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PREDICTED DRUG LIKENESS AND MOLECULAR DOCKING STUDIES OF SOME TRANSITION METAL COMPLEXES AS INHIBITORS OF PLASMEPSIN II AND IV ENZYMES

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ABSTRACT

Despite tremendous growth in malaria chemotherapy, the malaria parasite had developed resistance to most antimalarial drugs including artemisinin, the core compound of the best antimalarial drugs available. Hence, there is a need to develop new antimalarial drugs. Malaria proteases have proven to be effective potential targets for malaria chemotherapy. Most antimalarial drugs developed, target the erythrocytic stage of the *Plasmodium falciparum* life cycle, however, plasmepsins II and IV act on the pre-erythrocytic liver stage and so are attractive drug targets. Recent advances in inorganic chemistry have shown transition metal complexes to be effective therapeutic agents. A library of 18 metal complexes was collected from the literature. The chemical structures of 18 metal complexes (ligands) were generated and optimized using Chemdraw ultra12 and chem3D. *In-silico* pharmacokinetics parameters were evaluated using SwissADME online server. Crystal structures of plasmepsin II (PDB ID: 5YIB) and IV (PDB ID: 1IS5) were collected from the protein data bank. They were prepared using Chimera and Autodock tools. Cygwin64 terminal was used to dock the ligands into the active site of the two targets. Post-docking analysis was performed with Chimera and Discovery Studio. All the metal complexes have shown favorable pharmacokinetics profile. One of the 'druggable' ligands coded Lig6 was found to have a higher binding affinity (-8.6 kcal / mol) to plasmepsin II than the native ligand, pepstatin A (-8.4 kcal / mol). For plasmepsin IV, Lig12 has the best binding affinity (-8.2 kcal / mol) compared with its native ligand (-5.6 kcal / mol). Therefore, Lig6 and Lig12 are potential antimalarial drug candidates and can be further investigated.

Keywords: Drug likeness, Malaria, Molecular docking, Plasmepsin II, Plasmepsin IV

INTRODUCTION

Transition metal complexes have shown intriguing properties as potentially effective medicinal agents. These substances have demonstrated a wide range of potential therapeutic uses, including the treatment of diabetes, typhoid, cancer and malaria (Selvaganapathy and Raman, 2016; Ravinderpal and Satya, 2019; Jaiswal *et al.,* 2020). Malaria is a disease caused by *Plasmodium* parasite. Humans often contract this parasite by the bite of an infected female Anopheles mosquito. Five specie of Plasmodium have been identified the malaria-causing agent, they are; *Plasmodium falciparum*, *Plasmodium* malariae, *Plasmodium* ovalae, sometimes known as *Plasmodium* vivax, and *Plasmodium* knowlesi (Kumar *et al.,* 2009). On the African continent, *Plasmodium falciparum* is known to be the deadliest and most common parasite.

An estimated 627, 000 deaths were recorded due to malaria in 2020 (WHO, 2021), most of them occurred on African continent. Malaria is a major public health concern in Nigeria, with an estimated 68 million cases

and 194 000 deaths due to the disease in 2021. Nigeria has the highest burden of malaria globally, accounting for nearly 27% of the global malaria burden with pregnant women and children being the most affected (WHO, 2022). Apart from being highly fatal, the disease is also associated with significant socio-economic burden (Onwujekwe *et al.,* 2013; Alonso *et al.,* 2019; Tefera *et al.,* 2020).

Unfortunately, most of the world's antimalarial medications are losing their effectiveness due to parasite resistance. This resistance to antimalarial medications results from spontaneous mutations that alter the drug target's molecular structure and function in the malaria parasite, or they alter the drug's ability to reach the target (Lindsay *et al.,* 2021). This necessitates the need for continuous search for new antimalarials. Recent strategies for the development of new antimalarial drugs involve identification of new targets (Arendse *et al.,* 2021; Forte *et al.,* 2021). Among those targets are various enzymes of *Plasmodium* including the proteases (Kumar *et al.,* 2018). These are enzymes responsible for the degradation of host haemoglobin, to provide the much-needed amino acids for the survival of the parasite, and for keeping the osmotic equilibrium of the infected erythrocyte during the intra-erythrocytic life cycle (Mauritz *et al*., 2009). Plasmepsins II and IV have been found in the acidic food vacuole of the parasite that digest hemoglobin and have been implicated in the degradation of the native heamoglobin (Rosenthal *et al.,* 2002),

