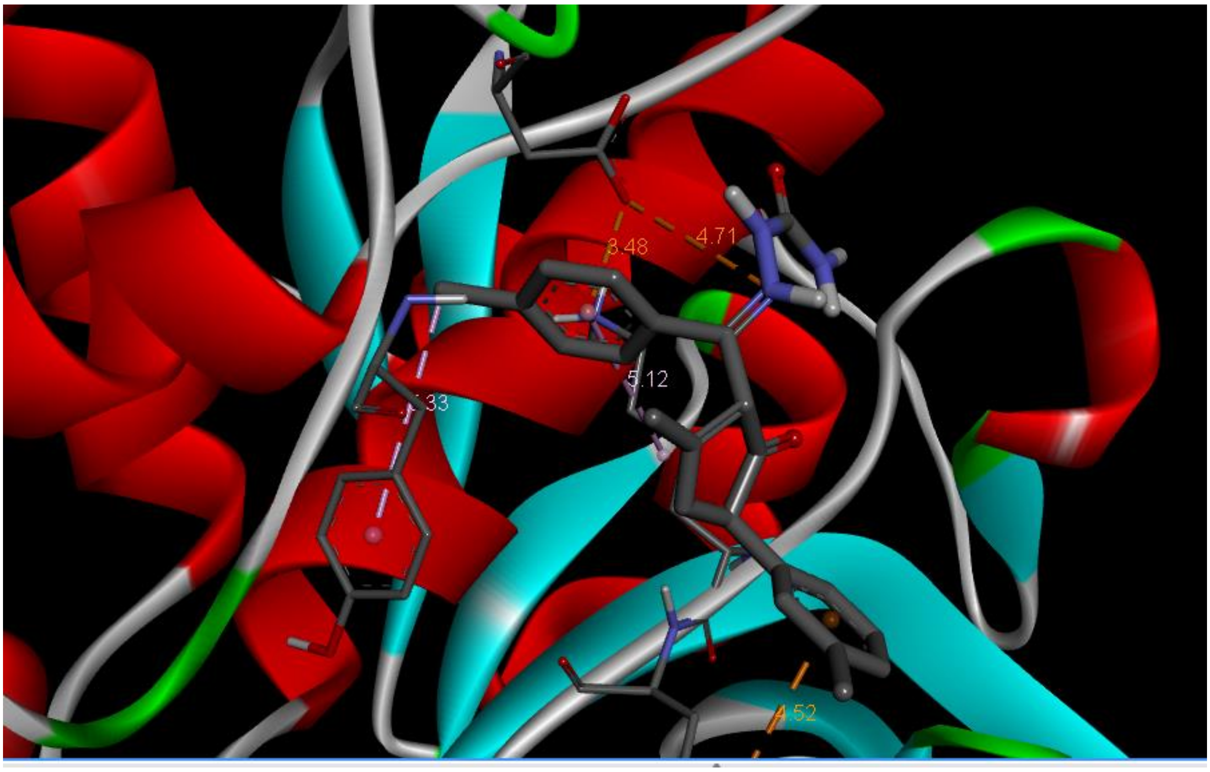
Chapter 5

Molecular Docking and Antimalarial Activity of Synthesized Ligands and Zinc(II) Metal Complexes



5.1. Introduction

Malaria

As per the WHO 2020 report, worldwide an estimated 229 million malaria cases in 2019 in 87 malaria endemic countries, declining from 238 million in 2000. At the Global technical strategy for malaria 2016–2030 (GTS) baseline of 2015, there were 218 million estimated malaria cases. The proportion of cases due to *Plasmodium vivax* reduced from about 7% in 2000 to 3% in 2019. Although there were fewer malaria cases in 2000 (204 million) than in 2019 in the WHO African Region, malaria case incidence reduced from 363 to 225 cases per 1000 population at risk in this period, reflecting the complexity of interpreting changing disease transmission in a rapidly increasing population. The population living in the WHO African Region increased from about 665 million in 2000 to 1.1 billion in 2019. In the WHO South-East Asia Region, malaria deaths reduced by 74%, from about 35 000 in 2000 to 9 000 in 2019. India accounted for about 86% of all malaria deaths in the WHO South-East Asia Region.[1,2] Quinoles, Chloroquine and Artemisine derivatives can resistance to *Plasmodium*, which is a causative agent of malaria and possibilities to create novel drug target and drug [3]. Some compound were analyzed to find better treatment, but it is important to further evaluate compounds synthesized worldwide. Medicinal plants and Phytochemicals should also provide effective treatment for combinatorial drug discovery approach and *in vitro* analysis provides effective strength to *in silico* drug discovery studies.[4,5]

All cases of suspected malaria should have a parasitological test (microscopy or Rapid diagnostic test (RDT)) to confirm the diagnosis. Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended artemisinin-based combination therapies (ACT) are artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, dihydroartemisinin + piperaquine, artesunate + sulfadoxine–pyrimethamine (SP), artesunate + pyronaridine. Currently, triple ACTs (TACTs) i.e. a combination of artemisinin with two exiting clinical drugs could be come first-line-treatment for malaria until new antimalarial drugs are available.[6,7]

Based on the fact, in the present study *in silico* and *in vitro* studies of organic ligands and their complexes have been carried out. The virtual screening was performed with Autodock Vina 4.2 software for molecular docking based approach and other hand structure-based and ligand-based pharmacophore virtual screening was carried out with Liganscout Software.

Several parasite surface proteins, for example, MSP-1 [8], were isolated by immune precipitation from parasite culture supernatants with human sera containing parasite-specific antibodies. Hybridoma technology was also applied to produce antibodies that recognise specific parasite antigens, for example, CSP.[9] The antibodies were subsequently used to purify parasite proteins by affinity chromatography and for immunolocalization experiments to determine the exact location of the proteins, such as AMA-1 in the parasite.[10]

In 2700 BC, several characteristic symptoms of what would later be named malaria were described in the Nei Ching in the book "The Canon of Medicine". Nei Ching was edited by Emperor Huang Ti. Malaria became widely recognized in Greece by the 4th century BCE, and it was responsible for the decline of many of the city-state populations. After a long period of time, there were extensive references to malaria in the literature and depopulation of rural areas were recorded.

In the Susruta, a Sanskrit medical treatise, the symptoms of malarial fever were described and attributed to the bites of certain insects. Several Roman writers attributed malarial diseases to the swamps. But due to lack of effective treatment, until the early 17th century, no truly effective cure for malaria had found forcing the patients to die. However, in China, during the second century BCE, the Qing-hao plant (*Artemisia annua*) was described in the medical treatise, 52 Remedies, found in the Mawangdui Tomb to have the antimalarial ability.

Spanish Jesuit missionaries, learned from indigenous Indian tribes of a medicinal bark used for the treatment of fevers. The bark from the tree was then called Peruvian bark and the tree was named Cinchona. Later on, it was found that the effective molecule in the bark of Cincona was Quinine.[11,12]

Quinoline antimalarials are derived from quinine, the active component extracted from the bark of cinchona trees (*Quinona Officinalis*). The ground tree bark has been used since the beginning of the 17th century to treat malaria.

