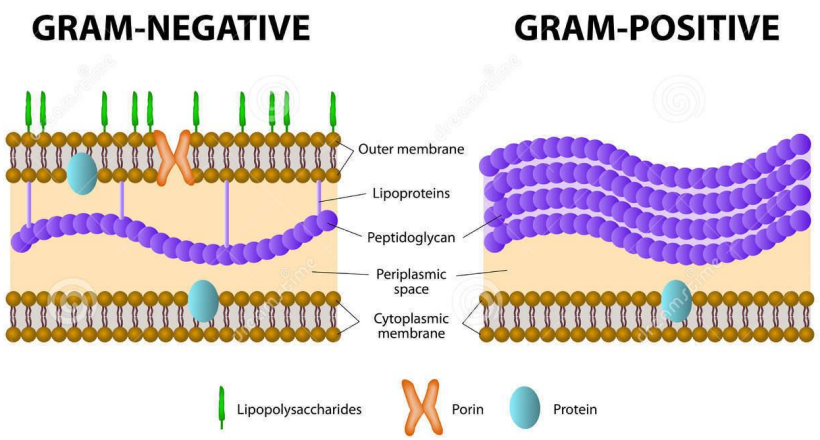
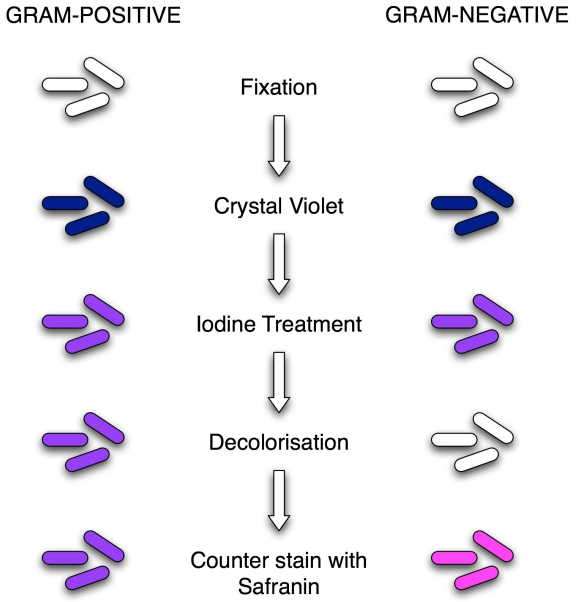
Classification of Bacteria



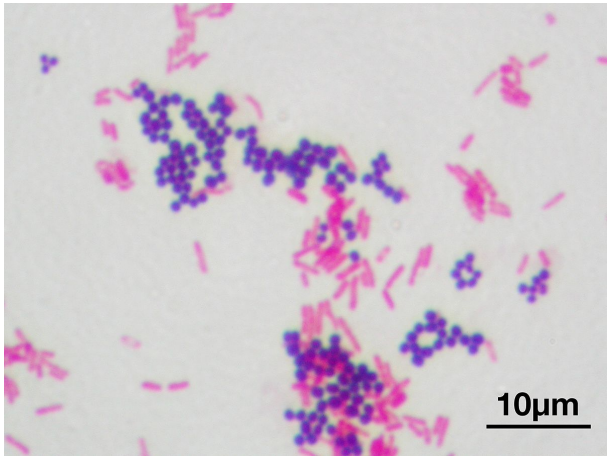


- Gram-positive cells have a thick layer of peptidoglycan in the cell wall that retains the primary stain, crystal violet

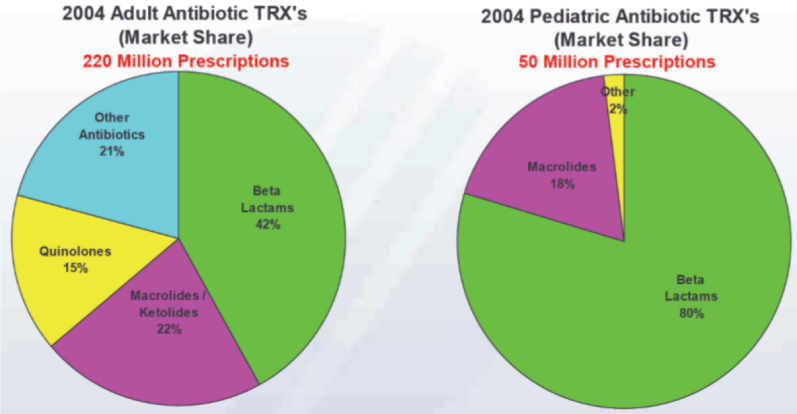
- Gram-negative cells have a thinner peptidoglycan layer that allows the crystal violet to wash out on addition of ethanol

What is covered: A brief history of drug development, common classes of antibiotics and their mechanisms of action, subsequent bacterial drug-resistance mechanisms and how scientists counteract these.

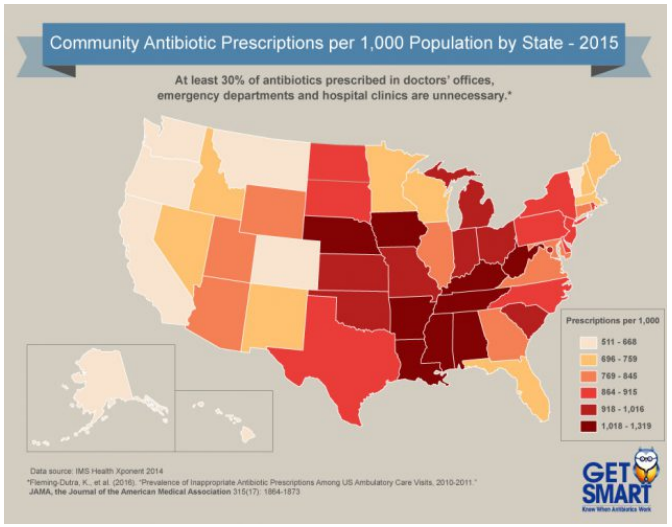
What is not covered: Synthesis of existing antibiotics and exploration of new chemical scaffolds for drug development (next time).



Prescriptions at a Glace



Geographic Prescription Distribution



Mechanisms of Action

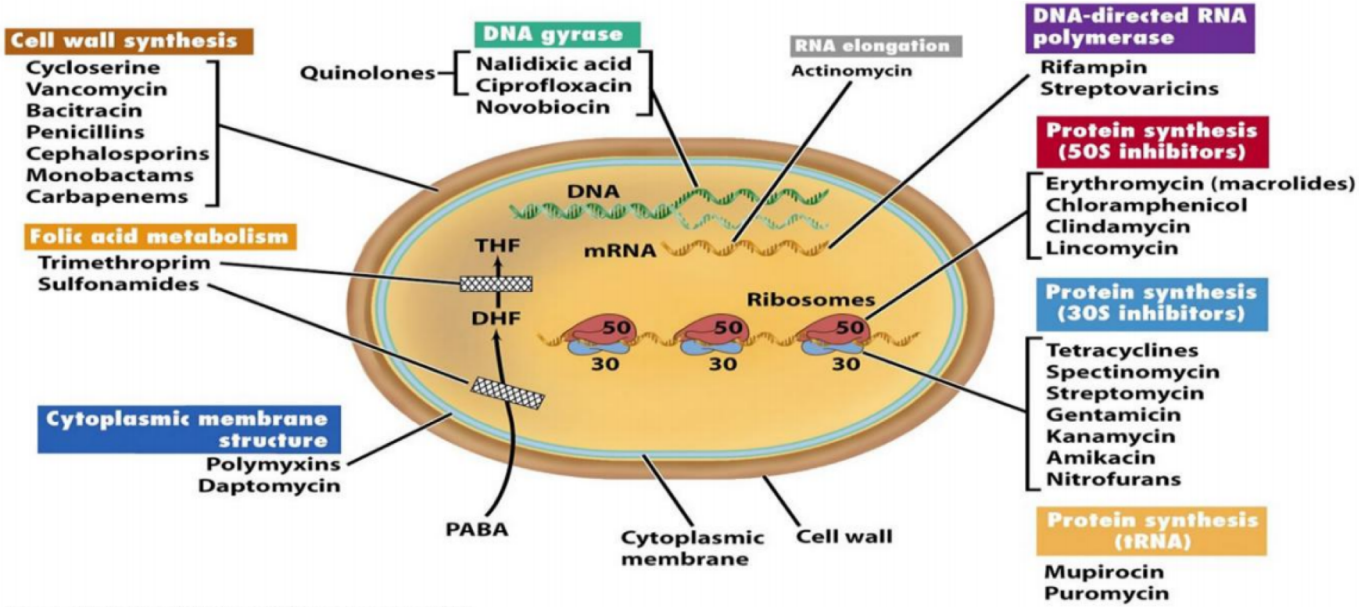


Figure 20-14 Brock Biology of Microorganisms 11/e
© 2006 Pearson Prentice Hall, Inc.

