

# DESIGN, 2D QSAR STUDIES, MOLECULAR DOCKING AND ADMET PREDICTION AND TOXICITY STUDIES OF AMINOQUINOLINES AS POTENT ANTI-MYCOBACTERIAL AGENTS

Submitted in fulfillment of the requirements for the award of

**Bachelor of Pharmacy**

By

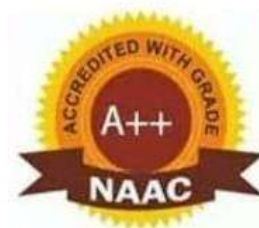
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SCHOOL OF PHARMACY

SATHYABAMA INSTITUTE OF SCIENCE & TECHNOLOGY

(Institution deemed of be University-U/s of the UGC Act, 1956)

Rajiv Gandhi Salai, CHENNAI – 600119

April 2024



# SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY  
(DEEMED TO BE UNIVERSITY)  
CATEGORY - 1 UNIVERSITY BY UGC



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### Bonafide Certificate

This is to certify that the thesis entitled "DESIGN, 2D OSAR STUDIES, MOLECULAR DOCKING AND ADMET PREDICTION AND TOXICITY STUDIES OF AMINOQUINOLINES AS ANTIMYCOBACTERIAL AGENTS", submitted by

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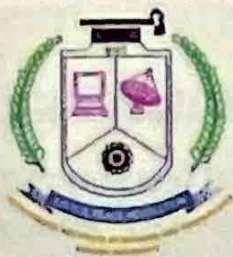
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## Declaration

We do hereby declare that the work presented in the thesis entitled "DESIGN, 2D OSAR STUDIES, MOLECULAR DOCKING AND ADMET PREDICTION AND TOXICITY STUDIES OF AMINOQUINOLINES AS ANTIMYCOBACTERIAL AGENTS", submitted by us to the Sathyabama Institute of Science & Technology, Chennai-600119 for the award of the degree of Bachelor of Pharmacy (2020-2024) by research is original record of research work carried out by under the supervision of **Dr. S. ARUNKUMAR, M.Pharm, Ph.D., Professor, School of Pharmacy**. The contents if this dissertation , in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma, membership, fellowship , association etc. In keeping with the general practice in reporting scientific observation, due acknowledgment has been made whenever the work described is based on the findings of other investigators.

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## ABSTRACT

### DESIGN, 2D QSAR STUDIES, MOLECULAR DOCKING AND ADMET PREDICTION AND TOXICITY STUDIES OF AMINOQUINOLINES AS POTENT ANTI-MYCOBACTERIAL AGENTS

#### Introduction:

Mycobacterium tuberculosis mainly affects the respiratory tract, is the leading cause of death from a curable infectious disease. It is estimated to kill around about 7 to 8 millions of people mainly men. However, the emergence of drug-resistant strains poses a significant challenge in clinical management, undetermining efforts to reduce mortality and disease prevalence. To protect public health emergency, there is an urgent need for a new antimycobacterial drugs, with a single dose cures, broad therapeutic potential, and novel mechanism of action, aminoquinoline analogues are one such novel class which possess a potent antimycobacterial activity<sup>[1]</sup>.

#### Methodology:



#### Results and discussion:

QSAR models were generated for 27 aminoquinone analogues. 20 Novels aminoquinones derivatives were designed and their probable activities were predicted. Physiochemical parameters, Drug likeness, pharmacokinetics characteristics were derived and were subjected to docking. Binding affinity and the interactions between protein and ligand molecules were analysed.

**Conclusion:**

The discovered novel aminoquinoline analogues were highlighted as potent antimycobacterial agents with comparatively lower toxicity and a novel mechanism of these compounds have higher binding affinity. Thus proving multi targeted drug therapy against tuberculosis.

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## **ABBREVIATIONS**

|       |                                                    |
|-------|----------------------------------------------------|
| ADME  | Absorption, Distribution, Metabolism and Excretion |
| AMDR  | Antimalarial Drug Resistance                       |
| BBB   | Blood Brain Barrier                                |
| QSAR  | Quantitative Structure Activity Relationship       |
| SAR   | Structure Activity Relationship                    |
| MDR   | Multi Drug Resistance                              |
| HIA   | Human Intestinal Absorption                        |
| MIC   | Minimum Inhibitory Concentration                   |
| Q2LOO | Variance in Leave one out                          |
| Q2LMO | Variance in Leave many out                         |
| CCC   | Concordance Correlation Coefficient                |
| CNS   | Central Nervous System                             |
| RMSE  | Root Mean Squared Error                            |
| MAE   | Mean Absolute Error                                |
| MIC   | Minimum Inhibitory Concentration                   |
| SEE   | Standard Error of Estimate                         |
| PSA   | Polar Surface Area                                 |

|                |                                                                            |
|----------------|----------------------------------------------------------------------------|
| R              | Correlation Coefficient                                                    |
| R <sup>2</sup> | Coefficient of Determination                                               |
| F              | Fischer's Value                                                            |
| AD             | Application Domain                                                         |
| TPSA           | Topological Polar Surface Area                                             |
| ROS            | Reactive Oxygen Species                                                    |
| GSH            | Glutathione                                                                |
| GSSG           | Glutathione disulfide                                                      |
| 2AQ8           | Enyl-ACP(CoA) reductase                                                    |
| 2IDZ           | Enoyl-ACP (CoA) reductase                                                  |
| 1F0N           | mycolyl transferase                                                        |
| 1DG5           | Dihydrofolate reductase                                                    |
| 1E9X           | Cytochrome P450 14 alpha-sterol demethylase                                |
| VIF            | Variance Inflation Factor                                                  |
| RCSB-PDB       | Research Collaboratory for Structural Bioinformatics-<br>Protein Data Bank |

# **DESIGN, 2D QSAR STUDIES, MOLECULAR DOCKING AND ADMET PREDICTION AND TOXICITY STUDIES OF AMINOQUINOLINES AS POTENT ANTIMYCOBACTERIAL AGENTS**

## **CHAPTER 1**

### **1. INTRODUCTION**

Medicinal chemistry integrates chemistry, biology, and medicine to discover and develop new drugs, aiming for safer and more effective treatments, thus advancing healthcare <sup>[2]</sup>. Medicinal/Pharmaceutical chemistry involves drug discovery, design, development, pharmacological and analytical characterisation, with chemists crucial in preclinical stages and quality control <sup>[3]</sup>. Medicinal chemistry, a rapidly evolving scientific discipline, has become a bridge between related disciplines, synthesizing bioactive molecules and forming the foundation of various scientific fields <sup>[4]</sup>. Medicinal chemistry is a scientific discipline that combines chemistry and pharmacy, focusing on drug design, synthesis, and study of existing drugs' biological properties and QSAR <sup>[5]</sup>. Drug discovery is a multifaceted task that involves finding and developing new therapeutic compounds. In recent years, the field has seen significant advancements, adopting diverse approaches and methodologies to improve the process. This evolution is driven by the need to meet the increasing demand for innovative treatments and tackle a wide range of medical conditions <sup>[6]</sup>. Drug discovery, spanning over a decade on average, varies in cost and time based on the drug and disease targeted. Recent decades have seen rapid advancements in scientific research and technology, facilitating the generation of novel drug candidates and their swift translation into usable treatments. Therapeutic agents targeting specific proteins or biological activities within living cells have revolutionized the field. High-throughput methods and technologies like genome sequencing have further transformed the drug discovery process <sup>[7]</sup>.

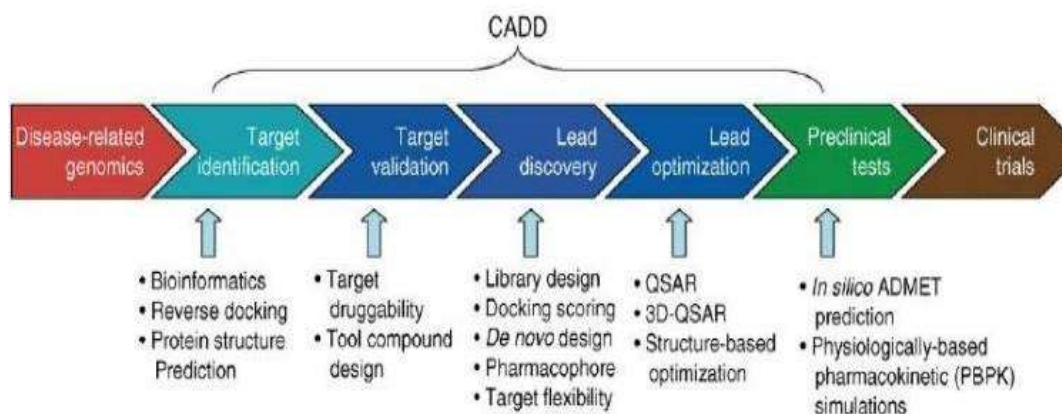
In drug discovery, ADME strategies are tailored to clinical goals, disease severity, and competitive factors. The focus is on creating safe and effective products, prioritizing drug potency and selectivity early on. For oral drugs, ideal ADME properties include good bioavailability and minimal risk of adverse effects. Rapid in vitro screening

methods help integrate ADME assessment into high-throughput processes <sup>[8]</sup>. Understanding molecular phenomena has enabled the discovery of new drugs through molecular strategies derived from biochemistry, pharmacology, or disease physiology. These approaches require input from organic, physical, and theoretical chemists, as well as biological contributions <sup>[9]</sup>. Small molecules from living creatures offer valuable insights into molecular structures, nature's strategy, and biological processes, inspiring organic chemistry, biologists, and pharmacologists in developing modern therapeutic agents <sup>[10]</sup>.

Molecular modeling systems are robust tools for constructing, visualizing, and analyzing complex molecular systems, aiding in interpreting structure-activity relationships. They assess interactions between prototype molecules and known receptor sites or evaluate a compound's ability to mimic three-dimensional features of active compounds. Both approaches strive to optimize receptor fit for selectivity and binding affinity, while also considering critical factors such as log P, solubility, and metabolic stability <sup>[11]</sup>. Computer-aided drug design (CADD) utilizes various tools and techniques such as molecular mechanics, quantum mechanics, molecular dynamics, and structure-based drug design (SBDD). It aims to foster collaborations between biologists, biophysicists, structural biologists, and computational scientists to discover novel therapeutic agents. CADD centers focus on utilizing available structure and other information to initiate research projects for developing potential therapeutic entities. Recent advancements in drug discovery technology encompass techniques such as Quantitative Structure-Activity Relationship (QSAR) and Quantitative Structure-Property Relationship (QSPR), structure-based design, and bioinformatics. Computer-Aided Drug Design (CADD) plays a pivotal role in accelerating drug development by employing computational methods. Active compounds engage with their targets through surface interactions, electrostatic forces, hydrophobicity, and hydrogen bonds, which are crucial aspects considered in drug design and optimization <sup>[12]</sup>. The concepts and techniques for performing quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) studies, focusing on empirical and theoretical parameters, data preprocessing, and machine learning techniques <sup>[13]</sup>.

Structure-activity relationships (SAR) are crucial in drug discovery, enabling structural modifications to optimize properties or activities, and rationally exploring chemical space without "sign posts" <sup>[14]</sup>.

The evolution of interpretation approaches for quantitative structure-activity relationship (QSAR) models, highlighting the potential for interpreting these models regardless of machine learning methods or descriptors <sup>[15]</sup>. Recent advancements in QSAR, particularly in 2D and 3D modeling, have enhanced accuracy and understanding of structure-activity relationships, facilitating improved drug discovery and toxicology predictions, alongside gaining regulatory acceptance <sup>[16]</sup>. New techniques and software have revolutionized the use of Structure-Activity Relationships (SARs) in the pharmaceutical industry and toxicological sciences. SARs involve characterization of chemicals and chemometric approaches to explore data patterns and relationships <sup>[17]</sup>. Molecular modelling is a crucial tool for analyzing biological macromolecules' three-dimensional structure, addressing structural biology problems, and is expected to become increasingly useful in the future <sup>[18]</sup>. Drug design is a creative act, influenced by computer software and hardware. Two novel tools, a haptic device and SCULPT, generate solid molecular models at atomic resolution <sup>[19]</sup>. High-performance computing applications in drug discovery, focusing on atomic and molecular scales, and demonstrates their applications in drug delivery, DNA translocation, and drug design<sup>[20]</sup>. Structure-based ligand design utilizes computational techniques to understand the three-dimensional characteristics of molecules and receptors, aiding in targeted drug development by optimizing molecular interactions <sup>[21]</sup>. The use of computational tools in chemical biology research, discussing their impact on understanding biomolecules, drug discovery, glycobiology, protein design, and molecular recognition, and discussing their limitations and future development <sup>[22]</sup>.



Tuberculosis predominantly impacts men. In areas where *Mycobacterium tuberculosis* transmission has remained stable or increased, the highest incidence rates occur among young adults. Most cases stem from recent infection or reinfection. However, with decreasing transmission rates, the number of cases among older adults increases. In this scenario, a greater proportion of cases result from the reactivation of latent infection. Tuberculosis remains the primary cause of death from a curable infectious illness. In 2004, an estimated 8.9 million new cases occurred worldwide, with only around half being reported to health authorities. Approximately 3.9 million cases were sputum-smear positive, the most contagious form of the disease. The World Health Organization (WHO) African region experiences the highest incidence rate, while populous Asian countries like Bangladesh, China, India, Indonesia, and Pakistan collectively account for half of all new cases each year <sup>[23]</sup>. The global tuberculosis situation in 1990 was reviewed, showing a high global magnitude. Approximately one-third of the world's population is infected, with an estimated 8 million people developing and 2.6-2.9 million dying. The review calls for urgent revitalization of control program<sup>[24]</sup>. *Mycobacterium tuberculosis* infections vary in clinical expression, with symptoms including weight loss, weakness, and malaise. HIV infection may show typical radiographic findings. Extra pulmonary tuberculosis is common, particularly in lymph nodes and pleura, with meningitis being the most frequent form. Chest pain can mimic angina <sup>[25]</sup>.

Over time, human communities grew, leading to environmental changes and a shift in the balance between humans and the tubercle bacillus. The epidemic spread to Eastern Europe, Asia, Africa, and South America, causing significant deaths and microepidemics <sup>[26]</sup>. A critical review of literature on tuberculosis treatment in Brazil aims to inform healthcare workers' recommendations and inform decision-making to minimize morbidity and interrupt disease transmission <sup>[27]</sup>. Tuberculosis resurgence in nonendemic populations due to migration and HIV epidemic. Early diagnosis is crucial for severe morbidity. Less than 50% of cases have active tuberculosis, and radiologists must understand typical symptoms <sup>[28]</sup>.

Isoniazid (INH), a pivotal anti-TB medication, disrupts mycobacterial cell wall formation by inhibiting mycolic acid synthesis through targeting InhA. Resistance to INH typically arises from mutations in *katG* or *InhA* genes, with *NAT* gene mutations also playing a role. INH metabolism generates reactive oxygen species, promoting bacterial cell death. Ongoing efforts aim to develop INH derivatives with improved efficacy and reduced side effects. Enhancing drug penetration into the bacterial cell wall can be achieved by incorporating hydrophobic moieties, while functionalizing the hydrazine group helps counter NAT2-mediated INH inactivation <sup>[29]</sup>.

The reemergence of tuberculosis has prompted the search for novel drugs targeting different mechanisms. Screening initiatives have employed diverse mycobacteria models, including *M. tuberculosis* strains H37Rv and H37Ra, as well as safer alternatives like *M. bovis* and *M. smegmatis*. High-throughput screenings have identified promising compounds, resulting in the development of new drug candidates. Notably, bedaquiline and delamanid have received approval from the European Medicines Agency (EMA), representing substantial advancements in the fight against tuberculosis <sup>[30]</sup>.

Two primary strategies for new antibiotic discovery are whole-cell screening and target-specific screening. Whole-cell screening presents several advantages, including the discovery of novel modes of action, identification of prodrugs like INH and PZA, and ensuring compounds can effectively penetrate bacterial membranes. However, there is a risk of discovering compounds that may be toxic to human cells or target human proteins <sup>[31]</sup>.

## **AMINOQUINOLINES**

Quinolines have garnered substantial interest as a versatile scaffold due to their broad applications in synthetic and medicinal chemistry, offering potential in the discovery of new drug compounds. These compounds showcase diverse biological effects, including anticancer, antitubercular, antileishmanial, anti-inflammatory, and cardiovascular activities. Notably, bedaquiline, a medication derived from quinoline, received approval from the United States Food and Drug Administration (FDA) in 2013 for the treatment of multidrug-resistant tuberculosis (TB) <sup>[32]</sup>. Subsequently, two new drugs were approved for the treatment of patients infected with strains resistant to clinically available drugs: delamanid and pretomanid. Delamanid was approved in 2014, followed by pretomanid in 2019 <sup>[33]</sup>. 8-aminoquinoline therapy for latent malaria offers unique scientific, clinical, and public health insights. Initially used in 1926, it became the sole therapeutic option in 2018 but has hemolytic toxicity. This review explores the history of 8-aminoquinolines <sup>[34]</sup>. Antimalarial agents often contain derivatives of 8-aminoquinoline, which can be toxic to certain human subjects, especially those deficient in glucose-6-phosphate dehydrogenase. Studies show that adult dogs excrete 16% of injected radioactivity in urine, with some metabolites potentially methaemoglobin-forming <sup>[35]</sup>.

- MOLECULAR FORMULA=  $C_9H_7N$
- MOLECULAR WEIGHT= 129.16 g/mole
- INDEX OF REFRACTION= Typically ranges from 1.60 to 1.70.
- DENSITY= Varies based on the specific isomer or derivative, typically in the range of 1.0 to 1.2 g/cm<sup>3</sup>.
- POLARIZABILITY= Specific value depends on the isomer or derivative, influenced by the molecular structure.
- MONOISOTOPIC MASS= 129.06 g/mole
- AVERAGE MASS= 129.16 g/mole

## ***DOCKING***

Molecular docking is a computational modeling technique used to predict the preferred binding orientation of molecules, such as ligands and receptors, to form stable complexes. By analyzing energy profiles and binding strength, it provides insights into molecular interactions, facilitating rational drug design. Docking predicts optimized orientations and different binding modes, thus guiding the development of potent and selective drug candidates <sup>[36]</sup>.

Virtual compound screening via molecular docking is a commonly employed method in the discovery of new lead compounds for drug design. This technique involves computationally evaluating the interactions between small molecules (ligands) and target proteins (receptors) to predict potential binding affinities and identify promising candidate compounds for further experimental validation. By efficiently screening large libraries of compounds, molecular docking accelerates the drug discovery process and aids in the identification of novel therapeutic agents <sup>[37]</sup>. Molecular docking is a crucial tool in structural molecular biology and drug design, predicting binding modes between ligands and proteins, aiding in virtual screening, lead optimization, and software usage <sup>[38]</sup>. Molecular docking methodology is increasingly used in drug discovery, analyzing small molecules' behavior in target protein binding sites. It helps calculate drug ability and specificity, aiding in lead optimization processes and identifying complementary interactions <sup>[39]</sup>.

## ***CHEMDRAW***

ChemDraw Ultra is a sophisticated tool designed for chemists, offering precise manipulation of chemical structures within queries and graphics. It serves as a crucial conduit for electronic molecule representations, facilitating modeling activities. Enhanced by prediction tools, it provides valuable insights into chemical behavior. With seamless web integration, it offers unparalleled accessibility. Renowned as the epitome of chemical drawing, ChemDraw Ultra sets the gold standard for publication and query preparation in the field of chemistry <sup>[40]</sup>.

## **DRUG DISCOVERY FILTERS**

### **1.4.1 SWISS ADME**

Swiss ADME is an online platform designed to analyze chemical compounds by computing various important parameters related to drug discovery and development. It takes the SMILES formula of a compound as input and provides a comprehensive analysis of its physicochemical properties, pharmacokinetic profile, drug likeness, and medicinal chemistry attributes.

