Chapter 1
Introduction

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1.1. X-ray Crystallography and Drug Design

X-ray crystallography is a method of determining the arrangement of atoms within a crystal, in which a beam of X-rays strikes a crystal and diffracts into many specific directions. The angles and intensities of these diffracted beams produce a three dimensional picture of the density of electrons resulting into atomic positions, chemical bonds, disorder if any and informations regarding the inter and intra molecular interactions therein **[1]**. It produces high-resolution 3D structures. Griffin et al. stated that "X-ray crystallography has become the sine qua non for elucidating the 3-dimensional structures of biologically interesting large and small molecules, providing the well-known picture that is worth a thousand words" **[2]**. Results from X-ray crystallographic studies provide unambiguous, accurate, and reliable 3-dimensional structural parameters, at time even before complete chemical characterization is available. In addition, crystallography is the only method for determining the "absolute" configuration" of a molecule, a critical property in biological system as changes in this may alter the response of the biologic system.

The results of crystallographic studies allow pharmacophore parameters to be calculated from the 3-dimensional coordinates. These can be used along with data on biological activity to guide future development. The use of 3-dimensional data allows comparison of the relative position of groups thought to be important in binding to the receptor even in structurally dissimilar compounds. As it is well recognized fact that pharmaceutical action of a drug molecule is an involved function of its structure, a small change in the structure can alter its pharmaceutical action drastically and it is therefore, very essential to acquire the knowledge of the structure of the molecule to modify the drug molecule in terms of its effectiveness, selectivity and mode of action leading to predict a safer and more effective drug.

In this context author has attempted to determine the structures of few sulfonamide derivatives especially of novel N-substituted sulfonamide derivatives by X-ray diffraction technique and DFT studies have been carried out using Schrodinger software to compare experimental result (by X-ray) with theoretically predicted optimized structure. In order to understand the role of intermolecular interaction in stability of crystal structure, PIXEL energy calculation and Hirshfeld surface analysis have been studied. To correlate the structure-function relationship, antimicrobial activity and molecular docking study have been carried out.

1.2. Introduction to Sulfonamides

Sulfonamides are defined by Paul Ehrlich, long time ago, as: the use of chemical agents to harm an attack organism or cell without harming the host. Sulfonamide, commonly known as 'Sulfa drugs' discovered in 1930s, are the first effective 'chemotherapeutic' agents which are employed systematically for the prevention and cure of bacterial infections in human beings. There are thousands of sulfonamide-based groups of drugs. The original antibacterial sulfonamides are synthetic antimicrobial agents that contain the phenyl – SO_2NH- group. After the introduction of penicillin and other antibiotics, the populations of sulfonamides decreased. However, they are still considered useful in certain therapeutic fields, especially in the case of ophthalmic infection in urinary and gastrointestinal tract **[3]**. The evolution of chemotherapy can be broadly divided into three eras:

1. Pre-Ehrlich era before 1891.
2. The period of Paul Ehrlich-the father of chemotherapy.
3. The period after 1935-highlighting the discovery of 'sulfonamides'

Sulfonamide is an antibacterial consisting of any of several synthetic organic compounds capable of inhibiting the growth of bacteria that require PABA (para-amino benzoic acid) **(Figure 1.1)** which is structurally similar to sulfonamide **(Figure 1.2)**.