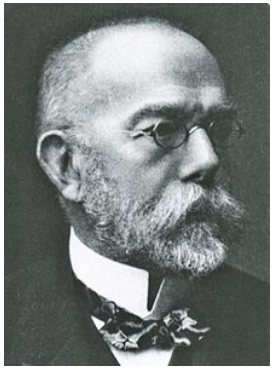
Prelude to Antibiotics: The Germ Theory of Disease

Louis Pasteur (Strasbourg, 1822 - 1895)
"Father of Microbiology"

Best known for the development of pasteurization

Disproved the theory of spontaneous generation

Concluded that microorganisms also infected animals and humans



Robert Koch (Berlin, 1843-1910)
Nobel Prize in Medicine (1905)

Awarded Nobel Prize for work on Tuberculosis

developed "Koch's Postulates"

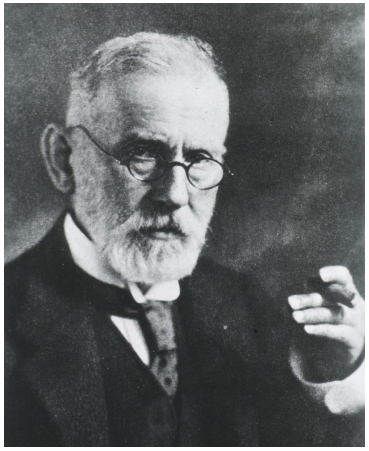
first to link a specific microorganism with a specific disease (*Bacillis anthracis*)

Koch's Postulates (1884-1890)

To establish that a microorganism is the cause of a disease, it must be:

1) Found in all cases of the disease.
2) Isolated from the host and maintained in pure culture.
3) Capable of producing the infection after multiple generations.
4) Recoverable from an experimental host.

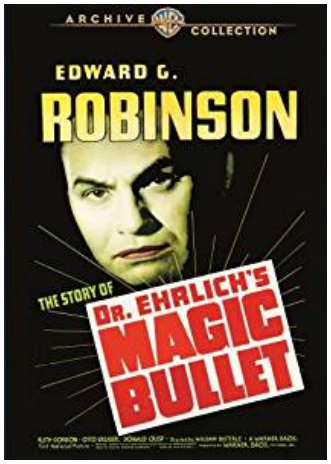
Modern Drug Discovery: Ehrlich and the Zauberkugel (Magic Bullet)



Paul Ehrlich (Frankfurt, 1854 - 1915)
Nobel Prize in Medicine (1908)

Ehrlich hypothesized that just as a bullet can be fired at a target, there could be a way specifically to target invading microbes.

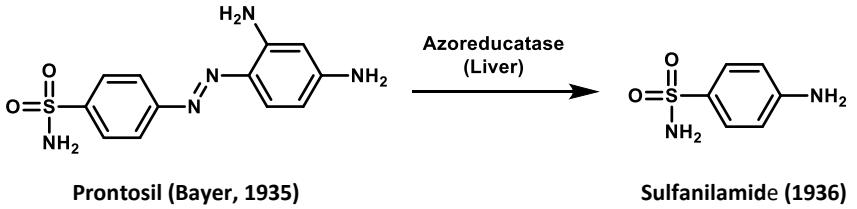
His search for the magic bullet resulted in the development of Arsphenamine (Salvarsam), the first modern treatment for syphilis.



Chem. Ber. **1912**, 45 (1), 756 - 766

The First Class of Antibiotics: Sulfonamides ("Sulfa-drugs")

Gerhard Domagk
Nobel Prize in Medicine (1939)



Prontosil (Bayer, 1935) **Sulfanilamide (1936)**

Sulfa-Drugs revolutionized medicine in the 1930's, however, within a few years bacteria developed resistance to the drugs

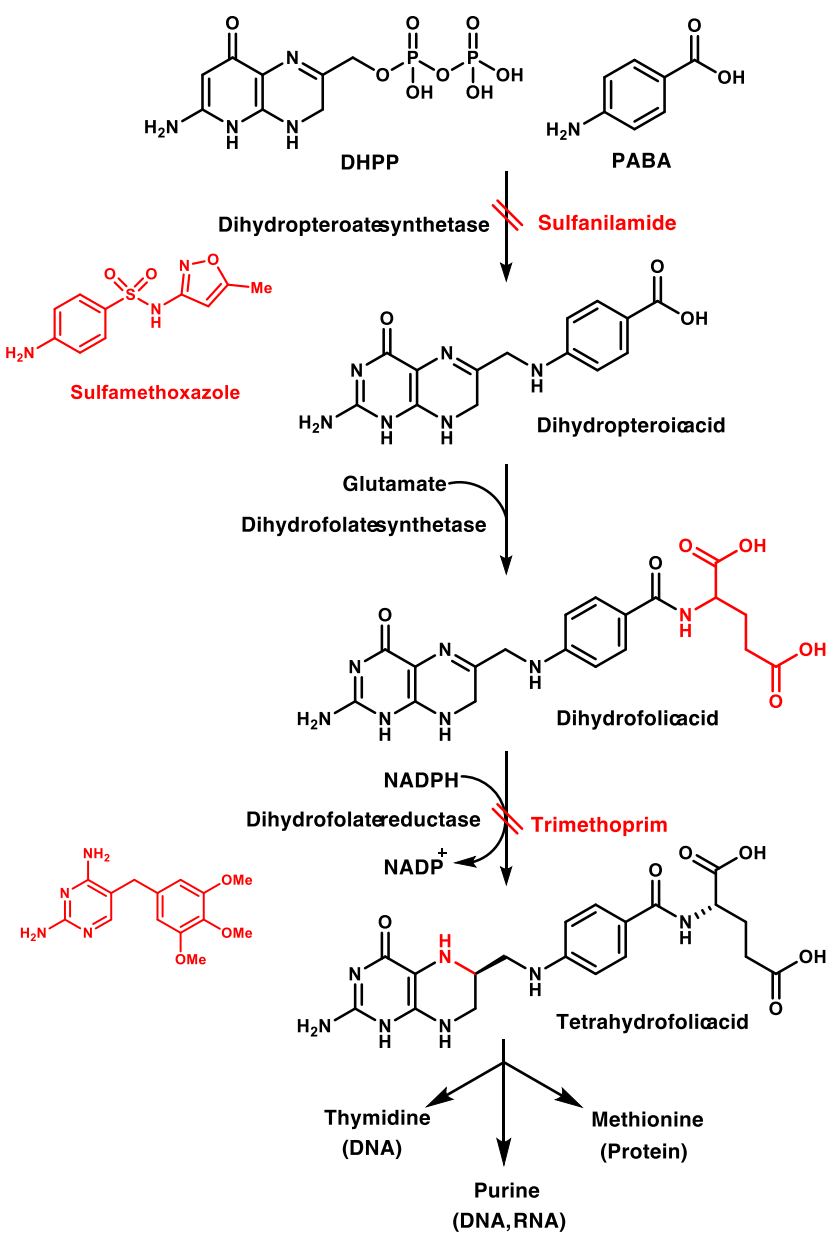
Domagk – allegedly - treated his own daugther with prontosil to fight a severe streptococcal infection and eventually saved her life

