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Proposing *de-novo* Generated, Iteratively Optimized New Lead Molecules Targeting *Plasmodium falciparum* Plasmepsin-II

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Abstract: Hemoglobin degradation pathway is the major source of nutrition of *Plasmodium falciparum*. Plasmepsin II is the key enzyme in hemoglobin degradation with two catalytic aspartates D34 and D214. The energy refined structure of Plasmepsin II of *Plasmodium falciparum* modeled by modeller 9v8 using PDB entry 1PFZ as template. Ligsite[csc] program revealed three potential ligand binding sites where the **pkt-364** is found to be more favorable containing critical aspartic residues (D34 and D214). Urea and its derivative, benzamide, are considered as seed molecules for *de-novo* generation of structurally complimentary lead molecules. LigbuilderV1.2 growing strategy was used for *de-novo* generation with 10 population cycles for each seed molecules. Binding energies were examined by Quantum3.3.0 for all the designed ligand molecules and the best molecules with -20KJ/mole and -18KJ/mole were found with some undesired activities. Iterative ***in-silico*** optimization of the examined molecules were done and finally *de-novo* generated ***N*** **- [(*R*) – hydroxy { [2 - (1 , 3 – oxathiol – 4 –yl) phenyl] amino } methyl] acetamide** and ***N*** **- (2 – amino – 2 – oxoethyl) – 3 - (1 - { [2 - (methylamino) ethyl] (phenyl) amino } vinyl) benzamide** were found as the best fit over rule of 5 and other ADME parameters. Binding studies suggest the direct interaction of designed molecules with catalytic aspartates (D34 and D214) of Plasmepsin II having 2HB and 1 ionic bond and good number of VdW interactions. Thus, the designed molecules could possibly inhibit the action of PM II preventing the degradation of hemoglobin and thereby kill the parasite by starvation.

Keywords: Malaria, *Plasmodium falciparum*, Aspartic protease, Plasmepsin II.

Abbreviations: D34 and D214: Aspartic acid 34 and Aspartic acid 214 respectively, PM II: Plasmepsin II, VdW: Vander wall, IC50: Inhibitory concentration.

Introduction:

Malaria remains a human disease of global significant and a major cause of high infant mortality in endemic nations [(1)] and nearly all malarial deaths are caused by *Plasmodium falciparum*, a protozoan parasite that lives in human red blood cells during its asexual life cycle. Drug resistance in *P.falciparum* is an enormous problem and new antimalarial agents focussing towards novel mechanism of action [(2)] are needed desperately. In the effort to develop new treatments for malaria, hemoglobin degradation by aspartic proteases in acidic digestive vacuole of the parasite has generated substantial interest because it is a major metabolic pathway of the parasite and is believed to be essential for the survival of the parasite[(3)].

PM II is the best studied member of aspartic proteases[(4)] responsible for initial hemoglobin degradation in intraerythrocytic *Plasmodium*

falciparum and it has become an attractive drug target for novel therapeutic compound to treat malaria[5]. Research regarding its importance in the *P.falciparum* metabolism and life cycle, makes it the target of choice for structure based drug designed [6].

PM II is translated as inactive zymogen where a large shift between the N-domain and the Central and the C-domain of proplasmepsin II opens the active site cleft, preventing the formation of a functional aspartic proteinase active site [7]. Activation of PM II involves the cleavage of its N-terminal part and this transition (maturation) brings about a domain shift in the enzyme's N-domain, which enables the formation of the catalytic site. The catalytic residues are D34 and D214 one of which is protonated while the other is negatively charged in the digestive vacuole (pH≈5) where hemoglobin cleavage takes place [8]. PM II has a remarkable stringent specificity towards native hemoglobin [3] and is involved in the early steps of hemoglobin degradation. They are able to recognize intact hemoglobin and make an initial cleavage in the β-helix of the hemoglobin α-chain between **Phe33** and **Leu34**. The cleavage is believed to unravel the globin chain, facilitating subsequent proteolytic cleavages [4]. (Figure 1 represents the hemoglobin degradation pathway in *P. falciparum*).

Methodology:

Energy refinement of PM II was modeled by Modeller 9v8 [9] using PDB entry 1PFZ as a template. The predicted models were evaluated for geometry, stereochemistry checks and energy distribution using PROCHECK [10]. The models were systematically analyzed using ProSA [11, 12] for various structural properties and the best modeled structure containing 94.6% residues in the core region of the Ramachandran plot was selected as the docking target enzyme.

Three potential binding sites of modeled PM II were revealed by Ligsite^{csc} [13] program where **pkt-364** is found to be the most favorable and conserved region containing critical aspartic residues (D34 and D214) and has a better binding affinity.

