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IN SILICO MOLECULAR DOCKING, SYNTHESIS AND EVALUATION OF IMIDAZOLE DERIVATIVES AS ANTIMALARIAL AGENTS

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ABSTRACT

This work aims to identify and optimize leads for Imidazole derivatives as *Pf* dihydrofolatereductase and farnesyltransferase inhibitors. We designed 30 Imidazole derivatives and synthesized 18 best compounds on the basis of glide score. Glide score was obtained using GLIDE module (version 9.1, Schrodinger, LLC, New York, 2010). Molecular docking study of the designed ligands was performed to study the binding pattern of the structure with the enzyme. Total 18 compounds showed a very good binding pattern with the enzyme and were synthesized. The characterization of all the synthesized compounds were performed by TLC and various spectroscopic techniques. *In vitro* antimalarial evaluation of all the synthesized compounds (S_1-S_{18}) was performed. Biological activity mean IC_{50} (µg/ml) of all synthesized compounds (S_1-S_{18}) were determined. The activity of S_{15} compound is 0.023µg/ml which is comparable to chloroquine and the activity of S_7 compound is 0.26 µg/ml which is comparable to quinine as antimalarial agent.

KEYWORDS

Pf dihydrofolatereductase, Farnesyltransferase, Drug design, Docking, Glide and Antimalarial Activity.

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INTRODUCTION

Malaria is one of the leading causes of death and disease worldwide. Its severity is a function of the interaction between the parasite, the Anopheles mosquito vector, the human host and the environment. Four species of *Plasmodium* can infect humans. These are: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae*. Almost all the deaths are generally caused by *Plasmodium Falciparum*[1].

Plasmodium Falciparum Dihydro Folate Reductase as emerging target

Dihydrofolatereductase, or DHFR, is an enzyme that reduces dihydrofolic acid to tetrahydrofolic

acid. Dihydrofolatereductase converts dihydrofolate into tetrahydrofolate, a methyl group shuttle required for the de novo synthesis of purines, thymidylic acid, and certain amino acids[2,3].

Farnesyltransferase as an emerging target

Farnesyltransferase's targets include members of the Ras super family of small GTP-binding proteins critical to cell cycle progression. For this reason, several FTase inhibitors are undergoing testing as anti-cancer agents but FTase inhibitors have shown efficacy as anti-antimalarial agents, as well[4].

Resistance

The main limitation of available therapy of malaria is development of resistance depending upon the different factors. Artemisinin, contained in the decoction prepared from the aerial parts of a plant Artemisia annua, used for over thousand years for fever resolution has been re-discovered as the most potent antimalarial drug. Artemisnin is the only drug for which no clinical resistance has been shown. Chloroquine, the only synthetic antimalarial drug which cured malaria for decades, rather than centuries, has fallen to resistance (WHO guidelines for treatment of malaria, 2006). So one of the strategies to delay the resistance of malaria is use of combination drug therapy instead of mono therapy. Artemisinin based combination therapy (ACT) are being suggested and support by WHO. Second problem with available therapy is scarcity, cost and unavailability of synthetic route for novel antimalarials (Artemisnin derivatives). Resistance for malaria may occur by different biochemical mechanisms. Resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization[5].

For some drugs resistance may occur due to spontaneous mutation that cause reduced effectiveness for that drug. This mutation may be single or multiple points for a drug to be resistant. Drugs, such as sulfadoxine and pyrimethamine, act through blockade of enzymes dihydropteroate synthase (DHPS) and dihydrofolatereductase (DHFR) respectively involved with folate synthesis. Specific gene mutations encoding for resistance to both DHPS and DHFR have been identified[6].

Atovaquone acts through inhibition of electron transport at the cytochrome *bc1* complex. Although resistance to atovaquone develops very rapidly when used alone, when combined with a second drug, such as proguanil or tetracycline, resistance develops more slowly. Resistance is conferred by single-point mutations in the cytochrome-b gene[6].