Johann "Hans" Andersag and colleagues synthesized and tested some 12,000 compounds, eventually producing Resochin® as a substitute for quinine in the 1930s.[13–15]

It is chemically related to quinine through the possession of a quinoline nucleus and the dialkylaminoalkylamino side chain. Resochin (7-chloro-4-4-(diethylamino)-1–methyl butyl amino quinoline) and a similar compound Sontochin (3-methyl Resochin) were synthesized in

1934.[16] In March 1946, the drug was officially named Chloroquine.[17] Chloroquine is an inhibitor of hemozoin production through biocrystallization. Quinine and chloroquine affect malarial parasites only at life stages when the parasites are forming hematin-pigment (hemozoin) as a byproduct of hemoglobin degradation.

Molecular docking tools utilized for antimalarial drug discovery virtual screening

Nowadays widely used and useful to predict the binding of a large database of ligands to a particular target, to identify the most promising compounds from the database for further study (Kitchen *et al.,* 2004; Kolb *et al.,* 2009). Numbers of compounds can be tested in very less time depending upon the available computational power for the software. Popular programs for molecular docking for insilico include AutoDock [18], Vina [19], Dock [20], FlexX [21], Glide [22,23] and Gold [24].

Autodock was used for Molecular Dynamics Simulations and Free Energy Calculations for binding of seven synthesized di-amide compounds for Plasmepsin II target by Erskmark *et al.,* in year 2005.[25]

Warner *et al.* used Glide software package for Falcipain inhibitors of *Plasmodium falciparum* [26]. The DOCK was utilized for FK506 immunophilin BCL6, oncogene in B-cell lymphomas [27]. Gutierre-De-Teran *et al.,* performed docking using GOLD version 2.2 allowing full flexibility for the structure-based approach for 20 independent runs of using the default genetic algorithm (GA) to calculate Gold score [28].

Kasam *et al.* reported WISDOM based design of new plasmepsin inhibitor with used of the open-source Autodock 3.2.[29] S Bjelic *et al.,* used two empirical scoring functions to predict the inhibitor binding affinity and ranking (Chemscore and Goldscore) initially to select the most potent stereoisomer.[30]

FlexX was utilized for Plasmepsin II and IV inhibitors [31] and malaria Anthrax edema factor [32]. Along with that, Structure and mechanism of a transmission blocking vaccine candidate protein Pfs25 from *P. falciparum*: a molecular modelling and docking study.

SEED algorithm based software was used for Plasmepsin.[33] FLEXX was used for docking and QSAR based study for identification of New benzimidazole derivatives as antiplasmodial agents and plasmepsin inhibitors by Saied *et al.*[34] Nag *et al.,* reported Autodock 4.0 and MGL

Tool package for the screening of heterocyclic compound libraries to identify novel inhibitors for PfRIO-2 kinase through docking [35].

The open source Autodock Vina 4.2 has been used for finding the inhibitory effect of Thiazides on Plasmepsins.[36] Sarfar Alam has used Autodock Vina for molecular docking studies to validate the LibDock score in his research work [37]Autodock also was used for identification of dietary flavonoids fisetin and myricetin for inhibition study of falcipain-2 and plasmepsin II of *Plasmodium falciparum*.[38]

Molecular docking is a well-established computational technique which predicts the interaction energy between two molecules. This technique mainly incorporates algorithms like molecular dynamics, Monte Carlo stimulation, fragment based search methods which are mentioned in details in later part. Recent advances in computer models and algorithms for biomolecular docking have made it feasible to analyze biomolecular interactions with the aid of the computer, to gain insights into biological processes and obtain ideas for the design of new drugs.[39]

Comprehensively utilized docking tools employ search algorithms such as genetic algorithm, fragment-based algorithms, Monte Carlo algorithms and molecular dynamics algorithms. Besides this, there are some tools such as DOCK, GOLD, FlexX and ICM which are mainly used for high throughput docking simulations. There are various kinds of molecular docking procedures involving either ligand/target flexible or rigid based upon the objectives of docking simulations like flexible ligand docking (target as a rigid molecule), rigid body docking (both the target and ligand as rigid molecules) and flexible docking (both interacting molecules as flexible).[40,41]

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

5.2. Experimental

5.2.1. Materials and Methods

All compounds (ligands and zinc(II) complexes) have been synthesised and method has been depicted in previous chapters **2-4**. All necessary reagents an2d chemicals have commercially purchased from the Loba chem, Mumbai and spectrochem Ltd, Mumbai.

5.2.2. *P. falciparum in vitro* growth inhibition assay

All the synthesized compounds and Quinine as a control molecule were tested for activity against Chloroquine sensitive Plasmodium *falciparum* isolated from the Clinical samples from Gujarat. The strain was grown *in vitro* with a modification of the method suggested by Trager and Jensen.[42] The cultures were maintained in fresh O-positive human erythrocytes with 5% haematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, 0.23 % sodium bicarbonate and 10% heat-inactivated human O+serum at pH 6.74. The stock solution for each compound was prepared by dissolving 5mg compounds in 1 ml DMSO. The stock solution was subsequently diluted using DMSO for further study. 20µl volume added to the test wells to obtain final concentrations (at fivefold dilutions) ranging between 0.4 µg/ml to 100µg/ml in well containing parasitized cell preparation. Along with that, 100µl *P. falciparum* parasite culture suspension was added to 96-well microtitre plate to a final haematocrit of 2% and parasitemia of 0.5%.[43] 20µl DMSO as a negative control as well as Quinine used as a positive control in this assay. After incubation, 20 µl 7.5mg/ml MTT solution was added to each well and the plate incubated again for 2 h as discussed in MTT Assay.[44] The plates were then kept at room temperature in the dark for 24 h and the optical densities of the wells were read at 570 nm on the plate reader. The Minimum Inhibitory Concentration (MIC) was calculated based on the cytotoxicity observed in microtiter plate.

5.2.3. *in silico* antimalarial activity

In present work, we have investigated in silico interaction of thirty-two test compounds along with co-crystallized control molecules against protein target Plasmepsin-1 (PDB code: 3QS1), Falcipain-2 (PDB code: 3BPF) and M1 aminopeptidases (PDB code: 4ZW3). Along with that, the control molecule extracted and also include in the Ligand Library for comparative binding pattern analysis. The proteins structures and Ligands were converted to pdbqt format using Openbabel software followed by energy minimization using python script provided the Autodock Vina Tool. The docking was performed using Autodock Vina 4.1 with default parameters provided by developers. The interaction analysis of docking results done using the LigPlus and Discovery Studio 4.0 Client Software. All test complexes were further analysed for ADME and Lipinski's rule of five using SWIS-ADME online web server (http://www.swissadme.ch/).[45]

5.3. Results and Discussions

5.3.1. In vitro results

Minimum Inhibitory Concentration against *P. falciparum*

All synthesized 32 compounds and 1 Control molecule were subjected to *in vitro* minimum inhibitory concentration (MIC) against Chloroquine sensitive Plasmodium falciparum using MTT assay[44] at Veer Narmad South Gujarat University, Surat a collaborative institute. Amongst total 32 compounds, 78% (25) compounds have shown higher MIC than Quinine (Control Molecule). While 22% (7) compounds shown lesser MIC than control molecule used in the present study. (Fig. 5.1) The inhibition potential of complexes shown anti-*P.falciparum* activity was considered to current commercial drugs used to treat Malaria.

