CHAPTER 1
INTRODUCTION

“Sulfonamide drug” is a common term used for any member of a class of synthetic antibacterial drugs with a particular chemical structure including both sulfur and nitrogen atoms.

Sulfonamide drugs are the first antimicrobial drugs [1] and paved the way for the antibiotic revolution in medicine. The first sulfonamide, trade-named Prontosil, was a prodrug. Experiments with Prontosil began in 1932 in the laboratories of Bayer. The Bayer team believed that coal-tar dyes that are able to preferentially bind to bacteria and parasites might be used to target harmful organisms in the body. Many years of fruitless trial and error work on hundreds of dyes, by a team led by the physician Gerhard Domagk, finally found the solution. A red dye synthesized by Bayer Chemist Josef Klarer had remarkable effects on stopping some bacterial infections in mice. The first official communication about the breakthrough discovery was not published until 1935, more than two years after the drug was patented by Klarer and his research partner Fritz Mietzsch. Bayer named the new drug as Prontosil. It was the first medicine discovered that could effectively treat a range of bacterial infections inside the body. It had a strong protective action against infections caused by streptococci, including blood infections, childbed fever, erysipelas and had a lesser effect on infections caused by other cocci. However, it had no effect at all in the test tube, exerting its antibacterial action only in live animals. Later, a French scientist Ernest Fourneau discovered that the drug was metabolized into two parts inside the body, releasing a smaller, colourless, active compound called sulfanilamide from the inactive dye portion. This discovery helped to establish the concept of “bioactivation” and dashed the German corporation's
dreams of enormous profit. The active molecule sulfanilamide was first synthesized in 1906 and was widely used in dye-making industry.

The result caused a sulfa craze. For several years in the late 1930s, hundreds of manufacturers produced thousands of tons of myriad forms of sulfa. Due to the lack of proper testing, the elixir sulfanilamide compounds faced disaster during 1937. During that period, more than 100 people were poisoned with diethylene glycol. This led to the passage of the Federal Food, Drug and Cosmetic Act in 1938. Since sulfa drug was the only antibiotic available before peniciline, it saved the lives of many people during World War II. Sulfanilamide had a crucial role in preventing wound infections during the war. First-aid kits containing sulfa pills were issued to the American soldiers and powders were told to sprinkle on any open wound. During 1942 to 1943, Nazi doctors conducted sulfanilamide experiments on prisoners in concentration camps. Sulfanilamide was credited with saving the lives of Franklin Delano Roosevelt Junior, the son of President Franklin Delano Roosevelt and Winston Churchill.

Sulfanilamide is more active in the protonated form. The solubility of the drug is very low and crystallizes in kidneys due to its first pKₐ of around 10. This will be a very painful experience and hence patients are advised to take the medication with more amount of water. Newer sulfonamide compounds have a pKₐ of around 5–6, which avoids the above problem of crystallization in the kidney.

Many thousands of molecules containing the sulfanilamide structure have been created since its discovery yielding improved formulations with greater effectiveness and less toxicity. Sulfanilamide is still widely used for conditions such as acne and urinary tract infections and are receiving renewed interest for the treatment of infections caused by bacteria resistant to other antibiotics.

Sulfonamides comprise an important class of therapeutic agents, that are known to exhibit a broad spectrum of biological activities like antibacterial [2], diuretic [3], hypoglycemic [4] etc. A number of sulfonamides have also been screened particularly for their antitumor activity [5] and they have a common
chemical motif of an aromatic/heterocyclic sulfonamide. A large number of structurally novel sulfonamide derivatives have been reported to show substantial antitumor activity \textit{in vitro} and \textit{in vivo} [6].

1.1 MECHANISM OF ACTION OF SULFONAMIDE ANTICANCER COMPOUNDS

There are variety of mechanisms for the anticancer activity of sulfonamides, such as CDK inhibition, PI3K/mTOR, Bcr-Abl, EGFR inhibition, c-MET, ALK inhibition and Carbonic anhydrase inhibition.