Computer aided drug design: Drug design

Sometimes referred to as rational drug design or more simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. Drug design frequently but not necessarily relies on computer modelling techniques. This type of modelling is often referred to as computer-aided drug design. Computer-aided drug design uses computational chemistry to discover, enhance, or study drugs and related biologically active molecules[7].

Structure-based drug design (Molecular Docking)

When sufficient information is available or inferable about the structure of the biological target and its binding site, then it is possible to invoke a structure based approach, wherein specific ligand-receptor interactions are studied to help identify new molecules with activity toward the target is called structure-based drug design[8].

Ligand-based drug design (Pharmacophore development)

If knowledge about the structure of the target is limited, but a sufficient number of actives have already been identified, then ligand-based drug design provide alternative ways of leveraging the available information into models that can help identify new actives. While ligand-based design formally includes any number of computational methods that rely only on the structure of known and potential ligands, it has become largely synonymous with pharmacophore modelling and quantitative structure-activity relationships (QSAR). The relative success of any ligand-based methodology may be attributed to a combination of factors, including demonstrated scientific validity,

novelty, ease of use, and integration with other software and experimental workflows.

Glide

Grid-based Ligand Docking with Energetics approximates is a complete systematic search of the conformational, orientational and positional space of the docked ligand. In this search, an initial rough positioning and scoring phase that dramatically narrows the search space is followed by torsionally flexible energy optimization on an OPLS-AA non-bonded potential grid for a few hundred surviving candidate poses. The very best candidates are further refined via a Monte Carlo sampling of pose. Selection of the best docked pose uses a model energy function that combines empirical and force-field based terms.

GScore = 0.065*vdW + 0.130*Coul + Lipo + Hbond + Metal + BuryP + RotB + Site

ADME screening as drug design approach

Prior to experimental studies one of the most important aspects in the drug discovery and development of drug molecules is the prediction of ADME parameters. The important ADME properties are:

1. Molecular weight (150–650)
2. Octanol/water partition coefficient (log P o/w) (-2 to6.5)
3. Aqueous solubility (QPlogS) (-6 to 0.5)
4. Brain/blood partition coefficient (QPlogBB) (-3.0 to1.2)
5. Percent human oral absorption (80 % is high, < 25 % are poor)

The first three properties are based on Lipinski rule of five, molecular weight, and partition coefficient between octanol and H_2O solubility. Brain/blood partition coefficient (Qlog BB) parameter indicates the ability of a drug to pass through BBB.

Biological evaluation

The antimalarial screening involves the assessment of the inhibitory effect of the candidate compound on the growth of different *Plasmodium* species causing malaria. The antimalarial biological evaluation involves different *in vivo* and *in vitro* assays. The *in vitro* assays include quantitative assessment of the effects of drugs on parasite growth, especially different strains of *Plasmodium falciparum.* Methods used for the in vitro assessment of antimalarial biological activity[9, 10].

- SYBR Green I-based assay
- 3H Hypoxanthine uptake method
- Giemsa stained slide method
- Flow cytometry using hydroethidine dye method
- Lactate dehydrogenase activity measurement method

MATERIAL AND METHODS

Computational study with Glide

Ligand preparation

The structures of the substituted imidazoles were drawn by using chemdraw ultra 8.0 and converted to 3D structure with the help Lig Prep version 2.4. All the ligands were subjected to full minimization with OPLS-2005 force field to correct its bond length and bond order.

Preparation of Protein

The X-ray crystal structures of the proteins human protein farnesyltransferase PDB id (1SA4) and *P. falciparum* dihydrofolate reductase-thymidylate synthase (PFDHFR-TS) with PDB id (3JSU) were obtained from the RCSB protein data bank. Protein preparation wizard of maestro from Schrodinger has been used to prepare the proteins. The A chain was selected of the protein of PDB id 1SA4 and the A chain of the protein of PDB id 3JSU treated to add missing hydrogen, assign proper bond orders.