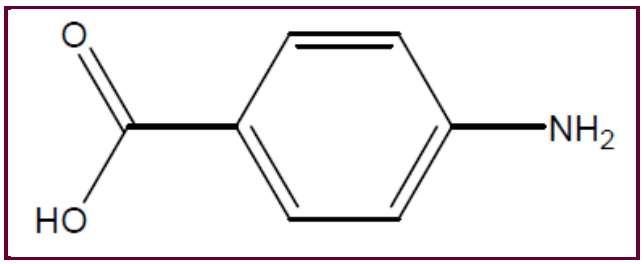


Figure 1.1 Structural formula of PABA

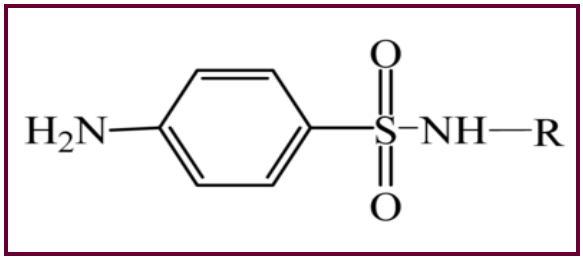
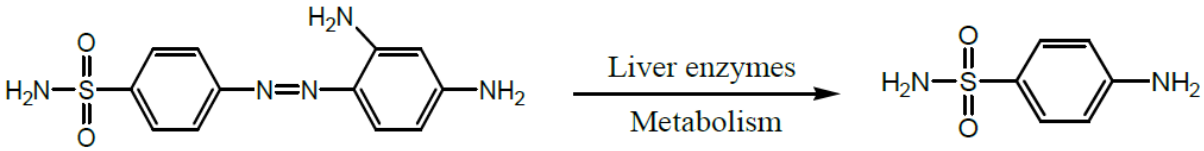


Figure 1.2 Structural formula of sulfonamide

The substituent –NH2 group has variable effect **[4]** on the antibacterial activity of the molecule.

1.3. History of Sulfonamides

Exactly in 1935, Gerhard Domagk discovered a red dye, 4'-sulfamyl-2,4- diaminoazo-benzene, which is later named prontosil **[5]**. Trefouel made the important observation that the antibacterial activity was not due directly to prontosil, but rather to a metabolite formed in the animal by the reduction of the diazyl bond of the prontosil **[6]**. This metabolite was identified as sulfanilamide in 1939 (Scheme I), Domagk was awarded the Nobel prize in medicine for his classic discovery of what was termed in 1940 "the only known chemicals capable of curing serious systemic bacterial infections in man in doses allowing a satisfactory margin of safety" **[7]**. *Domagk's* discovery quickly resulted in the development of a variety of sulfonamides, all of which were essentially substituted sulfanilamide. Sulfa drugs were found to be effective against such grave bacterial infections as meningitis, pneumonia and blood poisoning, and saved thousands of lives in World War II (1939-1945).



Prontosil Sulfanilamide

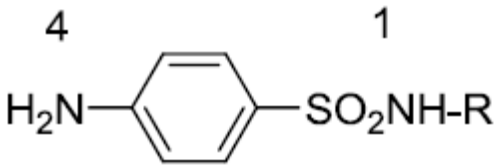
Scheme 1 Structural formulas of Prontosil and its metabolite in human body sulfanilamide

The molecule of sulfanilamide is a simple one. In order to come up with potent derivatives of this molecule, three effective approaches have been generalized (1) modification of the sulfonamide group (2) modification of the amino group, or (3) simultaneous modifications of both groups. Using this strategy, a number of molecules with considerably less toxicity and better formulations have surfaced over the years.

Several drugs containing sulfonamide functionality are in clinical use which covers carbonic anhydrase inhibitors **[8-9]**, antibacterial and antifungal drugs **[10-11],** anticonvulsant agents **[12]**, antimigraine agents **[13]**, anti-inflammatory agents **[14]** and agents acting against diabetic mellitus **[15]**. Some sulfonamides have proved to be useful as herbicides **[16]** and fungicides **[17]** and are also found to be useful as antitumor **[18]**.

1.4. Classification of Sulfonamides

The general term ‘’sulfonamides’’ has been used for derivatives of *p*-amino benzenesulfonamide (sulfanilamide), whereas specific compounds are described as N1 or N4-substituted sulfanilamide, depending on whether the substitution is on the sulfonamide amino group or aromatic amino group, respectively.



The classification of sulfonamides is based on chemical structure, duration of action, spectrum of activity and therapeutic applications. The classification rate of absorption and half-life appears to be clinically relevant **[19]**.

Classification of Sulfonamides based on half life

Short Acting	Intermediate Acting	Long Acting
Half life less than 10 hours e.g. Sulfacetamide, sulfisoxazole,sulfadiazine which are being used for the treatment of urinary tract infections.	**Half life between 10-20 hours** e.g. Sulfamethoxazole, sulfadoxine, sulfadimidine have been used for various infections especially active against invasive aspergillosis in AIDS patients.	**Half life more than 24 hours** e.g. Sulfamonomethoxine and sulfamethoxypyridazine have been used for the treatment of ulceration

Further classification of N1 derivatives based on the structural variations is given by Johnson **[20]**.