In this study, **urea** moiety and its derivative, **benzamide**, are considered as seed molecules for the *de-novo* generation with a final output of twenty structurally complimentary potential lead molecules using LigbuilderV1.2 [14]. All the twenty *de-novo* designed and selected ligand molecules were docked into the target enzyme using Quantum3.3.0 [15]. The ligands block the active site by directly interacting with either one or both of the catalytic aspartates, D34 and D214. The binding energies for all the twenty designed ligand molecules as examined by Quatum3.3.0 ranges between -26 to -7KJ/mole and -21 to -11KJ/mole (Result summarized in table I and II).

Lead optimization:

Bioisosteric replacement concept was employed over the examined lead molecules to circumvent undesirable ADME profiles and simultaneously optimizes lead compounds. Finally *de-novo* generated derivatives in-silico optimized ***N* - [(*R*) – hydroxy { [2 - (1 , 3 – oxathiol – 4 – yl) phenyl] amino } methyl]acetamide** and ***N*-(2-amino-2-oxoethyl)-3-(1-{[2-(methylamino)ethyl] (phenyl)amino}vinyl) benzamide** were found as the best fit over rule of five and other ADME parameters.

Binding studies suggest the direct interaction of the designed molecules with the catalytic aspartates dyads (D34 and D214) of PM II with the maximum IC_{50} value of 2.67e-004 mole/L and 6.4e-006 mole/L for *N* - [(*R*) – hydroxy { [2 - (1 , 3 – oxathiol – 4 – yl) phenyl] amino } methyl] acetamide and *N* - (2 – amino – 2 – oxoethyl) – 3 - (1 - { [2 - (methylamino) ethyl] (phenyl) amino } vinyl) benzamide respectively.

Result and Discussion:

Five modeled structures of PM II generated by Modeller 9v8 contains 93% to 94% residues in the core region of Ramachandran plot and the overall G-factors ranges between -0.09 to -0.04. Z-scores were within the range and energy functions of the residues were at minimum as analyzed by ProSA.

Binding pocket determination of modeled PM II by Ligsite^{csc} program revealed three potential binding sites **pkt-364**, **pkt-29** and **pkt- 15** where pkt-364 was found to be the major cleft with critical D34 and D214.

Ligand docking predicted the binding of generated derivatives at the substrate binding cleft (Figure 3(i) & 3(ii), Figure 4(i) & 4(ii)) with negative interaction energy and efficient binding. Pharmacokinetic properties analysis of the optimized lead molecules (Result summarized in Table III(a) & III(b)) performed by MolsoftLLC program predicted minimum number of Hydrogen bond acceptors, Hydrogen bond donors and molecular weight of 266.07Dalton and 352.19Dalton for urea derived ligand and benzamide derived ligand respectively. The partition co-efficient CLogP and Solubility CLogS were found to be minimal for both the designed ligands. These observed properties suggested good absorption and easy transportation of the molecule across the membrane, which according to the rule of five; a compound could possibly behave as a drug. Pharmacodynamically, the designed molecules *N*-[(*R*)-hydroxy{[2-(1,3-oxathiol-4-yl)phenyl]amino}methyl]

acetamide, exhibited 2 of 6 possible pharmacological effects (Uric acid excretion stimulant and Diuretic) and 2 of 3 side effects and toxicity (Embryotoxicity and Teratogen). Whereas, *N* - (2 – amino – 2 – oxoethyl) – 3 - (1 - { [2 - (methylamino) ethyl] (phenyl) amino } vinyl) benzamide designed molecule was found to exhibit 1 of 6 possible pharmacological effects (Vasodilator) and 1 of 3 possible side effects and toxicity (Embryotoxicity). The pharmacodynamic properties were calculated using PASS program[16] at Pa>Pi.

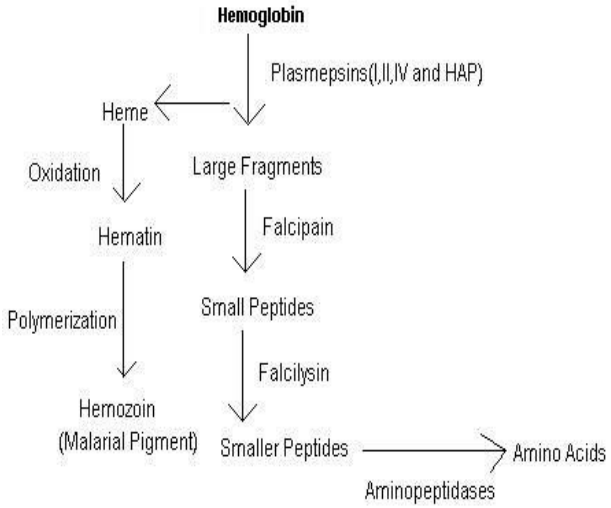

Figure 1: Schematic representation of hemoglobin degradation pathway in *P.falciparum*.