Physicochemical parameters include properties like molecular weight, lipophilicity ( $\log P$ ), water solubility, and the number of hydrogen bond donors and acceptors. These parameters are crucial for understanding how a compound interacts with biological systems and its potential as a drug candidate.

The pharmacokinetic profile prediction involves estimating key parameters related to absorption, distribution, metabolism, and excretion (ADME), such as gastrointestinal absorption, blood-brain barrier penetration, and cytochrome P450 metabolism. These predictions help assess the compound's likelihood of reaching its intended target in the body and its overall pharmacological profile.

Additionally, Swiss ADME evaluates the compound's drug likeness based on established rules derived from known pharmaceutical compounds. This assessment helps researchers gauge the compound's potential to become a successful drug candidate, considering factors like structural features and chemical properties associated with favorable pharmacological effects and safety profiles.

Furthermore, Swiss ADME provides insights into the compound's medicinal chemistry aspects, offering information on its synthetic accessibility and presence in natural products databases.

The accuracy of predictions provided by Swiss ADME typically ranges between 72% and 94%, making it a valuable tool for researchers and drug developers in the early stages of compound evaluation and optimization <sup>[41]</sup>.

#### **1.4.2. Pre ADMET:**

18 free web servers for predicting ADMET properties in molecular entities (NMEs) to increase the success rate of compounds in lead optimization. It analyzes their advantages, disadvantages, model-based calculations, and accuracy using experimental data from 24 FDA-approved TKIs <sup>[42]</sup>.

## CHAPTER II

### 2. REVIEW OF LITERATURE:

1. Christopher Dye et al., proposed that global epidemiology of tuberculosis stating that Tuberculosis is the top cause of death from a curable infection. In 2004, there were about 8.9 million new cases worldwide. Shockingly, less than half of these cases were reported to health authorities and the World Health Organization.<sup>[1]</sup>
2. Antimo Gioiello et al, stated that Medicinal chemistry is important for finding safe drugs. It uses cycles of designing, making, testing, and analyzing compounds. Traditional methods are slow, but new technologies like continuous flow systems and automation speed things up. In this Perspective, we talk about how these new tools are helping in drug discovery.<sup>[2]</sup>
3. Imming P et al., stated that Medicinal/Pharmaceutical chemistry involves discovering, designing, and developing new drugs, characterizing their properties, and ensuring quality control throughout the process. Chemists play crucial roles in preclinical stages, aiming to optimize efficacy and safety for eventual clinical use.<sup>[3]</sup>
4. Holbrook et al., proposed that Medicinal chemistry acts as a vital link between different scientific areas, creating bioactive molecules and laying the groundwork for progress in various fields.<sup>[4]</sup>
5. Davis et al., highlighted that Medicinal chemistry blends chemistry and pharmacy to specifically craft and analyze drugs, including designing new ones and understanding the properties and relationships of existing medications.<sup>[5]</sup>

6. George Mihai Nitulescu et al., highlighted that Drug discovery is intricate and constantly evolving. Despite efforts and occasional luck, there's no surefire formula for success. Creativity and innovation remain crucial, drawing substantial interest from scientists. The need for novelty and efficiency is paramount.<sup>[6]</sup>
7. Nurken Berdigaliyev et al., discussed that Drug discovery takes a long time and costs a lot. It started with natural products, then moved to high-throughput synthesis and combinatorial chemistry. New technologies aim to speed things up, but challenges persist. Drug repurposing offers a potentially faster and cheaper alternative. This review discusses various drug discovery methods.<sup>[7]</sup>
8. Suresh K. Balani et al., proposed that High-throughput ADME screening is now standard in drug discovery. This report discusses using ADME tools to optimize properties and a discovery assay by stage (DABS) paradigm for timing studies. DABS helps teams track compound progress efficiently.<sup>[8]</sup>
9. Suckling et al., stated that the discovery of new drugs relies on molecular strategies rooted in biochemistry, pharmacology, and disease physiology. This interdisciplinary approach involves organic, physical, and theoretical chemists collaborating with biologists to understand molecular phenomena, leading to innovative treatments.<sup>[9]</sup>
10. Clardy et al., proposed that Small molecules from living organisms inspire organic chemists, biologists, and pharmacologists in developing modern therapies by offering insights into molecular structures and biological processes.<sup>[10]</sup>

11. N. Claude Cohen et al., stated to introduce medicinal chemists to molecular modeling software and methods, with a focus on tools integrating various techniques. Emphasis is placed on software that combines graphical and computational tools, focusing on familiar systems for the committee.<sup>[11]</sup>
12. Injamul Hoque et al., reviewed that Computer-aided drug design (CADD) uses various methods like molecular mechanics, molecular dynamics, and virtual screening to discover new drugs. CADD centers foster collaboration between scientists to find novel therapeutic agents.<sup>[12]</sup>
13. Isarankura et al., reviewed that concepts and techniques for quantitative structure-activity relationship (QSAR) and quantitative structure-property relationship (QSPR) studies. It covers empirical and theoretical parameters, data preprocessing, and machine learning techniques.<sup>[13]</sup>
14. Guha et al., highlighted that Structure-activity relationships (SAR) play a pivotal role in drug discovery by allowing for strategic structural modifications to enhance properties or activities. They enable a rational exploration of chemical space, guiding the optimization process without predetermined "sign posts."<sup>[14]</sup>
15. Polishchuk et al., stated that this paper explores the evolution of interpretation approaches for quantitative structure-activity relationship (QSAR) models, emphasizing the potential for interpreting these models irrespective of the machine learning methods or descriptors used.<sup>[15]</sup>
16. Perkins et al., highlighted that Quantitative structure-activity relationships (QSARs) are statistical tools correlating chemical structure with activity, garnering attention in drug discovery, toxicology, and environmental science. Advances in 2D and 3D QSARs enhance drug target identification and statistical validation, underscoring their critical role in scientific progress.<sup>[16]</sup>

17. Tong et al., proposed new techniques and software have transformed the use of Structure-Activity Relationships (SARs) in pharmaceuticals and toxicology. SARs involve characterizing chemicals and employing chemometric approaches to uncover data patterns and relationships.<sup>[17]</sup>
18. Forster et al., stated that Molecular modeling is vital for understanding the three-dimensional structures of biological macromolecules and tackling issues in structural biology. Its importance is expected to grow further in the future.<sup>[18]</sup>
19. Meyer et al., proposed that Drug design is a creative process, increasingly influenced by computer software and hardware. Two innovative tools, a haptic device and SCULPT, produce solid molecular models at atomic resolution, enhancing the design process.<sup>[19]</sup>
20. Aminpour et al., highlighted that this paper explores high-performance computing applications in drug discovery, emphasizing their utilization at atomic and molecular scales. It showcases their effectiveness in drug delivery, DNA translocation, and drug design.<sup>[20]</sup>
21. Tollenaere et al., discussed that Structure-based ligand design in drug development uses computational methods to analyze molecular properties and behavior, focusing on characterizing three-dimensional molecule and receptor structures.<sup>[21]</sup>
22. Genheden et al., stated the book explores computational tools' role in chemical biology research, impacting biomolecule understanding, drug discovery, and more, while also discussing limitations and future prospects.<sup>[22]</sup>

23. Christopher Dye et al., proposed that global epidemiology of tuberculosis stating that Tuberculosis is the top cause of death from a curable infection. In 2004, there were about 8.9 million new cases worldwide. Shockingly, less than half of these cases were reported to health authorities and the World Health Organization.<sup>[23]</sup>
24. Sudre et al., stated that In 1990, a review showed a high global burden of tuberculosis, with around one-third of the world's population infected, resulting in 8 million cases and 2.6-2.9 million deaths. Urgent revitalization of control programs was urged.<sup>[24]</sup>
25. Hopewell et al., proposed that Clinical expression of Mycobacterium tuberculosis varies by site, with symptoms such as weight loss, weakness, and malaise. Early HIV infection can show typical radiographic findings. Extra pulmonary TB is common, notably in lymph nodes and pleura, with meningitis being the most frequent form. Chest pain may mimic angina.<sup>[25]</sup>
26. Daniel et al., stated that as human communities expanded over time, environmental changes occurred, altering the balance between humans and the tubercle bacillus. This led to the epidemic spreading across Eastern Europe, Asia, Africa, and South America, resulting in substantial mortality and microepidemics.<sup>[26]</sup>
27. Rabahi et al., reviewed that Analyzing tuberculosis treatment research in Brazil helps healthcare workers make better recommendations and decisions to lessen illness and prevent disease spread.<sup>[27]</sup>
28. Burrill et al., highlighted that Tuberculosis is re-emerging in nonendemic areas due to migration and HIV. Early diagnosis is vital to prevent severe illness. Radiologists need to recognize typical symptoms as less than 50% of cases show active tuberculosis.<sup>[28]</sup>

29. Anu Rani et al., stated that a group of compounds was made and tested for fighting tuberculosis. Most of them worked well against *M. tuberculosis* and were safe for cells. Compounds without certain parts didn't work, confirming the design's effectiveness.<sup>[29]</sup>
30. André Campaniço et al., highlighted that TB is a global health threat. Drug-resistant strains are increasing, requiring new antibiotics. However, converting promising compounds into effective treatments is challenging due to their limited efficacy against TB and unclear targets within the body.<sup>[30]</sup>
31. J. Liu et al., stated that Mycobacterial infections, including TB, are global concerns. Long treatments and drug resistance complicate control. Promising new drugs like diarylquinolines are emerging. Genetic studies offer insight into potential drug targets. Efforts to repurpose existing drugs for TB treatment are ongoing, and antibiotic resistance mechanisms are being studied.<sup>[31]</sup>
32. Eman F. Khaleel et al., discussed that TB is a global issue with drug-resistant strains. Hybrid compounds were tested against TB and InhA. Compounds 7a and 5g showed strong activity against TB and effectively inhibited InhA.<sup>[32]</sup>
33. Estevao Silveira Grams et al., proposed that new treatments are needed for tuberculosis. 27 compounds were tested against *M. tuberculosis* H37Rv strain. Two showed effectiveness comparable to isoniazid, with no harm to cells. They hold promise for tuberculosis treatment development.<sup>[33]</sup>
34. Baird et al., reviewed that historical journey of 8-aminoquinoline therapy for latent malaria, offering insights into its scientific, clinical, and public health significance, despite concerns about hemolytic toxicity.<sup>[34]</sup>

35. Strother et al., stated that antimalarial drugs with 8-aminoquinoline derivatives can be harmful, especially to individuals lacking glucose-6-phosphate dehydrogenase. Studies suggest adult dogs excrete 16% of injected radioactivity in urine, with metabolites possibly causing methemoglobinemia.<sup>[35]</sup>
36. Shweta Agarwal et al., highlighted that Molecular docking predicts how molecules interact computationally, generating possible structures ranked by a scoring function based on binding properties.<sup>[36]</sup>
37. Noriaki Okimoto et al., stated that this study used molecular dynamics simulations to enhance virtual compound screening for drug discovery. By combining docking poses with simulations, the approach improved enrichment performance compared to standard methods.<sup>[37]</sup>
38. Morris et al., stated that Molecular docking predicts how molecules bind, aiding in drug design and virtual screening.<sup>[38]</sup>
39. Pagadala et al., reviewed that Molecular docking in drug discovery analyzes how small molecules bind to proteins, aiding in lead optimization and identifying interactions.<sup>[39]</sup>
40. Karthikeyan Lakshmanan et al., proposed that molecular docking to confirm the hepatoprotective properties of flavones in the Gentianaceae family. Genkwanin exhibited the highest affinity with a specific target, suggesting its potential for hepatoprotection.<sup>[40]</sup>

41. Diana Larisa Roman et al., examined that the biomedical potential of chitosan, focusing on its use in drug delivery and tissue engineering. Computational analysis of chito-oligomers predicts favorable pharmacological profiles and minimal toxicological effects, supporting their suitability for biomedical applications.<sup>[41]</sup>
42. Dulsar et al., stated that 18 free web servers for predicting ADMET properties in new molecular entities (NMEs) to improve compound success in lead optimization. It assesses their strengths, weaknesses, model-based calculations, and accuracy using data from 24 FDA-approved TKIs.<sup>[42]</sup>

## CHAPTER III

### 3. AIM AND OBJECTIVE

The primary objective of this study is to explore the potential of N-(4-(benzyloxy)benzyl)-4-aminoquinolines as inhibitors of *Mycobacterium tuberculosis* (Mtb) growth, particularly focusing on their application in combating drug-resistant strains of tuberculosis. The study aims to address several key aspects:

- **Structural Activity Relationship (SAR) Evaluation:**

Investigate the impact of various substituents on both the quinoline and benzyloxybenzyl groups to discern their influence on the compounds' efficacy against *Mycobacterium Tuberculosis*. By systematically altering these chemical moieties, the study aims to elucidate structural features critical for potent anti-tuberculosis activity.

- **Selective Toxicity Assessment:**

Utilize viability assays with Vero and HepG2 cells to evaluate the selective toxicity of the most promising compounds. This entails determining the compounds' ability to selectively target *Mycobacterium Tuberculosis* while minimizing adverse effects on host cells, a crucial consideration for therapeutic development.

- **ADME Profiling:**

Conduct preliminary in vitro tests to profile the compounds' Absorption, Distribution, Metabolism, and Excretion (ADME) properties. Specifically, assess chemical stability, permeability across biological membranes, and metabolic stability to gain insights into the compounds' pharmacokinetic behavior and potential for clinical application.

- Compound Characterization:

Identify and characterize the most effective compounds, with a particular focus on compounds 9g, 9h, 9n, and 9o, based on their anti-tuberculosis activity, selective toxicity, and favorable ADME profiles. Comprehensive characterization of these compounds will inform their potential as promising candidates for further preclinical and clinical development as anti-tuberculosis agents.

Overall, this study aims to contribute to the ongoing efforts to combat drug-resistant tuberculosis by identifying novel chemical entities with potent anti-Mycobacterial tubercular activity, favorable toxicity profiles, and physiochemical properties conducive to therapeutic efficacy.

The work is aimed,

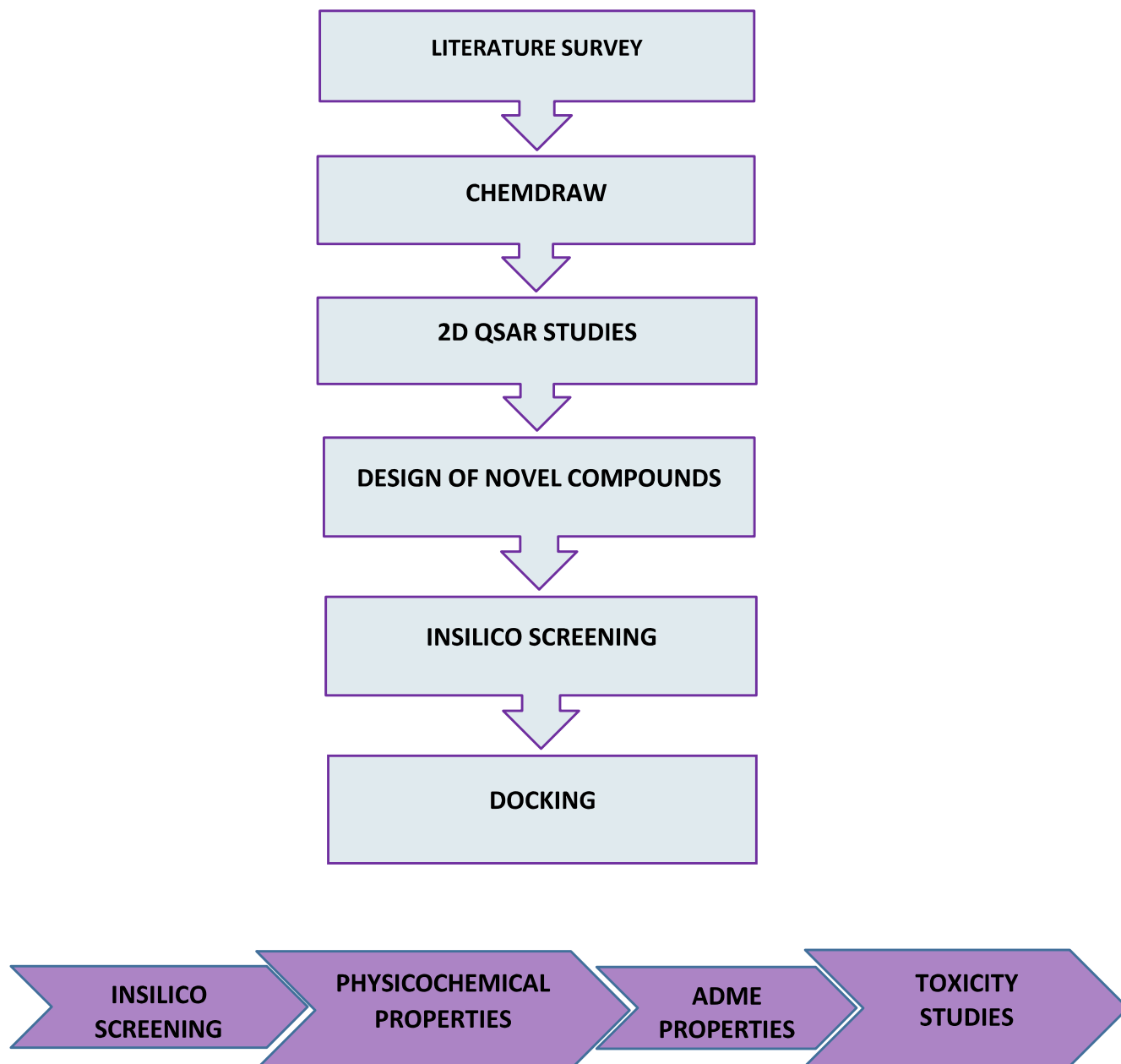
- To select the most active compounds showing better physiochemical and biological properties as a hit compound
- To develop a 2D-QSAR model using QSARINS software.
- To design novel compounds using the best model.
- To evaluate physiochemical properties, drug likeliness, ADME properties and toxicity level of newly designed compounds.
- To perform molecular docking studies for the designed compounds using specific protein.
- To assess the 2D and 3D interactions of designed compounds with the amino acids of selected target.

This study is aided with authenticated applications and softwares such as:

- Chemdraw
- Swiss ADME
- PreADMET
- PaDEL Descriptor
- BIOVIA Discovery Studio Visualizer etc.
- PyRX

## CHAPTER IV:

### 4. PLAN OF STUDY:



## CHAPTER V

### 5. MATERIALS AND METHODS:

#### *2D QSAR MODEL*

##### ***DATASET SELECTION AND DIVISION***

A Dataset of reported 27 quinoline derivatives as potent antimycobacterial activity were gathered from the literature. MIC values were taken from the literature and excluding the compounds having not well defined biological activities. All experimental minimum inhibitory concentration (MIC) were converted to negative logarithmic values ( $\text{pMIC} = -\log_{10}(\text{MIC})$ ) to provide numerically greater data values and it is taken as dependent variable in 2D-QSAR model. For developing 2D-QSAR model, this dataset was divided into training and prediction set in the ratio of 5:1 based on order by response.

##### ***GENERATION OF 2D-QSAR MODEL***

The structure of the compounds was drawn using Chemdraw freeware and saved in mol. Format. The molecular descriptors for the studied compounds were calculated using Padel-descriptor. Totally 797 descriptors including 2D and 3D properties can be calculated using Padel descriptor. Various descriptors like Autocorrelation, AlogP, atom count electro topological state descriptors, Crippen MR and Log, extended topological atom descriptors, McGowan volume, Molecule linear free energy relation descriptors, ring counts, count of chemical substructures identified by Laggner and binary fingerprints. The variables were organized and pre- filtered by excluding all zero values, missing values and constant value (>50%) descriptors. The perfect model should be with minimum number of descriptors to avoid over-fitting. QSAR models were generated by using QSARINS (QSAR-Insubria) software developed at the University of Insubria. QSARINS employs the application of both generic algorithm and multiple linear Regression were used to select the descriptors and to generate the correlation models, where multiple linear regression is used to calculate the relationship between the descriptors of interest and pharmacological activity.