Classification based on structural variations

NI-acyl derivatives e.g. Sulfacetamide	NI-heterocyclic derivatives containing six-membered rings e.g. pyridine, pyrimidines, pyridazines and pyrazines	NI-heterocyclic derivatives containing five-membered rings (e.g. thiazole, oxazole, isoxazole, 1,3,4-thiadiazole and pyrazole).

1.5. Mechanism of Antibacterial Action

The activity of the sulfa drugs has been extensively studied and can be explained in the following manner. Sulfonamides are typically administered in doses that are bacteriostatic **[21, 22]**, 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 p-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 through the synthetic sequence. Folic acid is essential for DNA synthesis, thus lack of folic acid will prevent replication that requires DNA. Humans do not synthesize folic acid. Thus, the hosts of these bacteria needed ready-made folic acid in their diets, and the hosts are unaffected by sulfonamides. This difference between bacteria and animals gives sulfonamides the properties of the magic bullet that would hit the target without harming the host. Therefore, Sulfonamides act as antimicrobial agents by inhibiting bacterial growth and activity. Some recent classes of sulfonamides and related sulfonyl derivatives disclosed as effective antibacterial agent, A. M. Badawi et al. **[23]** has synthesized and studied series of cresols disulfonamide derivatives and evaluate their antibacterial activity. More research develops to look upon different sulfonamides with noticeable antibacterial activity.

1.6. Applications of Sulfonamides

The discovery of sulfanilamide leads to the development of all these types of pharmacological agents that have a wide variety of biological action. From 1950s, more potent high-ceiling diuretics have been developed as well as to the systemic antiglucoma drugs such as Acetazolamide, Methazolamide, Ethoxzolamide and Dichlorophenamide. Moreover, the antibacterial agent Sulfathiazole, the carbonic anhydrase inhibitor Acetazolamide (clinically used for more than 45 years) **[24]**, the widely used diuretic Furosemide, the hypoglycemic agent Glibenclamide, the anticancer sulfonamide Indisulam (in advanced clinical trials), the aspartic HIV protease inhibitor Amprenavir used to treat AIDS and HIV infection and the metalloprotease (MMP) inhibitors of the sulfonyl amino acid hydroxamate type **[25-27]**.

Antitumor Activity: A large number of structurally novel sulfonamide derivatives have ultimately been studied to show substantial antitumor activity in vitro and in vivo **[28-33]**. Although Sulfonamides have a common chemical motif of aromatic / heterocyclic or

amino acid sulfonamide, there are a variety of mechanisms of their antitumor action, such as cell cycle perturbation in the G1 phase **[34, 35]**, carbonic anhydrase inhibition **[36-38]**, disruption of microtubule assembly and angiogenesis (matrix metalloproteinase, MMP) **[39]** inhibition among others. More researches are established to synthesize sulfonamide derivatives and study their antitumor activity. Z. Huang et al. **[40]** has obtained a novel kind of antitumor drug sulfonamide derivatives with low toxicity from parent compound, Sulfapyrazine has been shown to concentrate selectively in the Walker carcinoma growing in rats **[32]**.

Anticancer Activity: Another work was concerned with the structure-activity relationships for a series of potent, systemically available as matrix metalloproteinase, MMP and was studied by Sellarajah et al. **[41]**. They synthesized series of biphenyl bis-sulfonamide derivatives from the readily available biphenyl-4,4`- disulphonyl chloride and the appropriate amine utilizing DMAP and pyridine and evaluated for the treatment of different types of cancer.

Antimalarial Activity: A new series of benzene and isoquinoline sulfonamide derivatives were synthesized and evaluated for antimalarial activity against Plasmodium falciparum in vitro by M. Kumar Parai et al. **[42]**. Antimalarial activity of several benzene sulfonamides was reported **[43-48].**

Anti-AIDS Activity: Study that is more interesting, introduced certain biphenyl bissulfonamide of naphthalene sulfonic acid analogues by Prem Mohan et al. **[49]** as potential anti-AIDS agent. These compounds have been synthesized and evaluated for their inhibitory effect on HIV-1- and HIV-2- induced cytopathogenicity. Zhijian Zhao et al. **[50]** introduced novel indole-3-sulfonamides as potent HIV non-nucleoside reverse transcriptase inhibitors.