Receptor grid generation

Receptor was defined and the co-crystallized ligand was differentiated from the active site of receptor "A" chain. The active site was defined as an enclosing box at the centroid of the workspace ligand as selected in the receptor folder. The ligands similar in size to the workspace ligand were allowed to dock into the active site.

Ligand docking

Ligand docking was performed using OPLSAA force field. The receptor grid defined in the receptor grid generation folder was selected for the docking of ligands prepared using Ligprep*V*2.4. Flexible docking was performed using the Extra Precision (XP) feature of Glide module.

The general structure of the Imidazole derivatives synthesized (S_1- S_{18}) are mentioned in the table 8. It showed the structure of the compounds prepared from scheme No.1 and scheme No.2 given in the chemistry of the reaction. It also states the glide scores of the compound and showed that compound S_6 from scheme I and S_{10} from scheme No.2 showed the highest glide score whose interactions are shown in the figs 9,10,11,12. The substitution of 4 chlorobenzaldehyde and 3 nitro benzaldehyde showed maximum interaction.

CHARACTERIZATION

In order to ascertain whether the compounds were actually synthesized, the prepared compounds were the identified and characterized. Characterization of synthesized product was done by following methods:

Thin Layer Chromatography (TLC)

The $R_f$value of all product is determined on aluminum-foil silica gel as stationary phase and hexane/methanol (4:1) as mobile phase and running in closed container. The spots on the plate were visualized in UV chamber and R_f value was calculated.

Melting Point Determination (MP)

The melting point determinations were done in open capillary using liquid paraffin and are uncorrected.

Solubility Determination

The solubility of compounds were determined qualitatively in different solvents like dimethylsulphoxide, methanol and ethanol.

Clog P Determination

The Clog P values are the indicative of hydrophobicity. These values were predicted for all the derivatives using CS ChemOffice-2004 version 8.0.

Ultraviolet-Visible Spectroscopy (UV)

UV-Visible spectra were studied on SHIMADZU (UV-1700) double-beam spectrophotometer. All the synthesized compounds were dissolved in methanol and were scanned in UV-Visible light of range 200 - 800 nm.

Fourier Transform Infrared Spectroscopy (FTIR)

The determination of Infrared spectra were done as KBr pellets on FT/IR spectrophotometer.

^{1}H Nuclear Magnetic Resonance Spectroscopy (^{1}HNMR)

A Bruker ADVANCE II 400 NMR spectrometer was used to record ^{1} H NMR spectra and is reported in δ downfield from TMS (tetramethylsilane) as internal standard. All the NMR spectra were obtained in ($CDCl_3$).

Mass Spectrometry (MS)

The mass analysis was performed by Bruker Micro TOF QII High Resolution Mass Spectrometer data analysis.

***In Vitro* Biological Evaluation**

The SYBR Green I-based assay method was used to evaluate the antimalarial activity of the compounds which were synthesized on *Plasmodium falciparum* 3D7 strain at Microcare Laboratories, Surat (Gujarat). The results are reported in table 2.

Sybr Green I-Based Assay

This assay is a new technique for analyzing the activities of antimalarial drugs against intraerythrocytic *P. falciparum*. This assay is considerably faster, less labor-intensive, and less expensive than conventional radiotracer techniques. The principle used in the technique is based on the fact that:

- SYBR Green I is a DNA-intercalating dye that prefers G and C base pairs.
- The dye is highly fluorescent when it is intercalated into DNA but is poorly fluorescent when it is not.
- Typical laboratory growth of malarial parasites requires propagation in human red blood cells and red blood cells do not contain DNA, so not interfering with the assay.
- Thus, the use of SYBER Green I and its derivatives provides attractive advantages for malaria parasite growth detection assays.

RESULTS AND DISCUSSION

Out of eighteen evaluated compounds, five compounds (S_5, S_6, S_{15}, S_{16} and S_{18}) have shown potent antimalarial IC_{50} values in the range of 0.020-0.099 µg/ml comparable against chloroquine as antimalarial agent. Seven compounds (S_3, S_4, S_7, S_9, S_{10}, S_{11} and S_{13}) displayed antimalarial activity in the range 0.15-0.45 µg/ml comparable against quinine as antimalarial agent. Compounds S_6 and S_{10} exhibited good Gscore, good ranks on pharmacophoric features and very good antimalarial activity. ADME studies was performed on 18 synthesized compounds, All the compounds showed pharmacokinetic parameters within the acceptable range defined for human use, thereby indicating their potential for use as drug-like molecules.