Docking	IC50 Mol/L	GbindKJ/Mol	E es KJ/Mol	Evdw KJ	TdsKJ/Mol	Etor KJ/Mol	Gprot	Charge	Mass	Flex bond	RMS
Lig_1;	1.86e-003;	-15.89;	-2.86;	-32.44;	23.18;	42.59;	0.00;	0;	326;	1;	39.38
Lig_2;	4.54e-005;	-25.28;	-3.18;	-34.45;	-13.82;	-1.47;	0.00;	0;	387;	1;	42.29
Lig_3;	5.48e-005;	-24.80;	1.67;	-38.65;	-5.07;	7.10;	0.00;	0;	346;	1;	41.85
Lig_4;	1.31e-002;	-10.96;	18.46;	-27.96;	9.00;	7.55;	0.00;	-1;	368;	0;	42.91
Lig_5;	1.39e-002;	-10.80;	8.07;	-25.68;	14.53;	21.33;	0.00;	-1;	325;	1;	42.91
Lig_6;	1.83e-002;	-10.12;	-3.14;	-16.73;	5.25;	15.00;	0.00;	0;	296;	0;	45.94
Lig_7;	1.01e-003;	-17.44;	5.81;	-30.60;	1.32;	8.67;	0.00;	0;	361;	0;	44.36
Lig_8;	3.00e-005;	-26.33;	-1.91;	-40.78;	-1.32;	15.05;	0.00;	0;	332;	0;	40.21
Lig_9;	3.27e-004;	-20.29;	-8.15;	-24.91;	-12.16;	0.62;	0.00;	0;	244;	0;	39.07
Lig_10;	5.64e-002;	-7.27;	6.67;	-15.93;	4.28;	6.27;	0.00;	0;	310;	1;	43.09

Table I: Quantum 3.3.0 output for urea derived ligands.