## **MODEL VALIDATION**

Validation is the important criteria for the evaluation of QSAR predictive models. To demonstrate the different validation measures have included: internal validation, external validation and Y -randomization test. Internal validation including variance in leave -one-out ( $Q^2$  LOO) and leave many out ( $Q^2$  LMO), Concordance Correlation Coefficient (CCC), Root Mean squared error (RMSE). Mean absolute error (MAE), y-scrambling etc and external validation including  $Q^2$   $R^2$  metrics were applied on the selected models.

Several statistical parameters have been used to improve the goodness of fit of the QSAR model. They are correlation coefficient(R), the coefficient of determination ( $R^2$ ), the variance inflation factor (VIF) which identifies the collinearity between the descriptors presented in a statistical model.

The best model was selected on the idea of statistical parameters through higher observed squared correlation coefficient  $R^2$  value close to ( $R^2 > 0.6$ ) which may be relative measure of quality of fit. Standard error of estimate ( $SEE < 0.3$ ) gives the absolute measure of quality of fit and low MAE values indicate that good fit of the QSAR model with relevant correlation. Fischer's value (F) is the Fischer ratio, reflects the ratio of the variance explained by the model and the variance due to error in the regression. High values of the F-test indicate that the model is statistically significant.

Internal validation is to predict the training molecule by applying the LOO (Leave-one-out) cross validation technique, in which greater value of the correlation coefficient ( $Q^2_{cv}$ ) is considered as evidence of the predictive quality of the model. Y scrambling procedure by randomized the bioactivities and the new model is created. In this case, if the model in the validation process was good, the values of  $R^2$  and  $Q^2$  of each iteration, and their averages ( $R^2_{vs}$  and  $Q^2_{vs}$ ) must be lesser with respect to the values of the model, by this one could test the validity of developed QSAR models and ensure the selected descriptors are not random. Applicability domain is the surface space in which the model makes reliable prediction for the activity/toxicity of the compounds. The prediction is valid only if the compound is within the applicability domain of the model and can be employed in further computer aided compound design such as

ligand-based design. The boundary of applicability domain was evaluated as  $h^* = 3(m=1)k$ , where  $m$ =row descriptor value and  $k$ =columns of training set. The defined applicability domain (AD) was then visualized via Williams plot, the plot of the standardized domain, but it doesn't appear to be an outlier because its standardized residual may be small. Moreover, a value for accepting predictions, because points that lie within  $\pm 3$  standardized residuals given dataset  $h_i$ , is defined by this mathematical expression

$$HI = x_i (x^T x)^{-1} X^T i$$

Where  $x_i$  the descriptor row is vector of the considered compound  $i$ ,  $h_i$  is the  $n \times k$  descriptor matrix of the training set compound used to generate the model.

## ***DESIGN OF COMPOUNDS***

20 compounds were designed using chemdraw software. Physicochemical properties were calculated for all the compounds.

### **MOLAR REFRACTIVITY**

Molar refractivity,  $A$ , is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as  $A = 4\pi N_A \alpha^*/3$  where  $N_A = 6.0221023$  is the avogadro constant and  $\alpha^*$  is the mean polarizability of the molecules.

### **MOLAR VOLUME**

The molar volume, symbol  $V_m$ , is the volume occupied by one mole of a substance (chemical element or chemical compound) at a given temperature and pressure. It is equal to the molar mass ( $M$ ) divided by the mass density ( $\rho$ ). It has the SI unit cubic meters per mole ( $m^3/mol$ ), although it is more practical to use the unit's cubic decimeters per mole ( $dm^3/mol$ ) for gasses and cubic centimeters per mole ( $cm^3/mol$ ) for liquids and solids.

The molar volume of a substance can be found by measuring its molar mass and density then applying the relation

$$V_m = M/\rho$$

## INDEX OF REFRACTION

The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium

It is defined as  $n = c/v$

Where,  $c$  is the speed of light in vacuum and  $v$  is the phase velocity of light in the medium.

## SURFACE TENSION

Surface tension is the elastic force of a fluid surface which makes it acquire the least surface area possible. Surface tension has the dimension of force per unit length, or of energy per unit area

## POLARIZABILITY

Polarizability is the ability to form instantaneous dipoles. It is a property of matter. Polarizabilities determine the dynamical response of a bound system to external fields and provide insight into a molecule's internal structure. In a solid, polarizability is defined as the dipole moment per unit volume of the crystal cell.

## PARACHOR

It is an empirical constant for a liquid that related the surface tension to the molecular volume and that may be used for a comparison of molecular volumes under conditions such that the liquids have the same surface tension and for determinations of partial structure of compounds features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the

$$\text{Formula } P = V^{1/4} M/d$$

Where,

$\gamma^{1/4}$  is the fourth root of surface tension M is the molar mass D is the density.

#### DENSITY

The density, or more precisely the volumetric mass density, of a substance is its mass per unit volume.

$$\rho = m/V$$

#### DIELECTRIC CONSTANT:

Substances have capacity to produce dipoles in another molecule. Dielectrical constant is a measure of this capacity and it is a physical property. It is affected by both the attractive forces that exist between atoms and also molecules. It is denoted by  $\epsilon$ .

### **5.3. INSILICO ADMET PREDICTION STUDIES**

In silico is an expression meaning "performed on computer or via computer simulation" in reference to biological experiments. When lead molecules have been identified, they must be optimized in terms of potency, selectivity, pharmacokinetics (i.e.) absorption, distribution, metabolism and excretion (ADME) and toxicology before they can become candidates for drug development.

The Physicochemical properties and ADME properties were calculated for all the designed compounds using CHEMDRAW software, SWISS ADME and PreADMET online web server, drug likeness properties were evaluated by Mol inspiration web server. This had an aim to identify the molecule which satisfies the optimum requirements to exhibit drug like molecules based on the following criteria: Lipinski rule, Ghose rule, Veber rule, Egan rule, Muegge rule and the bioavailability score.

Toxicity studies of the designed compounds were performed by using PreADMET web server. The study of ADMET properties is necessary because they determine oral bioavailability, cell permeation, metabolism and elimination of drug molecules.

## **PHYSICOCHEMICAL PROPERTIES**

### **Lipinski's rule of five**

Lipinski's rule of five also known as the Pfizer's rule of five or simply the rule of five (RO5) is a rule of thumb to evaluate drug likeness or determine if a chemical compound with a certain pharmacological or biological activity has chemical properties and physical properties that would make it a likely orally active drug in humans.

#### **The rule**

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria.

No more than 5 hydrogen bond donors (the total number of nitrogen hydrogen and oxygen hydrogen bonds)

No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms) a molecular mass less than 500 daltons.

An octanol-water partition coefficient log P not greater than 5.

### **Ghose Filter**

This filter defines drug-likeness constraints as follows:

Calculated log P is between -0.4 and 5.6

Molecular weight is between 160 and 480

Molar refractivity is between 40 and 130

The total number of atoms is between 20 and 70.

## **Veber Filter**

The molecules fitting to these two properties have a high probability of good oral bioavailability.

Rotatable bond: max. 12

Polar Surface Area: max. 140Å.

## **Egan Rule**

Predicts good or bad oral

Bioavailability.  $0 \geq \text{TPSA} \leq 132$

$-1 \geq \log P \leq 6$ .

## **Molar Refractivity**

It is a measure of the total polarizability of a mole of a substance and is dependent on the temperature, the index of refraction, and the pressure.

The molar refractivity is defined as  $A = 4/3\pi T N A_a$

Where  $N_A = 6.022 \times 10^{23}$  is the Avogadro constant and is the mean polarizability of a molecule.

## **Polar surface area (PSA) or topological polar surface area (TPSA)**

It is a measure of apparent polarity of a molecule is defined as the surface sum overall polar atoms, primarily oxygen and nitrogen, also including their attached hydrogen atoms. PSA is commonly used for the optimization of a drug's ability to permeate cells. Molecules with a polar surface area of greater than 140 angstroms squared tend to be poor at permeating cell membranes.

For molecules to penetrate the blood-brain barrier (and thus act on receptors in the central nervous system), a PSA less than 90 angstroms squared is usually needed.

Topological PSA (TPSA, fast 2D calculation).ADME Guideline

TPSA < 140 Å<sup>2</sup> good intestinal absorption TPSA < 70 Å<sup>2</sup> good brain penetration.

## **Lipophilicity**

Lipophilicity is the ability of a molecule to mix with an oily phase rather than with water, is usually measured as partition coefficient,  $P$ , between the two phases and is often expressed as  $\log P$ . Lipophilicity has also been found to affect a number of pharmacokinetic parameters: higher lipophilicity ( $\log P > 5$ ) gives, in general, lower solubility, higher permeability in the gastrointestinal tract, across the blood-brain barrier and other tissue membranes, higher affinity to metabolizing enzymes and efflux pumps, and higher protein binding. Low lipophilicity can also negatively impact permeability and potency and thus results in low BA and efficacy.

### **Partition coefficient, $P$**

It is defined as a particular ratio of the concentrations of a solute between the two solvents (a bi phase of liquid phases), specifically for un-ionized solutes, and the logarithm of the ratio is thus  $\log P$ . When one of the solvents is water and the other is a non-polar solvent, then the  $\log P$  value is a measure of lipophilicity or hydrophobicity.

$\log P_{oct/wat} = \log [\text{solute}]_{\text{unionized octanol}} / [\text{solute}]_{\text{unionized water}}$   
 $\log P_{oct/wat} - \log C_O / C_W$

Lipophilicity not only impacts solubility but also influences permeability, potency, selectivity, absorption, distribution, metabolism, and excretion (ADME) properties and toxicity. A desired  $\log P$  value (octanol-water partition coefficient) is no more than 5.

### **Rotatable Bonds**

The bioavailability of a drug like molecule is related with its rotatable bond number. Less than seven rotatable bonds are essential for good bioavailability. Many highly potent molecules carried more than 10 rotatable bonds and still administered through oral route. Hydrogen bond acceptors and donors 12 or fewer H-bond donors and acceptors will have a high probability of good oral bioavailability.

## **Drug-Likeliness properties.**

Drug likeness is a qualitative concept used in drug design for how "druglike" a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested. The most well-known rule relating the chemical structures to their biological activities is Lipinski's rule and it is called the 'rule of five'. Another well-known rule is the Lead-like rule. PreADMET contains drug-likeness prediction module based on these rules.

## **ADME Prediction**

Numerous in vitro methods have been used in the drug selection process for assessing the intestinal absorption of drug candidates. Among them, Caco2- cell model has been recommended as a reliable in vitro model for the prediction of oral drug absorption. In absorption, this module provides prediction models for in vitro Caco2- cell. Additionally, in silico HIA (human intestinal absorption) model and skin permeability model can predict and identify potential drug for oral delivery and transdermal delivery. In distribution, BBB (blood brain barrier) penetration can give information of therapeutic drug in the central nervous system (CNS), plasma protein binding model in its disposition and efficacy. In order to build these QSAR models, genetic functional approximation is used to select relevant descriptors from all 2D descriptors that calculated by Topomol module, followed by Resilient back-propagation (Rprop) neural network to develop successful nonlinear model.

## **Toxicity prediction**

In silico toxicity prediction will have more and more importance in early drug discovery since 30% of drug candidates fail owing to these issues.

## **Toxic doses and Toxicity classes**

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethal dose meaning the dose at which 50% of test subjects die upon exposure to a compound. Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals (GHS). LD50 values are given in (mg/kg):

Class I: fatal if swallowed ( $LD50 \leq 5$ )

Class II: fatal if swallowed ( $5 < LD50 \leq 50$ )

Class III: toxic if swallowed ( $50 < LD50 \leq 300$ )

Class IV: harmful if swallowed ( $300 < LD50 \leq 2000$ )

Class V: may be harmful if swallowed ( $2000 < LD50 \leq 5000$ )

Class VI: non-toxic ( $LD50 > 5000$ ).

## **MOLECULAR DOCKING STUDIES**

### **Retrieval of the target protein**

The crystal structure of protein Enoyl-ACP (CoA) reductase (PDB ID: 2AQ8), Enoyl-ACP (CoA), Reductase (2IDZ), Mycolyl transferase (1F0N) Dihydrofolate reductase (PDB ID: 1DG5), Cytochrome P450 14 alpha-sterol demethylase (CYP51) (PDB ID: 1E9X) with the resolution 1.80-2.0 Å was downloaded as pdb. Format from the Research Collaboratory for Structural Bioinformatics- Protein Data Bank (RCSB-PDB) database.

### **Pre-processing of Protein**

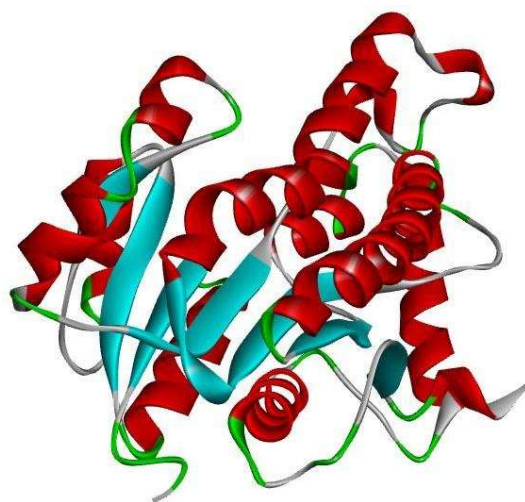
Pre-processing of the target protein was performed using Molegro Molecular Viewer Software. For the docking study, Water and heteroatoms were removed from the target protein. The target protein was saved in 'pdb' format. The prepared protein is shown in Figure 5. 1, 5. 2, 5.3, 5.4, 5.5.

### **Ligand Preparation**

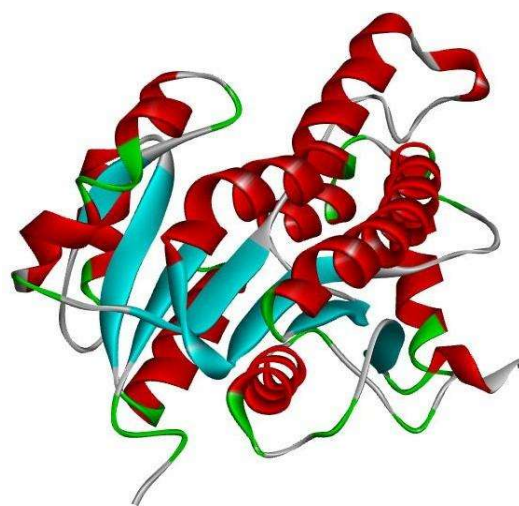
The chemical structure of the designed compounds for the target protein was drawn in Chemdraw software. Three-dimensional geometry of the Compounds was generated using Discovery studio and saved as .pdb format in order to be recognized by the PyRx software.

### **Molecular docking**

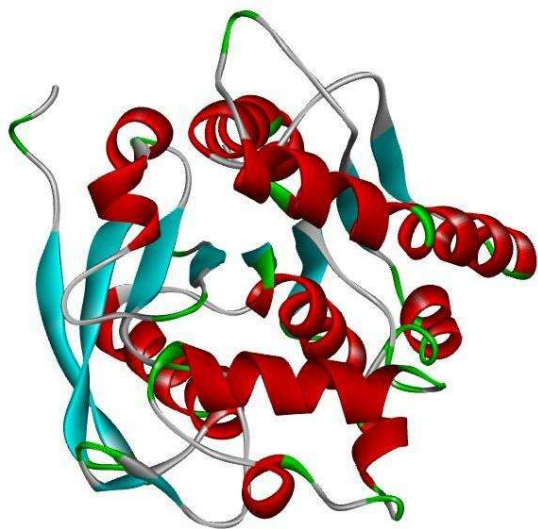
PyRx 0.8 software is a virtual screening tool that uses Autodock vina to conduct molecular docking simulation studies. The designed compounds were docked against Enoyl-ACP (CoA) reductase, Dihydrofolate reductase, Cytochrome P450 14 alpha-sterol demethylase using PyRx software, where binding affinity are calculated. Analysis and Visualization of amino acid residues in the protein were identified using BIOVIA Discovery studio visualizer version 19.1.0.18287 (Dassault systems, Paris, France).



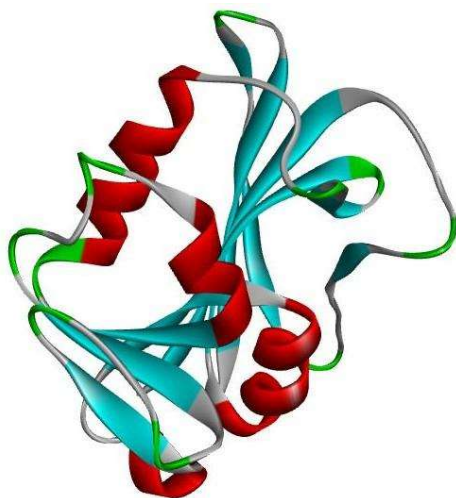
**Fig 5.1: Structure of pre-processed protein Enoyl-ACP (CoA) reductase (2AQ8)**



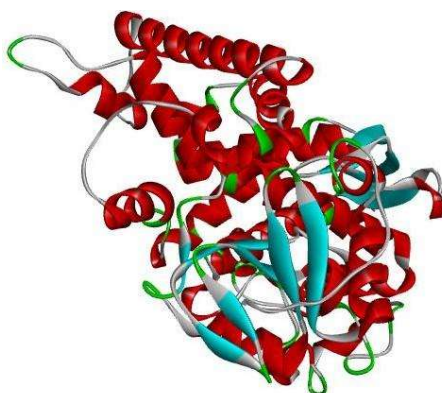
**Fig 5.2: Structure of pre-processed protein Enoyl-ACP (CoA) reductase (2IDZ)**



**Fig 5.3: Structure of pre-processed protein mycolyl transferase (1F0N)**



**Fig 5.4: Structure of pre-processed protein Dihydrofolate reductase (1DG5)**



**Fig 5.5: structure of pre-processed protein Cytochrome P450 14 alpha-sterol demethylase (CYP51) (1E9X)**

## CHAPTER VI

### 6. RESULTS AND DISCUSSION

#### *2D-QSAR Model Information*

In an attempt to determine the role of structural features of compounds which appear to possess an effect of antimycobacterial activity, QSAR models were generated. Data set compounds from the literature survey were mentioned in Table 6.2. To develop the statistical correlation, the descriptors were taken as independent variables and antibacterial activity as dependent variables. The validity, robustness and predicting ability of built models are ascertained by subjecting the model to certain validation tests.

#### **Best model equation:**

$$\text{pIC}_{50} = 1313.5814 - 2.7027(\text{Alogp2}) + 29.542(\text{GATS5m}) - 52.5251(\text{GATS6m}) \\ - 626.6435(\text{SpMin1\_Bhs}) + 309.1209(\text{ETA\_dAlpha\_A})$$

The best model developed with aforementioned options gave statistical parameters as

#### **(Fitting criteria)**

$R^2$ : 0.8703       $R^2_{\text{adj}}$ : 0.8322       $R^2 - R^2_{\text{adj}}$ : 0.0381      F: 22.8219

#### **(Internal validation criteria)**

$Q^2_{\text{LOO}}$ : 0.8045       $R^2 - Q^2_{\text{LOO}}$ : 0.0658       $Q^2_{\text{LMO}}$ : 0.7738

#### **(External validation criteria)**

$R^2_{\text{ext}}$ : 0.4638

#### **Predictions by LOO:**

Exp(x) vs. Pred(y):  $R^2$ : 0.8055

#### **External predictions by model equation:**

Exp(x) vs. Pred(y):  $R^2$ : -1.0000

Many equations were predicted out of that 3 equations showed better predicted activity and the model 3 is considered as the best model for which the statistical parameters are mentioned above. The descriptors Alogp2, GATS6m, SpMin1\_Bhs, contributes negatively to the antimycobacterial activity and GATS5m, ETA\_dAlpha\_A contributes positively towards the antimycobacterial activity. In designing novel compounds Alogp2, GATS6m and SpMin1\_Bhs should be reduced when designing novel compounds.

