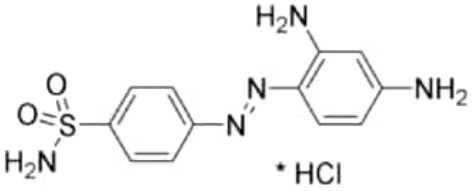
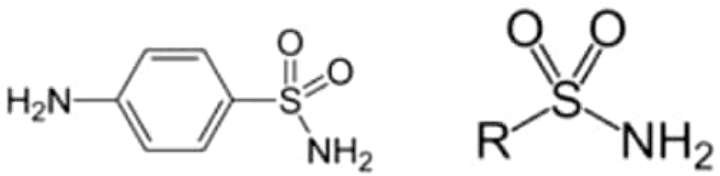
Sulfonamides: Classification, mode of action, uses and structure activity relationship of the following classes of drugs; Synthesis of Sulphadiazine, Sulphamethoxazole, Sulphacetamide sodium

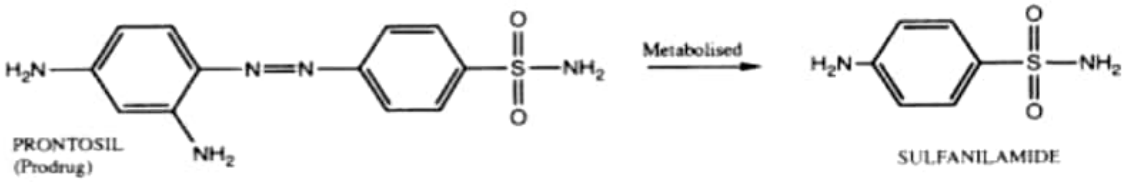
- Sulfonamide drugs were the first antimicrobial drugs. The first sulfonamide was trade named Prontosil, which is a prodrug **Prontosil**, the first commercially available antibacterial with a relatively broad effect (against Gram-positive cocci but not against enterobacteria).