Based on the literature study, docking studies and ADME screening it is expected that all of the new drug candidates will boost up the current Structure and ligand based drug design of new antimalarial discovery and lay down milestone in treatment of malaria.

The graphs show the comparison of the synthesized compounds with the potent drugs chloroquine and quinine.

Malaria is the most prevalent and most pernicious parasitic disease of humans which is caused by the protozoa of the genus *Plasmodium.* It is estimated to kill about 2-3 million people each year, especially children. Several new drugs are available currently but chloroquine, the first synthetically developed antimalarial drug proved to be a successful cure in the past 50 years. Emergence and spread of resistant parasites to some of the drugs and their combination has made it virtually ineffective in most parts of the world. Few newer drugs like artemether, lumefantrine retains efficacy but they have limitations, one of which is their high cost. Thus, it is indispensable need to discover, design and develop new drugs with different mechanisms. Resistance to chloroquine appears to arise mainly from mutations in a *Plasmodium* transmembrane protein, *Pf* CRT, mutant forms of which seem to lower the accumulation of CQ in the parasitized cell. Therefore, new drugs acting on the *Plasmodium falciparum* have every prospect of being active, even in CQ-resistant parasites, provided that they can circumvent the effects of *Pf* CRT.

Malarial dihydrofolatereductase (DHFR) is the target of antifolate antimalarial drugs. Design and discovery of new potential inhibitors of *Plasmodium Falciparum* dihydrofolatereductase (Pf DHFR), equally active against both the wild-type and mutant strains, was urgently needed. In this study, a computer-aided molecular design approach that involved ab initio molecular orbital and density functional theory calculations, along with molecular electrostatic potential analysis, and molecular docking studies was employed to design 15 1H imidazole-2, 4-diamine derivatives as potential inhibitors of Pf DHFR enzyme. It has been shown that Farnesyl Transferase (FT) inhibitors could cause significant lysis of *Plasmodium Falciparum* (the causative agent of critical malaria) in infected cells, and this was associated with a decrease in farnesylated proteins. The FT inhibitors showed substantial selectivity for the malarial parasites over mammalian cells. Some imidazole derivatives were designed using 3D QSAR and Molecular docking as potent FT inhibitors.

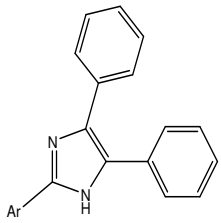
Several Imidazole derivatives of varied chemical structures with antimalarial activity have been shown to inhibition of *Pf* DHFR enzyme and FTPase enzyme. In view of the urgent need, this project was focused on designing and establishing of novel active molecules for malaria. As part of research, docking of 30 compounds were performed on *Pf* DHFR enzyme (Pdb Id-3JSU) and FTPase enzyme (Pdb Id-1SA4) was developed and on the basis of their Gscore, a series of eighteen imidazole derivatives were synthesized. The completion of reaction was determined by TLC. Finally, the structure of compounds was confirmed by IR spectroscopy ^{1}HNMR technique and Mass spectrometry. ADME screening of synthesized compounds was performed and compounds S_1-S_{18} were examined for *in vitro* antimalarial activity.

After confirmation of all the compounds (S_1-S_{18}) to anticipated structures, the antimalarial activity of the compounds was evaluated *in vitro* by SYBR green I assay method against *Plasmodium*

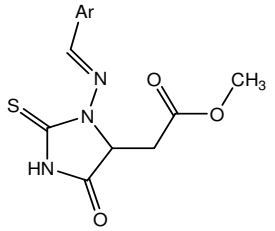
falciparum. The studies were carried out in the Microcare Laboratories, Surat (Gujarat).

