

DESIGN, 2D QSAR STUDIES, MOLECULAR DOCKING, ADMET PREDICTION AND TOXICITY STUDIES OF IMIDAZOLE DERIVATIVES AS POTENT ANTI-MALARIAL AGENTS

Submitted in partial fulfillment of the requirements for the award of

Bachelor of Pharmacy

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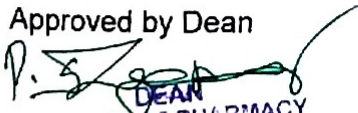
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ACKNOWLEDGEMENTS

We are pleased to acknowledge our sincere thanks to the management of Sathyabama Institute of Science and Technology, our founder chancellor **Colonel. Dr. Jeppiaar**, Chancellor **Dr. Mariazeena Johnson**, President **Dr. Marie Johnson**, Vice President **Mr. J. Arun Selvan**, Vice President **Mrs. Maria Bernadette Tamilarasi**, Vice President **Ms. Maria Catherine Jayapriya**, Vice Chancellor **Dr. T. Sasipraba**, Pro Vice Chancellor **Dr. E. Logashanmugam**, Registrar **Dr. S.S. Rao**, Director Research **Dr. B. Sheela Rani** and Director Administration **Dr. G. Sundari** for their kind encouragement in doing this project and for completing it successfully. We are grateful to them. We convey our thanks to **Dr. P. Shanmuga Pandiyan**, Dean, School of Pharmacy for providing us necessary support and details at the right time during the progressive work.

We would like to express our sincere and deep sense of gratitude to our project Guide **Dr. S. Arunkumar**, M.Pharm, Ph.D for his valuable guidance, suggestions and constant encouragement paved way for the successful completion of our project work.

We wish to express our sincere thanks to professor **Dr. N. Ramalakshmi**, M.Pharm., Ph.D, Professor and Head, Department of Pharmaceutical Chemistry, C.L. Baid Metha college of pharmacy, her valuable suggestions and discussion have enabled us to execute the present work successfully and also for providing an uninterrupted service at the college campus during the work period.

We also thank Mr. Prabhakaran, B.Pharm, Research Scholar, C.L. Baid Metha college of pharmacy for providing the necessary guidance in this project work.

We thank all the teaching and non-teaching staff members of the School of Pharmacy who were helpful in many ways for the completion of the project.

ABSTRACT

DESIGN, 2D QSAR STUDIES, MOLECULAR DOCKING, ADMET PREDICTION AND TOXICITY STUDIES OF IMIDAZOLE DERIVATIVES AS POTENT ANTI-MALARIAL AGENTS

Introduction :

Malaria, the most prevalent and most pernicious parasitic disease of humans, is estimated to kill between one and two million people, mainly children, each year. Resistance has emerged to all classes of antimalarial drugs and is responsible for a recent increase in malaria-related mortality. To prevent a potential public health emergency, there is an urgent need for new antimalarial drugs, with single- dose cures, broad therapeutic potential, and novel mechanism of action. Imidazole analogues are one such novel class which possess a potent antimalarial activity.

Methodology :



Results and discussion :

QSAR models were generated for 26 reported imidazole analogues. 13 Novel imidazole derivatives were designed and their probable activities were predicted from the developed QSAR model. Physiochemical parameters, Druglikeness, pharmacokinetic characteristics were derived and were subjected to Docking. Binding affinity and the interactions between protein and ligand molecules were analysed. From the results 8 novel active compounds were designed as a heme- peroxidase and MRP1 inhibitors. Toxicity studies were also conducted. The compounds were predicted to be non-toxic.

Conclusion :

The discovered novel imidazole analogues were highlighted as potent antimalarial agents with comparatively lower toxicity and a novel mechanism of these compounds have higher binding affinity towards plasmodial enzymes. Thus proving a multi targeted drug therapy against malaria.

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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.
Q ² LOO	Variance in Leave one out.
Q ² LMO	Variance in Leave many out.
CCC	Concordance Correlation Coefficient.
CNS	Central Nervous System
CLT	Clotrimazole.
CQ	Chloroquine.
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 ²	Coefficient of Determination
F	Fischer's Value
AD	Applicability Domain
TPSA	Topological Polar Surface Area
ROS	Reactive Oxygen Species

GSH	Glutathione
GSSG	Glutathione disulfide
PfHpo	Heme-Peroxidase
pfcr1	<i>P.falciparum</i> chloroquine resistance transporter
H ₂ O ₂	Hydrogen Peroxide
VIF	Variance Inflation Factor
MRP1	Multi Resistance-associated Protein 1
RCSB-PDB	Research Collaboratory for Structural Bioinformatics- Protein Data Bank

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CHAPTER I

1. INTRODUCTION

Medicinal chemistry is discipline at the intersection of chemistry, especially synthetic organic chemistry, and pharmacology and various other biological specialties, where they are involved with design, chemical synthesis and development for market of pharmaceutical agents, or bioactive molecules (drugs). In particular, medicinal chemistry in its most common practice – focusing on small organic chemistry and aspects of natural products and computational chemistry in close combination with chemical biology, enzymology and structural biology, together aiming at the discovery and development of new therapeutic agents.

Discovery is the identification of novel active chemical compounds, often called “hits”, which are typically found by assay of compounds for a desired biological activity. It is important to develop and for the subsequent hit-to-lead selection process. Lead compounds are selected from a collection of hits by refining the screening criteria to enable the selection of the most promising molecules for further development. The secondary assays employed for further development. The secondary assays employed for lead selection may screen for off-target effects as well as physio-chemical and ADME (absorption, distribution, metabolism and excretion) characteristics. Once hit molecules are identified, their analogues can be tested in order to determine structure-activity relationships (SAR) of the compounds. SAR information used for the selection and design of structural analogs with improved activity, and for the confirmation of the core structures of the molecules. In general, the purpose of the hit-to-lead process is to improve the potency, selectivity and physicochemical properties (e.g. solubility and stability) of the compounds for further *in vitro* and *in vivo* testing and for subsequent lead optimization. The optimization can be made through chemical

synthesis, computational chemistry and/or combinatorial chemistry. Lead optimization can direct the research to the synthesis of a new pharmacophore or a more active molecule.

Molecular modeling, or more generally, computational chemistry, has become a well-established part of drug development. Computer aided drug design is a powerful tool in the search of promising drug candidates. Computational modulation of a lead molecule can increase the specificity when the structural 3D image of the target is available, fitting the molecule for the target. Computational chemistry helps to produce almost perfect fits for a target also reduce the probability of side effects and toxicity. Drug design with the help of computers may be used at any of the following stages of drug discovery: I) hit identification using virtual screening (structure or ligand based design), II) hit-to-lead optimization of affinity and selectivity (structure based design, QSAR, etc.), III) lead optimization of other pharmaceutical properties while maintaining affinity. Use of computational drug design methods in any stage of drug discovery process allows great information evolution that can lead to better and more desirable drug candidates. Quantitative Structure Activity Relationship (QSAR) studies and molecular docking techniques are valuable tool in computational chemistry and it gives valuable information in early steps of drug discovery process. The prediction of biological activity can be achieved through QSAR analysis, where QSAR model is generated and validation of the developed model by using different statistical parameters. These models help to optimize the lead compounds which provide information about the correlation between structure properties and biological activity and also mathematically predict the biological property of novel compounds. Virtual screening is employed to filter hits and eliminate compounds with undesirable qualities, where experimental evaluation of compounds is not viable option in terms of money and time, so this helps to quickly access the drug likeness of a lead compound prior to experimental testing. In particular, medicinal chemistry in its most common practice-focusing on small organic molecules-encompasses synthetic organic chemistry and aspects of natural products and computational chemistry in close combination with chemical

biology, enzymology and structural biology, together aiming at the discovery and development of new therapeutic agents.

Malaria is an acute, life-threatening parasite disease spread by the *Anopheles* mosquito that poses a danger to world health. Each year, malaria poses a risk to two billion people.^[1-3] Incidence and fatality rates for malaria have not significantly altered since 2015. In 2020, there were predicted to be 241 million malaria cases and 6,27,000 malaria-related fatalities.^[4,5] *Plasmodium falciparum* is linked to the greatest fatalities among the five parasite species affecting humans.^[6] Malaria incidence and fatalities continue to be at unacceptable levels and are on the rise.^[7] Artemisinin resistance was followed by ACT partner drug resistance, and fit multidrug-resistant has now become globally widespread.^[8] A huge catastrophe in the fight against malaria would result from the loss of artemisinin and its derivatives' efficacy due to drug resistance.^[9] Antimalarial Drug Resistance (AMDR) is the major public health problem of the 21st century that threatens the effective prevention, treatment and management of Malaria. Resistance may develop due to the irrational use of chloroquine or multidrug therapy. Chloroquine (CQ) was one of the most successful antimicrobial agents in the treatment of malaria, while artemisinin represents the most effective therapeutic option for the treatment of resistant malaria.^[10] Although, there is a concern about overgrowing evidence that, Variants of the *P.falciparum* chloroquine resistance transporter (pfcr) genetic diversity showing resistance to chloroquine are commonly observed in almost all parts of the world where malaria is endemic.^[11] Also, the evolutionary *in vitro* appearance of artemisinin-resistant plasmodia is seen. This highlights the necessity for the development of a new class of antimalarial agents that differ from those of existing agents.^[12] Clotrimazole analogues are known to possess favourable antimalarial potency. The de novo emergence of resistance can be prevented by the use of antimalarial medicine combinations.^[13-15] To help an implicit public health exigency, there's a critical need for new antimalarial medicines, with single- dose cures, broad therapeutic potential, and novel mechanisms of action.

Antimalarial drug development can follow several approaches ranging from variations of being agents to the design of new agents that act against novel targets.^[16] The development of multidrug resistance in the malarial parasite has sabotaged the majority of the eradication efforts by restraining the as well as alternate-line antimalarial drugs, thus necessitating the development of new pharmaceuticals constructed on appropriate scaffolds with superior potency against the drug-resistant and drug-susceptible *Plasmodium* species^[17] and discovery of medicines with new mechanisms of action is essential.^[18] In this hunt, the identification of antimalarial effects of drugs already in use for other therapies represents an attractive approach with the potentially rapid clinical operation.