Prontosil

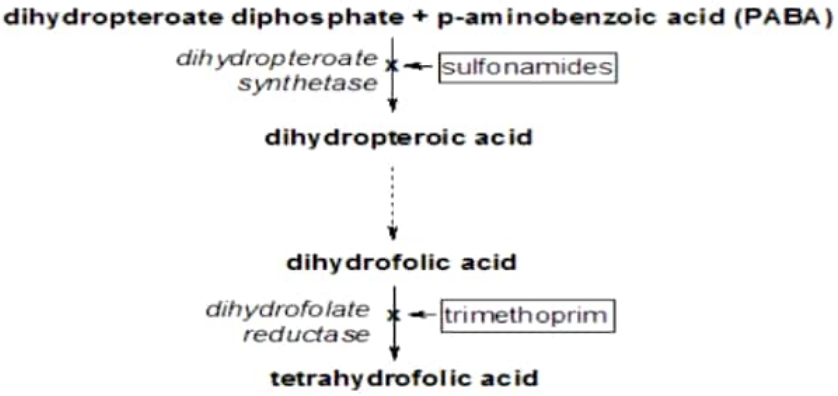


The structure of the sulfonamide group

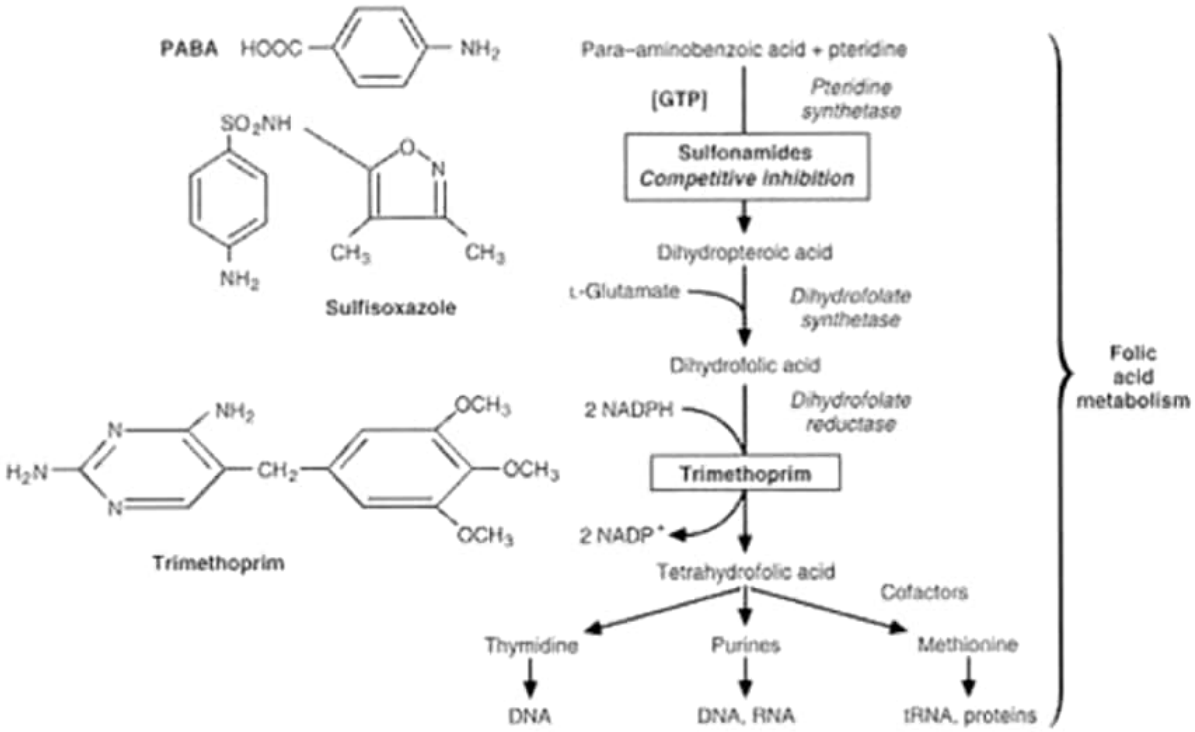


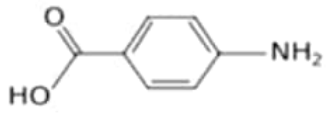
❖ Mechanism of action of Sulfonamide:

- PABA is an essential nutrient for some bacteria and is sometimes called Vitamin B_x However, PABA is not essential to human health, and is therefore not officially classified as a vitamin. Although humans lack the ability to synthesize folate from PABA, it is sometimes marketed as an essential nutrient under the promise that it can stimulate intestinal bacteria.
- PABA is an intermediate in bacterial synthesis of folate. Sulfonamides are chemically similar to PABA, and their antibacterial activity is due to their ability to interfere with conversion of PABA to folate by dihydropteroate synthetase, and subsequent utilization, by bacteria.
- ***Dihydropteroate synthetase*** is an enzyme.It produces dihydropteroate in bacteria, but does not function in humans. This makes it a useful target for sulfonamide antibiotics, which compete with the PABA precursor.
- It acts upon 4-Aminobenzoic acid (PABA} and dihydropteridine-hydroxymethyl-pyrophosphate.
- In bacteria, antibacterial sulfonamides act as competitive inhibitors of the enzyme dihydropteroate synthetase, DHPS. DHPS catalyses the conversion of PABA (*para*-aminobenzoate) to dihydropteroate, a key step in folate synthesis. Folate is necessary for the cell to synthesize nucleic acids (nucleic acids are essential building blocks of DNA and RNA), and in its absence cells will be unable to divide. Hence the sulfonamide antibacterials exhibit a bacteriostatic rather than bactericidal effect.
- **Folate** is not synthesized in mammalian cells, but is instead a dietary requirement. This explains the selective toxicity to bacterial cells of these drugs. These antibiotics are used to treat pneumocystis jiroveci pneumonia, urinary tract infections, shigellosis, and certain protozoan infections. The sulfonamide chemical moiety is also present in other medications that are not antimicrobials, including thiazide diuretics (including hydrochorothiazide, metolazone, and indapamide, among others), sulfonylureas (including glipizide, glyburide, among others), and acetazolamide.

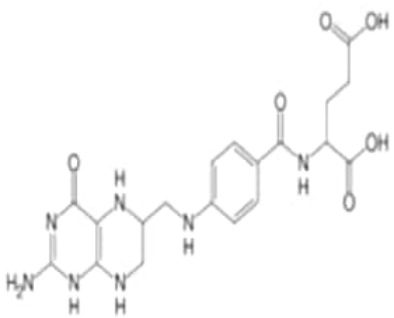


- Both trimethoprim and the sulfonamides interfere with folate metabolism in the bacterial cell by competitively blocking the biosynthesis of tetrahydrofolate, which acts as a carrier of one-carbon fragments and is necessary for the ultimate synthesis of DNA, RNA and bacterial cell wall proteins

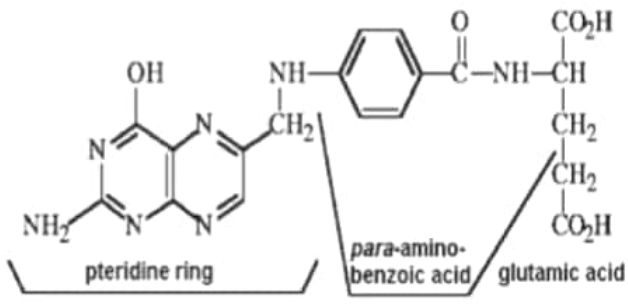




4-Aminobenzoic acid (PABA)



