مکانیسم‌های مولکولی مقاومت به آنتی‌بیوتیک‌ها در باکتری‌ها

مقاومت آنتی بیوتیکی (ذاتی یا اکتسابی)، فقط زمان کوتاهی پس از کشف آنتی بیوتیک توسط الکساندر فلمینگ (۱۹۲۸)، باکتری های مقاوم به آنتی بیوتیک پدیدار شدند.

اصطلاح مقاومت آنتی بیوتیکی: ابداع یک سپر دفاعی توسط میکروب که آنتی بیوتیک را بی اثر می کند.

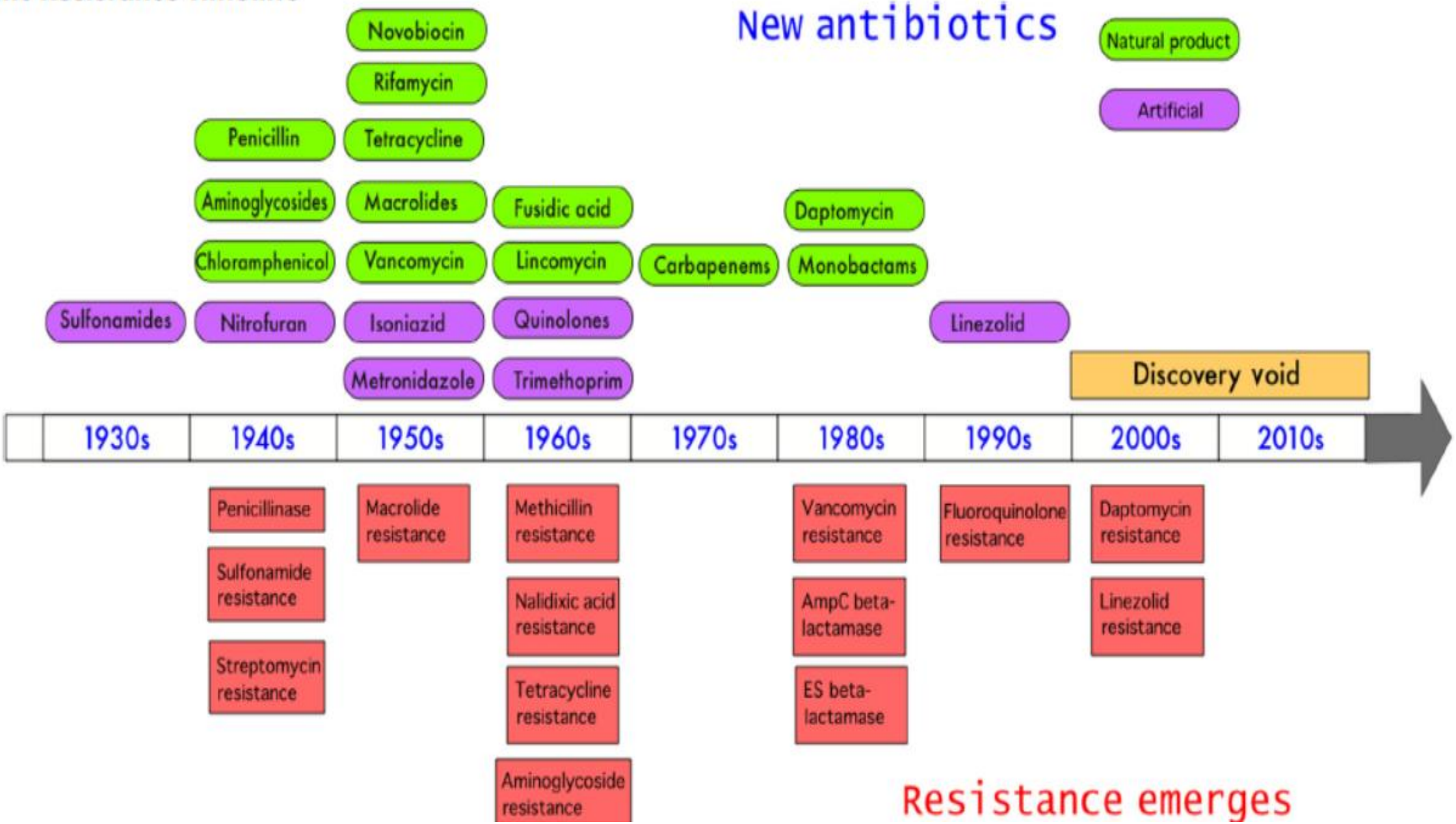
آنتی بیوتیک (دارو یا بمب)؟

ابر باکتری ها (باکتری های مقاوم به آنتی بیوتیک که از تروریسم جدی تر است).

کشف آنتی بیوتیک:1928 الکساندر فلمینگ

سرآغاز نگرانی از مقاومت میکروارگانیسم ها: 1940 سویه های استافیلوکوک مقاوم به پنی سیلین

Antibiotic Resistance Timeline



مکانیسم های کلاسیک مقاومت آنتی بیوتیکی:

1- مقاومت ذاتی (غیرفعال): غشای خارجی بصورت ذاتی نفوذپذیری کمی نسبت به آنتی بیوتیک ها دارد (سودموناس آئروژینوزا)

باکتری های بی هوازی اجباری بطور ذاتی نسبت به آمینوگلیکوزیدها مقاومند؟

ورود آمینوگلیکوزیدها نیازبه دو مرحله اساسی دارد:

الف- ازدیواره به فضای پری پلاسمیک بدون صرف ATP و ب- ورود به غشا با صرف ATP (فسفریلاسیون اکسید اتیو)

2- مقاومت اکتسابی (فعال): دراثرفشارتکاملی اتفاق می افتد.

مکانسیم های مقاومت اکتسابی :

1- جهش های کروموزومی

2- پلاسمیدهای قابل انتقال (R)

3- ترانسپوزون ها

Horizontal gene transfer=HGT , Vertical gene transfer=VGT

A bacterial antibiotic resistance gene with eukaryotic origins **Mupirocin=pseudomonic acid**

مکانیسم های عمده مقاومت آنتی بیوتیکی فعال شامل:

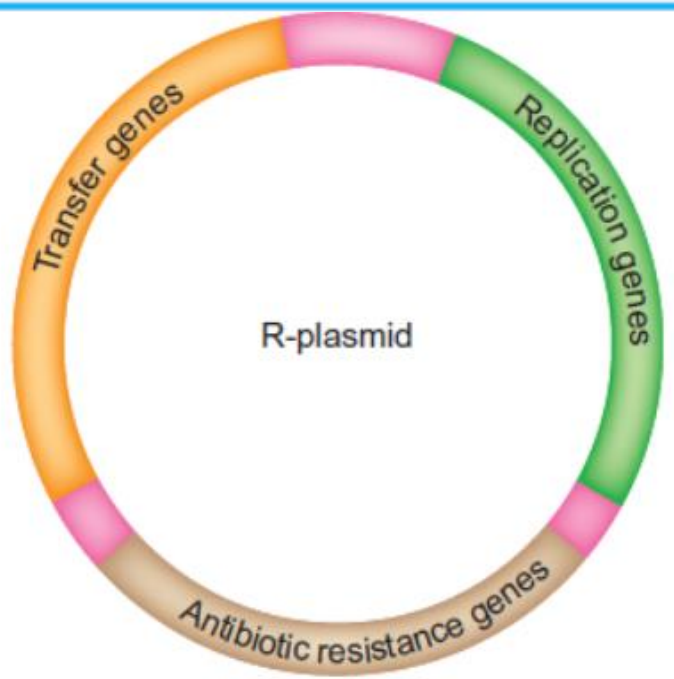
1- ممانعت از تجمع آنتی بیوتیک
2- تغییر جایگاه هدف (PBP در مورد پنی سیلین)
3- غیرفعال سازی آنزیمی آنتی بیوتیک
4- مسیر bypass (راه سنتز فرعی): سولفونامیدها
5- مکانیسم های جدید مقاومت در باکتری ها (تغییرات ژنتیکی، سیستم های تعمیری کارآمد، کروم سنسینگ، Riboswitch

Antibiotic Resistance Plasmids

R-plasmids make bacteria resistant to antibiotics.