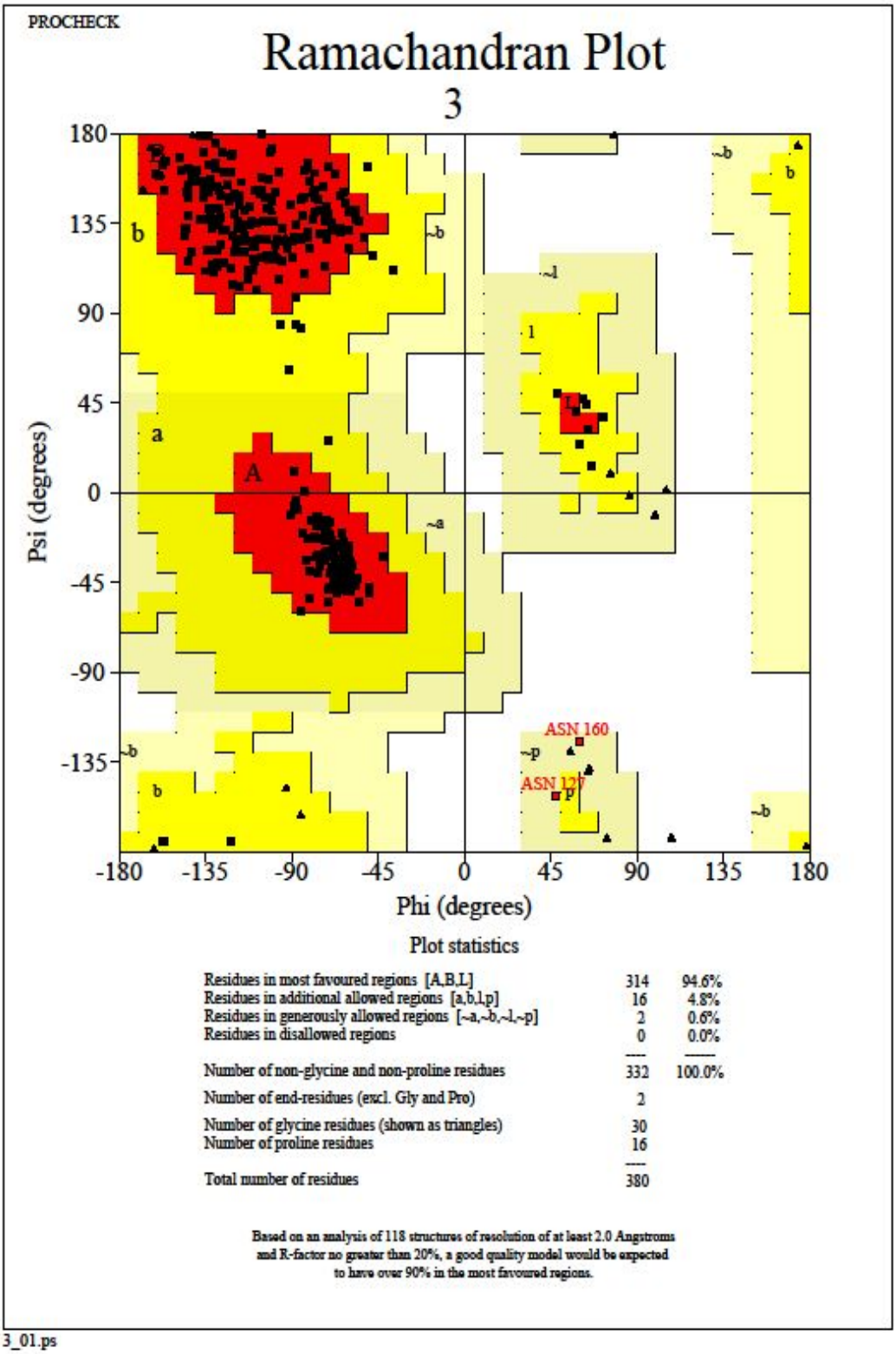

Figure 2: Representation of Ramachandran plot for modeled PM II.

Docking	IC50 Mol/L	G bind KJ/Mol	E es KJ/Mol	E vdw KJ	T dsKJ/Mol	E tor KJ/Mol	G prot	Charge	Mass	Flex bond	RMS
Lig_1;	7.71e-003;	-12.30;	9.71;	-31.24;	22.36;	31.60;	0.00;	-1;	326;	0;	42.14
Lig_2;	1.21e-003;	-16.98;	-0.85;	-25.19;	-4.54;	4.51;	0.00;	0;	326 ;	0;	43.27
Lig_3;	2.18e-004;	-21.31;	-1.32;	-40.32;	19.22;	39.55;	0.00;	0;	395;	1;	41.42
Lig_4;	9.66e-004;	-17.55;	-0.84;	-26.72;	-2.72;	7.29;	0.00;	0;	326;	0;	43.61
Lig_5;	5.23e-004;	-19.10;	10.00;	-34.45;	-1.04;	4.32;	0.00;	-1;	328;	2;	43.74
Lig_6;	6.19e-004;	-18.67;	-1.67;	-28.29;	-2.42;	8.86;	0.00;	0;	346;	0;	41.48
Lig_7;	5.30e-004;	-19.06;	-1.94;	-29.74;	0.29;	12.91;	0.00;	0;	376;	0;	42.98
Lig_8;	6.95e-004;	-18.38;	-4.38;	-27.99;	-0.38;	13.61;	0.00;	0;	369;	3;	44.35
Lig_9;	9.05e-003;	-11.89;	-1.62;	-26.08;	22.34	38.16;	0.00;	0;	322;	0;	44.46
Lig_10;	1.79e-003;	-15.99;	13.12;	-33.42;	7.74;	12.05;	0.00;	-1;	326;	0;	40.02

Table II: Quantum3.3.0 output for benzamide derived ligands.