1.1.1 CDK Inhibitors

A CDK (Cyclin-Dependent Kinase) inhibitor is a chemical that inhibits the function of CDKs. It is used to treat cancers by preventing over proliferation of cancer cells. Although there are no approved anti-cancer drugs that target CDKs yet, several compounds are on clinical trials now [7, 8]. In many human cancers, CDKs are overactive or CDK-inhibiting proteins are not functional. Therefore, it is rational to target CDK function to prevent unregulated proliferation of cancer cells.

However, the validity of CDK as a cancer target should be carefully assessed because genetic studies have revealed that knockout of one specific type of CDK often does not affect proliferation of cells or has an effect only in specific tissue types. For example, most adult cells in mice proliferate normally even without both CDK4 and CDK2 [9]. Furthermore, specific CDKs are only active in certain periods of the cell cycle. Therefore, the pharmacokinetics and dosing schedule of the candidate compound must be carefully evaluated to maintain active concentration of the drug throughout the entire cell cycle.

Till 2009, there are more than 10 CDK inhibitor compounds that have already gone through the clinical trials. Most of them are targeting multiple CDKs, but some are targeting specific CDKs. For example, P1446A-05 targets CDK4 and PD-0332991 targets CDK4 and CDK6. All compounds are either in phase I or phase II clinical trials. Various types of cancers, including leukemia, melanoma, solid
tumors and other types are being targeted. In some cases, very specific cancer types, such as 'melanoma positive for cyclin D1 expression' are targeted to maximize the efficacy. Sulfonamides in clinical trials [10] are given figure (Figure 1.1).

**Figure 1.1 Structures of R-547 and JNJ-7706621**

Cyclin-dependent protein kinases (CDKs) are key regulators of the cell division cycle, whose various checkpoints proliferating cells must traverse. Since CDK disregulation, either through direct or indirect means is found in most cancer cells, pharmacological CDK inhibition has become an attractive strategy towards mechanism-based and non-genotoxic therapies in oncology. Over the last decade, discovery and lead optimization efforts have provided a wealth of potential drug candidate molecules capable of inhibiting CDKs, blocking cell-cycle progression, modulating transcription and inducing apoptosis selectively in cancer cells. However, only few such agents have reached clinical evaluation.

1.1.2 PI3K/mTOR Inhibitors

Phosphoinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling are the most frequent events observed in solid tumors. The PI3K/mTOR pathway can be activated by overproduction of growth factors or chemokines, loss of INPP4B or PTEN expression, or by mutations in growth factor receptors, Ras, PTEN, or PI3K itself. Activation of this pathway contributes to cell growth, cell cycle entry, cell survival and cell motility, all important aspects of tumorigenesis. Rapamycin analogs have already been shown to have antitumor
efficacy in some tumor types. New generation PI3K, Akt and mTOR inhibitors have shown significant promise in preclinical and are now in clinical trials.

The PI3K/AKT/mTOR pathway is an intracellular signaling pathway important in apoptosis and hence cancers [11-16]. The figure (Figure 1.2) presents the mTOR signaling pathway. PI3K activation activates AKT which activates mTOR ('PDK1' in the figure is Phosphoinositide-dependent kinase-1). In many cancers, this pathway is overactive reducing apoptosis and allowing proliferation. Thus some experimental cancer drugs aim to inhibit the signaling sequence at some point. PI3K may be overactive because PTEN is faulty or deficient.
Sulfonamides [17] (Compound 3, 4; Figure 1.3) in clinical trials are shown below:

![Figure 1.3 Structures of GDC 0941 and Celecoxib](image)

### 1.1.3 Bcr-Abl Inhibitors

**Bcr-Abl** tyrosine-kinase inhibitors (TKI) are the first-line therapy for most patients with chronic myelogenous leukemia (CML). In more than 90% cases, CML is caused by chromosomal abnormality resulting in the formation of a so-called Philadelphia chromosome. This abnormality was discovered by Janet Rowley in 1972 and is due to the fusion between Abelson (Abl) tyrosine kinase gene at chromosome 9 and break point cluster (Bcr) gene at chromosome 22. This results in the chimeric oncogene Bcr-Abl and a constitutively active Bcr-Abl tyrosine kinase that has been implicated in the pathogenesis of CML. Compounds have been developed that selectively inhibit this tyrosine kinase. Before the U.S. Food and Drug Administration (FDA) approval of imatinib in 2001, no drugs were used that changed the natural progression of CML. Even though the first Bcr-Abl TK inhibitor was named as the magic bullet to cure cancer, a second generation of Bcr-Abl TKI has been developed as a cause of resistance to imatinib emerged. New forms of resistance in patients can arise as missense mutations within the Abl kinase domain, over-expression of Bcr-Abl, increased production of transmembrane plasma proteins or constitutive activating of downstream signaling molecules such as
Src-family kinases. Most of the drugs are adenosine triphosphate (ATP)-competitive inhibitors [18]. The common mechanisms of resistance to TKIs is represented in the figure (Figure 1.4).