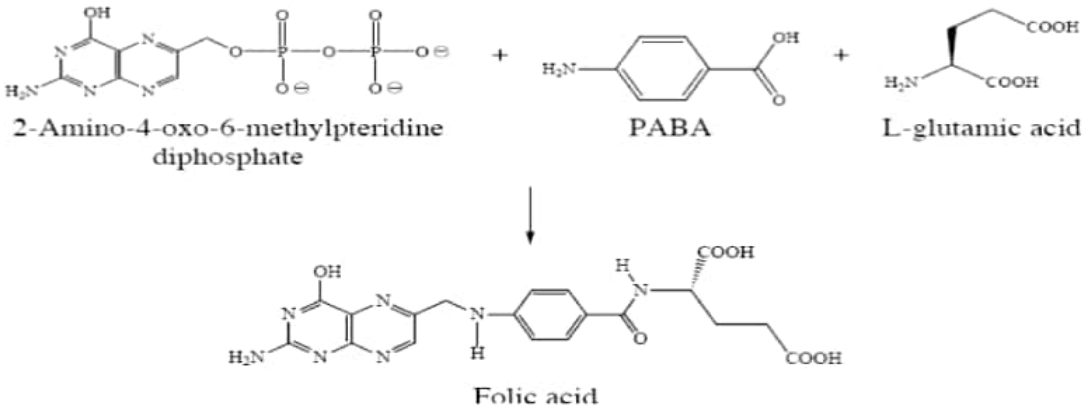
Tetrahydrofolic acid



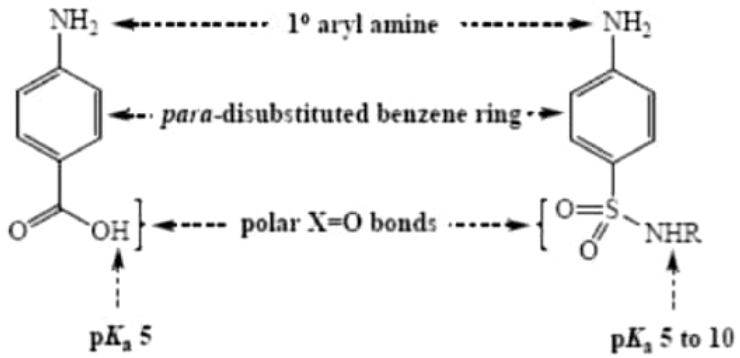
Folic acid

❖ *Why sulfanilide or sulfonamide inhibit folic acid synthesis mechanism*

- Cells use folic acid as a single-carbon atom building block for the construction of nucleic acids and other biological molecules. Inhibition of this process prevents growth and reproduction, but does not directly lead to cell death. Bacteria synthesize folic acid from 2-amino-4-oxo-6-methylpteridine diphosphate, *p*-aminobenzoic acid (PABA), and L-glutamic acid. Because sulfa drugs are structural mimics of PABA they may bind to dihydropteroate synthetase, one of the enzymes necessary for folic acid synthesis (reversible and competitive inhibition). With this enzyme inhibited, folic acid synthesis is prevented and cell growth and reproduction are halted.



- In addition, the two molecules are of nearly identical length (6.7 Å for PABA versus 6.9 Å for sulfanilide), both are roughly flat, and both have an equal distribution of charge (δ^+ on the NH_2 group and δ^- on the COOH or SO_2NHR groups). This effect can be seen more clearly by examining the electrostatic potential surfaces of these molecules.



❖ *Why bacterial dihydrofolate reductase is many times more sensitive to Trimethoprim than is equivalent enzyme in humans?*

- In microorganism human one of the key enzyme *dihydrofolate reductase* which reduces dihydrofolate to tetrahydrofolate is many more sensitive to folate antagonist trimethoprim in bacteria than in human because of **IC_{50}** values (the concentration causing 50% inhibition).

Inhibitor	**IC_{50} (micro mol/lit) for *dihydrofolate reductase* enzyme**		
	Human	**Protozal**	**Bacteria**
Trimethoprim	260	0.07	0.005
Pyrimethamine	0.7	0.0005	2.5
Methotrexate	0.001	0.1	Inactive

❖ Side effects:

- Sulfonamides have the potential to cause a variety of untoward reactions, including urinary tract disorders, haemopoietic disorders, and hypersensitivity reactions.
- When used in large dose, it may develop a strong allergic reaction. One of the most serious is *Stevens Johnson syndrome*(or toxic epidermal necrolysis).
- Some of the original sulfonamide drugs were derived from azo dyes and had the interesting effect of temporarily turning the patient red.
- **N.B- Stevens-Johnson syndrome** (SJS) is a life-threatening condition affecting the skin, in which due to cell death the epidermis separates from the dermis. The syndrome is thought to be a hypersensitivity complex affecting the skin and the mucous membranes.

❖ Adverse reactions:

i) The most common manifestation of a hypersensitivity reaction to sulfa drugs are rash and hives. However, there are several life-threatening manifestations of hypersensitivity to sulfa drugs, including Stevens-Johnson syndrome, toxic epidermal necrolysis, agranulocytosis, hemolytic anemia, thrombocytopenia, and fulminant hepatic necrosis, among others

ii) The sulfonamide antibiotic chemical structures are implicated in the hypersensitivity reactions associated with the class.

- The first is the **N^1 heterocyclic ring**, which causes a type I hypersensitivity reaction.
- The second is the **N^4 amino nitrogen** that, in a stereospecific process, forms reactive metabolites that cause either direct cytotoxicity or immunologic response.

❖ Note By:

Folic acid: Folic acid and folate (the anion form) are forms of the water-soluble Vitamin B_9. These occur naturally in food and can also be taken as supplements. Folate gets its name from the Latin word *folium*.