Drug design and development now starts with computational studies to screen molecules and predict the possible lead compound before undergoing the physical experiment. This method is particularly appealing as flexible, cost effective, saves time and environmentally friendly (Stanzione *et al.,* 2021). *One the tools* employed is molecular docking. It is an *in-silico* technique that forecasts where ligands or tiny molecules will end up in the target protein's (receptor's) active site. This method is mostly utilized for precise assessment of the most advantageous binding modes and binding affinities of ligands with their respective receptors. At the moment, it is widely used in virtual screening for lead compound optimization (Berry *et al.,* 2015). In light with the above information, this research is aimed at investigating the potential of some transition metal complexes as antimalarial drugs with the objective of utilizing in-silico druglikeness and molecular docking studies to achieve that.

MATERIALS AND METHODS

Materials

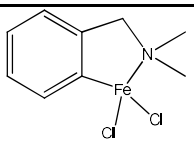
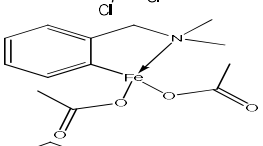
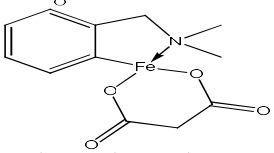
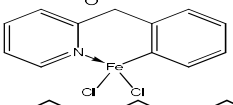
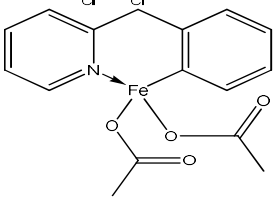
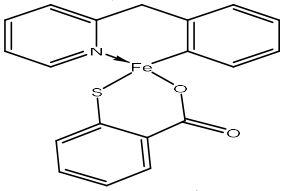
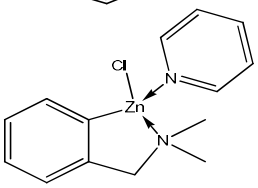
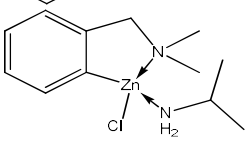
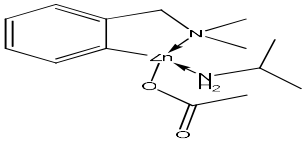
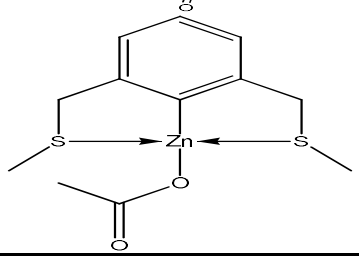
Hp computer system with the following specification: Intel® Pentium® CPU B960 @2.20GHz, @2.30GHz 4.00GB(RAM) with 64-bit operating system., Chemdraw professional 15.1 software, Chem3D 15.1, UCSF Chimera 1.11.2 software (Pettersen *et al.,* 2004), Autodock tools 1.5.6 (Trott and Olson, 2011), SwissADME online Server, Autodock Vina 1.5.6, Discovery studio 2020, Cygwin64 terminal, metal complexes (Fricker *et al*., 2008).

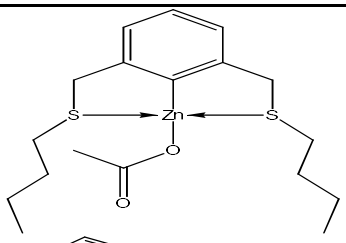
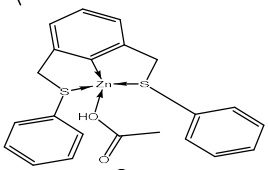
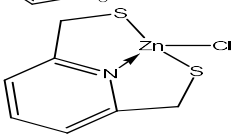
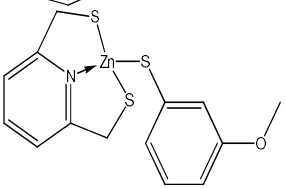
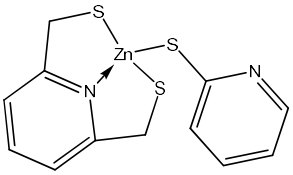
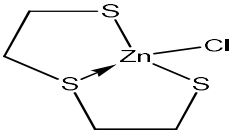
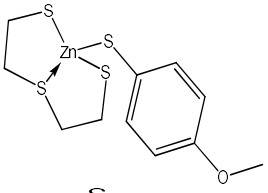
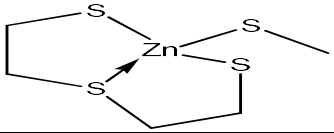
Methods

