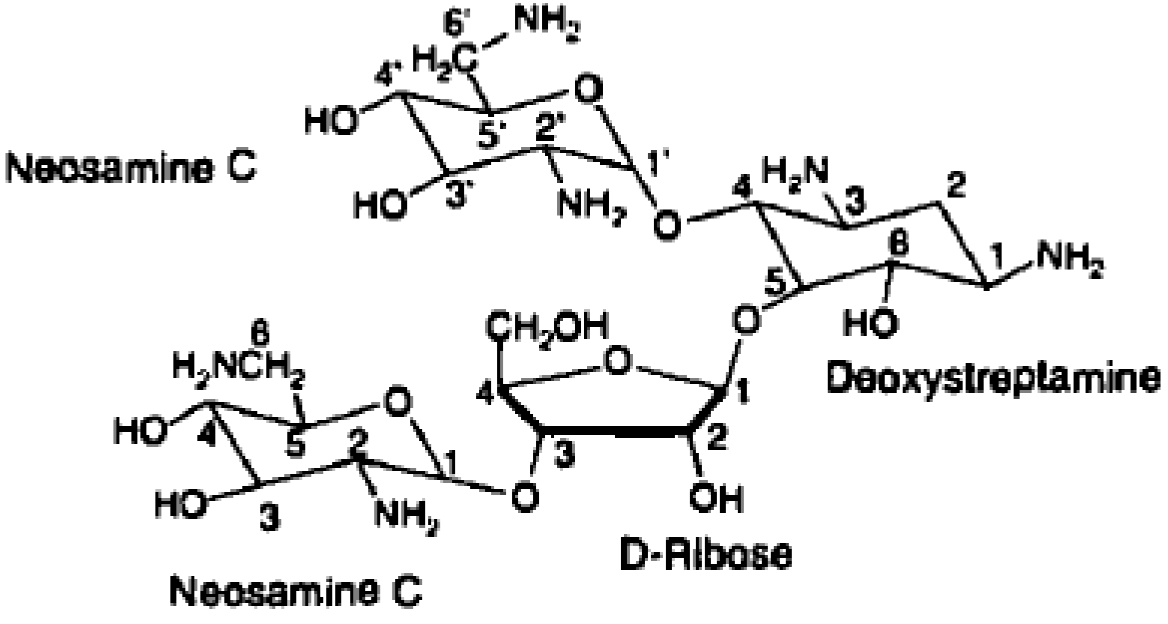
Gentamicin C1a



STREPTOMYCIN



Streptomycin


6'
NH2
4'
H2C
O
HO
5'
2'
Neosamine C
1'
HO
3'
NH2
4
H2N
3
2
O
6
1
NH2
5
CH2OH
O
HO
6
H2NCH2
O
Deoxystreptamine
4
1
HO
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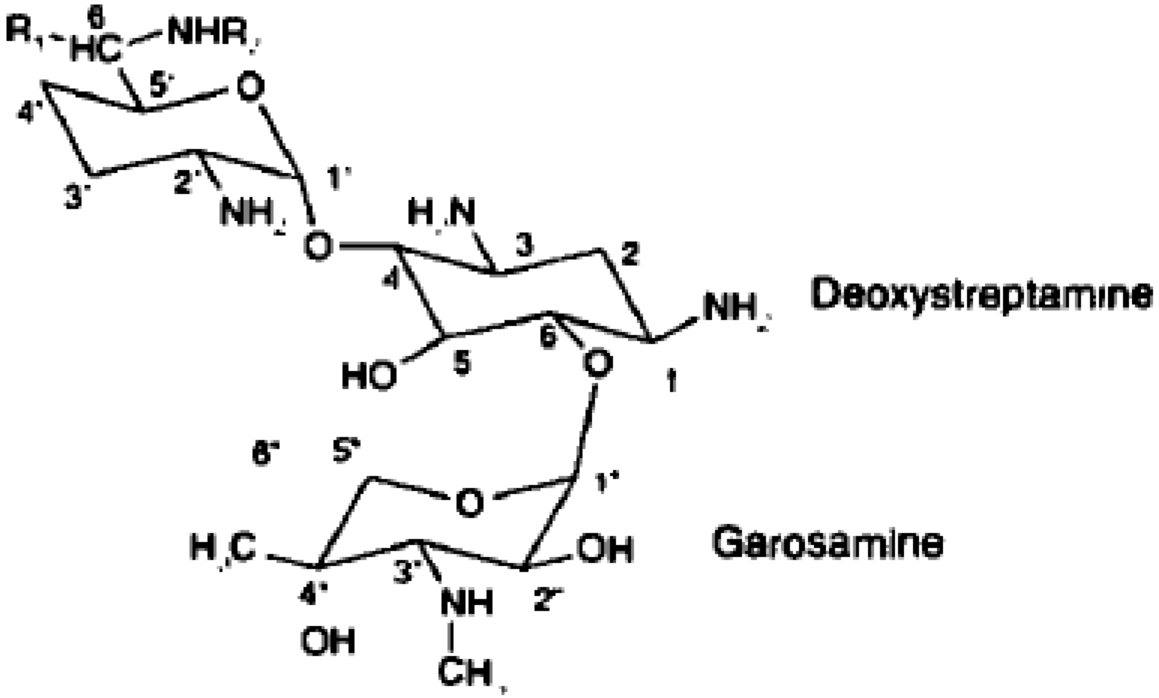
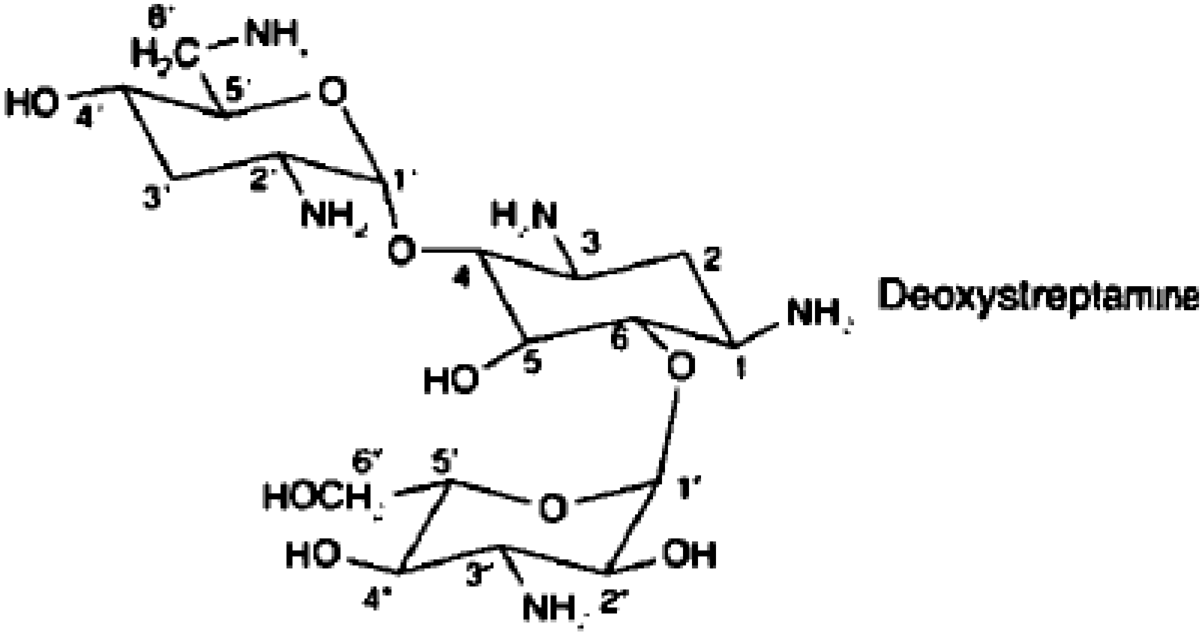
1
3
2
HO
3
NH2
O
OH
D-Ribose
Neosamine C

Neomycin



Gentamicin C_1: $R_1 = R_2 = CH_3$ Zetamycin amp®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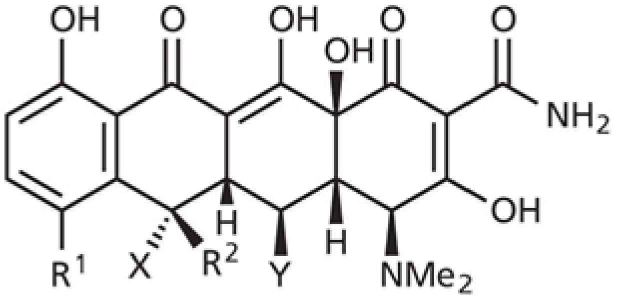
Tobramycin

2) TETRACYCLINES

- They are bacteriostatic antibiotics that have a broad spectrum of activity.

- one of the most known tetracyclines is chlortetracycline which was isolated in 1948. other are tetracyclin & doxycycline.