The best model showed good statistical values.

- a. The QSAR equation with  $R^2$  value greater than 0.6 is considered to have good predictability. We have value of 0.8 which shows better predictive activity.
- b. Higher the value of F more acceptable is the equation. We have good F value of 22.8219
- c. The difference between  $R^2$  and  $R^2_{adj}$  should be less than 0.1. We have the value of 0.0381

### **Internal validation**

Internal validation was carried out by LOO (Leave One Out) and LMO (Leave Many Out) method. In LOO one compound is randomly left and  $R^2$  value was determined which is named as  $Q^2_{LOO}$ . In LMO two or more compounds were randomly left and the  $R^2$  value is determined which is named as  $Q^2_{LMO}$ .  $Q^2_{LOO}$  and  $Q^2_{LMO}$  in the predicted model was found to be 0.8045 and 0.7738 respectively. So the equation is having better predictability. The difference between  $R^2$  and  $Q^2_{LOO}$  is found to be 0.0658 which is less than 0.1. Thus, internal validation passes the standard criteria for designing novel compounds.

## External validation

In external validation also  $R^2$  was found to be 0.8703 which is more than 0.6. Thus, external validation passes the standard criteria for designing novel compounds. The best model is passing all the fitting, internal validation and external validation criteria. So the best model has a relationship between the structure of aminoquinolines derivatives with Enoyl-ACP(CoA) reductase (2AQ8), Enoyl-ACP(CoA) reductase (2IDZ), Dihydrofolate reductase (1DG5), Mycolyl transferase(1F0N), Cytochrome P450 14  $\alpha$ -sterol demethylase (CYP51)(1E9X) protein inhibitory activity.

**Table 6.1: Description of molecular descriptors used in the study**

| Symbol       | Description                                                                             | Class |
|--------------|-----------------------------------------------------------------------------------------|-------|
| Alogp2       | Ghose-crippen octanol water co-efficient squared                                        | 2D    |
| GATS5m       | Geary autocorrelation - lag 5 / weighted by atomic masses                               | 2D    |
| GATS6m       | Geary autocorrelation - lag 6 / weighted by atomic masses                               | 2D    |
| SpMin1_Bhs   | Smallest absolute eight value of burden modified matrix-n1/weighted by relative 1-state | 2D    |
| ETA_dAlpha_A | ETA delta alpha (type: A)                                                               | 2D    |

**Table 6.2: pIC50 values of original dataset predicted by the model equation.**

| <b>COMPOUND ID</b> | <b>EXP. pIC50</b> | <b>PRED. pIC50</b> | <b>RESIDUALS</b> | <b>STATUS</b> |
|--------------------|-------------------|--------------------|------------------|---------------|
| 999a               | 7                 | 12.0669            | 0.0001           | Prediction    |
| 999b               | 12.9              | 16.209             | 3.6064           | Training      |
| 999c               | 26.8              | 19.149             | 0.1000           | Prediction    |
| 999d               | 25.6              | 25.6086            | 0.013            | Training      |
| 999e               | 27                | 23.5589            | -4.5477          | Training      |
| 999f               | 12.1              | 16.1206            | 4.5072           | Training      |
| 999g               | 6.4               | 2.907              | 0.0196           | Prediction    |
| 999h               | 5.9               | 0.993              | -6.0898          | Training      |
| 999i               | 6.1               | 5.7355             | -0.4164          | Training      |
| 999j               | 5.9               | 5.8805             | -0.0399          | Training      |
| 999k               | 5.8               | 8.1281             | 2.5664           | Training      |
| 999l               | 5.7               | 6.4037             | 0.8351           | Training      |
| 999m               | 5.8               | 4.6017             | -1.747           | Training      |

|       |      |         |         |          |
|-------|------|---------|---------|----------|
| 999n  | 2.7  | 2.6816  | -0.0338 | Training |
| 999o  | 2.8  | 3.5565  | 1.2056  | Training |
| 999p  | 2.7  | 4.3132  | 1.7702  | Training |
| 999q  | 5.3  | 8.2918  | 3.6178  | Training |
| 999r  | 5.1  | 4.5204  | -0.6797 | Training |
| 999s  | 6.4  | 2.1995  | 0.0012  | Training |
| 999t  | 5.2  | 6.9412  | 2.6794  | Training |
| 999u  | 13   | 10.5875 | -3.5951 | Training |
| 999v  | 11.9 | 7.3034  | -5.5852 | Training |
| 999w  | 23.4 | 19.3852 | -4.569  | Training |
| 999x  | 12.4 | 11.4847 | -1.0665 | Training |
| 999y  | 5.9  | 14.3202 | 9.2911  | Training |
| 999z  | 23.4 | 20.5485 | -3.4958 | Training |
| 999aa | 12.5 | 11.9259 | -0.7164 | Training |

**Table 6.3 Pic50 values of designed compounds predicted by the equation**

| Compound ID | ALogp2       | GATS5m       | GATS6m       | SpMin1_Bhs   | ETA_dAlpha_A | Predicted activity |
|-------------|--------------|--------------|--------------|--------------|--------------|--------------------|
| 9aa         | 0.012        | 1.039        | 1.031        | 2.034        | 0            | 15.28              |
| 9bb         | 0.400        | 0.749        | 0.961        | 2.026        | 0            | 14.02              |
| 9cc         | 0.135        | 0.971        | 1.034        | 2.026        | 0            | 17.38              |
| 9dd         | 0.718        | 1.023        | 0.977        | 2.019        | 0            | 24.99              |
| 9ee         | 0.212        | 1.007        | 0.966        | 2.022        | 0            | 24.40              |
| 9ff         | 0.046        | 1.025        | 1.154        | 2.026        | 0            | 13.29              |
| 9gg         | 0.400        | 0.806        | 1.033        | 2.041        | 0            | 2.80               |
| <b>9hh</b>  | <b>1.895</b> | <b>0.653</b> | <b>0.995</b> | <b>2.033</b> | <b>0.002</b> | <b>1.53</b>        |
| 9ii         | 1.236        | 0.776        | 1.046        | 2.033        | 0            | 3.58               |
| 9jj         | 2.497        | 0.868        | 0.982        | 2.034        | 0            | 6.05               |
| 9kk         | 1.452        | 0.792        | 0.969        | 2.030        | 0            | 9.54               |
| 9ll         | 0.279        | 0.819        | 1.099        | 2.033        | 0            | 4.66               |
| 9mm         | 0.603        | 0.670        | 0.996        | 2.043        | 0.016        | 4.20               |

|            |              |              |              |              |              |             |
|------------|--------------|--------------|--------------|--------------|--------------|-------------|
| 9nn        | 2.314        | 0.626        | 0.992        | 2.035        | 0.004        | 2.86        |
| <b>9oo</b> | <b>3.015</b> | <b>0.687</b> | <b>1.000</b> | <b>2.028</b> | <b>0.001</b> | <b>2.48</b> |
| 9pp        | 1.579        | 0.664        | 1.006        | 2.035        | 0.008        | 3.01        |
| 9qq        | 1.822        | 0.633        | 0.958        | 2.032        | 0.015        | 7.98        |
| 9rr        | 0.453        | 0.649        | 0.974        | 2.035        | 0.004        | 5.86        |
| 9ss        | 0.705        | 0.911        | 1.085        | 2.034        | 0            | 6.21        |
| 9tt        | 0.010        | 0.655        | 0.991        | 2.042        | 0.016        | 5.85        |
| 9uu        | 0.714        | 0.611        | 0.984        | 2.035        | 0.022        | 9.55        |
| 9vv        | 0.026        | 0.938        | 1.025        | 2.034        | 0            | 12.08       |
| 9ww        | 0.167        | 0.928        | 0.967        | 2.024        | 0            | 21.14       |
| 9xx        | 0.337        | 0.697        | 0.954        | 2.027        | 0            | 12.33       |
| 9yy        | 0.099        | 0.882        | 1.027        | 2.027        | 0            | 14.60       |
| 9zz        | 0.633        | 0.928        | 0.975        | 2.020        | 0            | 21.73       |
| 9aaaa      | 0.071        | 0.946        | 1.134        | 2.027        | 0            | 11.06       |

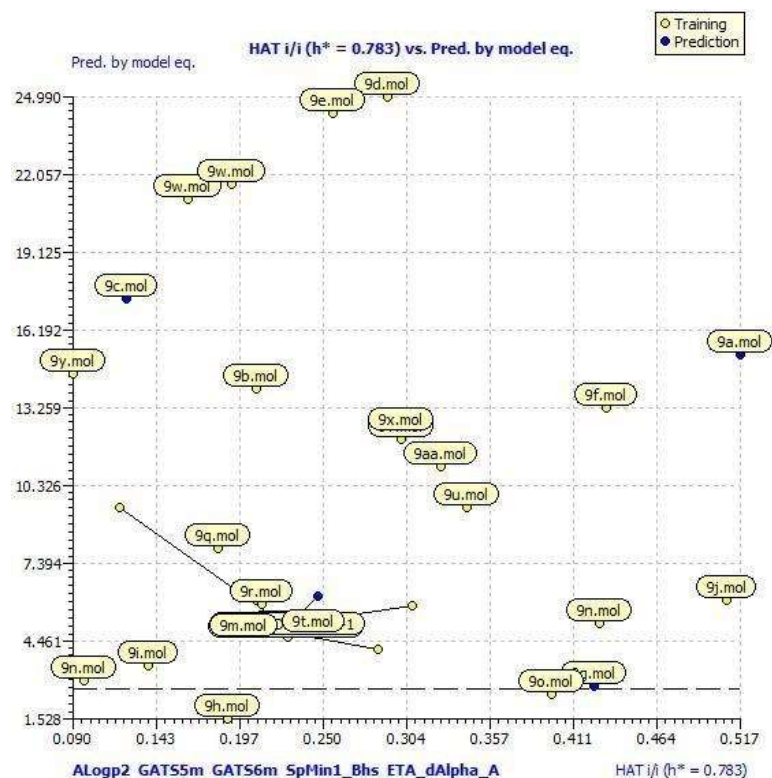


Fig 6.1: Williams plot of the best model with warning values of HAT ( $h^*=0.783$ )

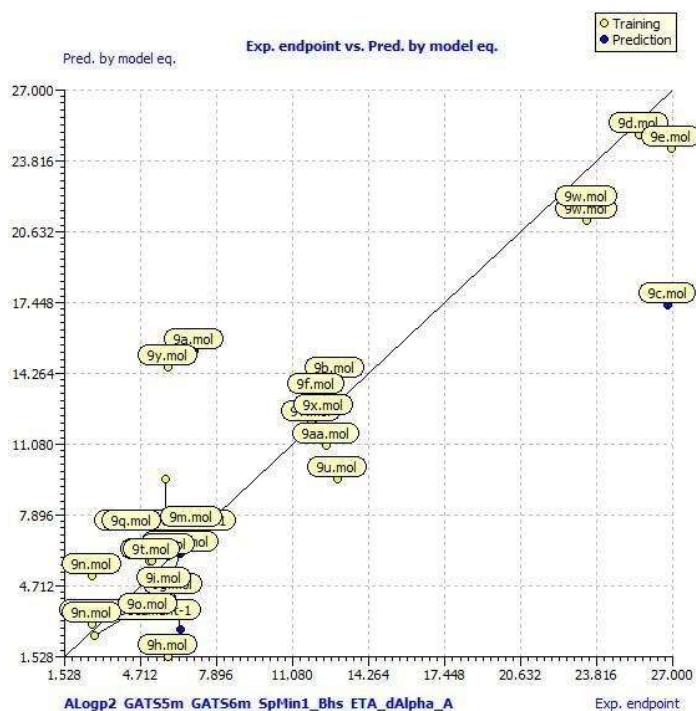


Fig 6.2: Scatter plot of dataset compounds obtained from experimental values and model

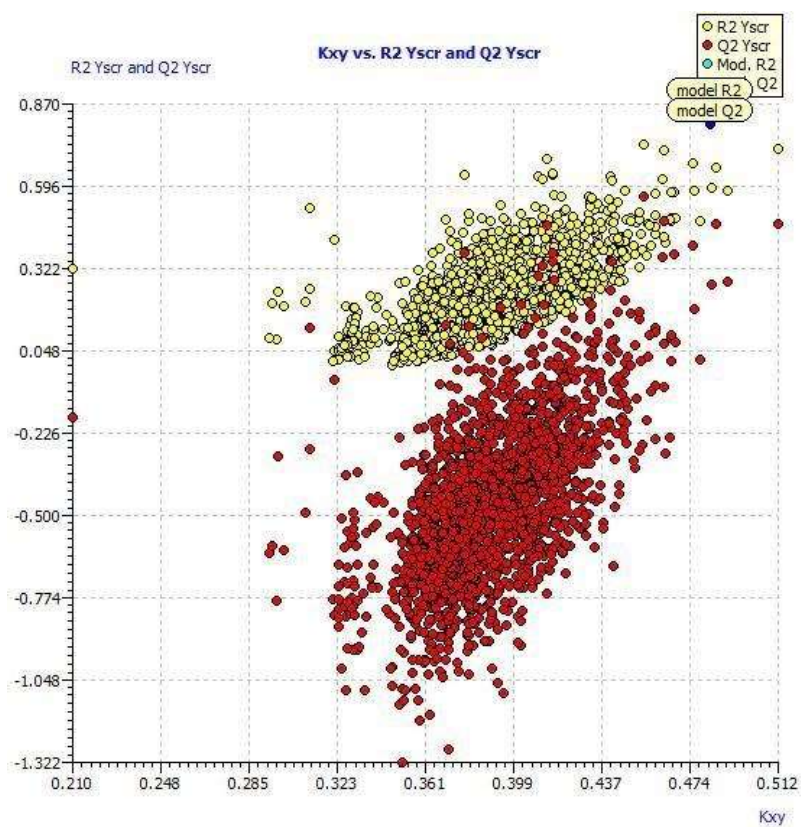


Fig 6.3: Y-scramble plot for best model

(Plot of  $k_{xy}$  vs  $R^2$  and  $Q^2$  Loo from Y-scrambling procedure).

## **DESIGN OF NEW COMPOUNDS**

Novel quinoline derivatives were designed based on predicted QSAR Model. Their activity were evaluated using same model and their activity are mentioned in table 6.5. Among these derivatives, 9g,9h,9n,9o compounds in the data set. The descriptors SpMin1\_bhs and ETA\_dAlpha\_A contributes positively towards the biological activity. The predicted pIC<sub>50</sub> derivatives were summarized in table 6.3. The structure of 20 newly designed compounds are given in table 6.8. .

## **INSILICO ADMET PREDICTION STUDIES**

In silico ADMET prediction is required to quality the compounds as lead candidates against activity. Quinoline derivatives were designed as antimalarial agents using computational methods. physicochemical properties, drug likeness properties and toxicity properties were calculated for all the designed compounds and compared with the standard drug in isoniazid. Physicochemical properties were shown in Table 6.4. All the designed compounds have less than hydrogen bond donors and equal to hydrogen bond acceptor. Molecular weight of all the designed compounds were around 550 – 590 Dalton's. Maximum of the designed compounds obey Lipinski rule of in comparison with standard drug.

All our designed compounds show log P values in the range of 1 to 6. It was found that lipophilicity plays a major role in determining whether drugs are distributed within the body after adsorption and a consequence, how rapidly they are metabolized and excreted. The activity of the synthesized compounds increases with increasing log P.

TPSA has been used as descriptor for characterizing absorption and passive transportation properties through biological membranes, allowing a good prediction of transport of candidate drugs in the intestines. TPSA (Total Polar Surface Area) of our designed compounds were found to be in the range of 90 – 120 Å<sup>2</sup>. Therefore, it is expected that our compounds might possess little intestinal absorption.

Synthetic accessibility score is normalized between 1 (easy synthetic) and 10 (very difficult synthetic). The designed compounds showed score 4 to 5. This indicates the designed compounds can be synthesized in use and values shown in **Table 6.5**.

Toxicity studies of the designed compounds were shown Table 6.6, Most of the designed compounds were inactive for hepatotoxicity and carcinogenicity.

**Table 6.4: Physicochemical properties of designed compounds.**

| Compound | Molecular Formula | Molecular weight (g/mol) | Molar Refractive | Number of rotatable bonds | Number of H bond acceptor | Number of H bond donors | TPSA A2 | Number of heavy atoms | Number of aromatic heavy atoms |
|----------|-------------------|--------------------------|------------------|---------------------------|---------------------------|-------------------------|---------|-----------------------|--------------------------------|
| 9aa      | C24H22N2O         | 354.44                   | 111.48           | 6                         | 2                         | 1                       | 34.15   | 27                    | 22                             |
| 9bb      | C24H21ClN2O       | 388.89                   | 116.49           | 6                         | 2                         | 1                       | 34.15   | 28                    | 22                             |
| 9cc      | C24H21FN2O        | 372.43                   | 111.44           | 6                         | 3                         | 1                       | 34.15   | 28                    | 22                             |
| 9dd      | C24H20F2N2O       | 390.43                   | 111.4            | 6                         | 4                         | 1                       | 34.15   | 29                    | 22                             |
| 9ee      | C27H28N2O         | 396.52                   | 126.06           | 7                         | 2                         | 1                       | 34.15   | 30                    | 22                             |
| 9ff      | C26H26N2O3        | 414.5                    | 124.46           | 8                         | 4                         | 1                       | 52.61   | 31                    | 22                             |
| 9gg      | C24H21ClN2O       | 388.89                   | 116.49           | 6                         | 2                         | 1                       | 34.15   | 28                    | 22                             |
| 9hh      | C24H20ClF2N2O     | 423.33                   | 121.5            | 6                         | 2                         | 1                       | 34.15   | 29                    | 22                             |
| 9ii      | C27H27ClN2O3      | 406.88                   | 116.45           | 6                         | 3                         | 1                       | 34.15   | 29                    | 22                             |
| 9jj      | C26H25BrN2O       | 410.84                   | 112.16           | 5                         | 4                         | 1                       | 34.15   | 29                    | 22                             |
| 9kk      | C24H20BrClN2O     | 430.97                   | 131.07           | 7                         | 2                         | 1                       | 34.15   | 31                    | 22                             |
| 9ll      | C24H20BrF2N2O3    | 448.94                   | 129.47           | 8                         | 4                         | 1                       | 52.61   | 32                    | 22                             |

**Table 6.5: Drug likeness properties of designed compounds.**

| Compound | Lipinski | Ghose | Veber | Egan | Muegge | Bioavailability score | Synthetic accessibility |
|----------|----------|-------|-------|------|--------|-----------------------|-------------------------|
| 9aa      | Yes      | Yes   | Yes   | Yes  | No     | 0.55                  | 2.41                    |
| 9bb      | No       | No    | Yes   | No   | No     | 0.55                  | 2.49                    |
| 9cc      | No       | No    | Yes   | Yes  | No     | 0.55                  | 2.5                     |
| 9dd      | No       | No    | Yes   | No   | No     | 0.55                  | 2.62                    |
| 9ee      | No       | No    | Yes   | No   | No     | 0.55                  | 2.76                    |
| 9ff      | Yes      | Yes   | Yes   | Yes  | No     | 0.55                  | 2.93                    |
| 9gg      | No       | No    | Yes   | No   | No     | 0.55                  | 2.49                    |
| 9hh      | No       | No    | Yes   | No   | No     | 0.55                  | 2.53                    |
| 9ii      | No       | No    | Yes   | No   | No     | 0.55                  | 2.57                    |
| 9jj      | No       | No    | Yes   | No   | No     | 0.55                  | 2.84                    |
| 9kk      | No       | No    | Yes   | No   | No     | 0.55                  | 2.840000                |