Data Collection

Eighteen transition metal complexes were taken from accessible scientific literature (Fricker *et al*., 2008). and their structures were created utilizing ChemDraw interface as shown in Table 1.

Table 1: 2D Structures of Transition Metal Complexes and their IUPAC Nomenclature

S/N	IUPAC Nomenclature	Structure
1	Dichloro (2-((2,2 dimethylamino) methyl) phenyl) iron (II)	
2	Diacetato (2-((2,2-dimethylamino) methyl) phenyl) iron (II)	
3	Malonato (2-((2,2-dimethylamino) methyl) phenyl) iron (II)	
4	Dichloro[2-[(2-pyridinyl-N) methyl] phenyl-C] iron (II)	
5	Diacetato[2-[(2-pyridinyl-N) methyl] phenyl-C] iron (II)	
6	[2-(mercapto-S) benzoato(2-)-O)] [2-[(2-pyridinyl-N) methyl] phenyl-C] iron (II)	
7	(Chloro)(pryidinyl)(2-((2,2-dimethylamino)methyl)phenyl)zinc(II)	
8	(Chloro)(isopropylamine)(2-((2,2-dimethylamino)methyl)phenyl)zinc(II)	
9	(Acetato)(isopropylamine)(2-((2,2-dimethylamino)methyl)phenyl)iron(II)	
10	Acetato[2,6-bis[(methylthio-S)methyl]phenyl-C]zinc(II)	

11	Acetato[2,6-bis[(butylthio-S)methyl]phenyl-C]zinc(II)	
12	Acetato[2,6-bis[(phenylthio-S) methyl] phenyl-C] zinc (II)	
13	(Chloro)[2,6-bis[(mercapto-S) methyl] pyridine-N] zinc (II)	
14	(M-methoxyphenylthiolato-S) [2,6-bis[(mercapto-S) methyl] pyridine-N] zinc (II)	
15	(2(1H)-pyridinethionato-jS2)[2,6-bis[(mercapto-S)methyl]pyridine-N]zinc(II)	
16	Chloro[2,2-(thio-S)bis[ethanethiolato-S)]]zinc(II)	
17	(P-methoxyphenylthiolato-S))[2,2-(thio-S)bis[ethanethiolato-S)]]zinc(II)	
18	(Methanethiolato)[2,2-(thio-S)bis[ethanethiolato-S)]] zinc(II).	

Pharmacokinetics and Drug Likeness Prediction

SwissADME free web server used to assess the pharmacokinetic, drug-likeness, and medicinal chemistry friendliness of small molecules, created and maintained by the Molecular Modelling Group of the Swiss Institute of Bioinformatics was utilized to predict the drug likeness of the complexes using the Lipinski rule of five.

Ligand Preparation

Chemdraw was used to create the 2D structures of the transition metal complexes. The 2D structures were transformed into 3D geometry using Chem3D software, they were then optimized and saved as lig.pdb files. Hydrogen and Gasteiger charges were added on Autodock tools. The pdb files were converted to pdbqt format for further

computational analyses using Autodock tools.

Protein Structure Preparation

Potential targets relevant to aspartic proteases were extracted from available published literature. The selected targets enzymes are plasmepsins II (PDB ID:5YIB) and IV (PDB ID: 1IS5). The 3D structures of the selected targets were obtained from RCSB- Protein Data Bank (www.rcsb/pdb). All the non- standard amino acids, crystallographic water molecules, ions and bonded ligands were removed using Chimera software (Pettersen *et.al*.,2004). The separated co-crystallized ligands were prepared on Chimera and saved as Lig.mol2, and the isolated receptors prepared too and saved as rec.pdb. The output files of Chimera were input to Autodock tools (Trott and Olson, 2011), were Lig.mol2 and rec.pdb were edited by adding polar hydrogen and Gastieger charges, then saved as pdbqt files and employed as receptors for the docking analysis.

