

Topic 4 Antibiotics Sept 27 2018

Antibiotics

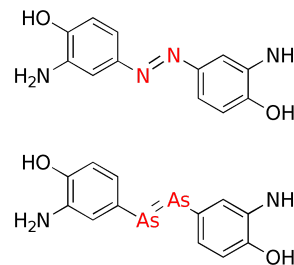
- “Anti-life”
 - Selective poisons for microbes
 - Bacteria
 - Viruses
 - Fungi
 - Protozoa
- Have had largest impact on human life of all medications except vaccines
- Infectious disease → leading cause of death throughout history
 - Middle ages - 25% killed by plague

Paul Ehrlich and the magic bullet 1907

- Antibiotics have been around for 70 years, but their history goes back to 1907
- Paul discovered that **trypanosome infection could be treated with Trypan red** (a dye) which was taken in by the trypanosomes cells but not blood cells
- **Coined the phrase “The Magic bullet” = chemical substance or therapy capable of targeting the infectious/pathogenic cell and providing a remedy for a disease without deleterious side effects** (in Paul’s case it was the trypan red).

Salvarsan 606

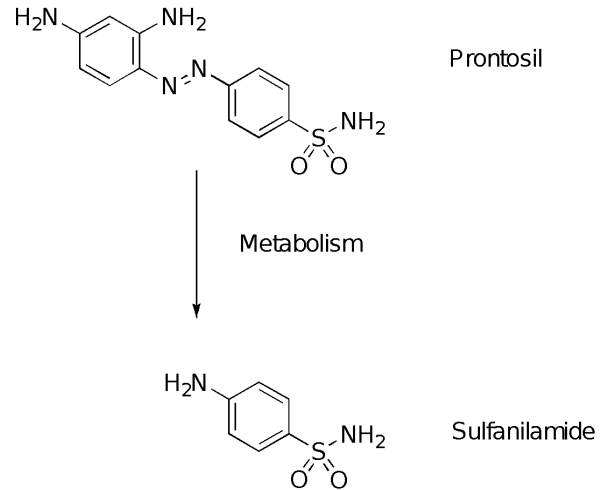
- Although a poor product, **Salvarsan 606 was the first effective drug to treat syphilis** (not very effective but more progress than before)
- **A simple SAR (Substitution** was used to replace the nitrogen and used As as the isostere) → **increased toxicity**
- **Was used to treat syphilis**
- Usage included weekly injections
 - Drug very insoluble
 - 600 mL each injection
 - Required 1 hour
 - Syringe was used (slow i.v. drip invented 1935)
 - Intramuscular injection produced necrosis



It did not have drug like properties but was ‘proof of principle’ = with testing and modifications, a better drug can be made to target the disease in the body

Gerhard Domagk at IG Farben 1932

- Produced the first commercially successful antibiotics through different testing called Prontosil
 - effective in vivo but ineffective in vitro (bacteria petri dish)
 - Effectiveness in vivo was due to metabolism (metabolism would activate it → **sulfonamide** meaning its a prodrug therefore only active inside the body)

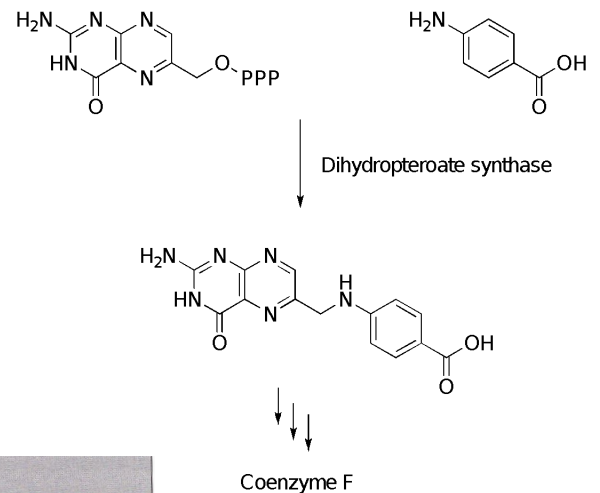


Mechanism of Action

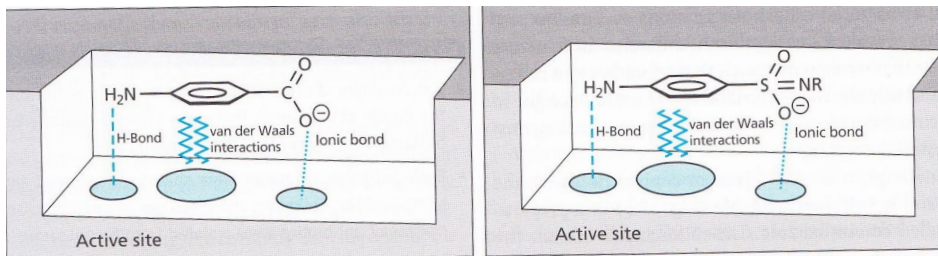
- Bacteriostatic, interferes with growth
 - Does NOT kill bacteria only stops growth
 - Only effective if patient has competent immune system
- Interferes with tetrahydrofolate biosynthesis (coenzyme F)
- Prevents bacteria from making coenzyme F
 - Bacterial growth stops

Sulfa drugs inhibit coenzymes F synthesis

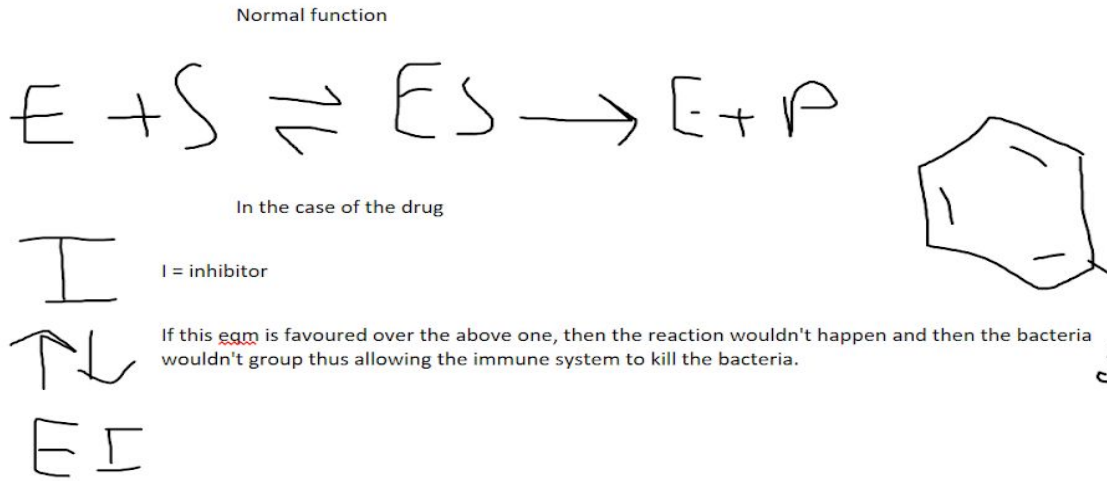
- Sulfonamides are competitive inhibitors of PABA
- Sulfonamide competes with p-aminobenzoic acid for active site
- Binding is in the active site
 - Substrate cannot bind → coenzyme F not produced