Figure 1.4 Common Mechanisms of Resistance to TKIs

Figure 1.5 represents the structure of Bcr-Abl molecules available for the therapy.

![Figure 1.5 Structures of Imatinib and Dasatinib](image)

1.1.4 EGFR Inhibitors

Epidermal Growth Factor was discovered by Stanley Cohen of Vanderbilt University along with Rita Levi-Montalcini for which both received Nobel prize in Physiology of Medicine in 1986. The Epidermal Growth Factor Receptor is a member of the ErbB family of receptors, a subfamily of four closely related receptor tyrosine kinases: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). Mutations affecting EGFR expression or activity could result in cancer [19, 20]. EGFR exists on the cell surface and is activated by binding of its specific ligands, including epidermal growth factor and transforming
growth factor α (TGFα). ErbB2 has no known direct activating ligand and may be in an activated state constitutively or become active upon heterodimerization with other family members such as EGFR. Upon activation by its growth factor ligands, EGFR undergoes a transition from an inactive monomeric form to an active homodimer [21]. In addition to forming homodimers after ligand binding, EGFR may pair with another member of the ErbB receptor family, such as ErbB2/Her2/neu, to create an activated heterodimer. There is also evidence to suggest that clusters of activated EGFRs form, although it remains unclear whether this clustering is important for activation itself or occurs subsequent to activation of individual dimers.

EGFR dimerization stimulates its intrinsic intracellular protein-tyrosine kinase activity. As a result, autophosphorylation of several tyrosine residues in the C-terminal domain of EGFR occurs [22]. This autophosphorylation elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains. These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to DNA synthesis and cell proliferation [23]. Such proteins modulate phenotypes such as cell migration, adhesion and proliferation. Activation of the receptor is important for the innate immune response in human skin [24]. The kinase domain of EGFR can also cross-phosphorylate tyrosine residues of other receptors it is aggregated with, and can itself be activated in that manner. The compound effective against EGFR inhibition is shown in the Figure 1.6.

Figure 1.6 Structure of Lapatinib
1.1.5 c-MET Inhibitors

c-Met is a receptor tyrosine kinase [25], which can cause a wide variety of different cancers, such as renal, gastric, small cell lung carcinomas, central nervous system tumors, as well as several sarcomas [26], when its activity is dysregulated. Targeting the ATP binding site of c-Met by small molecule inhibitors is one strategy for inhibition of the tyrosine kinase. c-Met inhibitors are a class of small molecules that inhibit the enzymatic activity of the c-Met tyrosine kinase. These inhibitors may have therapeutic application in the treatment of various types of cancers. c-Met stimulates cell scattering, invasion, protection from apoptosis and angiogenesis [27]. The compounds in clinical trials are shown in the figure (Figure 1.7).

![Figure 1.7 Structures of SU 11274 and MK2461](image)

1.1.6 ALK Inhibitors

ALK inhibitors [28] are potential anti-cancer drugs that act on tumours with variations of anaplastic lymphoma kinase (ALK) such as an EML4-ALK translocation. The most effective compound for ALK inhibition is crizotinib (10) and its structure is presented below in the figure (Figure 1.8).
1.1.7 Carbonic Anhydrase Inhibitors

Carbonic anhydrase inhibitors are a class of pharmaceuticals that suppress the activity of carbonic anhydrase. Their clinical use have been established as antiglaucoma agents, diuretics, antiepileptics, in the management of mountain sickness, gastric and duodenal ulcers, neurological disorders or osteoporosis [29].