Molecular Docking Analysis

The native (co-crystallized) ligands and the other ligands were docked on to the active site of the receptor using USCF Chimera, a grid box was applied on the receptor to indicate the interactions. After a successful docking protocol, re-formation of the complexes for further investigation was achieved utilizing Chimera. Discovery studio was used to visualize the interaction of the receptor and ligands. The grid box parameter for plasmepsin II is shown in Table 2.

Table 2: Grid Box Parameters for the Enzyme

Enzyme	Grid box size			Center		
	X	Y	Z	X	Y	Z
5YIB	66	34	52	20.168	-26.039	-24.719
1LS5	22.6832	10.2927	17.3983	-25.8948	37.0937	40.6325

Evaluation of Docking Methodologies

To ensure that the docking studies were valid, and represent the reasonable potential binding model, the docking methods and parameters were validated by re-docking experiment. The native ligand was docked into the protein to determine the ability of Auto dock program to reproduce the orientation and position of the ligand observed in the crystal structure. Procedure that gives conformation superimposable with geometrical conformation of the co-crystallized ligand in the active site was chosen.

RESULTS

Theoretical Oral Bioavailability

Table 3 present the molecular weight, hydrogen bond donor, hydrogen bond acceptor and MLogP value of the ligands (metal complexes) for predicting theoretical oral bioavailability based on Lipinski's rule of five.

Table 3: Theoretical Oral Bioavailability of some Metal Complexes Based on Lipinski 's Rule of Five

Compounds	Lipinski's rule of five					
	Mol. Wt.	HbA	HbD	BBB	MLogP	Inference
Lig1	260.95	0	0	Yes	3.06	Passed
Lig2	308.13	5	0	Yes	1.30	Passed
Lig3	292.09	5	0	Yes	0.63	Passed
Lig4	294.97	1	0	Yes	2.95	Passed
Lig5	342.15	5	0	Yes	1.43	Passed
Lig6	376.23	3	0	Yes	3,40	Passed
Lig7	314.13	2	0	Yes	2,28	Passed
Lig8	294,14	2	1	Yes	2.41	Passed
Lig9	317.73	4	1	Yes	1.66	Passed
Lig10	321.76	2	0	Yes	2.86	Passed
Lig11	405.92	2	0	No	4.38	Passed*
Lig12	445.90	2	0	No	5.38	Passed*
Lig13	285.13	1	0	Yes	1.80	Passed
Lig14	388.88	2	0	No	2.81	Passed
Lig15	359.84	2	0	No	2.03	Passed
Lig16	253.13	0	0	Yes	1.38	Passed
Lig17	401.98	1	0	No	4.14	Passed
Lig18	279.82	0	0	No	2.60	Passed

* = passed with one violation (MLogP>4.15)

Outcome of Validation of Docking Procedure

The docking procedures applied on the enzymes were well validated as shown in Table 4 all the co-crystallized ligands re-docked (cyan color) on their respective proteins are well super imposed on their original Protein Data Bank (PDB) structures.

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docked (cyan color) on their respective proteins are well super imposed on their original Protein Data Bank (PDB) structures.

Table 4: Crystal Structures of The Enzymes with Their Co-Crystallized Ligands and Those of Redocked Ligands