Former studies have demonstrated that antifungal azoles, ketoconazole, miconazole, and clotrimazole (CLT)^[19], effectively and rapidly inhibit the growth of CQ-resistant strains of *P. falciparum in vitro*^[20-24] the three azoles share the structural feature of possessing an imidazole group.^[25] CLT effectively and rapidly inhibits parasite growth in five different strains of *P. falciparum, in vitro*, irrespective of their chloroquine sensitivity.^[26,27]

1.1 CLOTRIMAZOLE

Clotrimazole (CLT, 3-(1-(2-chlorotriptyl)imidazole), anazole derivative, is effective against a wide range of fungal pathogens.^[28] Clotrimazole is extensively used as a topical treatment for tinea pedis (athlete's foot), as well as vulvovaginal and oropharyngeal candidiasis. It displays fungistatic antimycotic exertion by targeting the biosynthesis of ergosterol, thereby inhibiting fungal growth. The fungistatic medium of CLT is associated with the inhibition of sterol 14-demethylase and microsomal P-450-dependent enzymes.^[29,30] As well as its antimycotic exertion, clotrimazole has become a drug of interest against several other conditions similar to sickle cell disease, malaria and some cancers. It shows an antiproliferative effect by acting as a translation initiation inhibitor^[31], inhibits angiogenesis by blocking vascular endothelial growth factor expression^[32], decreases glycolysis of lung melanoma and colon adenoma-melanoma cells^[33], and induces apoptosis by altering calcium homeo-counterpoise of leukemic lymphoblasts.^[34] It's also known as an effective immunosuppressant.^[35,36] CLT inhibits cytochrome P450 monooxygenase and the growth of mycobacteria and streptomyces.^[37] It has also been combined with other molecules, such as metals, to produce clotrimazole complexes that show better pharmacological efficacy.^[38]

Molecular Formula = $C_{22}H_{17}ClN_2$

Molecular weight = 344.837 g/mol

Composition = C 76.63%, H 4.97%, Cl 10.28%, N 8.12%

Index of Refraction = 1.617

Density = 1.1 ± 0.1 g/cm³

Polarizability = 36.25 Å³

Monoisotopic Mass = 344.108026261 Da

Average Mass = 344

1.2 RECEPTOR

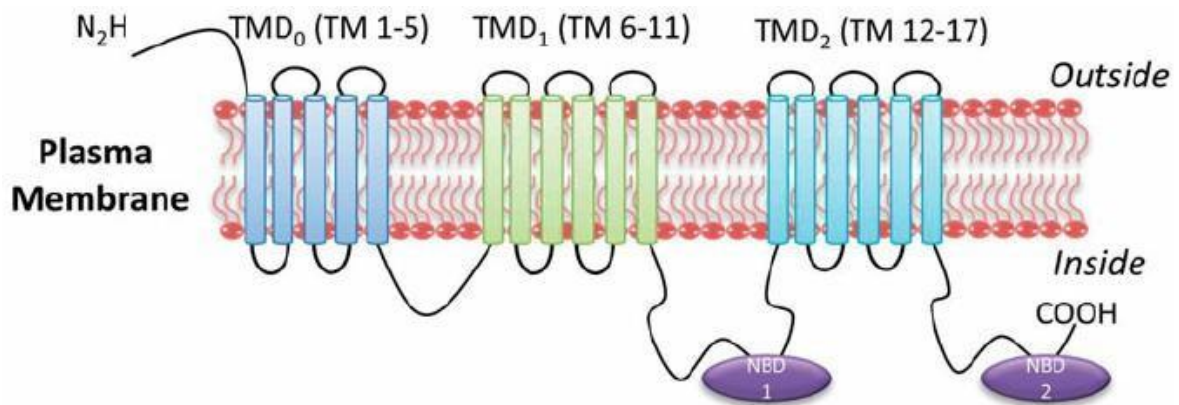


Fig1.1: Structure of Multidrug Resistance-associated Protein 1 (MRP1)

RECEPTOR 1 - Multidrug Resistance-associated Protein 1 (MRP1)

MRP1 is a unidirectional efflux transporter with ubiquitous tissue distribution and wide substrate specificity including important therapeutics. The main roles of this transporter are:

- (i) Efflux of xenobiotics and endogenous metabolites
- (ii) Transport of inflammatory mediators
- (iii) Defense against oxidative stress.

MRP1 has broad substrate specificity, transporting hydrophobic and anionic molecules, glucuronide and glutathione conjugates, as well as endogenous glutathione. Although many MRP1 substrates are conjugated to glutathione, co-transport of free glutathione is often observed. Cellular exposure to Reactive Oxygen Species (ROS) rapidly depletes GSH whilst increasing GSSG. GSSG is transported more efficiently by MRP1 than GSH, thus helps to maintain a healthy GSSG/GSH ratio.

RECEPTOR 2 - Heme-Peroxidase enzyme

Heme-peroxidases are enzymes with a heme prosthetic group in the catalytic site where the iron is bound by four coordination bonds to a protoporphyrin IX derivative. In the presence of H_2O_2 , heme-peroxidases are activated to highly reactive intermediates. H_2O_2 interacts with the Heme-peroxidase from *P. falciparum* (PfHpo) to form a catalytically active intermediate similar to compound II of peroxidases, where iron remains as a higher oxidation ferryl state Fe(IV)=O , and this active intermediate can oxidize aromatic substrate.

The mandatory requirements for the peroxidase activity of a heme-containing protein (i.e., its ability to form reactive intermediates) are:

- (1) The peroxidases operate having iron in ferric form Fe(3+) or Fe(III) .
- (2) The heme iron of a peroxidase has five coordination bonds: four with nitrogens of tetrapyrrole ring, and the fifth heme ligand on the proximal heme side is a highly conserved imidazole ring of histidine residue linking the heme to the protein. On the distal heme side, the iron binds a water molecule. This H_2O molecule is replaced by H_2O_2 upon activation of a peroxidase.
- (3) The peroxidases are characterized by the specific location of amino acids in the active center for effective coordination and use of H_2O_2 . On the distal heme site, a conserved histidine-arginine couple is involved in a hydrogen peroxide network.

If a catalytic center of a hemoprotein meets the requirements listed above, H_2O_2 is specifically in the peroxidase catalytic center and transfers its high oxidation potential to the heme. Two electrons are transferred from the enzyme to H_2O_2 , which is reduced into water, whereby the heme is oxidized to Compound I. Compound I has very high oxidizing ability and oxidizes many the peroxidase substrates through a one-electron mechanism via the intermediate Compound II. At the end of the reaction, the active center returns to the native form, completing the peroxidase cycle.

Mode of Actions and Targets for Current Antimalarial Drugs

The treatment of plasmodial infections is increasingly complicated by the ability of malarial parasite to develop resistance to antimalarial agents. Antimalarial agents used for the treatment of malaria are often categorized according to their principal mechanism of action. There are four major modes of action:

- 1) Generation of toxic metabolites
- 2) Oxygen radicals in Plasmodial mitochondria
- 3) Inhibit heme polymerase
- 4) Inhibit vacuolar degradation.

1.3 DOCKING

Docking is a procedural method to predict the preferred orientation of one molecule to another when bound forming a stable complex. Docking is important in Drug designing which is used for calculating the binding alignment of small molecular drugs or inhibitors to their protein targets and can predict affinity and activity of complexformed.

Molecular docking is an attractive scaffold to understand drug bio molecular interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule (ligand) into the preferred binding site of the target specific region of the DNA/protein (receptor) mainly in a non-covalent

fashion to form a stable complex of potential efficacy and more specificity. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complexes. At present, docking technique is utilized to predict the tentative binding parameters of ligand-receptor complex beforehand.

The main objective of molecular docking is to attain ligand-receptor complex with optimized conformation and with the intention of possessing less binding free energy.

Molecular docking can demonstrate the feasibility of any biochemical reaction as it is carried out before experimental part of any investigation. There are some areas, where molecular docking has revolutionized the findings. In particular, interaction between small molecules (ligand) and protein target (may be an enzyme) may predict the activation or inhibition of enzyme. Such type of information may provide a raw material for the rational drug designing. Some of the major applications of molecular docking are Lead optimization, Hit identifications, Drug- DNA interaction.

1.4 CHEMDRAW

ChemDraw Professional is the complete drawing tool of choice for chemists and biologists to create publication-ready, scientifically intelligent drawings for use in ELNs, databases and publications and for querying chemical databases, now including SciFinder®. It is used to quickly and effectively to draw molecules and reactions for use in documents and electronic lab notebooks, to search databases, and to generate accurate names from structures and to predict properties and spectra. It is a robust, comprehensive suite, purpose-built to simplify, facilitate, and accelerate chemistry communication. ChemDraw Professional adds access to a powerful set of tools to enable scientific research.

1.5 DRUG DISCOVERY FILTERS

1.5.1.Swiss ADME

A free webtool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. It allows to compute physicochemical descriptors as well as to predict ADME parameters, pharmacokinetic properties, druglike nature and medicinal chemistry friendliness of one or multiple small molecules to support drug discovery.

1.5.2. ProTox II

ProTox-II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. ProTox-II incorporates molecular similarity, fragment

propensities, most frequent features and (fragment similarity based CLUSTER cross-validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

1.5.3. PreADMET

PreADMET is a web-based application for predicting ADME data and building drug-like library using in silico method. It predicts permeability for Caco-2 cell, MDCK cell and BBB (blood-brain barrier), HIA (human intestinal absorption), skin permeability and plasma protein binding.

CHAPTER II

2. REVIEW OF LITERATURE

1. Nguyen Tien Huy et al. proposed that CLT offers an antimalarial effect by inhibiting heme catabolism in the malarial parasite and by enhancing heme-induced membrane damage.^[39]

2. Antonios Klokouzas et al. stated that one potential mechanism of the anti-malarial effect of CLT is the inhibition of oxidized glutathione (GSSG) export from the infected patient red cells or from the parasite itself. Multidrug resistance-associated protein (MRP1) and CLT inhibit the MRP1, which is present in human erythrocytes and is responsible for the efflux of GSSH from healthy erythrocytes.^[40]

3. Michael Foley et al. highlighted Free heme, which is produced by the haemoglobin breakdown in the malaria parasite's food vacuole, is toxic and redox-active, and it can produce a significant amount of H_2O_2 .^[41]

4. A. O. Wozencraft et al. discovered H_2O_2 decreases parasite viability and *P. falciparum* is known to be susceptible to oxidant-mediated killing.^[42]

5. Katja Becker et al. concluded A very promising justification for antimalarial chemotherapy is increased oxidative stress on the parasite.^[43]

6. H. B. Dunford et al. stated that Peroxidase, a common and widespread enzyme in both the plant and animal kingdoms, performs a broad range of biological functions and plays a crucial antioxidant role in the defence against oxidative insult.^[44]

7. Recently, a new heme-containing peroxidase (PfHpo) from *Plasmodium falciparum* was characterized by Vishal Trivedi et al.; however, its function in the antioxidant defence of the parasite is still unknown. The initial biochemical analysis demonstrates that H_2O_2 interacts with this hemoperoxidase to form a catalytically active intermediate that is similar to compound II of peroxidases.

Iron is left in a higher oxidation state (Fe(IV)O), and this active intermediate can oxidize aromatic substrate.^[45]

8. Michael A et al. provided concrete proof that CLT analogues successfully bind to and inhibit PfHpo. Also, the polyaromatic pharmacophore structure related to clotrimazole aids in antimalarial activity. SAR studies delineated several structural features suitable to modulate the *in vitro* and *in vivo* antimalarial activity. A named set of antimalarials was further biologically investigated and displayed low *in vitro* toxicity on a panel of human and murine cell lines. *In vitro*, the new composites proved to be selective for free heme. Composites also displayed *in vivo* exertion against *P. chabaudi* after oral administration and therefore represent promising antimalarial agents for further preclinical development.^[46]

CHAPTER III

3. AIM AND OBJECTIVE

Serious infections caused by microorganism's resistant to commonly used antimalarials have become a major healthcare problem worldwide in the 21st century. This is responsible for the significant increase in morbidity and mortality, longer hospitalization and increased health care costs. In recent years, the number of availability of new antimalarials for human use across the globe has been lower than in the recent past. No new potent classes of antimicrobials were developed between the introduction of chloroquine (1934) and artemisinin (1972).

