COMPUTATIONAL STUDIES OF THE SCHIFF BASES OF DAPSONE

BY

OLUBODUN DAVID TOLUWABORI

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CERTIFICATION

This is to certify that this research was carried out by OLUBODUN, David Toluwabori with the matriculation number CHM/2018/145 under my supervision, in partial fulfillment of the requirement for the award of Bachelor of Science (B.Sc. Honors) Degree in Pure Chemistry, Department of Chemistry, Faculty of Science, Obafemi Awolowo University, IleIfe, Osun State, Nigeria.

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Dr. O.F. Akinyele **Date**
(Supervisor)

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Professor A. Akinlua **Date**
(Head of Department)

DEDICATION

This project is dedicated to my parents and siblings.

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I appreciate God Almighty for the grace He bestowed upon me to start and finish this research work.

My sincere appreciation and gratitude goes to the Department of Chemistry for the opportunity to undergo this study, the head of the department, Prof. A. Akinlua and every lecturer for the knowledge they imparted on me and wisdom gained from them throughout my entire program.

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Thank you for everything.

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LIST OF ABBREVIATIONS

ABS: N-benzylidene-4-((4-((E)-benzylideneamino)phenyl)sulfonyl)aniline

ACS (NE)-N-(4-chlorobenzylidene)-4-((4-((4-chlorobenzylidene) amino)phenyl)sulfonyl)

ADME Adsorption, Distribution, Metabolism and Excretion

AFS: (NE)-N-(4-fluorobenzylidene)-4-((4-((4-fluorobenzylidene)amino) phenyl)sulfonyl)aniline

AIS: (NE)-N-(4-iodobenzylidene)-4-((4-((4-iodobenzylidene)amino) phenyl)sulfonyl)aniline

AmBS: (NE)-N-(4-methylbenzylidene)-4-((4-((4-methylbenzylidene)amino) phenyl)sulfonyl)aniline

AmoBS:(NE)-N-(4-methoxybenzylidene)-4-((4-((4-methoxybenzylidene)amino) phenyl)sulfonyl)aniline

ANS : (NE)-N-(4-nitrobenzylidene)-4-((4-((4-nitrobenzylidene)amino)

ANHS: A4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene)) bis(methanylylidene))dianiline

ANOS: (NE)-N-(4-nitrosobenzylidene)-4-((4-((4-nitrosobenzylidene)amino) phenyl)sulfonyl) aniline.

AOHS: 4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis (methanylylidene)) diphenolphenyl)sulfonyl)aniline

BBB Blood Brain Barrier

B3LYP Becke, 3-parameter, Lee-Yang-Parr

CACO-2 Colon Carcinoma cell line

DDS:	4,4'-sulfonyldianiline
DFT	Density Functional Theory
DM	Daphnia Magna
eV	Electron Volts
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HF-DFT	Hatree-Fock Density Functional Theory
HIA	Human Intestinal Absorption
MDCK	Madin-Darby Canine Kidney
PDB	Protein Data Bank
PPB	Plasma Protein Binding
PSA	Polar Surface Area
RB	Rotatable bonds
SCF	Self Consistent Field
SP	Skin Permeability
T.E	Total Energy

ABSTRACT

This study focused on investigating dapsone and its schiff bases as potential antibiotic agents. Dapsone is a well-established antibiotic drug used to treat leprosy. However, research on its schiff bases derivatives is limited. This study aimed to characterize these derivatives in terms of their properties and potential as antibiotic agents. A series of ten schiff bases of dapsone were computationally designed and subjected to in-depth analysis. These derivatives were evaluated across multiple parameters including physicochemical properties, pharmacokinetic behavior, toxicological profile, and potential interactions with a biological target. To achieve this, computational methodologies were employed.

Computational studies were conducted to evaluate the potential of the schiff bases of dapsone as drug candidates. The compounds' electronic properties and reactivity were assessed using the Spartan'14 software package. Density Functional Theory (DFT) calculations at the B3LYP/6-31G* level were employed for further refinement. Drug-likeness was evaluated based on Lipinski's Rule of Five, while ADMET properties and toxicity profiles were predicted using computational tools. Molecular docking simulations against reductase (PDB ID: 1zid) were performed to assess the compounds' potential as antibiotic agents. Several derivatives exhibited promising drug-like properties, acceptable toxicity profiles, and favorable binding affinities to the target protein, suggesting their potential as lead compounds for further development.

Physicochemical properties including partition coefficient (logP), total energy, dipole moment, and polarizability were computed for the compounds. Pharmacokinetic properties, encompassing absorption (HIA), distribution (including skin permeability and plasma protein binding), metabolism, excretion, and toxicity, were predicted using the Pre-ADMET online

platform. Additionally, toxicity endpoints such as LC_{50} were estimated using the T.E.S.T 4.2 software.This comprehensive approach enabled the identification of key structural features influencing the compounds' behavior and provided insights into their suitability as drug candidates.

CHAPTER ONE

INTRODUCTION

1.1 Introduction to Sulfone

In their 60-year history, dapsone and the sulfones have been used as both antibacterial and anti-inflammatory agents. Dapsone has been used successfully to treat a range of dermatologic disorders, most successfully those characterized by abnormal neutrophil and eosinophil accumulation. This article reviews and updates the chemistry, pharmacokinetics, clinical application, mechanism of action, adverse effects, and drug interactions of dapsone and the sulfones in dermatology (Zhu & Stiller, 2001).

Sulfones ($R-SO_2-R$) are versatile synthetic intermediates in organic chemistry.These molecules, containing a sulfur dioxide group (SO_2), are incredibly versatile and have found uses in everything from medicines to plastics (pharmaceuticals, agrochemicals, and polymers). The sulfone group can be employed as a temporary modulator of chemical reactivity. Therefore a variety of different transformations are feasible with this functional group, leading to the description of sulfones as 'chemical chameleons' (Trost & Chadiri, 1984). Sulfone groups can function as activating, electron-withdrawing substituents in Michael acceptors or as good leaving groups producing a sulfinate anion, a reactivity that often facilitates removal of the sulfone moiety after the desired transformation (Liu *et al.*, 2016).

Chemists have known for a while that sulfones can stabilize carbon-based structures with a negative charge (carbanions). This has been useful in creating new molecules. However, it's uncommon for sulfones to be replaced by carbon atoms in reactions (sulfone displacement by carbon nucleophiles). Surprisingly, despite extensive research on sulfones (long history of sulfone chemistry), no one has reported that these molecules can be affected by Lewis acids

(known for accepting electron pairs). This study reveals that certain sulfones are actually quite sensitive to Lewis acids. This new discovery, combined with the known stabilizing effect of sulfones on carbanions, opens up the possibility of transforming organosulfones into a special type of molecule called a 1,1-dipole. This finding paves the way for potentially new reactions and applications for sulfones (Trost & Chadiri, 1984).

1.2 Background of Study

Mycobacterium leprae is a fastidious, acid-fast, intracellular pathogen. In 2008, there were approximately 250,000 new cases reported, predominantly in India, Brazil, and Indonesia (Berrington *et al.*, 2010).

Decades ago, leprosy was widespread, but thanks to control efforts, new cases are now limited to a small number of countries (less than 20 reported over 1000 new cases in 2012).The World Health Organization (WHO) has refined its global leprosy control strategy to further reduce the disease burden. This strategy focuses on: finding all cases in a community, ensuring everyone with leprosy completes multidrug therapy (MDT) treatment,maintaining expertise and training more leprosy specialists,increasing participation of leprosy patients in their own care and reducing the stigma associated with leprosy. The strategy aims to achieve a 35% reduction by 2015 in the number of new cases with visible deformities or disabilities compared to 2010 (World Health Organization, 2013).

Finding cases early and providing effective multidrug therapy (MDT) remain the main ways to reduce leprosy's burden.This treatment not only cures patients but also shortens the infectious period, protecting others from getting infected.

Leprosy often strikes people in their prime working years. Visible deformities associated with advanced stages not only cause physical limitations but also lead to social isolation for

patients and their families, even after a cure. This can have a devastating psychological impact and cause significant economic hardship for individuals and the community as a whole.Tackling leprosy isn't just about treating a disease: it's about empowering individuals, families, and communities to reach their full potential (World Health Organization, 2013)

1.3 Leprosy

Even though *Mycobacterium leprae*, an etiological agent of leprosy, is not highly contagious and effective treatments have existed for six decades, this age-old disease continues to plague humanity. *Mycobacterium leprae* produces a broad spectrum of illness, and the host factors that regulate susceptibility to its diverse clinical forms are largely unknown. Leprosy is primarily a disease of the skin and peripheral nervous system. Less commonly, the eyes, bone, lymph nodes, nasal structures, and testes may also be involved (Nyamogoba *et al.*, 2020, Roberta *et al.*, 2011). Leprosy ancient disease also called Hansen's disease, is a chronic, progressive infectious disease caused by the bacterium *Mycobacterium leprae*. An obligate intracellular parasite, and a close relative of the Mycobacterium tuberculosis.

Recent years have witnessed a significant advancement in the identification of genes associated with leprosy susceptibility. A multifaceted approach has been employed, encompassing case-control and family-based association studies, complemented by large-scale linkage and gene expression analyses. The latest addition to this arsenal is genome-wide association studies (GWAS), which has further refined and prioritized candidate genes. These combined efforts have shed light on crucial innate and adaptive immune pathways implicated in leprosy susceptibility or resistance. Further, researchers delve deeper by investigating the impact of subtle genetic variations, primarily single nucleotide polymorphisms (SNPs) within these genes. These variations demonstrably influence an

individual's susceptibility to leprosy or the severity of the disease course. Elucidating the functional roles of these genes holds significant promise for revolutionizing leprosy prevention, treatment strategies, and ultimately, early diagnosis. Intriguingly, some of the genes implicated in leprosy pathogenesis also exhibit associations with autoimmune diseases (for example the Crohn's disease, rheumatoid arthritis, psoriasis) and neurodegenerative disorders (for example, Parkinson's disease, Alzheimer's disease). This observation suggests that knowledge gleaned from leprosy research, as a model system, could offer invaluable insights into the underlying mechanisms of these complex diseases (Cardoso *et al.*, 2011).

The emergence of drug resistance in *Mycobacterium leprae* presented a formidable obstacle to leprosy treatment. Dapsone monotherapy, implemented in the 1960s, demonstrated the rapid selection of resistant mutants by 1964. This trend repeated with rifampicin monotherapy, with resistance documented by 1976. In response to this challenge and to prevent the establishment of resistant populations in multibacillary leprosy cases, the World Health Organization (WHO) implemented multidrug therapy (MDT) regimens in 1981. Ofloxacin, a bactericidal fluoroquinolone antibiotic active against *Mycobacterium leprae*, has emerged as a potential candidate for inclusion in novel MDT combinations. However, the specter of acquired fluoroquinolone resistance, a well-documented phenomenon in numerous bacterial species, necessitates caution. While reports of ofloxacin resistance exist in *Mycobacterium tuberculosis* following monotherapy for cavitary disease, no such cases have been documented to date for *Mycobacterium leprae* (Emmanuelle *et al.*, 1997). A 2023 investigation identified drug resistance mutations or high frequency of ofloxacin resistance patterns of *Mycobacterium leprae* from India. These findings unveil a concerning trend of emerging resistance not only to established MDT medications (dapsone and rifampicin) but also to a potential future therapeutic option (ofloxacin).It is important to acknowledge the inherent limitations of this

study. The single-center design restricts generalizability, and the cross-sectional nature provides a static view, potentially not capturing the complete dynamic of drug resistance in the broader population (Seema *et al.*, 2023).

1.4 Introduction to Computational studies

Density functional theory (DFT) is a computational quantum mechanical modeling method used in physics, chemistry and materials science to investigate the electronic structure (or nuclear structure) (principally the ground state) of many-body systems, in particular atoms, molecules, and the condensed phases. Using this theory, the properties of a many-electron system can be determined by using functionals, i.e. functions of another function. In the case of DFT, these are functionals of the spatially dependent electron density. DFT is among the most popular and versatile methods available in condensed-matter physics, computational physics, and computational chemistry. (*Density Functional Theory*, 2024).

The B3LYP (Becke,3-parameter,Lee–Yang–Parr) approach belongs to the *hybrid (i.e., mixed) approximations* for the exchange-correlation functional. The approximation is famous, because it gives very good results and, therefore, is extremely popular. B3LYP is a functional that includes exact exchange and GGA corrections in addition to LDA electron-electron and electron-nuclei energy. The weights of the parts were fit to reproduce geometry of a test suite of small molecules. As such use of B3LYP for calculations with heavier atoms is questionable.B3LYP/6-31G (d, p) density functional theory has been employed to calculate the geometry optimization and energies of donor- bridge-acceptor molecular systems. The electronic state of the system has been calculated depending on Koopman's theorem under the orbital-vertical theory. Quantum mechanical calculations at the B3LYP theory level, together with the 6-31G* basis set, were employed to obtain the energy.Highest Occupied Molecular

Orbital (HOMO), Lower Unoccupied Molecular Orbital (LUMO), Molecular Energy Potentials (MEPs), and charge of dapsone is compared with other derivatives.

Molecular Docking is a computational procedure that aims to predict the favored orientation of a ligand to its macromolecular target (receptor), when these are bound to each other to form a stable complex.Molecular docking is used to predict the binding mode of small molecules within the binding site of a protein target.Computational docking is applied to structure-based drug design and can be utilized to predict bond conformations and free energies of binding for small molecule ligands to macromolecular targets. It is widely used for the study of biomolecular interactions and mechanisms (Sinosh & Shruthi, 2018, Manuela et.al.,2017).

The acronym ADMET stands for "absorption–distribution–metabolism–excretion–toxicity" and summarizes an array of critical concerns that still remain after one or more bioactive compounds have been identified. The Lipinski rule of five will be employed in order to investigate the potential of the compound as a drug.

1.5 Aim of Study

This aim of the study is to model dapsone, its schiff bases and investigate their mycobacterial inhibitory potential.

The specific obejectives of the study are to:

1. model dapsone and its schiff bases:
2. predict electronic and reactivity parameters of dapsone and its schiff bases using DFT:
3. investigate the mycobacteria inhibition potential of dapsone and its derivative using

molecular docking;

4. investigate the druglikeness of the compound using ADMET.

CHAPTER TWO

LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1 Schiff Bases

Schiff bases, named after their discoverer Hugo Schiff, constitute a well-defined class of organic compounds formed by the condensation reaction of primary amines with carbonyl compounds. Characterized by the presence of an azomethine group (C=N), these molecules possess a nitrogen atom bonded to a carbon chain on one side and a variable substituent (often a hydrocarbon) on the other.They are formed via the condensation reaction of primary amines with aldehydes or ketones (first reported by Hugo Schiff in 1864). Their formation is often facilitated by acids, bases, or heat. These crystalline solids exhibit weak basicity and can form insoluble salts with strong acids. Beyond their role as intermediates in amino acid synthesis, schiff bases hold significant value as ligands for the creation of metal complexes with diverse structural characteristics. Notably, their ability to bind through multiple functional groups (such as oxygen and nitrogen) makes them "flexi-dentate" ligands.(Edyta *et al.*, 2022, Srividhya & Xavier, 2014).

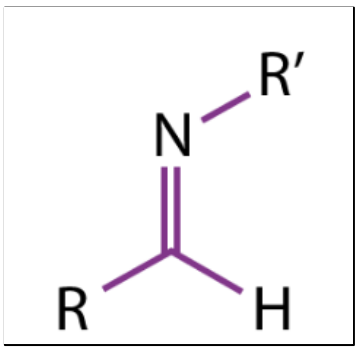

Fig 2.1 Structure of Schiff Base

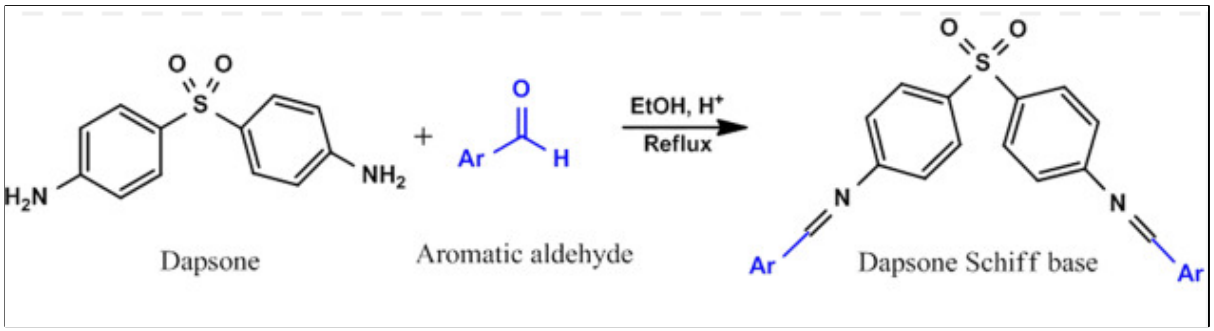

Fig 2.2 Reaction of Dapsone with Carbonyl to give Schiff Base of Dapsone

2.11 Mechanism of Schiff Base

The condensation reaction between primary amines and carbonyl compounds (aldehydes or ketones) yields Schiff bases (imines). This reaction exhibits reversibility, allowing for the hydrolysis of Schiff bases back to their constituent starting materials.