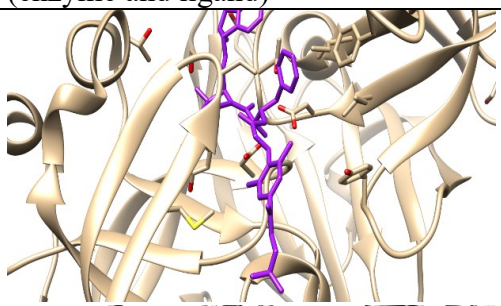
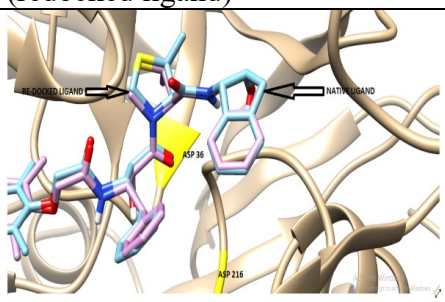
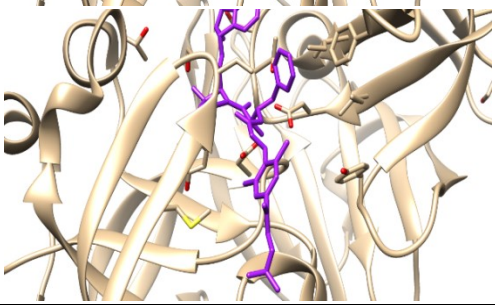
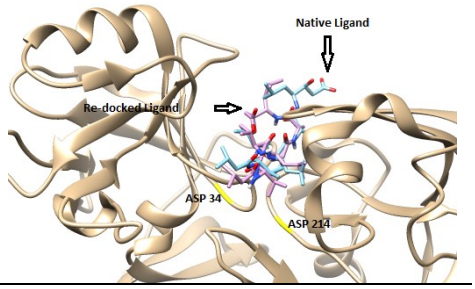
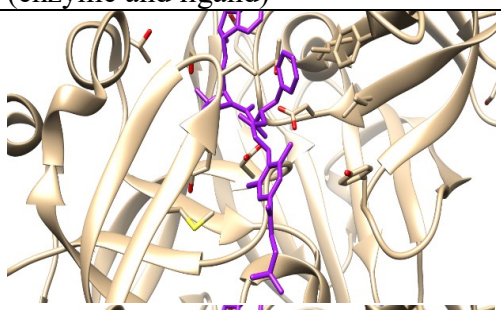
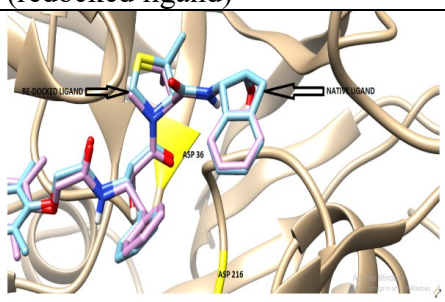
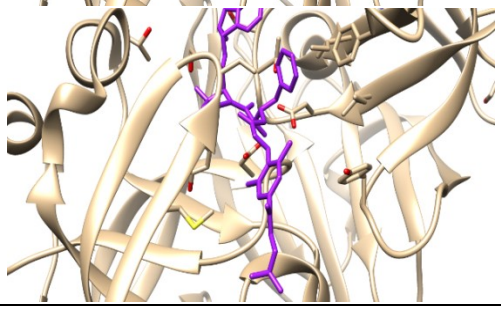
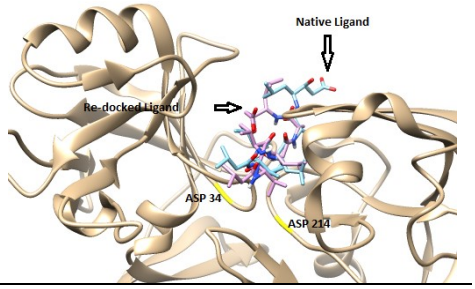
Enzyme	Crystal structure of complex (enzyme and ligand)	Crystal structure of complex (redocked ligand)
5YIB		
1IS4		

Table 4: Crystal Structures of The Enzymes with Their Co-Crystallized Ligands and Those of Redocked Ligands

Enzyme	Crystal structure of complex (enzyme and ligand)	Crystal structure of complex (redocked ligand)
5YIB		
1IS4		