Acetazolamide is an inhibitor of carbonic anhydrase. It is used for glaucoma, epilepsy, idiopathic intracranial hypertension and altitude sickness. It can act as a mild diuretic by reducing NaCl and bicarbonate reabsorption in the proximal tubule. However, the distal segment partially compensates for the sodium loss and the bicarbonaturia will produce a metabolic acidosis, further reducing the effect. Compound 11 given below (Figure 1.9) is a typical example for carbonic anhydrase inhibitor.

Methazolamide (Compound 12; Figure 1.10) is also a carbonic anhydrase inhibitor. It has a longer elimination half-life than acetazolamide and is less associated with adverse effects to the kidney.
Dorzolamide (Compound 13; Figure 1.11) is a sulfonamide and a typical carbonic anhydrase II inhibitor. It is indicated for the reduction of elevated intraocular pressure in patients with open-angle glaucoma or ocular hypertension and who are insufficiently responsive to beta-blockers. Inhibition of carbonic anhydrase II in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport.

Topiramate (Compound 14; Figure 1.12) is a weak inhibitor of carbonic anhydrase, particularly subtypes II and IV. It is a sulfamate-substituted monosaccharide, related to fructose. It has been approved by U.S. Food and Drug Administration (FDA) as an anticonvulsant to treat epilepsy, Lennox-Gastaut syndrome and migraine headaches. In rare cases, the inhibition of carbonic anhydrase may be strong enough to cause metabolic acidosis of clinical importance.
1.2 SULFONAMIDES AS ANTIBACTERIAL AND ANTIFUNGAL COMPOUNDS

Chohan and coworkers [30] have synthesized seven new indolenyl sulfonamides (Compounds 15-21; Figure 1.13) by the condensation reaction of indole-3-carboxaldehyde with different sulfonamides such as, sulphanilamide, sulfaguanidine, sulfathiazole, sulfisoxazole, sulfadiazine and sulfamethazine respectively. The synthesized compounds have been used as potential ligands for complexation with some selective divalent transition metal ions, such as cobalt, copper, nickel and zinc. All the compounds have also been assayed for their in vitro antibacterial activity against six pathogenic bacteria, such as Escherichia coli, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Bacillus subtilis and six fungi such as, Trichophyton longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium soloni and Candida glabrata. The results showed that all the compounds exhibit significant antibacterial and antifungal activities.

Patel and coworkers [31] have synthesized novel sulfonamide derivatives (Compounds 22-24; Figure 1.13) and screened the compounds for antibacterial activity against Gram-positive (S. aureus and S. pyogenes) and Gram-negative (P. aeruginosa and E. coli) bacteria, as well as antifungal activities against C. albicans, A. niger and A. clavatus. Some of the compounds were endowed with remarkable antibacterial as well as antifungal activities.
Figure 1.13 Sulfonamides as Antibacterial and Antifungal Agents
1.3 MECHANISM OF ACTION OF ANTIMICROBIAL ACTIVITY OF SULFONAMIDES

Microorganism requires p-amino benzoic acid (PABA) to synthesize dihydrofollic acid, which is required to produce purines and ultimately the nucleic acids. Sulfonamides, the chemical analogs of PABA, are the competitive inhibitors of dihydropteroate synthetase. Sulfonamides, therefore are reversible inhibitors of folic acid synthesis and bacterostatic not bacteriocidal [32].

Sulfonamides inhibit gram-positive and gram-negative bacteria. They also inhibit *E. coli, Klebsiella, Salmonella, Shigella* and *Enterobacter*. Resistance to sulfonamide may develop when bacterial mutations result. The following figure (Figure 1.14) explains the mechanism of PABA:

![Figure 1.14 Mechanism of Sulfonamides](image-url)
1.4 SULFONAMIDES AS DIURETICS

Selvaag [33] has synthesized several sulfonamide diuretics and investigated them for photohemolytic properties in vitro. Photohemolysis was induced in the presence of oral hypoglycemic drugs after exposure to UVA or visible light. UVB alone did not induce phototoxic hemolysis in the presence of the tested drugs. Compared to clinical reports on photosensitivity reactions, the photohemolysis model seems a good predictive model in recognizing potential photosensitizing sulfonamides. Some of the diuretic sulfonamide compounds are given below (Compounds 25-27; Figure 1.15):