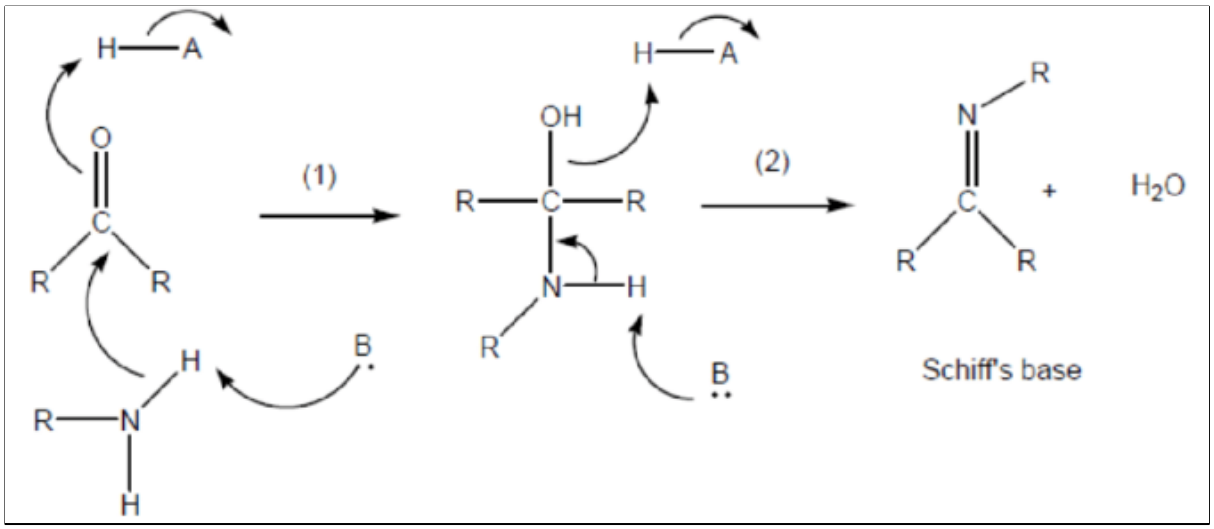

Fig 2.3 Mechanism of Schiff Base Formation

The formation of a Schiff base proceeds through a two-step sequence:

I. **Nucleophilic Addition:** The amine, acting as a nucleophile, attacks the electrophilic carbonyl carbon of the aldehyde/ketone. This interaction results in the formation of a transient intermediate known as a carbinolamine.

II. **Dehydration (Rate-Determining Step):** The carbinolamine undergoes dehydration, expelling a water molecule to form the final Schiff base product. This step is considered the rate-determining step in the overall reaction. Notably, dehydration can be accelerated by either acidic or basic catalysis.

2.1.2 Catalysis and Reaction Optimization:

I. **Acid Catalysis:** Mildly acidic conditions are preferred for acid-catalyzed dehydration. The acid aids in the removal of the hydroxyl group from the carbinolamine, facilitating its dehydration. However, excessively acidic environments can lead to the protonation of the amine, thereby diminishing its nucleophilicity and impeding the reaction's progress.

II. **Base Catalysis:** The base-catalyzed dehydration bears some resemblance to the E2 elimination mechanism observed in alkyl halides. However, unlike E2 elimination, it is not a concerted process. Instead, it proceeds through a two-step pathway involving an anionic intermediate.

2.1.3 Extensive Applications of Schiff Bases

Schiff base formation exemplifies a sequential process involving nucleophilic addition followed by dehydration.The dehydration step governs the reaction rate and can be

influenced by either acid or base catalysis.Maintaining a balanced reaction environment is crucial to avoid compromising the amine's nucleophilic character, which is essential for efficient Schiff base formation.

Schiff bases, a versatile class of organic compounds with a well-established repertoire of biological applications, have a long history of serving as chelating ligands in coordination chemistry.Notably, complexation with metals often leads to a synergistic enhancement of their beneficial properties.Schiff bases exhibit a remarkable ability to chelate transition metal ions (Saso & Kostova, 2013). This complexation process leads to the formation of stable coordination complexes, particularly advantageous for metals in higher oxidation states. These complexes offer significant advantages due to the versatility of donor atoms within the Schiff base ligand. Nitrogen, oxygen, and sulfur atoms can all participate in coordination with the metal ion, enabling the formation of bidentate, tridentate, and tetradentate complexes with diverse binding arrangements.

A thorough understanding of the ligand-metal interaction is paramount for the design of highly active compounds. The influence of specific metals on the biological activity of these complexes, coupled with their inherent chemical appeal as multidentate ligands, has fueled a significant surge in research efforts dedicated to elucidating their coordination behavior. This heightened interest has paved the way for the development of novel chemotherapeutic agents based on Schiff bases and their metal complexes, signifying a promising new frontier in antioxidant discovery. The development of metal-derived antioxidants has become a critical area of research, driven by the need to combat free radicals implicated in various oxidative stress-related disorders. While synthetic antioxidants are widely employed for their efficacy and cost-effectiveness, a growing focus lies on exploring novel alternatives. In this context, Schiff base metal complexes have emerged as potent free radical scavengers, showcasing

promising potential as antioxidants.

The field of medicinal chemistry has witnessed a growing interest in Schiff base-metal ion complexes as potential therapeutic agents. These complexes exhibit promising activity against a range of diseases, including bacterial and fungal infections, inflammation, and cancer (Debdulal, 2019, Ikechukwu & Peter, 2015). Recent research explores the utilization of novel metals like silver and gold in these complexes, further expanding their potential therapeutic applications. Beyond the field of medicine, Schiff bases also demonstrate catalytic activity in various reactions, including acid catalysis, reduction processes, and oxidation reactions.

There are extensive body of research on Schiff bases due to their:

I. Diverse structural features and inherent flexibility.
II. Capacity to form highly stable bi-, tri-, or tetra-dentate chelate ligands.
III. Applications in various scientific fields, including the identification, protection, and determination of aldehydes/ketones; purification of specific compounds; and production of complex or sensitive molecules.

Due to their broad spectrum of biological effects, Schiff bases are actively explored for the development of drugs with properties like:Antibacterial and antifungal activity,Anti-inflammatory and analgesic properties,Antimalarial and anticancer potential

Industrial Applications:

Schiff bases form complexes with metal ions, making them valuable in fields like material science and industrial sectors:

I. **Dyes and Pigments:** Schiff bases contribute vibrant colors to various materials, finding use in dyes for textiles, paints, and plastics.

II. **Catalysis:** They act as efficient catalysts in numerous organic synthesis reactions, accelerating reaction rates and improving process efficiency.

III. **Polymer Chemistry:** Schiff bases serve as stabilizers for polymers, enhancing their resistance to degradation and improving their overall performance.

Schiff Base Complexes in Catalysis and Medicinal Chemistry

The role of Schiff base complexes in:

I. Forming stable complexes with metal ions.

II. Demonstrating excellent catalytic activity in various reactions under high temperatures and moisture-tolerant conditions.

III. Finding applications in both homogeneous and heterogeneous catalysis, as evidenced by the numerous recent reports.

IV. Playing a central role in coordination chemistry, particularly with metal ions like Co(II), Cu(II), and Zn(II).

V. Possessing potential as DNA binding and cleavage agents under physiological conditions, making them attractive for further research as potential therapeutic tools.

2.2 Dapsone

Dapsone's history exemplifies the fascinating interplay between planned scientific inquiry and fortuitous discovery. In 1908, chemists Fromm and Wittmann synthesized the compound while exploring novel azo dyes, a testament to the often unpredictable nature of basic research. This initial foray, however, was far removed from the therapeutic applications dapsone would later find.

Concurrently, the late 1930s witnessed a surge in research on sulfonamides as potential

antibacterial agents. Despite predating this surge, dapsone's potential remained unexplored. Independent investigations by Buttle *et al.* and Fourneau *et al.* in the late 1930s finally revealed dapsone's ability to combat various pathogenic bacteria, including those responsible for leprosy (*Mycobacterium leprae*) and tuberculosis (*Mycobacterium tuberculosis*). However, early trials were hampered by unacceptable side effects associated with excessive initial dosages. This toxicity overshadowed the initial promise, leading to dapsone's temporary abandonment as a viable therapeutic option.

Fortunately, research on dapsone and related sulfones continued. The 1940s witnessed a remarkable turnaround as scientists rediscovered dapsone's efficacy in treating leprosy. This success story underscores the importance of perseverance in drug development. What appears ineffective at first glance can yield significant results with further investigation and optimized dosing strategies.

Dapsone's journey took another unexpected turn in 1950. Mistakenly believing dermatitis herpetiformis to be infectious, Portuguese researchers serendipitously discovered that dapsone effectively alleviated the condition. This fortuitous finding, later confirmed by others, paved the way for dapsone's use in dermatology.

The landscape of dapsone's application in medicine shifted dramatically with the introduction of penicillin and a wave of powerful antibiotics in the following years. While its role in combating bacterial infections diminished, dapsone found new purpose in the treatment of non-infectious inflammatory, autoimmune, and bullous diseases. Today, it remains a valuable tool in a dermatologist's armamentarium.

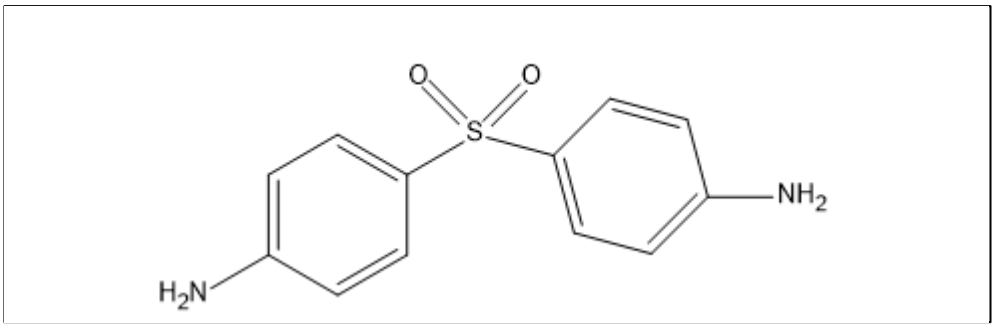

Fig 2.4 Structure of 4,4'-sulfonyldianiline

Dapsone (*4,4'-sulfonyldianiline*) is structurally the simplest of the sulfones, all of which share the characteristic structure: a sulfur atom linking to two carbon atoms. Dapsone, an antibacterial, is primarily and effectively used in the leprosy treatment which is also used in combination with rifampicin and clofazimine for the treatment of *Mycobacterium leprae* infections (leprosy), malaria, and pneumocystis pneumonia (PCP). Specifically, dapsone stops the bacterial dihydrofolic acid synthesis through the process of binding itself in the active site of the enzyme named 6-hydroxymethyl-7,8-dihydropteroate synthase (DHPS), which takes part in the condensation of para-aminobenzoic acid (pABA) with 6-hydroxymethyl-7,8-dihydropterin-pyrophosphate to form 7,8-dihydropteroate and pyrophosphate. Moreover, dapsone competes with para-aminobenzoate on the active site of DHPS and inhibits the bacterial dihydrofolic acid synthesis.

2.3 Clofazamine

Clofazimine, a lipophilic riminophenazine antibiotic discovered in 1957, stands out for its dual action: it possesses both antimycobacterial and anti-inflammatory properties. While its in vitro activity against multidrug-resistant Mycobacterium tuberculosis strains is impressive, its efficacy has only been established in the treatment of leprosy (Singh, R. K., *et al.*, 2008).Clofazimine (CFZ), a riminophenazine antibiotic with a characteristic dye-like

structure, is marketed under the brand name Lamprene (*Leprosy*, 2023). This active pharmaceutical ingredient (API) possesses both antibiotic and anti-inflammatory properties. It finds primary use in the treatment of leprosy caused by *Mycobacterium leprae*.

Beyond leprosy, CFZ demonstrates efficacy against various pathogens, including *Mycobacterium tuberculosis*, *Clostridium difficile*, and even multidrug-resistant (MDR) strains of *M. tuberculosis*.

Since 1962, clofazimine has been a cornerstone of leprosy treatment, particularly in multibacillary forms. It forms a crucial component of the World Health Organization (WHO) -recommended triple drug regimen. The effectiveness of clofazimine in leprosy goes beyond its direct antimicrobial activity. Its lipophilic nature allows it to accumulate within the skin and nerves, which are primary targets in leprosy (*Leprosy*, 2023). Additionally, clofazimine's anti-inflammatory properties play a vital role in managing complications like *erythema nodosum leprosum* (ENL) and reversal immunity reactions that can arise during antimicrobial therapy.

However, a major challenge associated with clofazimine is its poor water solubility. In vitro bioactivity assays often rely on dimethyl sulfoxide (DMSO) to overcome this limitation. This creates a discrepancy between in vitro and in vivo activity, as the poor solubility of clofazimine in vivo hinders its effectiveness. With a high partition coefficient (log P > 7), clofazimine is categorized as a highly hydrophobic molecule.Major consequences of Poor Solubility: Bioaccumulation which involves low water solubility coupled with high lipophilicity leads to clofazimine accumulating in the body's fatty tissues, and In Vivo Precipitation involving limited solubility can cause clofazimine to precipitate in vivo, particularly in its chloride salt form. This salt exhibits extremely low water solubility and can complex with

intracellular membranes, forming crystalline aggregates. These aggregates are subsequently phagocytosed by the phagocytic mononuclear system.

2.4 Theoretical Background

2.4.1 Semi-Empirical

In theoretical chemistry, semi-empirical methods are a type of computational tool that combines elements of both theory and experiment.These methods start with established theoretical frameworks, often based on quantum mechanics. For example, they might utilize the Hartree-Fock method for calculating electronic structure (Sara Tortorella *et al.*, 2016).To simplify calculations or improve accuracy, semi-empirical methods incorporate experimental data. This data can be in the form of: Values obtained from experiments (like ionization energies) used to adjust the theoretical framework for the specific system being studied or Experimental results are used to compare and validate the predictions made by the semi-empirical method (Amorim Madeira *et al.*, 2013).

By simplifying complex theoretical models and incorporating experimental data, semi-empirical methods can be computationally faster than more rigorous ab-initio methods. This allows them to handle larger and more complex molecules.The use of experimental data can sometimes lead to better accuracy for the types of molecules used to parameterize the method (Freed, 1974, Martin & Freed, 1996).

Since they rely heavily on parameterization, semi-empirical methods may not be as accurate for systems very different from those used for parameterization.Also, compared to ab-initio methods, semi-empirical methods offer less insight into the fundamental principles governing the system. Due to their computational efficiency, semi-empirical methods are useful for

studying large molecules like polymers, biological molecules, and materials (Nicholls *et al.*, 2021).They can provide insights into reactivity trends and reaction mechanisms, particularly for organic and inorganic molecules.These methods can be used for initial calculations to guide further investigation with more rigorous, but computationally expensive, methods.

Examples of Semi-empirical Methods:

I. **Extended Huckel Theory (EHT)**: A popular method for studying bonding and reactivity in coordination chemistry.
II. **Modified Neglect of Differential Overlap (MNDO)**: A versatile method used for studying structures, energies, and vibrational frequencies of various molecules.
III. **Austin Model 1 (AM1)**: Another widely used method known for its good balance between accuracy and computational cost.

2.4.2 Density Functional Theory (DFT)

Traditionally, quantum mechanics described electronic structure using the wavefunction, which provides the probability of finding an electron at a specific location around the nucleus.Calculating the exact wavefunction for complex systems with many electrons is often computationally expensive or even impossible.DFT takes a different approach. It focuses on the electron density ( ), which represents the probability of finding an electron anywhere in space around the nuclei. The ground-state properties of a system (its most stable state) are uniquely determined by its electron density (Eschrig, 1996). DFT utilizes a functional, a mathematical object that takes a function (in this case, the electron density) and returns a single numerical value (often the total energy of the system).The accuracy of DFT

calculations relies on the choice of this functional. Common functionals include local density

Unlike ab-initio methods that focus on the wavefunction, DFT calculates the total energy based on the electron density distribution around the nuclei. Both the Hamiltonian (the system's energy operator) and the expression for electron density are approximations in DFT (Palumbo, 2017).

DFT exhibits exceptional reliability in predicting geometries, vibrational frequencies, and total energies. This method offers a distinct advantage over wavefunction-based approaches by achieving rapid convergence to the basis set limit, leading to faster and more efficient calculations (Bursch *et al.*, 2022).

Semi-empirical MO methods were the primary tool before DFT.These methods relied on symmetry arguments to explain structural and reactivity features in coordination chemistry. Their accuracy was limited, especially for complex molecules. The introduction of DFT methods significantly improved MO technique applications.