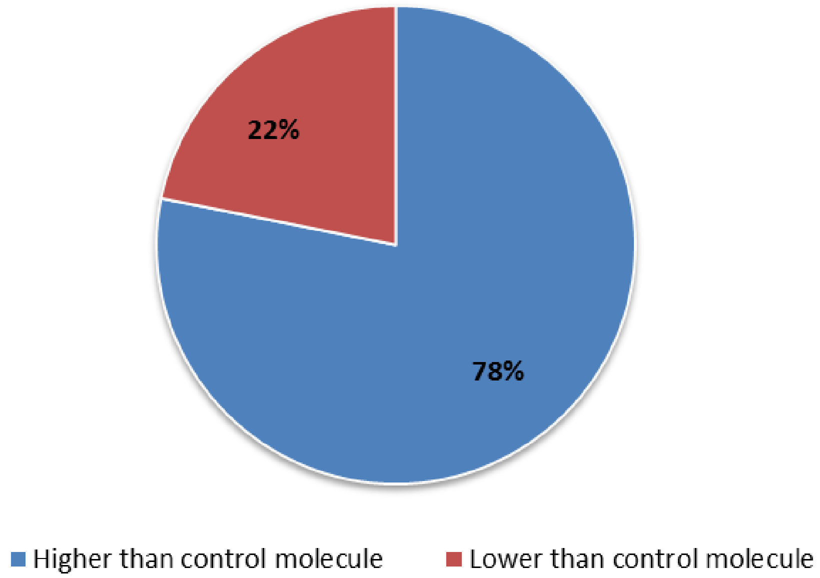


Fig. 5.1. Comparison of MIC of compounds against the control molecule.

MIC of 4-acylpyrazolone and their zinc(II) complexes against *P. falciparum*

Three 4-acylpyrazolones and their nine zinc(II) complexes displayed significant inhibitory effect against *plasmodium falciparum* in the *in vitro* cytotoxicity assay (Fig. 5.2). The lowest minimal inhibitory concentration of 0.11 µmol/L was obtained for complex **6**, followed by 0.51, 0.84, 0.9, 1.15, 1.24, 1.42, 1.56, 2.55, 2.97, 4.49, 4.54 µmol/L for complex **5, 3, 9, 2, 7, 8, 1, 4, L3, L2** and **L1** respectively. While wel-known drug Quinine shown 0.8 µmol/L. The similar analysis for another drug Chloroquine showing 0.09 μmol/L reported by Shaikh *et al.,2019.*[46] *In vitro* result reveals zinc(II) complexes show better activity than their parent ligands against *p. falciparum*.

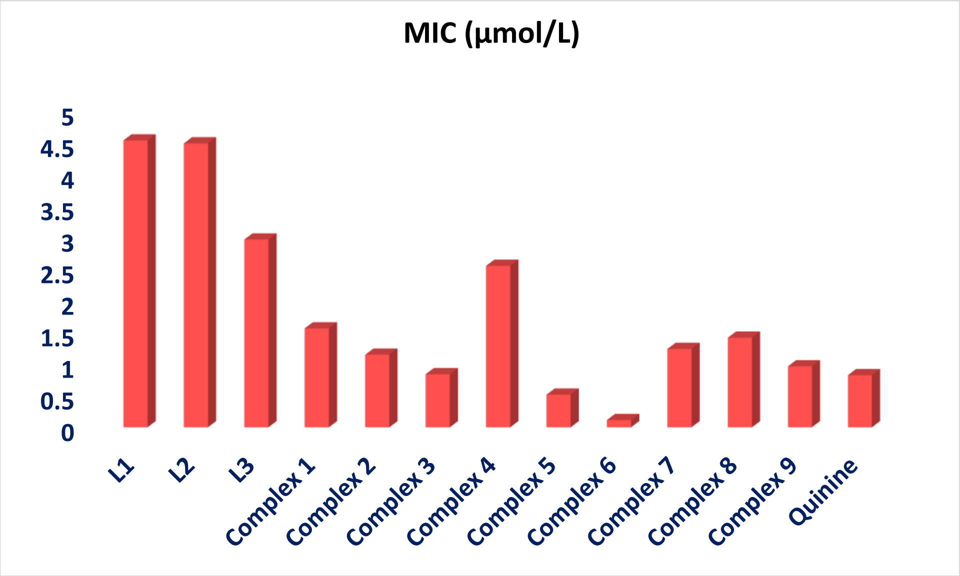


Fig. 5.2. Minimum Inhibitory Concentration (μmol/L) against *P.falciparum.*

MIC of 4-acylhydrazone-5-pyrazolones (L4-L6) and their nine zinc(II) complexes against *P. falciparum*

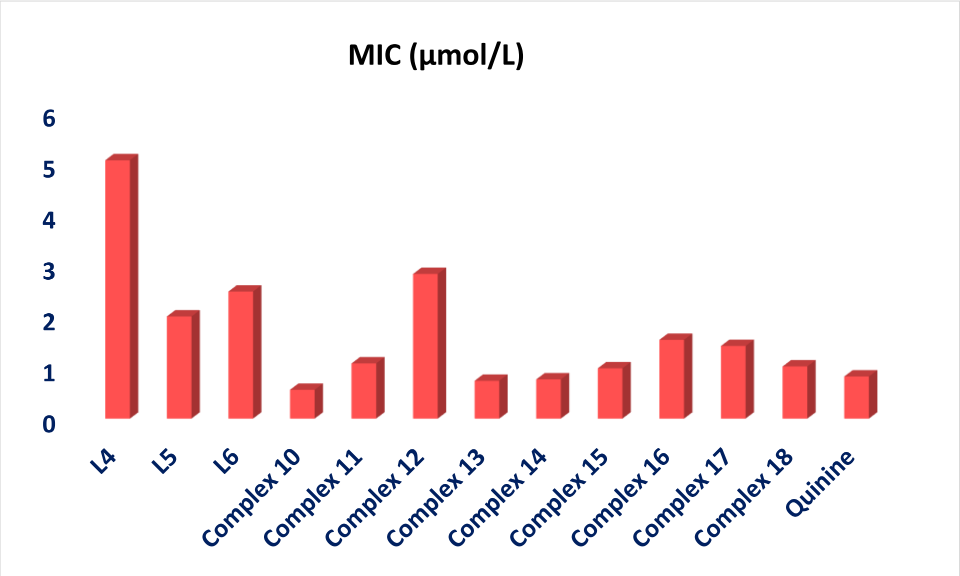


Fig. 5.3. Minimum Inhibitory Concentration (μmol/L) against *P.falciparum.*

Three 4-acylhydrazone-5-pyrazolones and their nine zinc (II) complexes displayed significant inhibitory effect against *plasmodium falciparum* in the *in vitro* cytotoxicity assay (Fig. 5.3). The lowest minimal inhibitory concentration of 0.56 µmol/L was obtained for complex **10**, followed by 0.74, 0.77, 0.98, 1.02, 1.08, 1.42, 1.54, 2.00, 2.49, 2.83 and 5.06 µmol/L for complex **13, 14, 15, 18, 11, 17, 16, L5, L6, 12** and **L4** respectively. While wel-known drug Quinine shown 0.8 µmol/L. The similar analysis for another drug Chloroquine showing 0.09 µmol/L reported by Shaikh *et al.,2019.*[46] *In vitro* result reveals zinc(II) complexes show better activity than their parent ligands against *p. falciparum*.