He was forced by the Nazi regime to refuse the prize and was arrested by the Gestapo and detained for a week

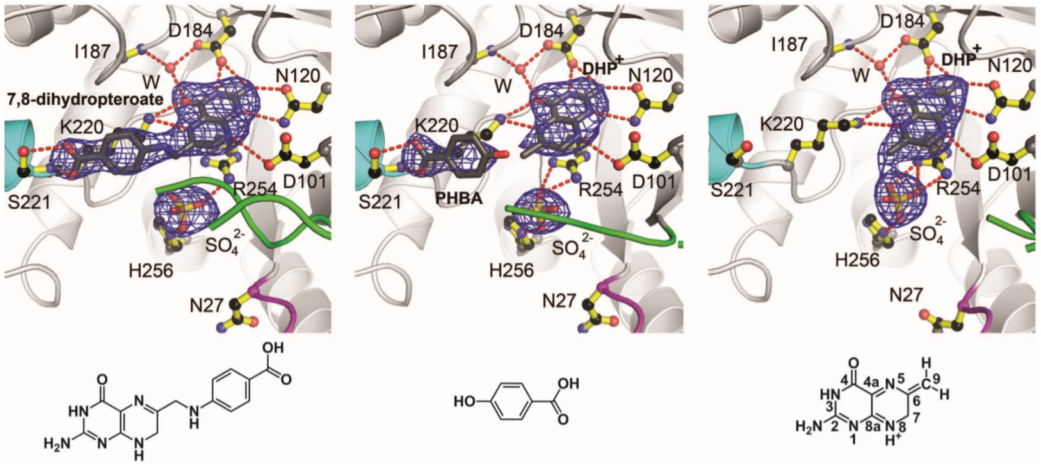
Elixir Sulfanilamide was a raspberry flavored solution of sulfanilamide in diethylene glycol. The product killed more than 100 people in 1937.

J. Antimicrob. Chemother. **1986**, 17 (6), 689-690

Mechanism of Action: Inhibition of Folic Acid Pathway



Crystal Structures of DHPS Active Site:



Common Mechanisms of Resistance:

Sulfonamides:

Increased production of PABA

Transferable genes that encode a drug-resistant DHPS via reduced affinity for sulfonamide (*sul1, sul2, sul3)*

Trimethoprim:

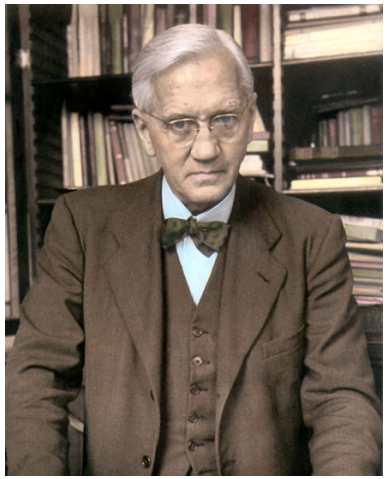
Increased production of dihydrofolate reductase (DHFR)

Mutations in the DHFR structural gene (described in streptococci, staphylococci)

High level of resistance is often derived from exogenous gene uptake that encodes an altered trimethoprim- resistant active site

Science **2012**, 335 (6072), 1110-1114
Drug Resistance Updates **2000**, 3, 155–160

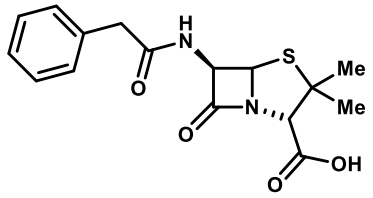
The First Natural Antibiotic: Fleming and β-Lactams



United Kingdom (1881 – 1955)
Nobel Prize in Medicine (1945)

Voted third “greatest Scot” by STV

One sometimes finds, what one is not looking for. When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did.
— Alexander Fleming



Penicillin

β-Lactam drugs are widely used today due to their efficacy and safety

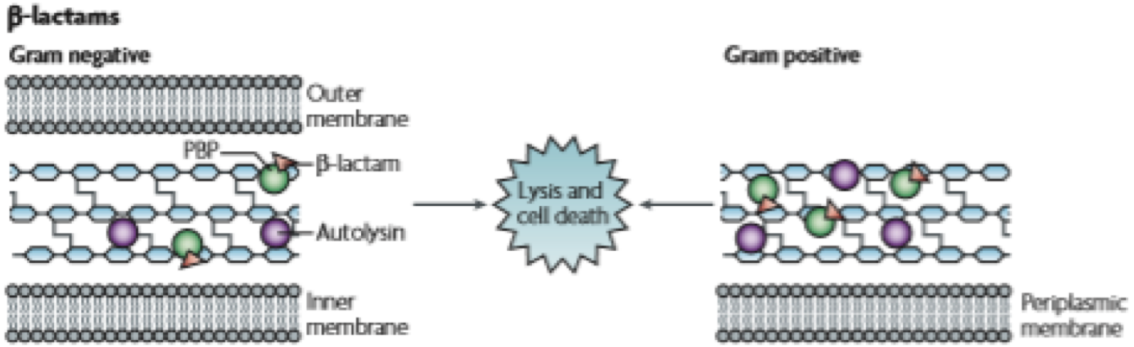
His discovery of penicillin can be attributed to the untidiness of his workspace!

http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html

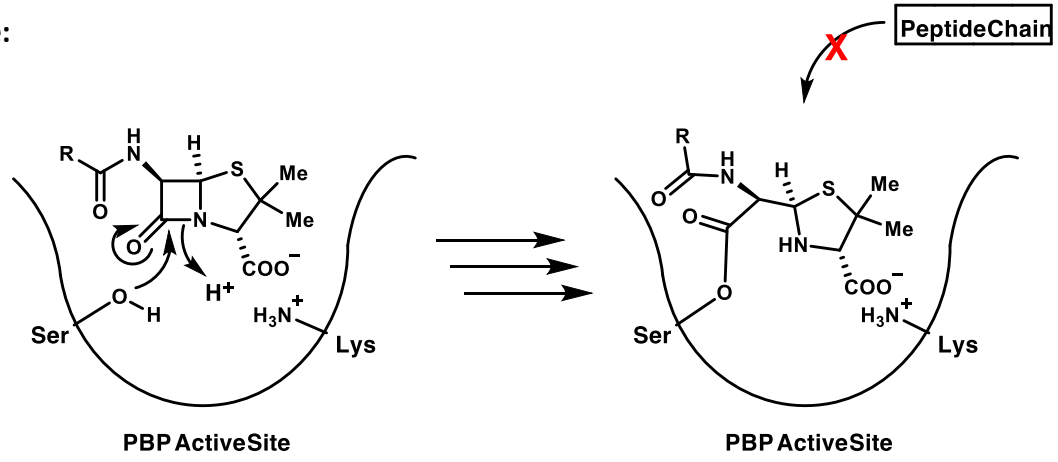
β-Lactam Mechanism of Action: Cell Membrane Disruption

β-Lactam antibiotics interfere with peptidoglycan synthesis by inhibiting enzymes called pencillin-binding proteins (PBP's)

PBP's are responsible for cross-linking peptidoglycan chains to form the bacteria's cell wall



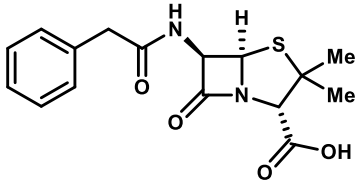
In The Active Site:



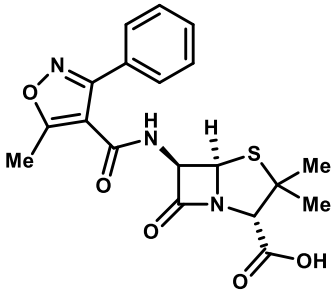
Chem. Rev. **2005**, 105 (2), 390-393
Infectious Diseases **2017**, 4th ed.