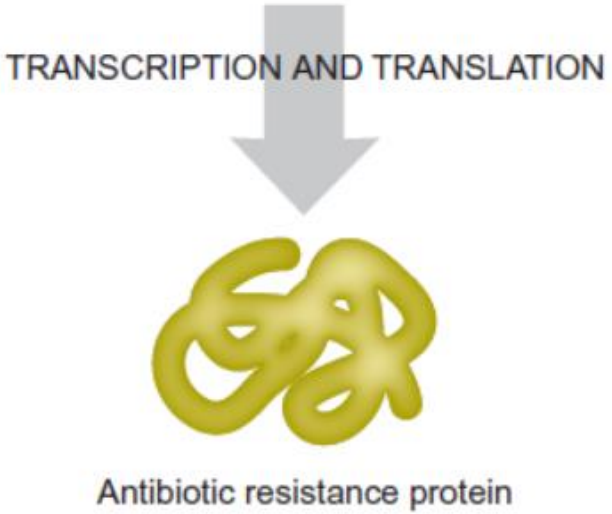
Bacterial resistant to antibiotics is becoming a major threat because resistance is spreading rapidly while few new antibiotics have been developed.

R-plasmid Plasmid that carries genes for antibiotic resistance.



Antibiotic Resistance Plasmids

Plasmids carry genes for replicating their DNA, transferring themselves from one host cell to another, and for a variety of phenotypes. Many plasmids carry genes that confer antibiotic resistance on the host cell when the genes are expressed.

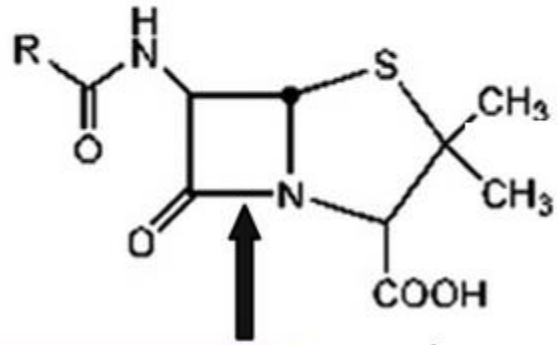


Resistance to Beta-Lactam Antibiotics

The **β-lactam** family includes the **penicillins** and **cephalosporins** and is the best-known and most widely used group of antibiotics. All contain the β-lactam structure, a four-membered ring containing an amide group, which reacts with the active site of enzymes involved in building the bacterial cell wall. Crosslinking of the peptidoglycan is prevented, so causing disintegration of the cell wall and death of the bacteria. Since peptidoglycan is unique to bacteria, penicillins and cephalosporins have almost no side effects in humans, apart from occasional allergies.