MIC of 4-semicarbazone-5-pyrazolones, 4-thiosemicarbazone-5-pyrazolones and their zinc(II) complexes against *P. falciparum*

Two 4-semicarbazone-5-pyrazolones, two 4-thiosemicarbazone-5-pyrazolones and their zinc (II) complexes displayed significant inhibitory effect against *plasmodium falciparum* in the *in vitro* cytotoxicity assay (Fig. 5.4). The lowest minimal inhibitory concentration of 0.65 µmol/L was obtained for **L7**, followed by 0.72, 1.05, 1.45, 1.90, 2.34, 2.84 and 4.35 µmol/L for **20, L8, 22, 19, L10** and **L9** respectively. While wel-known drug Quinine shown 0.08 µmol/L. *In vitro* result reveals zinc(II) complex **20**, **21** and **22** show better activity than their parent ligands against *p. falciparum*.

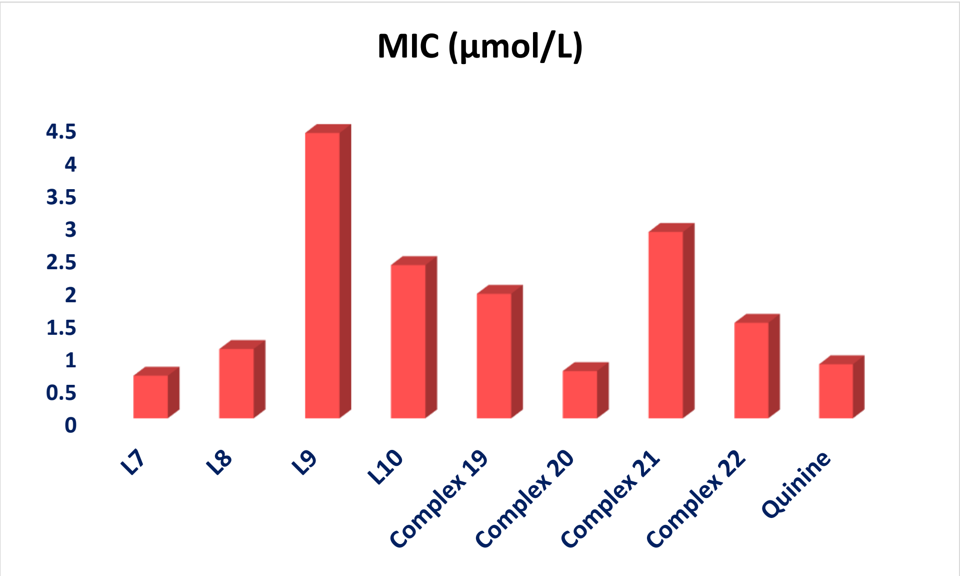


Fig. 5.4. Minimal Inhibition Concentration against *P. Falciparum*.

5.3.2. Multi target *in silico* virtual screening

Virtual screening is a computational technique used in drug discovery to search libraries of small molecules to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme [47,48]. This method of screening is widely used to predict the binding of a large database of ligands to a particular target, to identify the most promising compounds from the database for further study (Kitchen *et al.*, 2004; Kolb *et al.*, 2009). Millions of compounds can be tested in very less time depending upon the available computational power for the software. In the present study, we applied a similar approach to evaluate anti-malarial potentials of compounds synthesized.

All synthesized 32 compounds and 1 Control molecule from each three protein molecules were converted to PDB file format using Openbabel software Tool. The protein molecules were prepared using automated python script "prepare_molecule.py". The script provides the removal of water molecules and stabilization of structure for Docking study. Whereas, all the 32 compounds and control molecule for each protein were prepared using "prepare_ligand.py" python script in Linux Environment. The resulting Ligand Library of 32 compounds and 1 control molecules were subjected docking study using Autodock Vina 4.0. The virtual screening has shown significant results for all three protein molecules used in the present study. The comparative analysis of binding energy indicated all 32 compounds were shown lower binding energy compared to control molecules for 3BPF and 4ZW3. While from docking against 3QS1, 9 compounds had higher binding energy compared to control ligand.

Virtual screening against Plasmpesin-I of *P. falciparum*

The genome sequencing of *P. falciparum* has led to the identification of ten different genes encoding plasmepsins.[49] There are ten aspartic proteases or plasmepsin reported in the genome of *P. falciparum* and four of them (Plm I, Plm II, HAP and Plm IV) have been localized in the food vacuole and shown to be involved in Hb degradation and as such constitute possible drug targets. [50,51] The degradative process, taking place in an acidic food vacuole of *P. falciparum*, provides a source of amino acids and helps maintain intracellular osmolarity during rapid parasite growth.[52] Plasmepsin V, IX and X are expressed concurrently with Plm I–IV but are not transported to the food vacuole. The remaining plasmepsins (Plm VI, VII, VIII) are not expressed during the intraerythrocytic stage.[53] Plasmepsin-I is a key molecule in Food Vacuole pathway. This enables us to select Plasmepsin-I as one of the target protein in our study. The protein structure (PDBID:3QS1) was downloaded from the Protein Data Bank. The protein structure includes 4 chains of peptides and KNI-10006 as ligand molecule. The KNI-10006 was used as a control molecule for 3QS1.

The results of virtual screening against all compounds indicate control ligand have -9.1 kcal/mol as binding energy. Concerning that, 23 molecules from compounds had shown lower binding energy than the control molecule. The overall range for binding energy observed in the present study ranging from -11.3 to -7.9 kcal/mol. The lowest binding energy was observed for complex 3 having a binding energy of -11.3kcal/mol followed by complex 6, complex 5, complex 7 and complex 4 having a binding energy of -11.2, -11.1, -11 and –10.9 kcal/mol. (Fig. 5.10) The top 7 molecules observed in the virtual screening against 3QS1 were amongst to 4-acylpyrazolone and their zinc(II) complex library followed by 1 compound complex 18 from 4-acylhydrazone-5-pyrazolone and their zinc(II) complexes library and 2 compounds complex 19 and complex 21 from 4-semicarbazone-5-pyrazolones, 4-thiosemicarbazone-5-pyrazolones and their zinc(II) complexes library. These results indicated significant anti-malarial activity of compounds from library 4-acylpyrazolone and their nine zinc(II) complexes. The best compound from docking against 3QS1 able to create 2 H-bonds and non-conventional bonds with the protein structure (Fig. 5.5). Agrawal *et al.* reported the specific binding residues for 3QS1 receptor molecule.[36] The binding pattern observed for Gly34, Gly217, Tyr189, Asp32, Asp215 and Ser77 amino acid residues for Plasmepsin 1 receptor.