Examples of β-Lactam Structure Diversification

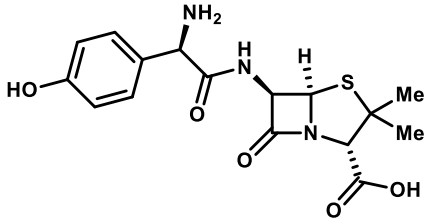
Penams



Benzylpenicillin
Narrow Spectrum
β-Lactamase sensitive

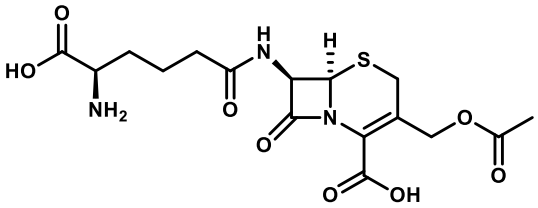


Oxacilin
Narrow Spectrum
β-Lactamase resistant

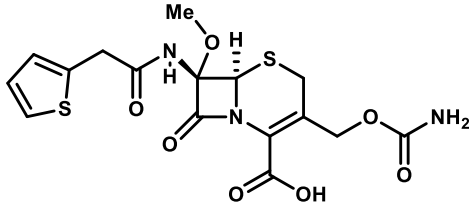


Omaxacilin
Narrow Spectrum
β-Lactamase resistant

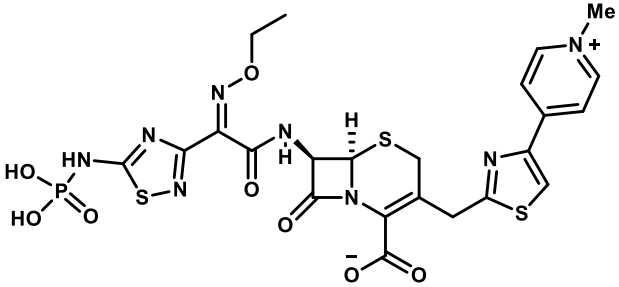
Cephems (Five Generations)



Cephalosporin C
Moderate Spectrum
1st Generation

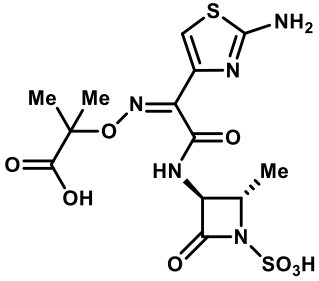


Cefoxitin
Moderate Spectrum
2nd Generation



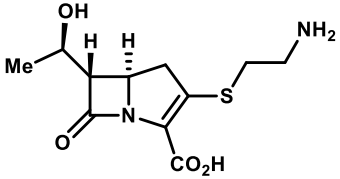
Ceftaroline Fomasil
Moderate Spectrum
5th Generation

Monobactams



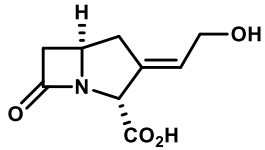
Aztreonam
only aerobic Gram-negative bacteria

Carbapenems

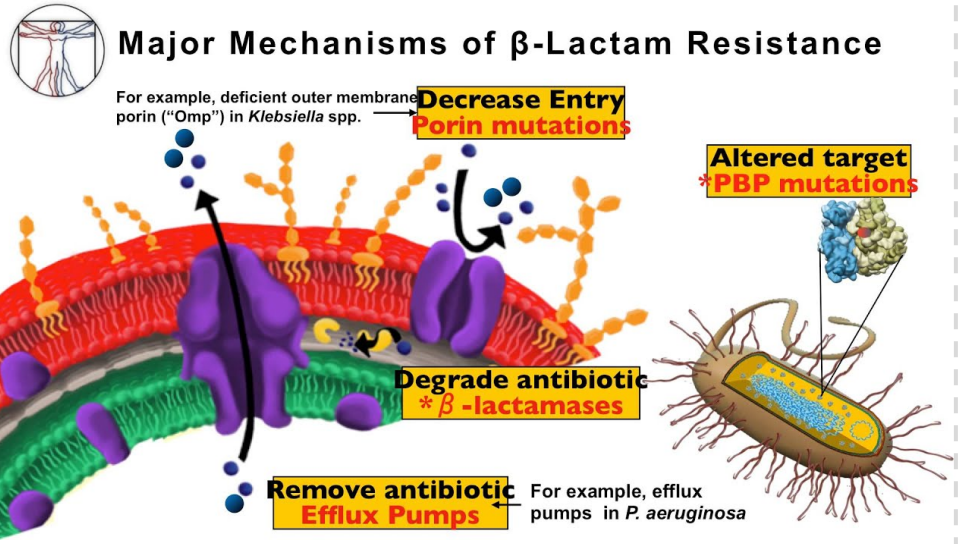


Thienamycin
Broad Spectrum
Extremely potent

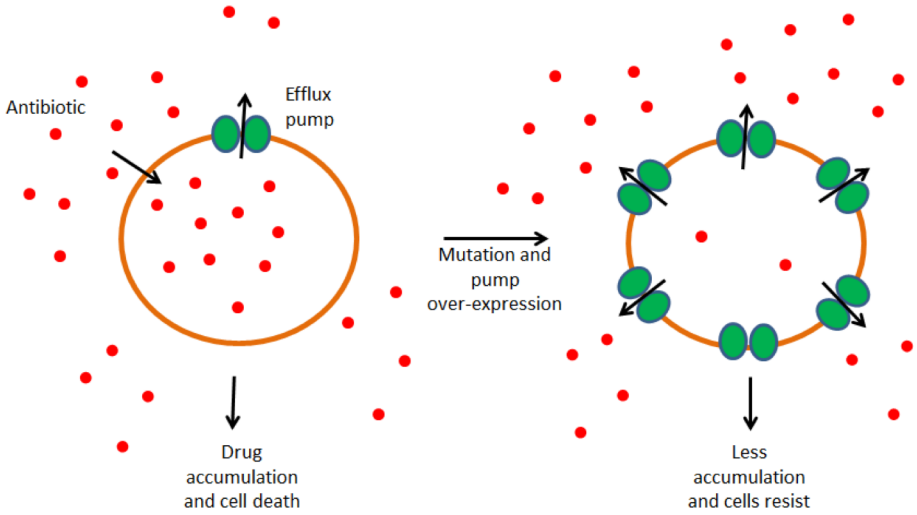
β-Lactamase Inhibitors



Clavulanic Acid



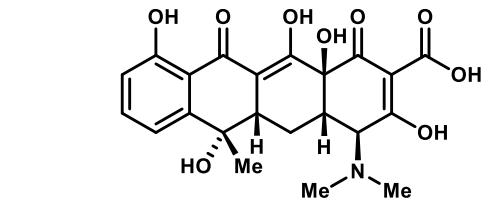
Over-expression of Efflux Pumps



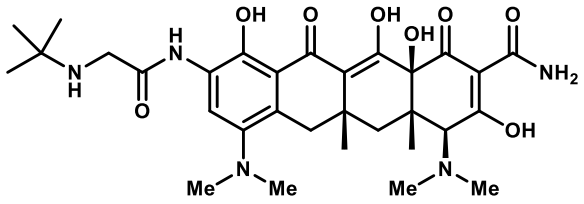
Efflux Pump

Individual pump recognizes a broad scope of substrates. This is due to selectivity based on physical properties and not chemical structure.

Efflux systems function via an energy-dependent mechanism to pump out undesired substances.