Chloroquine (CQ) was one of the most successful antimicrobial agents in the treatment of malaria, while artemisinins represent the most effective therapeutic option for the treatment of resistant malaria. Although, there is concern of overgrowing evidence that, variants of the *P. falciparum* chloroquine resistance transporter (pfcr) genetic diversity showing resistance to chloroquine are commonly observed in almost all parts of the world where malaria is endemic. Also, evolutionary *in vitro* appearance of artemisinin resistant plasmodia is seen.

We describe herein the design, synthesis, QSAR studies and biological evaluation of an innovative class of antimalarial agents by analyzing the analogues of azole antifungal agents. The articles of azole antifungal agents showing antimalarial activity were studied which led to the overarching purpose of QSAR and Molecular docking studies in delineating the potency of azole anti-fungal agents in malarial treatment approach. The reviewed literature focused on, Identification of the clotrimazole analogues which can be a novel hit endowed with potent antimalarial activity. The synthesis and the biological evaluation of two series of compounds (3a-h and 4a-r) based on the innovative polyaromatic pharmacophore.

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

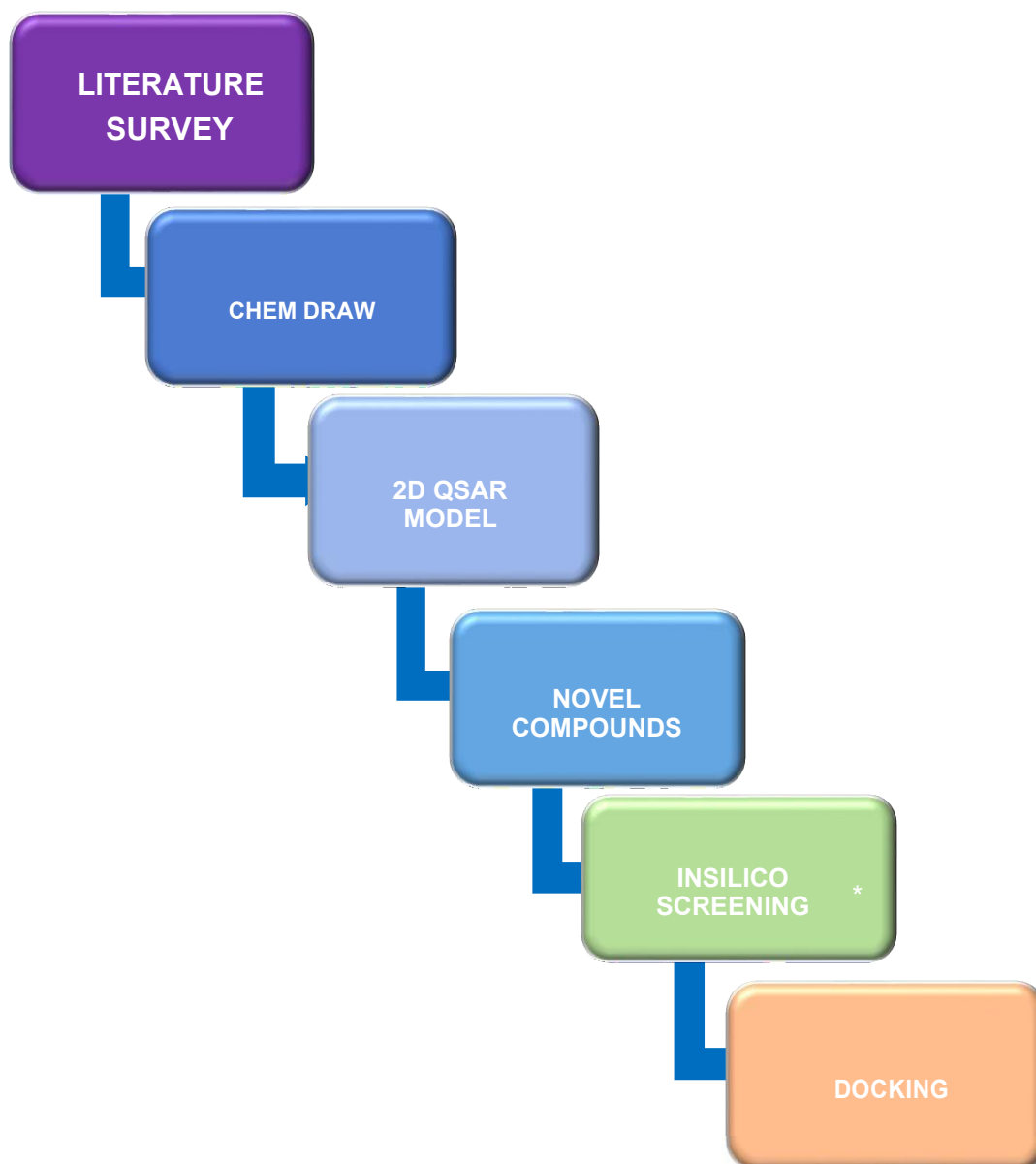
- a. Chemdraw
- b. SwissADME
- c. preADMET
- d. PaDEL Descriptor
- e. BIOVIA Discovery Studio Visualizer etc.
- f. PyRX
- g. Protox

The work is aimed,

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

CHAPTER IV

4. PLAN OF THE STUDY



CHAPTER V

5. MATERIALS AND METHODS

5.1 2D-QSAR MODEL

5.1.1 Dataset Selection and Division

A dataset of reported 26 molecules of imidazole derivatives as potent antimalarial 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 concentrations (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.

5.1.2 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 2.2 software. 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 logP, extended topological atom descriptors, McGowan volume, molecular 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 Genetic 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.

5.1.3 Model Validation

Validation is the important criteria for the evaluation of QSAR predictive models. To demonstrate this 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 1 ($R^2 > 0.6$) which may be a 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 was performed to check the descriptors used in the model. It is performed 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 that 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 residual versus the leverage values (h). A compound with $h_i > h^*$ seriously influences the regression performance and may be excluded from the applicability domain, but it doesn't appear to be an outlier because its standardized residual may be small. Moreover, a value of 3 for standardized residuals is commonly used as a cut-off value for accepting predictions, because points that lie within ± 3 standardized residuals from the mean cover 99% of the normally distributed data. Leverage of a given dataset h_i is defined by this mathematical expression

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

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.

5.2. DESIGN OF COMPOUNDS

13 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 $N_A \approx 6.0221023$ is the Avogadro constant and α is the mean polarizability of the molecule.

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 (ρ). 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 gases 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 relates 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 by adding values obtained for constituent atoms and structural features called also molar parachor, molecular parachor.

Parachor is a quantity defined according to the

formula $P = Y^{1/4} M/d$

Where,

$Y^{1/4}$ 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. Dielectric 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 E.

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 molecule have been identified, it 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 satisfy 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 ProTox II 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. 140 Å.

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 $A = \frac{4}{3}\pi N_A \alpha$

Where $N_A = 6.022 \times 10^{23}$ is the Avogadro constant and α 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 a 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 Å² good intestinal absorption TPSA < 70 Å² 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 (logP>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.

$\log P_{oct/wat} = \log \frac{[\text{solute}]_{\text{unionized octanol}}}{[\text{solute}]_{\text{unionized water}}}$
 $\log P_{oct/wat} = \log CO/CW$

Lipophilicity not only impacts solubility but also influences permeability, potency, selectivity, absorption, distribution, metabolism, and excretion (ADME) properties and toxicity. A desired logP 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.

ProTox II

ProTox II, a virtual lab for the prediction of toxicities of small molecules. The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals, but can also help to reduce the amount of animal experiments.

ProTox II incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity based CLUSTER cross- validation) machine- learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets.

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 ($LD50 > 5000$).

5.4 MOLECULAR DOCKING STUDIES

5.4.1. Retrieval of the target protein

The crystal structure of protein Heme-Peroxidase (PDB ID: 1CKK) and MRP1 (PDB ID: 2CBZ) with the resolution 1.9 Å⁰ was downloaded as pdb.format from the Research Collaboratory for Structural Bioinformatics- Protein Data Bank (RCSB-PDB) database.

5.4.2. 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** .

5.4.3. 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.

5.4.4. 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 Heme-Peroxidase and MRP1 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).

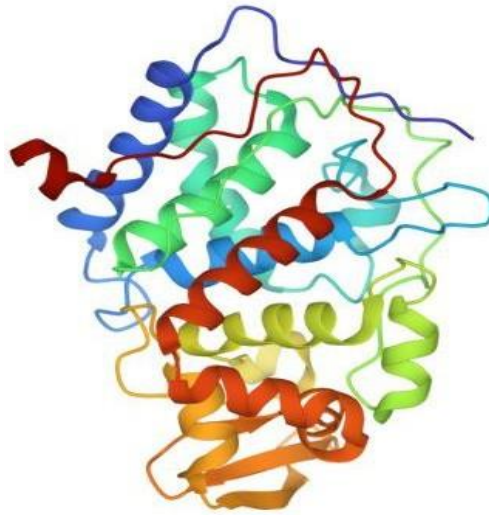


Fig 5.1: Structure of pre-processed protein Heme-peroxidase

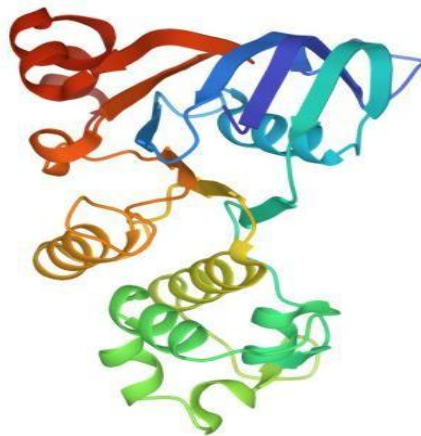


Fig 5.2: Structure of pre-processed protein MRP1

CHAPTER VI

6. RESULTS AND DISCUSSION

6.1 2D-QSAR Model Information

In an attempt to determine the role of structural features of compounds which appear to possess an effect of antimalarial 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.

Model 1 equation (Best model)

pIC_{50}

$$=18.0417-1.6687(nN)-0.0338(AATS8v)-9.3307(GATS4e)+2.4301 (SpMax6_Bhs)$$

The model 1 developed with aforementioned options gave statistical parameters as

(Fitting criteria)

R^2 : 0.8457 R^2_{adj} : 0.8071 $R^2-R^2_{adj}$: 0.0386 F: 21.9216

(Internal validation criteria)

Q^2_{LOO} : 0.7461 $R^2-Q^2_{LOO}$: 0.0995 Q^2_{LMO} : 0.6811

(External validation criteria)

R^2_{ext} : 0.6839

Predictions by LOO:

Exp(x) vs. Pred(y): R^2 : 0.7491

External predictions by model equation:

Exp(x) vs. Pred(y): R^2 : 0.6839

Many equations were predicted out of that 3 equations showed better predicted activity and the model 1 is considered as the best model for which the statistical parameters are mentioned above.