- The following figure shows the different derivatives of tetracyclines



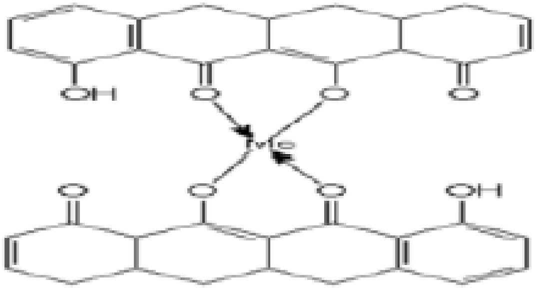
Chlortetracycline (Aureomycin) ($R^1 = Cl$, $R^2 = Me$, $X = OH$, $Y = H$)
Tetracycline ($R^1 = H$, $R^2 = Me$, $X = OH$, $Y = H$)
Doxycycline (Vibramycin) ($R^1 = H$, $R^2 = Me$, $X = H$, $Y = OH$)
Demeclocycline ($R^1 = Cl$, $R^2 = H$, $X = OH$, $Y = H$)

- the tetracyclines inhibit protein synthesis by binding to the 30S rubunit of ribosmes and preveting aminoacyl-tRNA from binding.

- this stops the further addition of amino acids to the growing protein chain. Protein release is also inhibited.

- selectivity is due to the ability to bacterial cells to concentrate these agents faster than human cells.

- **Tetracyclines form stable chelate complexes are formed by many metals, including calcium, magnesium, and iron.**



➢ These chelates are usually very insoluble in water causing the impaired absorption of most (if not all) tetracyclines:

- in the presence of milk
- calcium-, magnesium-, and aluminium containing antacids
- Iron salts.
- Soluble alkalinizers such as sodium bicarbonate, also decrease the gastrointestinal absorption of the tetracyclines.

➢ The affinity of tetracyclines for calcium cause them to be incorporated into newly forming bones and teeth.

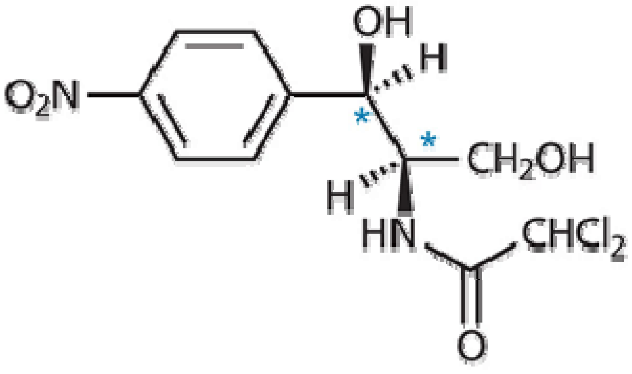
– They form tetracycline—calcium orthophosphate complexes.
– Deposits of these antibiotics in teeth cause a yellow discoloration that darkens over time.

3) CHLORAMPHENICOL

- Chloramphenicol was originally isolated from *Streptomyces Venezuela*. It is now prepared synthetically.

- The drug should only be used with restriction since it is quite toxic, especially to bone marrow.

- Chloramphenicol possesses two chiral carbon atoms via the acylamidopropanediol chain. Only the R,R-isomer is active.



Chloramphenicol

❖ SAR studies:

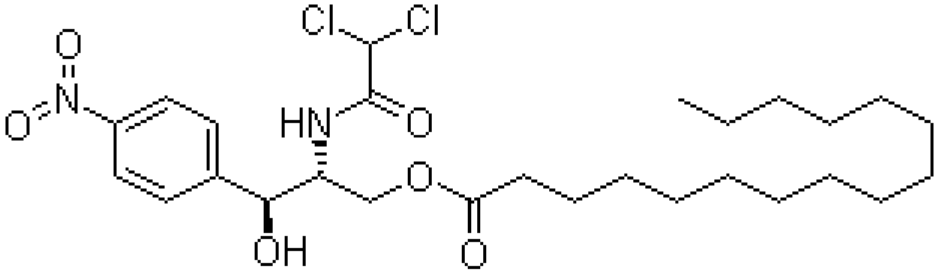
- There must be a NO_2 substituent on the aromatic ring.
- The R,R-propanediol group is essential.
- The OH groups must be free and presumably are involved in hydrogen bonding.
- The dichloroacetamide group is important, but can be replaced by other electronegative groups.