Inactivation of Penicillin by β-Lactamase

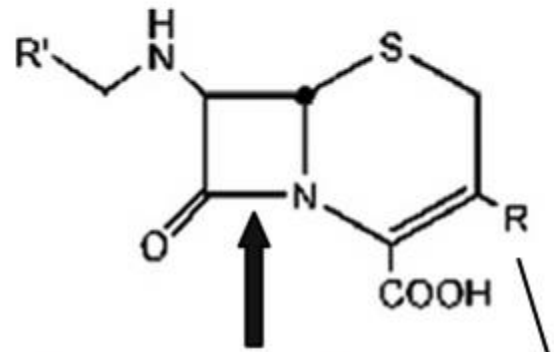
Penicillin



β-lactamases

Thiazolidine

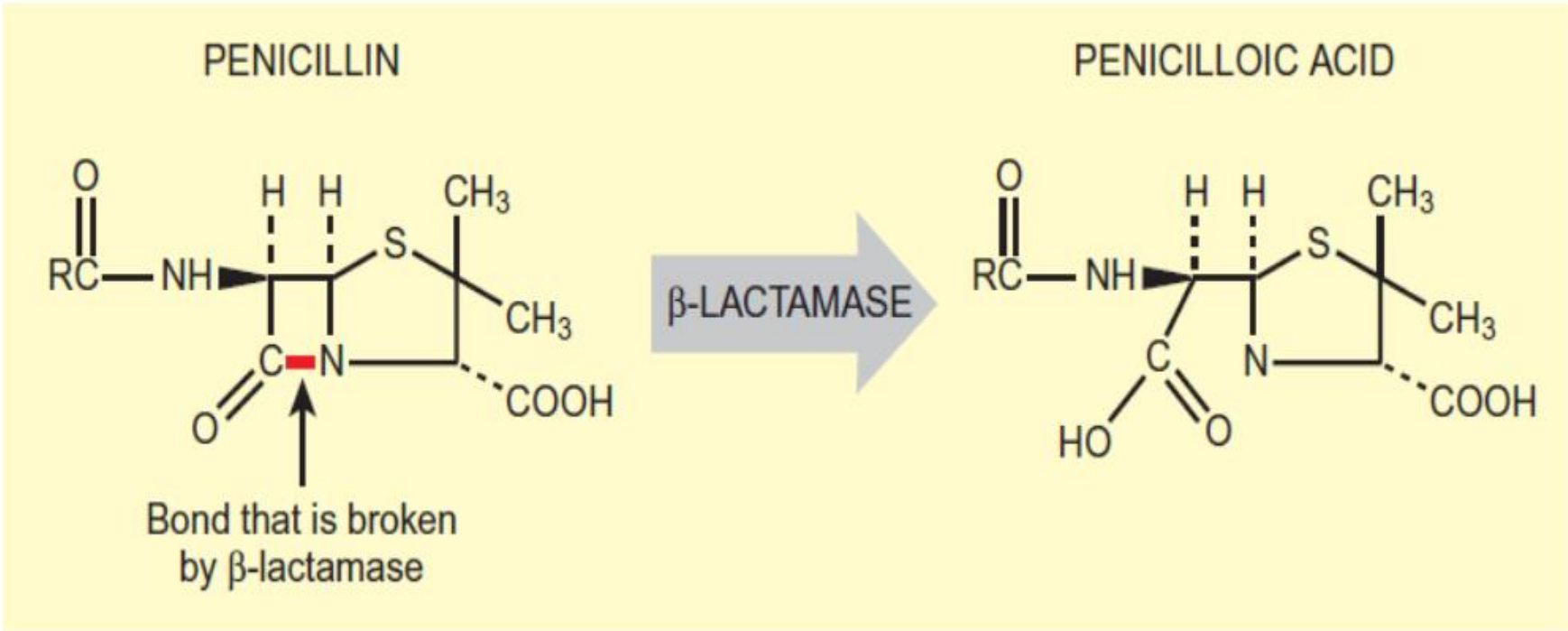
Cephalosporin



β-lactamases

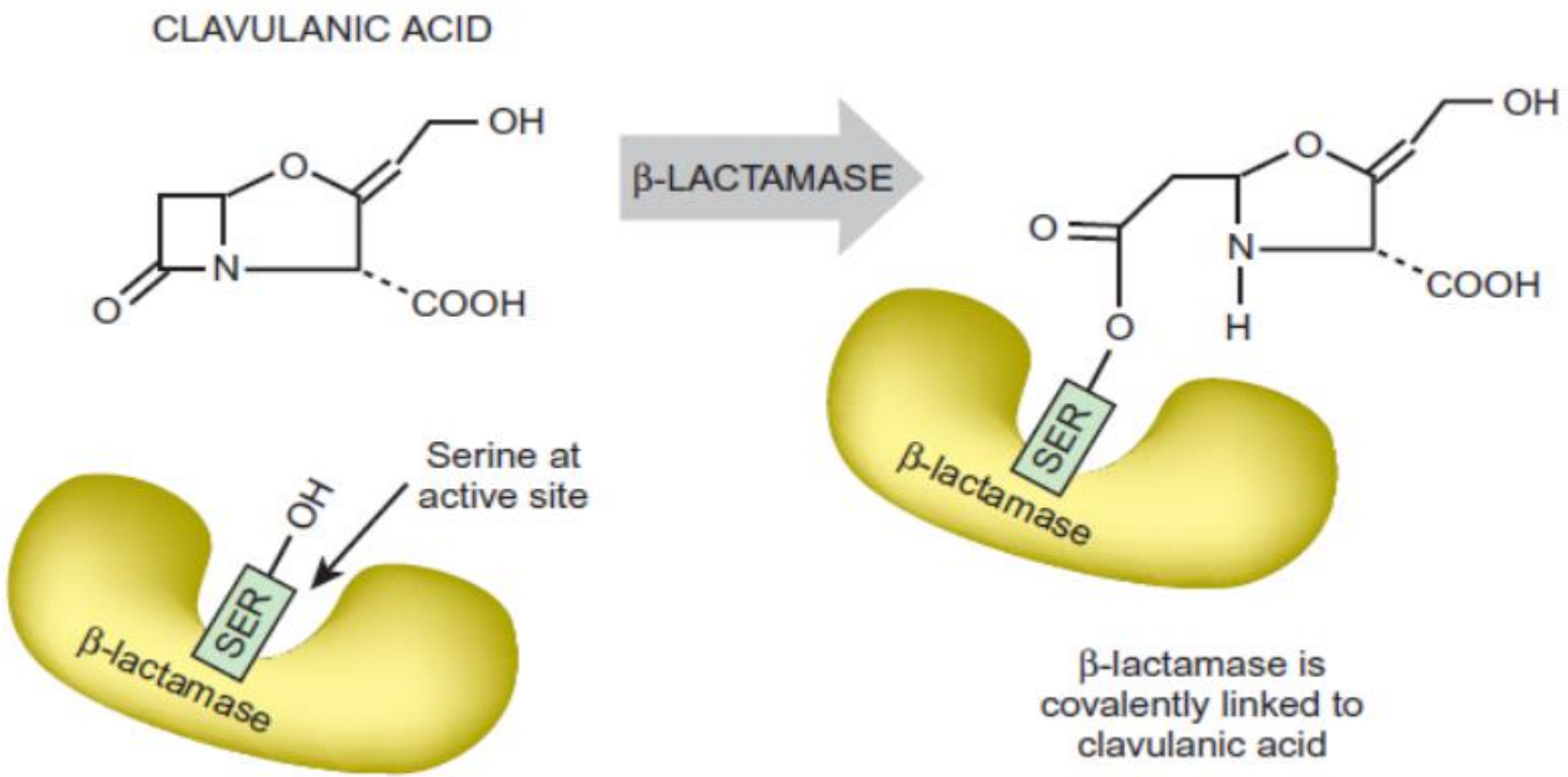
Dihydrothiazine

Inactivation of Penicillin by β-Lactamase



Penicillin is an antibiotic that attacks the cell wall of bacteria, preventing the cells from growing or dividing. The antibiotic has a four-membered β-lactam ring that binds to the active site of the enzymes that assemble the cell wall. The enzyme β-lactamase cleaves the β-lactam ring of penicillin (red bond). The penicillin is inactivated.

Inactivation of β-Lactamase by Clavulanic Acid



In order to inactivate β-lactamase, analogs of penicillin such as clavulanic acid are added along with the antibiotic. Clavulanic acid has a four-membered ring similar to penicillin. Consequently, β-lactamase will bind and cleave this ring. When this happens, clavulanic acid is covalently bound to β-lactamase rendering it useless against penicillin. Added penicillin can now kill the bacteria, even though they contain the resistance gene.

راههای مقاومت به پنی سیلین:

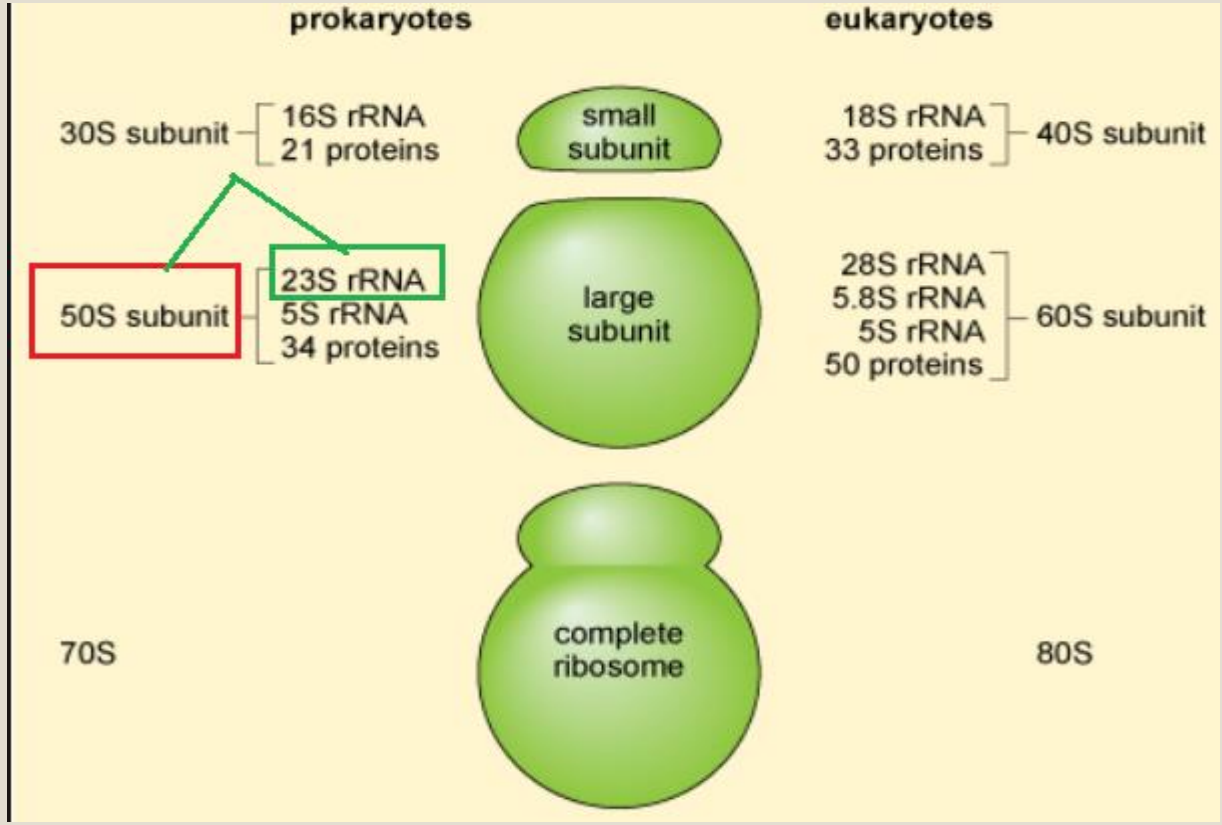
1- غیرفعال سازی آنزیمی توسط پنی سیلیناز

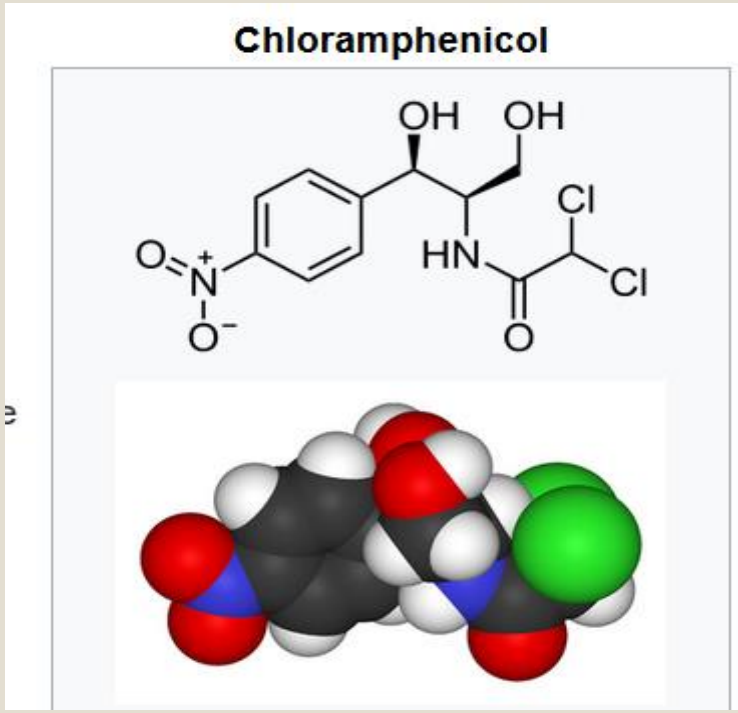
2- تغییرجایگاه هدف (PBP ها که با تغییردادن آن دیگرپنی سیلین متصل نمی شود) وبنابراین باید از نیمه سنتزی ها مانند آموکسی سیلین یا آمپی سیلین ویا سنتزی ونکومایسین استفاده نمود.

سوال: دریک کارخانه داروسازی می خواهیم یک روش علمی شناسایی آلودگی میکروبی آنتی بیوتیک های خانواده بتالاکتام را مورد بررسی قراردهیم، چه روش/روش هایی را پیشنهاد می دهید؟

Resistance to Chloramphenicol

An antibiotic first isolated from cultures of *Streptomyces venequelae* in 1947 but now produced synthetically. It has a relatively simple structure and was the first broad-spectrum antibiotic to be discovered. It acts by interfering with bacterial protein synthesis and is mainly bacteriostatic.