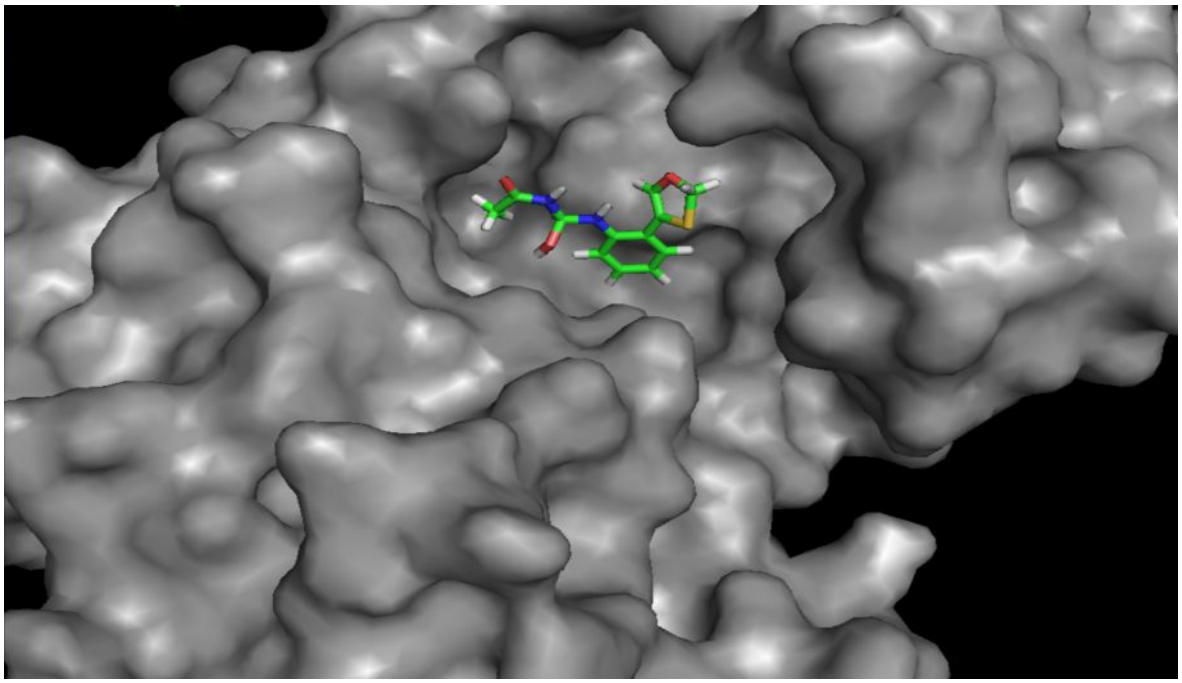

Figure 3(i): Surface representation of PM II in complex with *N*-[(*R*)-hydroxy {[2-(1, 3-oxathiol-4-yl) phenyl] amino} methyl] acetamide.

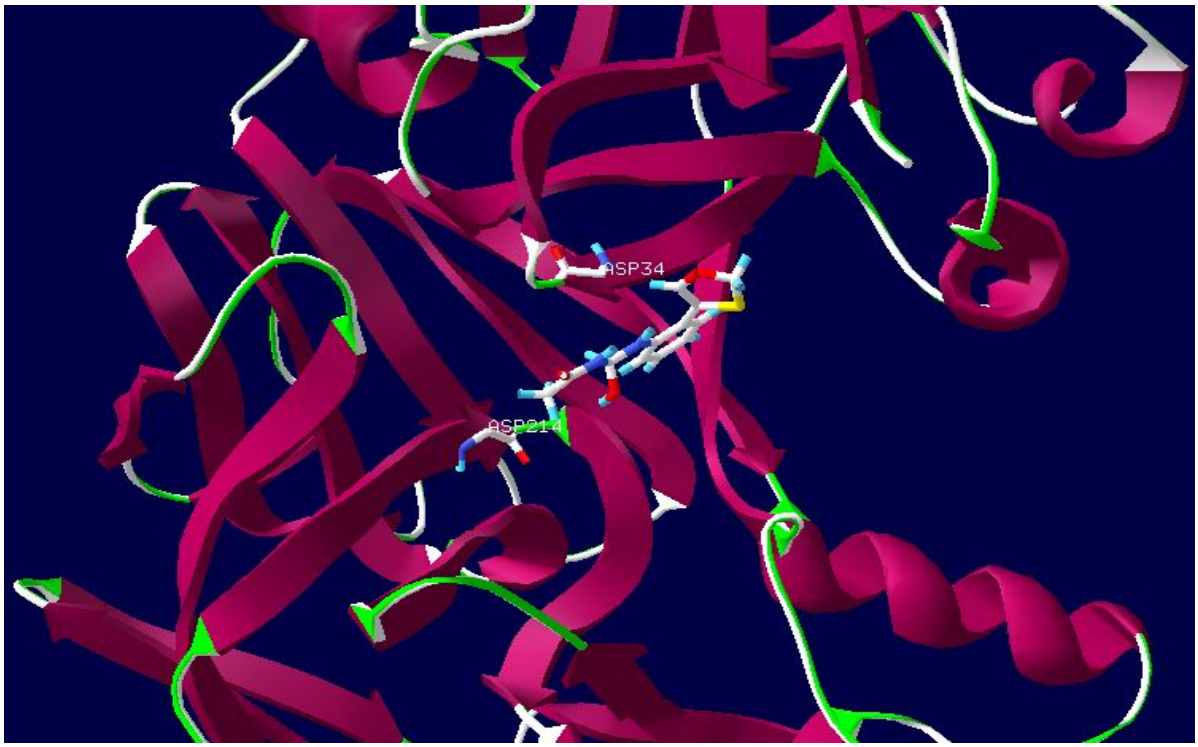

Figure 3(ii): Ribbon representation of PM II in complex with *N*-[(*R*)-hydroxy {[2-(1, 3-oxathiol -4-yl) phenyl] amino} methyl] acetamide.