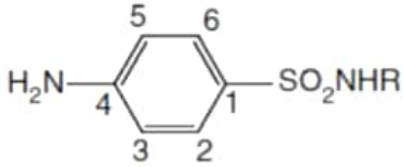
- Biological roles of folate-

i) Folate is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to synthesize DNA bases (most notably thymine, but also purine bases) needed for DNA replication. Thus folate deficiency hinders DNA synthesis and cell division, affecting most notably bone marrow and cancer, both of which participate in rapid cell division.

ii) In the form of a series of tetrahydrofolate (THF) compounds, folate derivatives are substrates in a number of single- carbon-transfer reactions, and also are involved in the synthesis of dTMP (2′-deoxythymidine-5′-phosphate) from dUMP (2′-deoxyuridine-5′-phosphate). It is a substrate for an important reaction that involves vitamin B_{12} and it is necessary for the synthesis of DNA, required for all dividing cells.

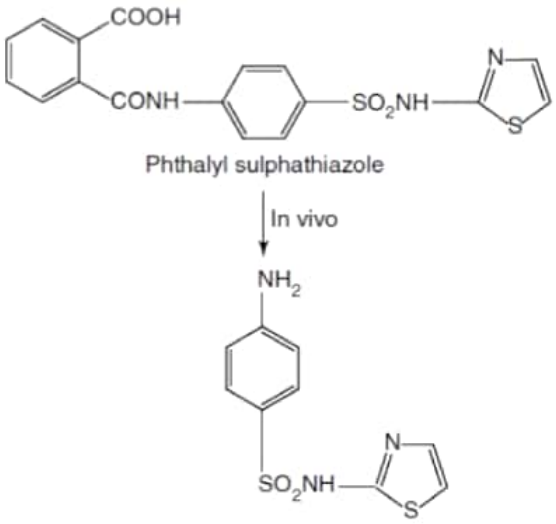
The pathway leading to the formation of tetrahydrofolate (FH_4) begins when folate (F) is reduced to dihydrofolate (DHF) (FH_2), which is then reduced to THF. Dihydrofolate reductase catalyses the last step.Vitamin B_3 in the form of NADPH is a necessary cofactor for both steps of the synthesis

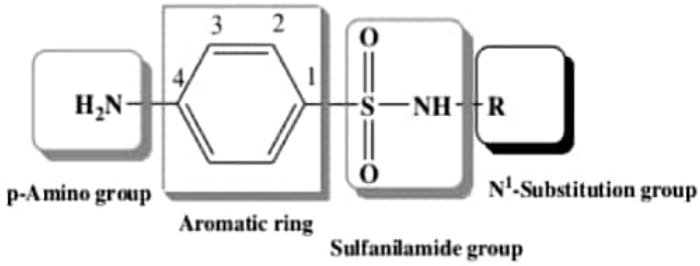
❖ SAR of Sulphonamides



- The major features of SAR of sulphonamides include the following:

- Sulphanilamide skeleton is the minimum structural requirement for antibacterial activity.
- The amino- and sulphonyl-groups on the benzene ring are essential and should be in 1 and 4 position.
- The **N-4** amino group could be modified to be prodrugs, which are converted to free amino function in vivo.





- Sulphur atom should be directly linked to the benzene ring.
- Replacement of benzene ring by other ring systems or the introduction of additional substituents on it decreases or abolishes its activity.
- Exchange of the **–SO_2NH** group by **–CONH** reduces the activity.
- On **N-1**-substituted sulphonamides, activity varies with the nature of the substituent at the amino group. With substituents imparting electron-rich characters to **SO_2 group**, bacteriostatic activity increases.
- Heterocyclic substituents lead to highly potent derivatives, while sulphonamides, which contain a single benzene ring at **N-1 position**, are considerably more toxic than heterocyclic ring analogues.
- The free aromatic amino groups should reside para to the sulphonamide group. Its replacement at ortho or meta position results in compounds devoid of antibacterial activity.
- The active form of sulphonamide is the ionized, maximum activity that is observed between the pKa values **6.6–7.4.**
- Substitutions in the benzene ring of sulphonamides produced inactive compounds.
- Substitution of free sulphonic acid (**–SO_3H**) group for sulphonamido function destroys the activity, but replacement by a sulphinic acid group (**–SO_2H**) and acetylation of **N-4** position retains back the activity.
- *Meta-Sulphonamides* bind to the basic centres of arginine, histidine, and lysine sites of proteins. The binding groups are alkyl, alkoxy, and halides. The binding affects the activity of sulphonamides; protein binding appears to modulate the availability of the drug and its half-life.
- The lipid solubility influences the pharmacokinetic and antibacterial activity, and so increases the half-life and antibacterial activity in vitro.

❖ Sulphonamides can be classified in various ways:

1. On the basis of the site of action

(i) ***Sulphonamides for general infection:*** Sulphanilamide, Sulphapyridine, Sulphadiazine, Sulphamethoxacine, Sulphamethoxazole.