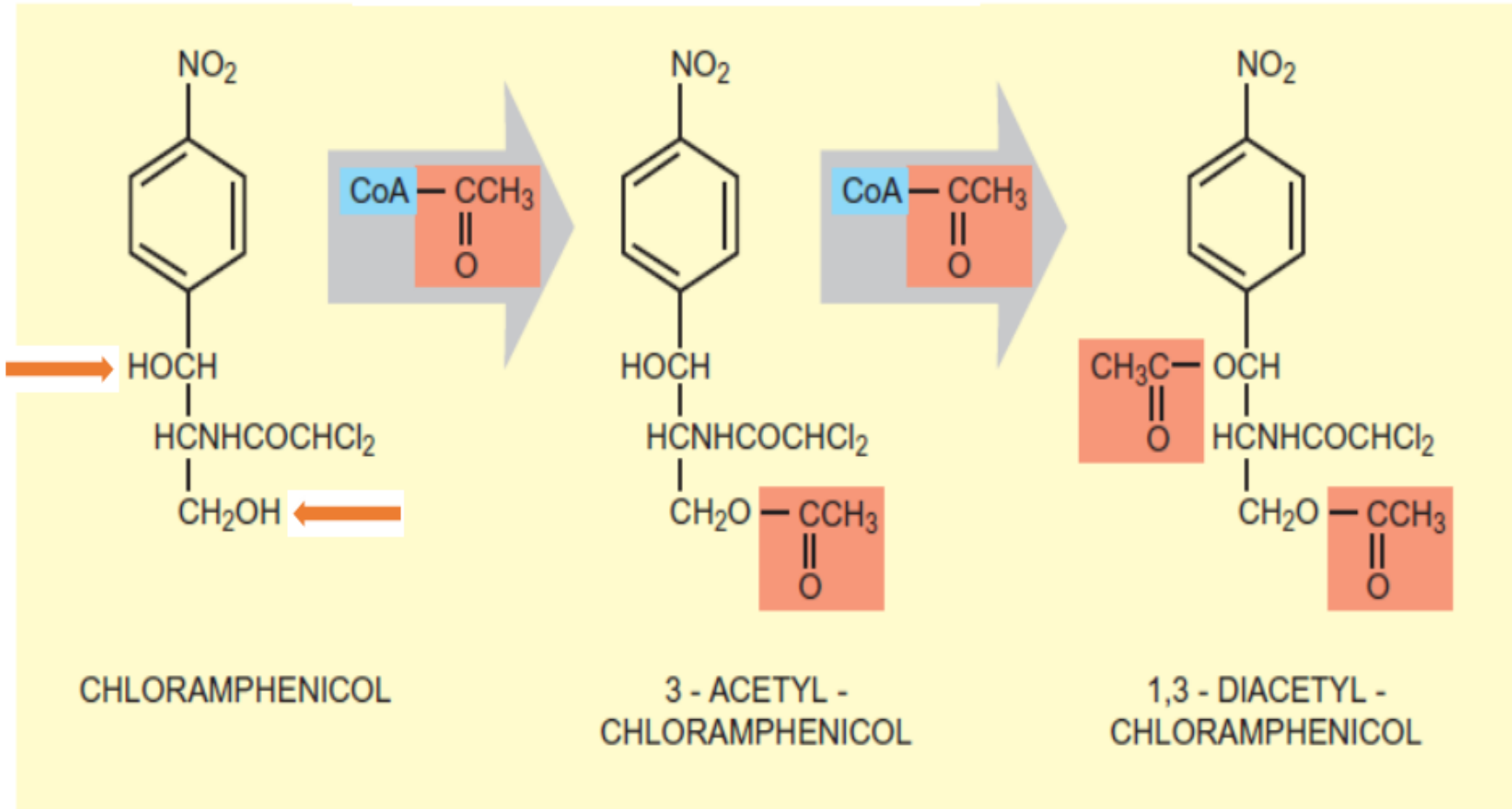


Resistance to Chloramphenicol

Chloramphenicol binds to the **23S rRNA of the large subunit** of the bacterial ribosome and inhibits the peptidyl transferase reaction (Protein Synthesis). R-plasmids protect the bacteria by producing the enzyme **chloramphenicol acetyl transferase (CAT)**. CAT transfers two acetyl groups from acetyl CoA to the side chain of chloramphenicol. This prevents binding of the antibiotic to the **23S rRNA**. Replacement of the terminal OH of chloramphenicol with fluorine results in nonmodifiable yet still active derivatives. There are two major groups of chloramphenicol acetyl transferase: one from gram-positive and one from gram-negative bacteria. The two groups differ greatly from each other except for the chloramphenicol-binding region.

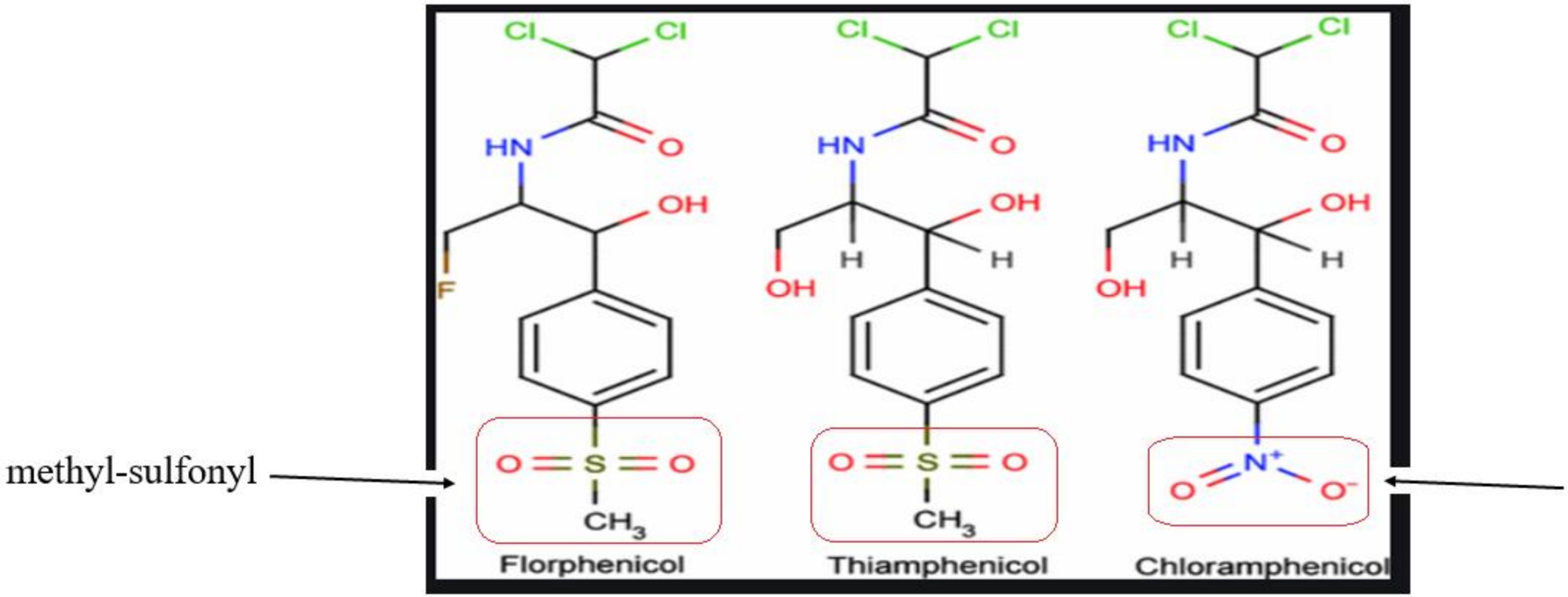
Chloramphenicol is inactivated by addition of acetyl groups.

Inactivation of Chloramphenicol



The side chain of chloramphenicol has two OH groups that are important for binding to the bacterial ribosomes. Chloramphenicol acetyl transferase, produced by R-plasmids, catalyzes the addition of two acetyl groups to chloramphenicol. The enzyme uses acetyl-CoA as a source for the acetyl groups. The resulting 1,3-diacetyl-chloramphenicol can no longer bind to the ribosomes.