The descriptors nN, AATS8v, GATS4e contributes negatively to the antimalarial activity and SpMax6_Bhs contributes positively towards the antimalarial activity. In designing novel compounds nN, AATS8v and GATS4e 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 21.9216.
- c. The difference between R^2 and R^2_{adj} should be less than 0.1. We have the value of 0.0386.

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 0.7461 and 0.6811 respectively. So the equation is having better predictability. The difference between R^2 and Q^2_{LOO} is found to be 0.09 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 0.6836 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 imidazole derivatives with heme-peroxidase enzyme and MRP1 protein inhibitory activity.

Table 6.1: Description of molecular descriptors used in the study

Symbol	Description	class
nN	Number of nitrogen atoms	2D
AATS8v	Average Broto-Moreau autocorrelation - lag 8/ weighted by van der Waals volumes	2D
GATS4e	Geary autocorrelation - lag 4/ weighted by Sanderson electronegativities	2D
SpMax6_Bhs	Largest absolute eigenvalue of Burden modified matrix - n 6/ weighted by relative I-state	2D

COMPOUND ID	EXP. pIC ₅₀	PRED. pIC ₅₀	RESIDUALS	STATUS
1111111a	8.3010	7.6839	-0.6172	Training
1111111b	6.5498	6.0965	-0.4533	Training
1111111c	5.4317	5.6331	0.2014	Training
1111111d	5.9072	6.2074	0.2371	Training
1111111e	6.4510	6.3262	-0.1248	Training
1111111f	5.3678	5.4269	0.0591	Training
1111111g	6.4214	5.4269	0.0591	Training
1111111h	6.1278	7.0765	0.9486	Training
1111111i	6.7011	6.6575	-0.0436	Training
1111111j	9.3188	9.0619	-0.2568	Training
1111111k	5.3717	6.0822	0.7105	Training
1111111l	5.3104	9.2810	3.9706	Prediction
1111111m	6.4202	6.5398	0.1196	Training

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

COMPOUND ID	EXP. pIC ₅₀	PRED. pIC ₅₀	RESIDUALS	STATUS
1111111n	7.9318	7.5840	-0.3479	Training
1111111o	5.8969	5.8998	0.0029	Training
1111111p	7.1759	6.8467	-0.3292	Training
1111111q	5.3077	5.5246	0.2169	Training
1111111r	6.8097	6.3336	-0.4761	Training
1111111s	6.2924	6.5447	0.2523	Training
1111111t	5.7501	5.0399	-0.7102	Training
1111111u	6.7447	5.8515	-0.8933	Training
1111111v	7.2218	6.7539	-0.4679	Training
1111111w	7.1739	5.7480	-1.4259	Training
1111111x	7.6383	8.1153	0.4770	Training
1111111y	5.4547	7.4172	1.9625	Prediction
1111111z	7.6326	7.6742	0.0416	Training

COMPOUND ID	nN	AATS8v	GATS4e	SpMax6-Bhs	PREDICTED ACTIVITY
2aa	6	184.7493988	0.975686497	3.410170354	0.968187303
2bb	6	185.3298744	0.927903113	3.531940063	1.690332218
2cc	6	185.656553	0.595527286	3.856572358	5.569478545
2dd	7	187.7539444	0.923855347	3.805825243	0.643035515
2ee	7	183.6345863	0.919452532	3.416889102	-0.121802554
2ff	6	190.6836526	0.9477284	3.416848884	1.044707629
2gg	6	183.0972862	0.925852222	3.413735642	1.49768138
2hh	6	187.0994871	0.954058598	3.416833681	1.106750303
2ii	6	187.6666819	0.982146723	3.416828386	0.825484386
2jj	7	185.3460445	0.922379567	3.413281385	-0.215728237
2kk	6	194.3103636	0.832104645	3.423617718	2.017424316
2ll	6	189.6409474	0.966557247	3.416836279	0.904234111
2mm	7	185.3335194	0.935572457	3.41708026	-0.32917214

Table 6.3: pIC₅₀ values of designed compounds predicted by the equation

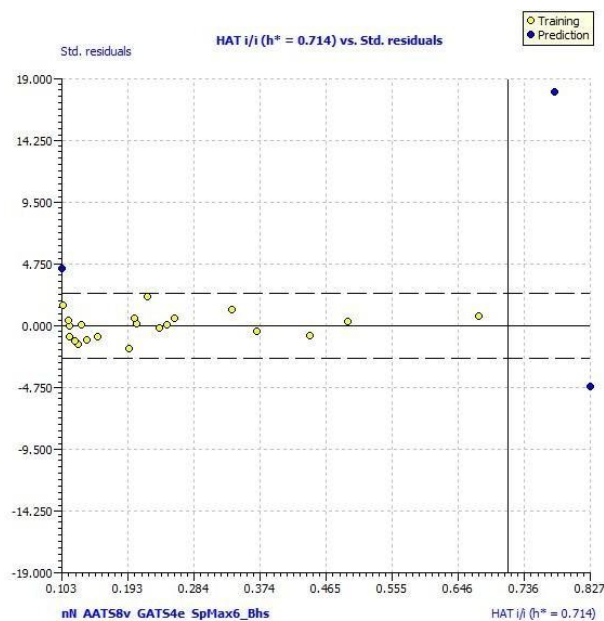


Fig 6.1: William's plot of the best model with warning values of HAT ($h^* = 0.714$)

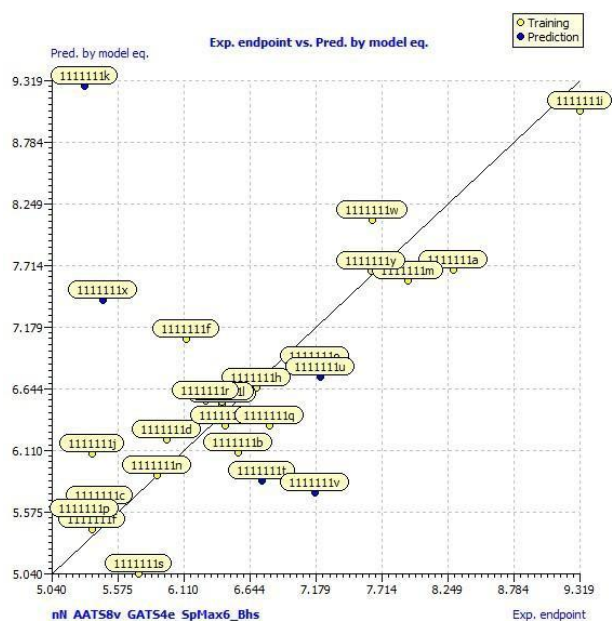


Fig 6.2: Scatter plot of dataset compounds obtained from experimental values and model

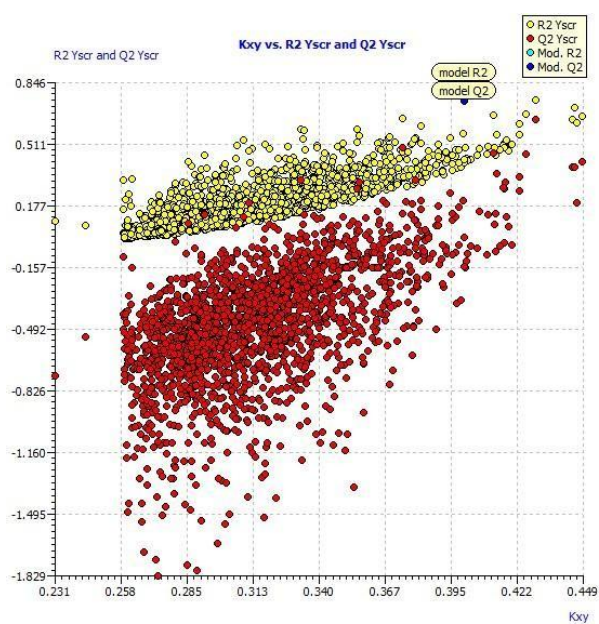


Fig 6.3: Y-scramble plot for best model (plot of K_{xy} vs R^2 and Q^2 Loo from Y-scrambling procedure).

6.2 DESIGN OF NEW COMPOUNDS

13 novel imidazole derivatives were designed based on predicted QSAR Model. Their activity were evaluated using same model and their activity are mentioned in Table 6.4. Among these derivatives, **2cc** and **2kk** compounds showed better predicted activity than the most active compounds in the data set. The descriptors nN, AATS8v, GATS4e and SpMax6_Bhs contributes positively towards the biological activity. The predicted pIC₅₀ derivatives were summarized in Table 6.3. The structure of 13 newly designed compounds are given in table 6.8.

6.3 *INSILICO* ADMET PREDICTION STUDIES

In silico ADMET prediction is required to qualify the compounds as lead candidates against activity. Imidazole derivatives were designed as antimalarial agents using computational methods. Physicochemical properties, drug-likeness properties, pharmacokinetics and toxicity properties were calculated for all the designed compounds and compared with the standard drug Artemisinin. Physicochemical properties were shown in **Table 6.4**. All the designed compounds have less than 5 hydrogen bond donors and equal to 5 hydrogen bond acceptors. Molecular weight of all the designed compounds were around 550-590 Dalton's. Maximum of the designed compounds obey Lipinski rule of five 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. Compounds with TPSA values in the range 150 Å² have good intestinal absorption. TPSA (Total Polar Surface Area) of our designed compounds were found to be in the range of 90–120 Å². Therefore, it is expected that our compounds might possess little

intestinal absorption.

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

Pharmacokinetics values of designed compounds were shown in **Table 6.6**. CaCo2 cell permeability of all compounds lies in the range of 1-60. This range indicates all the designed compounds have moderate permeability. Plasma protein binding of all the compounds are in the range of 90-100, which indicates that the chemicals are strongly bound. Human intestinal absorption (HIA) values of all designed compounds were found 80-100. This indicates that all the designed compounds are well absorbed in intestine.

Toxicity studies of the designed compounds were shown in **Table 6.7**, Most of the designed compounds were inactive for hepatotoxicity and carcinogenicity.