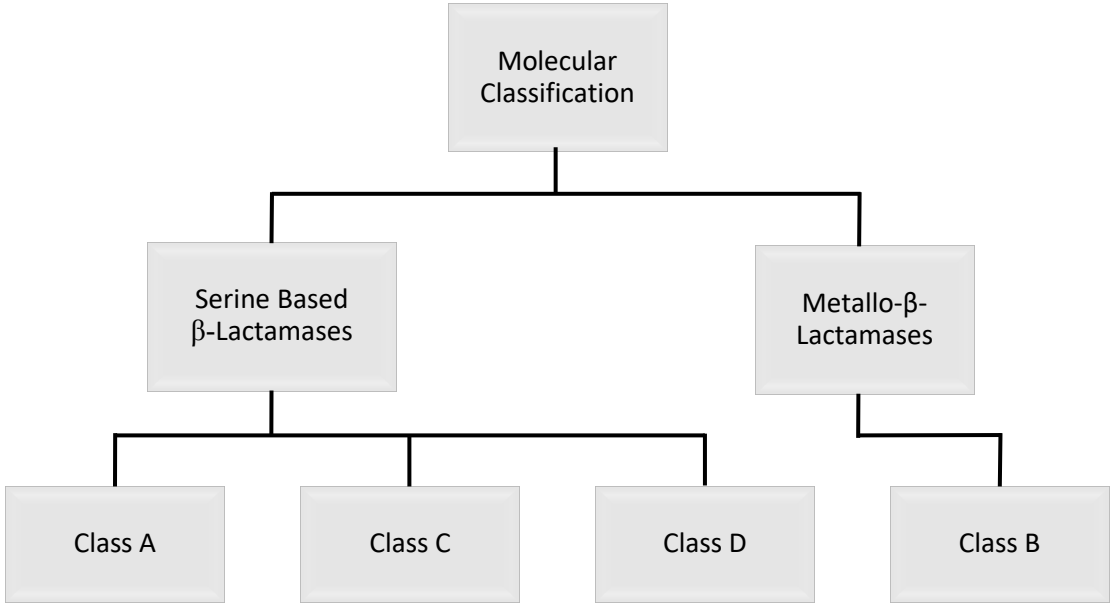
Tetracycline



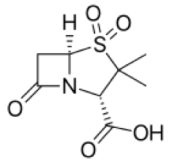
Tigecycline (glycycycline)

Less susceptible to Efflux

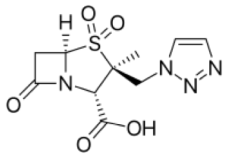
β-Lactamase



β-Lactamase Inhibitors



Sulbactam



Tazobactam

Inhibitors have no antibacterial properties, but they act as a sacrificial β-Lactams to suppress the β-Lactamase effects.

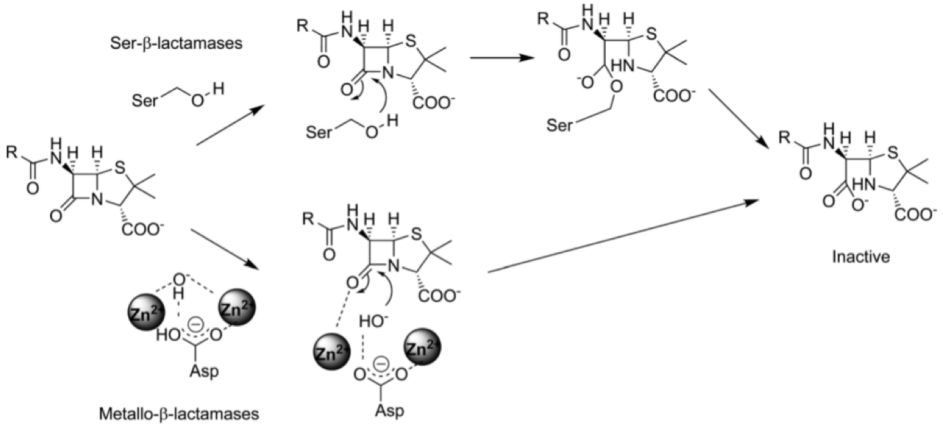
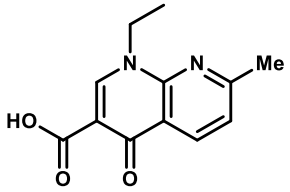


Fig. 4 General mechanism of Ser and metallo-β-lactamases.

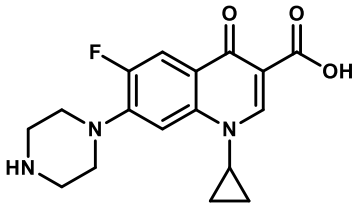
Chem. Commun. **2011**, 47, 4055–4061

Quinolones: DNA Gyrase Inhibition



Nalidixic Acid
Although not a quinoline, it is considered the predecessor of all quinolone drugs

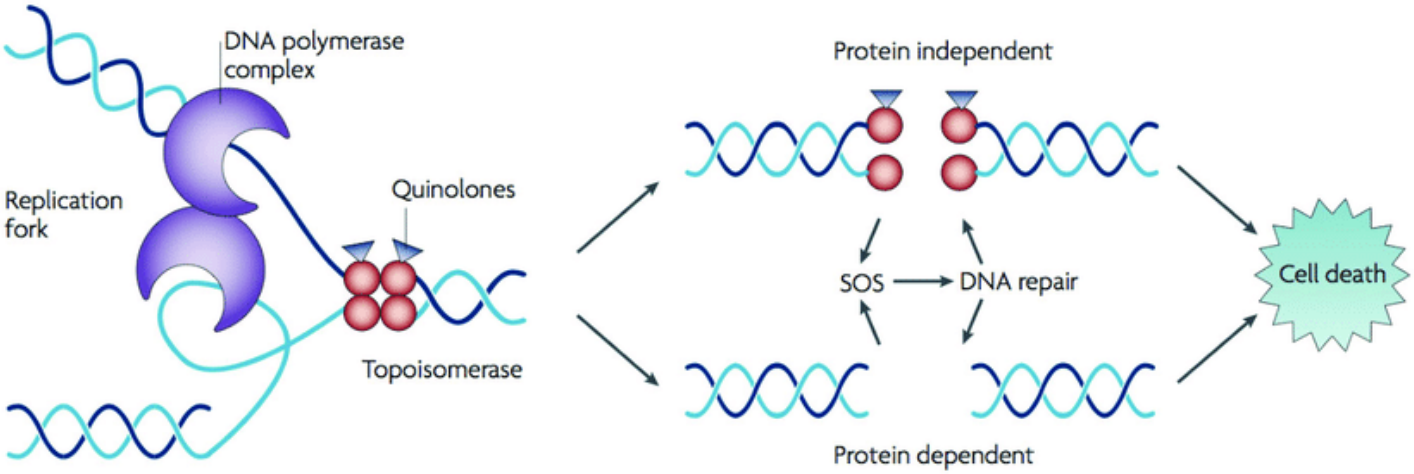
Most quinolones in clinical use belong to the second generation "fluoroquinolones"



Ciprofloxacin
2nd Generation

Today's action also follows a May 12, 2016, drug safety communication advising that fluoroquinolones should be reserved for these conditions only when there are no other options available due to potentially permanent, disabling side effects occurring together." -FDA (2016)