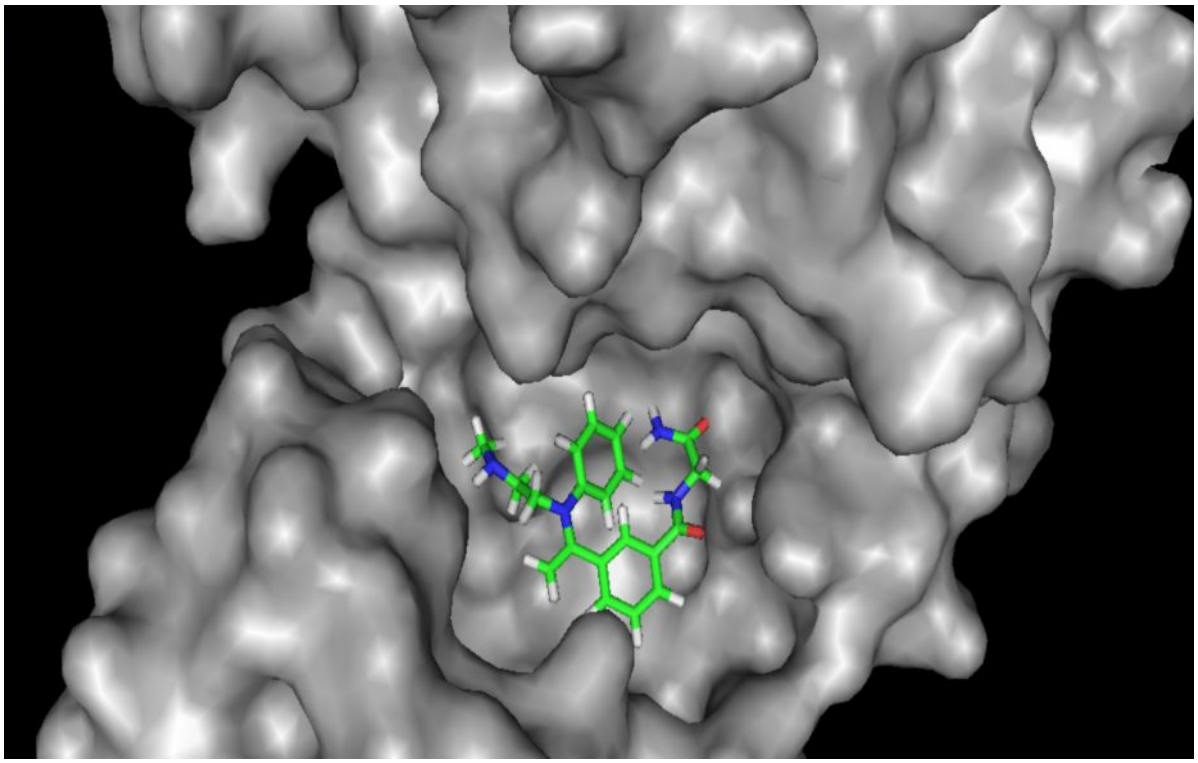

Figure 4(i): Surface representation of PM II in complex with *N*-(2-amino-2-oxoethyl) -3-(1-{[2-(methylamino)ethyl](phenyl)amino}vinyl)benzamide.

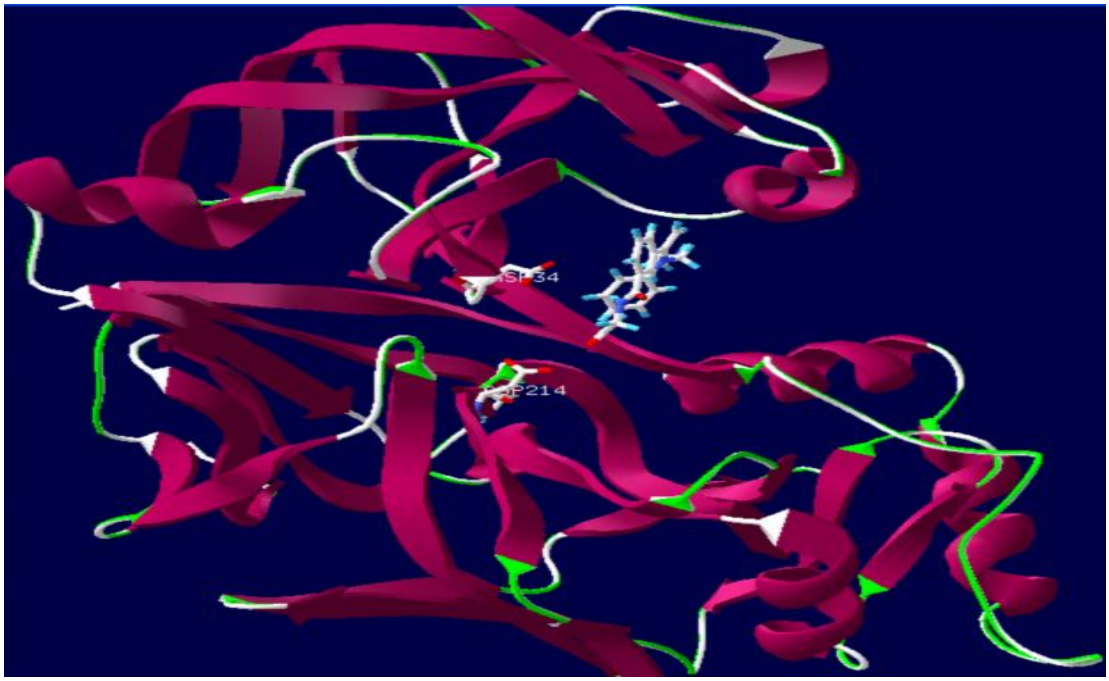

Figure 4(ii): Ribbon representation of PM II in complex with *N*-(2-amino-2-oxoethyl) -3-(1-{[2-(methylamino)ethyl](phenyl)amino}vinyl)benzamide.