2.5 Theoretical Parameters

2.5.1 Dipole moment

The molecular dipole moment, a well-established descriptor of the asymmetric electron distribution within a molecule, offers valuable insights into the overall charge distribution. However, a complete and quantitative description of the electron cloud remains computationally demanding due to the influence of higher-order charge distributions (multipoles) (Cisneros *et al.*, 2014).

High-order charge distributions, also known as multipoles, refer to the way electrical

charge is arranged in a molecule beyond the basic concept of a dipole moment. A dipole moment arises from the unequal sharing of electrons between atoms in a molecule, resulting in a positive and negative end. However, the charge distribution can be more complex, with regions of positive and negative charge that extend beyond a simple two-pole model.The presence of higher-order multipoles can influence various molecular properties, including: polarizability (the ease with which a molecule's electron cloud can be distorted by an external electric field) and intermolecular forces (the attractive or repulsive forces between molecules).

Nevertheless, the combined analysis of dipole moment and polarizability provides significant information regarding: solubility, chemical reactivity.

2.5.2 Electrophilicity(ω)

Electrophiles are electron-deficient species seeking to gain electrons in a chemical reaction. They act as Lewis acids according to the Bronsted-Lowry theory, often carrying a positive charge or a partial positive charge due to an incomplete octet of electrons (Ghosh, 2021).The concept of electrophilicity goes beyond simple charge and offers a quantitative measure. (Parr *et al.*, 1999) introduced the electrophilicity index ( ) as the stabilization energy gained by a molecule when it acquires electrons from its surroundings.This index reflects the molecule's ability to be "saturated" by electrons. Higher values indicate greater electrophilic character.

Electrophilicityindex ( ) = μ^2 /2

Calculating relies on other reactivity parameters like chemical potential (μ) and global

hardness ( ). These, in turn, are derived from the highest occupied molecular orbital (HOMO) energy (E_H) and the lowest unoccupied molecular orbital (LUMO) energy (E_L) using finite difference approximations.

2.5.3 Energy parameters

I E_{LUMO}

The lowest unoccupied molecular orbital (LUMO) energy refers to the energy level of the lowest available orbital that does not contain any electrons in a molecule. It's a crucial concept in chemistry, particularly in understanding molecular reactivity and electronic transitions.It plays a vital role in determining a molecule's reactivity, as it's the most likely orbital to accept electrons during chemical reactions. The LUMO is an empty orbital with the lowest energy level compared to other unoccupied orbitals.The LUMO shape and energy can indicate potential sites for electrophilic attack on a molecule. A low-energy LUMO suggests a higher propensity for electrophilic reactions.The symmetry and nodal structure of LUMO orbitals are essential for understanding the feasibility and stereochemistry of pericyclic reactions.

II E_{HOMO}

The Highest Occupied Molecular Orbital (HOMO) is a crucial concept in quantum chemistry, providing valuable insights into a molecule's electronic structure and reactivity.The HOMO energy is directly related to a molecule's ability to donate electrons. A higher HOMO energy indicates a greater tendency to lose electrons, making the molecule more nucleophilic.The energy required to remove an electron from the molecule (ionization potential) is closely related to the HOMO energy. A lower HOMO energy corresponds to a higher ionization

potential.The HOMO plays a role in forming chemical bonds by overlapping with the LUMO of another molecule.The electron density distribution of the HOMO can provide information about potential intermolecular interactions, such as hydrogen bonding and van der Waals forces.

III E_{GAP}

$E\text{-gap}(E_g) = E_{LUMO} - E_{HOMO}$

The energy gap is the difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) energies, and is a crucial parameter in computational chemistry. It provides valuable insights into a molecule's electronic structure and properties, with implications across various fields (Xiang *et al.*, 2016). A large energy gap often indicates a molecule with low reactivity, as it requires significant energy to either lose or gain an electron.A small energy gap suggests a molecule is more reactive, as it can more easily participate in electron transfer processes. The energy gap can influence a molecule's ability to interact with biological targets.Understanding the energy gap can help in designing molecules with optimal binding affinities.

IV Ionization Energy(I)

$\text{Ionization energy}(I) = -E_{HOMO}$

V Electron Affinity

$\text{Electron Affinity}(A) = -E_{LUMO}$

2.5.3 Reactivity Indices

I Electronegativity index(χ)

This represents the average pull a molecule exerts on its electrons. It's calculated as the change in energy with respect to the number of electrons.

Electronegativity ( )= –

The electronegativity, could be written as the partial derivative of the system's energy with respect to the number of electrons, N, at the fixed external potential ( ):

=(/) ( ) = –

Where is the electronegativity.

II Chemical Hardness(η)

The chemical hardness, , can be seen as the resistance to charge transfer:

= ½(² / ²) ( )

According to Mulliken, one has:

= = – ½(+)

And = ½(–)

Mulliken's equations relate these concepts to the system's electronic energy and external potential.Where E and ( ) are electronic energy and external potential of an N-electron system

$\approx -\frac{1}{2}(\quad + \quad) \approx \frac{1}{2}(\quad + \quad)$

$\approx \frac{1}{2}(\quad - \quad) \approx \frac{1}{2}((\quad - \quad)$

Finite difference approximations and Koopman's theorem are used to simplify the calculations. These approximations estimate the chemical potential and hardness based on the highest occupied molecular orbital (HOMO) energy (ε_H) and the lowest unoccupied molecular orbital (LUMO) energy (ε_L).

Chemical hardness ( ) = 1 /2 (ε_{LUMO} - ε_{HOMO})

III Chemical Potential

(Parr & Yang, 1989) define the chemical potential, μ as a measure of the escaping tendency of an electron from equilibrium in DFT.This reflects the tendency of an electron to escape from a molecule.

Chemical potential (μ) = 1/2 (ε_{HOMO}+ε_{LUMO})

IV The Electrophilicity Index ( ):

Electrophilicity index was introduced to quantify a molecule's reactivity. This explains the measure of a molecule's electron-accepting ability.This explains how DFT concepts, derived from a molecule's orbital energies, are used to calculate the electrophilicity index. Electrophilicity index provides a quantitative measure of a molecule's desire to gain electrons.Parr *et al.* (Parr *et al.*, 1999) proposed a new concept, , to quantify electrophilicity. It combines the chemical potential ( ) and global hardness ( ) which relates to the energy gap between HOMO and LUMO (ε_H - ε_L), offering a way to assess electron-accepting ability based on orbital energies.

$= \quad ^{2}/2 \quad = (\quad + \quad)^{2}/2(\quad + \quad) = (\quad + \quad)^{2}/2(\quad + \quad)$

where E_H and E_L are the energies of the highest occupied and the lowest unoccupied molecular orbital, I and A are the first ionization potential and electron affinity respectively

V Dipole Moment

A dipole moment is a measure of the polarity of a bond or molecule. It arises due to the unequal sharing of electrons between atoms in a bond. The greater the difference in electronegativity between two atoms, the larger the dipole moment. A dipole moment is a measure of the polarity of a bond or molecule (Coulson, 1942).It arises due to the unequal sharing of electrons between atoms in a bond. The greater the difference in electronegativity between two atoms, the larger the dipole moment

VI Polarizability

This is the ability of these molecules to acquire a dipole moment, p, in an electric field: E. The appearance of p is due to the displacement of electric charges in atomic systems under the influence of E.The moment, p, thus disappears when no electric field is present. The concept of polarizability is generally not applied to particles having permanent dipole moments such as polar molecules.

$=$

Where is a quantitative measure of polarizability, which is called molecular polarizability for some molecules. The value of may depend on the direction of E (Anisotropic polarizability). Polarizability is a measure of how easily the electron cloud of an atom or molecule can be distorted by an external electric field (Yakar *et al.*, 2020). In simpler terms,

it's how easily the electrons can be shifted away from their original positions.

2.6 Molecular Modeling

Molecular modeling has become an indispensable tool in various scientific disciplines, offering a powerful computational approach to simulate and comprehend the behavior of molecules and their interactions. In essence, it constructs a virtual representation of molecules and their interactions, allowing for the prediction or explanation of their properties and behavior.These methods leverage fundamental physical and chemical laws to describe molecules and their interactions. This category includes quantum mechanics-based techniques and statistical mechanics approaches. It can also involve relying on pre-existing experimental data and statistical relationships to predict properties.By simulating how potential drug candidates interact with target molecules, researchers can design more effective medications.The accuracy of simulations is inherently dependent on the chosen method and the quality of the underlying data used for model construction. To facilitate computational feasibility, models often involve simplifications. Therefore, they may not perfectly capture all real-world complexities. However, molecular modeling has revolutionized our understanding of molecules and their interactions

2.7 Pharmacokinetics(ADME/T) and Drug-Likeness Properties Evaluation

ADME properties determine how well a drug reaches its target in the body and how long it stays active.To address this issue, scientists now evaluate both a drug's effectiveness and its biopharmaceutical properties (ADME/T) early in development. This reduces the number of promising leads that fail later due to ADME/T problems, which are the main culprit behind clinical trial failures, not a lack of effectiveness. (Dong *et al.*, 2018).

Drug discovery uses a concept called "drug-likeness" to identify promising early-stage drug candidates. Experts traditionally assess drug-likeness through a voting process. This approach has evolved alongside concepts like Pfizer's "rule of five" or Lipinski rule of five and "lead-likeness," all aiming to distinguish between drug-like molecules and non-drug-like molecules. The rule of five is not a strict rule, and there are many successful drugs that violate one or more of its criteria. Scientists can now use various statistical tools and chemical descriptors to differentiate drug-like molecules from non-drug-like ones. This replaces the reliance on expert committees and makes the process more objective and efficient. The rule of five states that a drug-like molecule is more likely to have good oral bioavailability (absorption through the digestive system) if it follows all of these criteria:

I. **Molecular weight (MW) less than 500 Daltons (Da)**: Larger molecules may have difficulty passing through cell membranes and being absorbed.
II. **Number of hydrogen bond donors (HBD) less than or equal to 5**: Too many HBDs can lead to strong interactions with water molecules, hindering absorption. (Hydrogen bond donors are typically OH or NH groups).
III. **Number of hydrogen bond acceptors (HBA) less than or equal to 10**: Similar to HBDs, too many HBAs can increase interaction with water, affecting absorption. (Hydrogen bond acceptors are typically lone electron pairs on O and N atoms)
IV. **Calculated octanol-water partition coefficient (ClogP) less than or equal to 5**: ClogP is a measure of a molecule's lipophilicity (fat solubility). A very high ClogP suggests the molecule might be too fat-soluble and have difficulty dissolving in water for proper absorption.

Lipinski's Rule of 5 (RO5) identifies potential problems with a drug's absorption, distribution, metabolism, and excretion (ADME) properties early in the development process. Poor ADME

properties can hinder a drug's ability to reach its target site in the body and can lead to failure in clinical trials (Lipinski *et al.*, 1997, Lipinski, 2004, Mishra *et al.*, 2009)

CHAPTER THREE

COMPUTATIONAL DETAILS

3.1 Methodology and Software

By leveraging computational chemistry, scientists can explore molecular structures, properties, and reactions with unprecedented efficiency and accuracy. This approach complements traditional experimental methods, providing valuable insights into chemical phenomena that may be challenging to observe directly.Computational chemistry has become an indispensable tool for modern chemical research. This discipline involves employing mathematical models and computer simulations to study molecular systems. To execute these calculations, researchers rely on specialized software packages such as SPARTAN, HYPERCHEM, QCHEM, GAUSSIAN, and GAMESS. These platforms offer a range of functionalities and are continually updated to incorporate new methodologies.In this thesis we explored the SPARTAN molecular software package for our calculations (Spartan '14V 1.1.4)

3.2 General Description of SPARTAN

As such, we have developed spartan (Simulation Parameter Analysis R Toolkit Application), a toolkit of statistical techniques that aid understanding and analysis of results generated through simulation. *Spartan* is freely available, open-source, and implemented within the R statistical environment.Spartan is a versatile computational chemistry software package capable of modeling a wide range of molecular systems. It supports a variety of computational methodologies, encompassing molecular mechanics (using force fields such as

SYBYL and MMFF), semi-empirical, ab-initio, density functional theory (DFT), and post-Hartree-Fock levels of theory.For the present study, DFT calculations were conducted at the B3LYP/6-31G* level of theory within the Spartan environment to optimize the geometries of dapsone and its schiff bases.

3.3 Computation of Dapsone

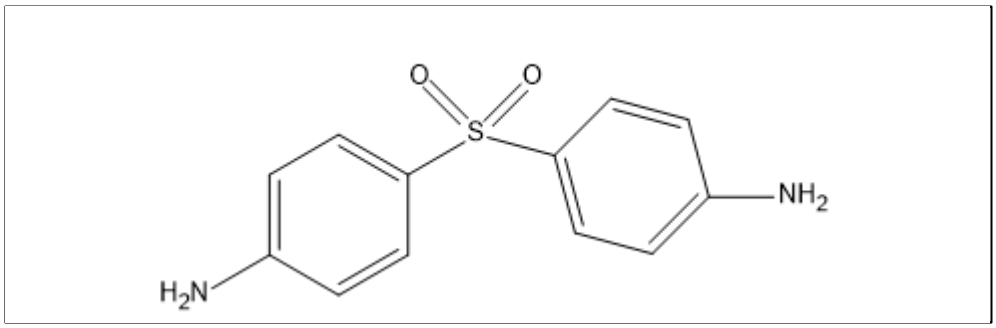

Fig 3.1 Structure of 4,4'-sulfonyldianiline

3.4 Structure of the Simulated Schiff Bases Derivatives of Dapsone

4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene)) dianiline

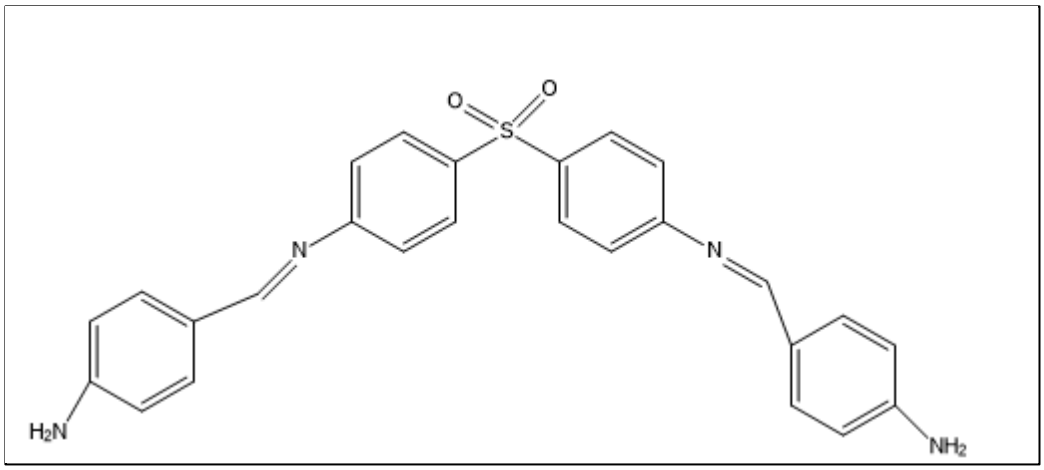

Fig 3.2 Structure of 4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))dianiline

(NE)-N-(4-methoxybenzylidene)-4-((4-((4-methoxybenzylidene)amino)phenyl)sulfonyl)aniline

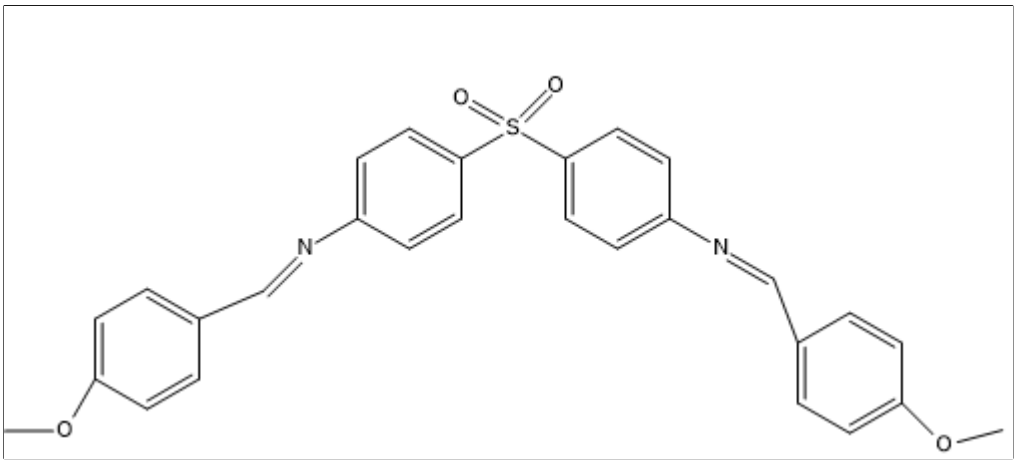

Fig 3.3 Structure of (NE)-N-(4-methoxybenzylidene)-4-((4-((4-methoxybenzylidene)amino)phenyl)sulfonyl)aniline