(ii) ***Sulphonamides for urinary tract infections***: Sulphaisoxazole, Sulphathiazole.

(iii) ***Sulphonamides for intestinal infections:*** Phthalylsulphathiazole, Succinyl sulphathiazole, Sulphasalazine.

(iv) ***Sulphonamides for local infections:*** Sulpahacetamide, Mafenamide, Silver sulphadiazine.

(v) ***Sulphonamides for dermatitis:*** Dapsone, Solapsone.

(vi) ***Sulphonamides in combination:*** Trimethoprim with Sulphamethoxazole.

2. On the basis of the pharmacokinetic properties

(i) ***Poorly absorbed sulphonamides (locally acting sulphonamides)*:** Sulphasalazine, Phthalylsulphathiazole, Sulphaguanidine, Salicylazo sulphapyridine, Succinyl sulpha thiazole.

(ii) ***Rapidly absorbed and rapidly excreted (systemic sulphanamides):*** Sulphamethoxazole, Sulphaisoxazole, Sulphadiazine, Sulphadimidine, Sulphafurazole, Sulphasomidine, Sulphamethiazole, Sulphacetamide Sulphachlorpyridazine.

(iii) ***Topically used sulphonamides:*** Sulphacetamide, Mafenide, Sulphathiazole, Silver sulphadiazine.

3. On the basis of the pharmacological activity

(i) ***Antibacterial agents:*** Sulphadiazine, Sulfi soxazole.

(ii) ***Drugs used in dermatitis***: Dapsone.

4. On the basis of the duration of action

(i) ***Extra-long-acting sulphonamides*** **(half-life greater than 50 h):** Sulphasalazine, Sulphaclomide, Sulphalene.

(ii) ***Long-acting sulphonamides*** **(half-life greater than 24 h):** Sulphadoxine, Sulphadimethoxine, Sulphamethoxy pyridazine, Sulphamethoxydiazine, Sulphaphenazole, Sulphamethoxine.

(iii) ***Intermediate-acting sulphonamides*** **(half-life between 10–24 h):** Sulphasomizole, Sulphamethoxazole.

(iv) ***Short-acting sulphonamides*** **(half-life less than 20 h):** Sulphamethiazole, sulphaisoxazole.

(v) ***Injectable*** **(soluble sulpha drugs):** Sulphafurazole, Sulphadiazine, Sulphamethoxine.

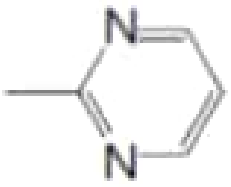
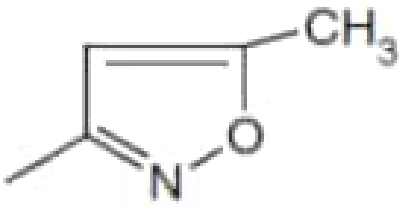
5. On the basis of the chemical structure

(i) ***N-1 substituted sulphonamide:*** Sulphadiazine, Sulphacetamide, Sulphadimidine.

(ii) ***N-4 substituted sulphonamides (prodrugs):*** Prontosil.

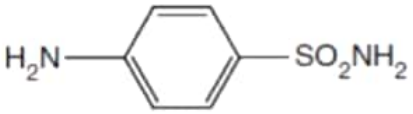
(iii) ***Both N-1 and N-4 substituted sulphonamides***: Succinyl sulphathiazole, Phthalylsulphathiazole.

(iv) ***Miscellaneous:*** Mefenide sodium.

Name	R	R^1
Sulphanilamide	–H	–H
Sulphacetamide	–H	$-COCH_3$
Sulphadiazine	–H	
Sulphamethoxazole	–H	