**Table 6.6: Toxicity studies of the designed new compounds**

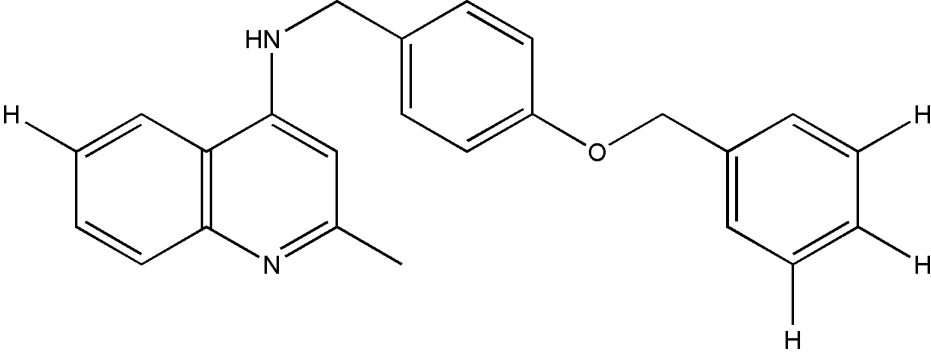
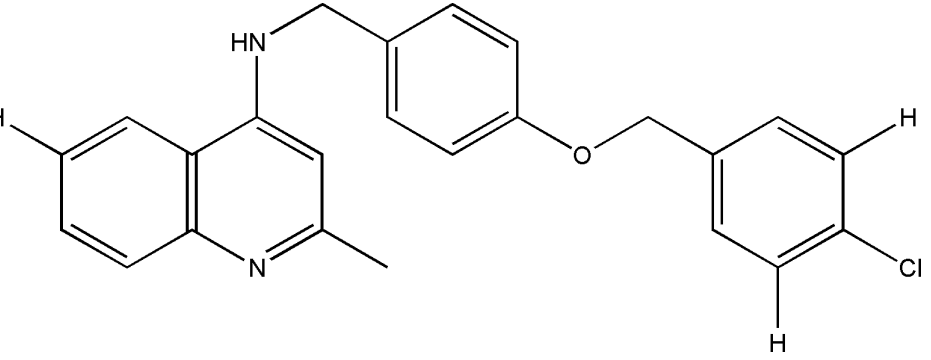
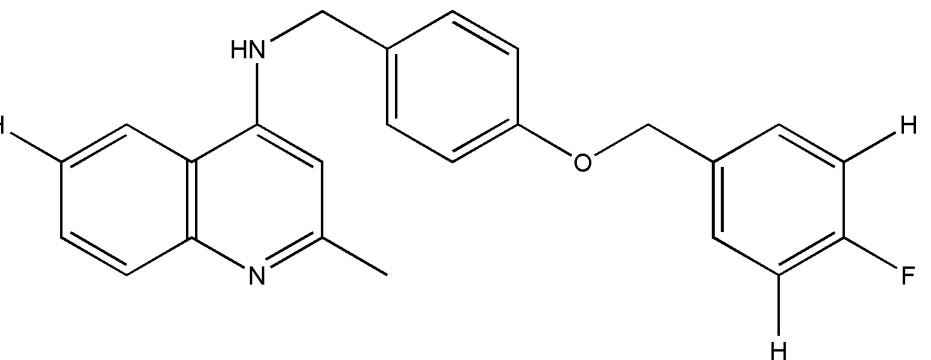
| Compound | Acute Oral Toxicity Class | Predicted acute oral toxicity | Carcinogenicity | Hepatotoxicity | Nephrotoxicity | Respiratory toxicity |
|----------|---------------------------|-------------------------------|-----------------|----------------|----------------|----------------------|
| 9g       | III                       | 2.2034                        | Negative        | Positive       | Positive       | Positive             |
| 9h       | III                       | 2.3677                        | Negative        | Positive       | Positive       | Positive             |
| 9h       | III                       | 2.2986                        | Negative        | Positive       | Negative       | Positive             |
| 9o       | III                       | 2.3252                        | Negative        | Positive       | Negative       | Positive             |

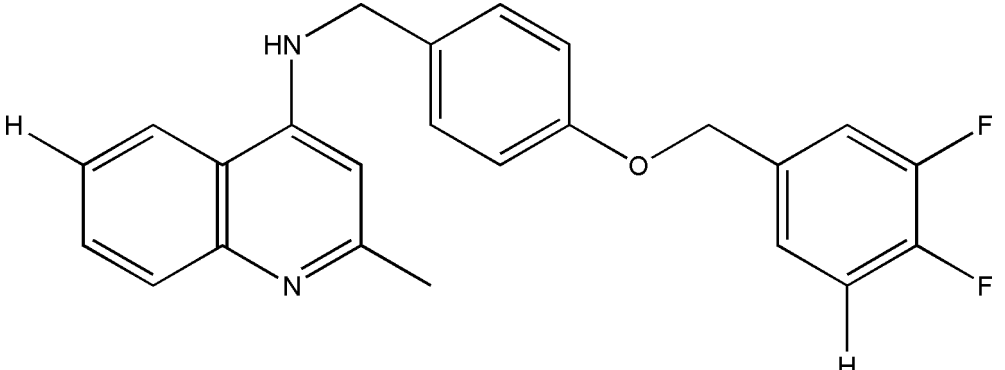
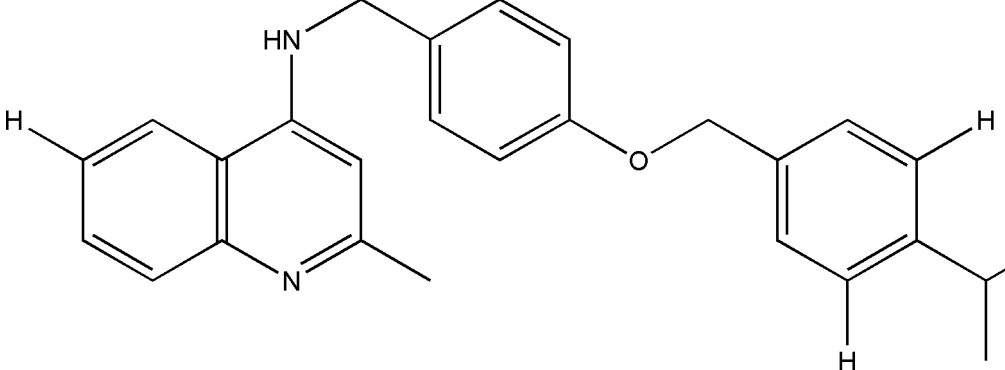
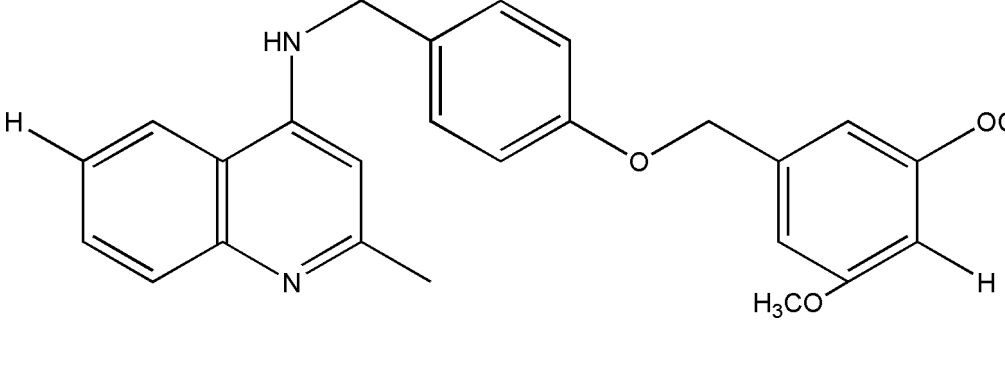
## **MOLECULAR DOCKING**

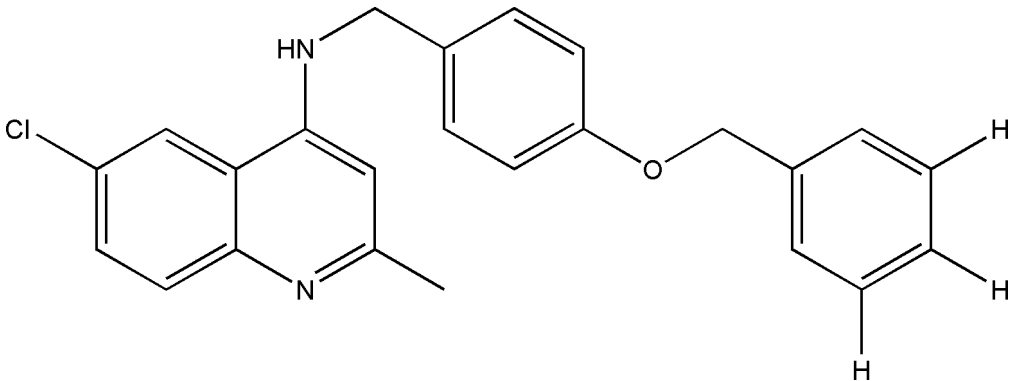
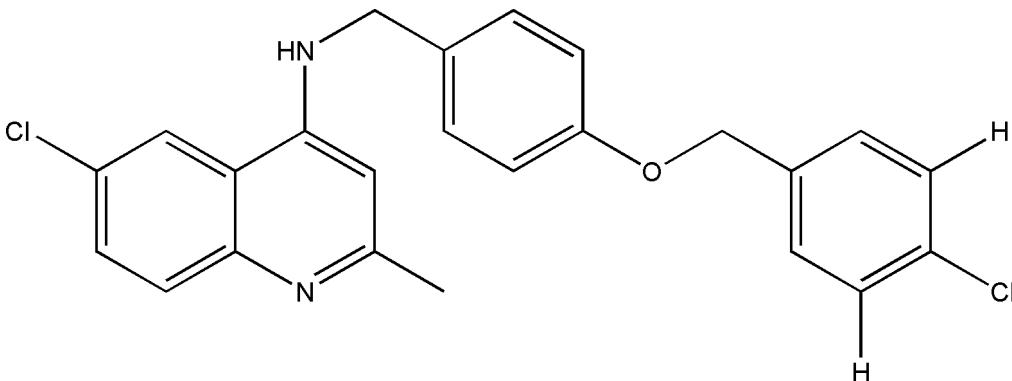
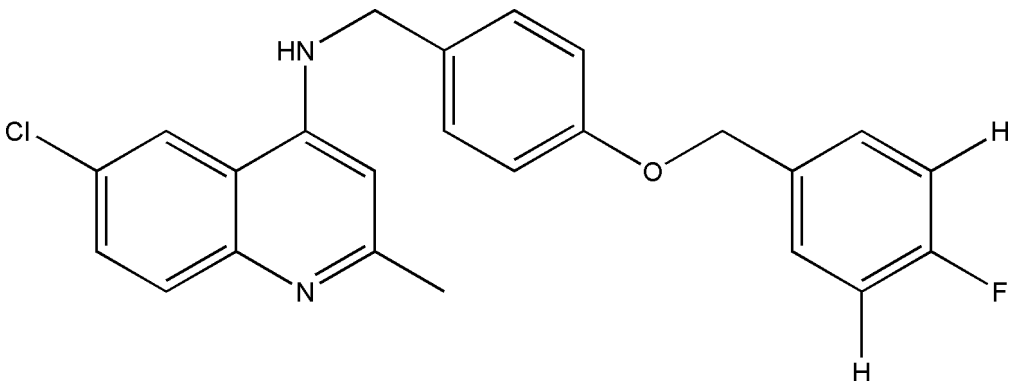
A molecular docking study investigated the binding affinities of compounds 9g, 9h, 9n and 9o to various enzymes, including Enoyl-ACP(CoA) reductase (PDB ID:2Aq8), Enoyl-ACP(CoA) reductase (PDB ID:2IDZ), Mycolyl Transferase (PDB ID:1F0N), and Dihydrofolate reductase (PDB ID:1Dg5).

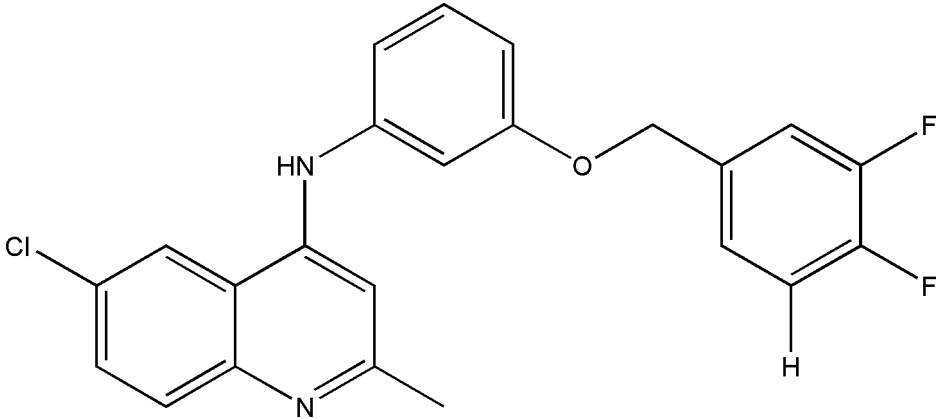
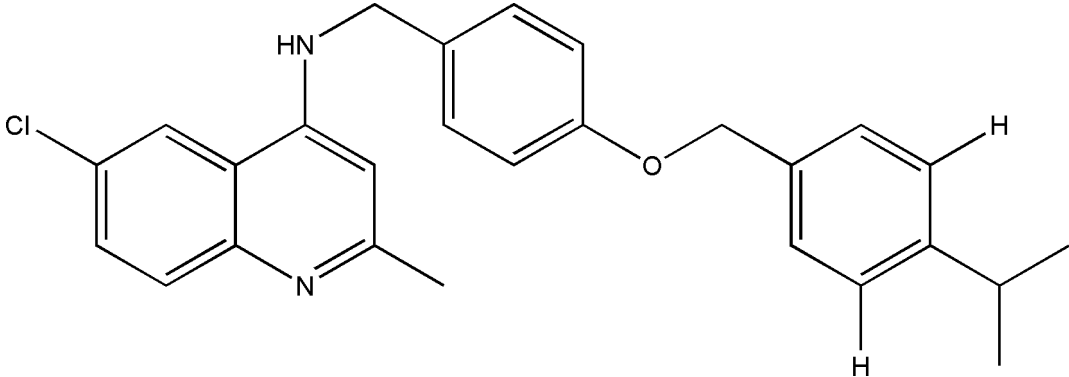
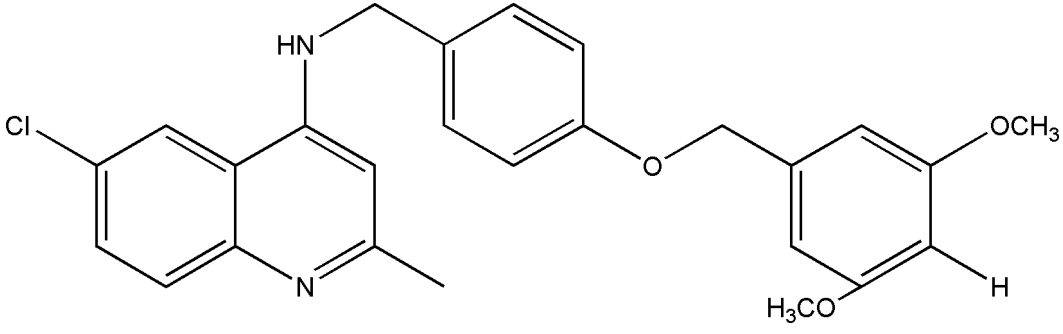
- Among the compounds tested, 9h exhibited the highest binding affinities with values of -9.9kcal/mol for Enoyl-ACP (CoA) reductase (2Aq8), -9.6kcal/mol for Enoyl-ACP(CoA) reductase (2IDZ), -9.4kcal/mol for Mycolyl Transferase (1F0N), and -9.7kcal/mol for Dihydrofolate reductase (1Dg5).
- Compound 9n also showed strong binding, with affinity values of -9.9, -9.5, -9.4 and -9.7kcal/mol for the respective enzymes.
- Compound 9g displayed affinity values of -9.6, -9.4, -9.1, and -9.6kcal/mol.
- Compound 9o exhibited affinities of -9.5, -9.7, -9.3 and -9.7kcal/mol.
- In particular, compound 9h demonstrated the highest binding affinity across all enzymes tested, indicating its potential as a potent inhibitor. Compound 9g also showed considerable affinity, especially towards Enoyl-ACP(CoA) reductase (2Aq8) and Mycolyl Transferase (1F0N).
- The formation of hydrogen bonds with the target molecules results in inhibition, whereas binding affinity is increased by Van der Waals, pi-pi and hydrophobic interactions.

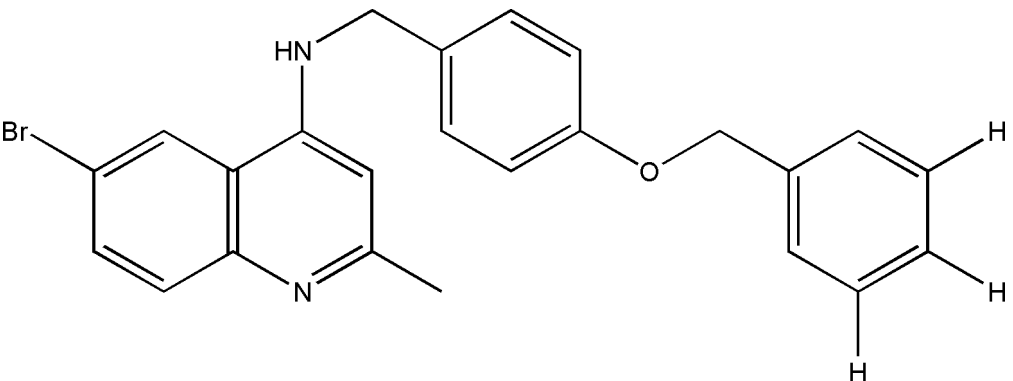
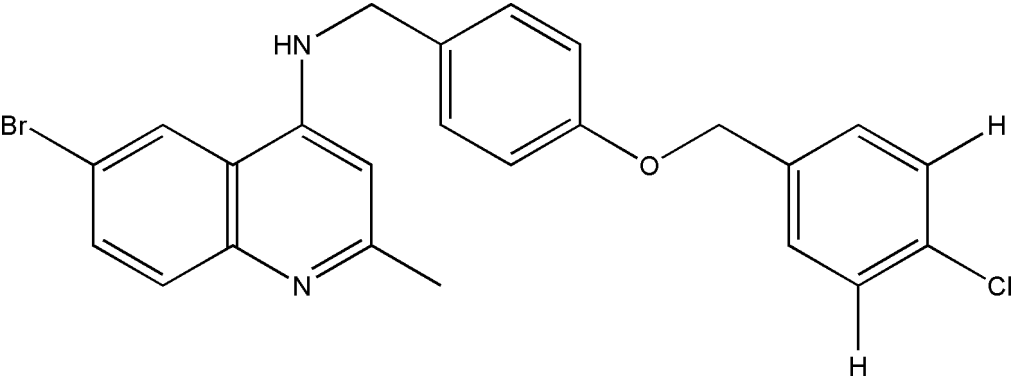
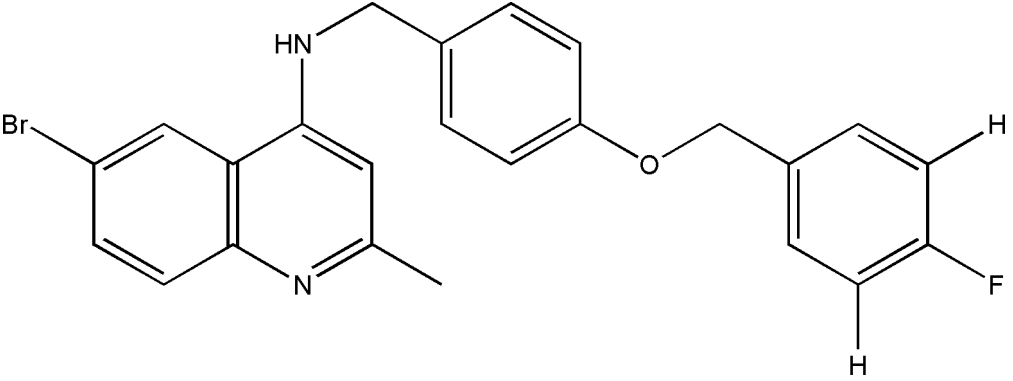
**Table 6.7: Structure of designed compounds**