4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene)) diphenol

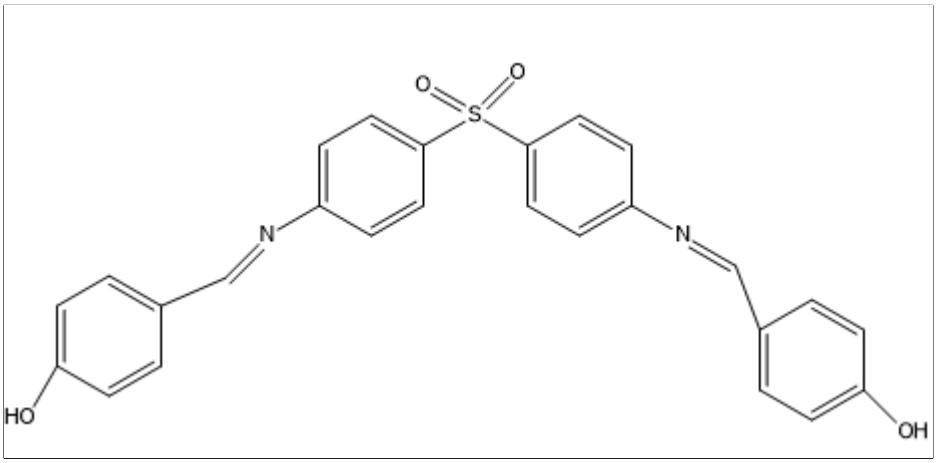

Fig 3.4 Structure of 4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene)) diphenol

(NE)-N-(4-nitrosobenzylidene)-4-((4-((4-nitrosobenzylidene)amino)phenyl)sulfonyl) aniline

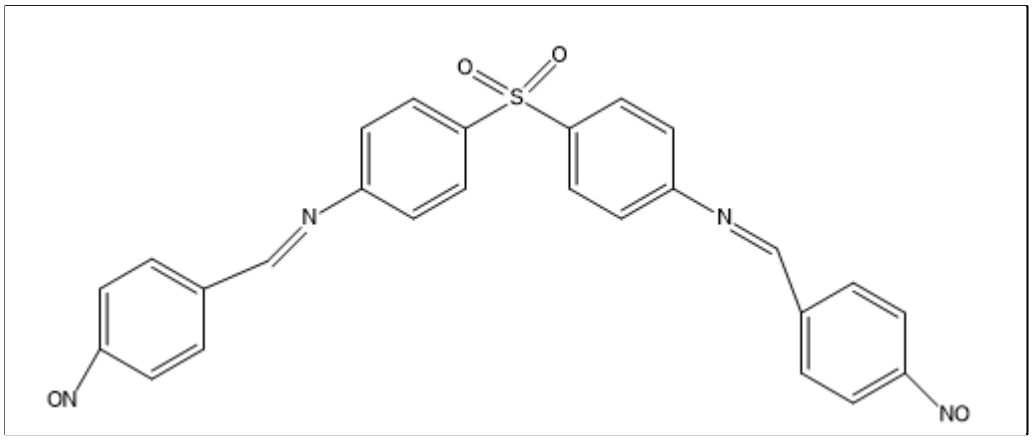

Fig 3.5 Structure of (NE)-N-(4-nitrosobenzylidene)-4-((4-((4-nitrosobenzylidene) amino)phenyl)sulfonyl) aniline.

(NE)-N-(4-methylbenzylidene)-4-((4-((4-methylbenzylidene)amino)phenyl)sulfonyl) aniline

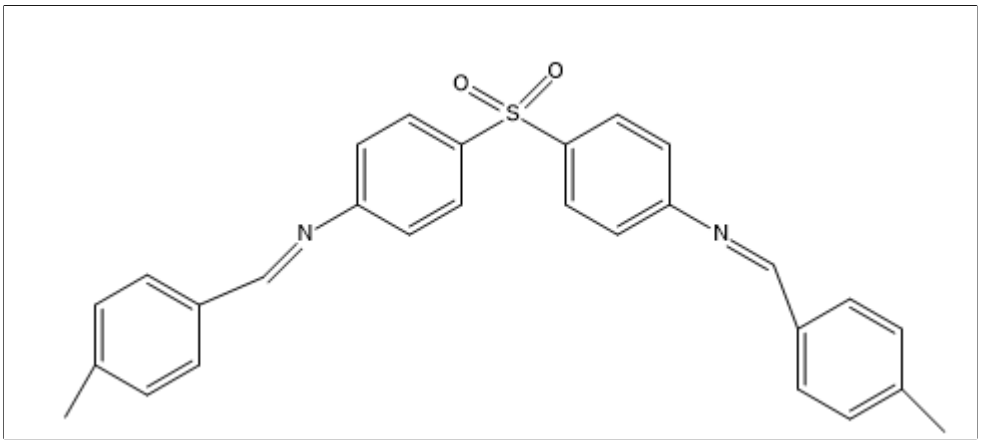

Fig 3.6 Structure of (NE)-N-(4-methylbenzylidene)-4-((4-((4-methylbenzylidene)amino)phenyl)

sulfonyl)aniline

N-benzylidene-4-((4-((E)-benzylideneamino)phenyl)sulfonyl)aniline

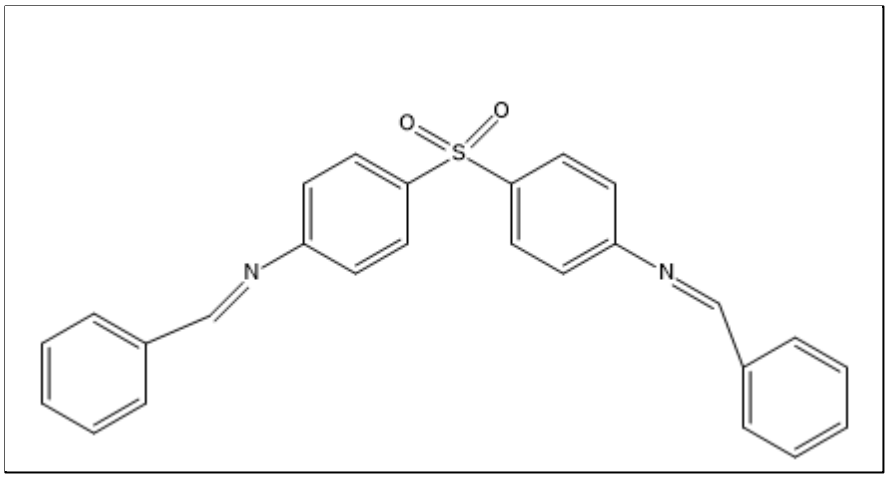

Fig 3.7 Structure of N-benzylidene-4-((4-((E)-benzylideneamino)phenyl)sulfonyl)aniline

(NE)-N-(4-fluorobenzylidene)-4-((4-((4-fluorobenzylidene)amino)phenyl)sulfonyl)aniline

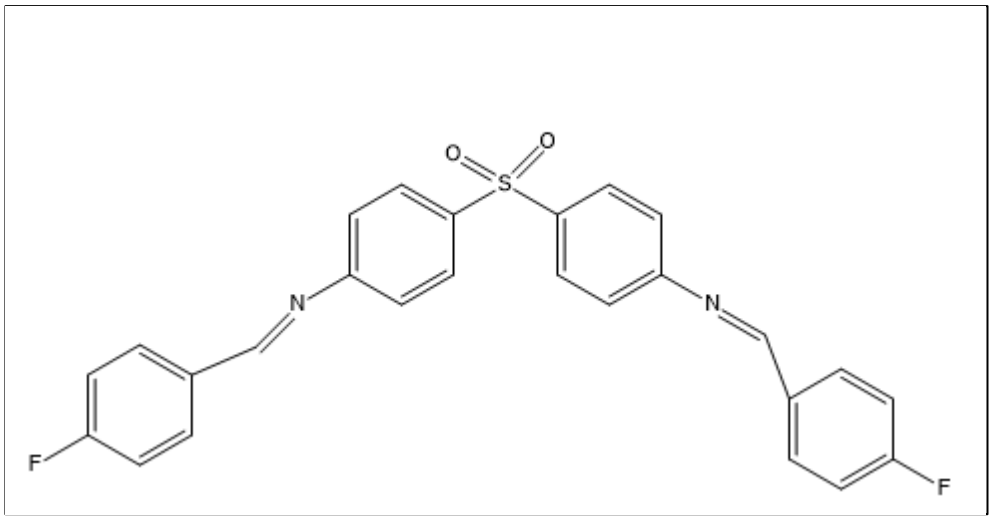

Fig 3.8 Structure of (NE)-N-(4-fluorobenzylidene)-4-((4-((4-fluorobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-iodobenzylidene)-4-((4-((4-iodobenzylidene)amino)phenyl)sulfonyl)aniline

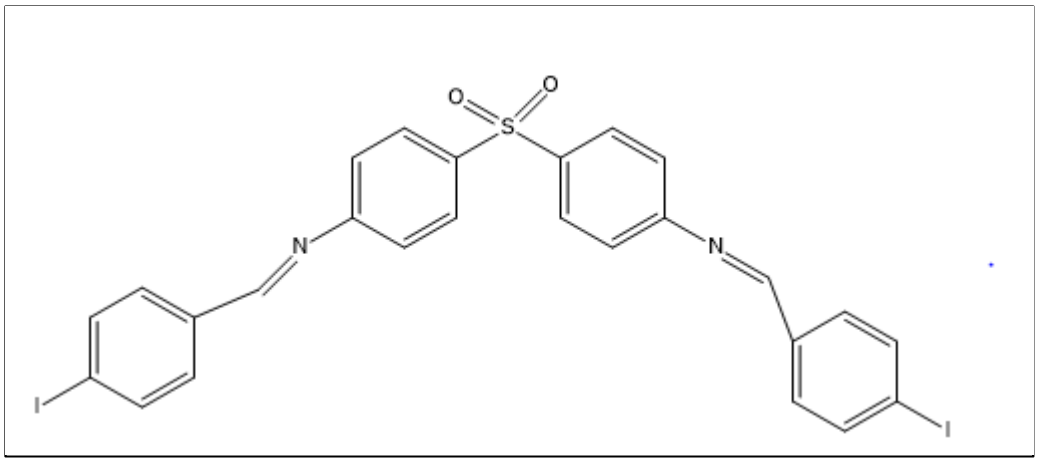

Fig 3.9 Structure of (NE)-N-(4-iodobenzylidene)-4-((4-((4-iodobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-chlorobenzylidene)-4-((4-((4-chlorobenzylidene)amino)phenyl)sulfonyl)

aniline

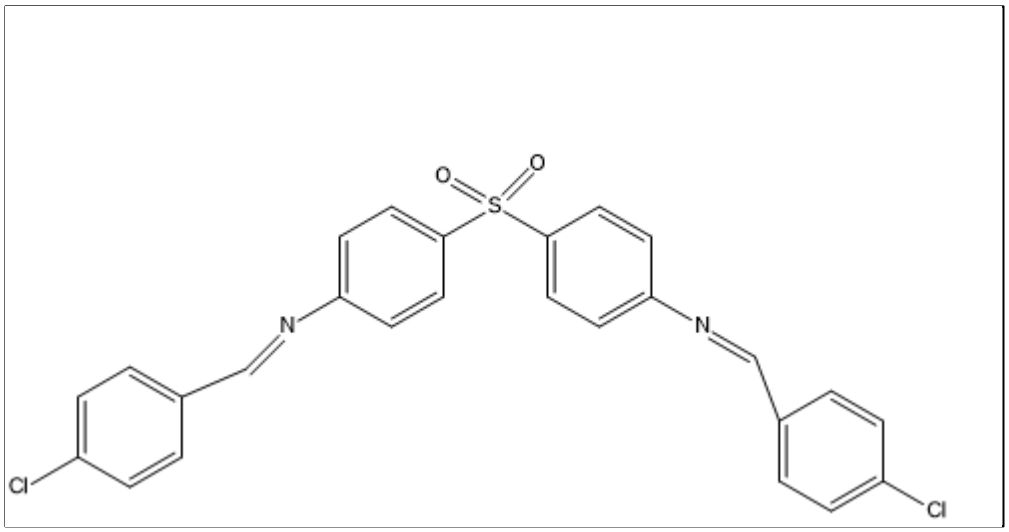

Fig 3.10 Structure of (NE)-N-(4-chlorobenzylidene)-4-((4-((4-chlorobenzylidene)amino)phenyl)sulfonyl)

aniline

(NE)-N-(4-nitrobenzylidene)-4-((4-((4-nitrobenzylidene)amino)phenyl)sulfonyl)aniline

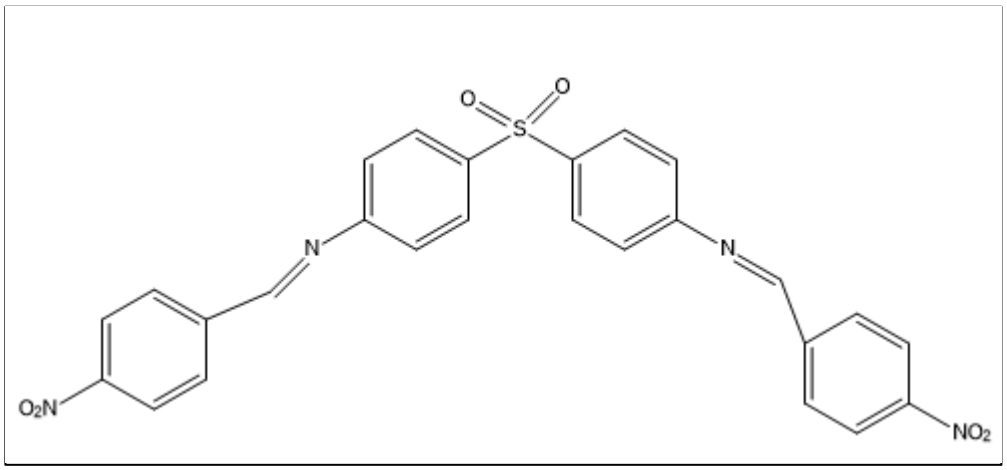

Fig 3.11 Structure of (NE)-N-(4-nitrobenzylidene)-4-((4-((4-nitrobenzylidene)amino)phenyl)sulfonyl)aniline

3.5 Optimized Structure of the Simulated Schiff Bases Derivatives of Dapsone

4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene)) dianiline

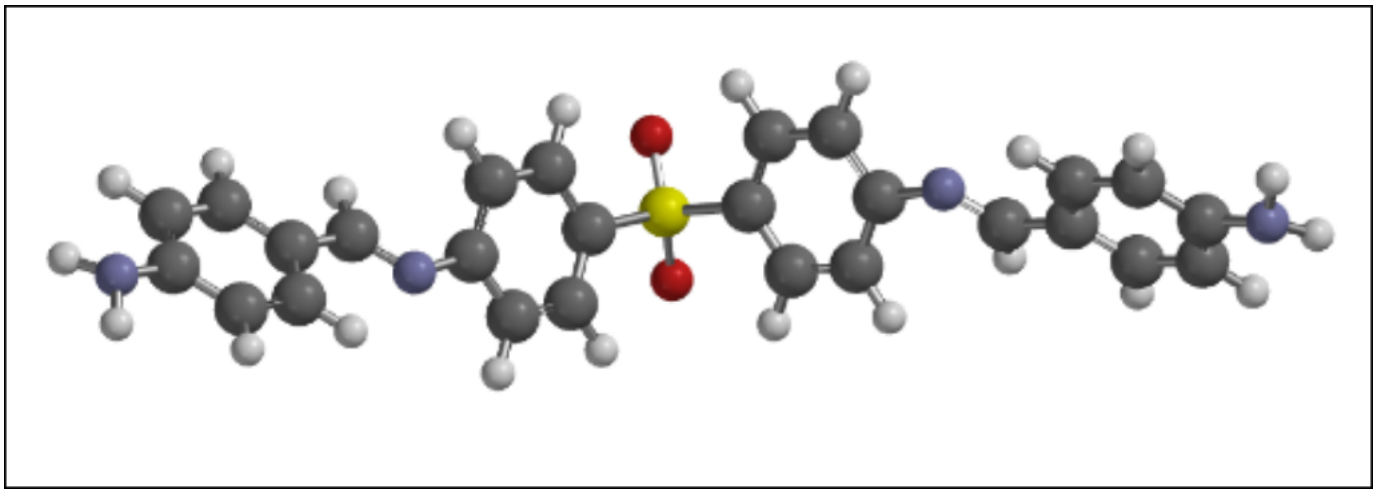

Fig 3.12 Optimized Structure of 4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))dianiline