N-1 and N-4 substituted suphonamic

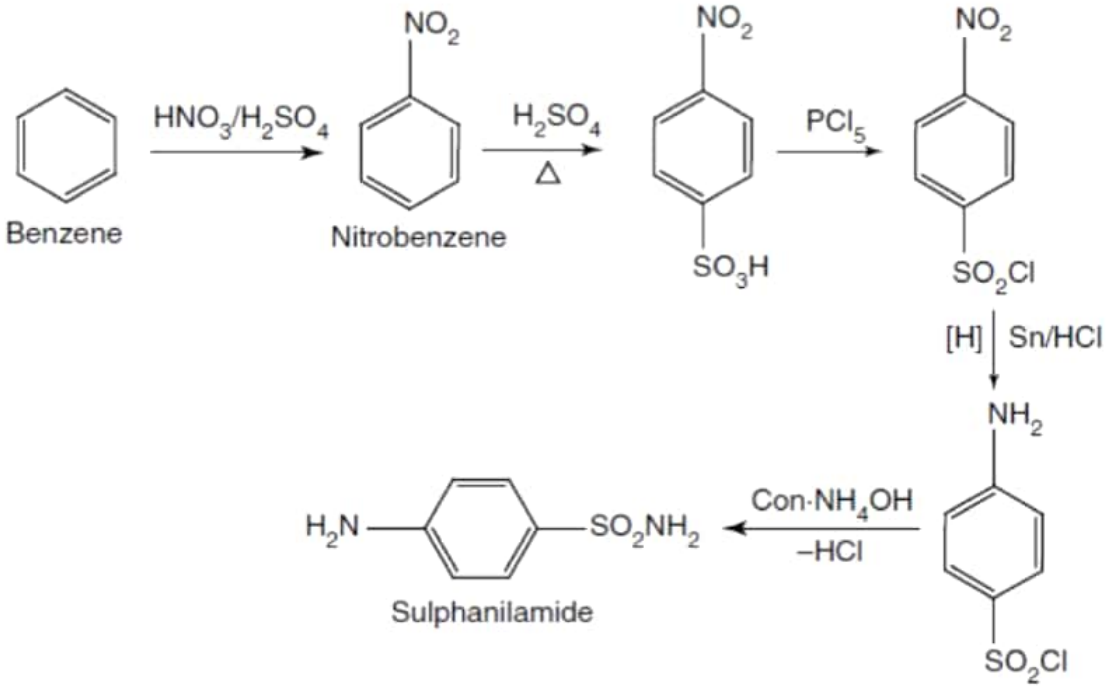
❖ **Synthesis of Sulphanilamide**



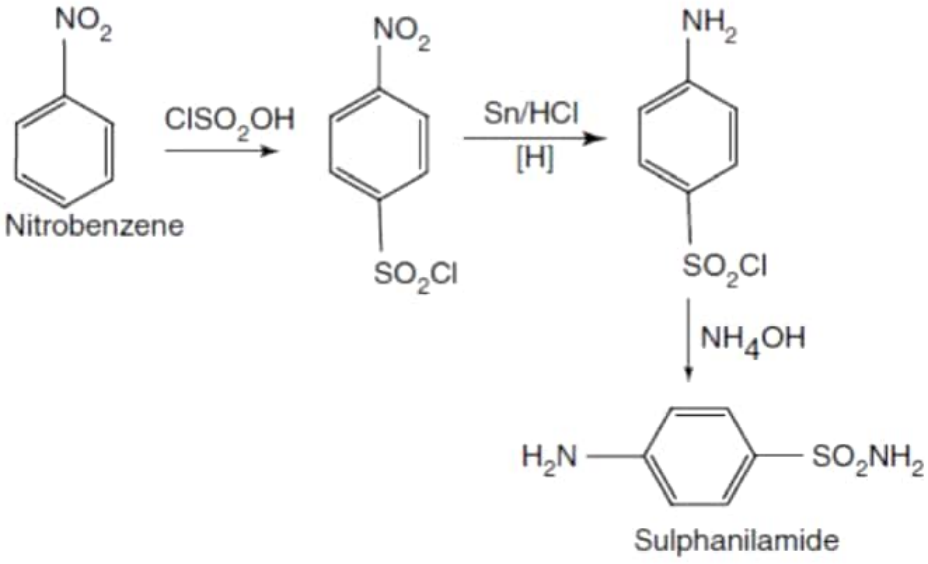
4-Aminobenzene sulphonamide

Synthesis

Route-I. From: Benzene

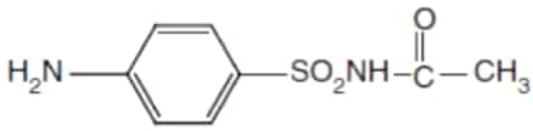


Route-II. From: Nitrobenzene



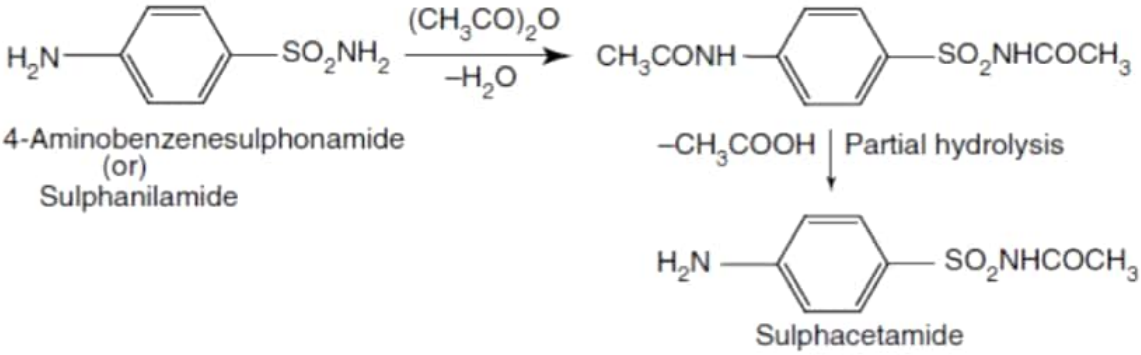
