LEAD DESIGN AND OPTIMIZATION AGAINST DISEASE MALARIA

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Submitted in partial fulfillment of the Degree of Bachelor of Technology

DEPARTMENT OF BIOTECHNOLOGY & BIOINFORMATICS

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CERTIFICATE

This is to certify that the work entitled, "LEAD DESIGN AND OPIMIZATION AGAINST DISEASE MALARIA" submitted by VIVEK (041505) & AVIN MAHAJAN (041517) in partial fulfillment for the award of degree of Bachelor of Technology in Bioinformatics of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

24.05.08

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Vivek(041505)

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LIST OF ABBREVIATIONS

1.	ECE	Embrace Constraint Energy
2.	EEE	Embrace Electrostatic Energy
3.	EMC	Embrace minimization converged
4.	ESE	Embrace Solvation Energy
5.	ETEWC	Embrace Total Energy without constraints
6.	EvdWE	Embrace vdW Energy
7.	EVE	Embrace Valence Energy
8.	PMCE	Prime_MMGBSA_Complex_Energy
9.	PMDB	Prime_MMGBSA_DG_bind
10.	PMLE	Prime_MMGBSA_Ligand_Energy
11.	PMRE	Prime_MMGBSA_Receptor_Energy
12.	FEB	Free Energy of Binding

ABSTRACT

With the increase in the resistance for various malarial drugs, it has become imperative to look out for the new drugs or for modified drugs with high efficacy. Thus looking for new target sites of proteins is the forefront in current drug research. Currently, drugs are designed on the basis of their active site properties.

There are many proteins involved in the lifecycle of malaria disease like, pfemp1, pf332, eba-175, MSP, AMP-1, RBL etc. but after analysis of all the above targets, pf332 emerged as the most important protein responsible for successful invasion and proliferation of the parasite. Pf332. This protein is unique in many aspects, but it also shows high degree of similarity to the DBL domain of the EBL family. The DBL domain of pf332 is conserved in all parasite clones/strains investigated. In addition, the expression level of Pf332 correlates with proliferation efficiency of the parasites in vitro. It displays sequence polymorphism (exon 2) among different isolates possibly attracting the host immune response and shielding the functional N-terminal region of the molecule. Subsequently, the DBLdomain of Pf332, although exposed in some way to the immune system, is able to retain a conserved sequence mediating the important first step of the process of RBC invasion. Thus, Pf332 is a molecule with a potential role to support merozoite invasion.

DBL domain of pf332 is modeled by homology modeling procedure and 3D structure of 120 antimalaria compounds whose target was not known is generated. These molecules are docked with the active site of DBL domain through Schrodinger Suite and the 18 molecules showed binding affinity to the target protein. The Molecule with id 3(68) showed the strongest binding.

CHAPTER-I

INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites. It is widespread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, it causes disease in approximately 650 million people and kills between one and three million, most of them young children in sub-Saharan Africa. Malaria parasites are transmitted by female Anopheles mosquitoes.

Species of parasite

Malaria in man is caused by 4 distinct species of malaria parasite

1. *Plasmodium vivax*
2. *Plasmodium falciparum*
3. *Plasmodium malariae*
4. *Plasmodium ovale*

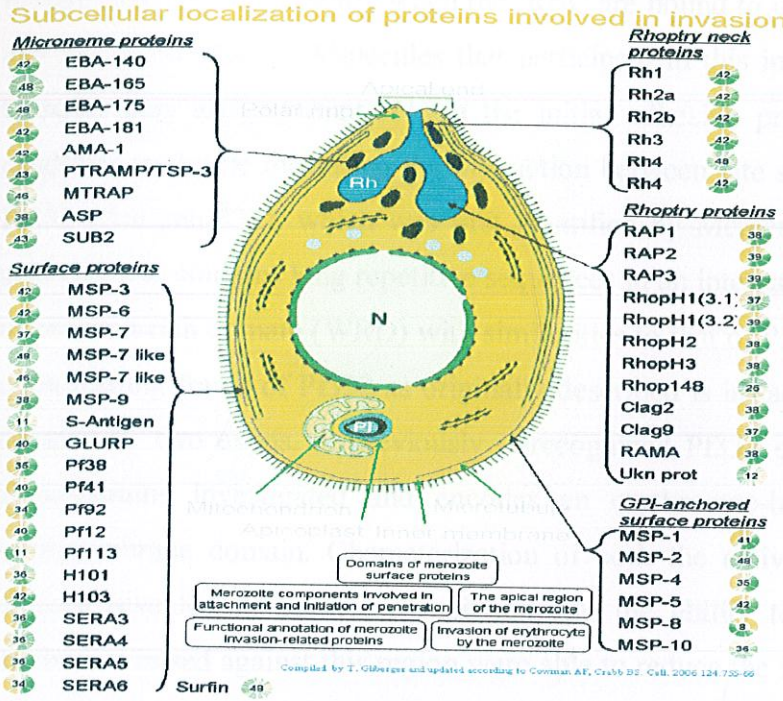
Plasmodium vivax- It has the widest geographic distribution throughout the world and causes much debilitating disease. In India, about 60% of the infections are due to *P. vivax.*

Plasmodium falciparum It is also wide spread, results in the most severe infections and is responsible for nearly all malaria-related deaths. 40% of infection is due to *P. falciparum.*

Plasmodium malariae It has restricted distribution and is said to be responsible for less than 1% of infections in India.

Plasmodium ovale It is very rare parasite of man, mostly confined to tropical Africa. In highly endemic areas, the patient may become infected with one, two or even more species of the malarial parasite. In India, 4-8% is due to mixed infection.

SUBCELLULAR LOCALIZATION OF PROTEINS INVOLVED IN INVASION



Source: Compiled by T.Gilberger and updated according to Cowman AF, Crabs BS Cells 2006 124:755.66

Known Drug/Vaccine Tagets

Other important protein involved is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), which is expressed at the infected erythrocyte surface — mediates parasite binding to all the various receptors. PfEMP1 is encoded by the large and diverse var gene family that has a central role in *P. falciparum* pathogenesis.

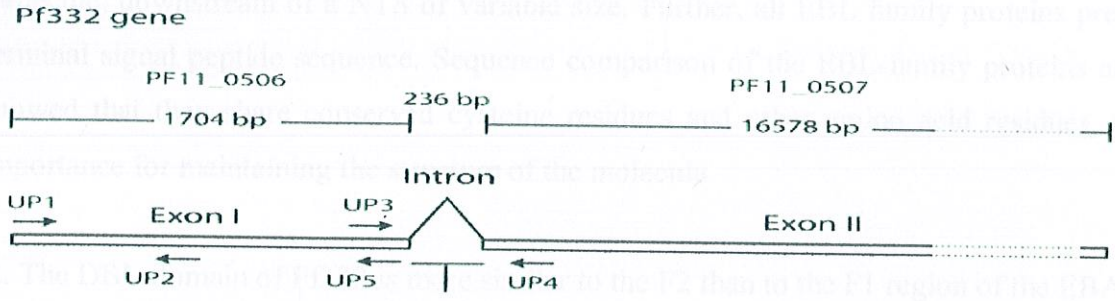
Target protein (pf332)

The Plasmodium falciparum antigen Pf332 is of potential interest for inclusion in a subunit vaccine against the malaria parasite. Pf332 is a 750-kD protein expressed by late stages of intra-erythrocytic asexual parasites. Its amino acid comprises repeat sequences with regularly spaced pairs of glutamic acid. Plasmodium falciparum malaria is brought about by the asexual stages of the parasite residing in human red blood cells (RBC). Contact between the erythrocyte surface and the merozoite is the first step for successful invasion and proliferation of the parasite.