Out of eighteen evaluated compounds, five compounds (S_5, S_6, S_{15}, S_{16} and S_{18}) have shown potent antimalarial IC_{50} values in the range of 0.020-0.099 µg/ml comparable against chloroquine as antimalarial agent. Seven compounds (S_3, S_4, S_7, S_9, S_{10}, S_{11} and S_{13}) displayed antimalarial activity in the range 0.15-0.45 µg/ml comparable against quinine as antimalarial agent.Compounds S_6 and S_{10}exhibited good Gscore, good ranks on pharmacophoric features and very good antimalarial activity. ADME studies was performed on 18 synthesized compounds, All the compounds showed pharmacokinetic parameters within the acceptable range defined for human use, thereby indicating their potential for use as drug-like molecules.

Table No.1: General structure of synthesized compounds (S_1- S_{18}) along with Glide Score

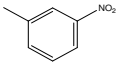
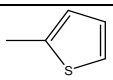
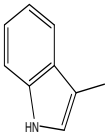
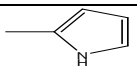
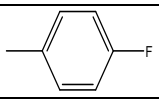
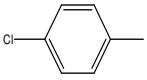
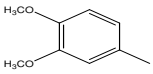


(S_1-S_9) Scheme I



(S_{10}-S_{18}) Scheme II

General structure of Imidazole derivatives (S_1-S_{18})

Compound Code	Ar	Glide Score
S_1		-9.15
S_2		-10.05
S_3		-9.98
S_4		-10.69
S_5		-10.39
S_6		-11.38
S_7		-10.21

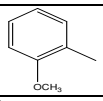
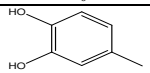
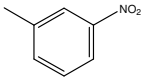
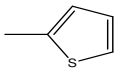
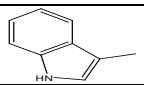
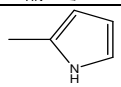
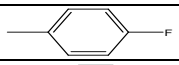
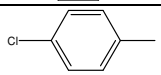
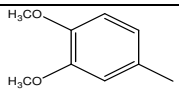
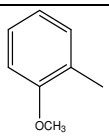
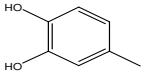
S_8		-9.13
S_9		-10.15
S_{10}		-11.65
S_{11}		-11.16
S_{12}		-10.06
S_{13}		-9.85
S_{14}		-9.79
S_{15}		-10.78
S_{16}		-9.44
S_{17}		-11.34
S_{18}		-10.65

Table No.2: *In-vitro* antimalarial activity of synthesized compounds against *P. falciparum*

Compounds	IC_{50} (µg/ml)
S_1	1.56 µg/ml
S_2	2.00µg/ml
S_3	0.40 µg/ml
S_4	0.42 µg/ml
S_5	0.073 µg/ml
S_6	0.057 µg/ml
S_7	0.26 µg/ml
S_8	0.098 µg/ml
S_9	0.15 µg/ml
S_{10}	0.42 µg/ml
S_{11}	0.56 µg/ml
S_{12}	2.05µg/ml
S_{13}	0.48 µg/ml
S_{14}	1.24 µg/ml
S_{15}	0.023 µg/ml
S_{16}	0.095 µg/ml
S_{17}	2.46 µg/ml
S_{18}	0.078 µg/ml