(NE)-N-(4-methoxybenzylidene)-4-((4-((4-methoxybenzylidene)amino)phenyl)sulfonyl)

aniline

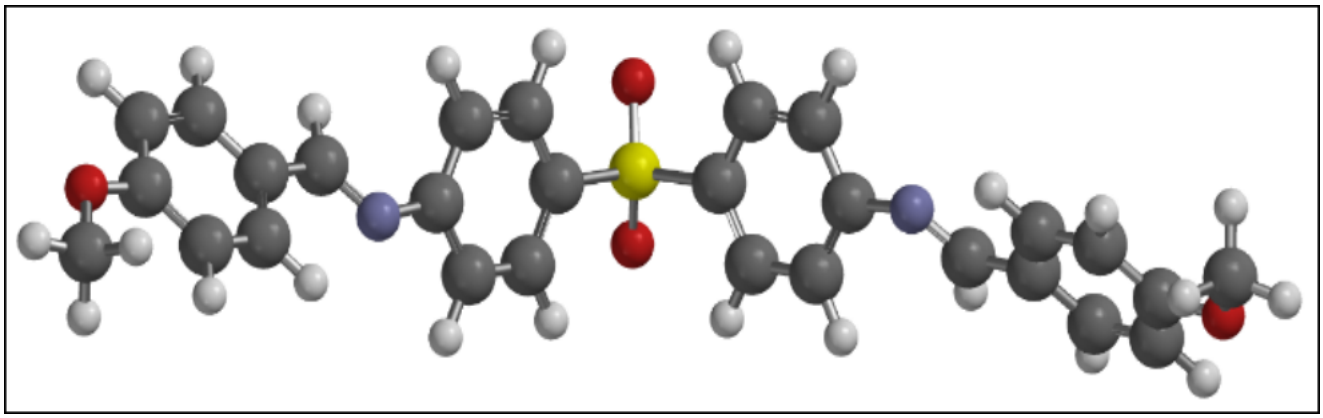

Fig 3.13 Optimized Structure of (NE)-N-(4-methoxybenzylidene)-4-((4-((4-methoxybenzylidene)amino)phenyl)sulfonyl)aniline

4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene)) diphenol

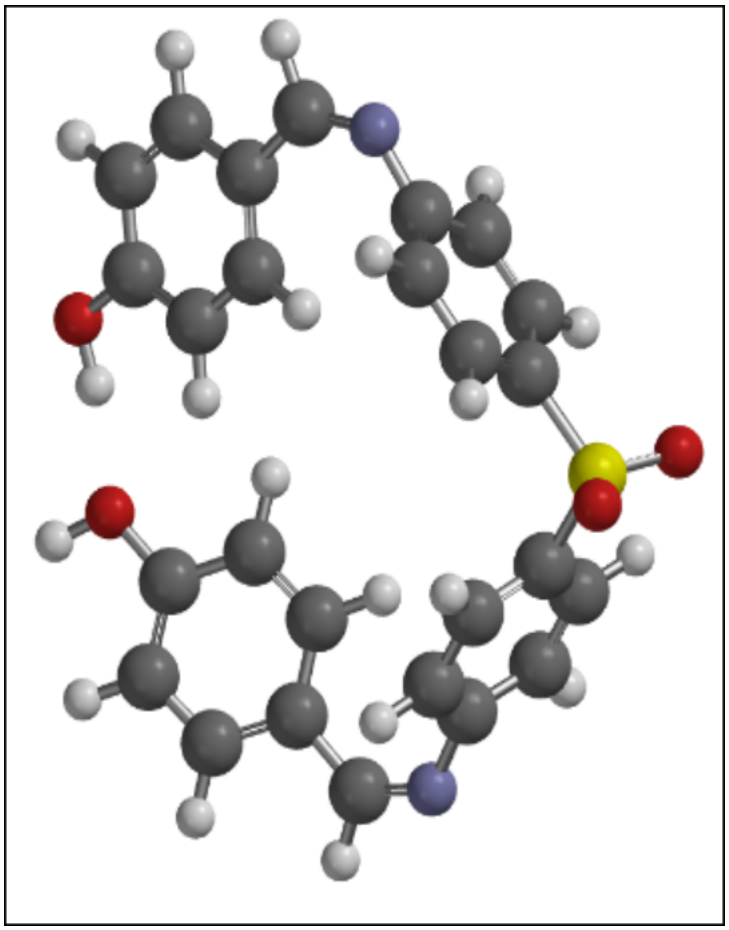

Fig 3.14 Optimized Structure of 4,4'-((1E)-((sulfonylbis(4,1-phenylene))bis(azanylylidene))bis(methanylylidene))diphenol

(NE)-N-(4-nitrosobenzylidene)-4-((4-((4-nitrosobenzylidene)amino)phenyl)sulfonyl)

aniline

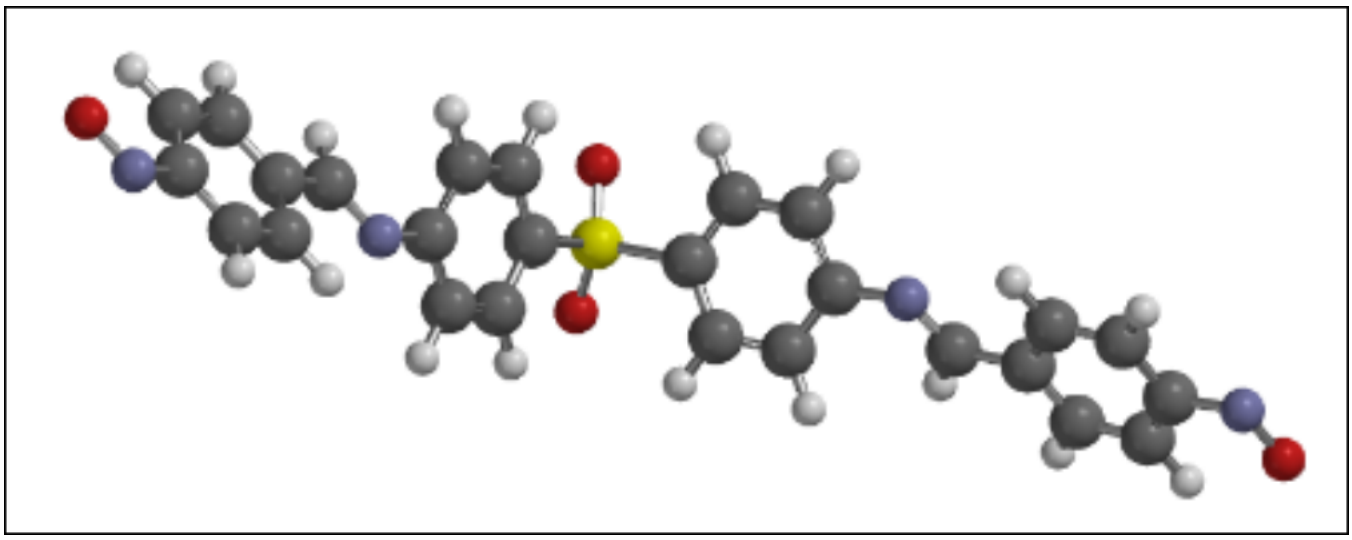

Fig 3.15 Optimized Structure of (NE)-N-(4-nitrosobenzylidene)-4-((4-((4-nitrosobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-methylbenzylidene)-4-((4-((4-methylbenzylidene)amino)phenyl)sulfonyl)

aniline

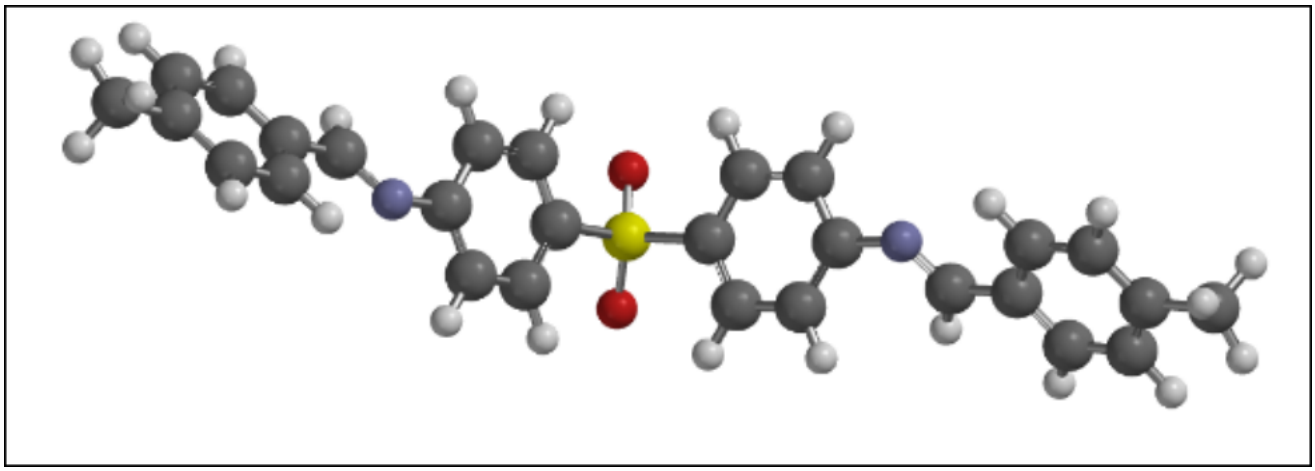

Fig 3.16 Optimized Structure of (NE)-N-(4-methylbenzylidene)-4-((4-((4-methylbenzylidene)amino)phenyl)sulfonyl)aniline

N-benzylidene-4-((4-((E)-benzylideneamino)phenyl)sulfonyl)aniline

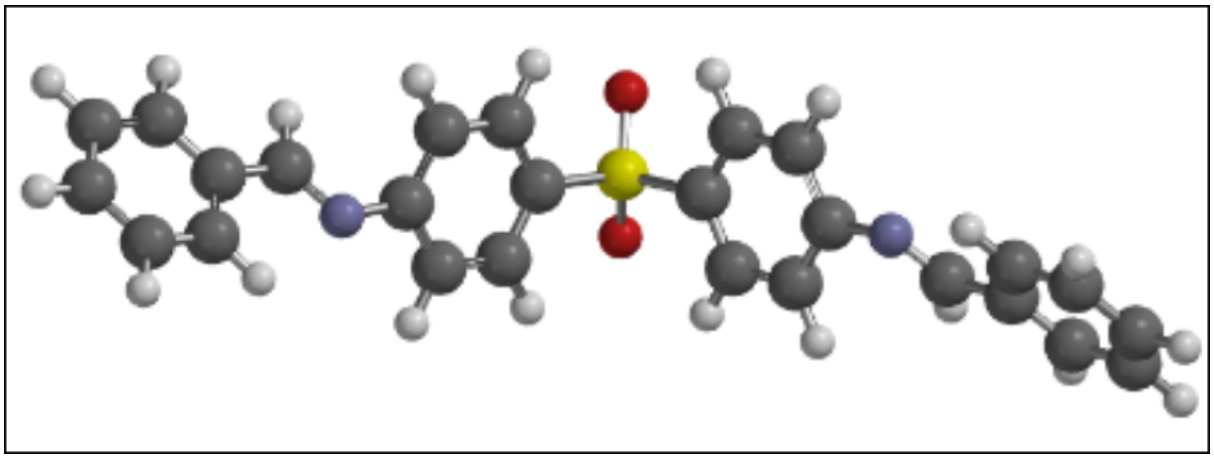

Fig 3.17 Optimized Structure of N-benzylidene-4-((4-((E)-benzylideneamino)phenyl)sulfonyl)aniline

(NE)-N-(4-fluorobenzylidene)-4-((4-((4-fluorobenzylidene)amino)phenyl)sulfonyl)aniline

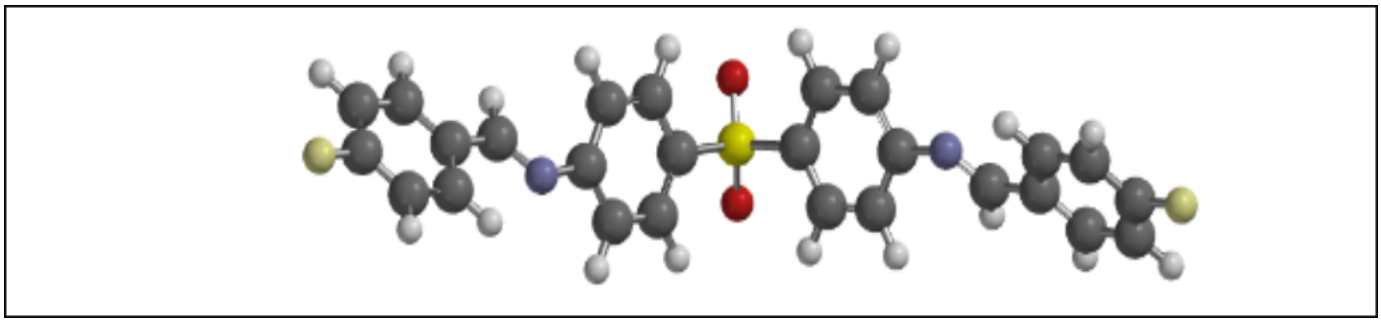

Fig 3.18 Optimized Structure of (NE)-N-(4-fluorobenzylidene)-4-((4-((4-

fluorobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-iodobenzylidene)-4-((4-((4-iodobenzylidene)amino)phenyl)sulfonyl)aniline

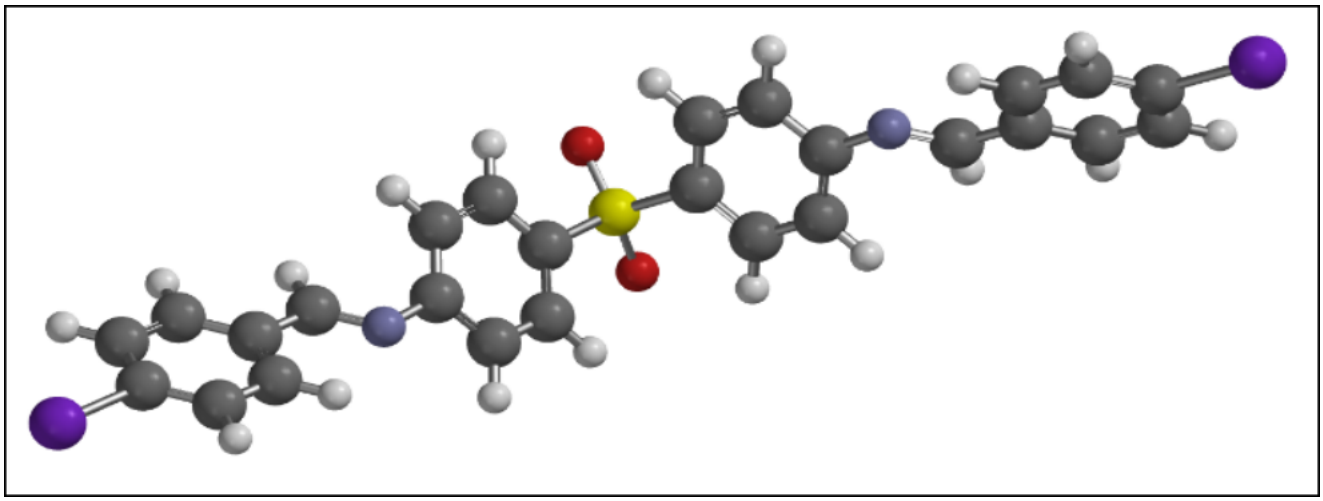

Fig 3.19 Optimized Structure of (NE)-N-(4-iodobenzylidene)-4-((4-((4-iodobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-chlorobenzylidene)-4-((4-((4-chlorobenzylidene)amino)phenyl)sulfonyl) aniline

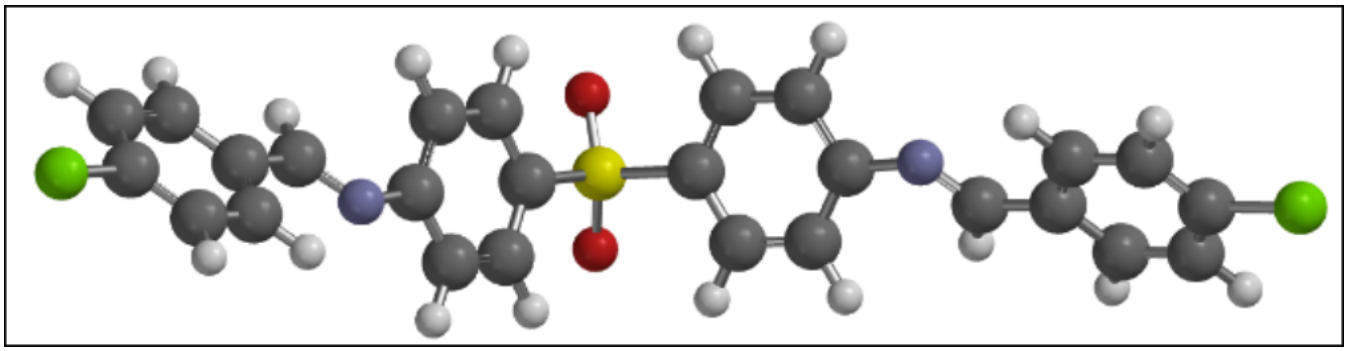

Fig 3.20 Optimized Structure of (NE)-N-(4-chlorobenzylidene)-4-((4-((4-chlorobenzylidene)amino)phenyl)sulfonyl)aniline