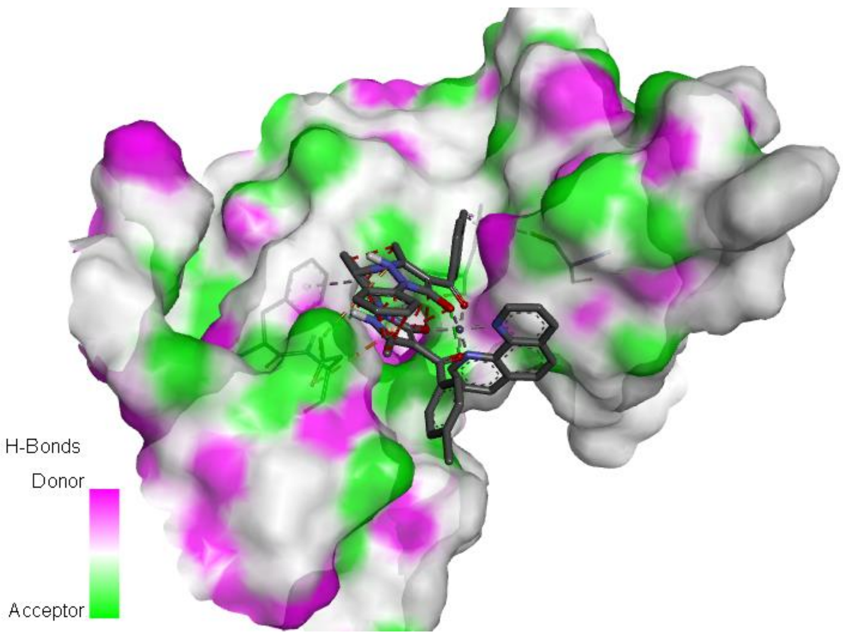


Fig. 5.5. Interaction analysis of 3QS1 with complex 3

Virtual screening against Falcipain-2 of *P.facliparum*

Similar to Plasmepsin-I, Falcipain-2 is involved in haem detoxification protein, which is essential for parasite viability in the Human body.[54] The degradation of haem in solution creates the space for replication in side host cell. This allows researchers to utilize Falcipain-2 as anti-malarial therapeutics target. A large number of FP2 inhibitors have been identified in several studies.[55,56] The most of which are peptide-based, although many peptidomimetics[57] and non-peptidic inhibitors [58] have also been found. Despite this proliferation of potential therapeutics, no antimalarials specifically targeting FP2 are currently available. Besides, some of the strongest Falcipain-2 inhibitors known are peptides, which limit their potential as drug candidates since they degrade rapidly in vivo and cannot be administered orally. In the present study, the structure of Falcipain-2 (PDB ID:3BPF) was downloaded from Protein databank. The structure contains 4 homologus protein and E64 Ligand Molecule.

Complex **17** with -10kcal/mol binding energy represents the lowest energy amongst the Libraries used in the present study. Similarly, complex 13, 15 and 19 had -9-9kcal/mol binding energy followed by complex **18**, complex **9**, complex **14** having a binding energy of -9.6 and -9.5kcal/mol (Fig. 5.11). The overall range of virtual screening ranging from -10 to -5.5kcal/mol binding energy.

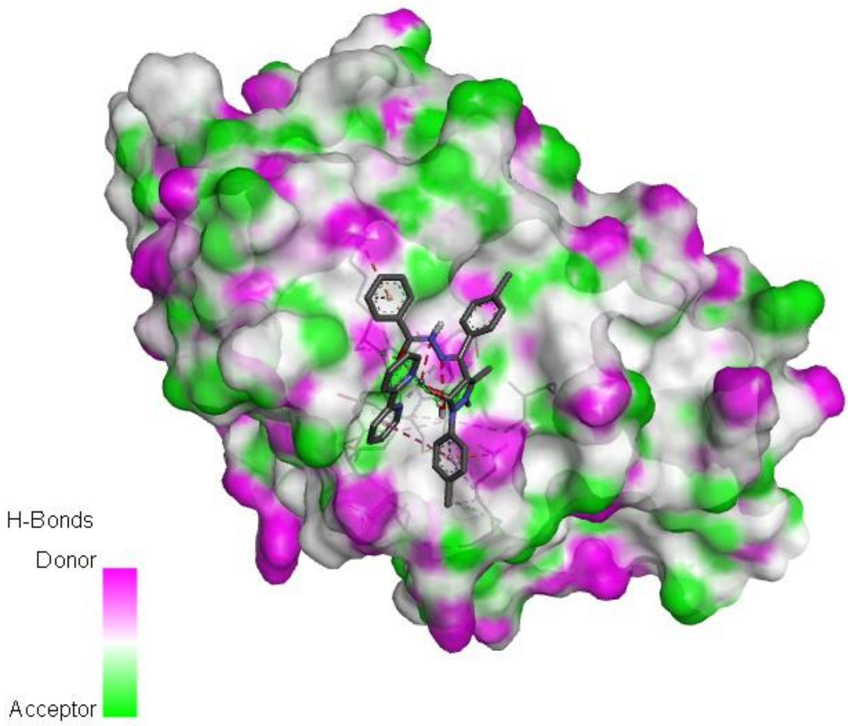


Fig. 5.6. Interaction analysis of 3QS1 with complex 17.

The results represent, complexes from 4-acylhydrazone-5-pyrazolone and their nine zinc(II) having significant binding efficiency against Falcipain-2. The 2 H-bond and 3 Van-der-Waals bonds were reported in the best docking pose of complex **17** with 3BPF receptor molecule. (Fig. 5.6) The h-bond was reported with Tyr55A-H and Lys122A. Whereas on amino acid Gln124A, Lys59A and Lys122A, van-der-Waals bond reported. Mendes *et al.* reported similar binding observed for Falcipain-2.[59] These results indicated the firm binding of the complex **17** with 3BPF protein structure.

Virtual screening against M1 aminopeptidase of *P.facliparum*

The *P. falciparum* malarial M1 aminopeptidase (PfA-M1) is an enzyme involved in the terminal stages of hemoglobin digestion and the generation of an amino acid pool within the parasite. The enzyme has been validated as a potential drug target since inhibitors of the enzyme block parasite growth *in vitro* and *in vivo*. Due to the emergence of drug resistance of parasite against sulfadoxine-pyrimethamine allow the doctors to prescribe the combination of artemisinin based therapy in uncomplicated malaria. In the present study, the 3D structure (PDB ID:4ZW3) was downloaded from the Protein Data Bank. The inhibitor molecule 4S9 was extracted and used as control Ligand present study. 4S9 inhibitor shown -8.3 kcal/mol binding energy whereas complex **2** and complex **7** shown the lowest binding energy of -13.5 kcal/mol followed by complex 4 -13.1 kcal/mol. While complex 1 and complex **9** having -13 kcal/mol binding energy. The overall binding energy observed for all compounds ranging from -13.5 kcal/mol to -8.3 kcal/mol (Fig. 5.12). The zinc derivatives were previously reported for inhibitory effect against aminopeptidases of *P.falciparum*.[60] The results indicated compounds from Library of 4-acylpyrazolone and their nine zinc(II) complexes might have good potentials as anti-malarial complex. This may further validated with comparing in vitro anti-malarial cytotoxicity assay. The 3 H-bond and 3 Van-der-Waals bonds were reported in the best docking pose of complex 17 with 3BPF receptor molecule. (Fig. 5.7) The h-bond was reported with Arg489A-NH_2, Arg489A-H and Thr492A-H amino acids chains/compound. Whereas, on amino acid Gln1038A, His496A and Thr492A, van-der-Waals bond reported. The X-ray crystal structure analysis revealed the H-bonds to Ala461A, Glu497A, Tyr580A and Tyr575A and Van-der-Waals bonds reported to Tyr575A and Val459A amino acid residues.[61]