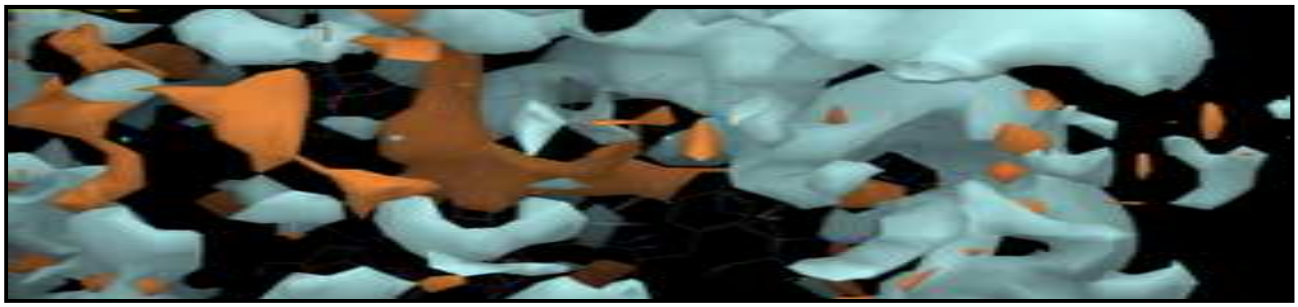


Figure No.1: Software images showing the Interaction Pattern of the Ligand molecule S_6 in Protein Pdb of Ligand molecule S_{10} with protein Pdb id (3JSU). id (1SA4)

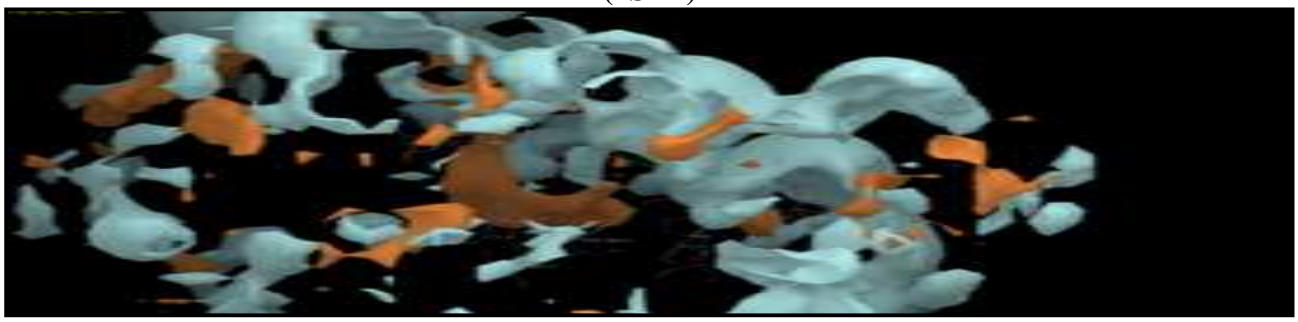


Figure No.2: Software images showing the Interaction Pattern of the Ligand molecule S_6 in Protein Pdb of Ligand molecule S_{10} with protein Pdb id (3JSU). id (1SA4)

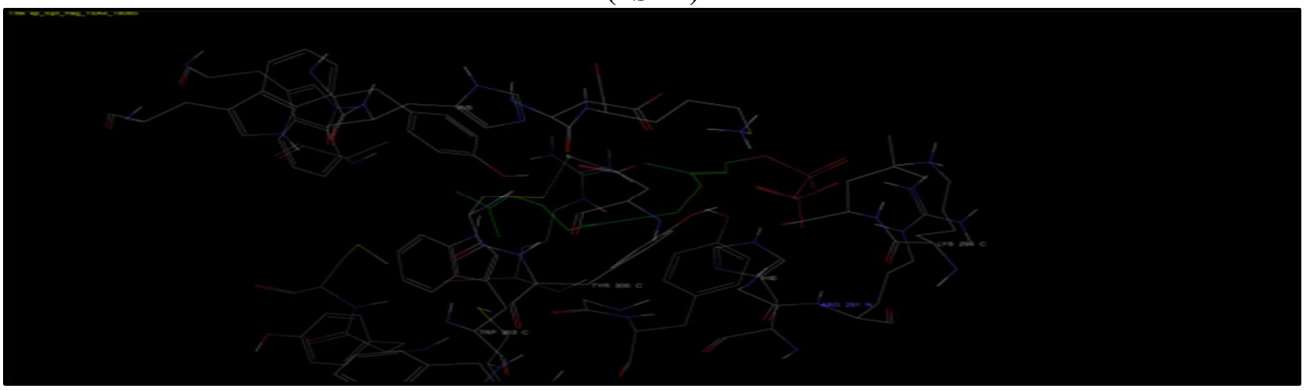


Figure No.3: Software Images showing the Interaction of Docked Ligand S_6 with amino acids