Compounds	Molecular formula	Molecular weight (g/mol)	Molar refractivity	Number of rotatable bonds	Number of H bond acceptor	Number of H bond donors	TPSA A ²	Number of heavy atoms	Number of aromatic heavy atoms
2aa	C ₂₈ H ₂₁ ClN ₆ OS	525.02	147.49	7	5	2	116.99	37	32
2bb	C ₂₈ H ₂₁ ClN ₆ S	509.02	145.47	7	4	1	96.76	36	32
2cc	C ₂₈ H ₂₁ ClN ₆ O ₃ S	557.02	151.54	7	7	4	157.45	39	32
2dd	C ₂₈ H ₂₀ ClN ₇ O ₂ S	554.02	154.29	8	6	1	142.58	39	32
2ee	C ₂₈ H ₂₂ ClN ₇ S	524.014	149.87	7	4	2	122.78	37	32
2ff	C ₂₈ H ₂₀ BrClN ₆ S	587.92	153.17	7	4	1	96.736	37	32
2gg	C ₂₉ H ₂₃ ClN ₆ S	523.05	150.44	7	4	1	96.76	37	32
2hh	C ₂₈ H ₂₀ Cl ₂ N ₆ S	543.47	150.48	7	4	1	96.76	37	32
2ii	C ₂₈ H ₂₀ BrClN ₆ S	587.92	153.17	7	4	1	96.76	37	32
2jj	C ₂₇ H ₂₀ ClN ₇ S	510.01	143.26	7	5	1	109.65	36	32
2kk	C ₂₈ H ₁₉ Cl ₃ N ₆ S	577.91	155.49	7	4	1	96.76	38	32
2ll	C ₂₈ H ₂₀ BrClN ₆ S	587.92	153.17	7	4	1	96.76	37	32
2mm	C ₂₈ H ₂₂ ClN ₇ S	524.04	149.87	7	4	2	122.78	37	32
Artemisinin	C ₁₅ H ₂₂ O ₅	282.33	70.38	0	5	0	53.99	20	0

Table 6.4: Physicochemical Properties of designed compounds

Compound	Lipinski	Ghose	Veber	Egan	Muegge	Bioavailabilty score	Synthetic accessibility
2aa	yes	no	yes	no	no	0.55	4.09
2bb	yes	no	yes	no	no	0.55	4.04
2cc	yes	no	no	no	no	0.55	4.20
2dd	yes	no	no	no	no	0.55	4.35
2ee	yes	no	yes	no	no	0.55	4.22
2ff	no	no	yes	no	no	0.17	4.13
2gg	yes	no	yes	no	no	0.55	4.24
2hh	no	no	yes	no	no	0.17	4.07
2ii	no	no	yes	no	no	0.17	4.07
2jj	yes	no	yes	no	no	0.55	4.04
2kk	no	no	yes	no	no	0.17	4.18
2ll	no	no	yes	no	no	0.17	4.14
2mm	yes	no	yes	no	no	0.55	4.16

Table 6.5: Drug likeness properties of designed compounds.

Compound	CaCo ₂	Plasma protein binding	Log P	HIA
2aa	51.3517	96.951043	6.315860	97.734711
2bb	35.5201	99.902643	6.122670	96.971963
2cc	14.3375	100.000000	5.462760	93.935255
2dd	7.26372	95.399436	6.312260	97.711284
2ee	29	98.248839	5.603340	96.937102
2ff	46.4078	100.000000	7.093940	98.163320
2gg	52.0875	94.379590	6.780670	97.798512
2hh	52.2412	100.000000	6.987320	98.011789
2ii	46.4953	100.000000	7.104720	98.163320
2jj	47.7024	95.420903	5.222700	97.369012
2kk	52.1161	100.000000	7.516650	98.220170
2ll	46.4078	100.000000	7.143560	98.163320
2mm	29	92.721302	5.652960	96.937268
Artemisinin	30.3276	93.368123	1.686120	96.314307

Table 6.6: Pharmacokinetic properties of the designed compounds

Compound	Predicted LD50 (mg/kg)	Predicted toxicity class	Hepatotoxicity	Carcinogenicity
2aa	1100	4	Inactive	Inactive
2bb	1100	4	Inactive	Inactive
2cc	1100	4	Active	Inactive
2dd	1100	4	Inactive	Active
2ee	1100	4	Inactive	active
2ff	1100	4	Inactive	Inactive
2gg	1100	4	Inactive	Inactive

Table 6.7: Toxicity studies of the designed compounds

Compound	Predicted LD50 (mg/kg)	Predicted toxicity class	Hepatotoxicity	Carcinogenicity
2hh	1100	4	Inactive	Inactive
2ii	1100	4	Inactive	Inactive
2jj	1100	4	Inactive	Inactive
2kk	1100	4	Inactive	Inactive
2ll	1100	4	Inactive	Inactive
2mm	1100	4	Inactive	Inactive

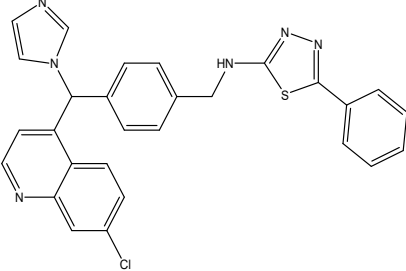
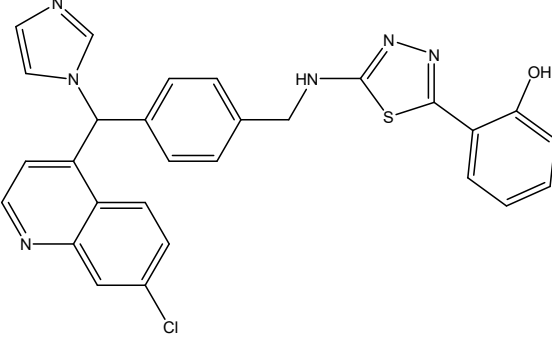
6.4 Molecular Docking

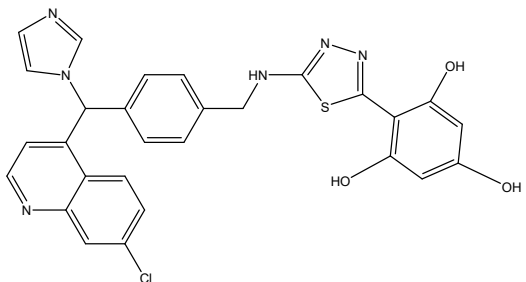
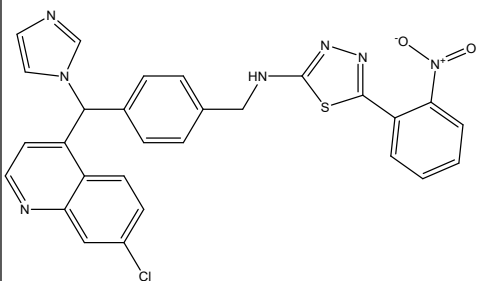
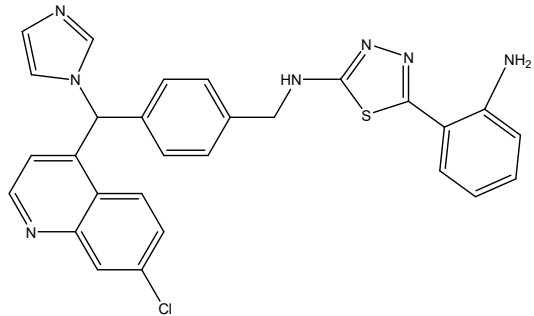
Molecular study was performed to further investigate binding modes of the synthesized compounds at the active sites of the target enzymes and correlate with experimental data. The compounds showed good docking scores and were found to be stabilized through hydrogen bonding and hydrophobic interactions at the active sites. In this study, Pyrx tool is used to perform docking studies. Docking studies were performed with the crystal structure of heme-peroxidase enzyme and MRP1 protein.

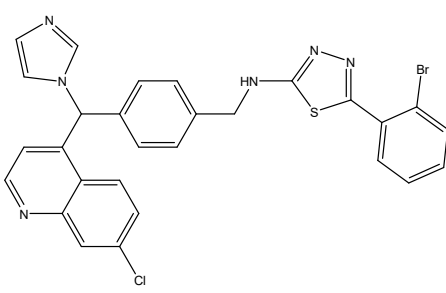
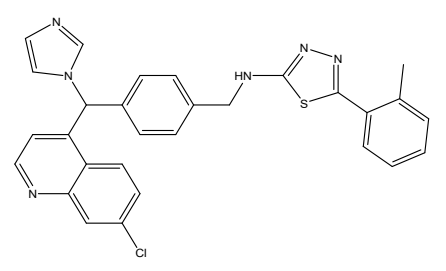
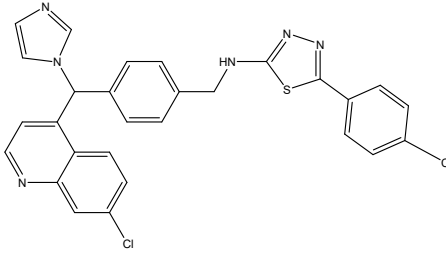
The docking studies for 13 designed compounds having good predicted activities were done along with 2cc and 2kk, which has high experimental biological activity. From the two best compounds (2cc and 2kk), 2kk showed better interaction with heme-peroxidase enzyme and MRP1 protein. The compound 2kk showed good binding affinity (-12.3kcal/mol) with heme-peroxidase enzyme due to hydrophobic interactions with HIS 175, PRO 145 in the 2,6 dichloro phenyl ring and with ALA 147, TYR 187 in the substituted phenanthrene ring. Also with MRP1 protein, 2kk showed good binding affinity (-8.8 kcal/mol) due to two hydrogen bond interactions with GLU 694 in 1,3,5 thiadiazole ring and three hydrophobic interactions with PRO 712, TYR 710, TRP 716 in the 2,6 dichloro phenyl ring and four hydrophobic interactions with TRP 653 in substituted phenanthrene ring. Standard drug Artemisinin showed binding affinity of -9.7 kcal/mol with MRP1 protein and -13.1 with heme-peroxidase enzyme. Compound 2ff with the high docking score of -8.6 kcal/mol has diazo group of 1,3,5 thiadiazole nucleus and imino group which form 2 hydrogen bond interactions with LEU 692 and VAL 708 respectively. Most of the compounds were found to be involved in both hydrogen bond and hydrophobic interactions with the receptor. The formation of hydrogen bond with target molecules results in inhibition, whereas binding affinity is increased by Van der Waals, pi-pi and hydrophobic interactions. The results indicate that these compounds can act as potent Heme-peroxidase and MRP1 protein inhibitors.

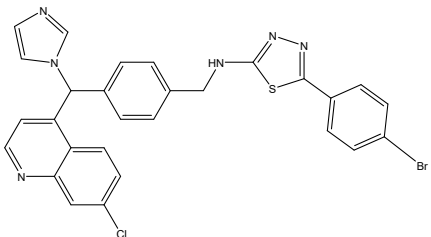
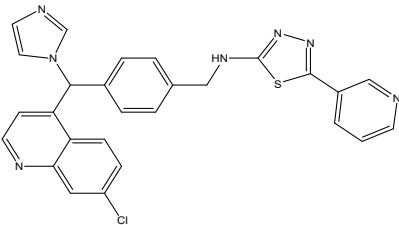
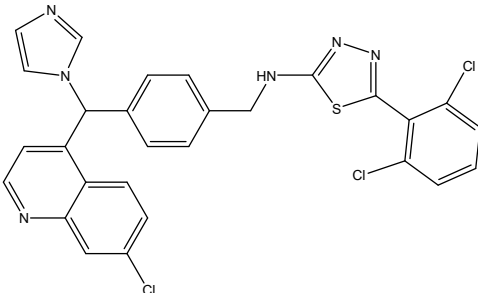
The 2D and 3D interaction of compounds 2aa, 2bb, 2dd, 2ff, 2gg, 2hh, 2jj, 2kk, 2ll, 2mm and standard drug artemisinin with protein 1ckk and 2cbz are shown in Figure 6.4 to 6.25 .