Interestingly, a majority of Plasmodium falciparum merozoites released from ruptured schizonts fail to invade new RBC in vitro. Further, P. falciparum purified merozoites do not consistently infect RBC while in contrast isolated merozoites of both murine and primate malaria parasites easily

invade and proliferate within new RBC. A recent study by Glushakova illustrates that merozoites invade RBC more efficiently when the iRBC are bound to uninfected RBC prior to schizont rupture and merozoite release. Molecules that participate in this interaction between iRBC and RBC may likewise play an important role in the initial adhesion process between merozoite and RBC. A candidate molecule mediating the interaction between late stage iRBC and RBC is the polypeptide Pf332 (Antigen 332), which was first identified by Mercereau-Puijalon. Pf332 is highly glutamic acid rich and contains long repetitive sequences in an internal region named EB200, and a conserved tryptophan-rich domain (WRD) with similarities to that of PfEMP-1, SURFIN, and PkSICAvar. The open reading frame of Pf332 as originally described is in fact only the second exon of a giant gene comprising two exons. A previously unrecognized Pf332 exon I, which is present in all parasite clones/strains investigated and encodes an erythrocyte-binding DBL-domain together with a transmembrane domain. Characterization of both the native and in vitro expressed Pf332 DBL-domain revealed its surface association and the ability to bind to human RBC. Significantly, antibodies raised against this region were able to reduce the invasion efficiency in vitro independent of geographical origin or receptor specificity of the P. falciparum clone/strain. This suggests the conserved polypeptide encoded by exon I of Pf332 exhibit a functional role during the RBC invasion process and is therefore a potential novel candidate for a blood stage vaccine.

Structural analysis of the gene encoding Pf332.



Source: doi:10.1371/journal.pone.0000477.g001

Pf332 consists of two exons separated by a short intron.

A

Source: doi:10.1371/journal.pone.0000479.g001

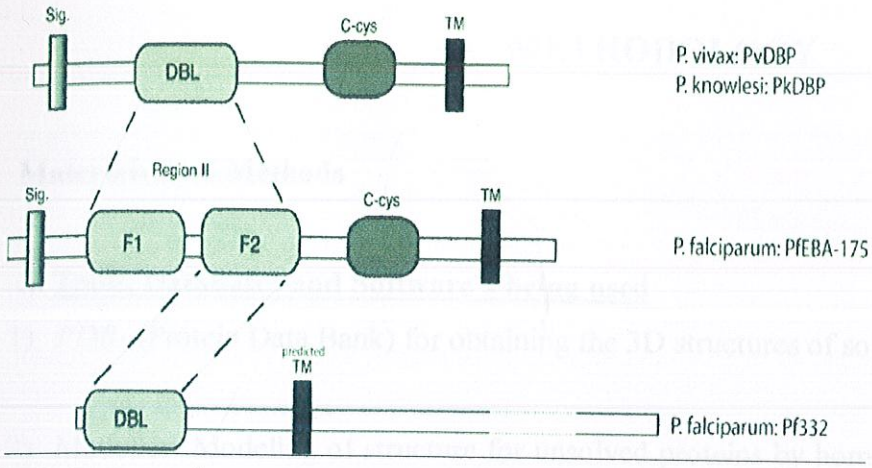
Target sequence

>gi|23496419|gb|AAN36075.1|AE014843_39 hypothetical protein PF11_0506 [Plasmodium falciparum 3D7]

Why Pf332?

1. Exon I of Pf332 encodes for a DBL-domain with high similarity to the DBL domains of the EBL-protein family BLASTP search and the N-terminal region (aa 1-250) possesses a domain homologous to the DBL-domains of the EBL-family including e.g. the EBA-175 of *P. falciparum* (although the overall protein structure of Pf332 differs from this protein family. Pf332 does neither contain an N-terminal segment (NTS) nor a signal leader sequence upstream of the DBL-domain. The DBL-domains of the other EBL-family members are located further to the centre of the molecule, downstream of a NTS of variable size. Further, all EBL family proteins presented an N-terminal signal peptide sequence. Sequence comparison of the EBL-family proteins and the Pf332 showed that they share conserved cysteine residues and other amino acid residues, which are of importance for maintaining the structure of the molecule.

2. The DBL-domain of Pf332 is more similar to the F2 than to the F1 region of the EBA-175 protein however, the absence of the WWXXXXXXXW sequence motif commonly found in other EBL family members suggests a distinct RBC-binding function for this domain. Alignment with the EBL-family also reveals that Pf332 does not have a C-terminal Cysteine-rich domain after the DBLdomain. In contrast, Pf332 has a putative transmembrane region, located close to the DBL-domain (aa 540–560). Four membrane targeting motifs (RxLxE/Q) were found in the Nterminal region (aa 77–81), in the middle (aa 2628–2632) and close to the end (aa 4537–4541, 4922–4926) of the molecule, indicating that Pf332 is a transmembrane protein with a similar



Source: doi:10.1371/journal.pone.0000477.g002

3. The first exon of Pf332 encodes a conserved erythrocyte binding domain homologous, but with a distinct sequence, to the DBL-domains of the EBL-family. This sequence is, in contrast to other erythrocyte-binding proteins, conserved among different *P. falciparum* strains and clones. Proteins expressed by the Plasmodium parasite often show sequence alterations in regions that are crucial for immunity or undergo antigenic variation, in particular surface related molecules, due to the constant pressure from the host immune system. This also includes members of the EBL-family, as for example the EBA-175, that are conserved only in function but show variation within their sequence. However, the extracellular region of Pf332 displays a conserved sequence, which might ensure the parasite's ability to adhere to RBC independent of the genetic background of the host.

4. The association of the DBL domain of Pf332 with the iRBC surface correlates with late stage resetting, indicating that Pf332 might facilitate invasion for released merozoites through providing close proximity of new host cells. In contrast to the DBL-domain, exon II displays sequence polymorphism among different isolates possibly attracting the host immune response and shielding the functional N-terminal region of the molecule. Subsequently, the DBL domain of Pf332, although exposed in some way to the immune system, is able to retain a conserved sequence mediating the important first step of the process of RBC invasion. Still, the lack of variation of the DBL-domain suggests that it is not overtly exposed to the immune system as PfEMP1 or the RIFINs, but concealed prior to exerting it function.

CHAPTER-II

METHODOLOGY

Materials And Methods

i). Tools, Databases and Software's being used

1) *PDB*- (Protein Data Bank) for obtaining the 3D structures of solved proteins.

2) Modeller- Modeling of structure for unsolved proteins by homology and generating missing co-ordinates.

3) ChemSketch- Used for generating 3D structures from 2D information.

4) CASTp – (Computed Atlas of Surface Topology of Proteins)used for area and volume calculation of pockets..

5) Schrödinger
   - Glide –Flexible Docking
   - QIKPROP – ADME Property
   - eMBrAcE – FEB Studies

ii). Template sequence

Our target structure is not known in RCSB protein data bank but related structures like was found by Blast-P search. Then the structure of former was modeled by using related structure as template.

iii) Modelling of protein

Modeller

A 3D model is obtained by optimization spatial restraints of a molecular probability density function (PDF). The molecular pdf for comparative modeling is optimized with the variable target function procedure in Cartesian space that employs methods of conjugate gradients and molecular dynamics with simulated annealing.

From Modeler we get the five best predicted structures for the protein.

Evaluation of models

PROCHECK

In procheck the protein structure is evaluated on the basis of the RAMACHANDRAN-PLOT .It is a way to visualize dihedral angles φ against ψ of amino acid residues in protein structure. It shows the possible conformations of φ and ψ angles for a polypeptide.

The aim of PROCHECK is to assess how normal, or conversely how unusual, the geometry of the residues in a given protein structure is, as compared with stereo chemical parameters derived from well-refined, high-resolution structures.

Binding site residues

Functional residues which are responsible for binding are determined from the literature and the complete residue information was taken from the results of CASTp.

