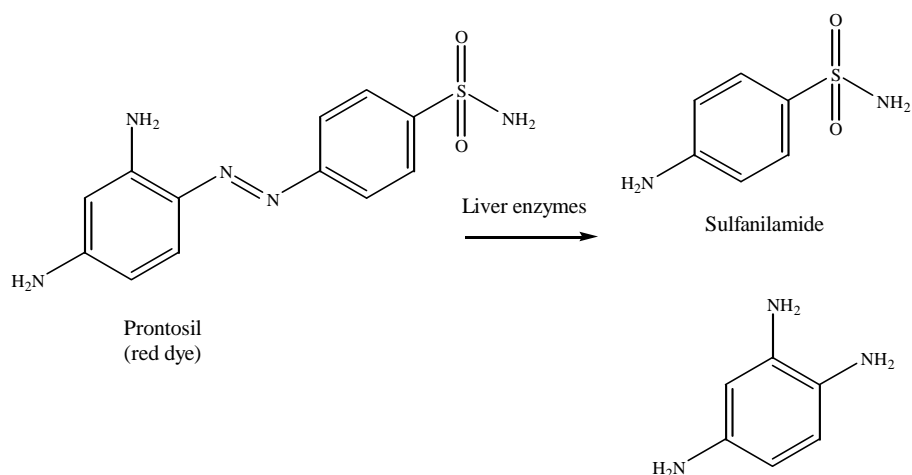


Background Information

History of Sulfonamides

Sulfonamides or “sulfa” drugs are synthetic antimicrobial agents that were discovered in the 1930s. In 1932, Domagk, a scientist working at Bayer in Germany on methods to stain pathogenic bacteria, found the red dye, Prontosil, cured streptococcal infections in mice, but was ineffective in cell culture. A few years later, Trefouel demonstrated that the azo linkage of Prontosil was metabolized *in vivo* to the active drug, sulfanilamide. Between 1935 and 1948, over 4500 sulfanilamide derivatives were synthesized and evaluated for antimicrobial activity, however only about 0.5% of these synthesized compounds have been used clinically. In the 1940s, penicillins began to replace many of the commonly used sulfa drugs due to the toxicity of some of these compounds, and the development of bacterial strains that were resistant to the sulfa drugs. Some sulfonamide antibacterials are still currently used today.



Sulfonamide	Therapeutic Indication
trimethoprim-sulfamethoxazole	Treatment and prophylaxis of pneumonia
pyrimethamine-sulfadiazine	Treatment and prophylaxis of cerebral toxoplasmosis
trimethoprim-sulfamethoxazole	First attack of urinary tract infection
silver sulfadiazine	Prevention and treatment of bacterial infection for burn patients
sodium sulfacetamide	Conjunctivitis and related superficial ocular infections
sulfadoxine and sulfalene in combination with quinine	Chloroquine-resistant malaria

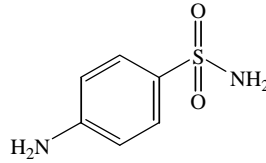
Table 1: Examples of Current Therapeutic Use of Sulfonamides

Sulfanilamide Structure and Mechanism of Action

Sulfanilamide was the first sulfonamide in this class of antimicrobial agents to be discovered, and its structure is considered to contain the minimum or “parent” pharmacophore. A “pharmacophore” is the structural component(s) in a drug molecule necessary for that drug to have biological activity. Sulfanilamide contains a benzene ring, para substituted with an amino group and a sulfonamide group. Second generation sulfa drugs (those developed after sulfanilamide was established as an effective antimicrobial agent) contain the essential pharmacophore. However, the structures of these second generation sulfa drugs have been manipulated to enhance activity, solubility and excretion.