Table 6.8: Structure of designed compounds

COMPOUND CODE	STRUCTURE AND IUPAC NAME
2aa	N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-5-phenyl-1,3,4-thiadiazol-amine 
2bb	2-(5-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methylamino)-1,3,4-thiadiazol-2-yl)phenol 

2cc	2-(5-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methylamino)-1,3,4-thiadiazol-2-yl)benzene-1,3,5-triol 
2dd	N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-5-(2-nitrophenyl)-1,3,4-thiadiazol-2-amine 
2ee	5-(2-aminophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 

2ff	5-(2-bromophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 
2gg	N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-5-o-tolyl-1,3,4-thiadiazol-2-amine 
2hh	5-(4-chlorophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 

2ii	-((4-bromophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 
2jj	N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-5-(pyridin-3-yl)-1,3,4-thiadiazol-2-amine 
2kk	N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-5-(2,6-dichlorophenyl)-1,3,4-thiadiazol-2-amine 

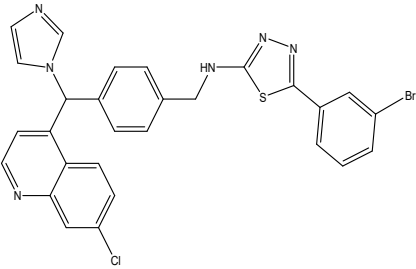
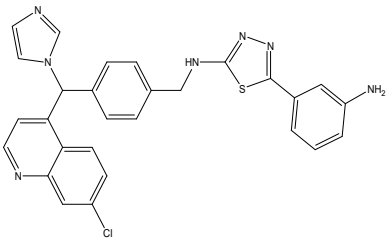
2II	5-(3-bromophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 
2mm	5-(3-aminophenyl)-N-((4-((7-chloroquinolin-4-yl)(1H-imidazol-1-yl)methyl)phenyl)methyl)-1,3,4-thiadiazol-2-amine 

Table 6.9: Binding affinity of designed compounds

COMPOUND CODE	BINDING AFFINITY	
	Heme-peroxidase	MRP1
2aa	-12.1	-9.1
2bb	-11.9	-8.9
2dd	-10.9	-9.5
2ff	-12.3	-8.6
2gg	-12.2	-9.1
2hh	-12.3	-9.4
2jj	-13	-9
2kk	-12.3	-8.8
2ll	-12.2	-9.3
2mm	-11.9	-9.1
Artemisinin (Standard)	-13.1	-9.7



Figure 6.4: 2D and 3D interaction of Standard Drug Artemisinin with heme-peroxidase

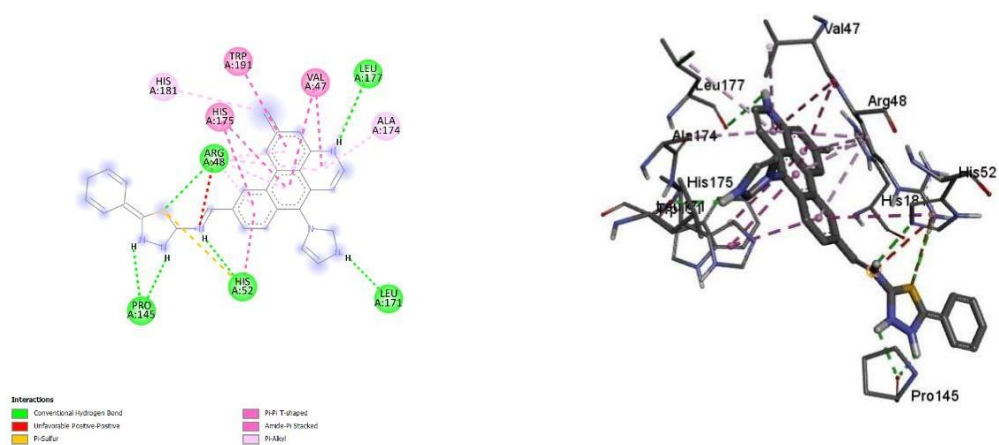


Figure 6.5: 2D and 3D interaction of compound 2aa with heme-peroxidase

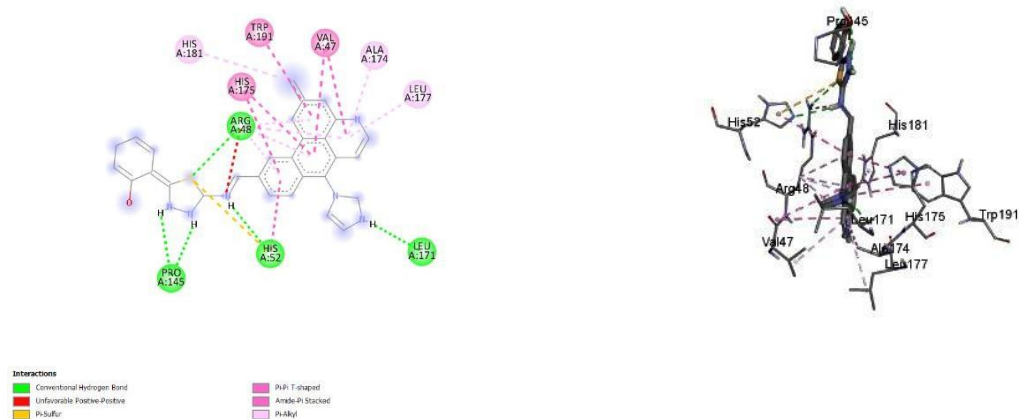


Figure 6.6: 2D and 3D interaction of compound 2bb with heme-peroxidase

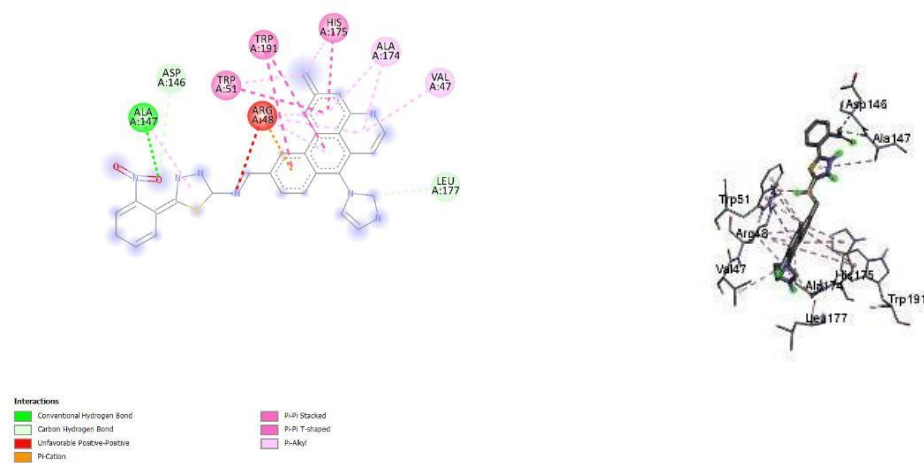


Figure 6.7: 2D and 3D interaction of compound 2dd with heme-peroxidase

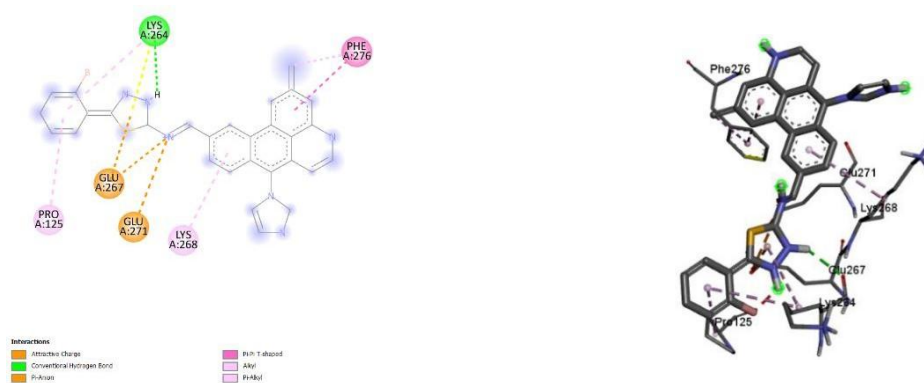


Figure 6.8: 2D and 3D interaction of compound 2ff with heme-peroxidase

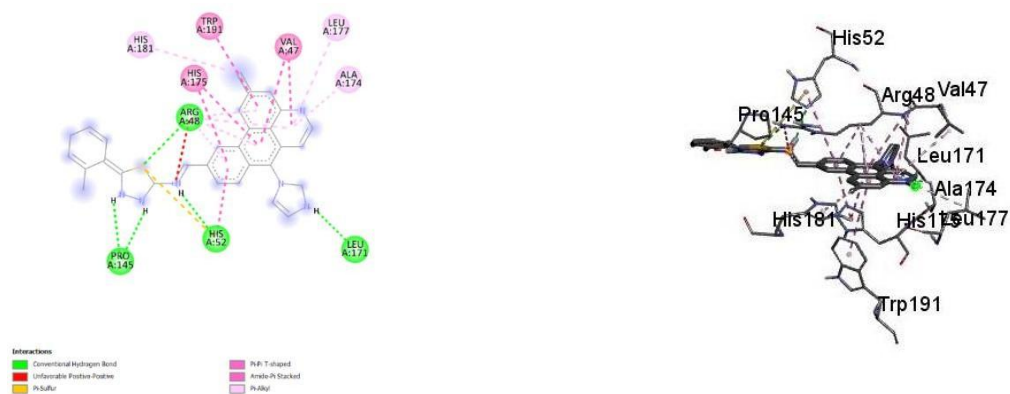


Figure 6.9: 2D and 3D interaction of compound 2gg with heme-peroxidase

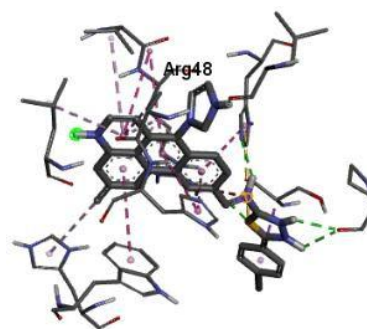
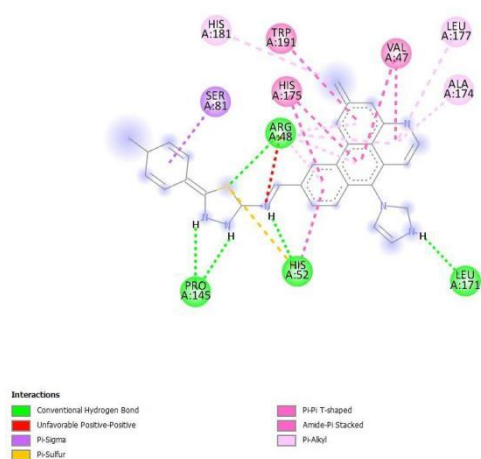