Model of sulfonamide competition



Blocking enzyme function



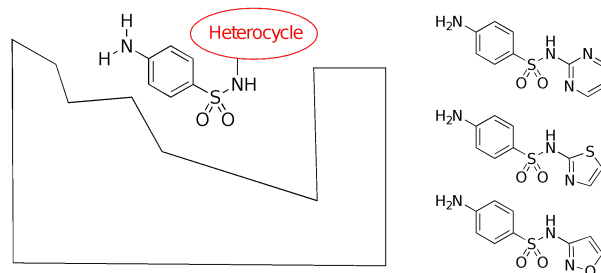
Competitive Inhibitors

- Binds better than the natural substrate
 - Nature does not make optimal systems, just good enough
 - If nature were optimal, drugs would not be possible
- Basis for selectivity in humans
 - Humans lack the enzyme
 - Get tetrahydrofolate from folic acid in our diet

need to make drugs that outcompete natural substrates

Common structural Modifications

- A common type of structural modification is the addition of heterocycles that carry nitrogen which are more susceptible to being protonated and soluble in water → easier entrance into the body



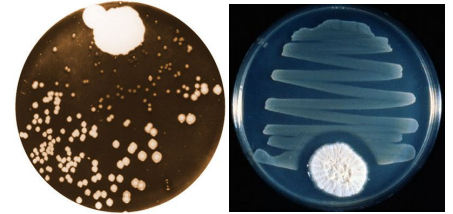
Penicillins

- 80 % of all antibiotics
- More than 30,000 synthesized
- More than 100 sold as drugs

- Most are semi-synthetic (artificial version of the original one)

Alexander Fleming contaminates culture in 1938

- The plate on the left is Fleming's whereas the one on the right is the modern representation of the model
- Fleming was growing two types of bacteria, 1) small colonies and 2) large colonies
- The mold growth (white) prevented bacterial growth from occurring by secreting a substance that was killing one type of bacteria over the other



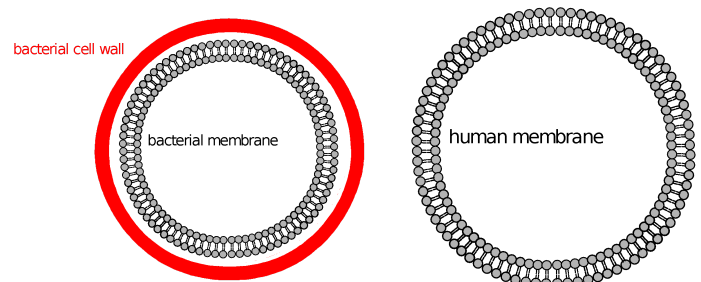
Howard Florey and Ernst Chain isolates penicillin in 1941

Discovery Timeline

- 1929 - Discovered mold by accident
 - Fleming
- 1940 – Active substance isolated, identified, tested
 - Chain & Ernst
- 1940 – First demonstration in animals
 - Chain & Ernst
- 1941 - First human use
 - Chain & Ernst
- 1942 – Production made war priority
 - Development of corn fermentation
 - Simplified extraction process
- 1943 – First widespread use by US Army (available for human use-- usually takes longer)
- 1944 – available to general public

Mechanism of Action

- **Bacteria have a rigid outer layer (bacterial cell wall) which imparts structure and resists osmotic pressure--distinct from human cells**
- The drug (penicillin) interferes with the production using the bacterial cell wall as a target making it very selective
- Human cells not affected because the drug is only specific to the bacterial cell wall

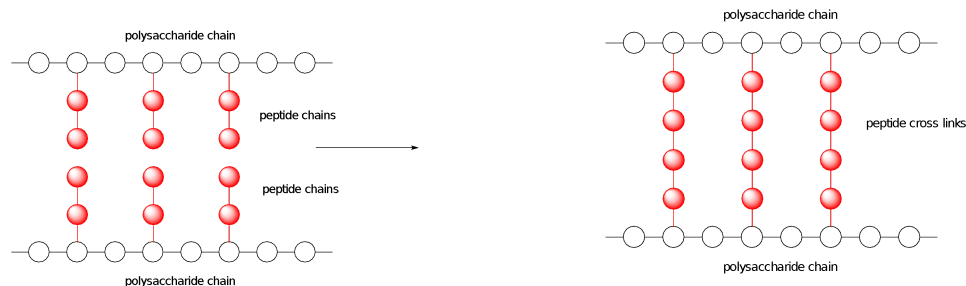


Role of Osmotic Pressure and difference between human cells

- In general to achieve eq in a cell, water moves from area of high [] to low [] = osmotic pressure (move towards area with highest solute concentration)
- In human cells, **the movements of water to area of higher solute [] will most likely cause it to rupture** because of the **fragile property if it was not big in size**(larger size = lower pressure)
- **Bacteria cells are smaller** and would not be able to compensate high osmotic pressure **but the cell wall is constructed in such a way that it is able to resist this issue.**

Cell wall structure

- **Polysaccharide chains linked together** and connected to other other chains **via peptide cross links** (short piece proteins around 10 amino acids) resulting in a **very rigid molecule that is similar to plastic**
- The polymer structure is formed through the linking of small molecules(monomers) = spaghetti like mixture
- The formation of the cell wall includes the enzyme transpeptidase which converts the liquid form of the polysaccharide chain into a solid form following the linking of another chain = **Cross link** → **a method of strengthening the structure**
- Formation of cross link is the last step in cell wall biosynthesis
- **Peptidoglycan structure**