Other Activities of Sulfonamides: Sulfonamides have wide variety of biological activities. More recent researches are directed to evaluate antithyroid activity, insulin-releasing antidiabetic **[51],** ulcerative colitis can be treated by sulfasalazine (salazopyrin), where mesalazine is joined to a sulfapyridine, and acts as an anti-inflammatory drug in the treatment of rheumatic fever and rheumatoid arthritis **[52-56]**. Similarly, dermatitis herpetiformis, a skin disorder, is treated with sulfapyridine and sulfones **[57]**. Reaction of aromatic/heterocyclic sulfonamides containing free amino, imino or hydrazino with series of amino acids, some dipeptides and tripeptides were performed by C. T. Supuran et al.

[58] and they evaluate carbonic anhydrase inhibition and antiglucoma activity giving very promising experimental data. The clinical use has been established as antiglucoma agents, diuretics, and antiepileptic in the management of mountain sickness, gastric and duodenal ulcers, neurological disorders or osteoporosis **[59-61]**. Schneider et al. **[61]** and Sprengeler et al. **[62],** studied the biocidal action of some aromatic sulfonamide derivatives which prove that sulfonamides are used successfully for the biocide action. B.N. Herbert **[63]** introduced various compounds known to possess microbiocidal properties including sulfonamide derivatives and some fatty acid salts of alkyl diamines.

1.7. Transition Metal Complexes

Transition metal complexes have attracted attention of inorganic, metallo-organic as well as bio-inorganic chemists because of their extensive applications in wide ranging areas from material to biological science. Biologically relevant metal complexes have several requirements in terms of their synthetic design. First, a biologically active metal complex should have a sufficiently high thermodynamic stability to deliver the metal to the active site. The metal-ligand binding should be hydrolytically stable. The kinetics with which the metal ion undergoes ligation or deligation reactions is of great importance. The molecular weight of the metal complex is also critical. The compounds of low molecular weight with neutral charge and some water solubility are soluble in almost any medium and may slip through biological membranes by passive diffusion. The introduction of metal ions into chemotherapy agents with the aim of increasing their efficacy has been an extensive research for more than three decades since the discovery of Cis-platin **[64-66]**. In the search for novel therapy against resistant organism, the modification of existing drug by combination to a metal centre has gained attention in recent years **[67]**. Metal complexes modify the pharmacology and toxicology of the sulfonamide based ligands **[68]**. The emergence of multidrug resistance to almost all known antibiotics makes the discovery of new agents a crucial necessity. Looking towards the importance of metal complexes of sulfonamides **[69-71**], author has worked out on the coordination of metal (silver) to different sulfonamides to obtain more active agents. The ability of the sulfonamides to act as ligand is based upon the acidity of the $–SO_2NH–$ function, allied with the presence of vicinage nitrogen atoms of the substituents as potential coordination sites **[72-74]**.

Heavy metals in traces are essential for all forms of life. They are taken up by the living cells as cations. Heavy metals like copper Cu(II), iron Fe(II), molybdenum Mo(II), cobalt Co(II) and occasionally manganese Mn(II) assist oxidation-reduction equilibrium while

those like zinc Zn(II), magnesium Mg(II) and manganese Mn(II) are concerned with hydrolytic processes . Some metal complexes are known to exhibit remarkable antitumor, antiviral and special biological activities **[75]**. Most of heavy metals play a vital role as co-factors for many important enzymatic reactions in human body. However, coordination metal complexes are gaining increasing importance in the design of respiratory, slow release and long acting drugs. Metal ions are therefore known to accelerate drug action. The efficacies of some therapeutic agents are known to increase upon coordination **[76]**. It is evident that the sulfonamides behave as bidentate ligands through the N-sulphanamido and the N-heterocyclic atoms **[77-80]**. Silver is present in the human body at very low concentrations (<2.3 μg l-1) and is absorbed through the lungs, gastrointestinal tract, mucus membranes and the skin **[81]**. It can be tolerated at high concentrations within the body, does not appear to be a cumulative poison and is eliminated from the body through the urine [**81]**. It is absorbed mainly in the form of silver protein complexes but has no physiological or biochemical role within the body **[82]**.