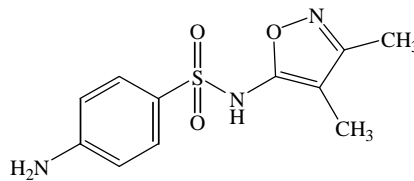
Synthesis of Sulfanilamide

1st generation

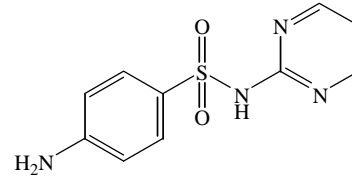


Sulfanilamide

2nd generation

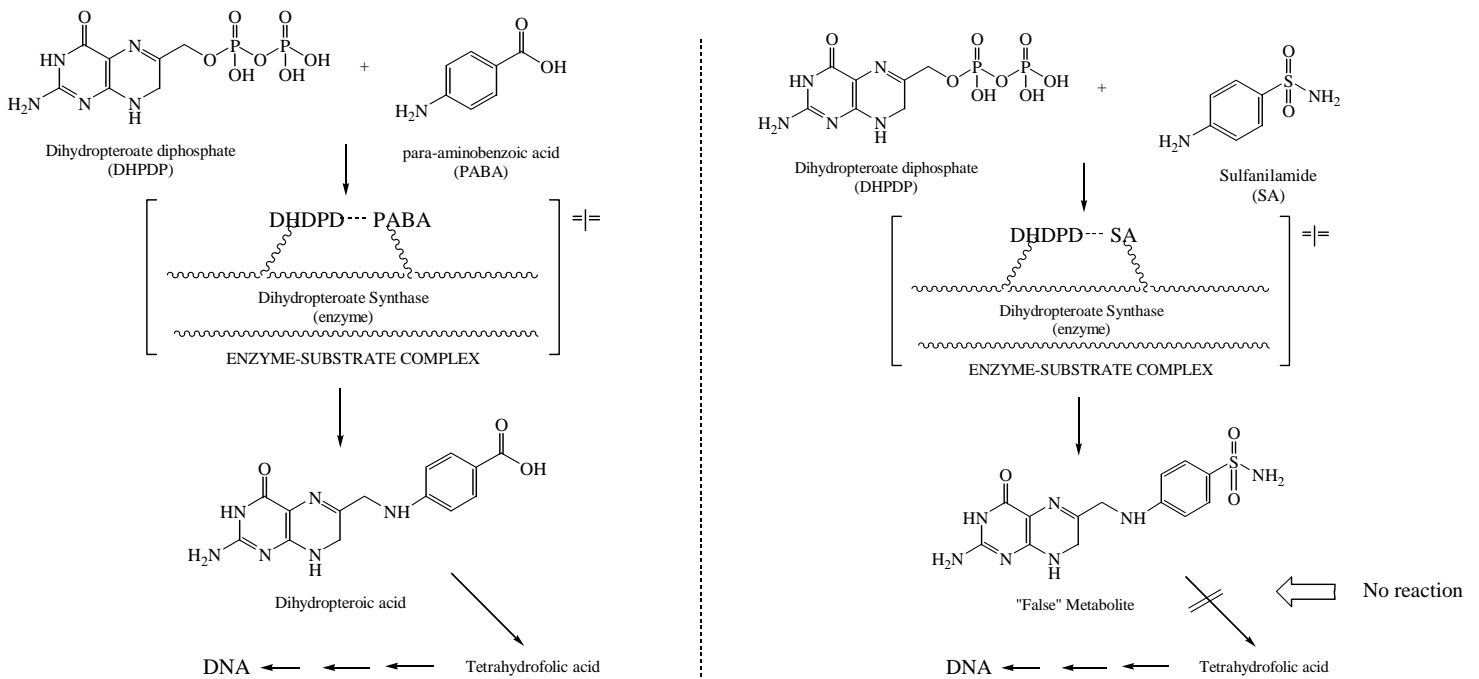


Sulfisoxazole



Sulfadiazine

Sulfonamides are typically administered in doses that are “bacteriostatic, meaning they prevent or limit bacterial multiplication. Sulfonamides achieve this bacteriostatic action (i.e., the mechanism of action) by inhibiting the synthesis of folic acid in bacteria. Bacteria synthesize their own folic acid using endogenous compounds and enzymes. (Endogenous compounds are those that occur naturally in the biological system.). Specifically, sulfonamides inhibit the enzyme dihydropteroate synthase, an enzyme that catalyzes the conversion of para-aminobenzoic acid (PABA) and dihydropteroate diphosphate to dihydropteroic acid, a precursor to folic acid and DNA. Sulfonamides compete with PABA for the “active site” in the dihydropteroate synthase enzyme, and are considered to be “competitive inhibitors” of this enzyme. The structural similarity of the sulfonamides to PABA “tricks” the enzyme into binding with the drug (sulfonamide) instead of the endogenous compound (PABA). The displacement of the PABA by the sulfonamide leads to the formation of a “false” metabolite in the folic acid synthesis which cannot continue on through the synthetic sequence.