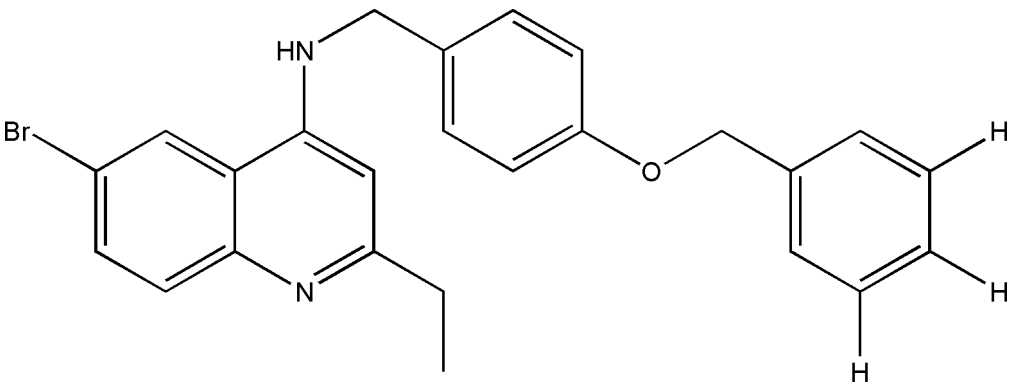
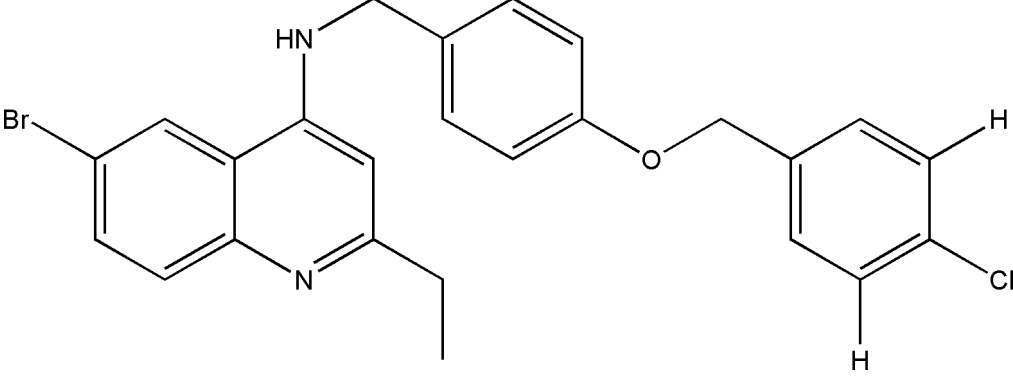
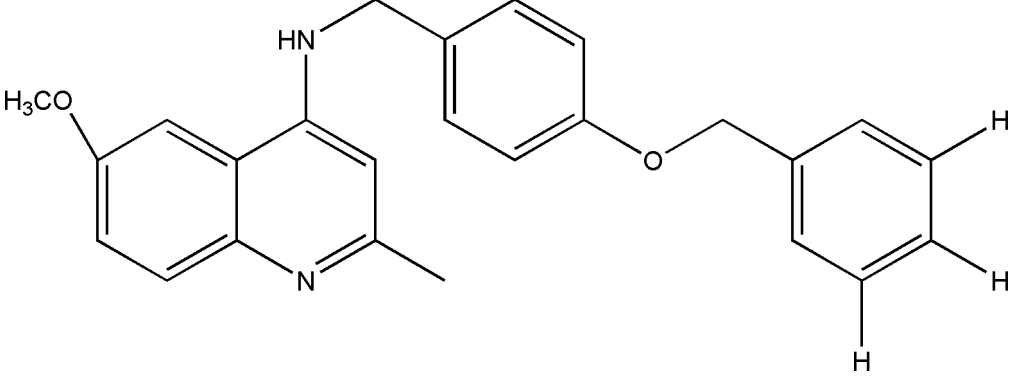
| COMPOUND<br>CODE | STRUCTURE AND IUPAC NAME                                                                                                                             |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9a               | <p>N-(4-(benzyloxy)benzyl)-2-methylquinolin-4-amine</p>            |
| 9b               | <p>N-(4-(4-chlorobenzyloxy)benzyl)-2-methylquinolin-4-amine</p>  |
| 9c               | <p>N-(4-(4-fluorobenzyloxy)benzyl)-2-methylquinolin-4-amine</p>  |

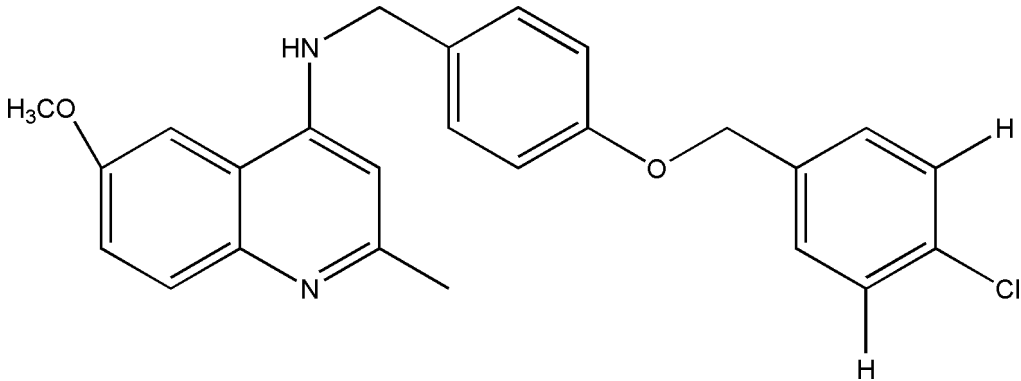
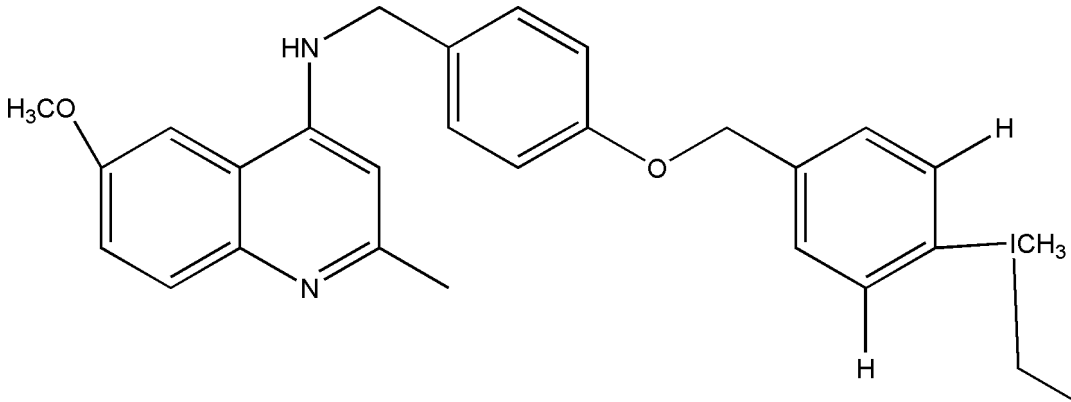
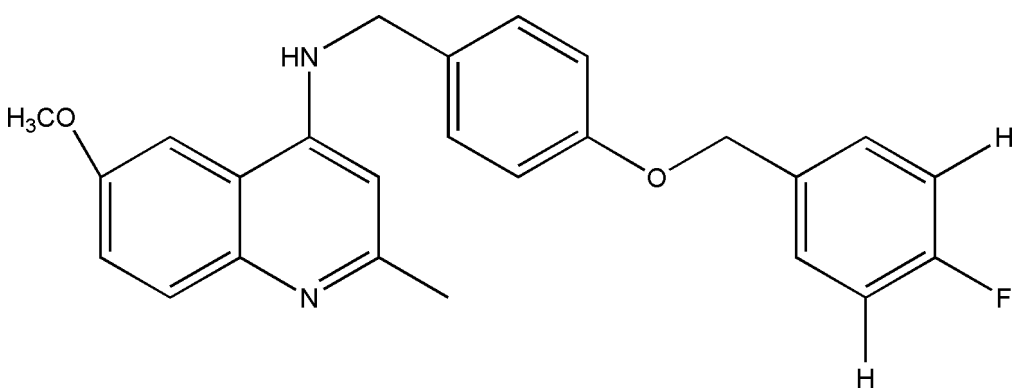
|    |                                                                                                                                                           |
|----|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9d | <p>N-(4-(3,4-difluorobenzyloxy)benzyl)-2-methylquinolin-4-amine</p>     |
| 9e | <p>N-(4-(4-isopropylbenzyloxy)benzyl)-2-methylquinolin-4-amine</p>     |
| 9f | <p>N-(4-(3,5-dimethoxybenzyloxy)benzyl)-2-methylquinolin-4-amine</p>  |

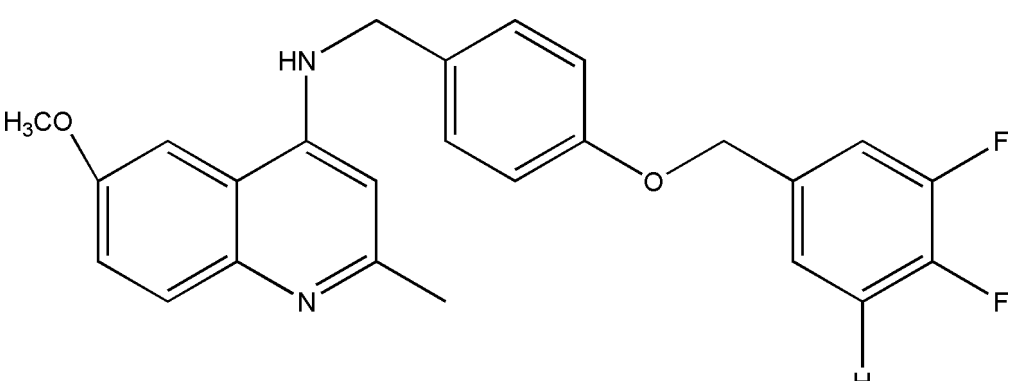
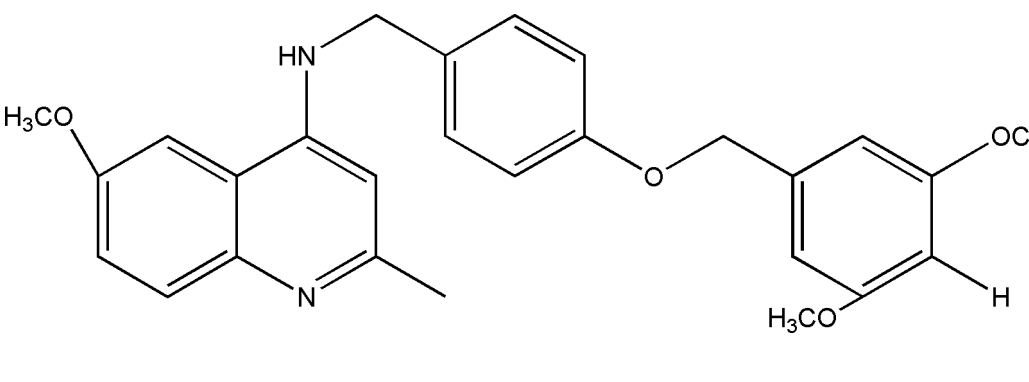
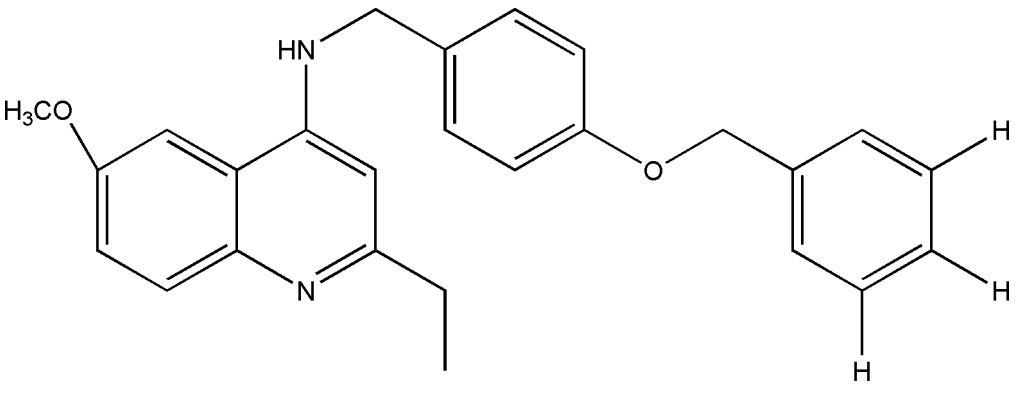
|    |                                                                                                                                                               |
|----|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9g | <p>N-(4-(benzyloxy)benzyl)-6-chloro-2-methylquinolin-4-amine</p>            |
| 9h | <p>N-(4-(4-chlorobenzyloxy)benzyl)-6-chloro-2-methylquinolin-4-amine</p>   |
| 9i | <p>N-(4-(4-fluorobenzyloxy)benzyl)-6-chloro-2-methylquinolin-4-amine</p>  |

|    |                                                                                                                                                                    |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9j | <p>N-(3-(3,4-difluorobenzyloxy)phenyl)-6-chloro-2-methylquinolin-4-amine</p>     |
| 9k | <p>N-(4-(4-isopropylbenzyloxy)benzyl)-6-chloro-2-methylquinolin-4-amine</p>     |
| 9l | <p>N-(4-(3,5-dimethoxybenzyloxy)benzyl)-6-chloro-2-methylquinolin-4-amine</p>  |

|    |                                                                                                                                                              |
|----|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9m | <p>N-(4-(4-benzyloxy)benzyl)-6-bromo-2-methylquinolin-4-amine</p>          |
| 9n | <p>N-(4-(4-chlorobenzyloxy)benzyl)-6-bromo-2-methylquinolin-4-amine</p>   |
| 9o | <p>N-(4-(4-fluorobenzyloxy)benzyl)-6-bromo-2-methylquinolin-4-amine</p>  |

|    |                                                                                                                                                            |
|----|------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9s | <p>N-(4-(benzyloxy)benzyl)-6-bromo-2-ethylquinolin-4-amine</p>           |
| 9t | <p>N-(4-(4-chlorobenzyloxy)benzyl)-6-bromo-2-ethylquinolin-4-amine</p>  |
| 9u | <p>N-(4-(benzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>      |

|    |                                                                                                                                                                |
|----|----------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9v | <p>N-(4-(4-chlorobenzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>    |
| 9w | <p>N-(4-(4-propylbenzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>   |
| 9x | <p>N-(4-(4-fluorobenzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>  |

|     |                                                                                                                                                                    |
|-----|--------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 9y  | <p>N-(4-(3,4-difluorobenzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>    |
| 9z  | <p>N-(4-(3,5-dimethoxybenzyloxy)benzyl)-6-methoxy-2-methylquinolin-4-amine</p>  |
| 9aa | <p>N-(4-(benzyloxy)benzyl)-2-ethyl-6-methoxyquinolin-4-amine</p>               |

**Table 6.8: Binding affinity of designed compounds**

| Compound code               | Binding Affinity                |                                |                           |                                |                                                            |
|-----------------------------|---------------------------------|--------------------------------|---------------------------|--------------------------------|------------------------------------------------------------|
|                             | Enoyl-ACP(CoA) reductase (2AQ8) | Enoyl-ACP(CoA) Reductase(2IDZ) | Mycolyl transferase(1F0N) | Dihydrofolate reductase (1DG5) | Cytochrome P450 14 alpha-sterol demethylase (CYP51) (1E9X) |
| <b>9g</b>                   | -9.6                            | -9.4                           | -9.1                      | -9.6                           | -10.0                                                      |
| <b>9h</b>                   | -9.9                            | -9.6                           | -9.4                      | -9.7                           | -9.3                                                       |
| <b>9n</b>                   | -9.9                            | -9.5                           | -9.4                      | -9.7                           | -9.6                                                       |
| <b>9o</b>                   | -9.5                            | -9.7                           | -9.3                      | -9.7                           | -10.0                                                      |
| <b>Isoniazid (standard)</b> | <b>-5.1</b>                     | <b>-5.1</b>                    | <b>-5.4</b>               | <b>-5.2</b>                    | <b>-5.4</b>                                                |

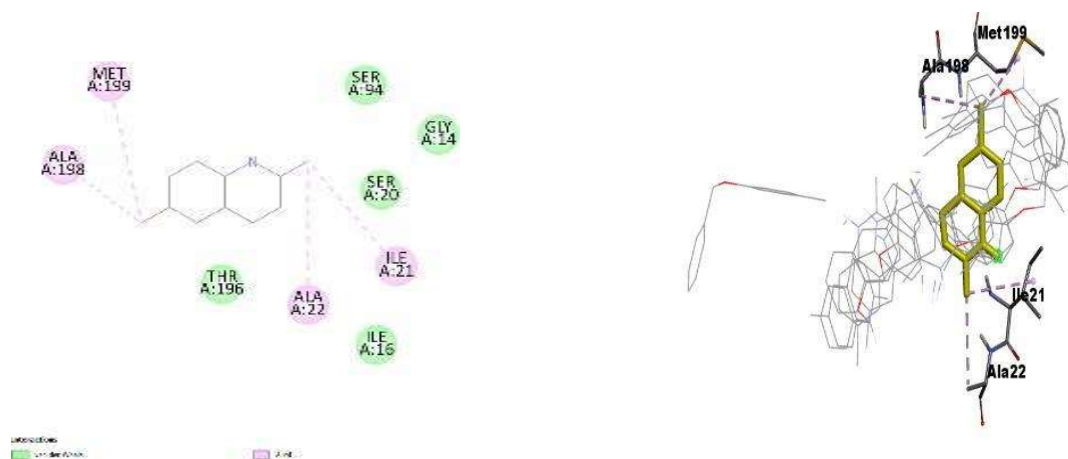


Fig 6.4: 2D and 3D interaction of compound 9g with Enoyl-ACP (CoA) reductase (2AQ8)

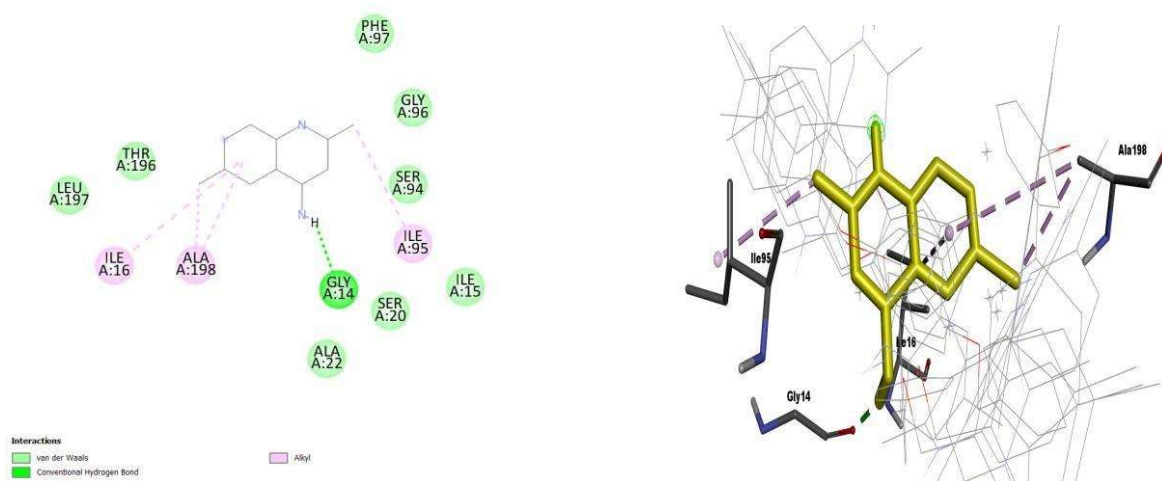


Fig 6.5: 2D and 3D interaction of compound 9h with Enoyl-ACP (CoA) reductase (2AQ8)