Outcome of Docking Studies

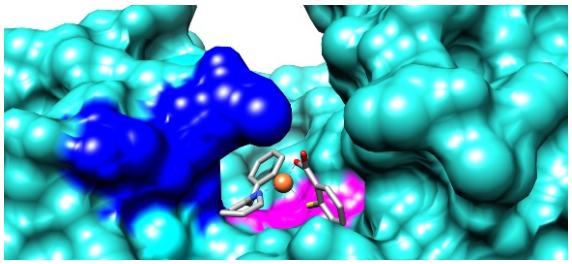
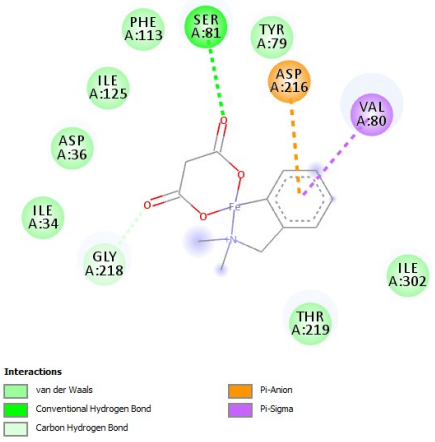
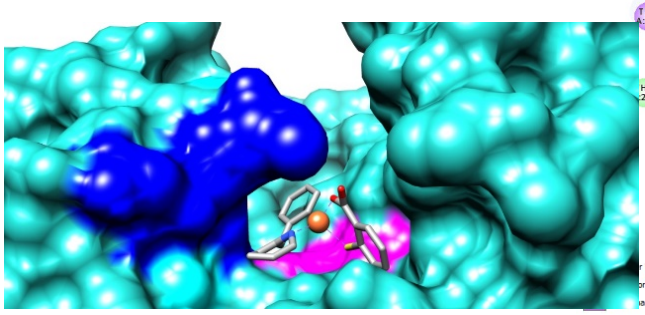
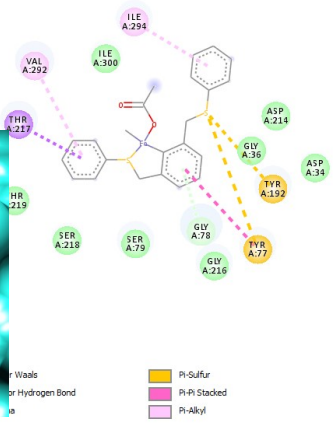
Docking procedure was employed to investigate the interaction between amino acid residues of the active pocket of these enzymes, with the ligands and also to estimate their binding affinities in kcal /mol. The binding affinities are presented in Table 5.

Table 5: The Binding Affinities in Kcal / Mol of The Co-Crystallized Ligands and Eighteen Transition Metal Complexes (Ligands) Against the Enzymes

S/N	Ligands	5YIB	1IS5
1	lig1	-5.6	-5-5
2	lig2	-5.5	-6.2
3	lig3	-6.7	-7.5
4	lig4	-6.6	-7.0
5	lig5	-6.4	-6.4
6	lig6	-8.6	-7.5
7	lig7	-6.2	-5.9
8	lig8	-5.5	-6.5
9	lig9	-5.9	-6.9
10	lig10	-5.8	-5.6
11	lig11	-6.2	-6.3
12	lig12	-7.4	-8.2
13	lig13	-4.7	-5.3
14	lig14	-6.0	-6.9
15	lig15	-5.8	-6.7
16	lig16	-3.6	-3.9
17	lig17	-4.5	-5.0
18	lig18	-3.4	-3.0
19	Native ligand	-8.4	-5.6

The binding poses and the types of interactions between the ligands and amino acid residues also determine the inhibitive effect of the ligands against the enzymes. Table 6 depicts the 3D and 2D binding poses and types of interactions in the studies complexes.

Table 6: 2D And 3D Binding Poses and Interactions of Lig6 and Lig12 in Relation To The Sites of The Enzymes

Complexes	3D binding poses (hydrophobic surface)	2D interactions
5YIB + lig6		
1IS5 + lig12		

DISCUSSION

Prediction of theoretical oral bioavailability is an important step in drug design process. This is to reduce the chances of drug failure at a later stage of the process due to poor pharmacokinetics. According to Lipinski rule of five orally active compounds should compose of not more than five hydrogen bond donors and ten hydrogen bond acceptors. The molecular weight of the compound must be <500g/mol, as increasing molecular weight reduces the compound concentration at the surface of the intestinal epithelium, hence lessens the absorption. An octanol-water partition coefficient MLogP

must not be greater than five to prevent poor absorption or permeation (Lipinski, 2004; Lipinski *et al.,* 2012). From Table 4.1, Sixteen of the selected metal complexes have passed the criteria put forward by Lipinski for a chemical compound to be orally bioavailable and thus are good candidate for further evaluation. However, two complexes (Lig11 and Lig12) passed the test with one violation (MLogP > 4.15). Violating one of the criteria does not disqualify the compound from being orally active.