❖ Synthesis of Sulphacetamide

Sulphacetamide



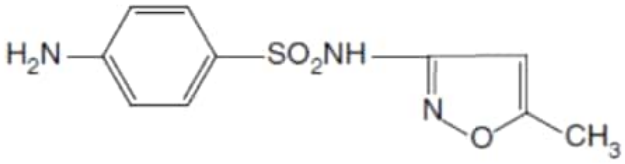
N-Sulphanilyl acetamide

Synthesis



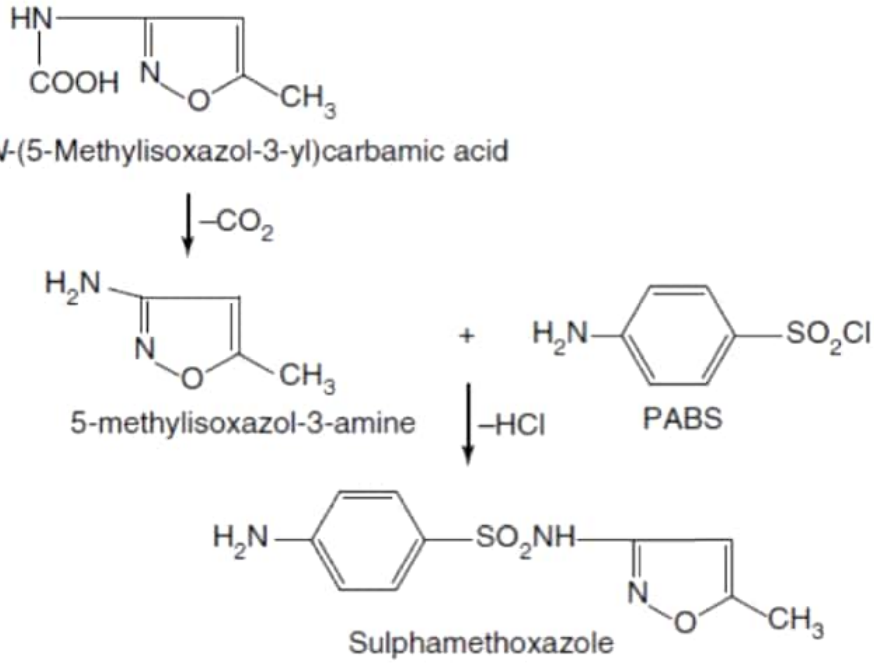
❖ Synthesis of Sulphamethoxazole

Sulphamethoxazole



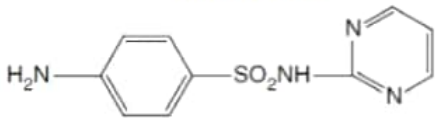
3-(4-Amino benzene sulphamido)-5-methyl isoxazole