Figure 6.10: 2D and 3D interaction of compound 2hh with heme-peroxidase

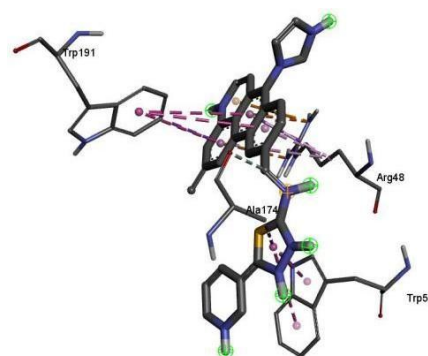
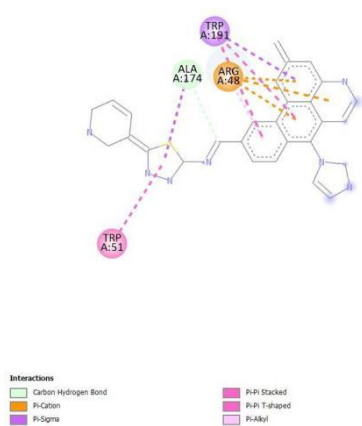


Figure 6.11: 2D and 3D interaction of compound 2jj with heme-peroxidase

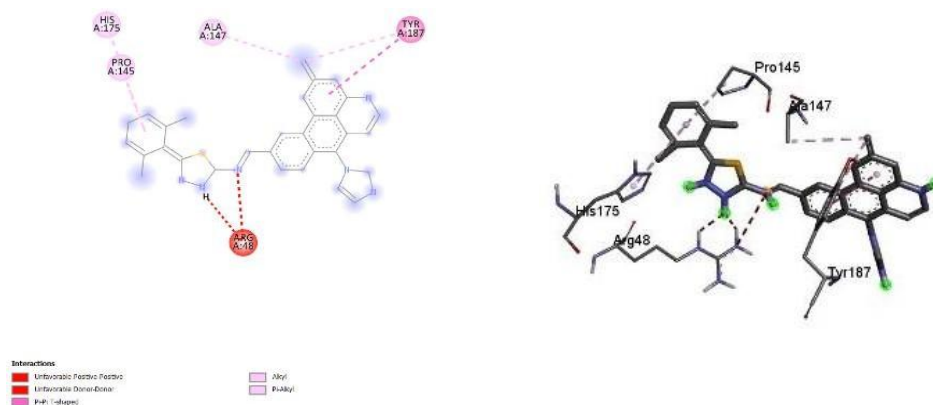


Figure 6.12: 2D and 3D interaction of compound 2kk with heme-peroxidase

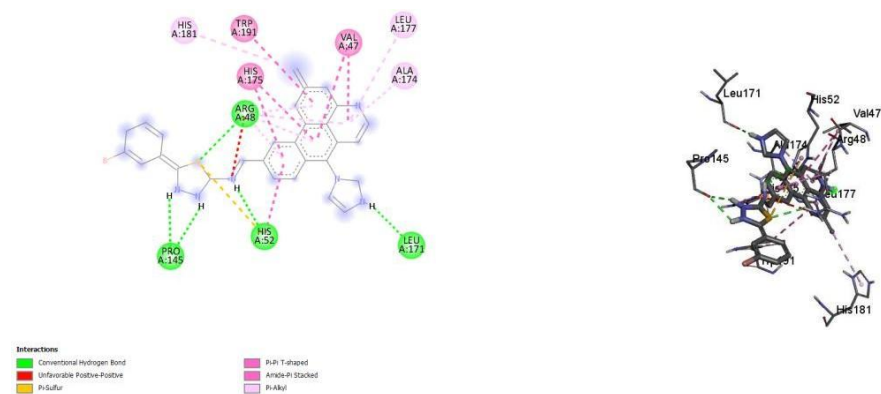


Figure 6.13: 2D and 3D interaction of compound 2ll with heme-peroxidase

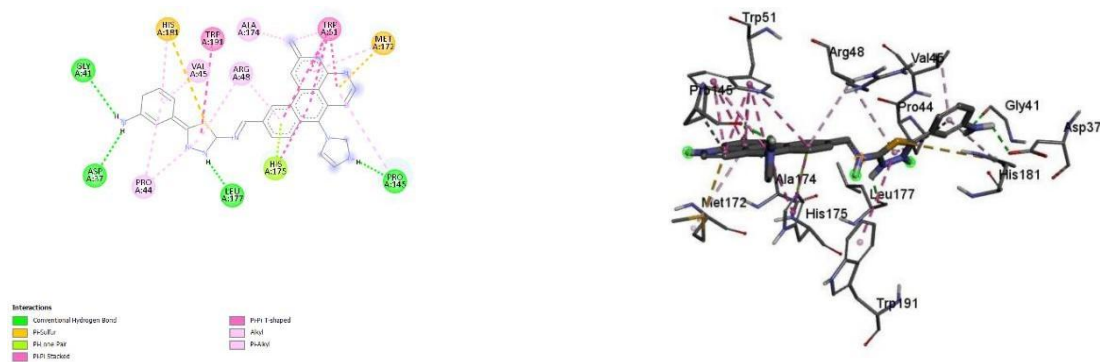


Figure 6.14: 2D and 3D interaction of compound 2mm with heme-peroxidase

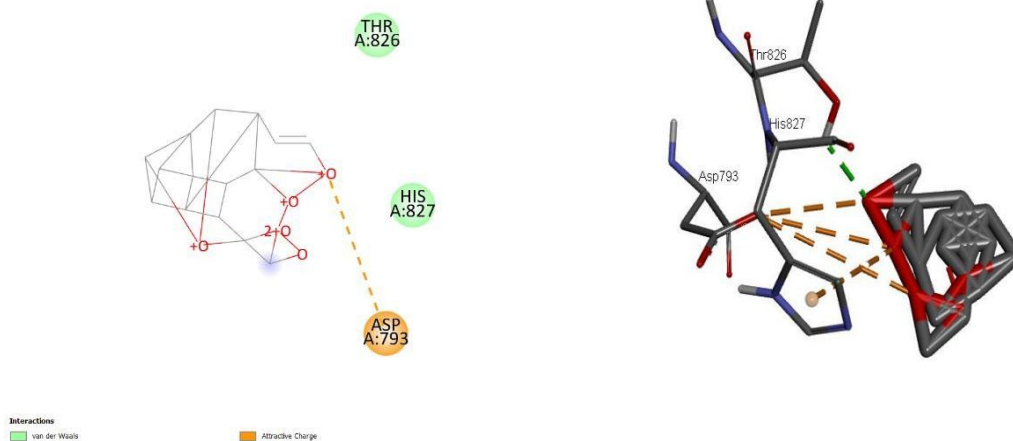


Figure 6.15: 2D and 3D interaction of standard drug artemisinin with MRP1

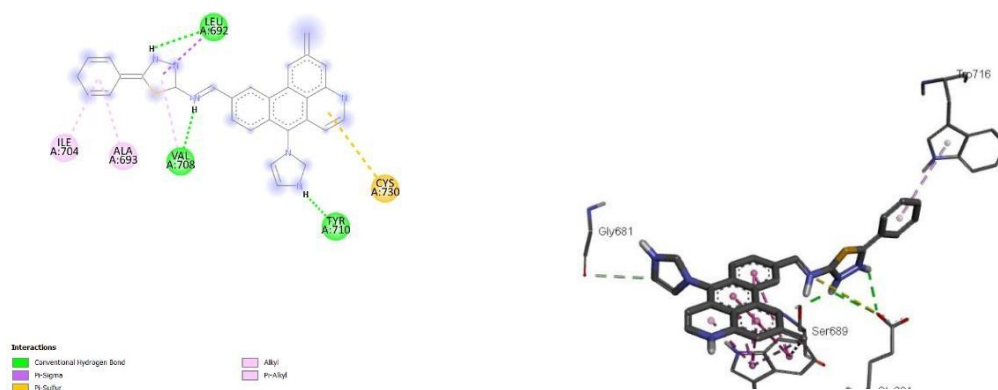


Figure 6.16: 2D and 3D interaction of compound 2aa with MRP1

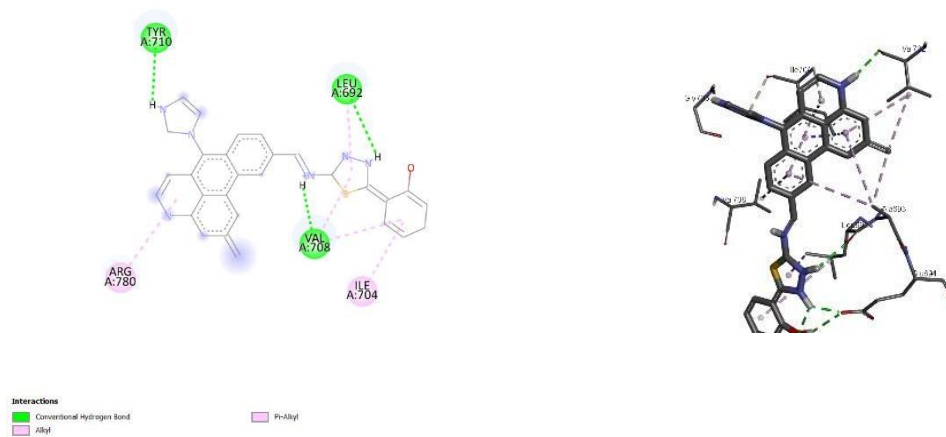


Figure 6.17: 2D and 3D interaction of compound 2bb with MRP1

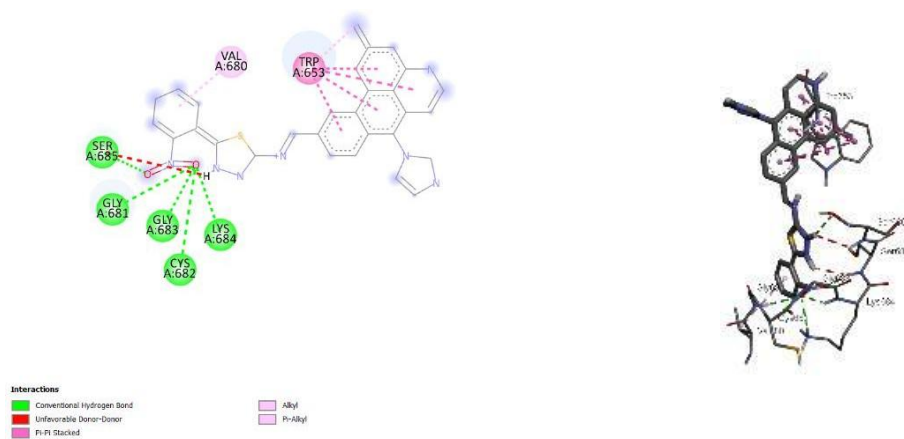


Figure 6.18: 2D and 3D interaction of compound 2dd with MRP1

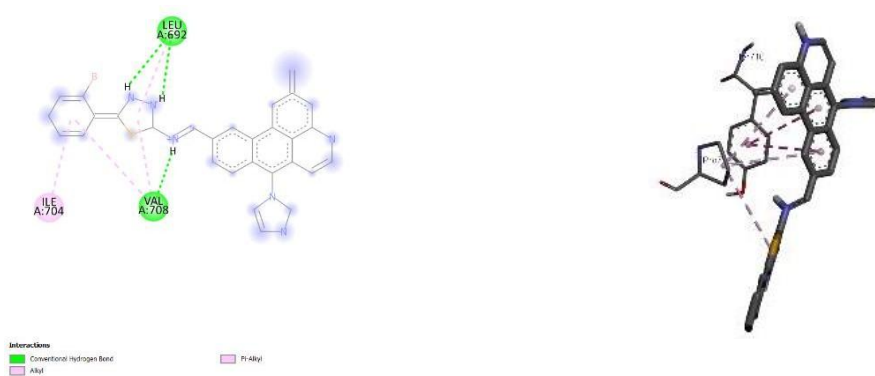


Figure 6.19: 2D and 3D interaction of compound 2ff with MRP1

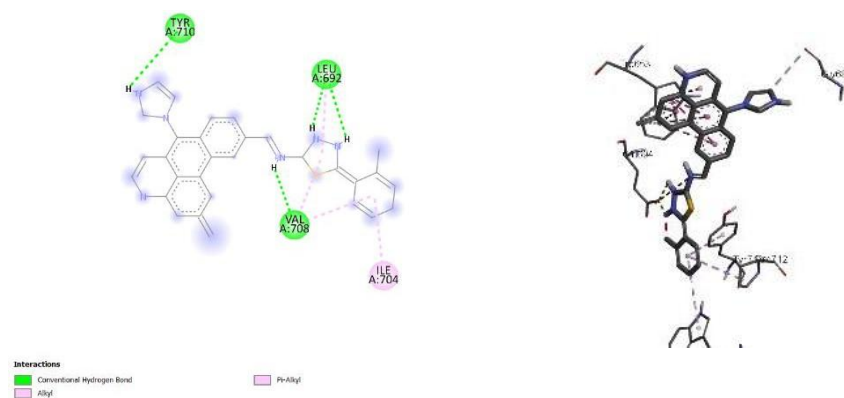


Figure 6.20: 2D and 3D interaction of compound 2gg with MRP1

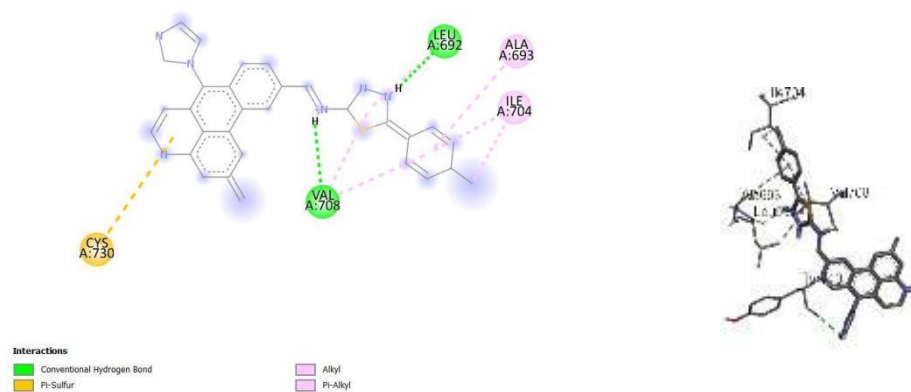


Figure 6.21: 2D and 3D interaction of compound 2hh with MRP1

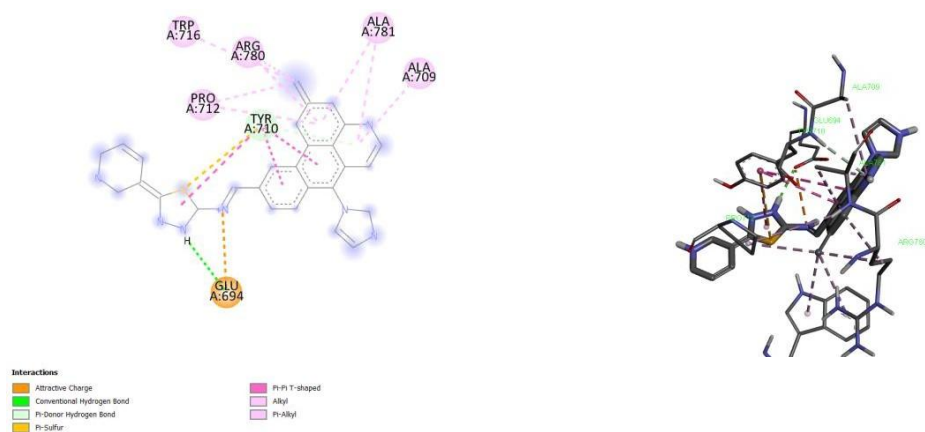


Figure 6.22: 2D and 3D interaction of compound 2jj with MRP1

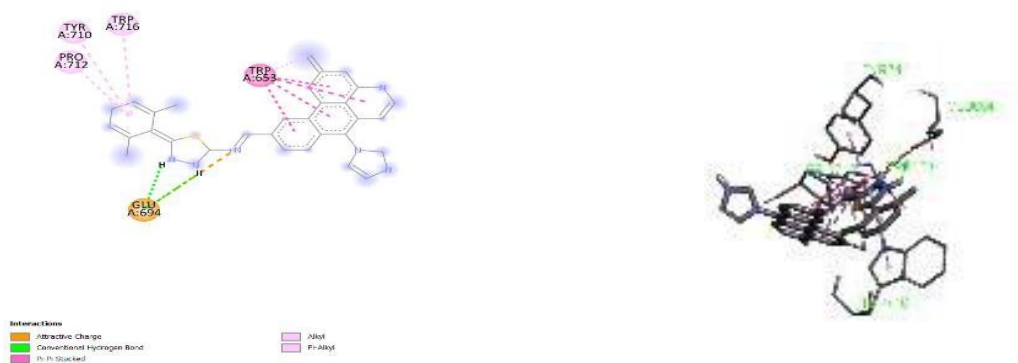


Figure 6.23: 2D and 3D interaction of compound 2kk with MRP1

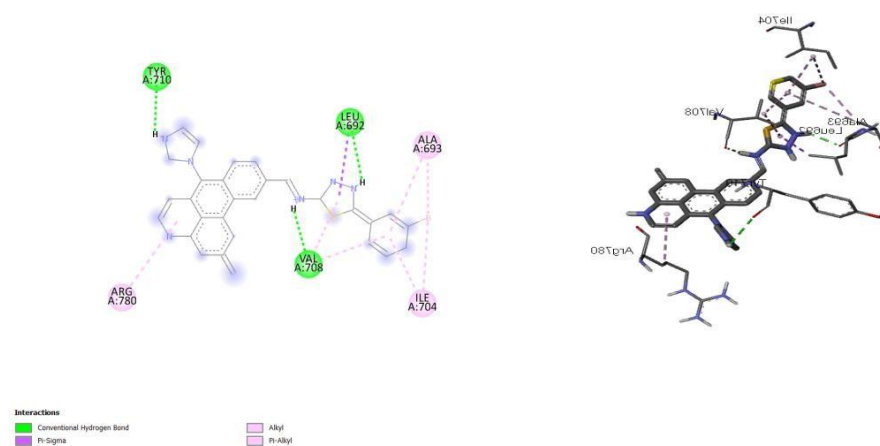


Figure 6.24: 2D and 3D interaction of compound 2II with MRP1

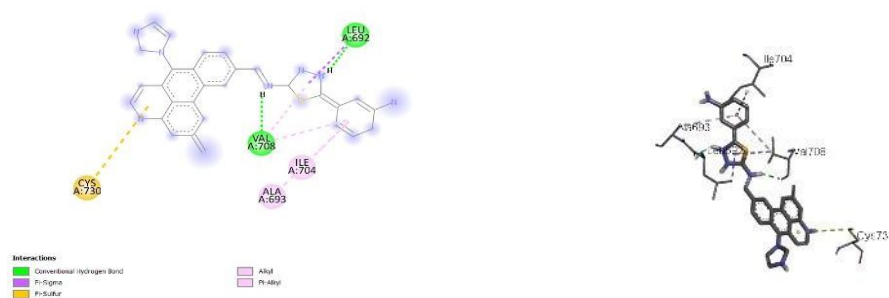


Figure 6.25: 2D and 3D interaction of compound 2mm with MRP1

CHAPTER VII

7. CONCLUSION