❖ Mechanism of action:

- Chloramphenicol binds to the 50S subunit of ribosomes

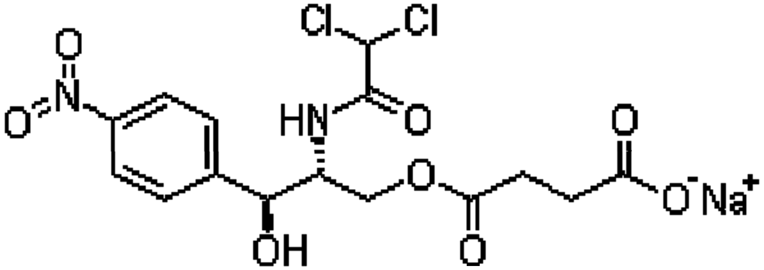
- It acts by inhibiting the movement of ribosomes along mRNA, probably inhibiting the peptidyl transferase reaction by which the peptide chain is extended.

Chloramphenicol Palmitate:



- Chloramphenicol palmitate is the palmitic acid ester of chloramphenicol.
- It is a tasteless prodrug of chloratnphenicol intended for pediatric use.
- The ester must hydrolyze in vivo following oral absorption to provide the active form.

Chloramphenicol Sodium Succinate



- Chloramphenicol sodium succinate is the water-soluble sodium salt of the succinate ester of chioramphenicol
- The sodium succinate is preferred for intravenous administration

4) MACROLIDES

- The best known example of this class of compounds is erythromycin

- A metabolite produced by the microorganism *Streptomyces erythreus*.

- The structure consists of a macrocylic lactone ring with a sugar and an aminosugar attached to it.

- The macrolide antibiotics have three common chemical characteristics:

- a large lactone ring (the name macrolide).
- a ketone group, and
- a glycosidically linked amino sugar.

- Usually, the lactone ring has 12, 14, or l6 atoms in it

- They may have, in addition to the amino sugar, a neutral sugar that is linked glycosidically to the lactone.

- Erythromycin binds selectively to a specific site on the 50S ribosomal subunit to prevent the translocation step of bacterial protein synthesis.

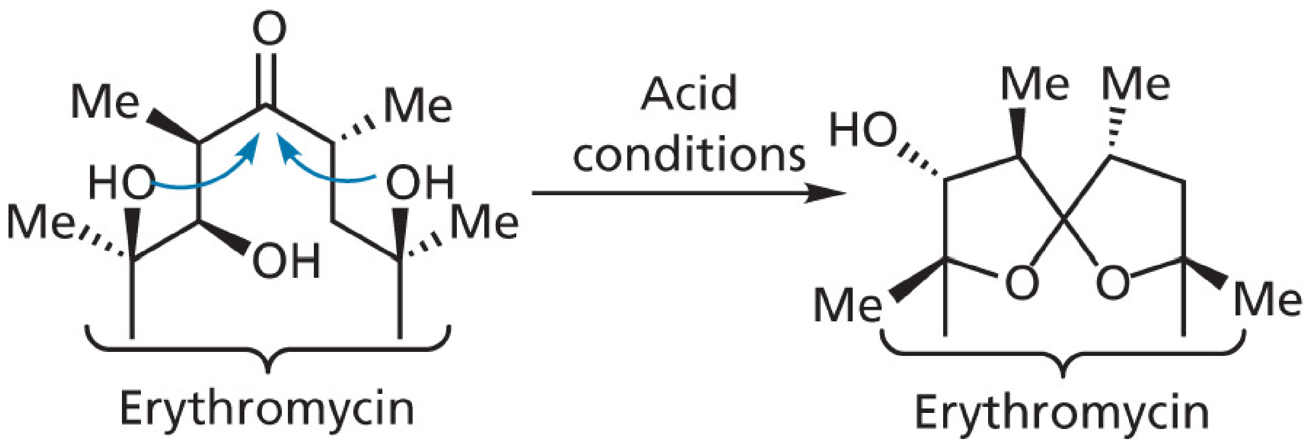
- It does not bind to mammalian ribosome

- Since macrolides and chloramphenicol bind to the same region of the ribosome they should not administered together as they will compete with each other and be less effective.

- Erythromcyin is unstable to somtach acids, but can be taken orally in a tablet form.

- The formulation of the tablet involves a coating that is designed to protect the tablet during its passage through the stomach, but is soluble once it reaches the intestines.