CASTp

Computed Atlas of Surface Topography of proteins (CASTp) provides an online resource for locating, delineating and measuring concave surface regions on three-dimensional structures of proteins. These include pockets located on protein surfaces and voids buried in the interior of proteins. The measurement includes the area and volume of pocket or void by solvent accessible surface model (Richards' surface) and by molecular surface model (Connolly's surface), all calculated analytically. CASTp can be used to study surface features and functional regions of proteins. CASTp includes a graphical user interface, flexible interactive visualization, as well as on-the-fly calculation for user uploaded structures.

iv). Anti malaria compounds

Since 1954 to 2004 number of ant malarial compounds has been discovered. We identified few of the anti-malarial compounds whose compound target was not known but could act as the potential lead on the basis of affinity to the protein.

We drew their 2-D structures in ISIS draw and minimized them in **Hyperchem** 7.

v). Docking using schrodinger

Once the structures were available we used the licensed software SCHRODINGER SUITE for the docking. Now we go for the high-throughput screening of potential ligands based on binding mode and affinity for a given receptor molecule. *GLIDE* is designed to do this. Comparing ligand scores with those of other test ligands, or compare ligand geometries with those of a reference ligands

Grid based ligand docking with energetics and searches for favourable interactions between one or more typically small ligand molecules and it typically large receptor molecule, usually protein. Schrodinger recommends the performance of test calculations with different scaling factors for the receptor and ligand atom vander wall radii , because stearic repulsive interactions might otherwise over emphasized , leading to rejection of overall correct binding modes of active compounds .After ensuring that protein and ligand are in correct form of docking , the receptor grid file are generated using grid-receptor generation program , that grid was generated at the centeroid of the active site

Following are the main steps that we followed→

1. Protein and ligand preparation
2. Receptor grid generation
3. Ligand docking
4. Examining Glide data

Ligand Docking

Typically, Glide standard-precision docking is used to find probable good binders in a large set; the top-scoring 10% to 30% can then be investigated more intensively using Glide extra-precision (XP) docking or other methods available from Schrödinger.

MMGBSA and Binding Free Energies

For the calculation of free energy of binding (FEB) of ligands with DBL domain of Pf332, XP and SP docking results have been taken and only the best scoring pose for each ligand was taken into consideration . Biomolecular association with Energetics (eMBrAcE) developed by Schrodinger

ADME Screening

The QuickProp program has been used to obtain the absorption ,distribution, metabolism and excretion (ADME)properties of analogues .It also evaluate the acceptability of the analogues based on tht Lipinski's Rule of 5 essential for Drug design

CHAPTER-III

RESULTS AND DISCUSSION

i). Template sequence

After running BLASTP, we got number of hits with our target sequence but maximum sequence identity was with 1ZRL. So we took 1ZRL as template. 1ZRL is 596 residues long. Analysis has shown that DBL domain of pf332 matches with F2 domain of 1ZRL. Domain study through CDD tool has indicated that F2 domain starts from 313th residue.

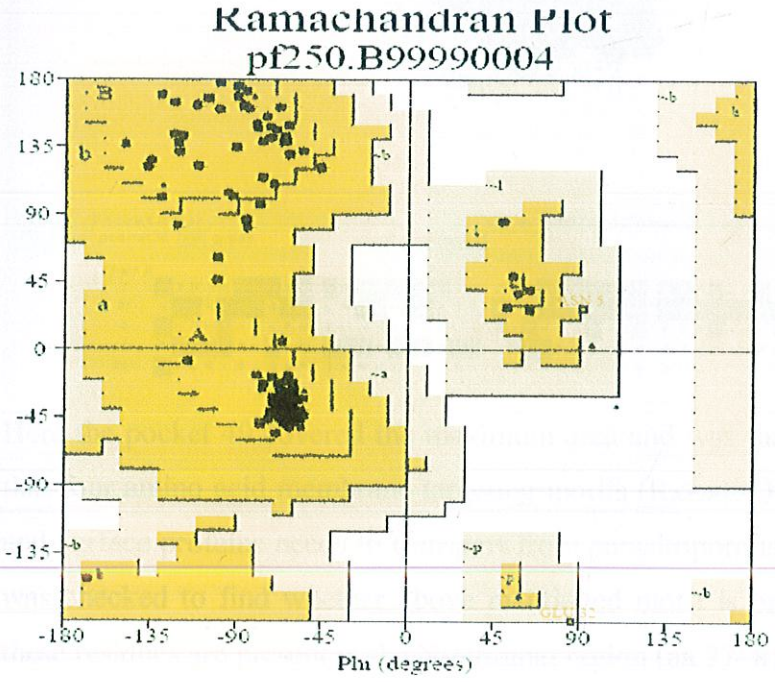
ii). Modelling of protein

Modeller

The output is a 3D model for the target sequence containing all main chain and side chain non hydrogen atoms .After optimization 5 best predicted structures of protein were obtained

Evaluation of models

Ramachandran plot evaluation



Plot Statistics

Residues in most favoured regions [A,B,L] 217	93.5%
Residues in additional allowed regions [a,b,l,p] 13	5.6%
Residues in generously allowed regions [~a,~b,~l,~p]	20.9%
Residues in disallowed regions	00.0%

---- ------

Number of non-glycine and non-proline residues 232	100.0%

Number of end-residues (excl. Gly and Pro) 2

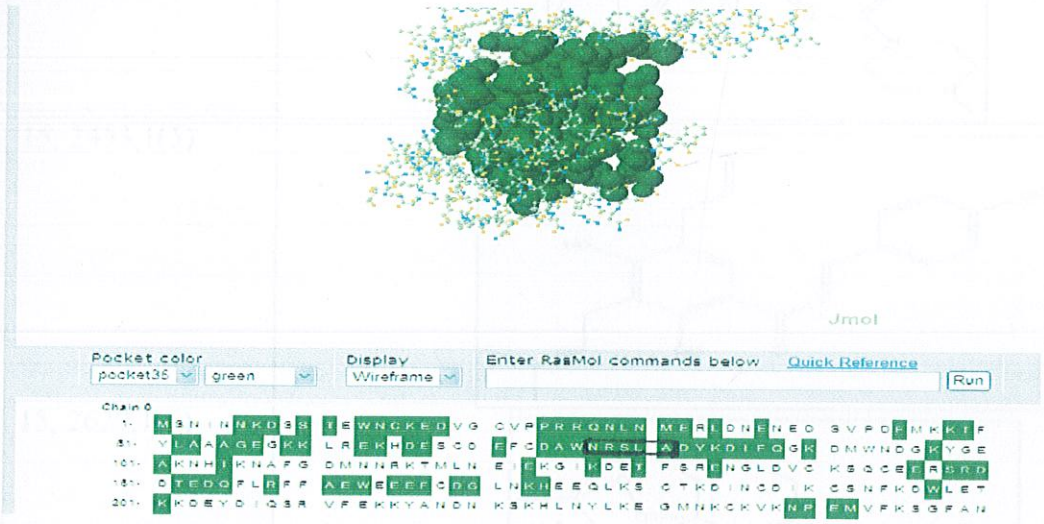
Number of glycine residues (shown as triangles) 12