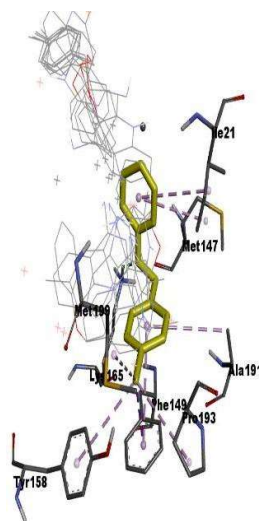
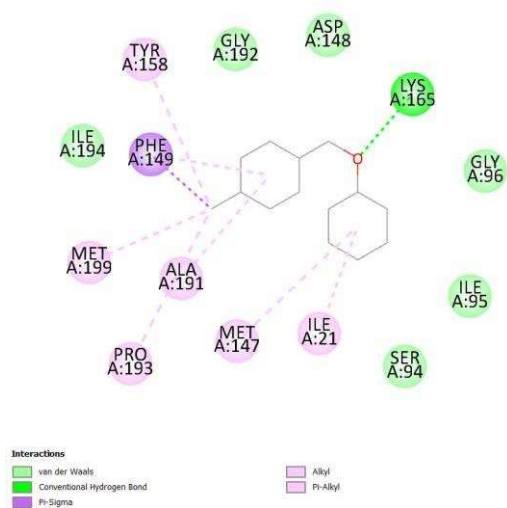


Fig 6.6:2D and 3D interaction of compound 9n with Enoyl-ACP (CoA) reductase (2AQ8)

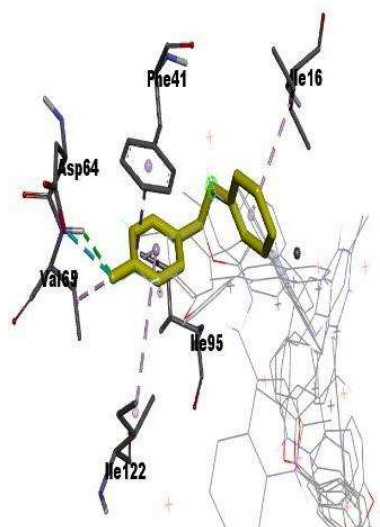
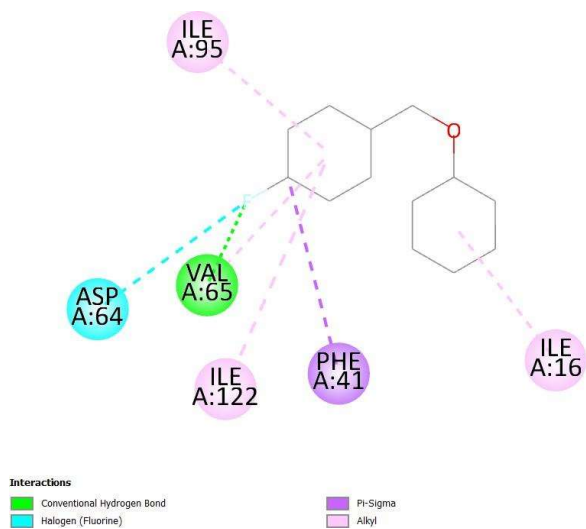


Fig 6.7:2D and 3D interaction of compound 9o with Enoyl-ACP (CoA) reductase (2AQ8)

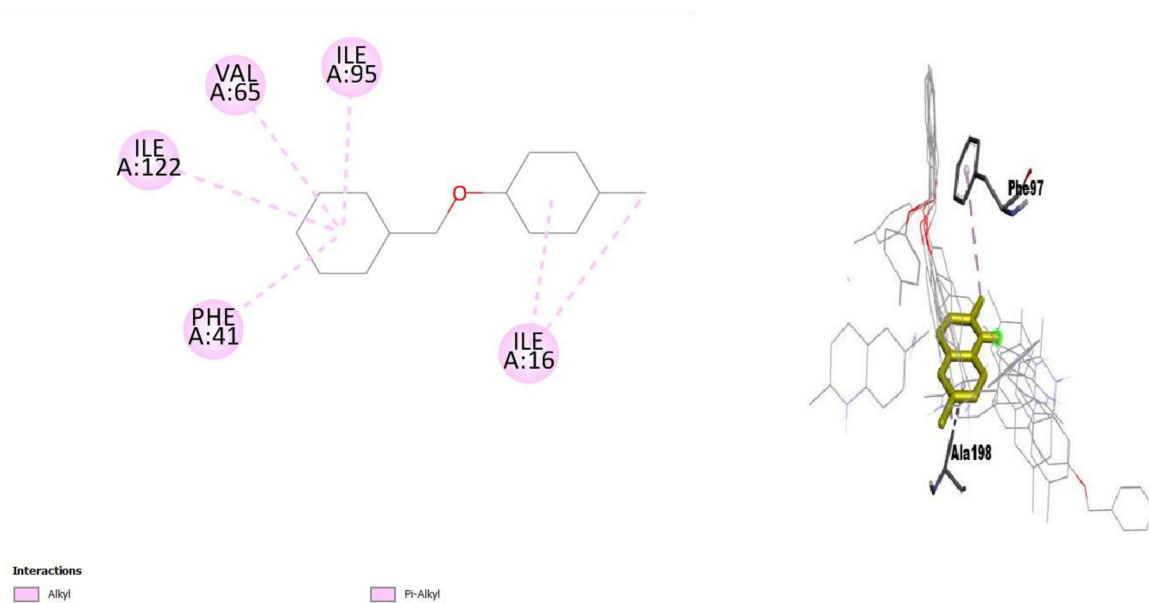


Fig 6.8: 2D and 3D interaction of compound 9g with Enoyl-ACP (CoA) reductase (2IDZ)

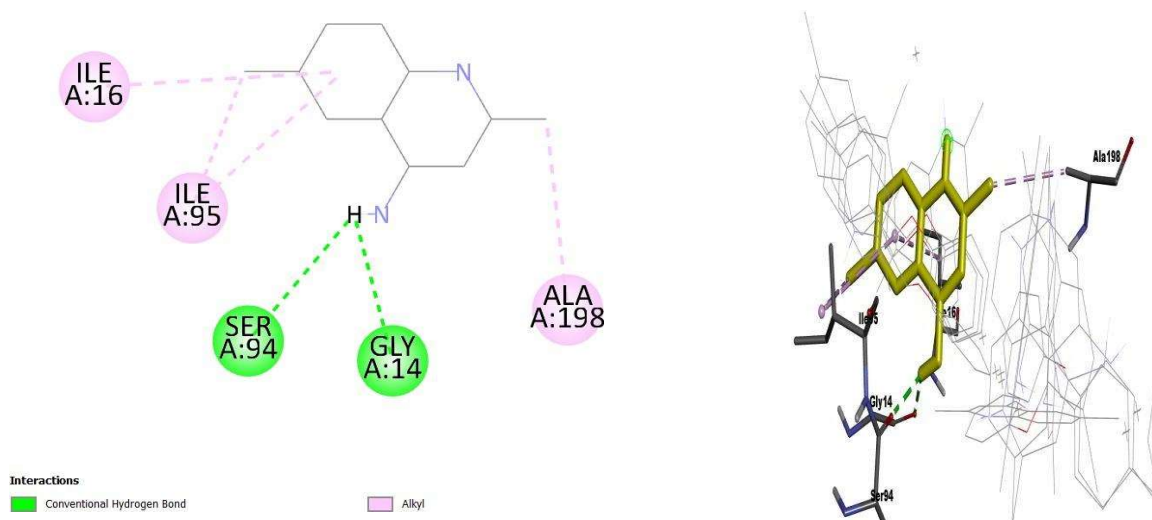


Fig 6.9: 2D and 3D interaction of compound 9h with Enoyl-ACP (CoA) reductase (2IDZ)

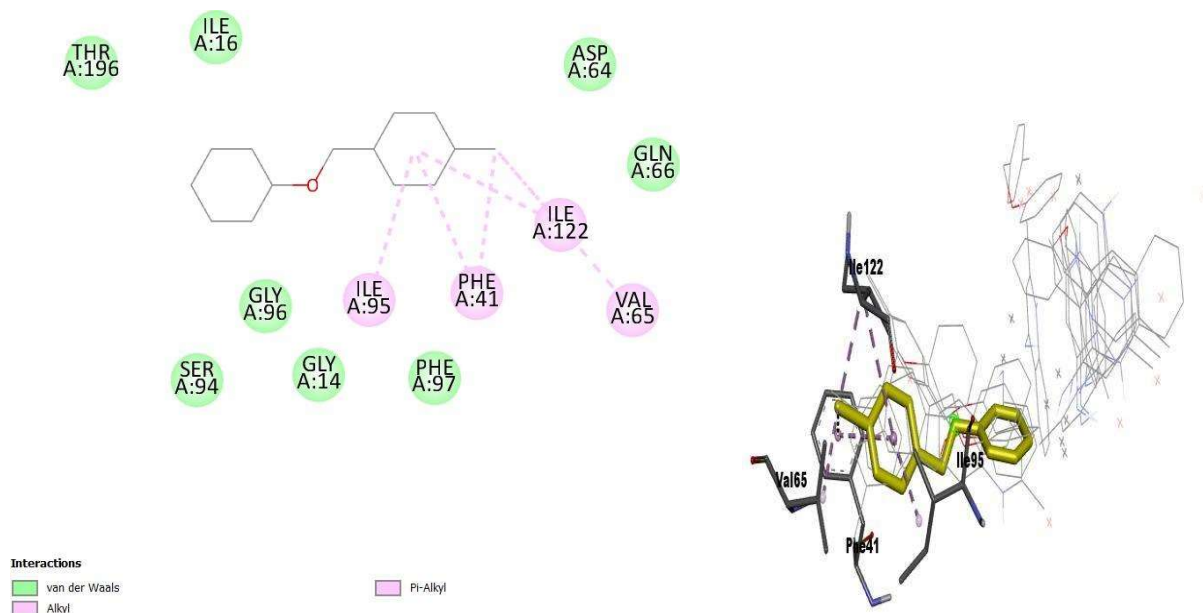


Fig 6.10:2D and 3D interaction of compound 9n with Enoyl-ACP (CoA) reductase (2IDZ)

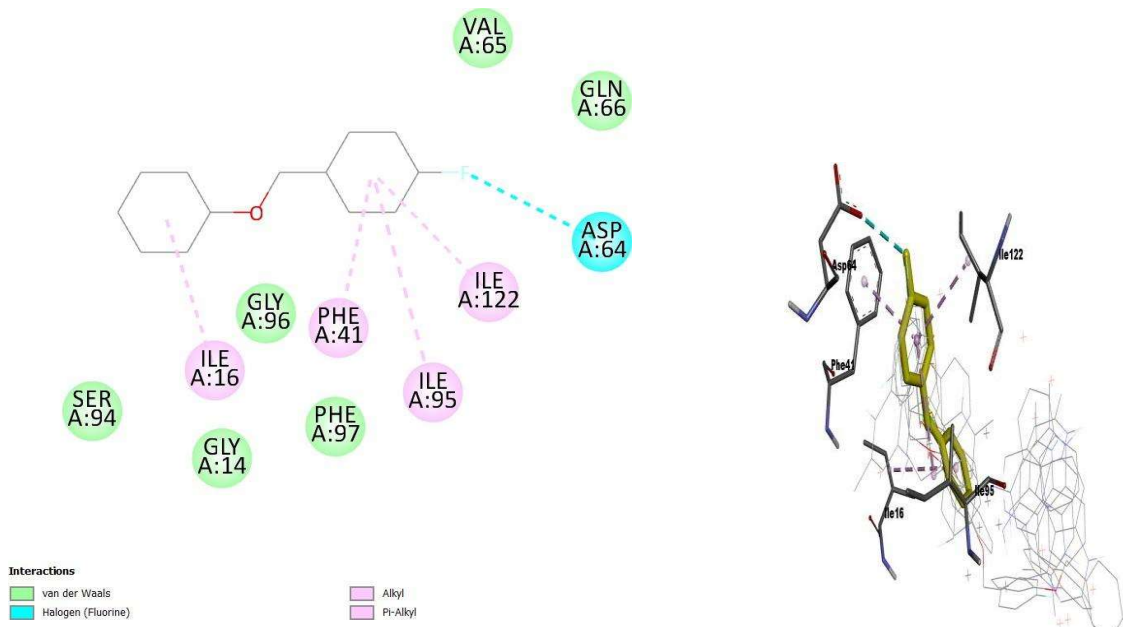


Fig 6.11:2D and 3D interaction of compound 9o with Enoyl-ACP (CoA) reductase (2IDZ)

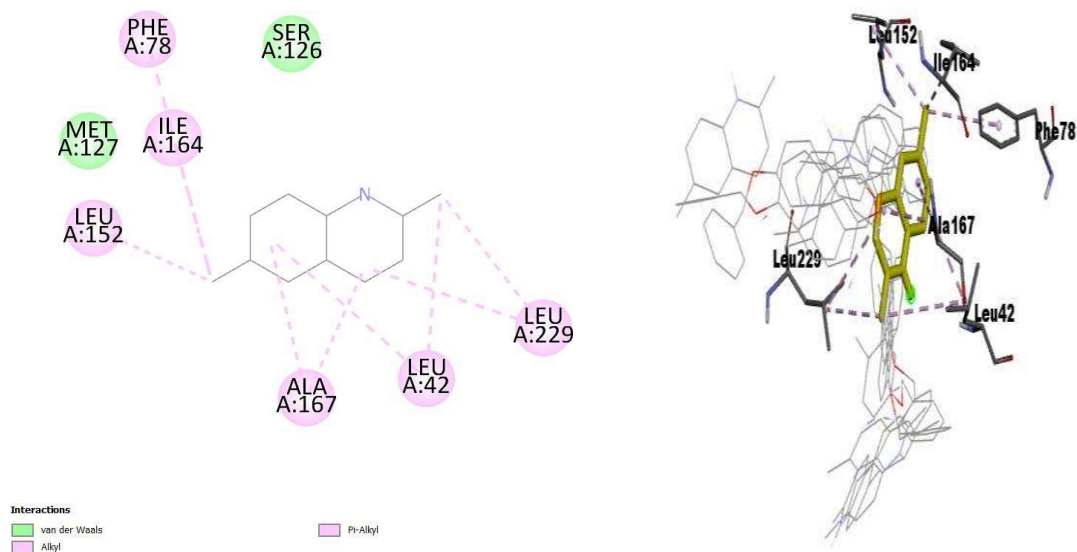


Fig 6.12: 2D and 3D interaction of compound 9g with mycolyl transferase (1F0N)

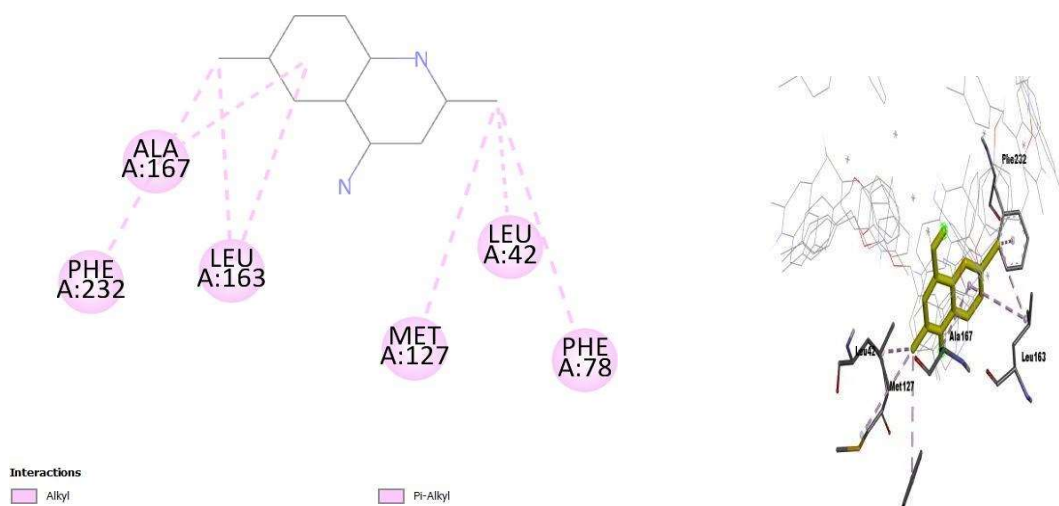


Fig 6.13: 2D and 3D interaction of compound 9h with mycolyl transferase (1F0N)

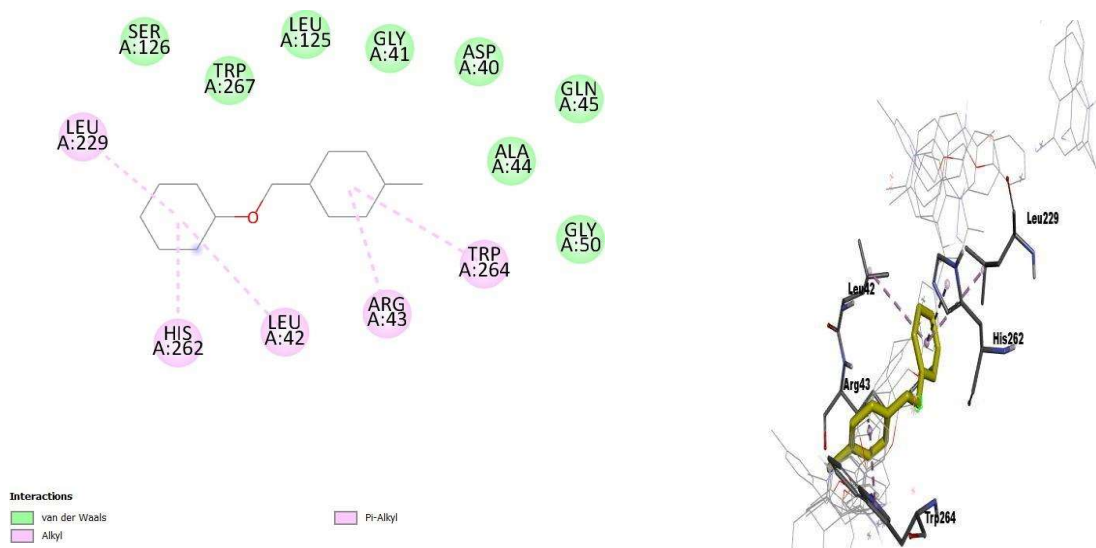


Fig 6.14: 2D and 3D interaction of compound 9n with mycolyl transferase (1F0N)

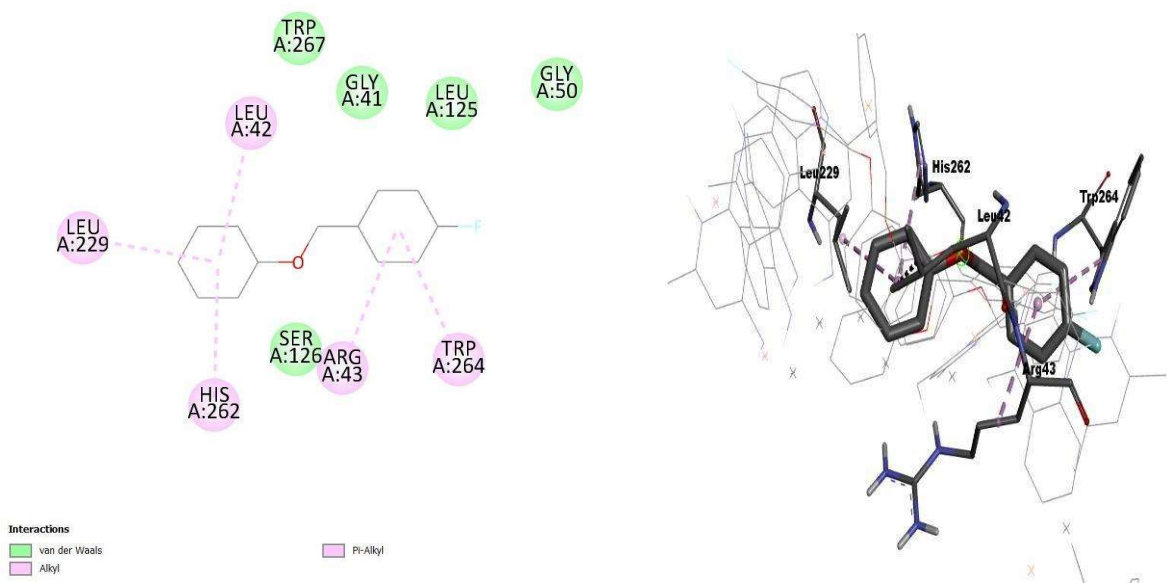


Fig 6.15: 2D and 3D interaction of compound 9o with mycolyl transferase (1F0N)