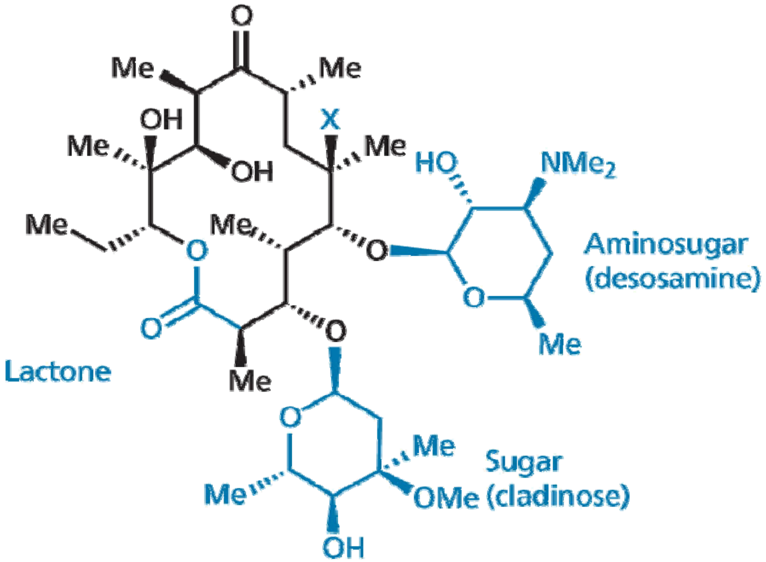
- this acid sensitivity is due to the presence of a ketone and two alcohol groups which are set up for the acid-catalyzed intramolecular formulation of a ketal.



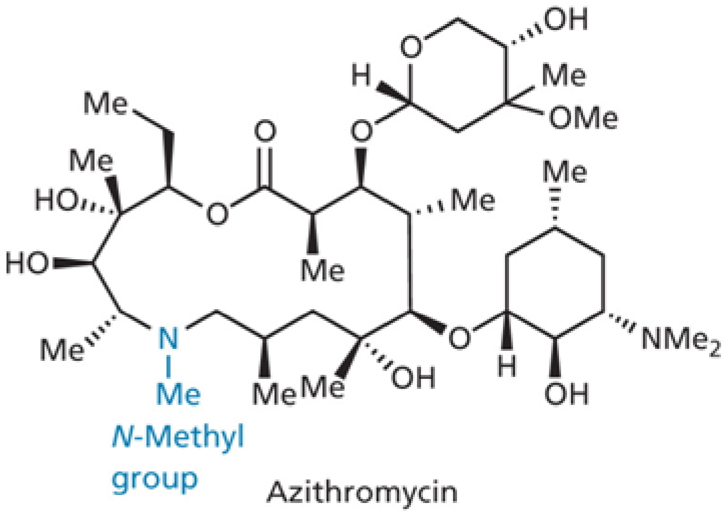
- One way of preventing this is to protect the hydroxy groups. E.g. clarithromycin is a methoxy analogue of erythromycin which is more stable to gastric juice and has improved oral absorption.

- Another method of increasing acid stability is to increase the size of the macrocycle to a 16-membered ring.

- Azithromycin contains a 15-membered macrocycle where an *N*-methyl group has been incorporated into the macrocycle.



Erythromycin; X = OH
Clarithromycin; X = OMe



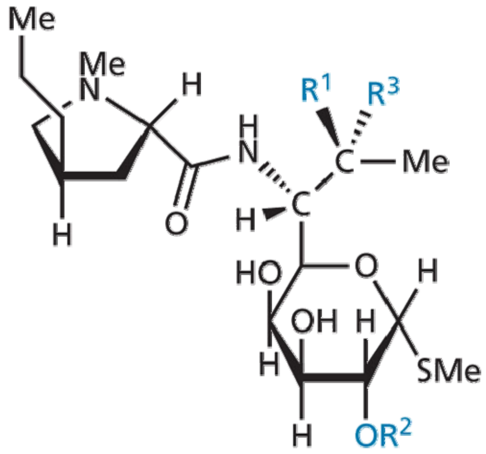
Azithromycin

5) LINCOSAMIDES

- They have similar antibacterial properties to the macrolides and act in the same fashion.

- Lincomycin was the first of these agents and was isolated in 1962 from Streptomyces lincolnensis found near Lincoln, Nebraska.

- Chemical modification led to the clinically useful clindamycin, which has increased activity.



Lincomycin $R^1=R^2=H$, $R^3=OH$
Clindamycin $R^1=Cl$, $R^2=R^3=H$
Clindamycin phosphate $R^1=Cl$, $R^2=PO_3^{2-}$, $R^3=H$

5) Agents which act on nucleic acid: transcription and replication

1) QUINOLONES AND FLUOROQUINOLONES

- They are useful in the treatment of urinary tract infections, and also in the treatment of infections which prove resistant to antibacterial agents.