Number of proline residues 4

Total number of residues 250

Binding site residues

CASTp RESULTS

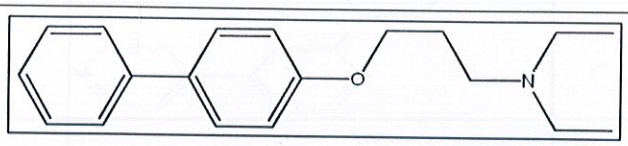
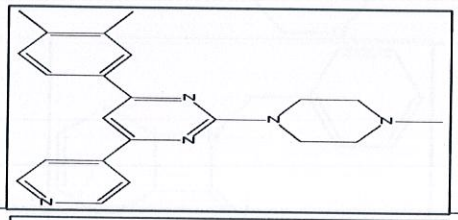
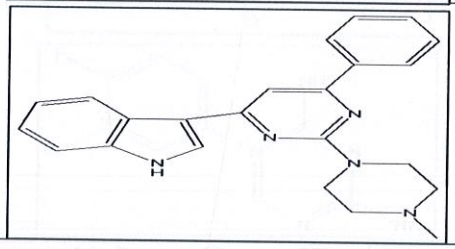
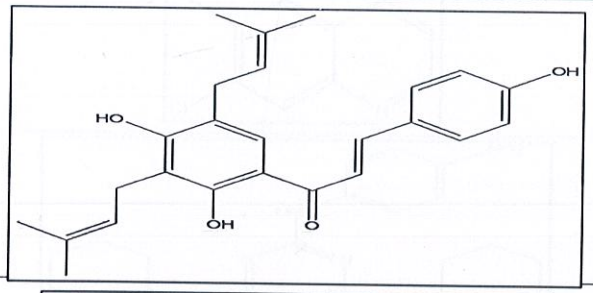
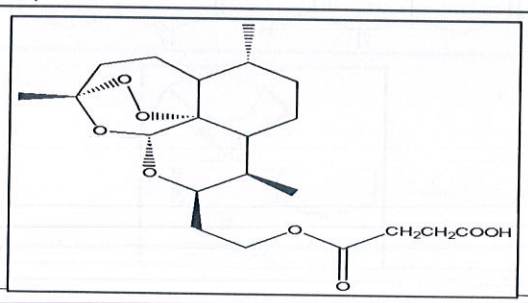


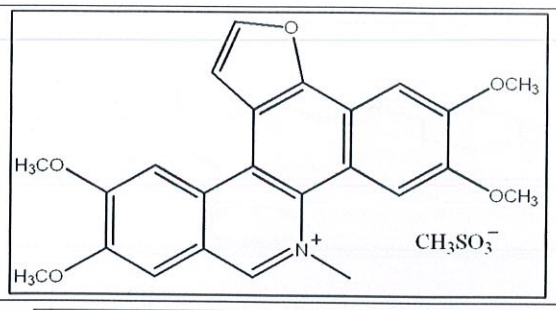
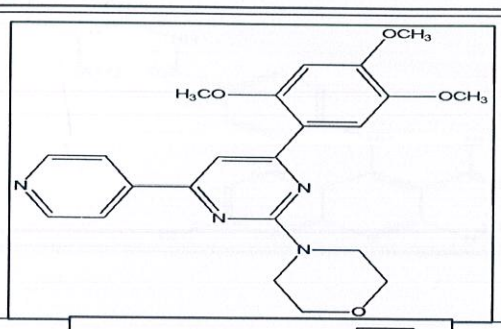
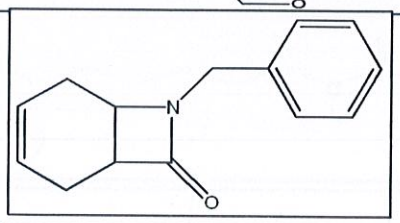
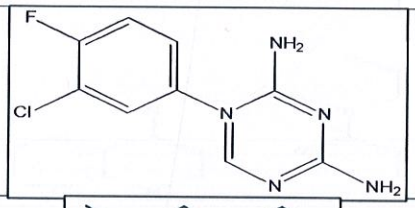
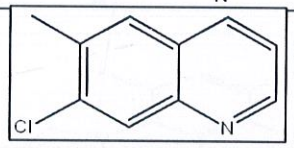
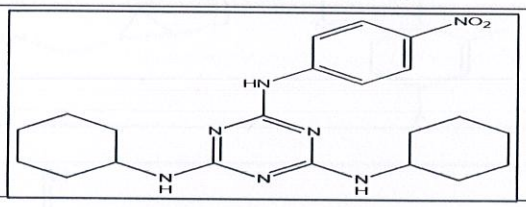
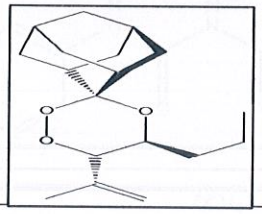
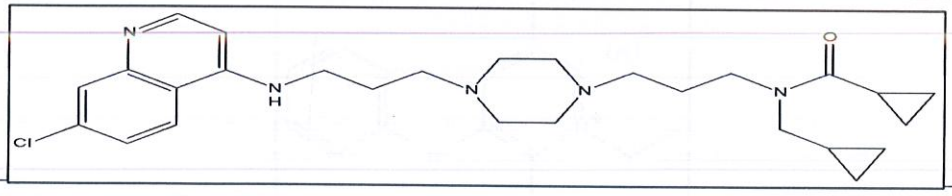
Here the pocket 49 covered the maximum area and was the best pocket. Since literature mentioned that four amino acid membrane targeting motifs (RxLxE/Q) were important in membrane transport; and surface proteins needs to transport from parasitoporous vacuole to surface; cavity from CASTp was checked to find whether above mentioned motif is present or not. In DBL domain of pf332, these residues are present in the N-terminal region (aa 77–81).

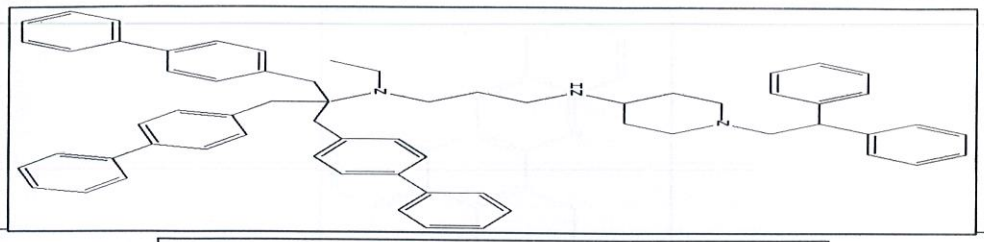
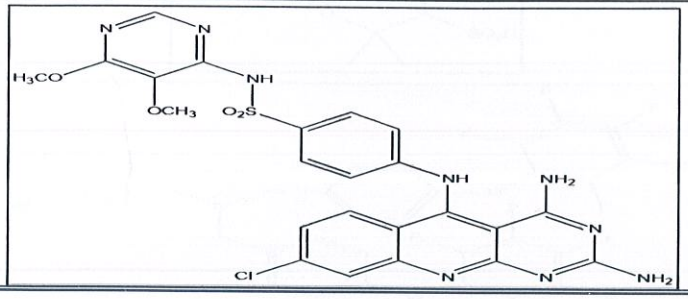
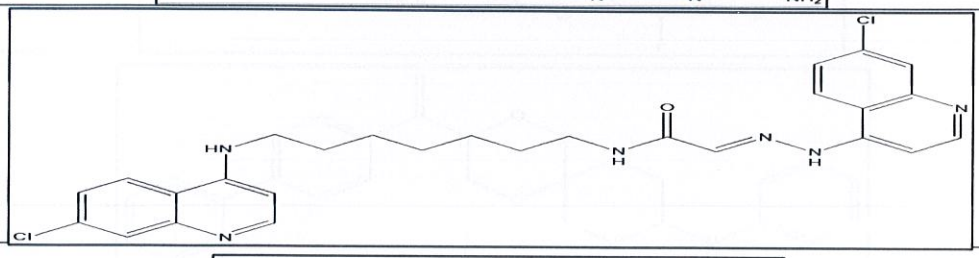
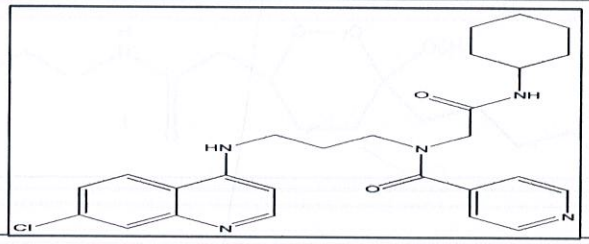
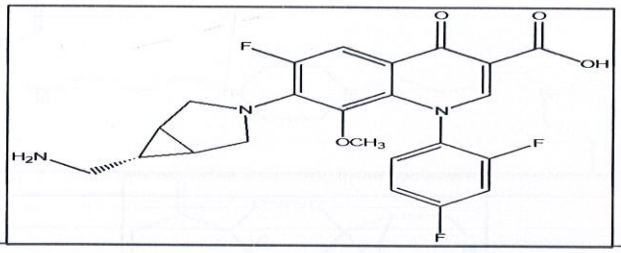
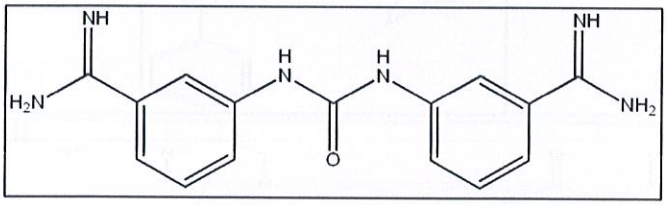
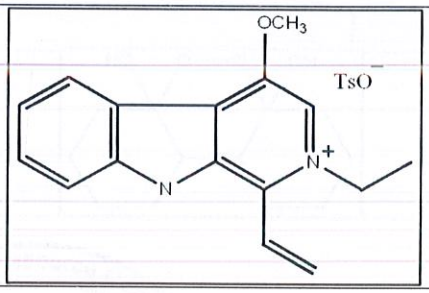
iii). Anti malaris compounds