Silver and its complexes have long been used as antimicrobial agents in medicine. Silver is active at low concentrations and has a low toxicity. Silver sulfadiazine is a widely used broad-spectrum antibiotic ointment, effective against a broad range of bacteria and some yeast. It is used to prevent and treat skin infections on the areas of burnt skin. Silver complexes of oxygen donor ligands such as, [Ag(hino)]2 (where hino=4-isopropyltopolone) and water-soluble silver(I) complexes of 2-pyrollidone-2-carboxylic acid displayed wide ranging and effective activities against some bacteria, yeasts and moulds **[83-84].** It has been found that the silver–oxygen bonding properties rather than the chiral helical or achiral polymer structure play a role in exhibiting antimicrobial activities. The magnitude of antimicrobial properties of silver complexes is related to the ease with which they participate in ligand exchange reactions. For example, it has been speculated that the weak Ag–O and Ag–N bond strength might play an important role in exhibiting wider spectrum of antimicrobial and antifungal activities and that the potential target sites for inhibition of bacterial and yeast growth by silver complexes might be the sulfur containing residues of proteins. Generally, Ag–S complexes have been shown to have a narrower spectrum of antibacterial activity than Ag–N complexes but no antifungal activity. Antimicrobial activities have been observed for sulfur bonded silver complexes of 2- mercaptonicotinic acid, $[Ag(Hmna)]_6 \cdot 4H2O$ (Hmna = 2-mercaptobenzoic acid), $[Ag(Hmba)]_n$ and $[Na\{Ag(Hmba)\} \cdot H2O]_n$ (Hmba=2-mercaptobenzoic acid) **[85-86]**. The key factor determining the spectra of antimicrobial activity is the nature of atom

coordinated to the silver(I) atom and its bonding properties, (i.e., the ease of ligand replacement), rather than the solid state structure, solubility, charge and degree of polymerization of the complexes.

1.8. Polymorphism

Polymorphs are crystalline solids with the same chemical composition but with different arrangements and/or conformation of the molecules in a crystal lattice. When polymorphism exists as a result of difference in crystal packing, it is called packing polymorphism. Polymorphism can also result from the existence of different conformers of the same molecule in conformational polymorphism. In pseudo polymorphism the different crystal types are the result of hydration or solvation. This is more correctly referred to as solvomorphism as different solvates have different chemical formula. The discovery and characterization of polymorphs are important in various fields, because different polymorphs exhibit significantly different physicochemical properties. In the pharmaceutical field, for example, the sudden appearance of a more stable polymorph which is not discovered at the early stage of pharmaceutical development can cause loss of time and resources **[87]**. Solvent-mediated polymorphic transformation is an efficient method to prepare more stable polymorphs **[88-89]**. It is found in literature that there are few well known sulfa compounds which exist in different crystalline form (Polymorph). e.g sulfathiazole**[90]**, sulfachloropyridiazine **[91-92]**, sulfadimethoxine **[93]**, sulfapyridine **[94-97]**, sulfasalazine**[98-99]**, sulfameter**[100]** and sulfamonomethoxine (present Study).

1.9. Quantitative Analysis of Sulfonamide

1.9.1. Ab-initio Calculation

The term “Ab initio” is a latin word which means “from the beginning”. This name is given to computation which is derived directly from theoretical principles, with no inclusions of experimental data. Most of the time this is referring to an approximate quantum mechanical calculation **[1]**. It requires a large amount of numerical computation. The term Ab initio was first used in quantum chemistry by Robert Parr and coworkers, in a semi empirical study on the excited states of benzene **[101]**. Its modern meaning is 'from first principles of quantum mechanics', the term was used by Chen **[102]**. The simplest or most common type of Ab initio electronic structure calculation is called Hartree-Fock calculation. Hartree-Fock method is central starting point for most Ab initio quantum chemistry method. This method offer variety of basis sets of varying complexity as the

basis set size increased, the energy and wave function tend towards a limit called Hartree-Fock limit. As the size of the atom or molecule increases the time required for computing also increases. Ab initio calculation can be used to determine bond length and bond angle of molecules by calculating the total energy of the molecule for a variety of molecular geometries and determining the lowest energy conformation.