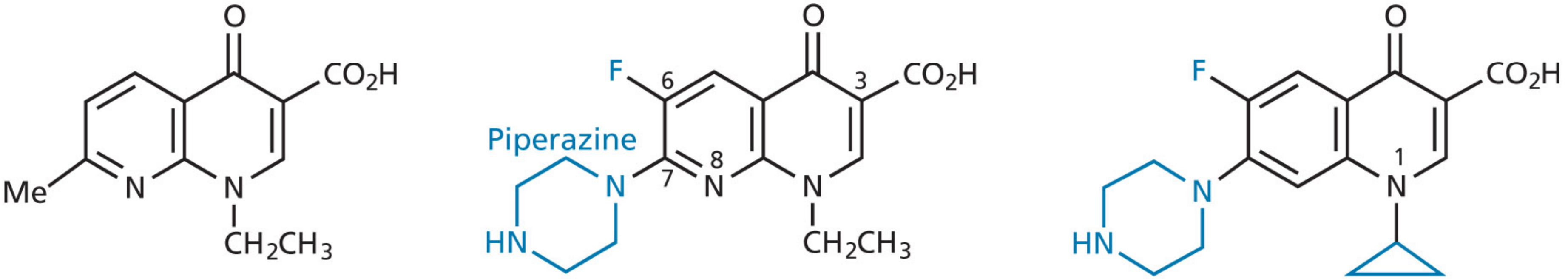
- **Nalidixic acid** was the first agent in this class of compounds and was synthesized in 1962.

- a breakthrough was made in the 1980s, with the development of **Enoxacin** was based on the discovery that a single flourine atom at position 6 greatly increased activity as well as increasing uptake into the bacterial cell.

- A basic substituent such as a piperazinyl ring at position 7 was beneficial for a variety of pharmacokinetic reasons due to the ability of the basic substituent to form a zwitterion with the carboxylic acid gp at position 3.

- The introduce of a cyclopropyl substituent at position 1 further increased a broad spectrum activity.

- while replacement of the nitrogen at position 8 with carbon reduced adverse reactions and increased activity against *S. aureus*. This led to **ciprofloxacin**.



Nalidixic acid Enoxacin Ciprofloxacin

❖ Mechanism of action

- They inhibit the replication and transcription of bacterial DNA by stabilizing the complex formed between DNA and topoisomerases.

- In gram +ve bacteria, the stabilized complexes between DNA and topoisomerase IV, with the drugs showing a 1000-fold selectivity for the bacterial enzyme over the corresponding enzyme in human cell.

- In gram –ve bacteria, the main target is the complex between DNA and a topoisomerase II enzyme called DNA gyrase. It has the same role as topoisomerase IV.

- Topoisomerase enzymes are required when the DNA double helix is being supercoiled after replication and transcription.

- A large number of fluoroquinolones have now been synthesized. These agents have a good activity.

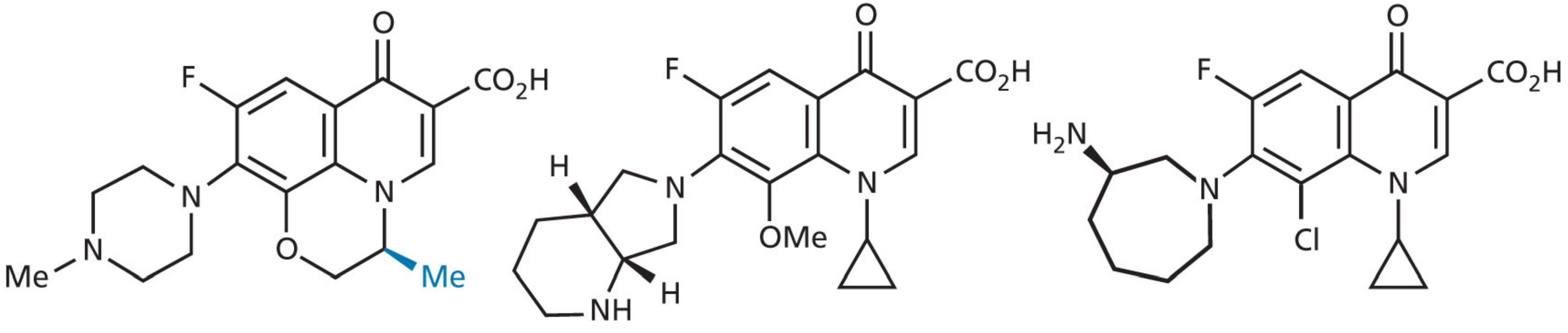
- All have a similar bicyclic ring system which includes a pyridone ring and a carboxylic acid at position 3.

- A problem with 1st and 2nd generation fluoroquinolones is that they show only moderate activity against S. aureus, with resistance being quick to arise.