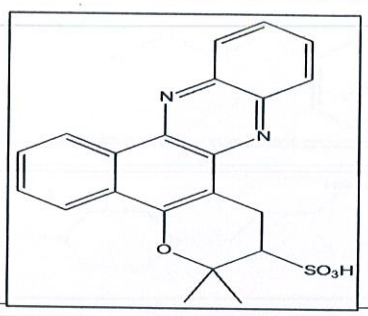
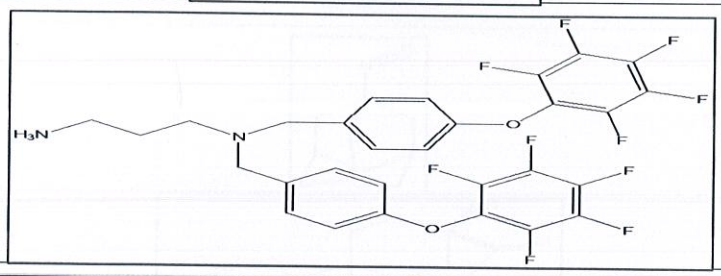
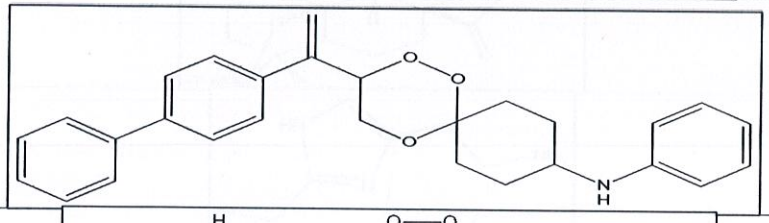
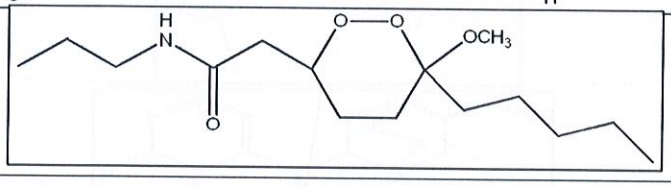
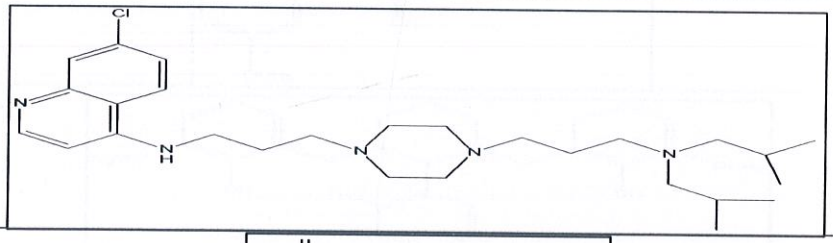
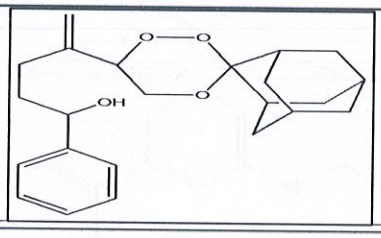
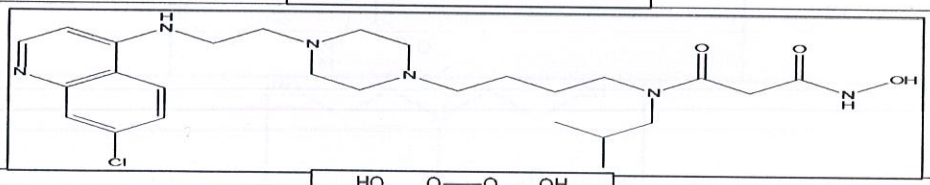
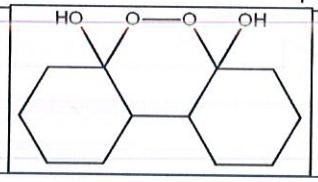
We have drawn and optimized all lead compounds (by Chemsketch). The analogues are optimized by means of the MMFF94 force field using default setting (Schrödinger Suite), then go for docking.

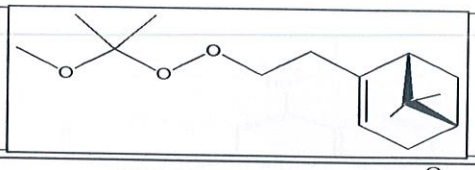
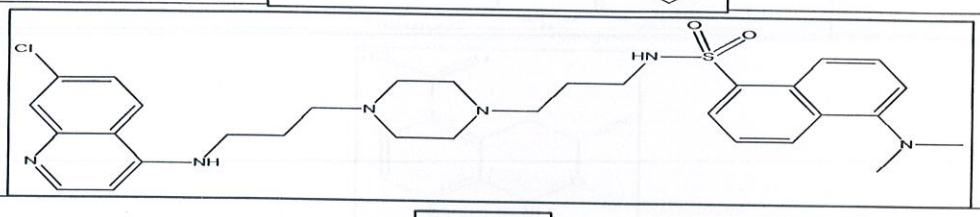
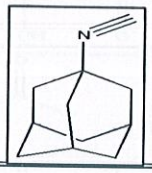
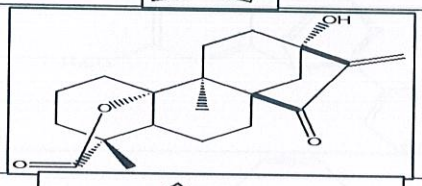
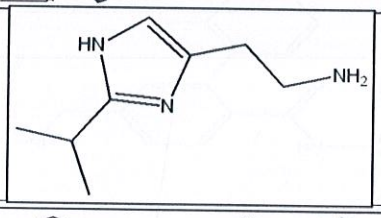
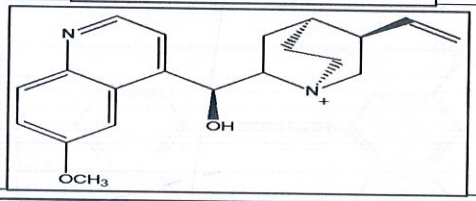
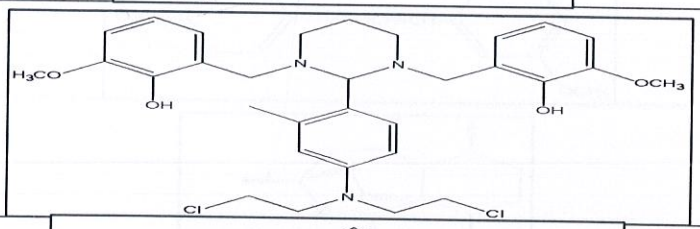
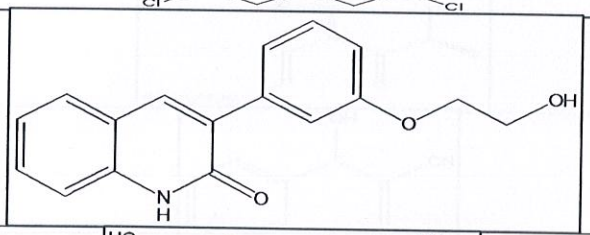
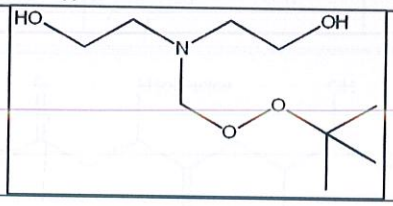
Table.1: Antimalaria compounds reported in literature used for docking against DBL domain of pf332