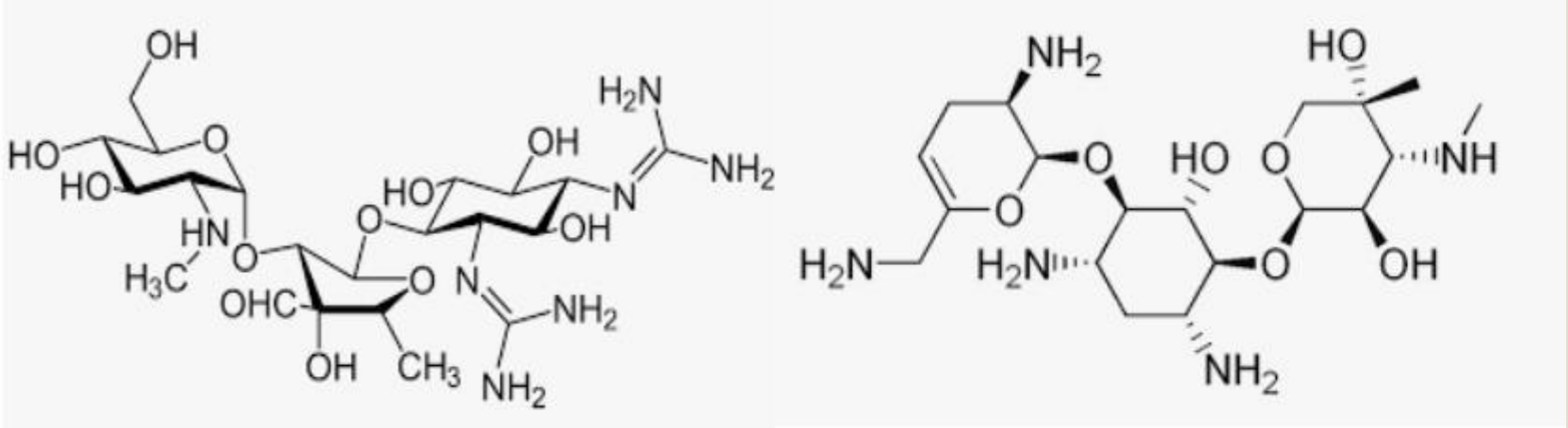
Chloramphenicol Derivatives



Aminoglycoside

potent bactericidal antibiotics

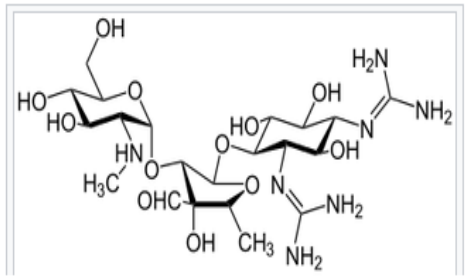
Gram-negative antibacterial medications



amino-modified glycoside (sugar)

Aminoglycoside

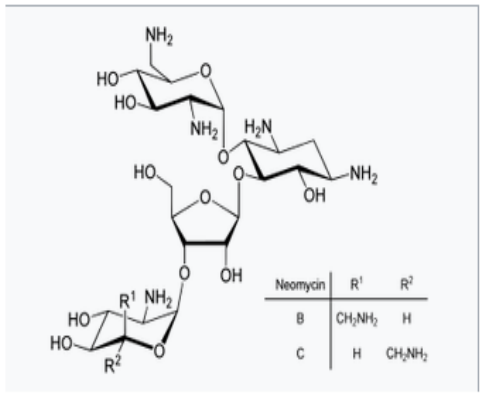
Streptomycin



▶1943 from *Streptomyces griseus*

tuberculosis, endocarditis, brucellosis, *Burkholderia* infection, plague, tularemia, and rat bite fever

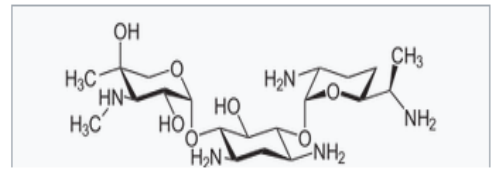
Neomycin



▶1949 from *Streptomyces fradiae*

Neosporin (neomycin/polymyxin B/bacitracin).

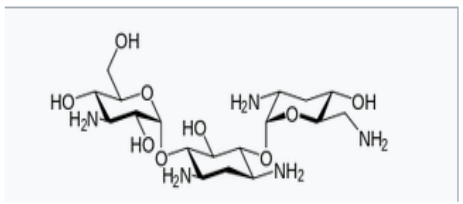
Gentamicin



▶1963 from *Micromonospora purpurea*

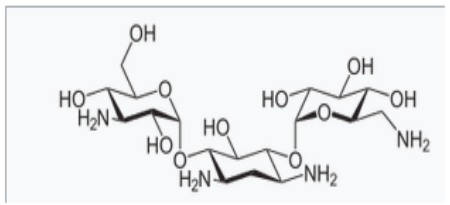
bone infections, endocarditis, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, and sepsis

Tobramycin



▶1974 from *Streptomyces tenebrarius*

Kanamycin A



▶1957 from *Streptomyces kanamyceticus*

treat severe bacterial infections and tuberculosis.

E. coli, *Proteus* species (both indole-positive and indole-negative), *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Acinetobacter* species.

Resistance to Aminoglycosides

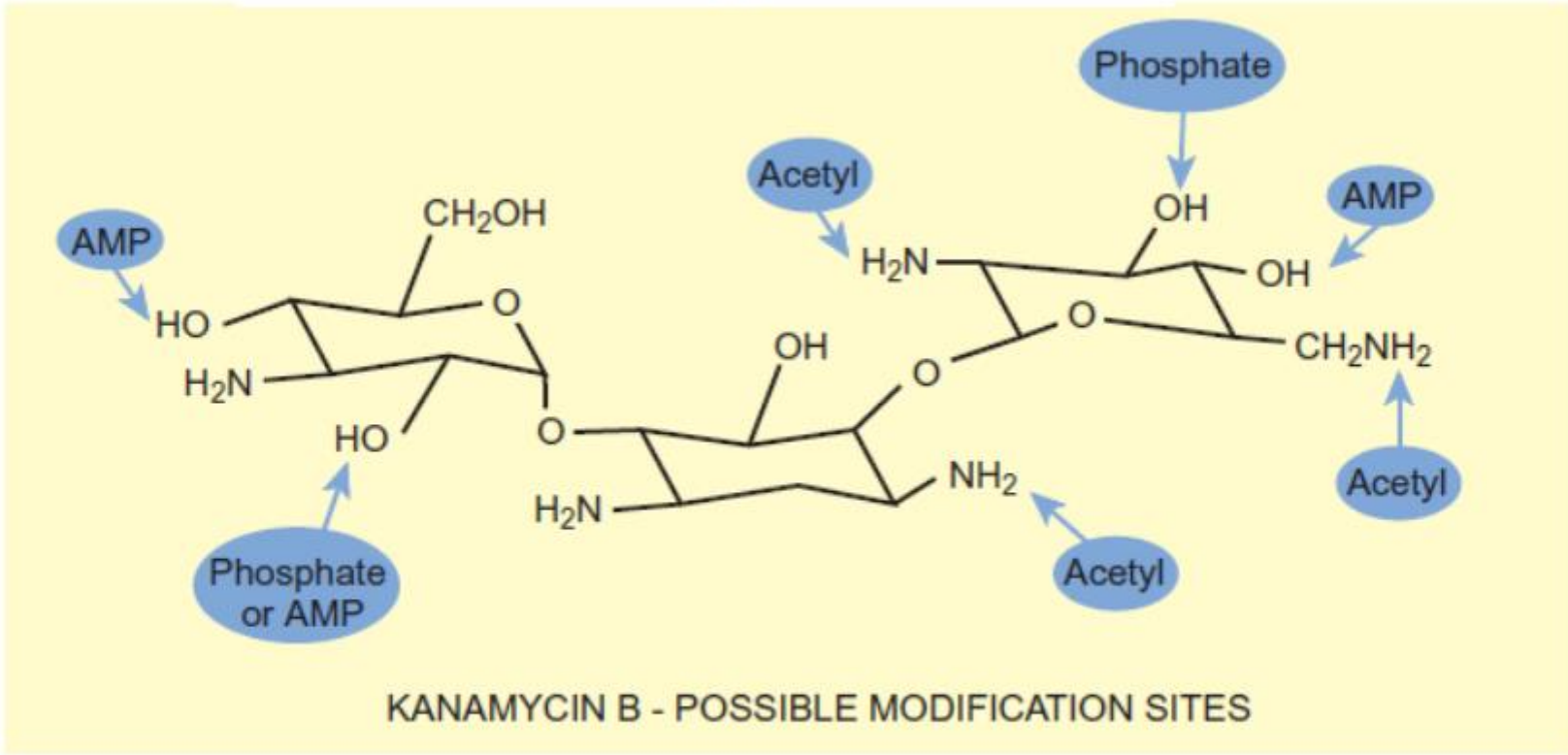
The aminoglycoside family of antibiotics includes streptomycin, kanamycin, neomycin, tobramycin, gentamycin, amikacin, and a host of others. Aminoglycosides consist of three or more sugar rings, at least one of which (and usually two or three) has amino groups attached. They inhibit protein synthesis by binding to the small subunit of the ribosome (Protein Synthesis). Plasmid-borne resistance is typically due to inactivation of the antibiotics. Several alternatives exist, including modification by phosphorylation of OH groups, adenylation (i.e., addition of adenosine monophosphate, AMP) of OH groups or acetylation of NH2 groups. ATP is used as a source of phosphate and AMP groups, whereas Acetyl-CoA is the acetyl donor. Modified aminoglycosides no longer inhibit their ribosomal target sites.