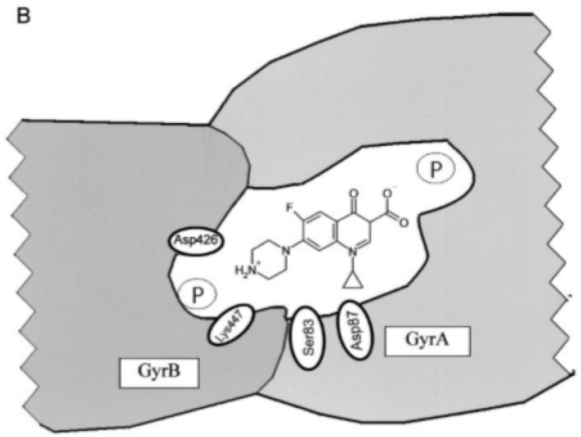
Quinolones: Inhibition Mechanism

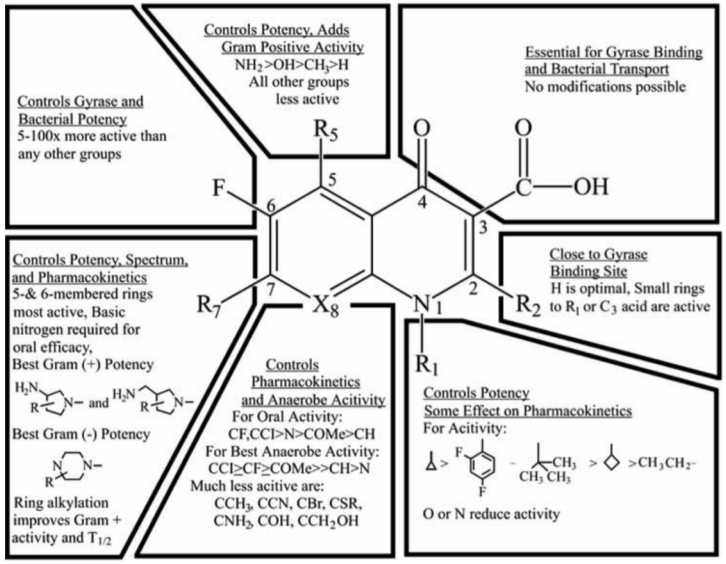


Quinolones binds to Topoisomerase (II and/or IV) and stabilizes the complex of topoisomerase and the recently cleaved DNA strands. This results in double-stranded DNA breaks.

Nat. Rev. Microbiol. **2010**, 8, 423-435
Infectious Diseases **2017**, 4th ed.

Quinolones: Mechanism of Action





Resistance to Quinolones

Topoisomerase mutations leading to decreased affinity for quinolones

Mutations leading to resistance are within discrete regions of the enzyme, called quinolone resistance determining regions (QRDRs)

Mutations typically involve replacing a hydroxyl group with a bulky hydrophobic residue which leads to an altered active site geometry

Active Efflux and Decreased Uptake

Changes in the outer membrane of gram-negative bacteria can lead to decreased uptake of the fluoroquinones

Active efflux quinones have been reported in various bacteria.

Target Protection

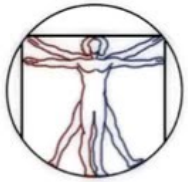
Small pentapeptide repeat proteins (Qnr proteins) bind to the topoisomerase and protect the enzyme from quinolone

This was the first example of plasmid-encoded transferable resistance mechanism against quinolones

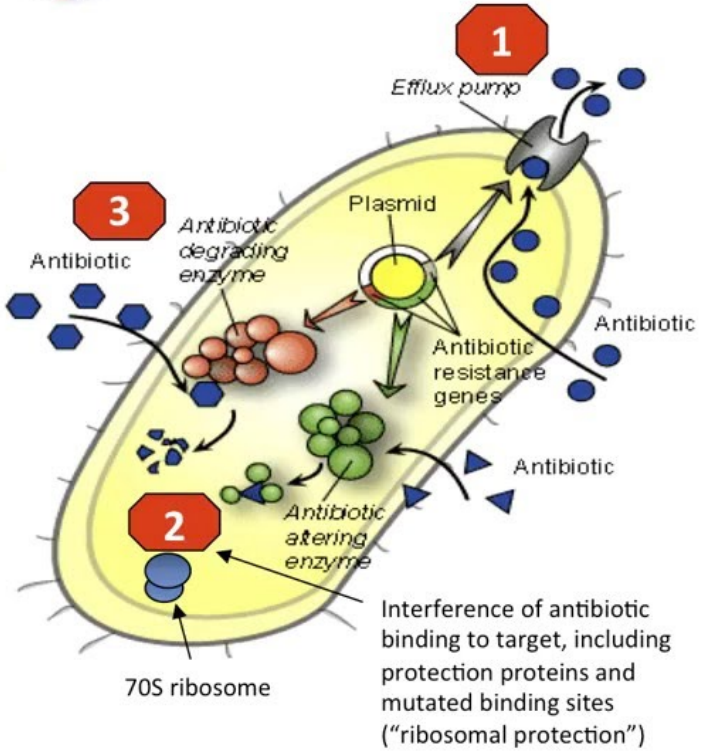
Drug Inactivation

Enzyme AAC(6')-lb-cr has evolved to acetylate secondary piperazinyl amines – decreasing the efficacy of certain quinolones

Infectious Diseases **2017**, 4th ed.
Antimicrob. Agents Chemother. **2007**, 51(1), 1–22



Major Bacterial Resistance Mechanisms to Protein Synthesis Inhibitors



3 Major Mechanisms:

1 Impaired influx or increased efflux

- E.g., Tet(AE) and Tet(K) efflux pumps (tetracyclines)
- E.g., altered active transporters (aminoglycosides)

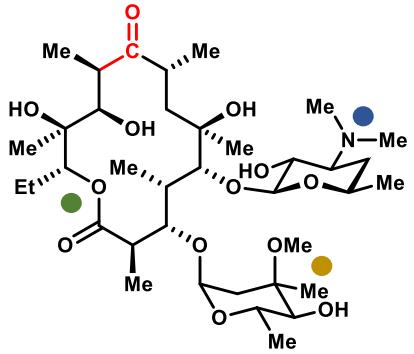
2 "Ribosomal protection"

- E.g., Tet(M) ribosomal protection protein (tetracyclines)
- E.g., "MLS_B resistance" vs. macrolides, lincosamides, and streptogramin B

3 Enzymatic inactivation (degradation, alteration)

- E.g., bacterial esterases (macrolides)
- E.g., acetyl-, phospho-, and adenylyltransferases (aminoglycosides)

Macrolides:



Erythromycin
Biosynthetic

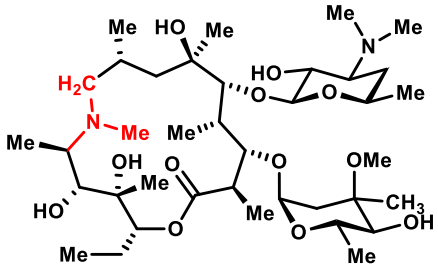
Reactive Structural Components:

Lactone ring and Desosamine sugar

Reactive groups of the desosamine and the lactone mediate all the hydrogen-bond interactions of erythromycin and its 2nd gen. derivatives clarithromycin and roxithromycin with the peptidyl transferase cavity.

Cladinose sugar

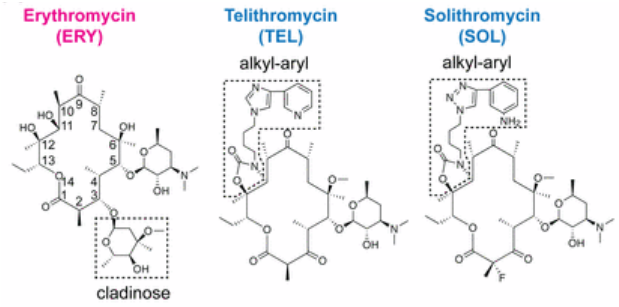
No significant hydrogen-bonding interactions. 4-''OH bond is disposable.