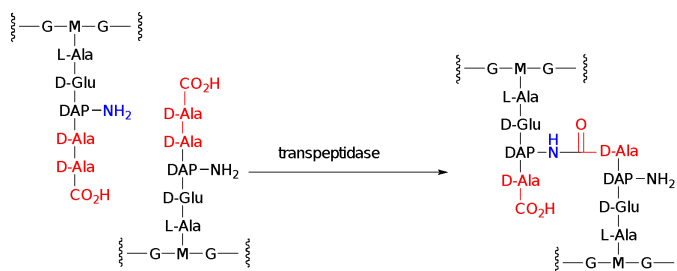


****Note in the first diagram the linking has not yet occurred and the two chains are in liquid form****

Transpeptidase

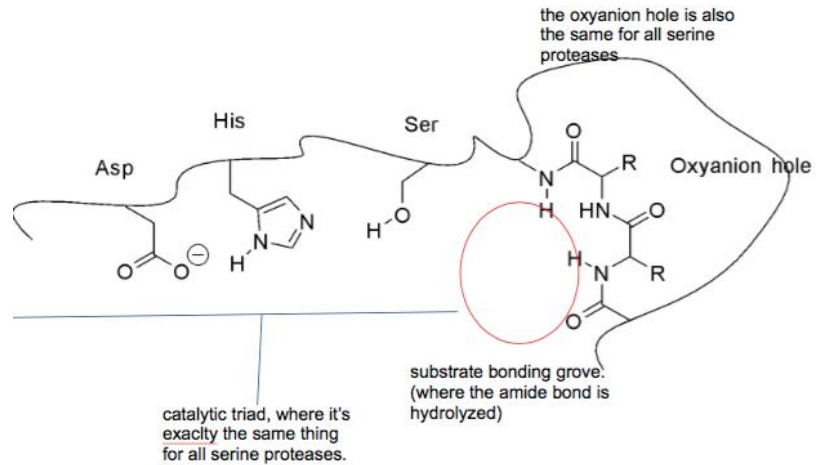
3 main functions

1. Cut the bond between the D-Ala from each chain
2. Form peptide bond between the NH₂ from one chain to the D-Ala of the other chain
3. ???



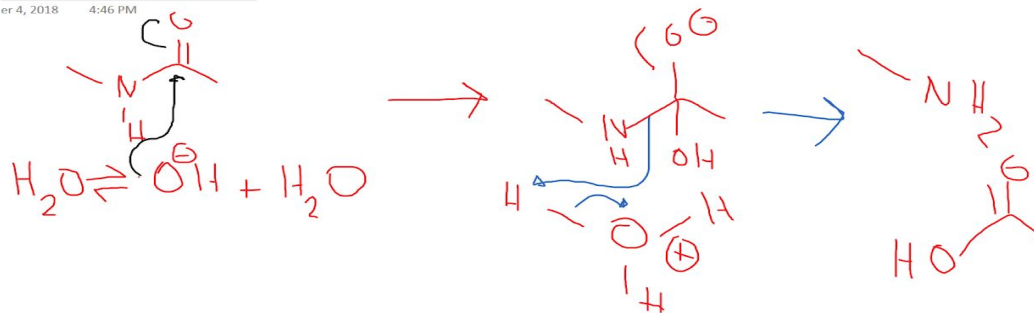
Serine Protease Active Site

- Protease cuts amide bonds (protein into little bits)
- Serine protease uses the amino acid serine as a catalytic portion of the enzyme.
- Serine proteases have a variety of shapes and structures, but all have the same active site shape



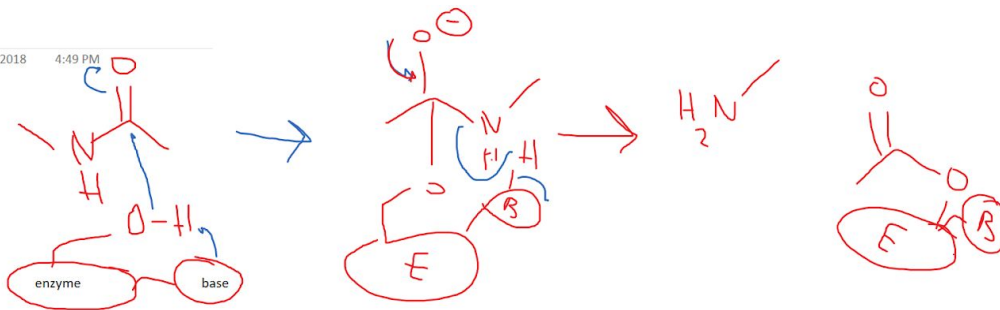
Amide Hydrolysis in Solution

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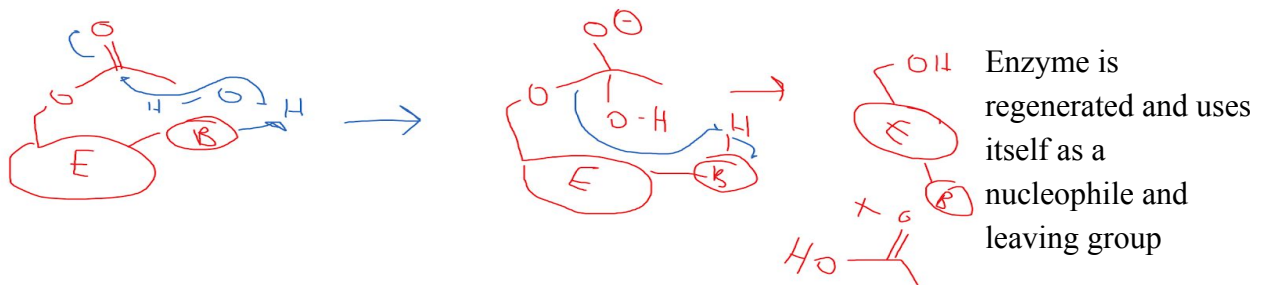
Amide Hydrolysis in Enzyme

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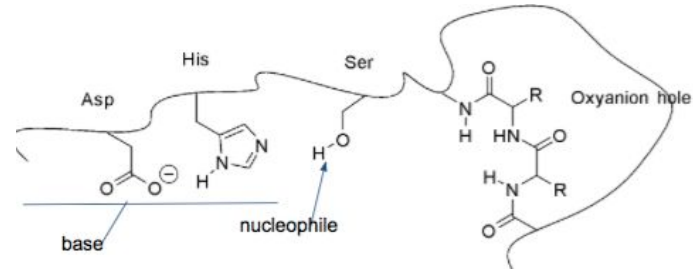
- NH₂ will diffuse out, and water molecules will process the enzyme complex

Role of water in the Reaction



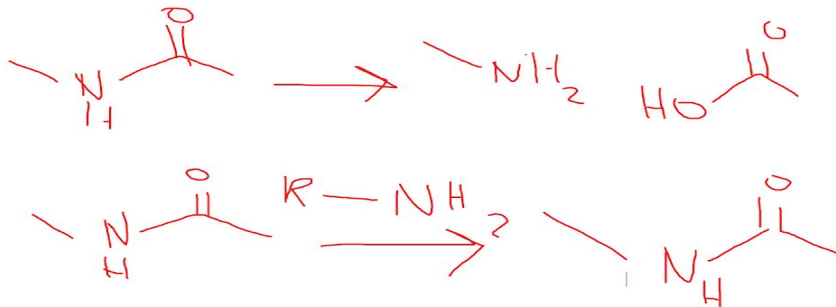
Role of the Oxyanion hole

- Role = stabilize the tetrahedral intermediate
- the tetrahedral has a negatively charged oxygen (which is high energy species).
- When this oxygen is in the active site it does hydrogen bonding with the hydrogens on the NH in the hole so that to stabilize the negative charge (reduce the energy)