- 3rd generation such as ofloxacin, levofloxacin, and moxifloxacin began to developed in 1990s to tackle these issues.

- Ofloxacin has an asymmetric centre and is sold as racemic mixture of both enantiomers, one of which is active and the other not.

- Levofloxacin is the active enantiomer of oflaxcin and is twice as active.



Levofloxacin
(Ofloxacin is the racemate)

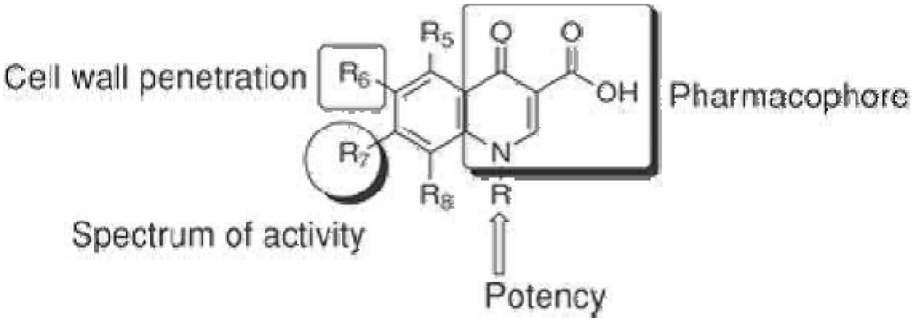
Moxifloxacin

Besifloxacin

- The incidence of CNS effects associated with the quinolones.

- Another property of the quinolone class is phototoxicity extreme sensitivity to sunlight.

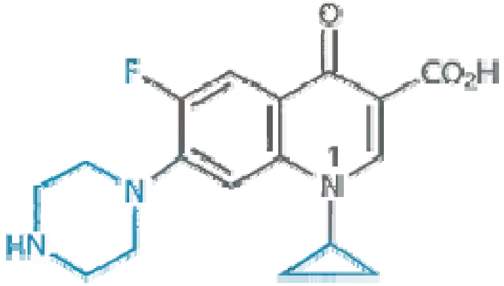
SAR of quinolones:



- The essential pharmacophore for activity is:
  1. The carboxy-4-pyridone nucleus .
     - The carboxylic acid and the ketone are involved in binding to the DNA/DNA-gyrase enzyme system.
     - Reduction of the 2,3-double bond or the 4-keto group inactivates the molecule.
     - Substitution at C-2 interferes with enzyme–substrate complexation.
  2. Fluoro substitution at the C-6 position:
     - Greatly improves antimicrobial activity:
       - By increasing the lipophilicity of the molecule thus improves the drugs penetration through the bacterial cell wall .
       - Fluoro group at C-8 further improves drug absorption and half-life
     - but also may increase drug-induced photosensitivity.

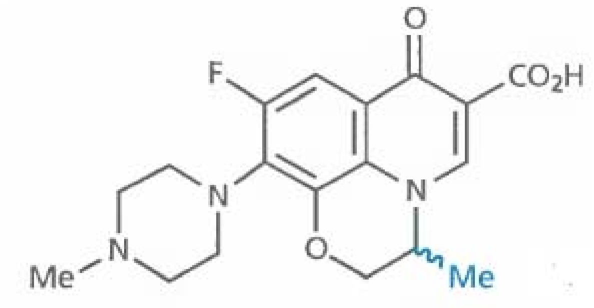
3. Heterocyclic (Piperzine)substitution at C-7:

- Improves the spectrum of activity especially against Gram-negative organisms.
- The piperazinyl (as in ciprofloxacin) represent the most significant antimicrobial improvement .
- But, the piperazinyl group at C-7 also increases binding to (CNS) γ-aminobutyric acid (GABA) receptors, which accounts for CNS side effects.
- Alkyl substitution on the piperazine (Ofloxacin **Taricin®**) is reported to decrease binding to GABA



Ciprofloxacin

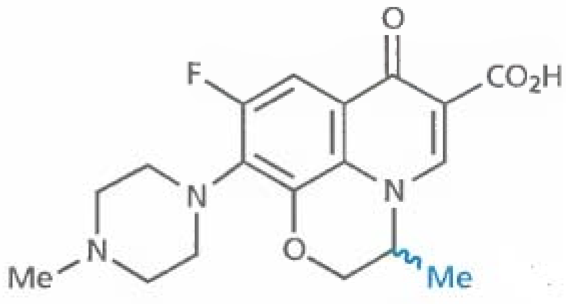
Ciprocare®, Floxin®



Ofloxacin

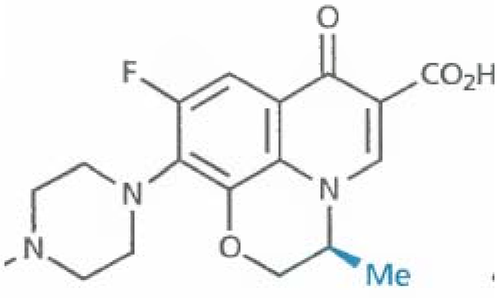
Ultracin ®Taricin®

- The introduction of a third ring to the nucleus of the quinolones:
  - Gives ofloxacin.
  - Ofloxaxin has an Chiral carbon.
    - The *S*- (–) -isomer (levofloxacin -Levox ®) is twice as active as ofloxacin