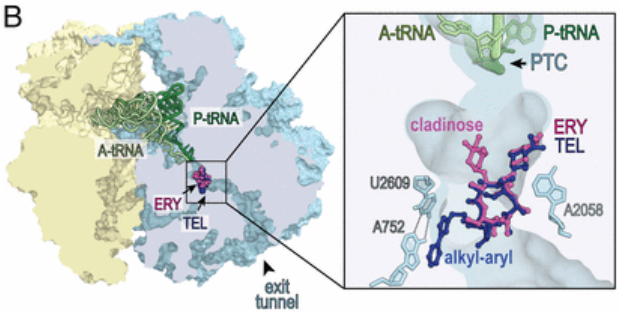


Azithromycin
Semi-Synthetic

Mechanism of Action:

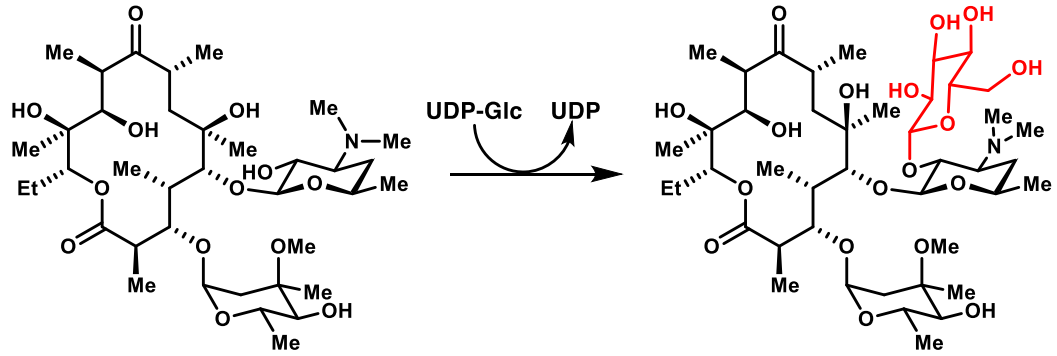
Binds to 50S ribosomal subunit blocking the tunnel that channels nascent peptides from the peptidyl transferase center





Inhibition Mechanism: Drug Deactivation

Glycosylation:

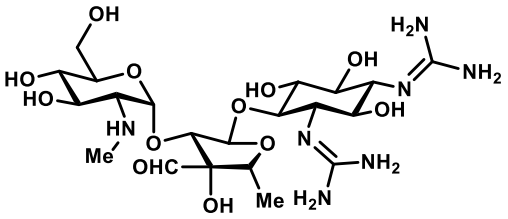


Methylase methylates adenine 2058 residue, which is present in the active site for macrolides

Additional Mechanisms: Active Efflux and Target Modification

Proc Natl Acad Sci USA. **2017**, 114(52), 13673-13678
Infectious Diseases **2017**, 4th ed.

Aminoglycosides

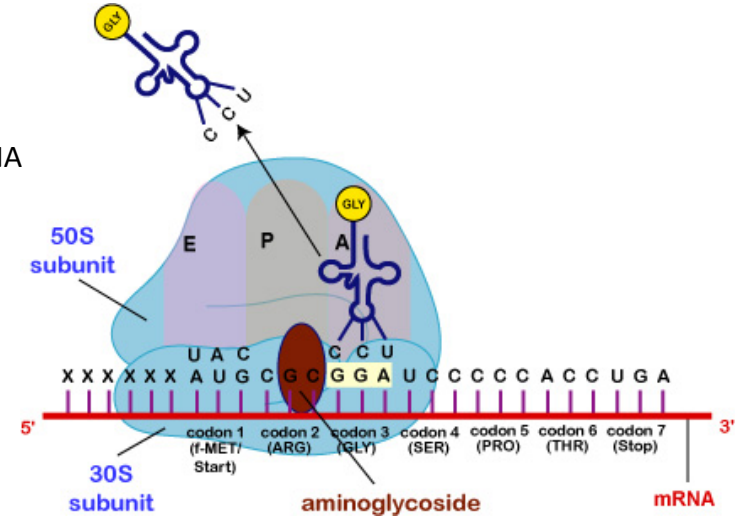


Streptomycin

Mechanism of Action:

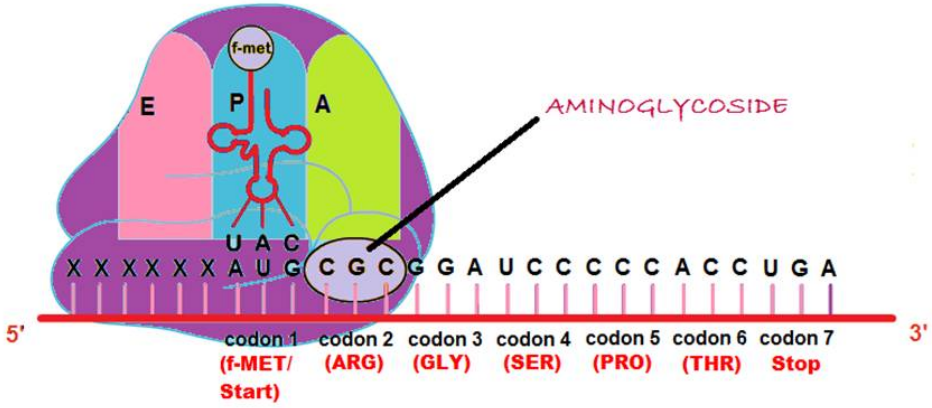
Interference with the translocation of tRNA from the A-Site to the P-Site

Protein sequence does not get elongated to the full sequence, leading to incomplete protein expression



Mechanism of Action:

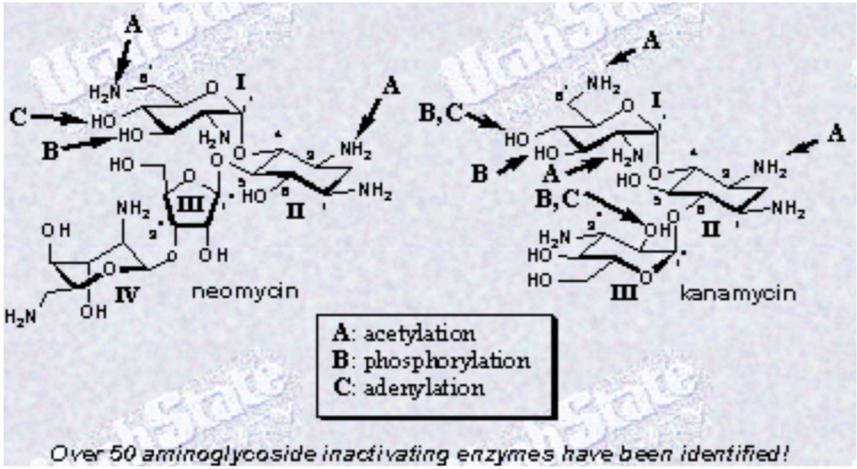
Interference with Translation by causing a Misreading of the Codons along the mRNA yielding improper protein expression



Binds irreversibly!

Inhibition Mechanism:

Aminoglycoside-modifying enzymes (AME's)



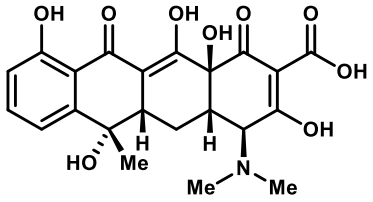
Additional Mechanisms:

Methyltransferase methylation of ribosome amino acids increase steric hinderance

Active Efflux and Target Modification

Pharmaceutical Sciences **2011**; 2(12), WMC002744

Tetracyclines



Tetracycline

Resistance Mechanism: Ribosomal target protection

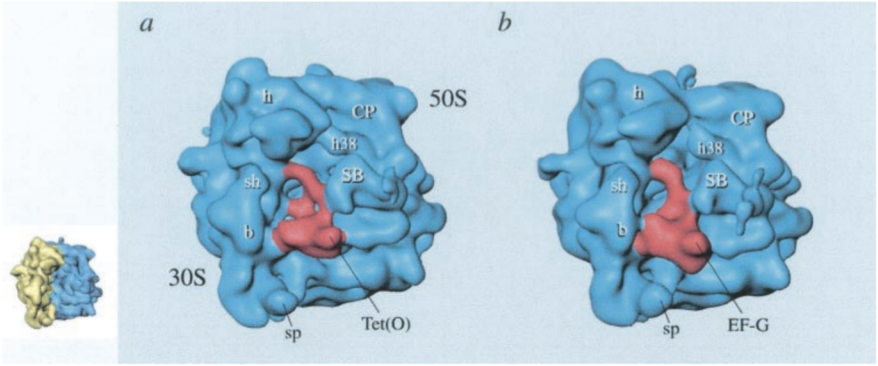
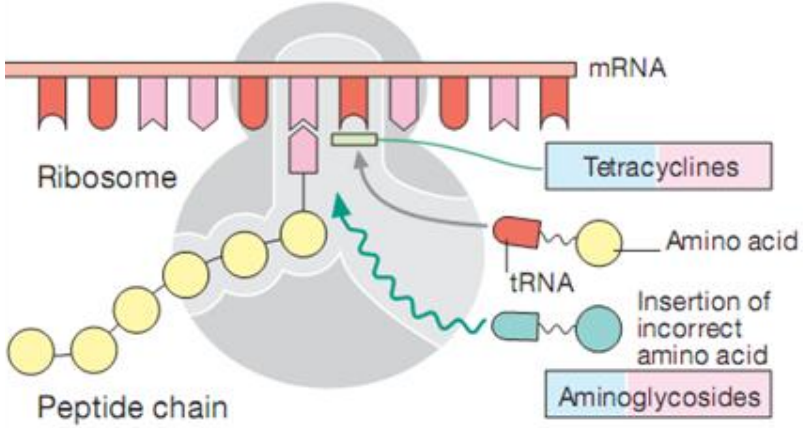


FIG. 3. Cryo-EM reconstructions of Tet(O)–GTPγS (45) (a) and EF-G–GMPPCP (1) (b) ribosomal complexes. The ribosome (blue density) is shown in the same orientation as seen in the insert on the left, where the 30S subunit is colored yellow and the 50S subunit is colored blue. Tet(O) and EF-G are shown as red densities. Ribosomal landmarks are indicated. h, head; CP, central protuberance; h38, helix 38 of 23S rRNA; SB, stalk base; sp, spur; sh, shoulder; b, body. This figure has been reproduced from reference 45 with permission of the publisher.

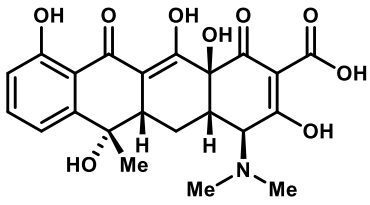
Antimicrob. Agents Chemother. **2003**, 47(12), 3675-3681

Mechanism of Action:

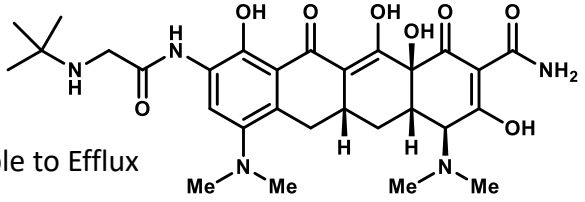
Competes with tRNA for active site on mRNA and the peptide chain fails to grow – bacteriostatic effect.



Resistance Mechanism: Active Efflux



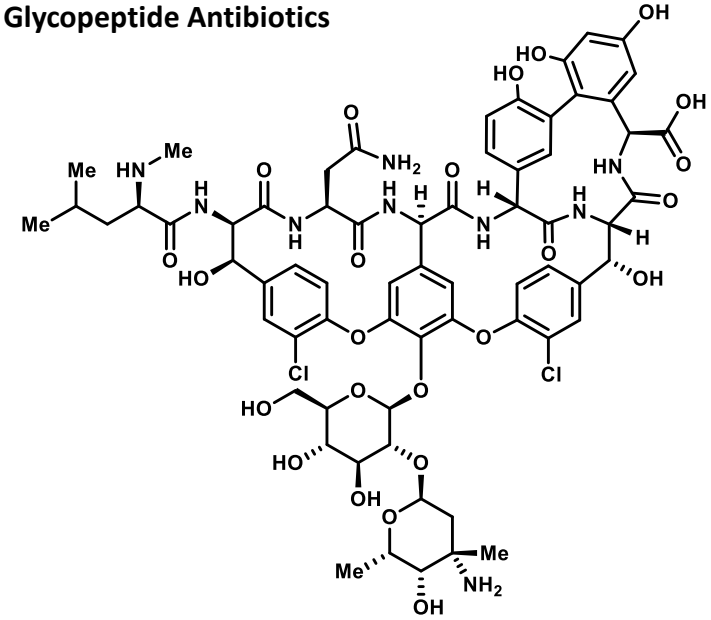
Tetracycline



Tigecycline (glycycycline)

Less susceptible to Efflux

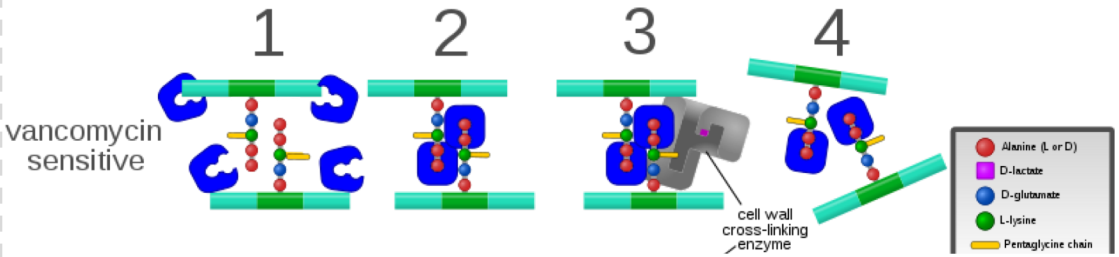
Glycopeptide Antibiotics



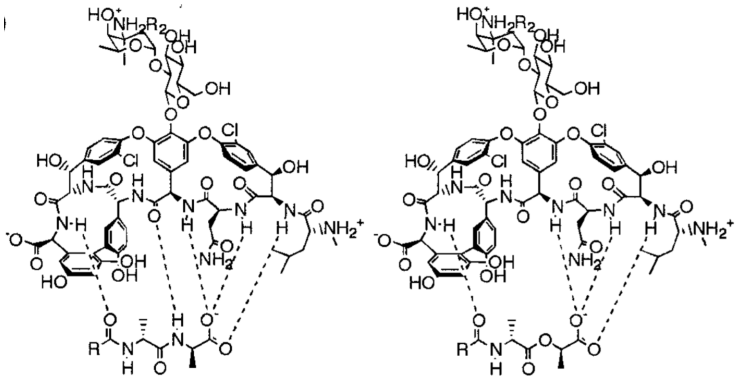
Vancomycin

Mechanism of Action:

Inhibition of cell wall synthesis in Gram-positive bacteria by binding to d-alanyl-D-alanine moieties of *N*-acetylmuramic acid (NAM) and *N*-acetylglucosamine (NAG) – which form the backbone strands of the bacterial cell wall.

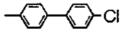


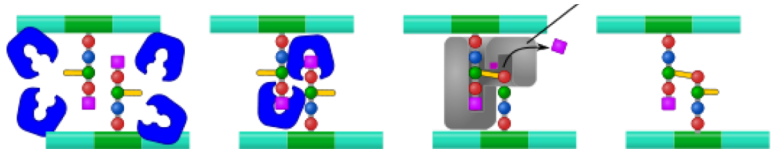
Resistance Mechanism: Target Modification



D-Ala-D-Ala D-Ala-D-Lac

Vancomycin R_2 = H

Chlorobiphenyl Vancomycin R_2= 



Mutation of the D-Ala-D-Ala moieties to D-Ala-D-Lac inhibits the glycopeptide's ability to bind to the moieties. The result is restored function of the cell wall cross-linking enzyme.

Microbiol Rev. **1987**, 51(3), 341–350
J. Am. Chem. Soc. **1999**, 121, 10004-10011