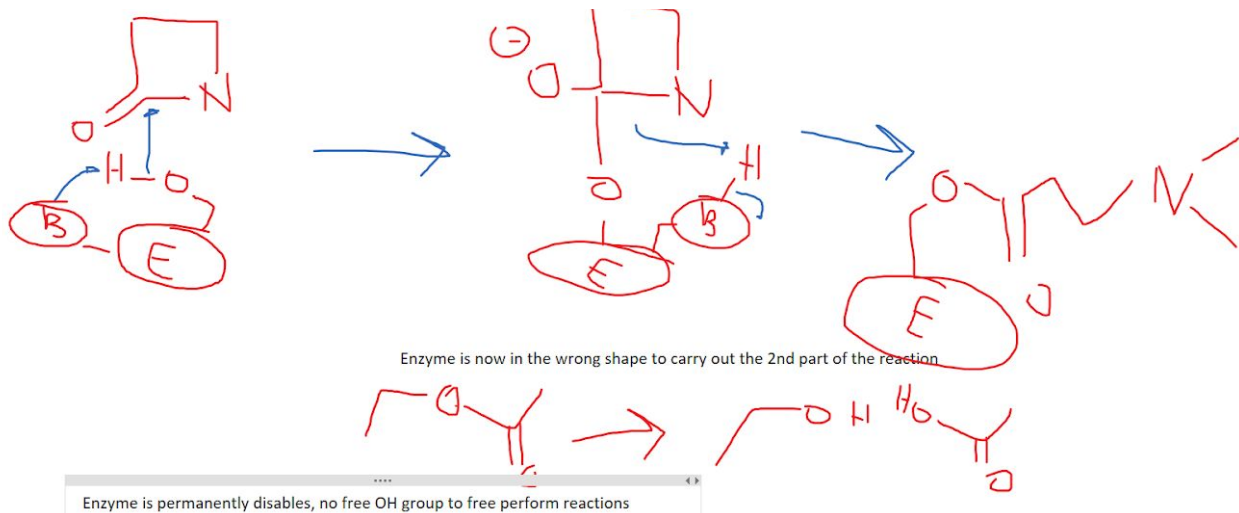


Reactions catalyzed by transpeptidase

- Two steps showing how transpeptidase works



β -lactam opening by enzyme



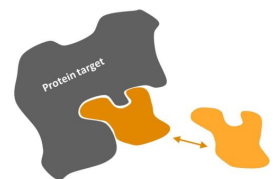
Covalent and irreversible inhibition

- Inhibition is permanent
 - Enzyme unable to hydrolyze the ester formed
- Cell wall formation is prevented

Covalent Irreversible Drugs Can Silence Proteins

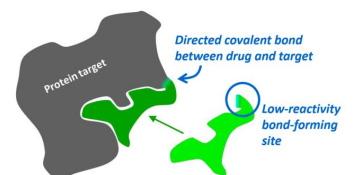
Reversible inhibitors

Traditional reversible drugs are in equilibrium with their target – continually binding, unbinding, & rebinding



Covalent inhibitors

Covalent irreversible drugs bind specifically to a drug target and form a precisely directed, permanent bond with their target



- Cells burst from turgor (internal pressure) when dividing (because of the incomplete cell wall the osmotic pressure changes and cell bursts)

Selectivity

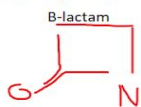
- Human cells do not have cell walls
 - No equivalent enzyme
 - D-Ala-D-Ala linkage is unique in nature
 - Bacteria only
 - Virtually all other amino acids have L configuration
- Very clean drug
 - Non-toxic
 - Very safe
 - Few side effects
 - Major side effect is allergy (~ 0.1 % incidence)

Source of Allergy

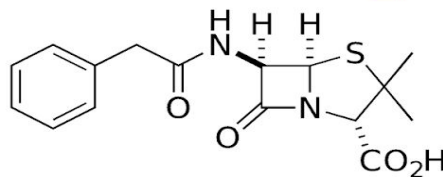
- Penicillin is a good electrophile
- Can acylate host serine proteases or other proteins
- Acylate host (human) serine proteases
 - “tagged” protein recognized by immune system
- Strongest reactions happen second time taking drug (allergy gets stronger as drug intake increases)

Mechanism and Structure

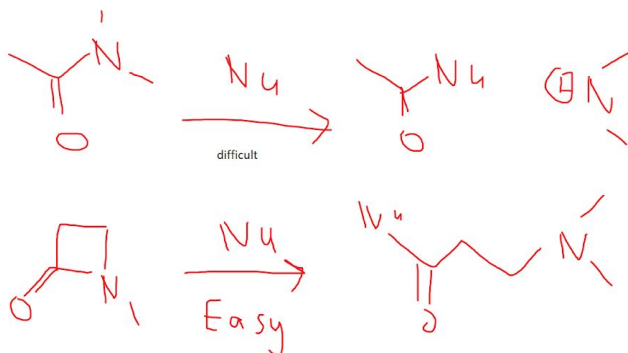
Chemically reactive



The ring structure has 3-d shape similar to D-Ala D:Ala

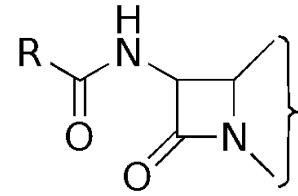


β -lactam carbonyl



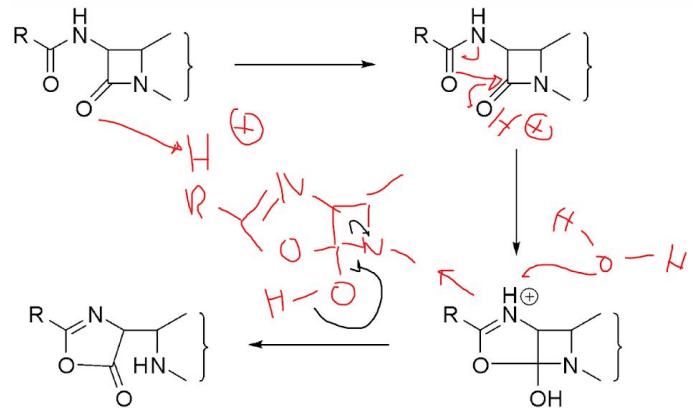
Penicillin G

- Isolated from mold (G is the natural drug)
- Natural form
- Has 4 significant limitations as a drug (as a mold)
 1. Acid sensitivity (IV only)
 2. Resistance
 3. Spectrum of action (works on G + bacteria only)
 4. Bioavailability (only a small % makes it into the body if ingested orally)