Aminoglycoside antibiotics are inactivated by addition of phosphate, AMP, or acetyl groups.

Resistance to Aminoglycosides

There are many different aminoglycosides and a correspondingly wide range of modifying enzymes. The npt gene (encoding neomycin phosphotransferase) is the most widely used in molecular biology and provides resistance to both kanamycin and the closely related neomycin. Aminoglycosides are made by bacteria of the Streptomyces group, which are mostly found in soil. These organisms need to protect themselves against the antibiotics they produce. Probably, therefore, the aminoglycoside-modifying enzymes came originally from the same *Streptomyces* strains that make these antibiotics. Recently, a second mechanism of aminoglycoside resistance has emerged and is starting to spread. This involves modification of the target site on the 16S ribosomal RNA by a methyl-transferase. This enzyme adds a methyl group to G1405 of 16S rRNA, which prevents binding of almost all aminoglycosides, except streptomycin.

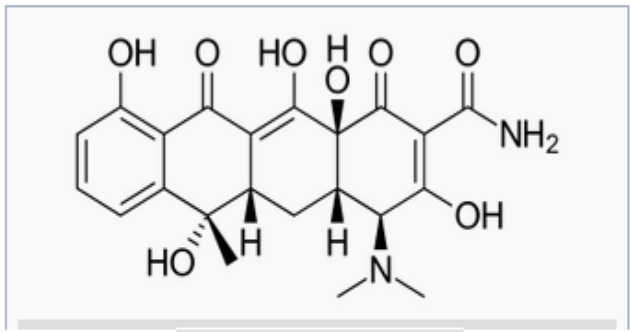
Inactivation of Aminoglycoside Antibiotics



KANAMYCIN B - POSSIBLE MODIFICATION SITES

Much like chloramphenicol, members of the aminoglycoside family are inactivated by modification. One member, kanamycin B, can be modified by a variety of covalent modifications, such as phosphorylation, acetylation, or adenylation. A variety of bacterial enzymes make these modifications to prevent kanamycin B from attaching to the small ribosomal subunit.

Tetracycline



$C_{22}H_{24}N_2O_8$

►acne, cholera, brucellosis, plague, malaria, and syphilis and pneumonia.

Tetracycline is a broad-spectrum naphthacene antibiotic produced semisynthetically from chlortetracycline, an antibiotic isolated from the bacterium Streptomyces aureofaciens. In bacteria, tetracycline binds to the 30S ribosomal subunit, interferes with the binding of aminoacyl-tRNA to the mRNA-ribosome complex, thereby inhibiting protein synthesis.

Resistance to Tetracycline

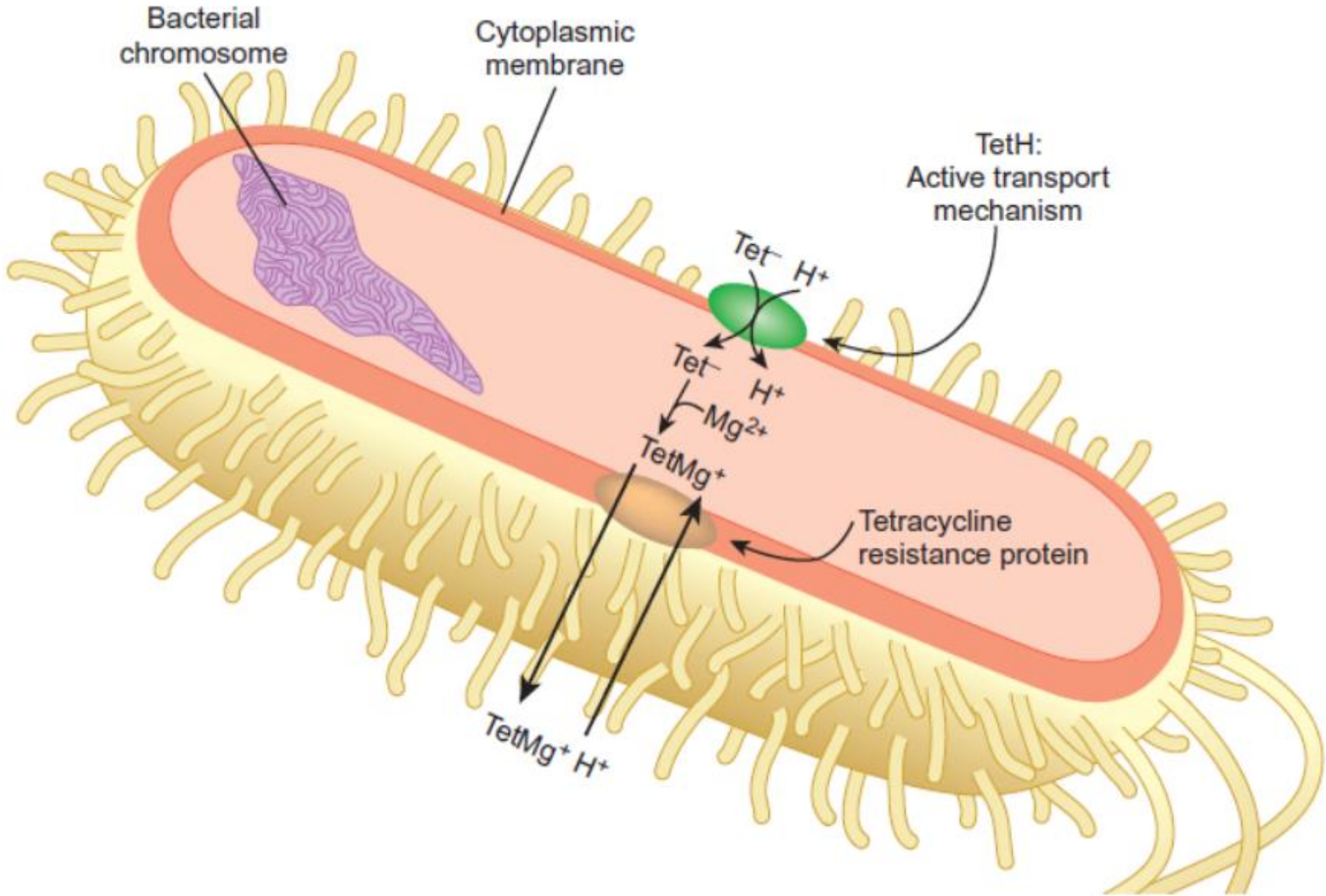
Tetracycline binds to the 16S rRNA of the small subunit and also inhibits protein synthesis. However, the mechanism of resistance is quite different from chloramphenicol and aminoglycosides. Rather than inactivating tetracycline by modification, R-plasmids produce proteins that pump the antibiotic out of the bacteria. Tetracycline actually binds to both prokaryotic and eukaryotic ribosomes. Bacteria are more sensitive than animal cells because tetracyclines are readily taken up by bacterial cells, but not by eukaryotic cells. In fact, eukaryotic cells naturally actively export tetracyclines. In tetracycline-resistant bacteria, the antibiotic is taken into the cell, but then pumped out again. The nature and mechanism of the bacterial uptake of tetracycline is still obscure. However, the Tet-resistance protein is part of a large family of sugar transporter proteins, and may have evolved from recognizing sugar to recognizing tetracycline.

Plasmid-encoded tetracycline resistance is typically in two stages. A basal constitutive level of resistance protects bacteria by 5_10-fold relative to sensitive bacteria. In addition, exposure to tetracycline induces a second higher resistance level. Both resistance levels are due to production of proteins that are found in the cytoplasmic membrane and actively expel tetracycline from the cell. Tetracycline enters the cell as the protonated form by an active transport system. Inside the cell, it binds Mg^{2+}. The Tet-resistance protein uses energy to expel the Tet-Mg^{21} complex by proton antiport .

Tetracycline resistance is due to energy-driven export of the antibiotic.

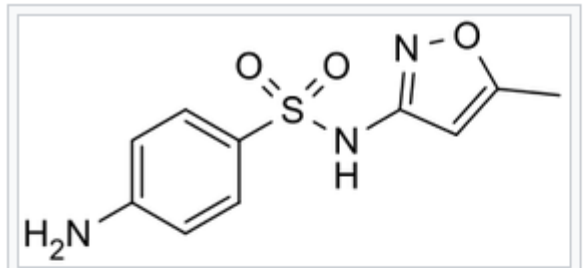
Expulsion of Tetracycline from Resistant Bacteria

The bacterial chromosome contains the gene for TetH, a protein that takes tetracycline from the environment and actively pumps the antibiotic and a proton into the cell. Once inside the cell, tetracycline complexes with Mg^{2+}, and may bind to the ribosome. In bacterial cells with an R-plasmid for tetracycline, another transport protein, called the tetracycline resistance protein, is manufactured. This protein allows a proton to enter the cell to produce energy for export of the Tet-Mg^{+} complex.