In computational physics and chemistry, the Hartree-Fock Method is an approximate method for the ground state energy of a quantum many body system. This method is also known as self consistent method. For most Ab initio quantum chemistry methods, it is the central starting point. In HF calculation, the wave function must be described by some functional form. The functions used are linear combination of Slater type orbital or Gaussian type orbital. Therefore, most of the HF calculations give the computed energy greater than the Hartree-Fock limit **[103].** There are two types of the Hartree-Fock methods:

1. Restricted Hartree-Fock method (RHF)
2. Unrestricted Hartree-Fock method (UHF)

In RHF method the atom or molecule is a closed shell system with all atomic or molecular orbital doubly occupied. In UHF method the atom or molecule is an open shell system where some electrons are not paired. The Hartree-Fock method is typically used to solve the time independent Schrodinger equation for a multi-electron atom or molecule as described in the Born-Oppenheimer approximation.

1.9.2. DFT Calculations

Density functional theory (DFT) is a quantum mechanical modeling method used in Physics and Chemistry to investigate the electronic structure (principally the ground state) of many-body system, particularly of atoms, molecules and the condensed phases. With this theory, the properties of a many-electron system can be determined by using functional, i.e. functions of another function, which in this case is the spatially dependent electron density. Hence the name density functional theory comes from the use of functional of the electron density. DFT is among the most popular and versatile methods available in Condensed Matter Physics, Computational Physics and Computational Chemistry. DFT has been very popular for calculations in Solid State Physics since the 1970s. However, DFT is not considered accurate enough for calculations in quantum chemistry until the 1990s, wherein the approximations used in the theory based on exchange and correlation interactions **[1]** to obtain a better model. The B3LYP

approach **[104-106]** belongs to the hybrid approximations for the exchange–hybrid correlation functional. The approximation is famous, because it gives very good result and therefore, is extremely popular. The distinguishing feature of such hybrid approximations is that they mix in a certain amount of the exact Hartree–Fock exchange energy into the exchange and correlation obtained from other functional. In the present study B3LYP **[107-108]** method is used for quantum chemical calculation with suitable basis set.

1.9.3. Application of DFT and Ab initio Energy Calculation

It is capable of predicting many properties of molecules and reactions, in the gas phase and solution, including molecular energies and structures, Bond and reaction energies, Molecular orbital, Atomic charges, Multipole moments, Polarizabilities and hyperpolarizabilities-both static and frequency dependent, Electrostatic potentials and electron densities. To correlate the experimental data with theoretical data, it is felt worth to work out the optimized geometry of the molecule by calculating the bond length and bond angle, HOMO-LUMO energy and Mulliken charge distribution.

1.9.4. Lattice Energy Calculation

PIXELC calculation, as incorporated in the CLP program, is performed to determine intermolecular interaction energies and lattice energy **[109]**. PIXEL calculations allow the analysis of lattice and intermolecular interaction energies between pairs of molecules in terms of coulombic, polarization, dispersion and repulsion contribution. The total PIXEL energy, which is the sum of these four energy contributions, gives an indication of the overall interaction energy for a particular dimer and for crystal packing. However, it is the separation of these energies into the four different terms that makes the PIXEL method a powerful tool for crystal structure analysis. It is a theoretical approach for the evaluation of intermolecular potentials. The fundamental assumption is that any intermolecular potential can be subdivided into a coulomb-polarization term, a dispersion term (London) and a repulsion term (depending on overlap, Pauli). The method has been developed and calibrated mainly for organic compounds containing C, H, N, O, S, Cl; it can also be applied to Br, I, P, Si, B. The CLP approach is implemented in two forms

a) The atom-atom form, AA-CLP: potentials depend on distance between atomic nuclei, and are totally empirical. The evaluation of the lattice energy of a large crystal takes less than one second, and the interaction energy of a molecular dimer perhaps a millisecond. Atom-atom potentials are a useful option for preliminary screening of large databases or

quick calculations, and are the only option for crystal structure generation and for Monte Carlo simulations **[110]**.

b) The PIXEL form: intermolecular energy is calculated as a numerical integral over a large number (20,000 for a typical medium-size organic molecule) of electron density pixels, obtained from a standard molecular orbital calculation. The method requires one Ab initio molecular orbital calculation, to prepare the molecular electron density.