A Quantitative analysis of the structure activity relationship (QSAR) was performed on a data set of 26 compounds of imidazole derivatives as antimalarial agents. The 2D-QSAR models for a series was established using QSARINS software that yielded a regression model with good predictive power. The predictability of the proposed models is validated both internally and externally using software statistical parameters. By the use of auto correlated descriptors and Lipinski parameters, structural features were used to relate with biological activity. The models were used for the design of 13 new imidazole derivatives.

The best predicted active compounds were subjected to molecular docking studies, *in silico* ADMET prediction for structural interaction, *in silico* swissADME prediction for drug like properties information supporting our QSAR model and protox studies for predicting toxicity. Based on the binding affinity and ADMET prediction results, Ten designed compounds 2aa, 2bb, 2dd, 2ff, 2gg, 2hh, 2jj, 2kk, 2ll and 2mm can potentially act as an inhibitor of the heme-peroxidase enzyme in plasmodial species and also an inhibitor of the Multi resistance-associated Protein (MRP1). From the obtained results, the designed compounds **2cc** and **2kk** showed better activity when predicted using generated QSAR equation.

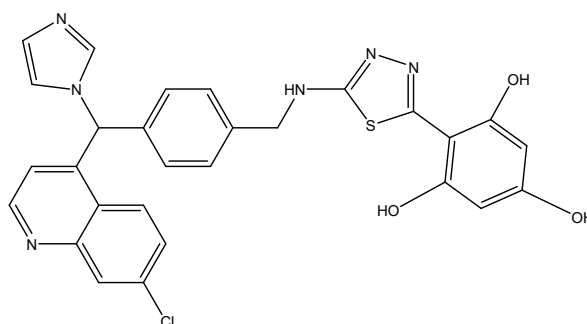


Figure 7.1: Structure of 2cc

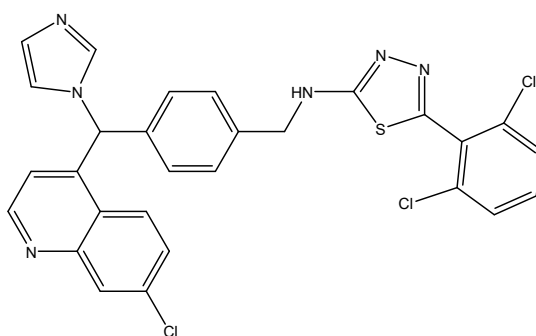


Figure 7.2: Structure of 2kk

The compound 2kk showed comparable binding energy of -12.3 and -8.8 with standard drug artemisinin which has -13.1 and -9.7 with heme-peroxidase enzyme and MRP1 protein respectively. The properties of 2kk are highlighted in the table 7.1.

Table 7.1: Properties of designed compound

COMPOUND	2kk
BBB	0.36615
TPSA (A²)	96.76
TOXICITY CLASS	4
BINDING ENERGY	Heme-peroxidase : -12.3 kcal/mol
	MRP1 : -8.8 kcal/mol
PREDICTED ACTIVITY	2.017424316

The results of binding affinity studies explain that the newly designed active compounds can serve as effective antimalarial agents and classified under class 4 toxicity which indicates a minimized toxic effects and is inactive against hepatotoxicity and carcinogenicity.

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