Docking results are shown in Table 4.3. From the docking scores, it can be deduced that the Lig6 has better binding affinity on 5YIB (-8.6 kcal / mol) which was higher than that of the co-crystallized ligand (-8.4 kcal / mol). Although few compounds have shown appreciable inhibition to the aspartic proteases, reports have shown that inhibition of plasmepsin is desirable in potential antimalarial to ensure clinical efficacy and combat resistance due to their complementary role (Coombs *et al.,* 2001; Liu *et al.,* 2005; 2006; Sriwilaijaroen *et al.,* 2006). Other compounds investigated particularly lig12, lig3, and lig4 have a reasonable binding affinity of -7.4, -6.7, and -6.6 kcal / mol respectively.

The ligand-receptor complex formed between lig6 and 5YIB was mainly due to electrostatic interactions between the ligand and the amino acid residues in the active site of the enzyme (Table 4.4). Notable of these interactions are the pi-anion interaction between benzene ring of Lig6 with the catalytic amino acids (Asp36 and Asp216) in the active pocket and on the flap region of the enzymes. Other important interactions include Van der Waal's interactions with PHEA:296, SER:39, TYR:79 and THR:219. Pi-alkyl interaction was also observed with VAL:80 and pi-sigma interaction occurred between ILE:214 and the benzene ring of the complex. These widespread interactions contribute significantly to the high binding affinity of this complex (Klenam *et al.*, 2021).

From Table 4.4, it can be observed that lig6 fit into the binding pocket of 5YIB, resting on the catalytic dyad and fully covered with the flap. This gives the ligand suitable conformation to interact with the catalytic amino acids of the enzyme (ASP26 and ASP216), and thereby hindering them from further interaction with another incoming substrate and hence inhibiting its action as earlier observed (Meliza *et al.,* 2024).

In the case of 1IS5, lig12 has the highest binding affinity (-8.2 kcal / mol), compared with the co-crystallized or native ligand (Pepstatin A) -5.6 kcal / mol. Other ligands; lig6 (-7.5 kcal / mol), lig3 (-7.5 kcal / mol), lig4 (-7.0 kcal / mol) and lig9 (-6.9 kcal / mol) showed appreciable binding affinity and the remaining thirteen compounds have lower binding affinity compared with the native ligands. From Table 4.4, lig12 situated itself inside the active cavity of the enzyme, thereby covering the catalytic enzymes and thus, prevent them from exerting their action. The ligand also of interact with the amino acid residues of this enzyme. Van der Waal's interaction was observed between the catalytic dyad (ASP:34 and ASP:214) and the ligand, other amino acid residues involve in Van der Waal's interaction include LEU:290, THR:219: SER:218, SER:79, GLY:216, GLY:36 and ILE:300. There is a hydrogen formation between GLY:78 and the complex. TYR:77, THR:217 and VAL:292 were involve in pi-pi stacked, pi-sigma and p-alkyl interactions with the complex. This has been implicated in the observed high binding affinities of some compounds (Nervall *et al.,* 2006; Prakoso *et al.,* 2021). The aforementioned observations imply that lig6 and lig12 are potential antimalarial

agents. The compounds have also passed the Lipinski rule for compound to have good oral bioavailability (although Lig12 passed with one violation) thus they are druggable candidate.

CONCLUSION

Eighteen metal complexes were investigated as potential antimalarials via *In silico* studies to assess their inhibitive effect towards plasmepsin II and IV enzymes of *Plasmodium falciparum* which are potential targets for antimalarial drug design and development. Pharmacokinetics properties of the compounds was also predicted using Lipinski rule of 5. The compounds also passed the rule of 5 (although Lig12 has one violation, MLogP > 4.15) indicating their good oral bioavailability and drug likeness. Molecular docking studies pointed out that lig6 and 12 are potential inhibitors of plasmepsins II and IV respectively having binding affinity higher than those of their co-crystallized or native ligand. This high binding affinity is attributed to widespread interactions between these complexes and the amino acid residues of the enzymes which include hydrogen bond, Pi-alkyl and Van der Waals interactions. Therefore, these compounds are potential lead candidate for antimalarial drug design and development.

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