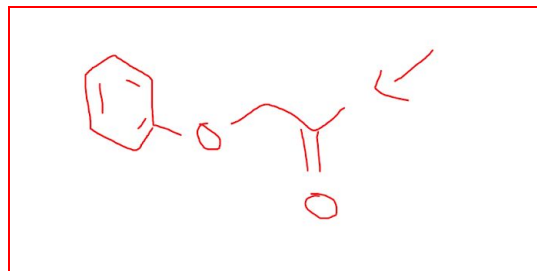
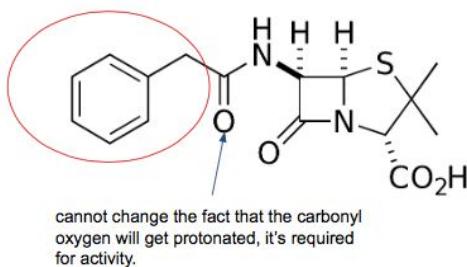
1) Acid Sensitivity

- **Stomach environment is very acid and may be ingested**
- **4 membered ring is the active form and once opened = inactive form**
- H₂O in the body donates a H⁺ to the structure causing opening of the ring
- Drug must be given by i.v. injection

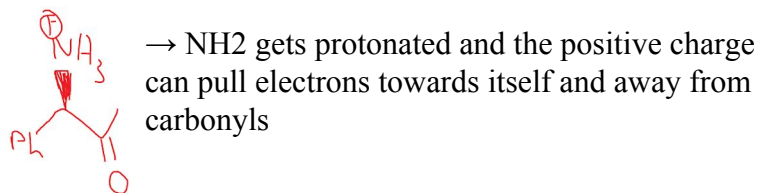
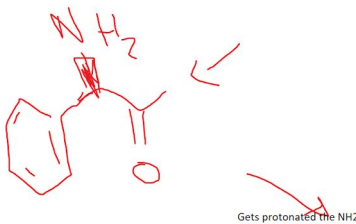


Engineering for Acid Resistance

- In order to make it more resistance to acid, need to reduce the nucleophilicity of the oxygen by making it into an electron withdrawing group
- This can be done by introducing electron withdrawing groups
 - Adding Heteroatoms
 - Charge



Oxygen is more EN and will pull electrons away from the carbonyl

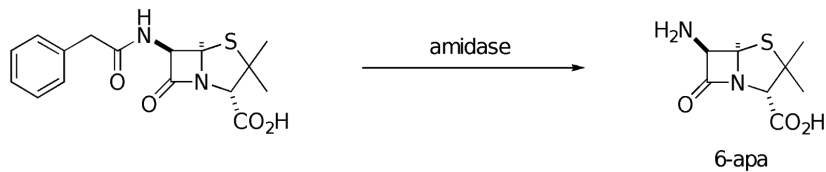


Penicillin Semi-synthesis

- Penicillin “core” is very complex and difficult to synthesize
- Strategy
 - Grow large amounts of the mold
 - obtain the core from biological sources
 - Attach the decorations with the properties you want on it
 - use synthetic transformation to prepare new drug

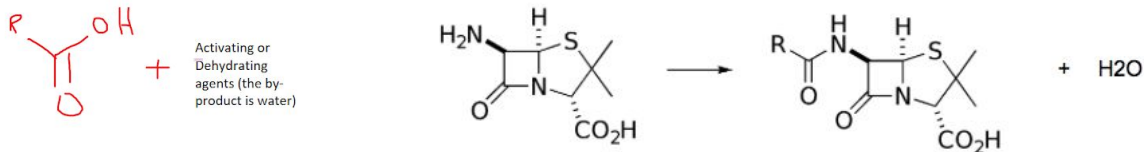
Biochemical preparation of 6-apa

- Grow penicillin mold
- Extract Penicillin G from mold
- Use amidase enzyme from *E. coli* to remove side chain so that the 6-apa can be used to add different decorations through chemical transformations to get the desired property
 - Modern versions have been optimized by genetic modification
- World-wide production over 20, 000 tons per year



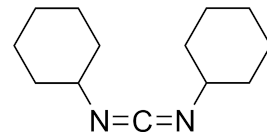
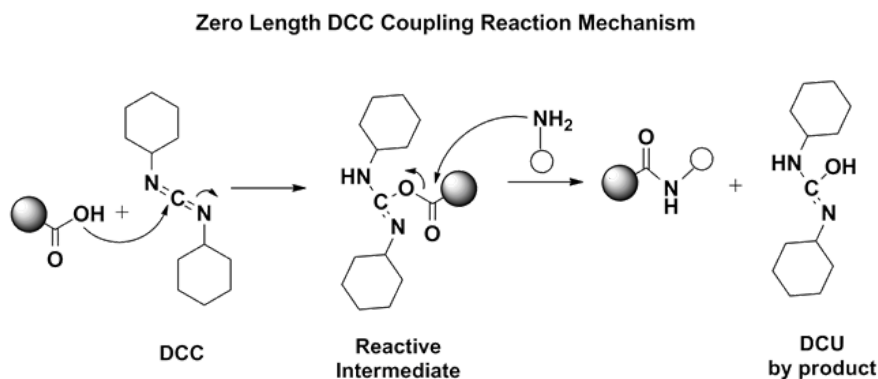
Acylation of 6-apa

- Attach acyl group to NH_2 of 6-APA



Peptide coupling reagents

- DCC was developed to manufacture different types of penicillin

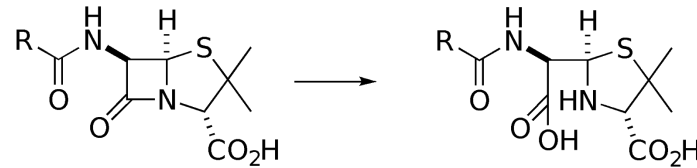


2) Resistance

- Many bacteria develop/evolve immune resistance to penicillin
 - Mutations in transpeptidase
- Bacteria evolve ways to get rid of the drug
 - Actively transport **out** of cell
 - **Destroy the drug before it destroys the bacteria**