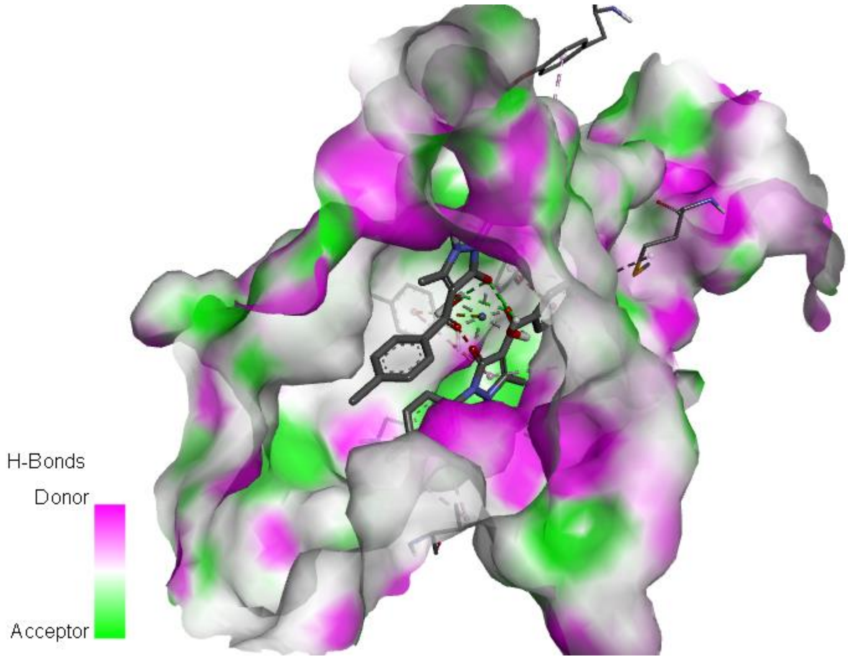


Fig. 5.7. Interaction analysis of 4ZW3 with complex *2*

Virtual screening for ADME-Toxicity

Lipinski's rule of five, also known as 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. Based on the observations of Christopher A. Lipinski in 1997 for orally administered drugs are relatively small and moderately lipophilic in nature.[62,63]

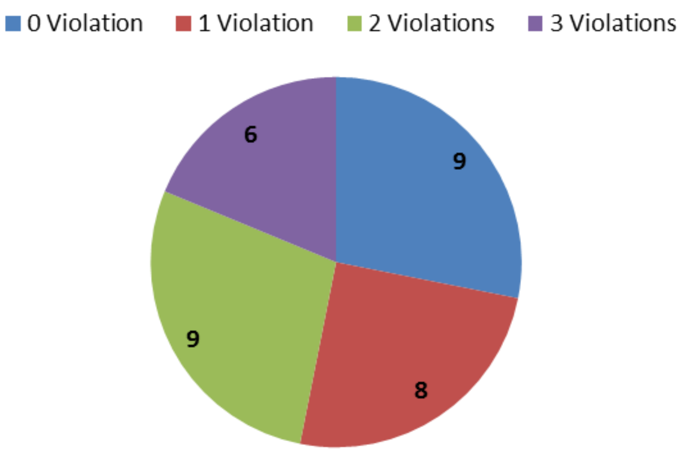


Fig. 5.8. Number of violations in Lipinski`s rule of five.

In the present study, all compounds were screened based on their pharmacokinetic properties using virtual screening protocols. In the case of Lipinski`s rule of five, 9 completely passed Lipinski's rule followed by 8 compounds with 1 violation. However, 9 compounds showed 2 violations and 6 compounds with 3 violations observed in the study. These compounds may need to lead optimisation to utilize for drug discovery (Fig. 5.8).

ADME (Absorption, Distribution, Metabolism and Excretion)

ADME is an abbreviation in pharmacokinetics and pharmacology for "absorption, distribution, metabolism, and excretion", and describes the disposition of a pharmaceutical compound within an organism. The four criteria all influence the drug levels and kinetics of drug exposure to the tissues and hence influence the performance and pharmacological activity of the compound as a drug. Some of the important factors were analysed in the present study.

The blood-brain barrier (BBB) permeability is an important criteria for drug discovery. It is the most important factor limiting the future growth of neurotherapeutics.[45,64] In the present study, out of total 32 compounds, 27 compounds were not able to cross Blood Brain Barrier. While 5 compounds can cross due to structural anomaly.

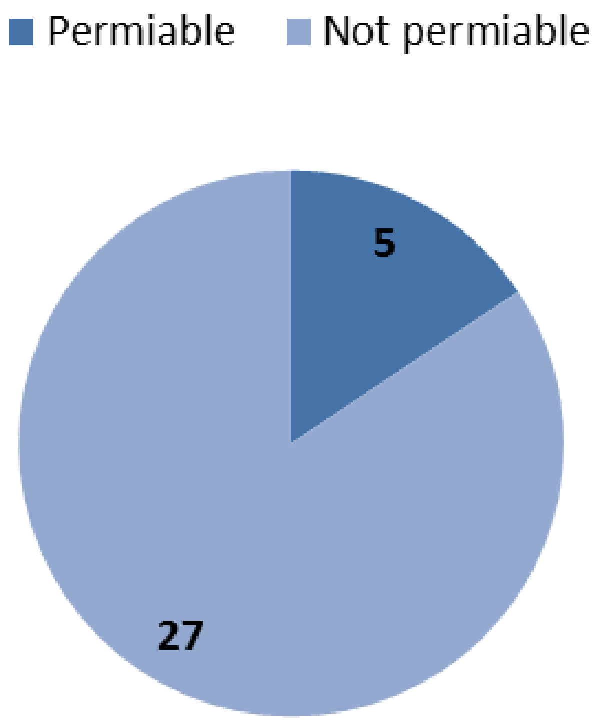


Fig. 5.9. Blood Brain Barrier (BBB) Permeability

Along with the BBB permeability, 16 compounds exhibited high Gastro intestinal absorption and remaining compounds possess a low level of GI absorption as per ADME-TOX results.