(NE)-N-(4-nitrobenzylidene)-4-((4-((4-nitrobenzylidene)amino)phenyl)sulfonyl)aniline

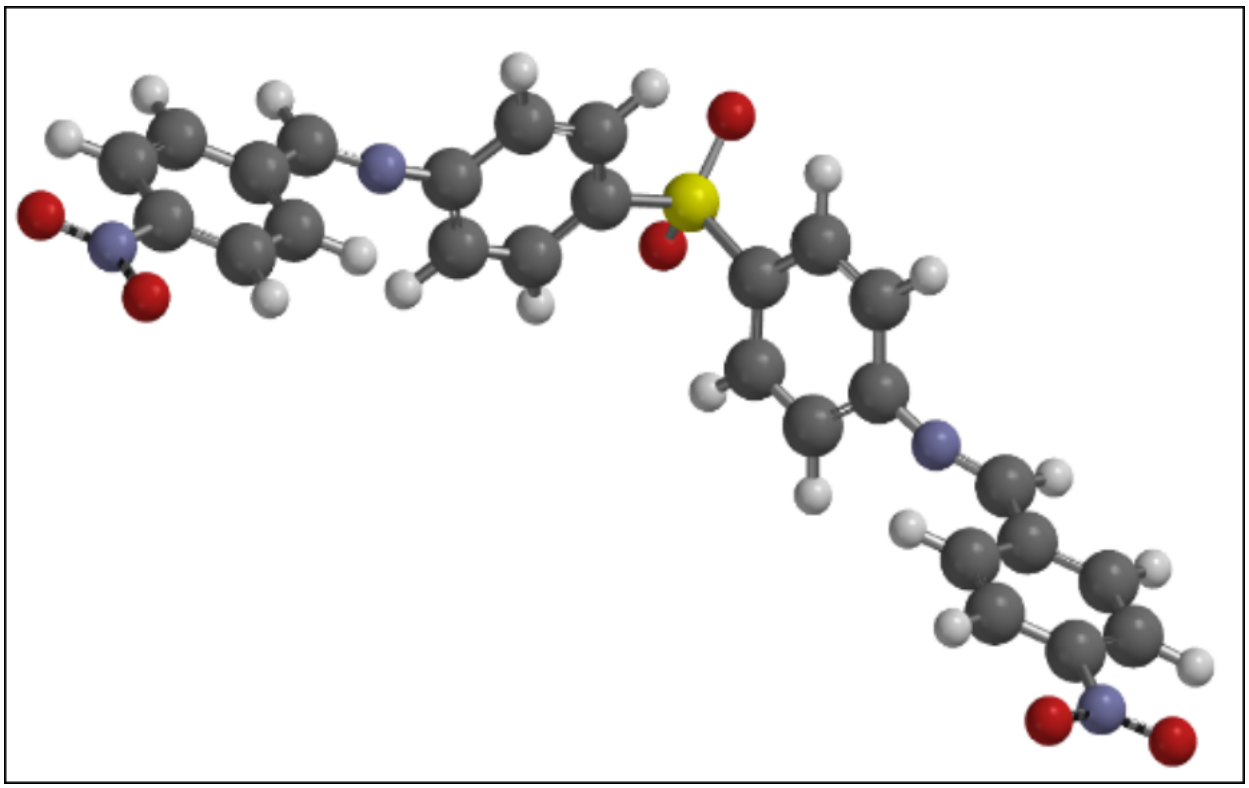

Fig 3.21 Optimized Structure of (NE)-N-(4-nitrobenzylidene)-4-((4-((4-nitrobenzylidene)amino)phenyl)sulfonyl)aniline

3.6 ChemDraw

ChemOffice is a comprehensive software suite that has become indispensable for chemists worldwide. Its core component, ChemDraw, excels in creating precise and aesthetically pleasing chemical structures and reactions.ChemDraw significantly accelerates the process of drawing complex molecules and reactions, saving researchers valuable time.The software ensures chemical structures are drawn correctly, adhering to established standards and conventions.ChemDraw seamlessly integrates with other components of the ChemOffice suite, such as Chem3D and ChemFinder, for a holistic chemical research experience.It allows for efficient organization and management of chemical information.ChemDraw supports various file formats, enabling compatibility with other software applications.

3.7 Molecular Docking Softwares

Molecular docking is a computational technique used to predict the orientation of one molecule (e.g., a drug) when bound to another (e.g., a protein). This information is crucial in drug discovery to identify potential drug candidates and understand their interactions with biological targets.Several software packages have been developed to perform molecular

docking simulations e.g AutoDock, AutoDock Vina, Glide,GOLD (Genetic Optimization for Ligand Docking)(employs genetic algorithms for ligand optimization),DOCK (one of the earliest docking programs, still widely used for its flexibility), MOE (Molecular Operating Environment), Schrodinger Suite.Different software packages use different docking algorithms, which can impact the results.The method used to evaluate the quality of the predicted binding poses can vary between software packages.Some software packages allow for protein flexibility during docking, which can improve accuracy.The software packages are preferred depending on the time required to perform docking simulations,the ability to predict correct binding poses and affinities,the ability to handle different types of molecules and receptors,the licensing fees associated with the software and access to technical assistance and updates

PyRx is a user-friendly software package designed for virtual screening in drug discovery. It allows researchers to efficiently screen large libraries of compounds against potential drug targets to identify promising lead molecules. It integrates popular docking engines like AutoDock and AutoDock Vina, providing flexibility in docking calculations.PyRx was used in this thesis.

3.8 SwissADME/T

SwissADME is a free online platform developed by the Swiss Institute of Bioinformatics (SIB). It is designed to predict various physicochemical, pharmacokinetic, and drug-likeness properties of small molecules. This tool is invaluable for researchers in the early stages of drug discovery as it helps assess the potential of compounds before progressing to more expensive and time-consuming experiments.

Physicochemical properties (like molecular weight, logP, number of hydrogen bond donors and

acceptors, polar surface area, and other relevant parameters).Pharmacokinetic properties (absorption, distribution, metabolism, excretion (ADME) properties, including blood-brain barrier permeability and P-glycoprotein substrate potential) and Drug-likeness of small molecules (evaluates compounds based on established drug-likeness rules, such as Lipinski's rule of five) were predicted using SwissADME. The medicinal chemistry was also predicted because it offers information on potential toxicity, mutagenicity, and other relevant properties.

3.9 Protein Data Bank

The protein structure was obtained from the Protein Data Bank (PDB), a repository of experimentally determined protein structures.Unnecessary components like water molecules and extraneous residues were removed to simplify the protein structure and improve computational efficiency.Gasteiger charges were calculated to assign partial charges to atoms, which is crucial for simulating electrostatic interactions during docking.Polar hydrogen atoms were added to the protein structure to complete its atomic representation.A grid box was defined within a specific coordinate space to specify the region where the ligand will be docked. This grid serves as the search space for the docking software.

The grid box with specific coordinate X= -4.49, Y=34.61, Z= 13.53

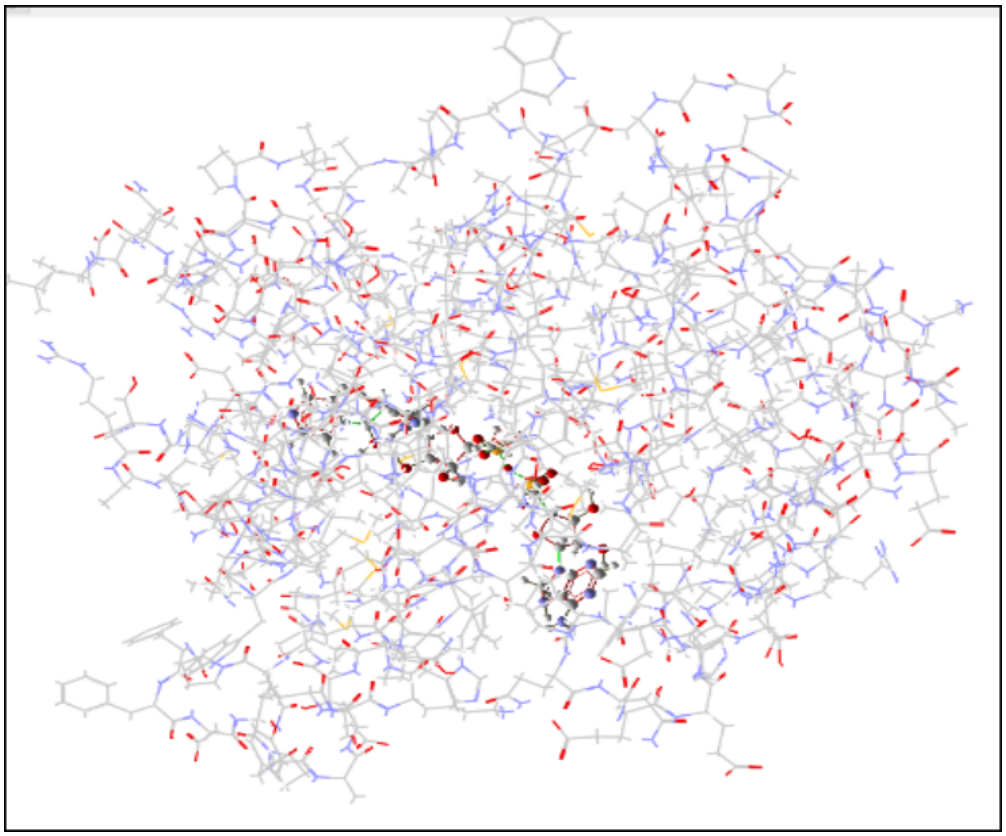

Fig 3.22 Surface structure of 1zid protein

3.10 Toxicity Estimation Software Tool (T.E.S.T)

T.E.S.T (Toxicity Estimation Software Tool) is a computational tool developed by the US Environmental Protection Agency (EPA) to predict the toxicity of chemicals. It utilizes Quantitative Structure-Activity Relationships (QSAR) models to estimate various toxicity endpoints based on a compound's molecular structure. Employs mathematical models to correlate chemical structure with toxicity. Allows users to input chemical structures and obtain toxicity predictions. Predicts endpoints such as acute toxicity, mutagenicity, carcinogenicity, and reproductive toxicity. Freely available for academic and research purposes.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Electronic properties

Density Functional Theory (DFT) calculations were performed on dapsone and its derivatives using the B3LYP method with a 6-31G* basis set to optimize their molecular structures. Subsequently, a range of physicochemical properties, including frontier molecular orbitals (HOMO and LUMO), ionization potential, electron affinity, electronegativity, chemical potential, hardness, electrophilicity, total energy, and dipole moment, were determined. These parameters provide essential insights into the compounds' chemical reactivity and potential biological activity.

A molecule's ability to donate or accept electrons is influenced by its electronic structure. A higher HOMO energy indicates a greater tendency to donate electrons to molecules with low-energy, empty orbitals (LUMO). A lower LUMO energy suggests a higher propensity to accept electrons. This energy difference between the HOMO and LUMO determines a molecule's reactivity. A smaller gap corresponds to higher reactivity and lower stability. Additionally, it represents the minimum energy required to excite an electron within the molecule. In essence, molecules with a smaller HOMO-LUMO gap are generally more reactive,less stable and prone to electron transfer processes (Kokalj, 2021).

An inhibitory candidate is a molecule or compound that is being studied for its potential to inhibit or block a specific biological process (this could be anything from enzyme activity to cell growth) (Bridges, 2001).A low E_{gap} indicates that it requires less energy to remove an electron from the HOMO of the molecule. This can make the molecule more reactive and potentially more likely to interact with a target molecule (like an enzyme or receptor) to inhibit its function. Therefore, molecules with lower E_{gap} are often considered better inhibitory candidates as they are more likely to participate in electron transfer processes, which are crucial for many biological interactions (da Silva *et al.*, 2024).