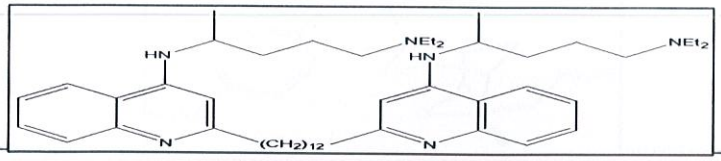
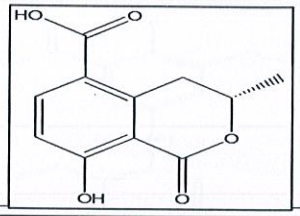
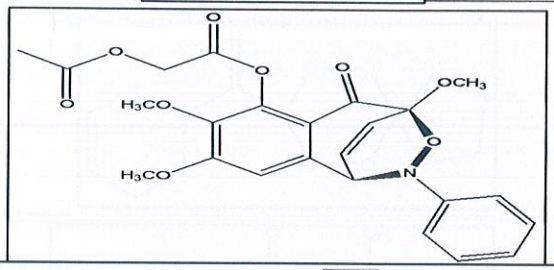
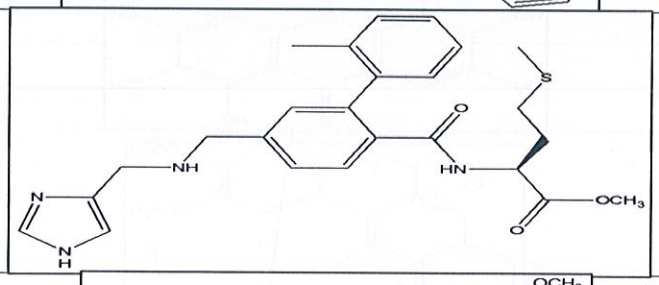
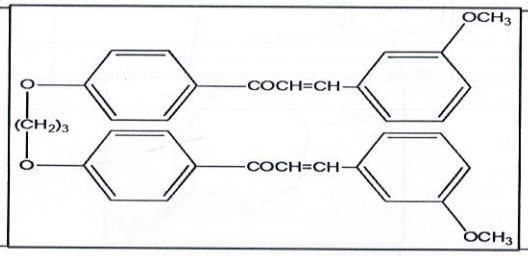
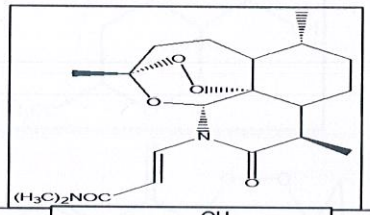
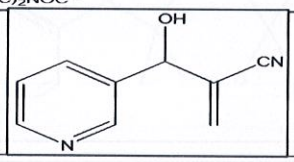
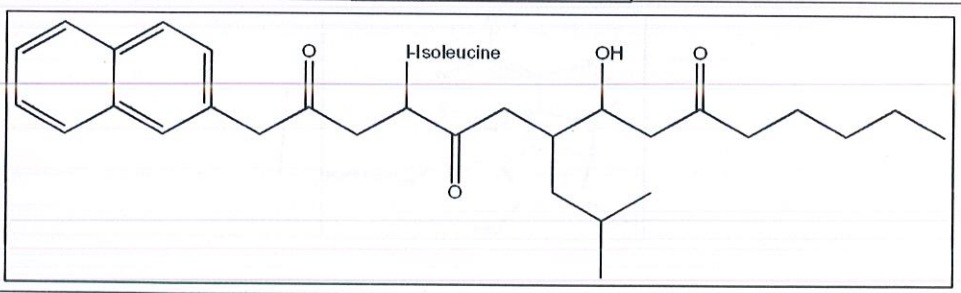
Entry	Compound structure
15, 3024,1(2)	
15, 3130,1(3)	
15, 3133,1(4)	
15, 2453,1(5)	
15, 2629,1(6)	

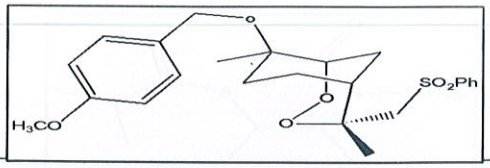
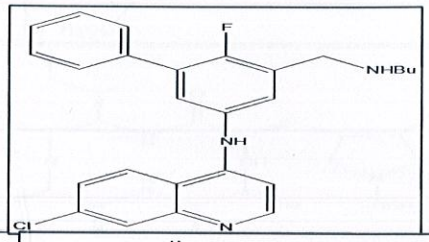
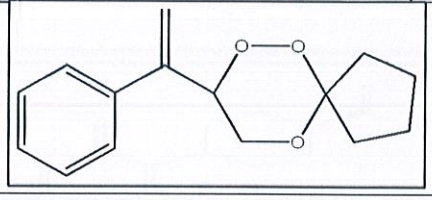
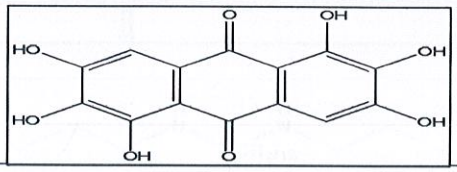
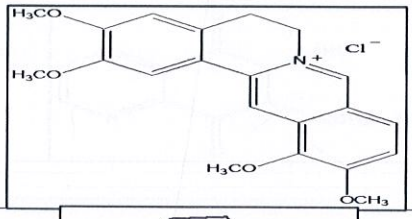
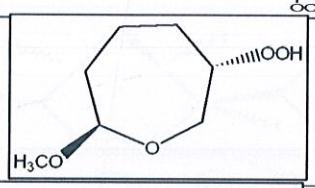
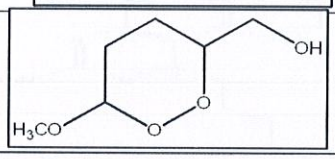
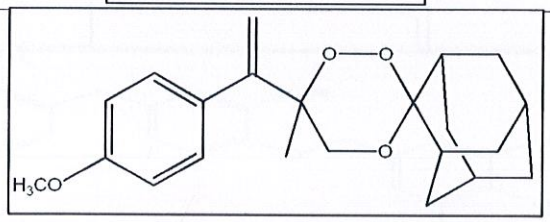
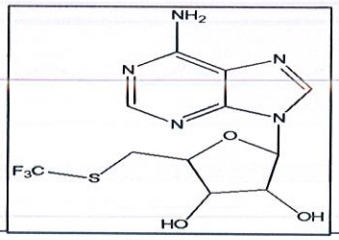
15, 2007,1(7)	
15, 18811(8)	
15, 1371,1(9)	
15, 915,1(10)	
15, 1015,1(11)	
15, 531,1(12)	
15, 595,1(13)	
15, 297,1(14)	