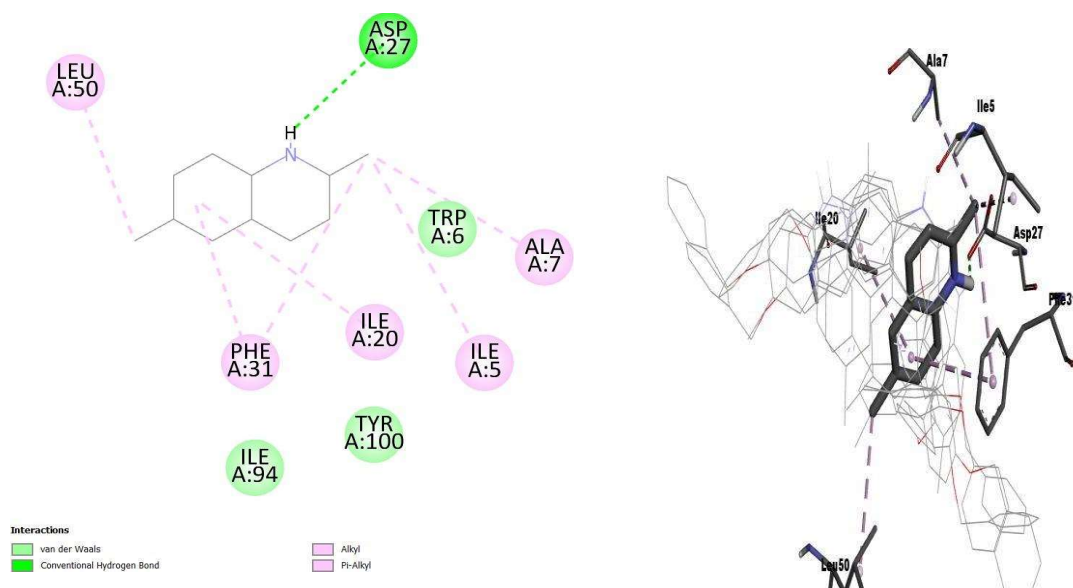


Fig 6.16: 2D and 3D interaction of compound 9g with Dihydrofolate reductase (1DG5)

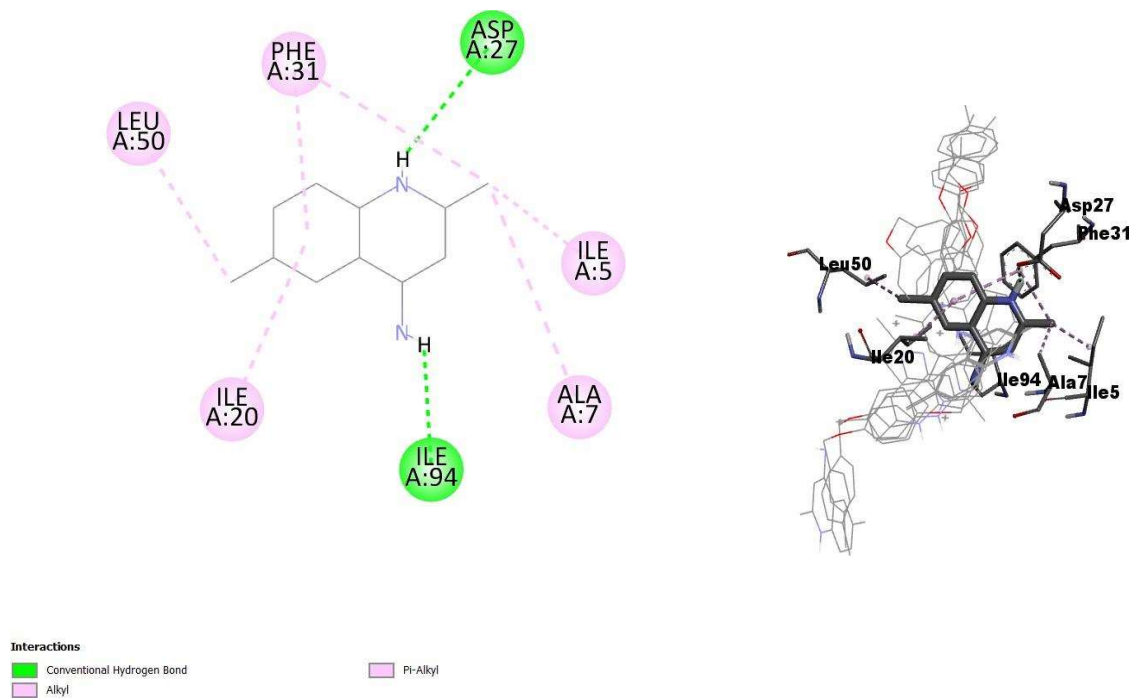


Fig 6.17: 2D and 3D interaction of compound 9h with Dihydrofolate reductase (1DG5)

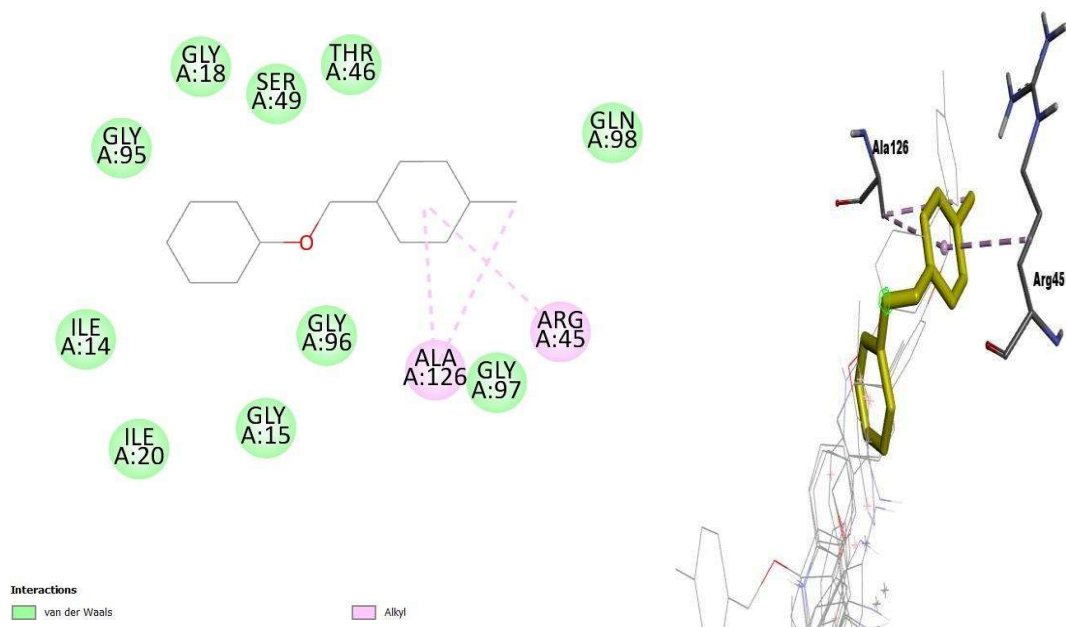


Fig 6.18: 2D and 3D interaction of compound 9n with Dihydrofolate reductase (1DG5)

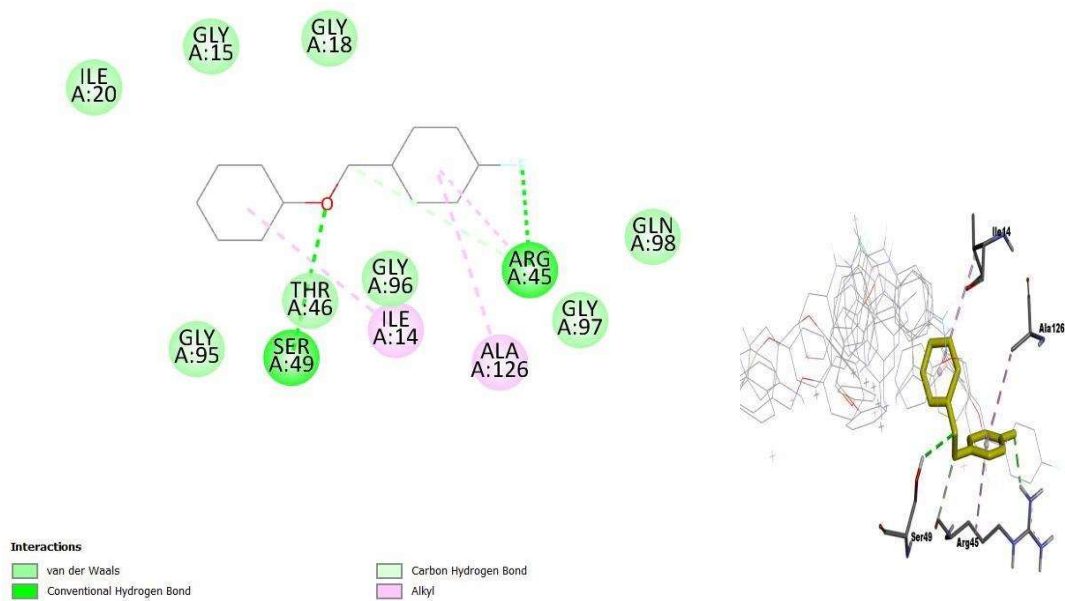


Fig 6.19: 2D and 3D interaction of compound 9o with Dihydrofolate reductase (1DG5)

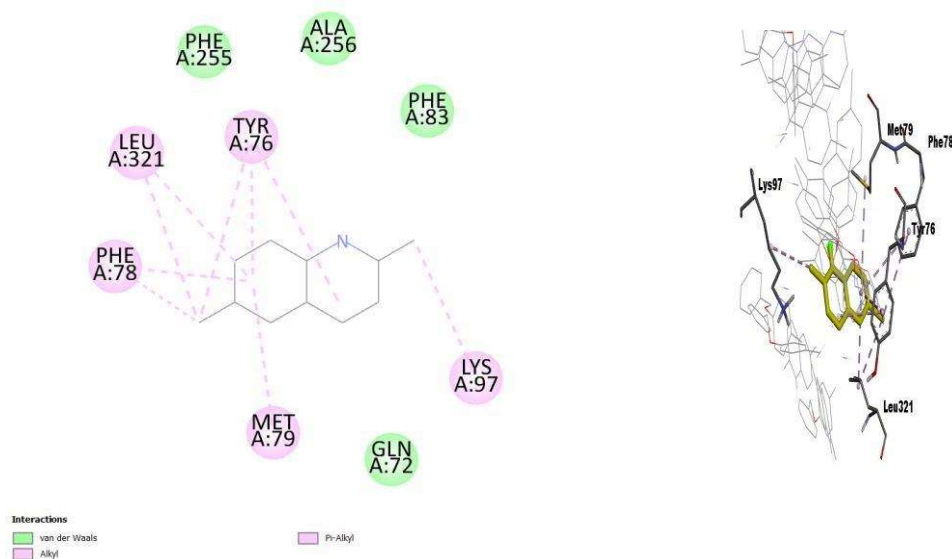


Fig 6.20: 2D and 3D interaction of compound 9g with Cytochrome P450 14 alpha-sterol demethylase (CYP51) (1E9X)

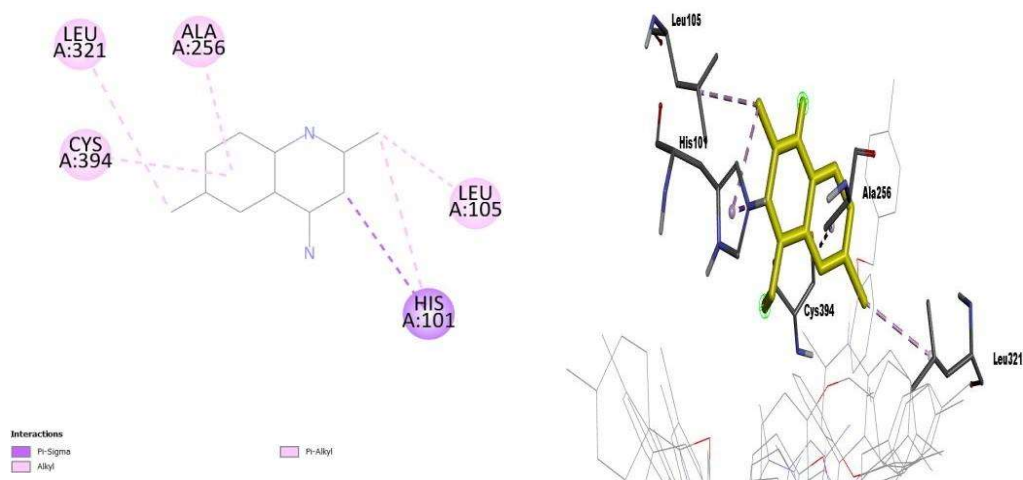


Fig 6.21: 2D and 3D interaction of compound 9h with Cytochrome P450 14 alpha-sterol demethylase (CYP51) (1E9X)

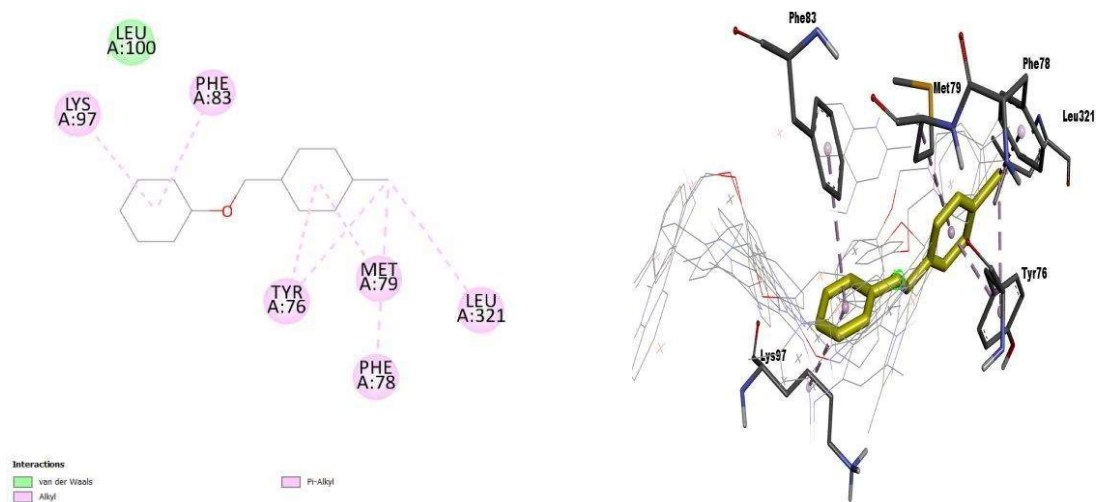


Fig 6.22: 2D and 3D interaction of compound 9n with Cytochrome P450 14  $\alpha$ -sterol demethylase (CYP51) (1E9X)

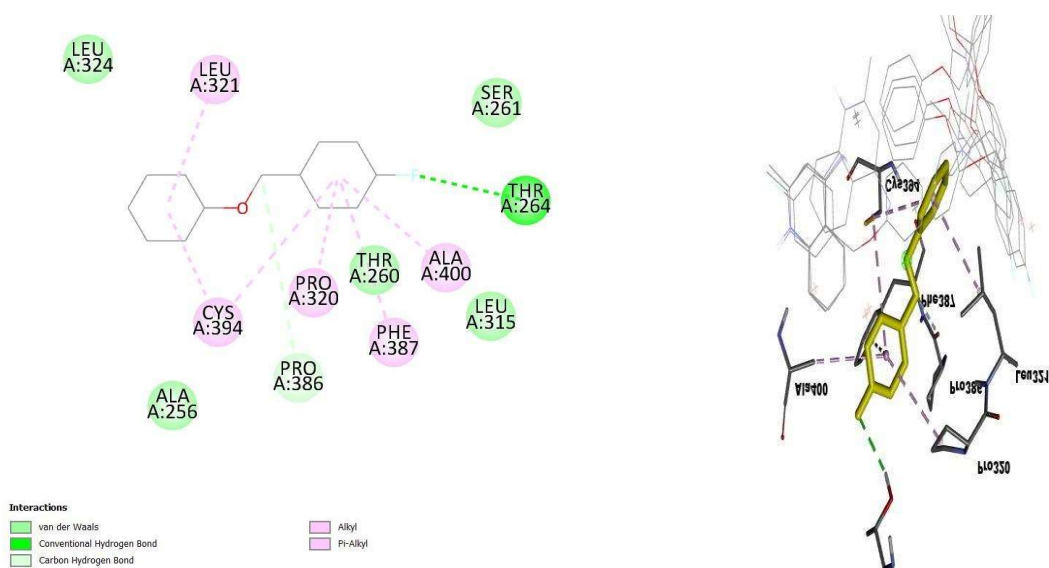


Fig 6.23: 2D and 3D interaction of compound 9o with Cytochrome P450 14  $\alpha$ -sterol demethylase (CYP51) (1E9X)

## CHAPTER VII

### 7. CONCLUSION

This is a comprehensive process involving computational chemistry techniques to design potential anti-tubercular compounds. Here's a breakdown of the steps involved:

- **Training Set Selection:** A training set of 27 compounds of aminoquinoline derivatives were chosen for the study, based on previous experimental data and literature review indicating their potency as anti-tubercular agents.
- **QSAR Studies:** Quantitative Structure-Activity Relationship (QSAR) studies were performed using QSARINS software. This involved establishing 2D-QSAR models to understand the relationship between the physicochemical properties of the compounds and their anti-tubercular activity. Multiple QSAR equations were generated and validated both internally and externally to ensure reliability.
- **Parameter Selection:** From the generated QSAR equations, the best equation based on statistical values were selected for further analysis. This likely involved identifying key structural features that significantly contribute to the compounds' anti-tubercular activity.
- **Structure-Activity Relationship Analysis:** Structural features identified through auto-correlation description and Lipinski parameters were used to relate to the observed biological activity of the compounds.
- **Design of New Compounds:** Utilizing the established QSAR models, From 20 new aminoquinoline derivatives four new compounds 9g,9h,9n,9o were designed with predicted anti-tubercular activity.
- **Molecular Docking Studies:** The best four predicted active compounds were subjected to molecular docking studies to understand their interactions with the target proteins associated with tuberculosis.

- In Silico ADMET Prediction: In silico Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) prediction was performed to assess the structural interactions and predict the compounds' pharmacokinetic and toxicological properties.
- Drug-likeness Prediction: In silico SwissADME prediction was likely utilized to assess the drug-like properties of the designed compounds, providing additional information to support the QSAR model.
- Toxicity Studies: Toxicity studies were conducted to evaluate the potential adverse effects of the designed compounds.
- Compound Selection: Based on the results obtained from molecular docking, ADMET prediction, drug-likeness assessment, and toxicity studies, the best four compounds 9g,9h,9n,9o were selected for further development as potential anti-tubercular agents.
- Multi-Target Approach: The results suggest that the designed compounds may have a multi-target approach, meaning they can interact with multiple biological targets associated with tuberculosis, potentially enhancing their efficacy.

Overall, Two designed compounds 9h, 9o showed promising results in terms of predicted anti-tubercular activity, minimized toxic effects, and potential for multi-target drug action, indicating their potential as candidates for further experimental validation and development into anti-tubercular drugs.

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