Synthesis



❖ Synthesis of Sulphadiazine

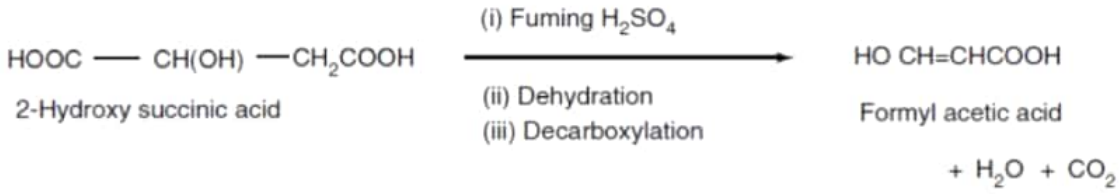
Sulphadiazine



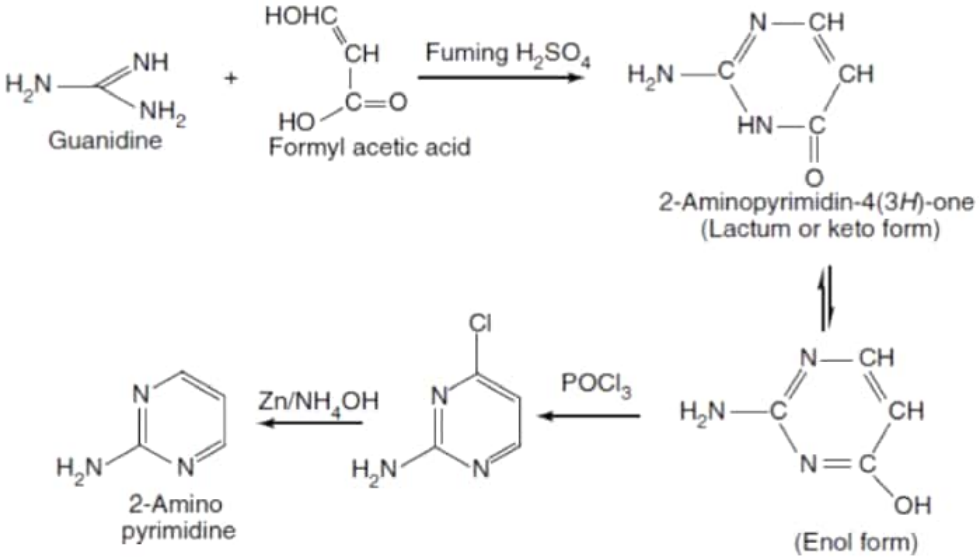
N'-2 Pyrimidinyl sulphanilamide

Synthesis

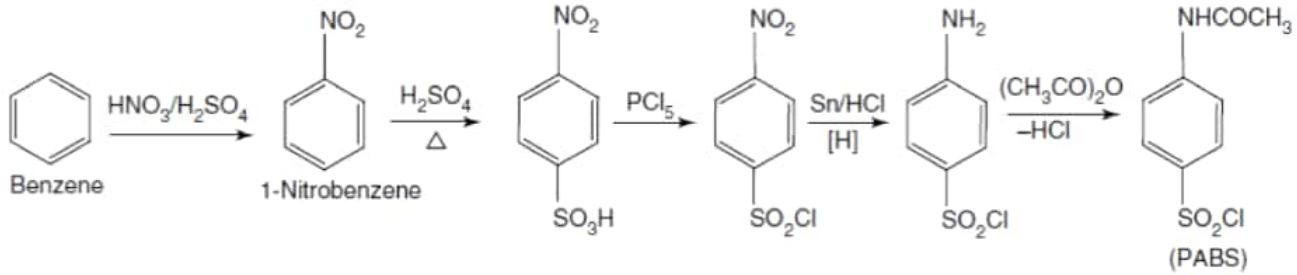
Step-I. Preparation of formyl acetic acid



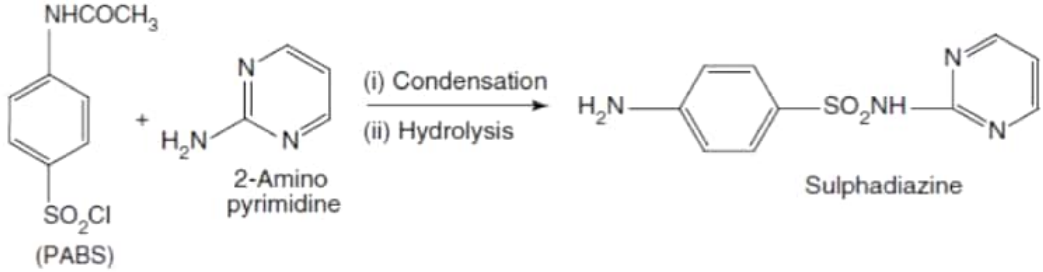
Step II. Synthesis of 2-Aminopyrimidine



Step III. Synthesis of *p*-acetamido benzene sulphonyl chloride (PABS)

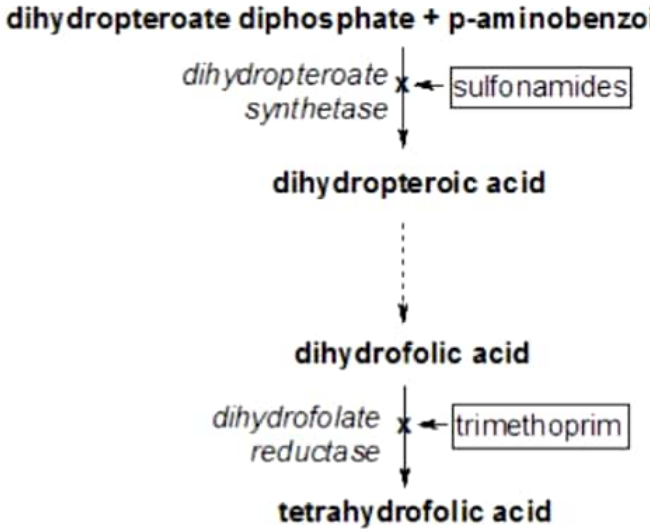


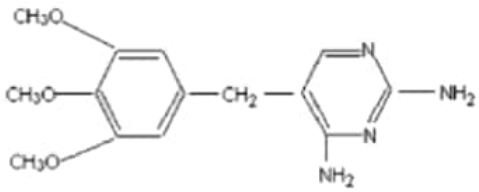
Step IV. Condensation of *p*-acetamido benzene sulphonyl chloride with 2-aminopyrimidine



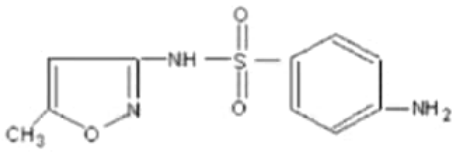
❖ Trimethoprim/Sulphamethoxazole

- Trimethoprim/Sulphamethoxazole (TMP/SMX), also known as co-trimoxazole.
- It is an antibiotic used to treat a variety of bacterial infections.
- It consists of one part trimethoprim to five parts sulfamethoxazole.
- It is used for urinary tract infections, skin infections, travelers' diarrhea, respiratory tract infections, and cholera, among others. It may be used both to treat and prevent pneumocystis pneumonia in people with HIV/AIDS.
- It can be given by orally or intravenously.
- Common side effects include nausea, vomiting, rash, and diarrhea.
- Severe allergic reactions and *Clostridium difficile diarrhea* may occasionally occur.
- Its use near the end of pregnancy is not recommended.
- It appears to be safe for use during breastfeeding as long as the baby is healthy.
- TMP/SMX generally results in bacterial death. It works by blocking the making of folate by the bacteria





trimethoprim



sulphamethoxazole