1.10. Hirshfeld Surface Analysis

The Hirshfeld surface **[111]** of a molecule in a crystal is constructed by partitioning space in the crystal into regions where the electron distribution of a sum of spherical atoms for the molecule (the promolecule) dominates the corresponding sum over the crystal (the procrystal). Following Hirshfeld, we define a molecular weight-function w(r):

$$w(r) = \frac{\sum_{A \in molecule} \rho_A(r)}{\sum_{A \in crystal} \rho_A(r)}$$

ρA(r) is a spherically-averaged atomic electron density centred on nucleus A, and the promolecule and procrystal are sums over the atoms belonging to the molecule and to the crystal respectively. The Hirshfeld surface is then defined in a crystal as that region around a molecule where w(r) >= 0.5. That is, the region where the promolecule contribution to the procrystal electron density exceeds that from all other molecules in the crystal **[1]**. Hirshfeld surface analysis is a technique to understand the nature of intermolecular interactions within a crystal structure using a fingerprint plot. This method uses visual recognition of properties of atom contacts through mapping of a range of functions (d_{norm}, shape index, curvedness, etc.) onto this surface **[112]**. The 2-D fingerprint plots can be decomposed into areas of specific contacts, enabling analysis and comparison of interactions in quantitative terms **[113]**. The shape of this plot is unique for each molecule. The Hirshfeld surfaces are generated for the structures using very high resolution. The 2-D fingerprint plots displayed by using the standard 0.6–2.8 Å view with the d_e and d_i distance scales displayed on the graph axes where, d_i is the distance from the surface to the nearest atom interior to the surface and d_e is the distance from the surface to the nearest atom exterior to the surface. The normalized contact distance (d_{norm}) based on both d_e, d_i and the vdw radii (r_i^{vdw} and r_e^{vdw}) of the atom, given by Eq. (1),

$$d_{norm} = (d_i - r_i^{vdw})/r_i^{vdw} + (d_e - r_e^{vdw})/r_e^{vdw} \quad \text{……..(1)}$$

which enables identification of the regions of particular importance to intermolecular interactions **[114]**. Because of the symmetry between d_e and d_i in the expression for d_{norm}, where two Hirshfeld surfaces touch, both will display a red spot identical in color intensity as well as size and shape.

1.11. Molecular Docking

Docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs **[115]**. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. The small molecule called ligand usually interacts with protein's binding sites. Binding sites are areas of protein known to be active in forming of compound. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. It also predicts the strength of the binding, the energy of the complex; the types of signal produced and calculate the binding affinity between two molecules using scoring function. The most interesting case is the type protein-ligand interaction, which has its applications in medicine. Molecular docking research focuses on computationally simulating the molecular recognition process. It aims to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized. Molecular docking can be referred as "lock and key" model **[116]**. Here the protein can be called as a lock and the ligand can be called as key, which describes the best fit orientation of the ligand which it goes and binds to a particular protein. To perform a docking, first one may require a suitable protein molecule which has been determined using a biophysical technique such as X-ray crystallography or NMR spectroscopy. The protein structure and ligand are the input for the docking.

1.12. Present Study

Due to broad biological applications of sulfonamides, the synthesis of several N-substituted sulfonamides and the study of their crystal and molecular structure and other physico-chemical and biochemical studies, continue to be an interesting field of research.

The non-covalent interactions responsible for generating supra-molecular structure with novel properties are the fundamental and prime importance for the tuning and prediction of crystal structure of sulfonamide molecules. At this context , author felt worth to work out three dimensional structures of polymorphs of sulfonamides and few metal complexes of sulfa derivatives, synthesized at the author's lab, to elucidate the non bonded intermolecular interactions responsible for the molecular stability and predict the molecular conformation. To compare the experimental data with those of optimized geometry, DFT studies have been performed. To further support the data, Hirshfeld surface analysis and Lattice energy calculations have been carried out. To correlate the structure–function relationship, Minimum inhibition concentration and molecular docking studies are carried out. The compounds picked up for the present study are:

1. **Silver complex of sulfaphenazole (AgSPZ)**
2. **Silver complex of sulfachloropyridazine (AgSCP)**
3. **Silver complex of sulfadiazine (AgSDZ)**
4. **Sulfamonomethoxine (SMM)**
5. **Polymorph-I of sulfamonomethoxine (SMM I)**
6. **Phthalyl sulfacetamide (PHSCA)**
7. **Polymorph-III of sulfapyridine (SP III)**