![Figure 1.15 Diuretic Sulfonamides](image)

1.5 SULFONAMIDES AS ANTIVIRAL AGENTS

Loh and coworkers [34] have reported that the host factors are the complementary strategy for the development of new antiviral drugs (Compounds 28-30; Figure 1.16). They screened a library of isoxazolidine and isoxazole sulfonamides and found compounds that inhibited HIV-1 infection in human CD4+ lymphocytic T cells with no toxicity at IC90 concentrations. Structure-activity relationship showed that benzyl sulfonamides and a halo-substituted aromatic ring on the heterocycle scaffold were critical for antiretroviral activity. The size and position of the incorporated halogen had a marked effect on the antiretroviral activity.
Boechat and coworkers [35] have synthesized a series of novel 1H-1,2,4-triazol-3-yl benzenesulfonamide derivatives (Compounds 31-34; Figure 1.17) with the aim of developing new antimalarial lead compounds. The authors have also done docking studies to identify the best lead compounds for new antimalarial drug development.
1.7 SULFONAMIDES AS ANTICONVULSANTS

Koller and coworkers [36] have reported a series Quinazoline-2,4-diones (Compounds 35-37; Figure 1.18) with sulfonamide group as a novel class of competitive AMPA receptor antagonists. One of the synthesized compounds, compound 35 shows nanomolar receptor affinity, whereas other compounds of the series display oral anticonvulsant activity in animal models.

![Figure 1.18 Anticonvulsant Sulfonamides](image1)

1.8 SULFA DRUGS IN THE MARKET

1.8.1 Antibiotics

Sulfamethoxazole and Sulfisomidine (Compound 38, 39; Figure 1.19) are the popular sulfonamide antibiotic drugs available in the market.

![Figure 1.19 Sulfamethoxazole and Sulfisomidine](image2)
1.8.2 Ophthalmologicals

Dichlorphenamide and Dorzolamide (Compound 40, 41; Figure 1.20) are the sulfonamide drugs which are used as ophthalmologicals.

![Dichlorphenamide and Dorzolamide](image)

**Figure 1.20 Sulfur Drugs – Ophthalmologicals**

1.8.3 Diuretics

There are many popular sulfonamide diuretic drugs available in the market and some of them are Acetazolamide, Bumetamide, Clopamide, Furosemide, Indapamide and Xipamide (Compound 42-47; Figure 1.21).

![Various Diuretics](image)

**Figure 1.21 Sulfur Drugs – Diuretics**
1.8.4 Anticonvulsants

The sulfonamide anticonvulsant drugs available in the market are Acetazolamide, Ethoxzolamide, Sultiam and Zonisamide (Compound 42 and 48-50; Figure 1.22).

\[ \begin{align*}
\text{Acetazolamide} & \quad \text{Zonisamide} \\
\text{Sultiam} & \quad \text{Zonisamide}
\end{align*} \]

Figure 1.22 Sulfa Drugs – Anticonvulsants

1.8.5 Dermatological

The most popular dermatological drug is Mafenide (Compound 51; Figure 1.23).

\[ \begin{align*}
\text{Mafenide}
\end{align*} \]

Figure 1.23 Sulfa Drug – Dermatological
1.8.6 **Anti-inflammatory**

Celecoxib (Compound 52; Figure 1.24) is the popular anti-inflammatory sulfonamide drug.

![Celecoxib](image)

**Figure 1.24 Sulfa Drug – Anti-inflammatory**

1.8.7 **Antiviral**

The most popular sulfonamide drug for the treatment of HIV infections is Darunavir (Compound 53; Figure 1.25).

![Darunavir](image)

**Figure 1.25 Sulfa Drug – Antiviral**

1.8.8 **Rheumatoid Arthritis**

Sulfasalazine (Compound 54; Figure 1.26) is the most popular drug used for Rheumatoid Arthritis.
1.8.9 Gout and Hyperuricemia

The most popular drug for Gout and Hyperuricemia is Probenecid (Compound 55; Figure 1.27).

1.8.10 Migraine Headache

Sumatriptan (Compound 56; Figure 1.28) is the drug available in the market for migraine headache.