E_{HOMO} E_{LUMO}

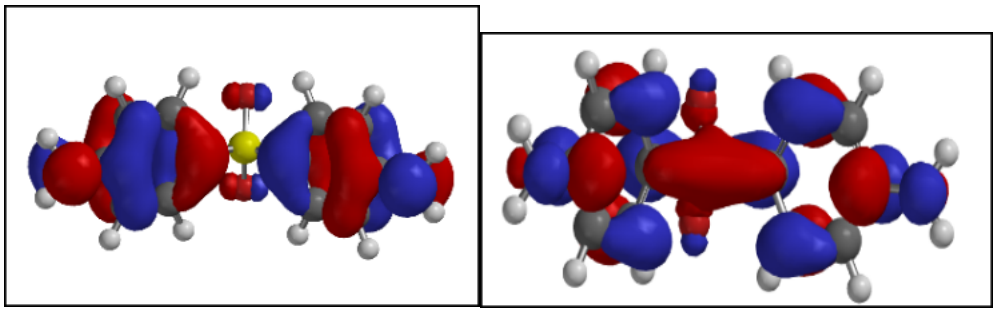

Fig 4.1 HOMO-LUMO Orbital Energy Diagram of DDS

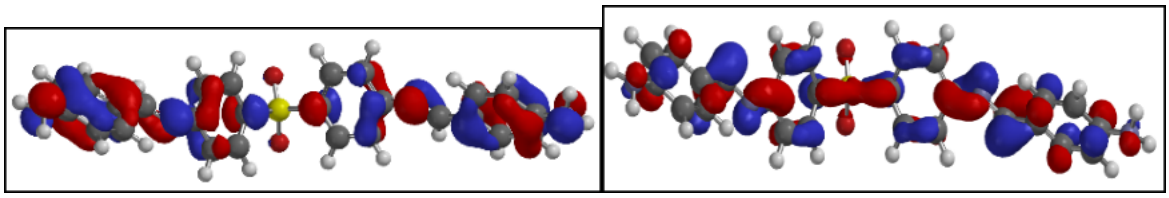

Fig 4.2 HOMO-LUMO Orbital Energy Diagram of ANHS

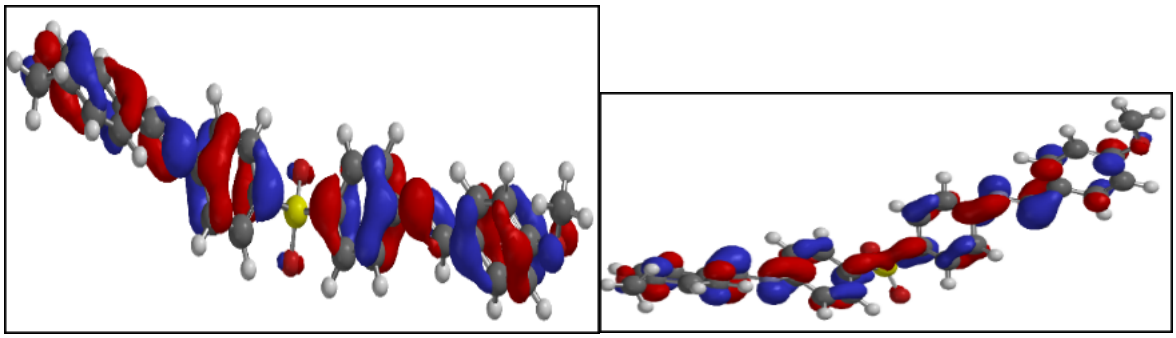

Fig 4.3 HOMO-LUMO Orbital Energy Diagram of AmoBS

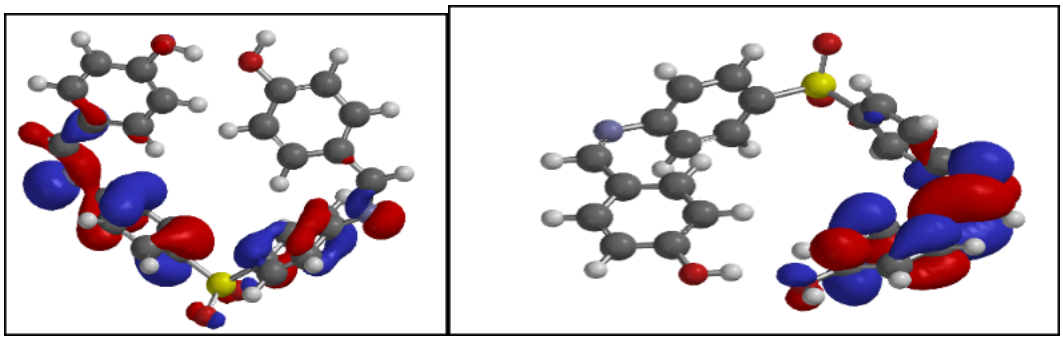

Fig 4.4 HOMO-LUMO Orbital Energy Diagram of AOHS

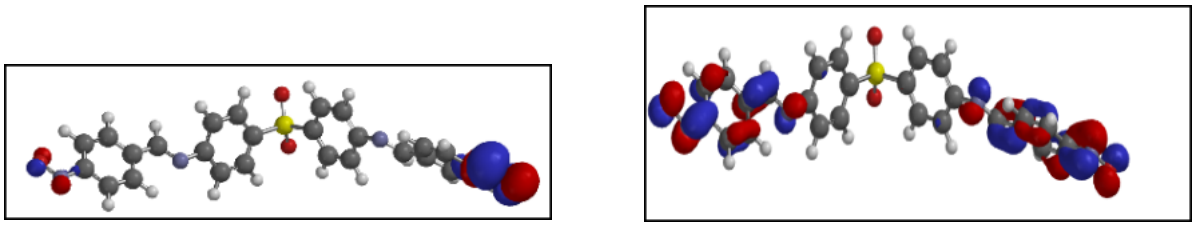

Fig 4.5 HOMO-LUMO Orbital Energy Diagram of ANOS

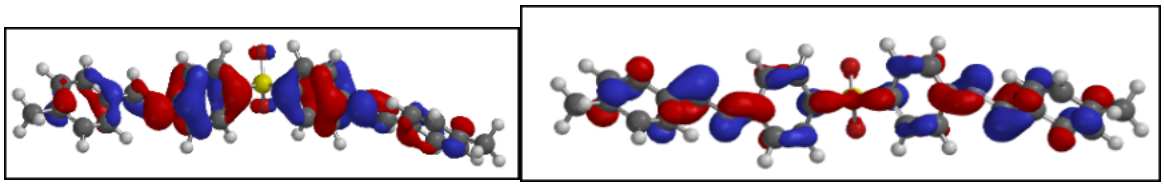

Fig 4.6 HOMO-LUMO Orbital Energy Diagram of AmBS

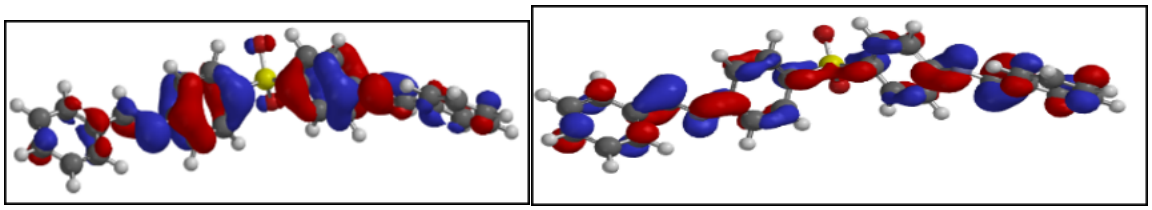

Fig 4.7 HOMO-LUMO Orbital Energy Diagram of ABS

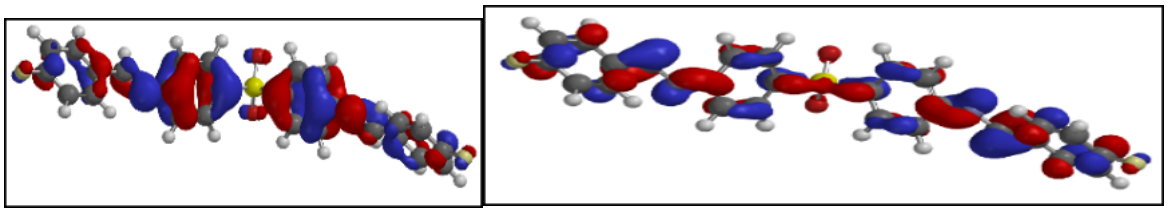

Fig 4.8 HOMO-LUMO Orbital Energy Diagram of AFS

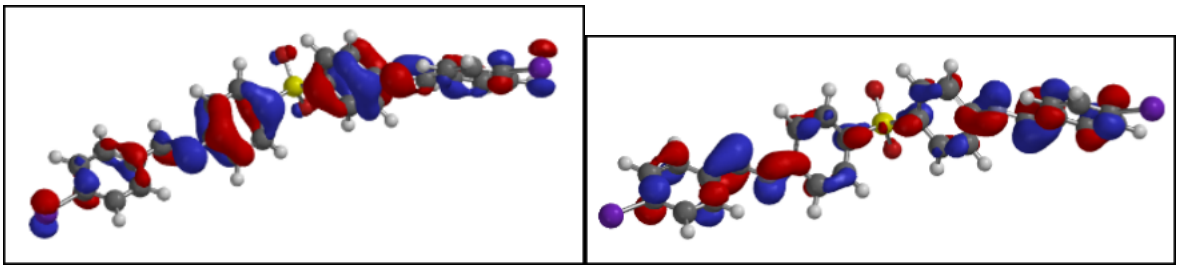

Fig 4.9 HOMO-LUMO Orbital Energy Diagram of AIS

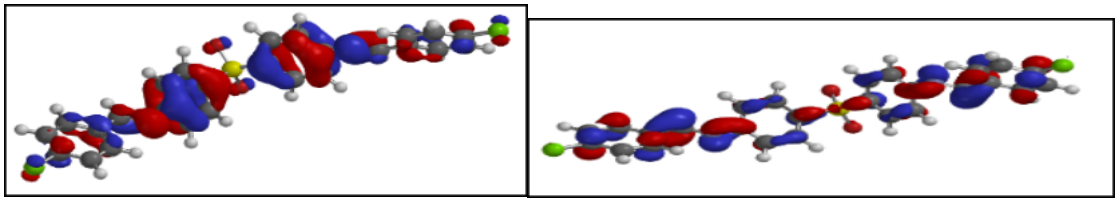

Fig 4.10 HOMO-LUMO Orbital Energy Diagram of ACS

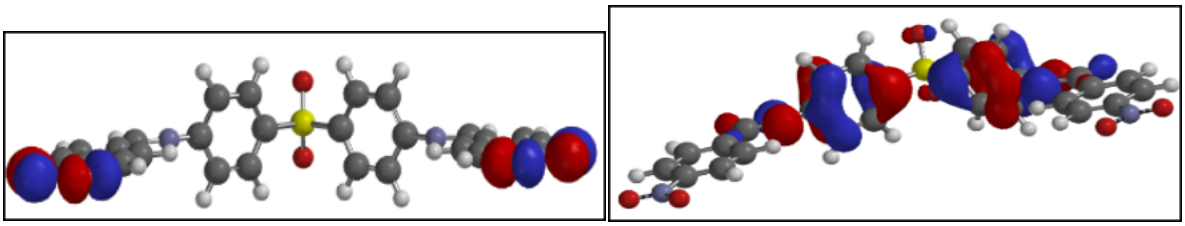

Fig 4.11 HOMO-LUMO Orbital Energy Diagram of ANS

Figure 4.1a-4.1k presents the frontier molecular orbitals (HOMO and LUMO distributions) of dapsone and its derivatives. The HOMO of dapsone is primarily localized on the conjugated double bonds, amino groups, and oxygen atoms. In contrast, the LUMO is concentrated on specific carbon, nitrogen, and sulfur atoms within the molecule.

The calculated E_{HOMO} and E_{LUMO}, dipole moment,total energy of the system, polarizability and band gap (E_{GAP}) of dapsone and its derivatives are recorded in Table 4.1

TABLE 4.1: Electronic property of DFT based DDS and its derivatives

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	E_{GAP}(eV)	Electronegativity(X)	Dipole Moment (Debye)	Total Energy (au)	Polarizability
DDS	-5.68	-0.67	5.01	-2.505	5.80	-1122.58162	59.51
ANHS	-5.51	-1.63	3.88	-1.94	7.96	-17751.5918	77.61
AmoBS	-5.82	-1.81	4.01	-2.005	9.09	-1889.92744	80.30
AOHS	-5.93	-1.86	4.07	-2.035	12.00	-1811.30334	76.88
ANOS	-5.99	-3.41	2.58	-1.29	8.44	-2069.20322	80.27
AmBS	-6.05	-1.93	4.12	-2.06	6.49	-1739.51586	78.85
ABS	-6.17	-2.02	4.15	-2.075	5.76	-1660.87825	75.88
AFS	-6.22	-2.08	4.14	-2.07	4.20	-1859.34542	76.62
AIS	-6.30	-2.25	4.05	-2.025	3.44	-1682.4420	79.97
ACS	-6.31	-2.24	4.07	-2.035	3.28	-2580.06944	78.12
ANS	-12.19	-11.27	0.92	-0.46	8.44	-2069.20322	80.27

An analysis of HOMO-LUMO energy gaps in dapsone and its derivatives in Table 4.1 above revealed a correlation between energy gap size and molecular stability and reactivity. *ABS*, with the largest HOMO-LUMO gap with 4.15eV, exhibited the highest stability and lowest reactivity. Conversely, *ANS*, characterized by the smallest energy gap with 0.92eV, demonstrated lower stability and higher reactivity compared to the other compounds studied, as shown in table 4.1 above.

However, polarizability and dipole moment are also indicators of reactivity,solubility and intermolecular forces, boiling point and melting point.

4.2 Reactivity parameters

HOMO and LUMO energies serve as the foundation for estimating key molecular properties such as ionization energy, electron affinity, electronegativity, chemical potential, molecular hardness, softness, and electrophilicity index.

Ionization Energy (I)= $-\varepsilon_{HOMO}$

Electron Affinity (A) = $-\varepsilon_{LUMO}$

Electronic Chemical Potential (μ) = –

Electronegativity ( )= 1/ 2(I + A)

Chemical Hardness ( ) = 1/ ¢2 (I – A)

Electrophilicity Index ( ) = ^{2}/ 2

This study examines the reactivity of the complexes using Density Functional Theory (DFT) and Hartree-Fock (HF) methods. Key reactivity descriptors, chemical potential, and global hardness, were calculated using finite difference and frozen orbital approximations. These approximations rely on the energies of the highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO).

While the accuracy of Koopmans' theorem within DFT remains contested, the discrepancies between Kohn-Sham and Hartree-Fock orbitals necessitate a comparative analysis of electronegativity, global hardness, and global electrophilicity index using both methods (Nalewajski, 2012). To enhance the reliability of these calculations, restricted hybrid HF-DFT computations incorporating Pulay DIIS and geometric direct minimization were employed. This approach strengthens the theoretical basis for determining vertical ionization energies

and electron affinities, calculated as energy differences between optimized neutral and ionized states, along with chemical potential and global hardness (Oyeneyin *et al.*, 2021).

Table 4.2: DFT based reactivity parameters of DDS and its derivatives

Compound	I(eV)	A(eV)	(eV)	(eV)	(eV)
DDS	5.68	0.67	2.505	-3.175	2.009
ANHS	5.51	1.63	1.94	-3.57	3.285
AmoBS	5.82	1.81	2.005	-3.815	3.6295
AOHS	5.93	1.86	2.035	-3.895	3.7275
ANOS	5.99	3.41	1.29	-4.70	8.5620
AmBS	6.05	1.93	2.06	-3.99	3.8641
ABS	6.17	2.02	2.075	-4.095	4.0407
AFS	6.22	2.08	2.07	-4.15	4.16
AIS	6.30	2.25	2.025	-4.275	4.5125
ACS	6.31	2.24	2.035	-4.275	4.4903
ANS	12.19	11.27	0.46	-11.73	149.5575

Given that inhibitors tend to adsorb on metallic surfaces with lower hardness, *ANS*'s lower hardness value of 0.46eV compared to *ABS*s 2.075eV is noteworthy. Additionally, the electrophilicity index, which measures a molecule's ability to accept electrons, was calculated. *ANHS*s low electrophilicity index of 3.285 eV suggests weak electron-accepting capacity, while *ANS*s higher value of 149.5575 eV indicates stronger electron-accepting potential, as shown in table 4.2 above.

4.3 Druglikeness parameters

Lipinski's Rule of Five outlines key physicochemical properties influencing a drug's oral bioavailability. These include molecular weight, lipophilicity (Log P), hydrogen bond donors and acceptors, and polar surface area. Compounds adhering to these parameters generally exhibit better membrane permeability and absorption.

Molecular weight: Compounds with higher molecular weights i.e 500 daltons tend to have poorer solubility, making it difficult for them to dissolve in biological fluids and reach their target site (Talevi & Bellera, 2022). A larger molecule has a harder time passing through the lipid bilayer of cell membranes. This is because the membrane is primarily composed of non-polar lipids, and larger molecules often have more polar groups, hindering their passage. All *DDS* derivatives possess molecular weight less than 500 daltons except *AIS* and *ANS* with 676.31 and 514.51respectively indicating poor solubility, as shown in table 4.3 below.

Partition Coefficient: The partition coefficient (log P), a measure of a compound's solubility in oil relative to water, is a critical factor in drug discovery and development (Arnott, & Planey, 2012).While typically determined using octanol and water, the principle can be applied to any immiscible solvent pair (Poole & Poole, 2003). Despite its importance, log P values are often estimated computationally rather than measured experimentally in early drug research. A comparison of predicted and experimental log P values for compounds revealed that derivatives of Dapsone with log P values below 5.6 are promising drug candidates.All the derivative of DDS have values below 5.6 except *ACSAIS* and *AmBS* with 6.22112O.5.925940 and 5.850940 respectively, as shown in table 4.3 below.

Polar Surface Area (PSA) is a crucial descriptor in medicinal chemistry for assessing a molecule's drug-like properties.It is calculated as the sum of the surface areas of polar atoms (primarily oxygen and nitrogen) and their attached hydrogen atoms. A higher PSA correlates with decreased cell membrane permeability. Molecules with a PSA greater than 140 A^2 often exhibit poor oral bioavailability. To access the central nervous system(CNS), a molecule typically requires a PSA below 90 A^2(Mannhold *et al.*, 2012). *ANS* indicates poor oral bioavailability with 143.42 while *ANOSANHS* and *AOHS* would have difficulty accessing the CNS due to values above $90A^2$ which are 126.1, 107.7and 119.28 respectively, as shown in table 4.3 below.

Rotatable bonds : are single bonds within a molecule that can freely rotate without breaking the molecule. They significantly influence a molecule's flexibility and three-dimensional shape.Compounds with a lower number of rotatable bonds tend to exhibit better oral bioavailability, as they are more likely to passively diffuse across cell membranes (Li, 2011). Rotatable bonds allow a molecule to adopt different conformations, increasing the likelihood of finding a favorable binding pose with a target protein. An excessive number of rotatable bonds can lead to increased conformational flexibility, which can negatively impact binding affinity.However, the number of rotatable bonds is a relatively simple descriptor and may not fully capture the complexity of a molecule's conformational space (Rai *et al.*, 2019). There are examples of drugs with high rotatable bond counts that exhibit excellent bioavailability and efficacy. While the number of rotatable bonds is a valuable parameter, it should be used in conjunction with other physicochemical properties to assess a molecule's drug-like potential.

A rotatable bond is any single non-ring bond, bonded to a nonterminal heavy (i.e., non-hydrogen) atom. Excluded from the count were amide C-N bonds because of their high rotational energy barrier (Maliar *et al.*, 2023.). This parameter is a measure of molecular

flexibility and has shown to be a very good descriptor of oral bioavailability of drugs. A large number of rotatable bonds (≥ 10) has been associated with poor oral bioavailability, particularly when associated with a high polar surface area (>140 $Å^2$) (Veber *et al.*, 2002). None of its derivatives has a rotatable bond greater than 10 hence they possess good oral bioavailability, as shown in table 4.3 below.

Hydrogen Bond Donors (HBDs) quantify a molecule's ability to form hydrogen bonds based on the number of hydrogen atoms directly attached to nitrogen or oxygen atoms. Molecules with more HBDs generally exhibit poorer membrane permeability due to the increased energy required to disrupt their interactions with water molecules when transitioning from an aqueous environment to a lipid membrane (Lomba *et al.*, 2021). The number of hydrogen atoms bonded to nitrogen or oxygen in a molecule. A higher number of HBDs often correlates with poor membrane permeability due to the energy required to break hydrogen bonds. HBDs are crucial for understanding a molecule's ability to interact with its environment, particularly in biological systems (Ijardar *et al.*, 2022). The number of HBDs is a significant factor influencing a compound's membrane permeability. A lower number of HBDs generally indicates better membrane permeability. Most of the *DDS*s derivatives possess low HBDs with value 0 except for *AOHS* and *ANHS* with slightly higher value of HBDs.2, as shown in table 4.3 below.