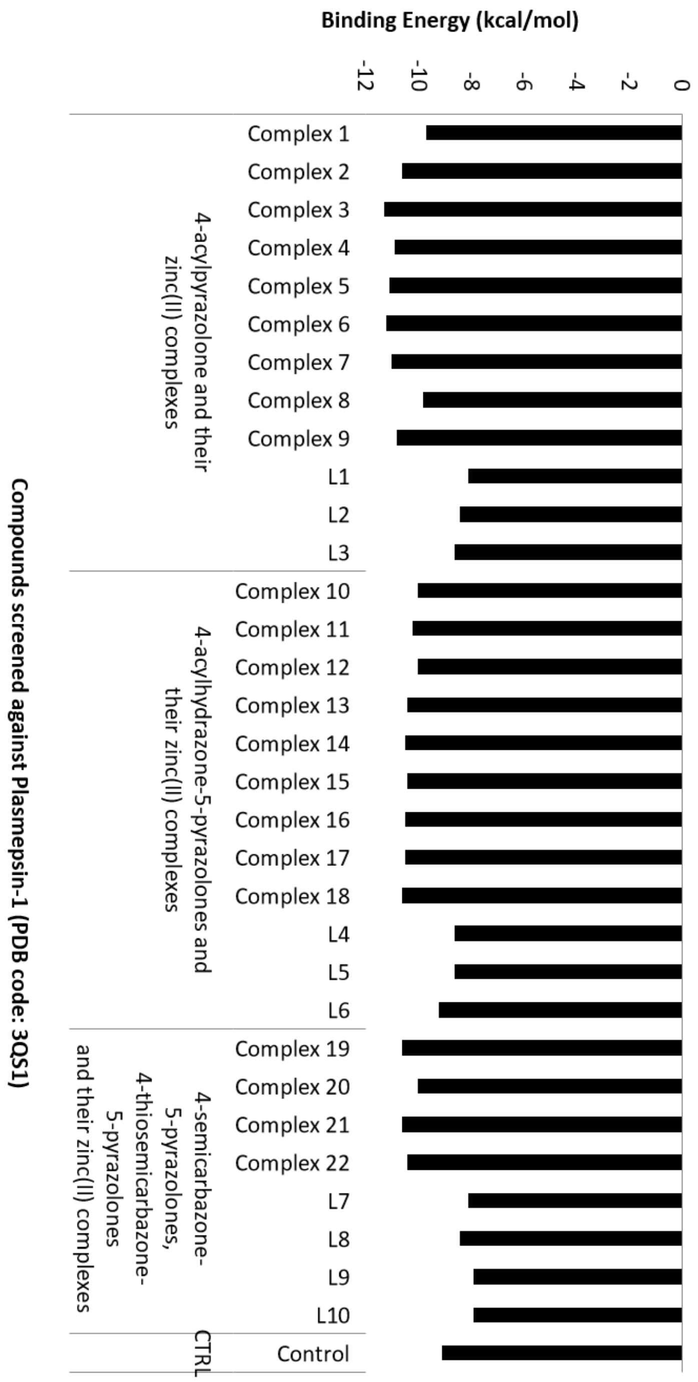


Fig. 5.10. The binding energy of ligand library against Plasmepsin- I of *P. falciparum*.

The compounds with a higher absorption rate provide excellent results in the drug delivery process. The Bioavailability of the compounds was ranging from 0.17 to 0.55 throughout the library. These results indicate poor to neutral Bioavailability in Human Models. Comparative Binding energy of the ligand library against 3 proteins of P. falciparum are shown in Fig. 5.13.

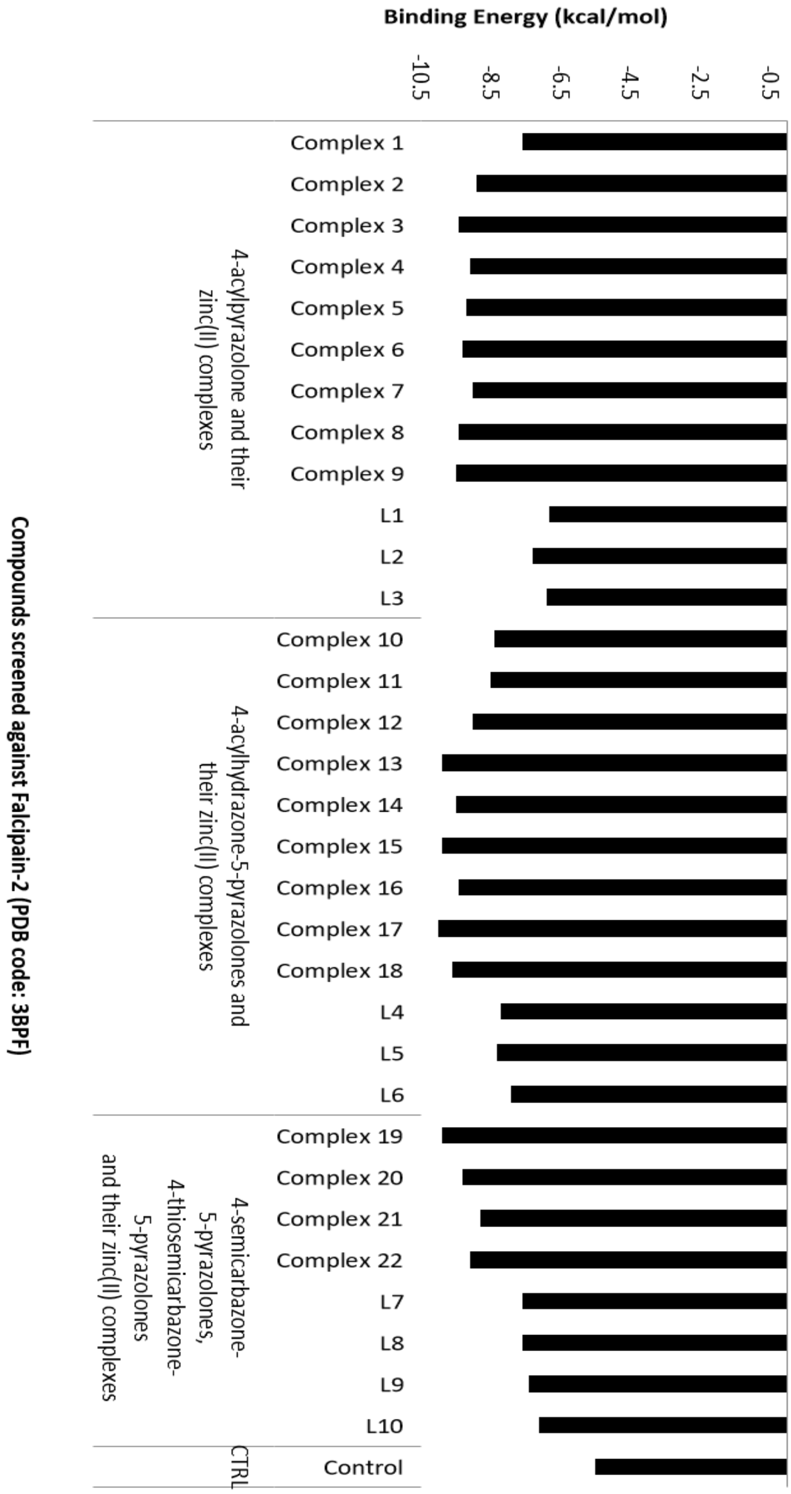


Fig. 5.11. The binding energy of ligand library against Falcipain-2 of *P. falciparum.*