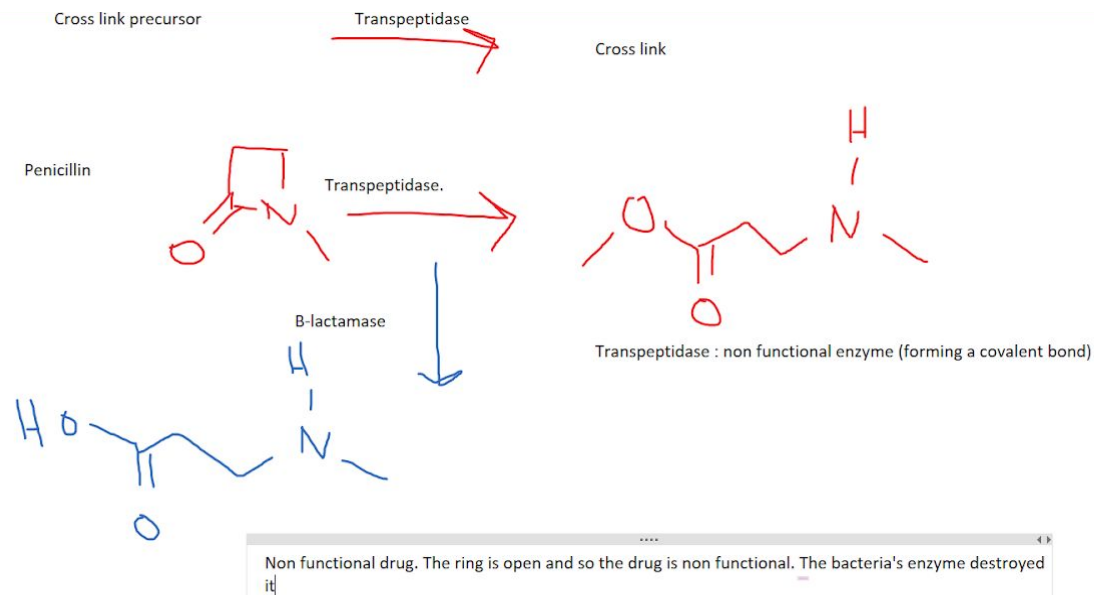
B-lactamase

- B-lactamase are enzymes produced by bacteria provide multi-resistance to β -lactam antibiotics such as penicillins
 - Example penicillinase \rightarrow enzyme evolved to open β -lactam ring of penicillin which is the functional part of the compound \rightarrow once opened cannot function to kill bacteria



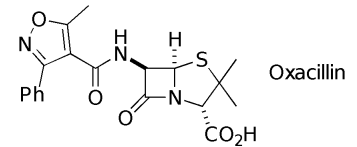
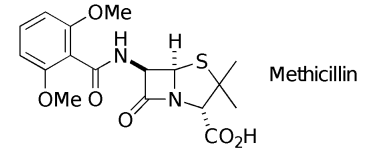
- Bacteria use transpeptidase to make cell walls and penicillinase to protect against penicillin
- First appeared in *Staph. aureus* in 1960's

Antibiotic resistance by B-lactamase



Regain activity using a “shield”

- Large substituted heterocycles
- OMe is located at the ortho position to carbonyl group.
- The double bond at the carbonyl with the double bond in the aromatic group like to be aligned following the addition of shield.
- The group at the ortho position, however, means that the carbonyl group will be at 90 degrees to the aromatic ring, thus changing the structure of the drug
 - Can now fit into the pocket of transpeptidase but NOT into the pocket of B-lactamase (see next slide)



“Shield” provides selectivity

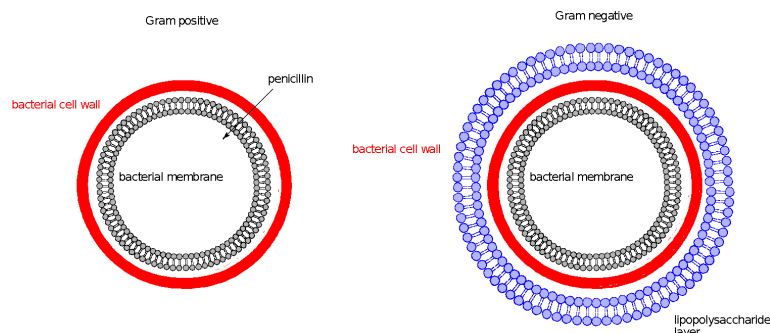
- The shield causes the size of the molecule to increase, transpeptidase is structured in a way where it is able to accommodate for this change (big pocket in active site), B-lactase is not able to do this.
- The drug is not able to fit in the beta lactase and cannot be destroyed by the bacteria
- The enzyme engineered is now able to allow the penicillin to attack bacteria instead of the other way around

3) Spectrum Activity

- Two general types of bacteria
 - Gram positive
 - Gram negative
- Original penicillins were only active against gram positive strains
 - Narrow spectrum of activity (idea that there's lots of bacteria but in principle only half of them are killed by penicillin either gram negative or positive)
- Desirable to have activity against gram negative strains

Gram positive vs Gram Negative

- Selectivity of drug is related to the difference in structure between gram +ve or -ve bacteria
- Gram + have less protective cell wall compared to gram -ve which exposes to drug allows for easy access killing the bacteria
- However gram -ve - has a lipopolysaccharide layer which prevents the drug from getting access to the cell wall and therefore penicillin doesn't work against -ve.

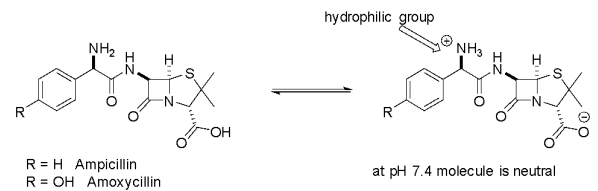
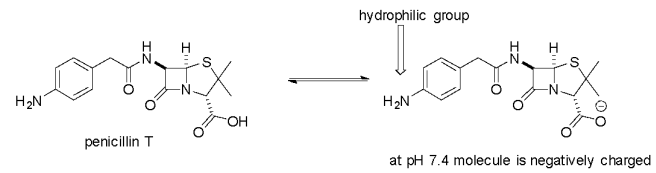
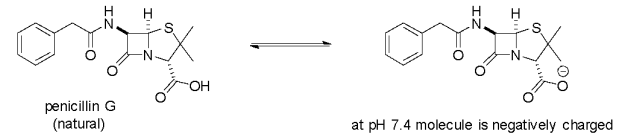
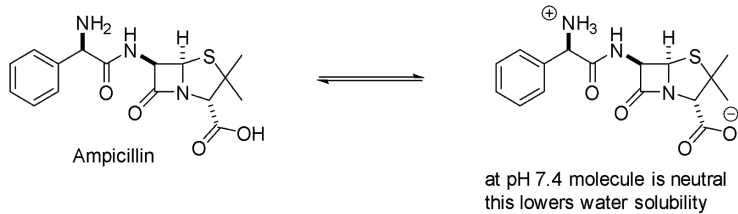