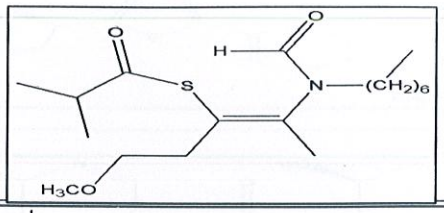
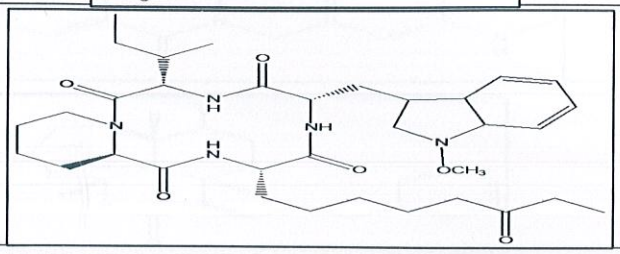
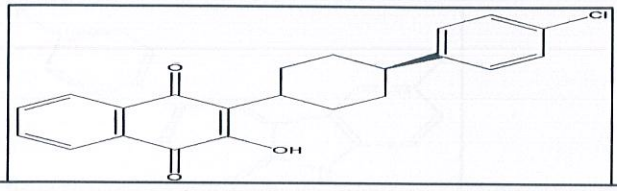
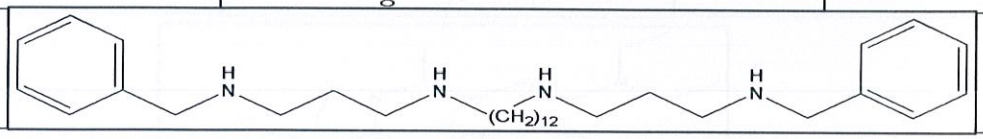
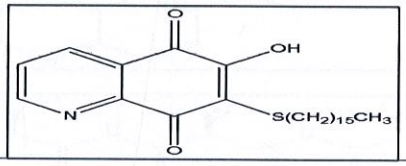
15, 345,1(15)	
15, 73,1(16)	
14, 4439,1(17)	
14, 3901,1(18)	
14, 2773,1(19)	
14, 1979,1(20)	
14, 1689,1(21)	

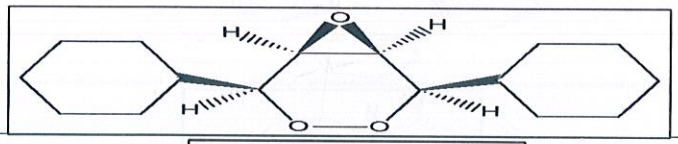
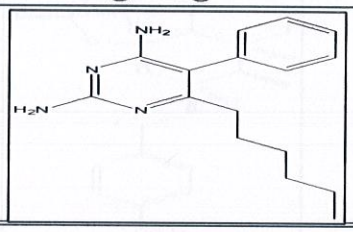
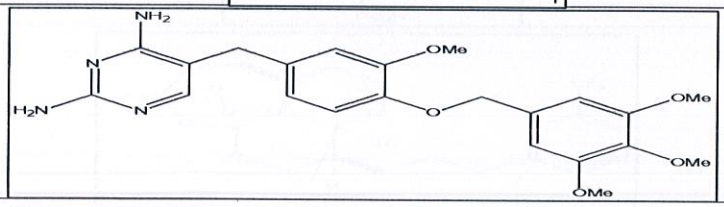
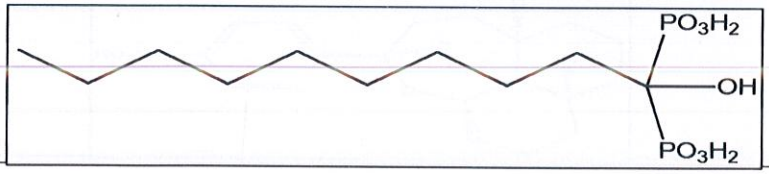
14, 1145,1(22)	
14, 615,1(23)	
14, 459,1(24)	
13, 4081,1(25)	
13, 3783,1(26)	
13, 3447,1(27)	
13, 2659,1(28)	
13, 2013,1(29)	

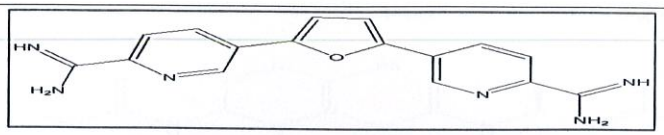
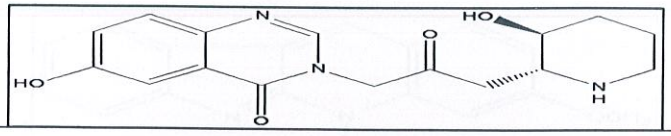
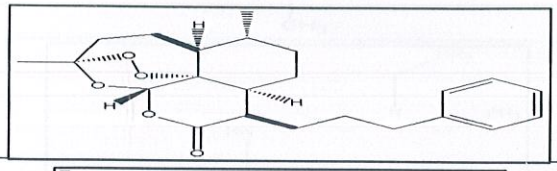
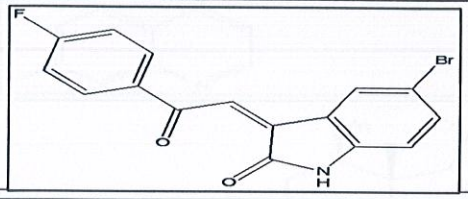
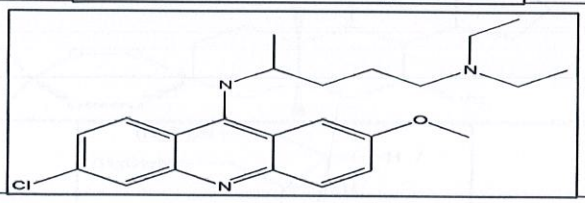
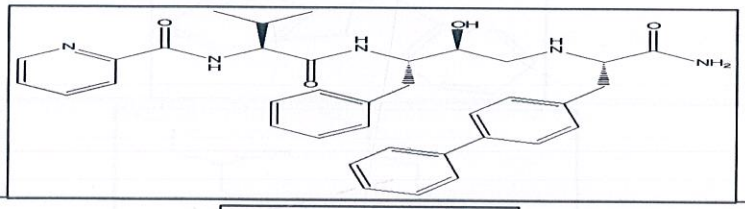
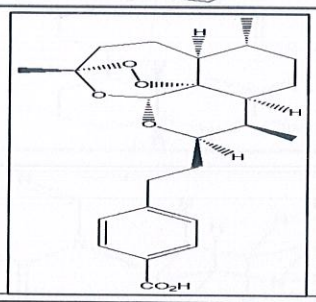
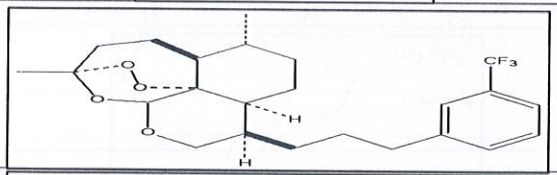
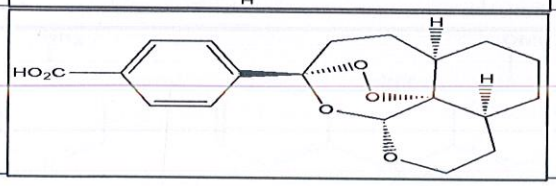
13, 75,1(30)	
12, 2595	
12, 2277	
12, 2167,1(33)	
12, 1701	
12, 1351	
12, 539	
11, 2875	
11, 2269	