Table III(a): Molsoft LLC output for *N* - [(*R*) – hydroxy { [2 - (1 , 3 – oxathiol – 4 – yl) phenyl] amino } methyl] acetamide.

Molecular formula: C12 H14 N2 O3 S
Molecular weight: 266.07
Number of HBA: 4
Number of HBD: 3
MolLogP : 2.17
MolLogS : -3.31 (in Log(moles/L)) 130.85 (in mg/L)
MolPSA : 62.57 A^2
MolVol : 244.51 A^3
Number of stereo centers: 1

TableIII(b):Molsoft LLC output for *N*-(2–amino–2–oxoethyl)–3–(1-{[2-(methylamino) ethyl](phenyl) amino}vinyl) benzamide.

Molecular formula: C20 H24 N4 O2
Molecular weight: 352.19
Number of HBA: 3
Number of HBD: 4
MolLogP: 2.01
MolLogS: -4.29 (in Log (moles/L)) 18.09 (in mg/L)
MolPSA: 74.55 A^2
MolVol: 374.05 A^3
Number of stereo centers: 0

Drug Likeness model score: -1.29

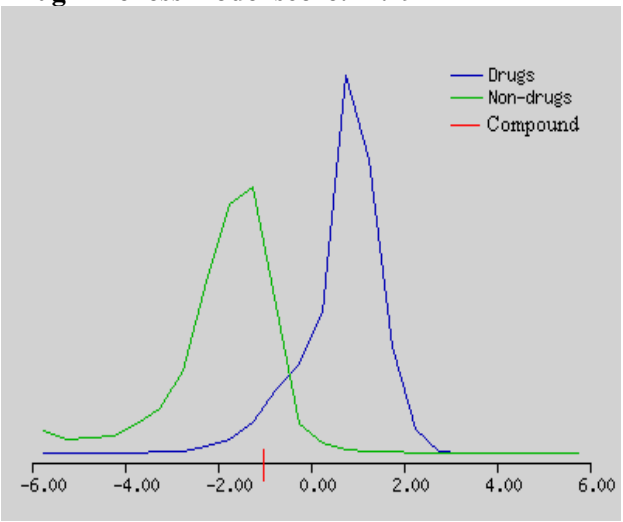

Figure 5(i): Representation of drug likeness score of *N* - [(*R*) – hydroxy { [2 - (1 , 3 – oxathiol – 4 – yl) phenyl] amino } methyl] acetamide.

Drug Likeness model score: -0.31

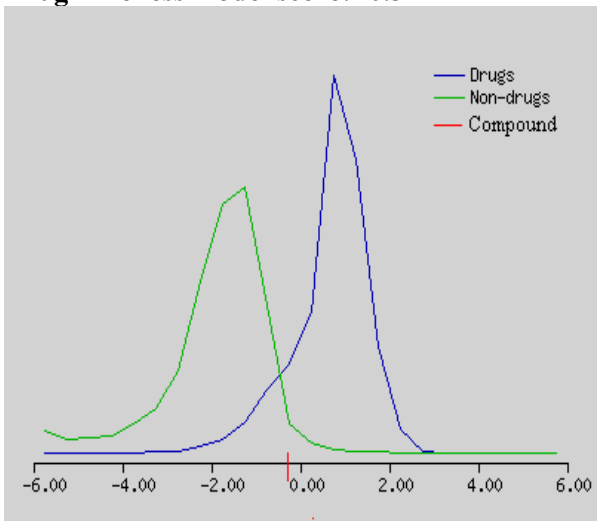

Figure 5(ii): Representation of drug likeness score of *N* - (2 – amino – 2 – oxoethyl) – 3 - (1 - {[2 - (methylamino) ethyl] (phenyl) amino} vinyl) benzamide.