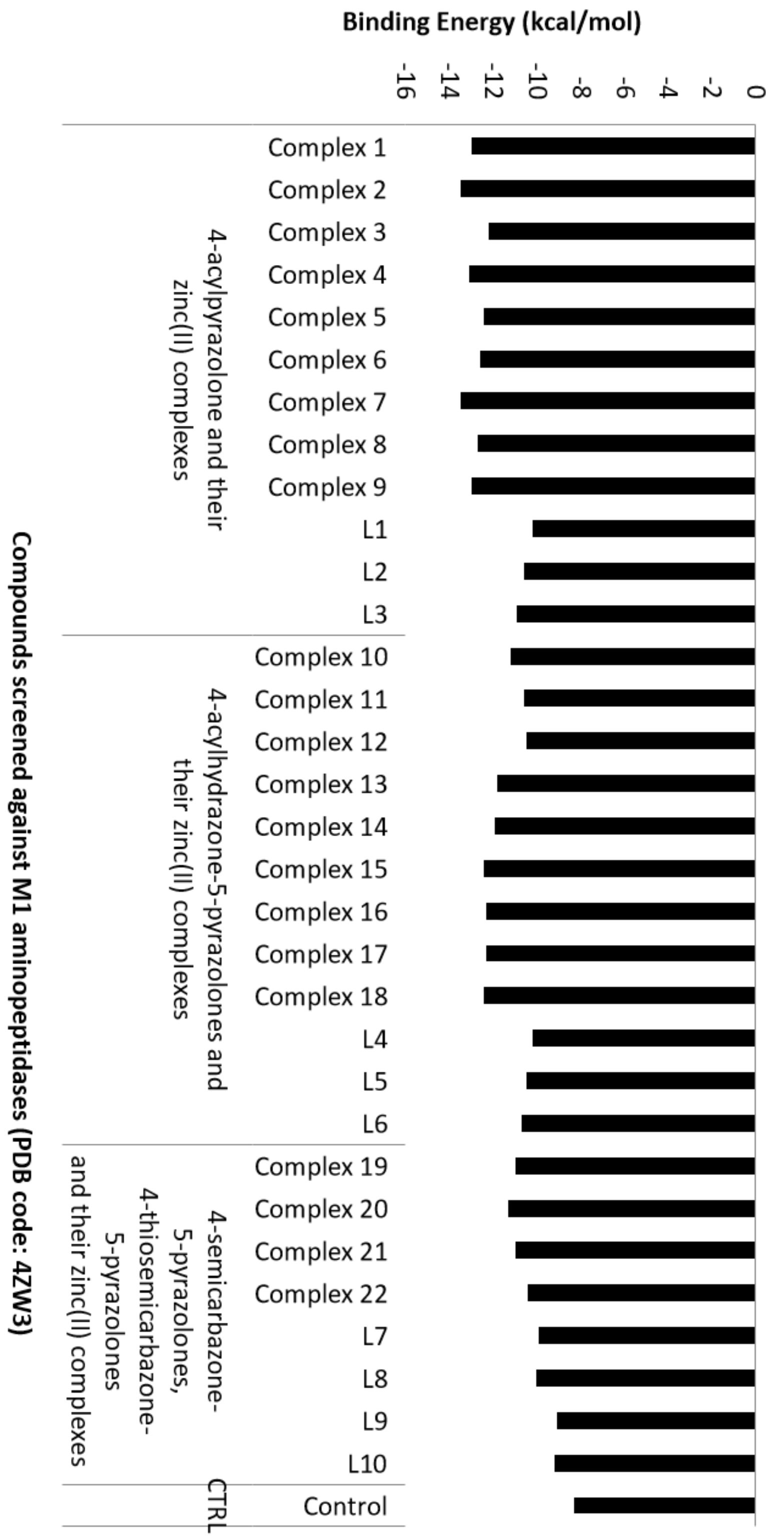


Fig. 5.12. The binding energy of ligand library against M1 Amino-peptidases of P. falciparum.

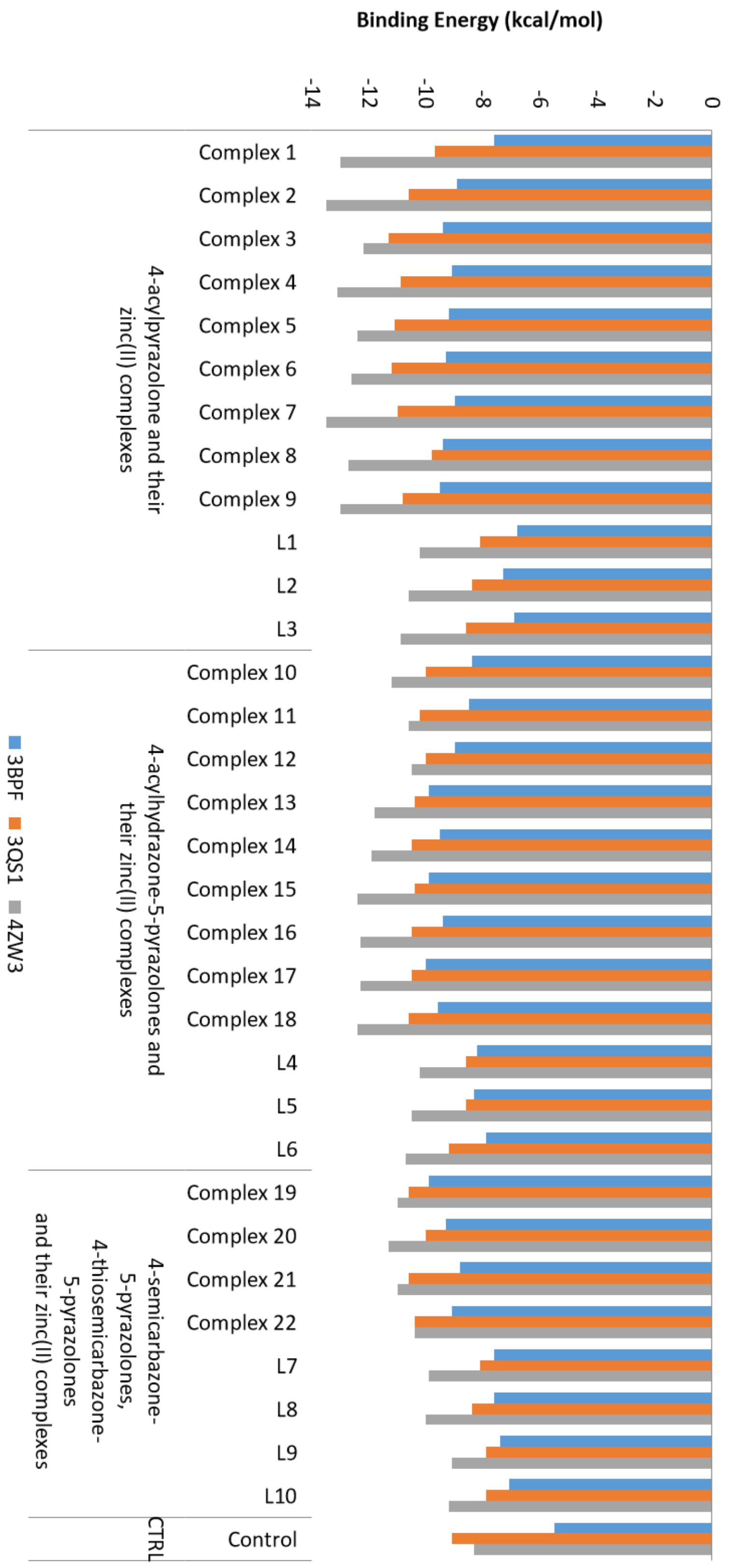


Fig. 5.13. Comparative Binding energy of the ligand library against 3 proteins of P. falciparum used in the present study

Conclusion

In the present work, we have experimented *in vitro and in silico* study for derivatives of pyrazolone compounds. The in vitro study was based on cytotoxic effects of compounds against the *P. falciparum* a deadly malarial parasite. While the molecular docking study was done against 3 different proteins downloaded from protein databank (PDB). Out of total 32, complex 2, complex 3 and complex 17 were found to have lowest binding affinity in *in silico* virtual screening. Whereas compound 6 found to have the lowest MIC value. The results indicated the potential of these compounds to have antimalarial activity. Besides, these compounds have zinc ant its derivatives, ADME-Toxicity study reported the number of Molecules having drug likeliness, Bioavailability and passed Lipinski`s rule of five, Which is prime. The results of in vitro and in silico anti-malarial study suggested some of the compounds have anti-malarial potential, these potent lead compounds could serve as advanced leads for further optimization and further exploration of antimalarial activity as well.

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