How to fix this Issue

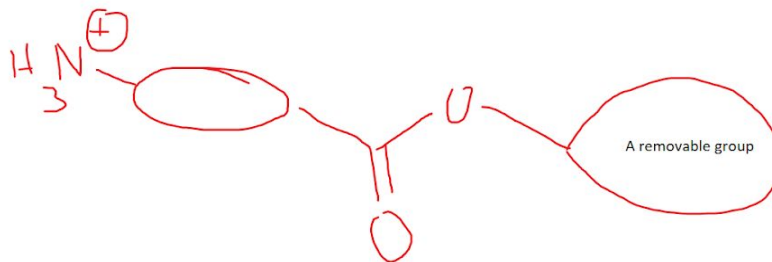
- Adding hydrophilic groups to the left hand side of penicillin allows it to penetrate the membrane and kill bacteria

3) Bioavailability

- Some forms not very water soluble
 - Results in poor bioavailability
 - Need to improve water solubility



Charge and Solubility

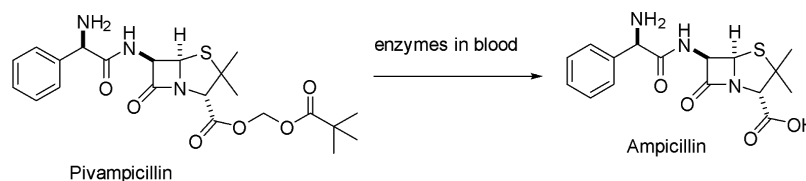


The removable group must be able to leave at some point of the enzyme so that it can react properly

Modify the structure so that on the left hand side is a group that's removable. So overall charge becomes 1 = high solubility.

Prodrug

- Add removable group to a drug to improve absorption into the body
- Once in the blood, this group is removed to deliver the drug in active form



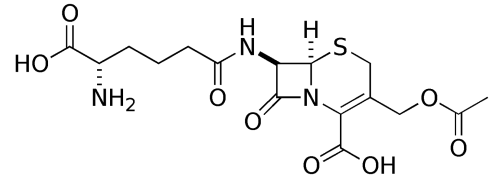
Pivampicillin
Bioavailability 87 - 94%

Ampicillin
Bioavailability 32 - 55%

- Using only ampicillin means only 32 -55 % makes it into the bloodstream
- But if pivampicillin (prodrug) is injected, the bioavailability has increased to 87-94% therefore it has a more effective dosage
- Enzymes in the blood stream move it from inactive form and convert to active form

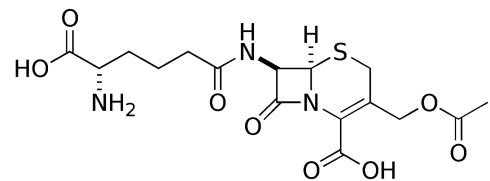
Cephalosporins

- Cephalosporin
 - Discovered 1948
 - Fungus growing in an Italian sewer



Advantages

- Lipophilic – broad spectrum
- [4,6] ring system less reactive than [4,5]
 - Activity against resistant strains
 - Less risk of allergy

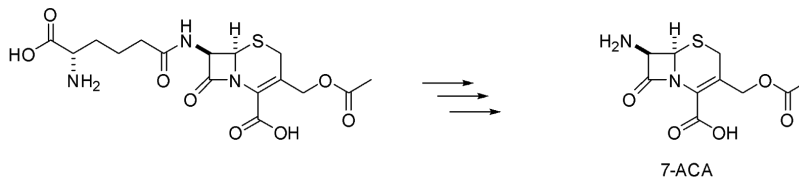


Disadvantages

- Not orally active
- Low potency (larger doses)
- Use Semi-synthesis to improve properties

Semisynthesis

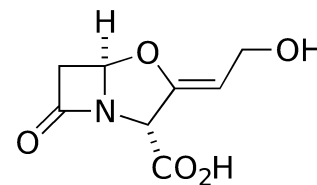
- Cephalosporin is converted into 7-ACA using the same chemistry as with penicillin



- Can modify properties by attaching different groups to the 7-ACA
- For example if bacteria begins to show resistance than addition of bigger group on LHS is a option

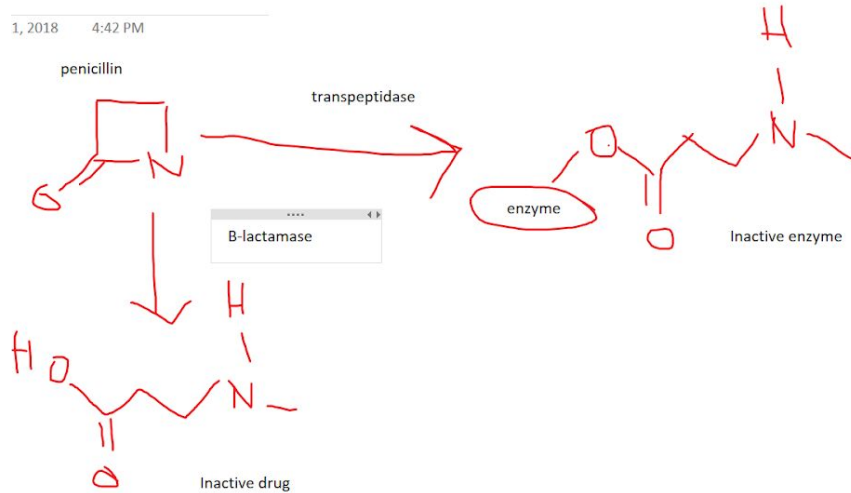
Clavulanic Acid

- Discovered in 1976
 - *Streptomyces clavuligerus*
- Not an antibiotic
 - Does not inhibit transpeptidase
 - Does not kill bacteria

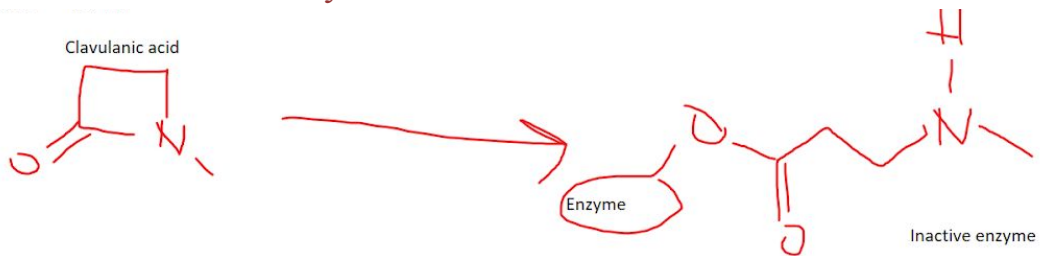


- Useful chemical compound in the case of drugs that are sensitive to beta lactamase