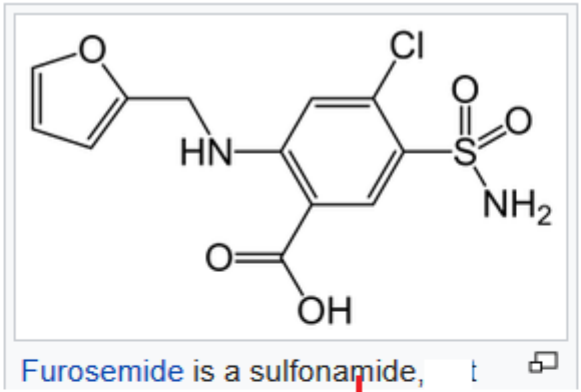


In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthase (DHPS), an enzyme involved in folate synthesis.

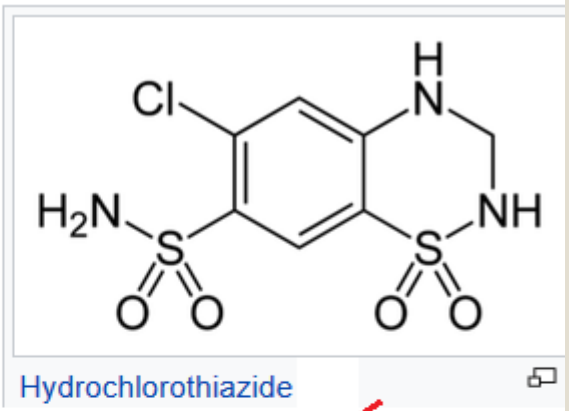
Sulfonamides are therefore bacteriostatic and inhibit growth and multiplication of bacteria, but do not kill them. Humans, in contrast to bacteria, acquire folate (vitamin B_9) through the diet.



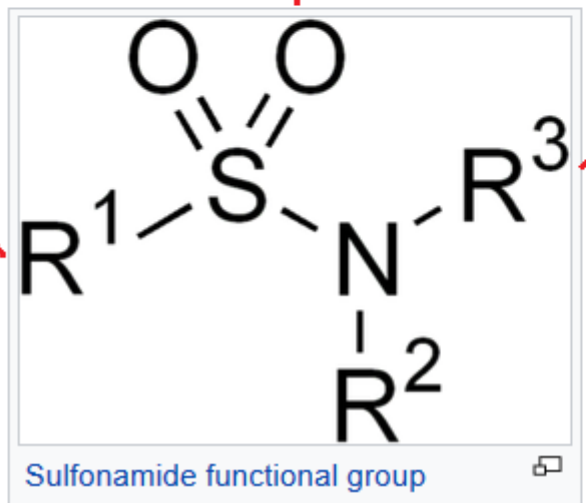
Sulfamethoxazole is an antibacterial sulfonamide