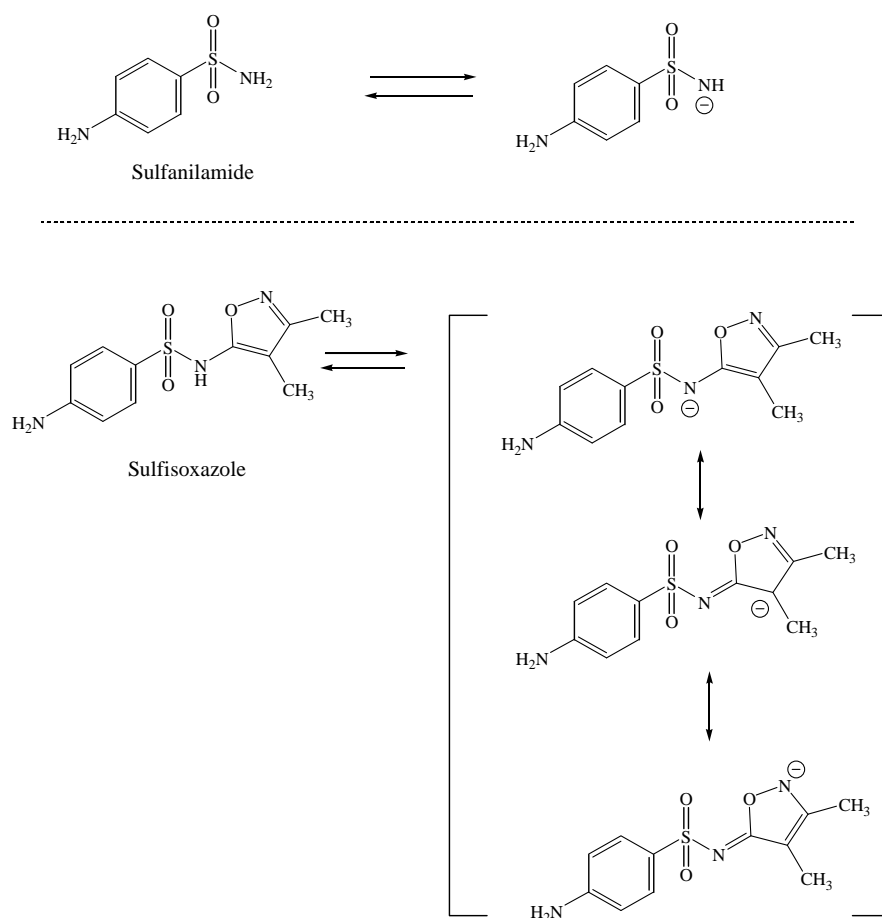


Synthesis of Sulfanilamide

Folic acid is essential for DNA synthesis, thus lack of folic acid will prevent replication that requires DNA. Humans do not synthesize folic acid. Instead, humans obtain folic acid from foods in their diets. Thus, using a sulfa drug to inhibit folic acid will not affect human DNA synthesis, but will affect DNA synthesis in organisms that synthesize their own folic acid.

Structure-Activity Relationships and Toxicity of Sulfonamides

Sulfonamides are effective in inhibiting folic acid synthesis due to their structural similarity to PABA. The common structural elements between the two compounds are the benzene rings, para substitution and an amino substituent. The acidic sulfonamide group mimics the carboxylic acid functional group of PABA. However, the pKa of the sulfonamide group of sulfanilamide is 10.4 where the pKa of the PABA carboxylic acid is ~6.5. Investigators predicted that developing a sulfonamide with a pKa closer to that of PABA would lead to a more effective drug. Indeed, substitution of the sulfonamide nitrogen atom with an electron-withdrawing substituent induced a significant drop in the pKa associated with the sulfonamide functional group. Sulfisoxazole is one of the most widely used sulfa drugs today with a pKa of ~5.0. The drop in pKa is attributable to the resonance stabilization of the sulfisoxazole conjugate base.



Dropping the pKa of the sulfonamide functional group also produced an additional advantage for this class of antimicrobials. The earliest sulfonamides, with high pKas tended to have poor water solubility, particularly in urine, that has a pH of ~6.0. The poor water solubility led to the precipitation of these compounds from urine, resulting in damage to the kidneys. Sulfonamides with lower pKa values are ionized at this pH, and their water solubility is significantly enhanced. Precipitation of these compounds from urine and kidney toxicity is rare for these compounds.

Synthesis of Sulfanilamide

Calculating Percent Yield

Organic reactions typically do not give 100% yields, meaning *all* of the starting material does not get converted to the product. The percent of starting material that is converted to product in a chemical reaction is referred to as the percent yield.

The percent yield can be calculated if the following information is known.

1. Weight of the starting material limiting reagent (*usually in grams or milligrams*)
2. Molecular weight of starting material (*g/mol*)
3. Weight of product (*in grams*)
4. Molecular weight of product (*g/mol*)

The limiting reagent is a starting material that is converted to product. If there are multiple starting materials, the limiting reagent is the reagent that is present in the smallest quantity (in moles not grams) in the reaction. If two or more starting materials are present in equal molar quantities, then any of these starting materials can serve as limiting reagent.

The calculations are done in the following way. An example involving the conversion of acetylsalicylic acid to salicylic acid is given.

1. Identify the limiting reagent. Convert the gram or milligram quantity of limiting reagent used in the reaction to moles.

Example: 5g of aspirin (acetylsalicylic acid) MW = 180.16g/mol

$$5g \text{ aspirin} \times \frac{1 \text{ mol}}{180.16g} = 0.027 \text{ mol}$$

2. Calculate the theoretical yield, the number of grams of product that would form if every mole of limiting reagent were converted 100% to product.

Example: Start with 0.027 moles of aspirin (limiting reagent) going to 0.027 moles of salicylic acid.

$$0.027 \text{ mol salicylic acid} \times \frac{138.12 \text{ g}}{\text{mol}} = 3.72g$$

3. Divide the number of grams of product obtained experimentally, by the number of grams obtained in the theoretical yield calculations and multiply by 100 to calculate the percent yield.

Example: Assume 2.96g of salicylic acid was obtained experimentally.

$$\frac{2.96}{3.72} \times 100 = 79.5\%$$