Bacteria uses Beta Lactamase to destroy antibiotics



Clavulanic Acid destroys B-lactamase



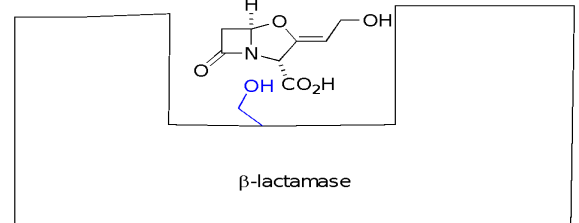
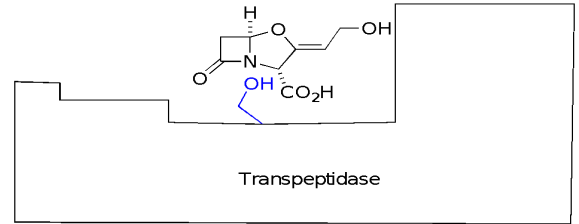
Clavulanic acid deactivates the B-lactamase, and so it allows old drugs that have bacteria resistant to them and restore their activity
Therefore it allows the antibiotic to kill bacteria.

Clinical Use

- Use in combination with β -lactam that is sensitive to β -lactamase
- Clavulanic acid
 - Inhibits β -lactamase
 - “protects” the antibiotic from the resistance enzyme
- β -lactam antibiotic
 - Inhibits transpeptidase
 - Kills bacteria

Selectivity for B-Lactamase

- Adding a big group to the penicillin side chain allows interaction between transpeptidase **but not** with clavulanic acid
- Opposite is true for the clavulanic acid
- There is a weak interaction between transpeptidase and an acid, while strong interactions between lactamase and acid



Drug - Drug Interaction

- One drug changes the bioavailability of another
 - Most common type of drug-drug interaction
 - Clavulanic acid increase the bioavailability of the penicillin (restore its destructive availability)

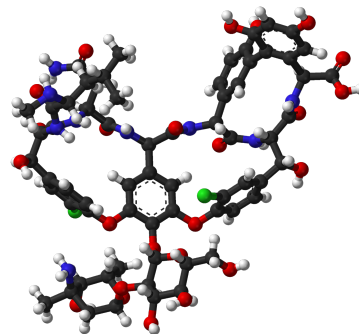
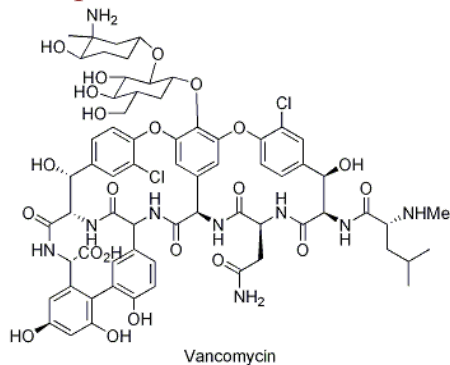
Grapefruit inhibits liver function

- Metabolized by liver
- Metabolite irreversibly binds a glutamine on CYP₄₅₀ \rightarrow CYP₄₅₀ is deactivated
- Bergamottin inhibits CYP in the liver and those drugs that were supposed to be broken down escapes because of this inhibition

Vancomycin

- Streptomyces orientalis
- Glycopeptide drug
 - Sugars (glycol)
 - Short protein (peptide) containing 7 amino acids
- Aromatic rings on peptide are linked forming a bowl-shaped structure

Complex three dimensional structure

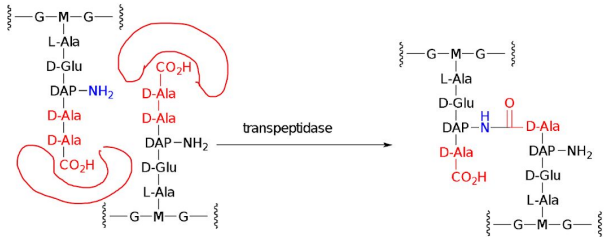


- Has a complex structure where the ortho groups are placed in the aromatic ring in such a

way where the drug is not planar but rather a curved shape.

Prevents cell wall cross linking by novel mechanism

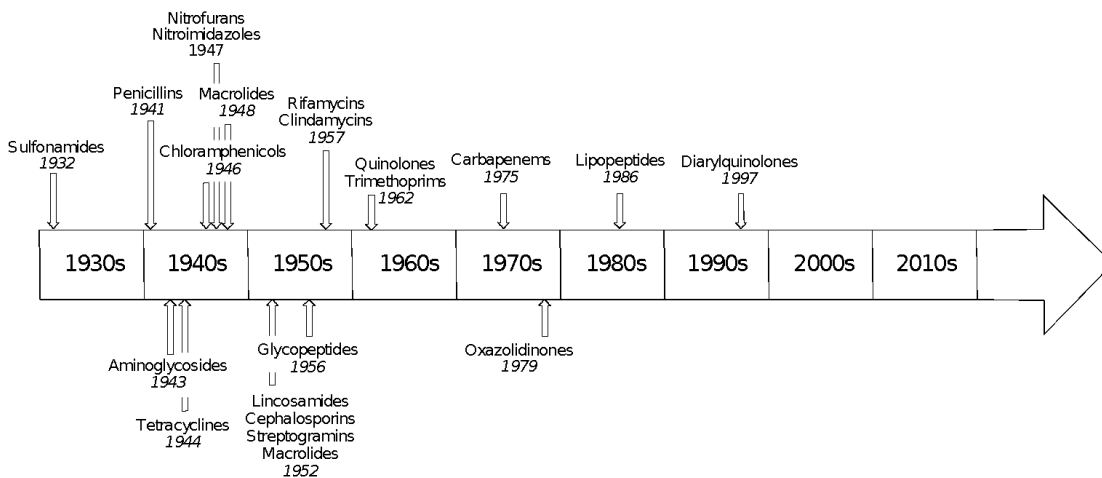
- Binds very strongly to D-Ala-D-Ala tail of peptide chains
- Prevents transpeptidase from binding



Vancomycin not widely used

- Considered a drug of last resort
- Used for infections with bacteria that are resistant to other antibiotics
- Works only on gram positive bacteria
 - Large molecule
 - Not lipophilic enough to cross lipopolysaccharide

Timeline of discovery for antibiotic classes



- Companies shut down the research for antibiotic after the 1990s because it wasn't cost effective.
- All of the other antibiotics are too cheap to be able to compete with.

Modern Antibiotic Research

- Very limited
 - Academic and small company only
- Antibiotics too cheap and readily available
 - New drugs will not make money
 - New drugs not likely to be used by doctors