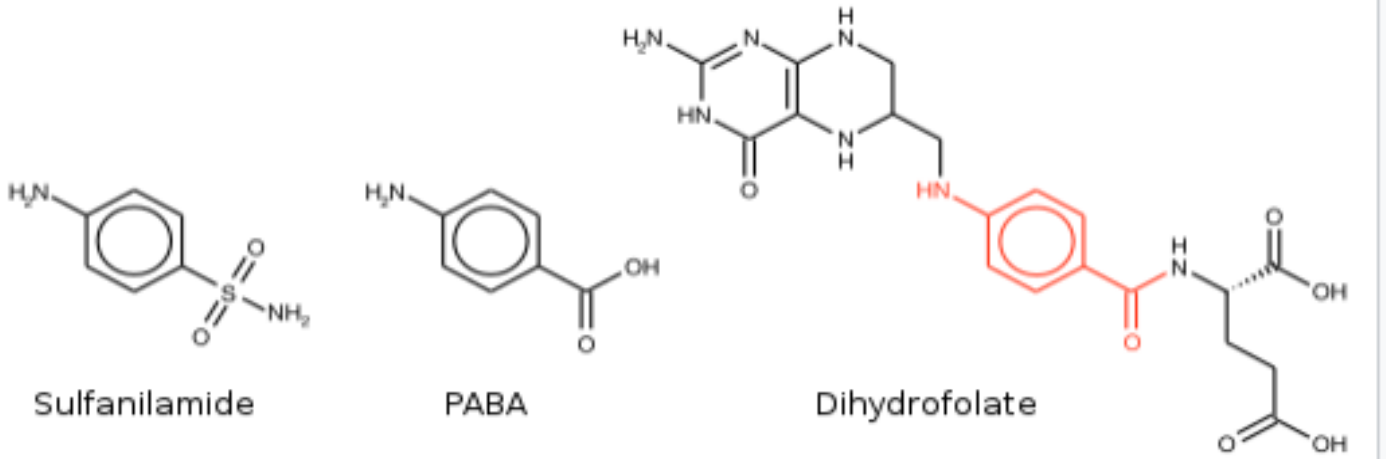
Furosemide is a sulfonamide,



Hydrochlorothiazide

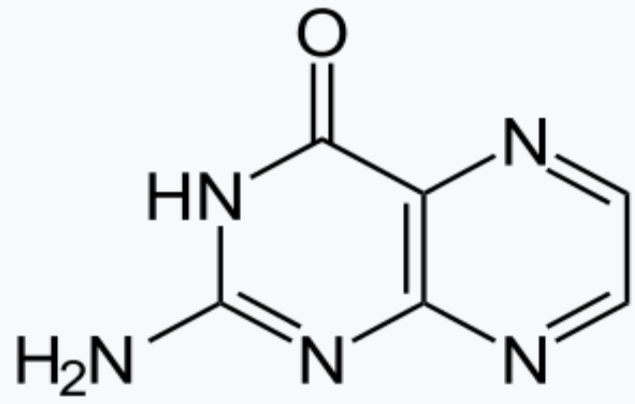


Sulfonamide functional group

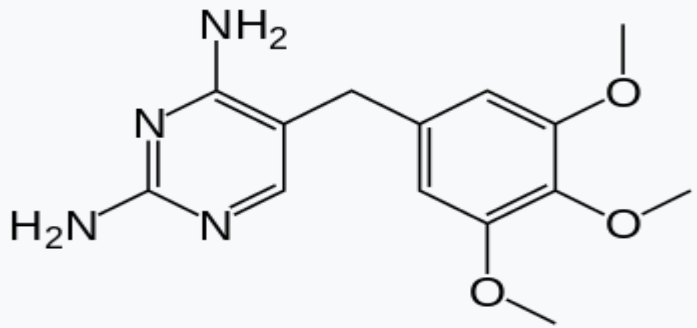


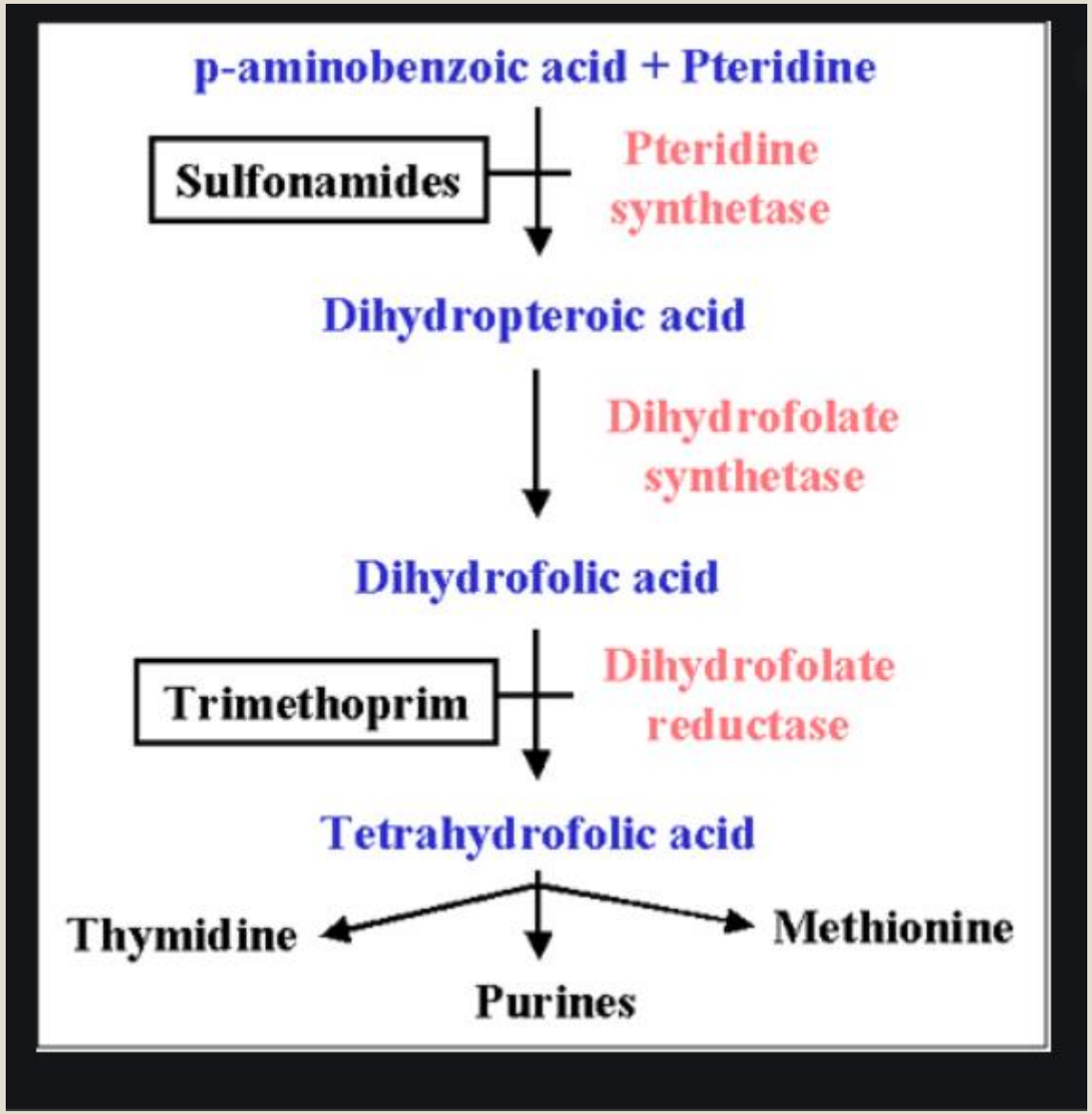
Structural similarity between sulfonilamide (left) and PABA (center) is the basis for the inhibitory activity of sulfa drugs on tetrahydrofolate (right) biosynthesis.

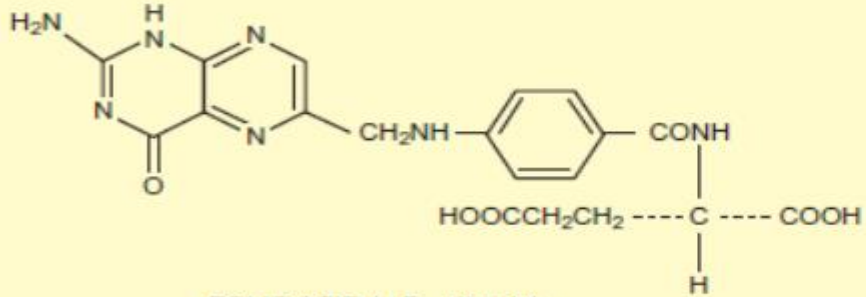
Pterin



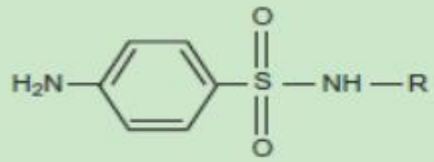
Trimethoprim



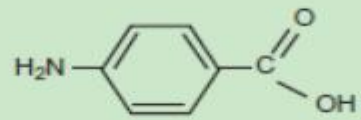

p-aminobenzoic acid + Pteridine
Sulfonamides
Pteridine synthetase
Dihydropteroic acid
Dihydrofolate synthetase
Dihydrofolic acid
Trimethoprim
Dihydrofolate reductase
Tetrahydrofolic acid
Thymidine
Purines
Methionine



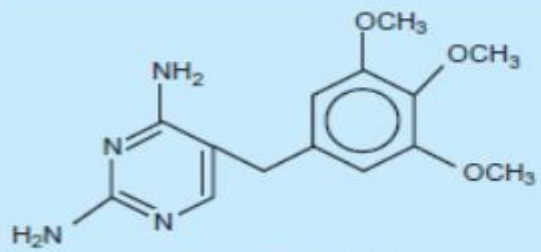
FOLIC ACID (a B - vitamin)



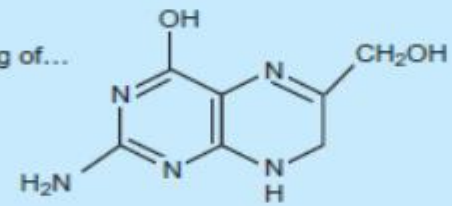
SULFONAMIDE is an analog of...



p-AMINOBENZOIC ACID
(part of folic acid)



TRIMETHOPRIM
is an analog of...



DIHYDROPTERIDINE
(part of folic acid)

Trimethoprim, Sulfonamides, and the Folate Co-factor

Bacterial cells make folic acid, whereas animal cells do not. The antibiotic sulfonamide is an analog of the *p*-aminobenzoic acid portion of folic acid. Trimethoprim is an analog of the dihydropteridine portion of folic acid. Both trimethoprim and sulfonamide bind to the biosynthetic enzymes and prevent synthesis of folic acid from its precursors.

Resistance to Sulfonamides and Trimethoprim

The mechanism of resistance to these two antibiotics is unusual—replacement of the sensitive target enzymes with resistant versions. Both **sulfonamides** and **trimethoprim** are antagonists of the vitamin **folic acid**. The reduced form of folate, tetrahydrofolate, is used as a co-factor by enzymes that synthesize methionine, adenine, thymine, and other metabolites whose synthesis involves adding a one-carbon fragment. Sulfonamides are completely synthetic antibiotics and are analogs of p-aminobenzoic acid, a precursor of the vitamin folic acid. Sulfonamides inhibit dihydropteroate synthetase, an enzyme in the synthetic pathway for folate. Trimethoprim is an analog of the pterin ring portion of tetrahydrofolate. It inhibits dihydrofolate reductase, the bacterial enzyme that converts dihydrofolate to tetrahydrofolate. Animal cells rely on folate in their food so these antibiotics are harmless to animals or humans but are effective against bacteria that normally manufacture their own tetrahydofolate. Plasmid-mediated resistance to both sulfonamides and trimethoprim involves synthesis of folic acid biosynthetic enzymes that no longer bind the antibiotic. R-plasmid-encoded dihydropteroate synthetase has the same affinity for p-aminobenzoic acid as the chromosomal enzyme but is resistant to sulfonamides. Similarly, R-plasmid-encoded dihydrofolate reductase is resistant to trimethoprim. Sulfonamides plus trimethoprim are often used in combination for double blockade of the folate pathway. As a result, sulfonamide and trimethoprim resistance are often found together on the same R-plasmid.

bypass=راه سنتز فرعی

Resistance to trimethoprim and sulfonamides is due to replacement of the target enzyme.

ampicillin A widely used antibiotic of the penicillin group.
beta-lactams or β-lactams Family of antibiotics that inhibit crosslinking of the peptidoglycan of the bacterial cell wall; includes penicillins and cephalosporins.
***bla* gene** Gene encoding β-lactamase thereby providing resistance to ampicillin. Same as *amp* gene.
clavulanic acid A beta-lactam derivative that does not act as an antibiotic but instead binds to β-lactamases and reacts forming a covalent bond to the protein that kills the enzyme.

aminoglycosides Family of antibiotics that inhibit protein synthesis by binding to the small subunit of the ribosome; includes streptomycin, kanamycin, neomycin, tobramycin, gentamycin, and many others.
chloramphenicol Antibiotic that binds to 23S rRNA and inhibits protein synthesis.
chloramphenicol acetyl transferase (CAT) Enzyme that inactivates chloramphenicol by adding acetyl groups.
kanamycin Antibiotic of the aminoglycoside family that inhibits protein synthesis.
neomycin Antibiotic of the aminoglycoside family that inhibits protein synthesis.
streptomycin Antibiotic of the aminoglycoside family that inhibits protein synthesis.

neomycin phosphotransferase Enzyme that inactivates the antibiotics kanamycin and neomycin by adding a phosphate group.
***npt* gene** Gene for neomycin phosphotransferase. Provides resistance against the antibiotics kanamycin and neomycin.
tetracycline Antibiotic that binds to 16S ribosomal RNA and inhibits protein synthesis.

sulfonamides Synthetic antibiotics that are analogs of *p*-aminobenzoic acid, a precursor of the vitamin folic acid. Sulfonamides inhibit dihydropteroate synthetase.
trimethoprim Antibiotic that is an analog of the pterin ring portion of the folate co-factor. It inhibits dihydrofolate reductase.