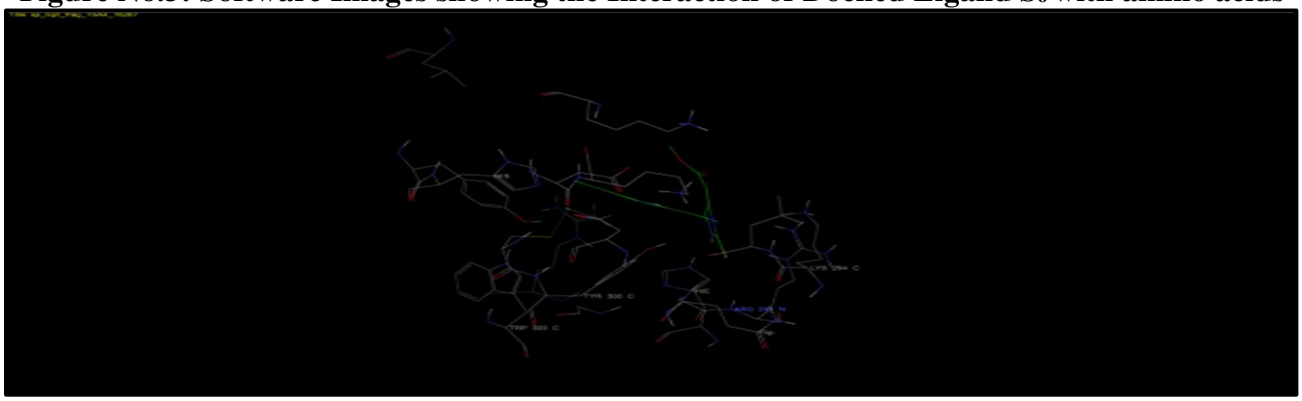
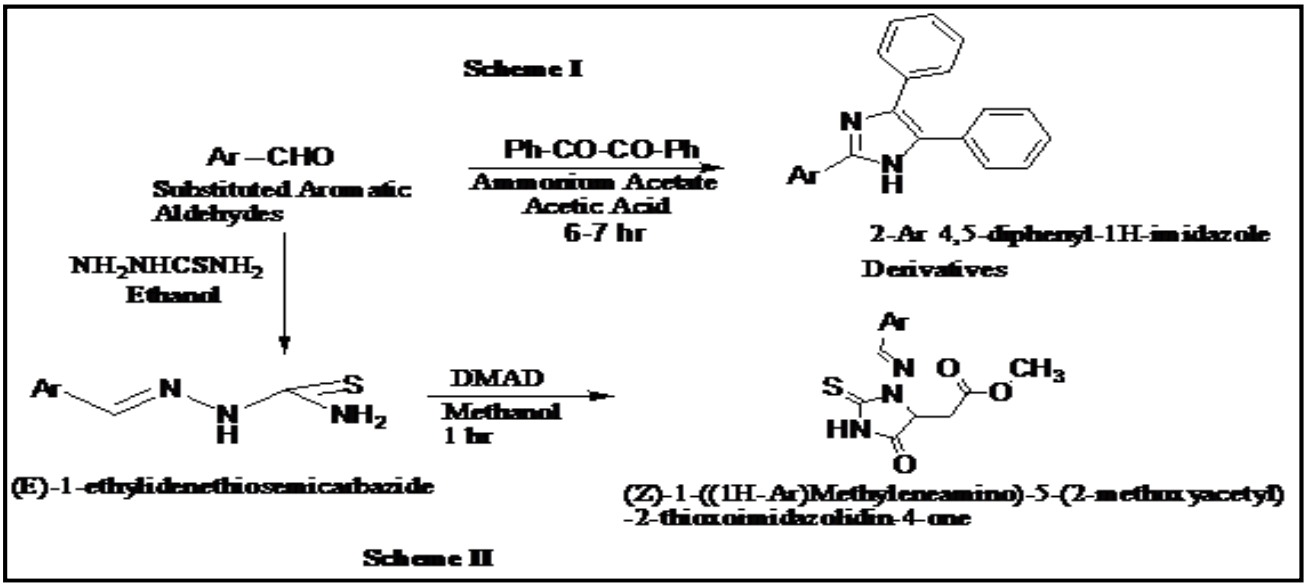