Hydrogen Bond Acceptors (HBAs) are atoms within a molecule capable of accepting a hydrogen bond from a donor molecule. Typically, these atoms are nitrogen, oxygen, or fluorine, excluding specific nitrogen configurations like positively charged or pyrrolic nitrogen. Like hydrogen bond donors, HBAs contribute significantly to a molecule's overall hydrogen bonding capacity. A molecule with a high number of HBAs often exhibits reduced membrane permeability (Kenny, 2022). This is because the molecule forms strong hydrogen

bonds with water molecules, creating a solvation shell. To penetrate the lipid bilayer, these hydrogen bonds must be broken, a process requiring substantial energy. As a result, molecules with fewer HBAs tend to have better membrane permeability (Rafi *et al.*, 2012). *DDS* and its derivatives reportedly have fewer than 10 HBAs, a characteristic that likely contributes to their ability to efficiently cross cell membranes. However, it's crucial to remember that membrane permeability is a complex property influenced by multiple molecular features, and the number of HBAs is just one factor among others, as shown in table 4.3 below.

Table 4.3: Druglikeness parameters for DDS and its derivatives

Compound	Mw (Daltons)	Log P	PSA(A^2)	RB	HBD	HBA
DDS	248.3	1.058380	94.56	2	2	4
ANHS	454.54	0.574900	119.28	6	2	6
AmoBS	484.57	4.79072	85.7	8	0	6
AOHS	456.51	4.030540	107.7	6	2	6
ANOS	482.51	4.154260	126.1	8	0	8
AmBS	452.57	5.850940	67.24	6	0	4
ABS	424.51	4.878200	67.24	6	0	4
AFS	460.5	5.152280	67.24	6	0	4
AIS	676.31	5.925940	67.24	6	0	4
ACS	493.4	6.221120	67.24	6	0	4
ANS	514.51	4.892560	143.42	8	0	8

4.4 ADME parameters

Drug disposition encompasses the processes by which a drug is absorbed, distributed, metabolised, and eliminated from the body. These processes, collectively known as ADME (Absorption, Distribution, Metabolism, and Excretion), significantly influence a drug's therapeutic efficacy and safety. The initial step in drug disposition, absorption, is the process by which a drug moves from its administration site (e.g., oral, intravenous, intramuscular) into the bloodstream. Factors such as the drug's physicochemical properties, formulation, and the physiological environment at the absorption site influence this process. Once in the bloodstream, a drug is distributed to various tissues and organs. Factors affecting distribution include regional blood flow rates, molecular size, polarity, binding to serum proteins, forming a complex.

Blood-brain barrier (BBB) is a highly selective semi-permeable membrane that separates the circulating blood from the brain's extracellular fluid. This protective barrier is composed primarily of tightly joined endothelial cells lining the brain's capillaries which restrict the passage of most molecules (Bors & Erdő, 2019).The BBB shields the brain from harmful substances circulating in the bloodstream, such as toxins, pathogens, and certain drugs.It allows the passage of essential nutrients like glucose and amino acids into the brain.The BBB facilitates the removal of waste products from the brain (Upadhyay, 2014). The BBB poses a significant challenge for drug development, as many potential therapeutic agents are unable to cross this barrier effectively. This limitation often hinders the treatment of brain diseases such as Alzheimer's, Parkinson's, and brain tumors. Astrocytes, pericytes, and other cells contribute to the BBB's function and integrity (Cabezas *et al.*, 2014). The BBB poses a significant challenge for drug development, as many potential therapeutic agents cannot cross it effectively. Overcoming this barrier is crucial for treating neurological

diseases.Smaller molecules are more likely to cross the BBB. Lipid-soluble molecules can more easily diffuse across the BBB. A higher polar surface area generally reduces BBB permeability. Compounds that can be transported by specific carriers can cross the BBB (Habgood, M., & Ek, J., 2010). Due to the BBB's stringent restrictions, only molecules with specific properties can cross it. These molecules typically possess high lipid solubility, a molecular weight below 400 Daltons, and are not substrates for efflux pumps that actively remove substances from the brain. As a result, the development of drugs that can effectively cross the BBB to treat central nervous system (CNS) disorders presents a significant challenge in the pharmaceutical industry.To quantify the ability of a compound to penetrate the BBB, a scale is often used, with higher values indicating better CNS absorption. Compounds with a BBB value exceeding 2.0 are considered to have high CNS penetration, while those below 0.1 exhibit low penetration.The table shows that the derivatives of *DDS* demonstrate lower BBB permeability with *ABS* exhibiting the lowest except *ANOS* with 2.11998. Consequently, these derivatives are expected to exhibit limited CNS absorption.All the derivatives show low CNS penetration, as shown in table 4.4 below.

Caco-2 Cell Line is a widely used in vitro model in the pharmaceutical industry to predict the absorption of orally administered drugs. Derived from human colon carcinoma cells, these cells exhibit characteristics similar to the small intestinal mucosa when cultured under specific conditions. Caco-2 cells form tight junctions, creating a barrier similar to the intestinal epithelium (Hidalgo *et al.*, 1989). These cells express enzymes found in the small intestine, enabling metabolism studies. Caco-2 cells express transporters involved in drug absorption, providing a more accurate prediction of intestinal permeability. The permeability coefficient is a quantitative measure of a compound's ability to pass through the Caco-2 monolayer (Van Breemen, R. B., & Li, Y., 2005). A higher permeability coefficient indicates

better absorption potential.

In the context of this research, the observation that *DDS* exhibits lower permeability than its derivatives except *AOHS* suggests that its derivatives have a better chance of being absorbed orally compared to its modified forms, as shown in table 4.4 below.

Human Intestinal Absorption (HIA) is a critical parameter in drug development, as it determines the extent to which an orally administered drug is absorbed into the bloodstream (Lin & Wong, 2017). Measured as a percentage of the drug reaching the hepatic portal vein, HIA significantly impacts a drug's bioavailability. Molecular weight, lipophilicity, solubility, and pKa influence a drug's ability to cross the intestinal barrier. Drug formulation (e.g., dosage form, excipients) can influence HIA. A high HIA value indicates that a significant portion of the administered drug is absorbed into the bloodstream, increasing the likelihood of reaching the target site and exerting its therapeutic effect (Hosseini *et al.*, 2024).

In the case of *DDS* and its derivatives, the reported HIA values ranging from 95.971666% to 100.00% suggest excellent intestinal absorption, which is a positive attribute for oral drug delivery, as shown in table 4.4 below.

MDCK (Madin-Darby Canine Kidney) cells are a widely used in vitro model to assess the permeability of compounds across cell membranes. They form tight junctions, mimicking the intestinal epithelium, making them a valuable tool in drug discovery. MDCK cells are employed to predict the intestinal absorption of compounds.A higher permeability value indicates a greater likelihood of the compound being absorbed from the intestine into the bloodstream (Wessel & Mente, 2001). The parent compound (*DDS*) exhibited significantly higher permeability across the MDCK cell monolayer compared to its derivatives with less than 3, as shown in table 4.4 below.

Plasma protein binding (PPB) refers to the extent to which a drug binds to proteins within the blood plasma. This interaction significantly influences a drug's pharmacokinetic and pharmacodynamic properties (Schmidt *et al.*, 2010).Only the unbound fraction of a drug can distribute to target tissues and exert its therapeutic effect. The unbound fraction is primarily metabolized and excreted from the body.A high degree of protein binding can reduce drug efficacy by limiting the amount of free drug available to interact with its target (Smith *et al.*, 2010).Competition for binding sites on plasma proteins can lead to drug-drug interactions. Compounds with high PPB values (close to 90%) have a larger proportion of the drug bound to plasma proteins(Hall *et al.*, 2009). This might lead to a slower onset of action and a longer duration of effect. However, a high PPB can also increase the risk of drug interactions. . It can be seen that all the derivatives of *DDS* show a binding affinity greater than 90. Only *ABS* and *AFS* also showed a bounding ability very close to 90. as shown in table 4.4 below.

Skin Permeability (SP): Skin permeability refers to the ability of substances to penetrate the skin and enter the bloodstream. It's a crucial factor in the development of transdermal drug delivery systems and in assessing the potential risks associated with skin exposure to chemicals (Schaefer *et al.*, 2013). *AmBS* have the highest skin permeability followed by *ABS* as compared to other derivatives hence they have a higher capability of penetrating and diffusing through the skin, as shown in table 4.4 below.

Table 4.4: ADME parameters of DDS and its derivatives

Compound	BBB	Caco-2	HIA(%)	MDCK	PPB	SP
DDS	0.41122	0.525127	95.808089	25.5011	62.747812	-2.96876
ANHS	0.073051	21.4501	97.134010	0.0865534	98.602861	-1.46641
AmoBS	0.943792	21.7117	97.672379	0.0488581	92.340493	-1.31429
AOHS	0.0730273	0.00067345	95.971666	0.0494539	95.996939	-1.73567
ANOS	2.11998	18.9824	100.000	0.0477411	93.537283	-1.49332
AmBS	0.147944	21.7135	97.909692	0.0573937	91.345558	-0.864806
ABS	0.00323683	21.7106	97.804994	0.0527791	89.091990	-0.960578
AFS	0.297552	21.7004	97.813251	0.0498759	89.453277	-1.41739
AIS	0.187793	22.9901	98.154478	0.517697	100.000	-1.13078
ACS	0.195916	21.4426	98.239063	0.0522383	94.614544	-1.12509
ANS	0.494505	9.05512	97.960940	0.047628	95.140616	-1.91051

4.5 Toxicity parameters

Toxicity refers to a substance's potential to harm or damage an organism. Individual responses to toxic substances can vary widely. To assess a compound's toxicity, in vivo studies are conducted using animals such as rabbits, rats, and mice. . These studies involve the test for parameters like algae_at,ames test,carcinogenicity,dalphnia magna test, and LC_{50}.

Algae-at is an efficient bioassay method that utilizes microalgae to evaluate the toxicity of various substances, including pure compounds, effluents, sediments, and water samples.It requires minimal resources for culture and maintenance (Hung, 2004). It's applicable to a

wide range of sample types and can detect toxicity *DDS* and its derivatives. A lower Algae-at value generally indicates higher toxicity. The results suggest that most derivatives exhibited lower toxicity compared to the parent compound as shown in table 4.5 below.

The Ames test is a widely used method to assess the mutagenic potential of chemicals. It employs bacteria to detect mutations induced by the test substance.The Ames test utilizes specific strains of *Salmonella typhimurium* that carry mutations affecting histidine biosynthesis.The test measures the ability of a chemical to cause these bacteria to revert to a histidine-independent state, indicating a mutation. A higher number of revertant colonies suggests increased mutagenicity of the test substance (McMahon *et al.*, 1979).

In the context of the provided information, the Ames test would be used to determine if the *DDS* and its derivatives have the potential to cause genetic mutations except *ANHS* and *ACS*as shown in table 4.5 below. A positive Ames test result would raise concerns about the compounds' safety and potential carcinogenicity while it doesn't definitively prove carcinogenicity, it warrants further investigation and careful risk assessment.

Carcinogenicity refers to the ability of a substance to cause cancer. In the context of drug development, it's crucial to assess the carcinogenic potential of compounds to ensure patient safety..These involve administering the compound to animals (typically rats and mice) over an extended period to observe tumor development.These tests also use cell cultures or isolated enzymes to evaluate a compound's potential to damage DNA or interact with cellular processes involved in carcinogenesis (Oliveira *et al.*, 2007). Predictive models can be used to estimate carcinogenicity based on a compound's chemical structure.Regulatory agencies, such as the FDA and EMA, have strict guidelines for assessing carcinogenicity during drug development. Compounds with a high carcinogenic risk are typically rejected.

In the context of this research, the use of a positive sign to indicate a carcinogenic tendency and a negative sign to indicate a non-carcinogenic tendency in table 4.5 provides a simplified representation of carcinogenicity assessment. However, it's important to note that carcinogenicity evaluation is a complex process involving multiple lines of evidence.

The Daphnia magna test is a widely used bioassay to assess the acute toxicity of substances. This small crustacean is sensitive to environmental contaminants and serves as a reliable indicator of potential harm to aquatic life. Daphnia magna is highly sensitive to a range of pollutants. The test is relatively inexpensive and easy to conduct. It provides valuable information about the potential ecological impact of chemicals (Tkaczyk *et al.*, 2021).

In the context of this research, the lower Daphnia_at value for most derivatives compared to the parent compound indicates that these derivatives are generally less toxic to aquatic organisms.

LC_{50} stands for Lethal Concentration 50. It is a measure of acute toxicity used to quantify the concentration of a substance required to kill 50% of a test population within a specified time period. This value is typically expressed in milligrams per liter (mg/L). Unlike LD_{50} (lethal dose), LC_{50} is used for substances in a liquid or gaseous form. A lower LC_{50} value indicates a higher toxicity of the substance. Common test organisms include fish, daphnia, and algae.

LC_{50} is used extensively in environmental toxicology and risk assessment to evaluate the potential hazards of chemicals (Gupta & Gupta, 2020).

The toxicity profiles of *DDS* and its derivatives were evaluated using the Pre-ADMET tool. The calculated toxicity parameters are summarized in Table 4.5below:

Table 4.5: Toxicity Parameters of DDS and its Derivatives

Compound	Algae_at	Ames.test	Carcino.mouse	Carcino.rat	Daphnia.at	LC_{50}
DDS	0.180636	mutagen	negative	negative	0.300425	250
ANHS	0.0104327	non-mutagen	negative	negative	0.00934319	3500
AmoBS	0.00477103	mutagen	negative	negative	0.00274594	3500
AOHS	0.00465976	non-mutagen	positive	positive	0.00420014	3500
ANOS	0.0416	mutagen	negative	positive	0.0014242	1345
AmBS	0.00309685	mutagen	negative	negative	0.00121026	3500
ABS	0.00755924	mutagen	negative	negative	0.00323683	3500
AFS	0.00482155	mutagen	positive	positive	0.00219951	3500
AIS	0.00160014	mutagen	negative	positive	8.92236e-005	3500
ACS	0.00210885	non-mutagen	negative	negative	0.000601562	3500
ANS	0.00628122	mutagen	positive	positive	0.00214232	1345

4.6 Docking Studies

Molecular docking simulations were employed to assess the potential of *DDS* and its derivatives as inhibitors of long fatty acid chain enoyl-acp reductase (INHA) in complex with an isonicotinic-acyl-NADH inhibitor protein. The protein target, 1zid, was retrieved from the

Protein Data Bank and prepared for docking by removing water molecules and adding hydrogen atoms. Computational analysis of the compounds' electronic properties and drug-likeness profiles was conducted using Spartan and Lipinski's Rule of Five, respectively. ADMET properties were predicted using Pre-ADMET, while toxicity endpoints were evaluated with T.E.S.T 4.2.

To prepare for docking simulations, the reductase (1zid) was obtained from the Protein Data Bank and processed by removing water molecules and unnecessary residues. The calculated binding free energies (G) for the best docking poses of the *DDS* and their derivatives indicated potential inhibitory activity against the reductase, as reflected by the negative G values.

Clofazimine, a well-established riminophenazine antibiotic inhibitor, served as a benchmark drug in this study. Its binding affinity to the reductase was determined to be -7.4. Docking results indicated that several derivatives exhibited comparable or even superior binding affinities to the reference drug, clofazimine. Compounds *ANOS, AOHS,AFS,ANS,ANHS* and ABS demonstrated the most promising inhibitory potential against the reductase. However, further studies are required to validate these computational findings experimentally.

Table 4.6: Binding Affinities of DDS and its derivatives with Reverse Transcriptase (PDB: 1zid)

Compound	Binding Affinity (kcal/mol)	In comparison to Cloazamine
DDS	-6.3	<
ANHS	-7.6	>
AmoBS	-7.2	<

AOHS	-7.9	>
ANOS	-7.9	>
AmBS	-7.1	<
ABS	-7.6	>
AFS	-7.7	>
AIS	-6.8	<
ACS	-7.2	<
ANS	-7.6	>

CHAPTER FIVE

SUMMARY AND CONCLUSION

5.1 SUMMARY

This research employed computational approaches to comprehensively characterize 4,4'-sulfonyldianiline and its derivatives. To elucidate the compounds' electronic structure and reactivity, density functional theory (DFT) calculations were undertaken utilizing the Spartan software package. A rigorous assessment of drug-likeness was conducted by applying Lipinski's Rule of Five to the compounds. To predict the compounds' absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles, the Pre-ADMET tool was employed. Furthermore, the toxicity endpoints of the compounds were estimated using the T.E.S.T 4.2 software.To evaluate the potential binding interactions of these compounds with a biological target, molecular docking simulations were performed using AutoDock. The protein structure, obtained from the Protein Data Bank, was meticulously prepared for docking by removing water molecules and adding hydrogen atoms.

5.2 CONCLUSION

Computational analysis revealed that the 4,4'-sulfonyldianiline derivatives possess reactive functional groups. Despite this, most compounds adhered to Lipinski's Rule of Five, suggesting potential oral bioavailability. Additionally, the derivatives exhibited favorable ADMET profiles and low toxicity, as indicated by toxicity parameter calculations. Docking studies demonstrated that certain derivatives exhibited superior binding affinities to the reductase compared to the reference drug, clofazimine. However, compounds with lower binding affinities might still exhibit antiviral activity but with reduced potenc

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