Ofloxacin

Ultracin ®Taricin®



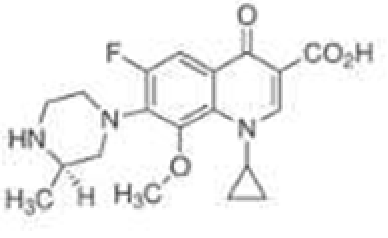
Levofloxacin

Tarvinic ®

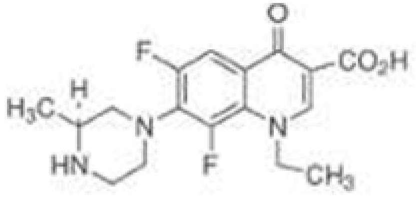
Levox ®

Volxal ®

- Several of the quinolones produce mild to severe photosensitivity.
  - C-8 halogen appears to produce the highest incidence of photosensitivity.
  - Lomefloxacin has been reported to have the highest potential for producing phototoxicity.
  - Substitution of a methoxy group at C-8 has been reported to reduce the photosensitivity (gatifloxacin, **Zymar ® E/D**).



Gatifloxacin (Tequin)



Lomefloxacin (Maxaquin)

2) RIFAMYCINS:

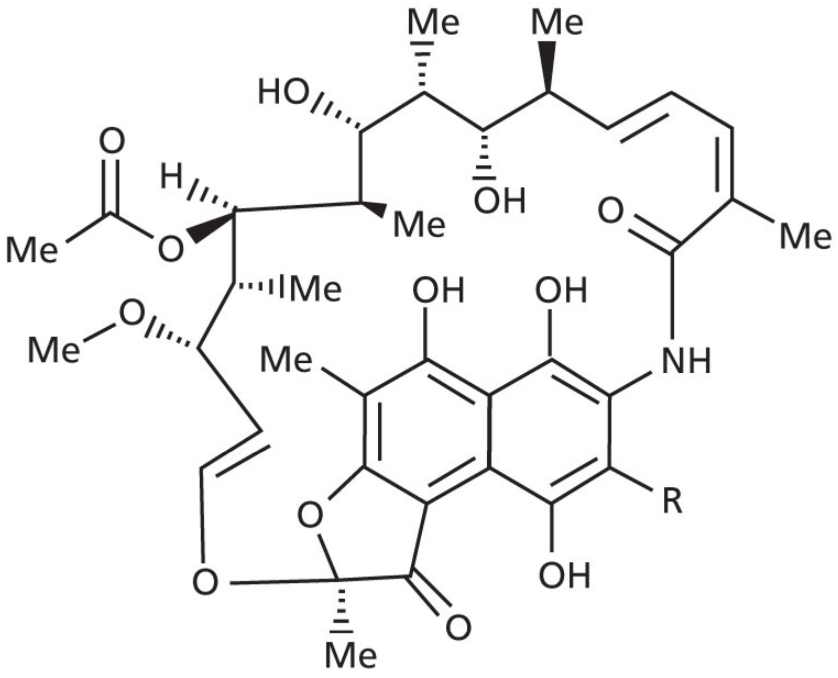
- **Rifampicin (Remactan®)**

➢ It is a semisynthetic rifamycinnmade from rifamycin B and isolated from *Streptomyces mediterranei* in 1957.

➢ It inhibits Gram-positive bacteria and works by binding non-covalently to RNA polymerase and inhibiting RNA synthesis.

➢ RNA polymerases in eukaryotic cells are unaffected since the drug binds to a peptide chain not present in the mammalian RNA polymerase. It is therefore highly selective.

➢ the flat naphthalene ring and several hydroxyl groups are essential for activity and the molecule exists as a zwittterion giving it good solubility both in lipids and aqueous acid.



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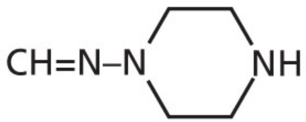
R =		Rifampicin