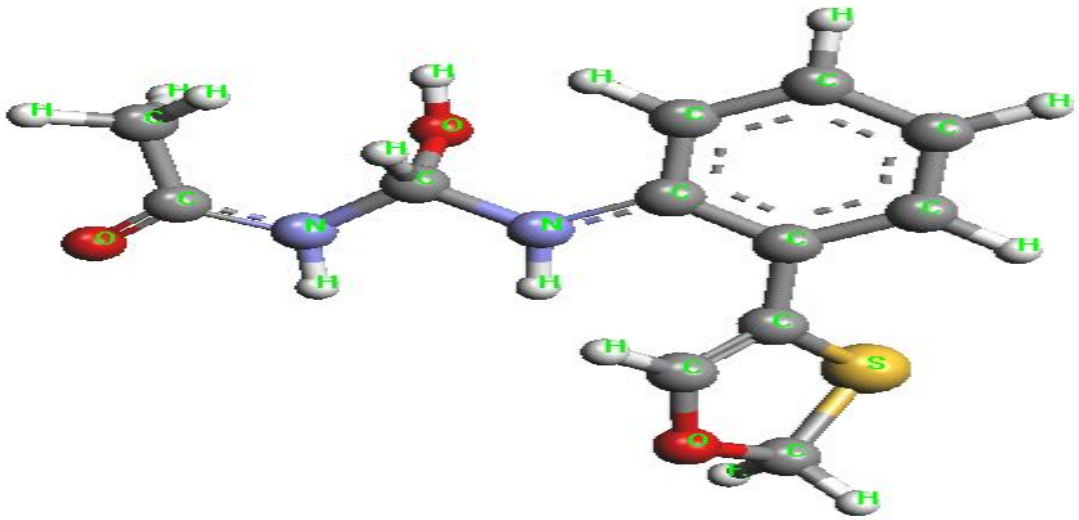

Figure 6(i): Molecular structure of *N* - [(R) – hydroxy { [2 - (1 , 3 – oxathiol – 4 – yl) phenyl] amino} methyl] acetamide.

SMILES Notation :(O=C (NC (O) Nc1ccccc1C=2SCOC=2) C)

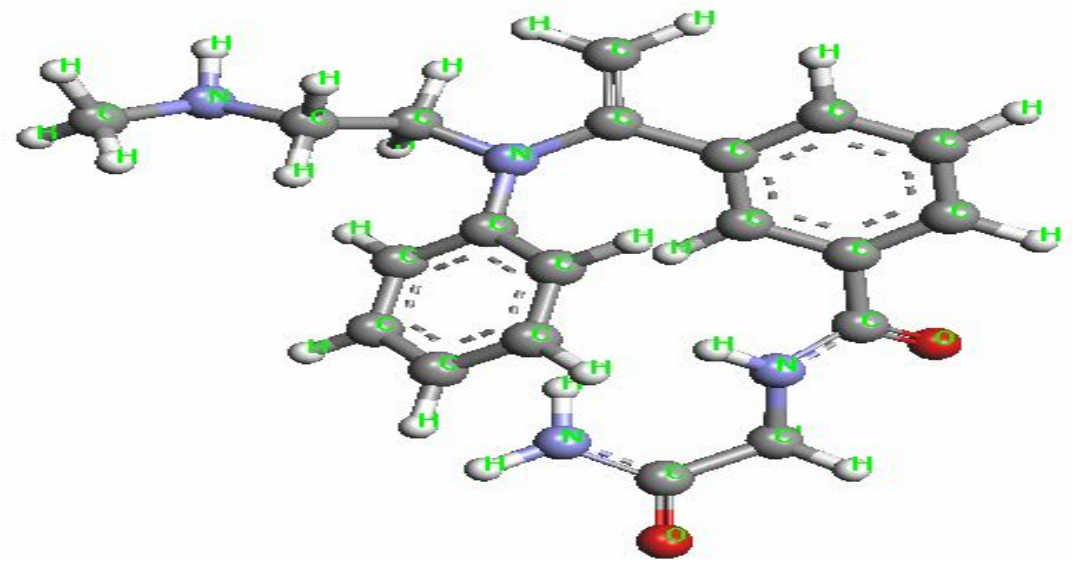

Figure 6(ii): Molecular structure of *N* - (2 – amino – 2 – oxoethyl)– 3 - (1 - { [2 - (methylamino) ethyl] (phenyl)amino}vinyl)benzamide.

SMILES Notation: O = C (N) CNC (=O) c1cccc (c1) C (\N (c2ccccc2) CCNC) = C

Conclusion:

Selective blockade of PM II impairs hemoglobin degradation and leads to parasite death. Two potent inhibitors *N*-[(*R*)-hydroxy{[2-(1,3-oxathiol-4-yl)phenyl]amino}methyl]acetamide and *N*-(2-amino-2-oxoethyl)-3-(1-{[2- (methylamino)ethyl] (phenyl)amino}vinyl)benzamide were designed *de-novo* to inhibit the action of PM II. Our results, suggested that, these two de-novo designed compounds could act as a potential drug and compete with the hemoglobin for the substrate binding site, hence prevents the process of hemoglobin degradation pathway by PM II.

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