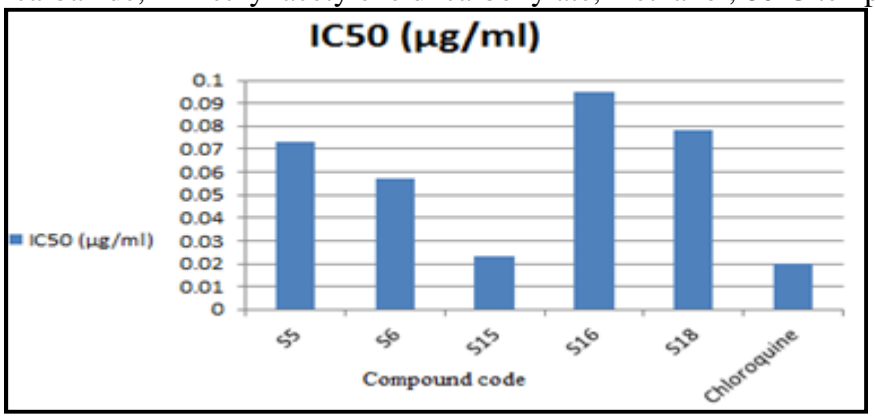
Figure No.4: Software Images showing the Interaction of Docked Ligand S_{10} with amino acids

CHEMISTRY

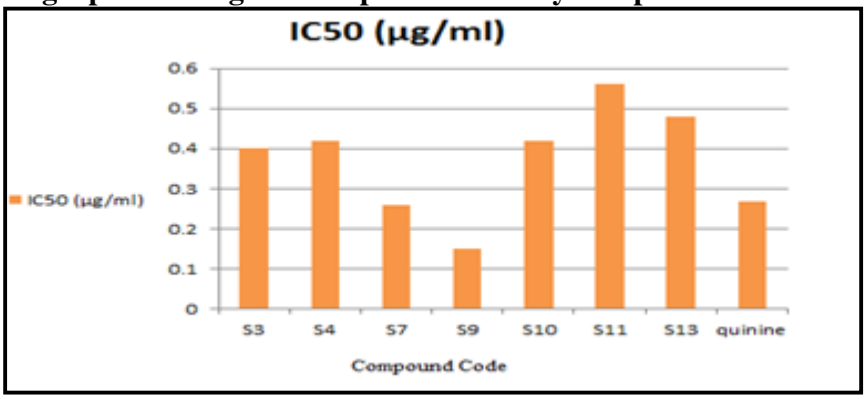


Scheme No.1: Synthesis of Imidazole derivatives (S_1-S_9): Reagent and condition: Heterocyclic aromatic aldehyde, Benzil, CH_3COONH_4, CH_3COOH, 120^0C temperature, 6-7 hr stirring.

Scheme No.2: Synthesis of Imidazole derivatives (S_{10}-S_{18}): Reagent and condition: Heterocyclic aromatic Aldehydes, thiosemicarbazide, Dimethyl acetylene di carboxylate, methanol, 80^0C temperature, 1hr reflux.



Graph No.1: graph showing the compounds activity comparable to the Chloroquine



Graph No.2: graph showing the compounds activity comparable to quinine

CONCLUSION

Based on the literature study, docking studies and ADME screening it is expected that all of the new drug candidates will boost up the current Structure and ligand based drug design of new antimalarial discovery and lay down milestone in treatment of malaria. The exploration and optimization of imidazole derivatives could furthur be used as antimalarial molecules, which will help to eradicate issues of cross resistance.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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