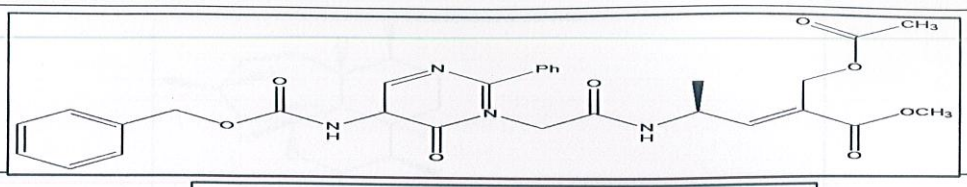
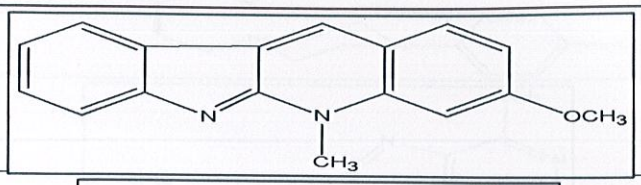
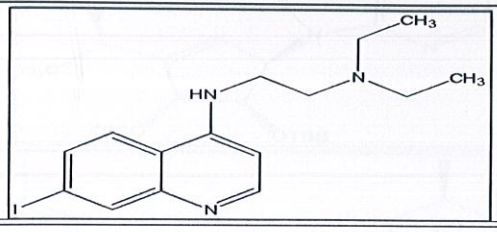
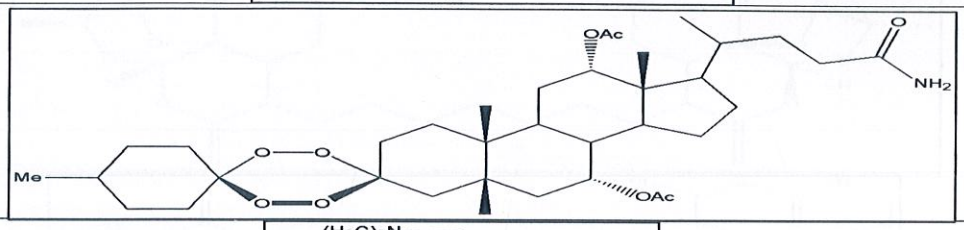
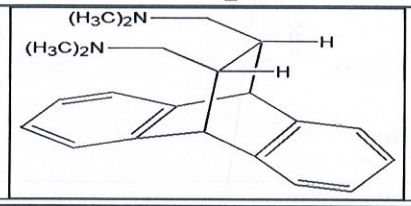
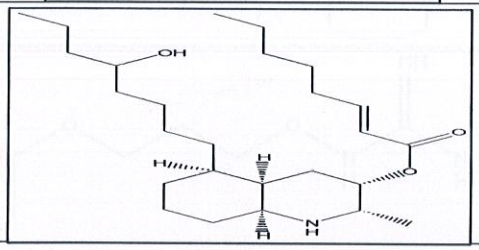
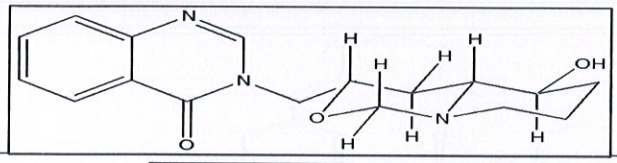
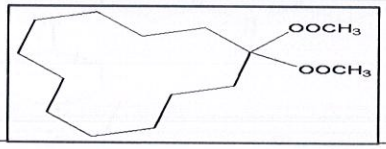
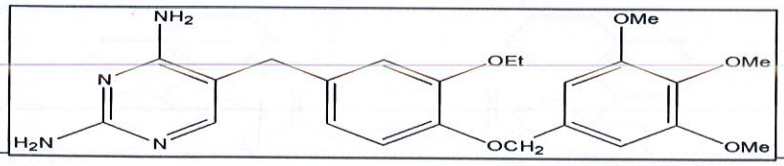
11, 2075	
11, 1965	
11, 1851	
11, 761	
10, 2159	
9, 2969	
9, 731	
8, 2315	

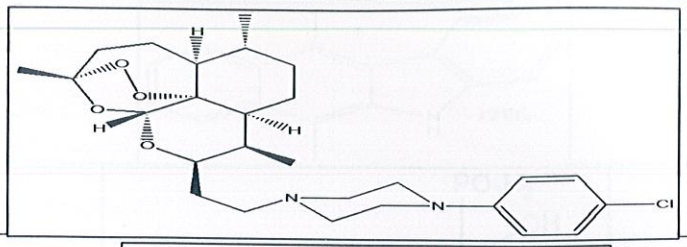
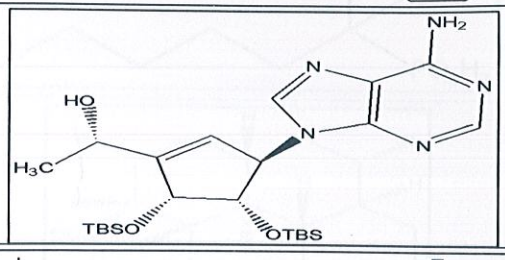
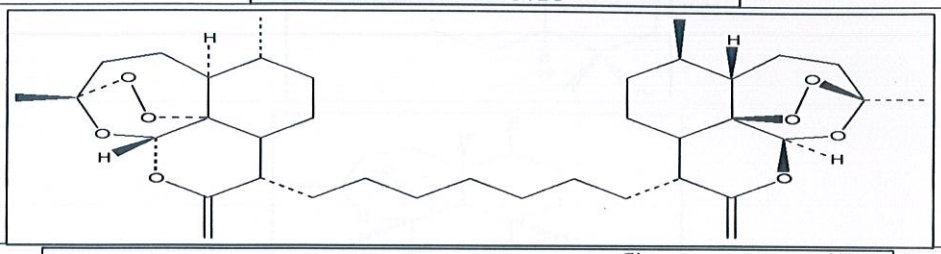
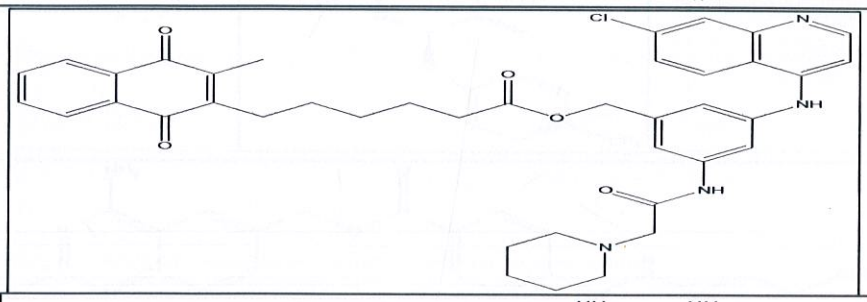
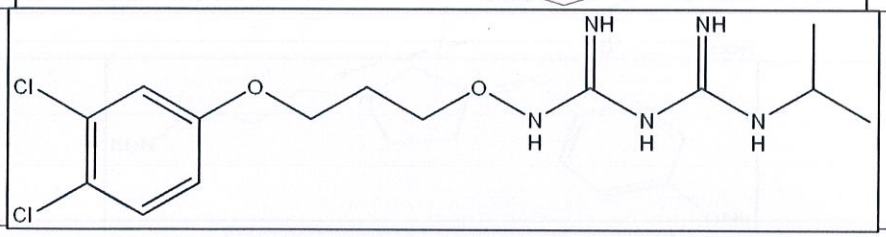
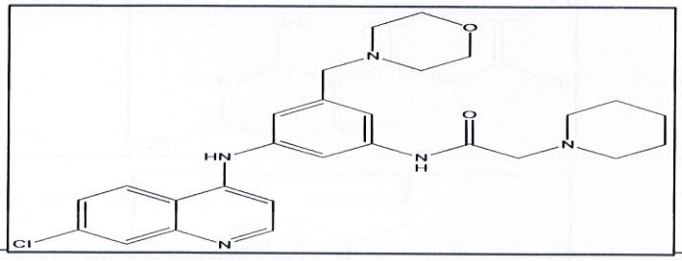
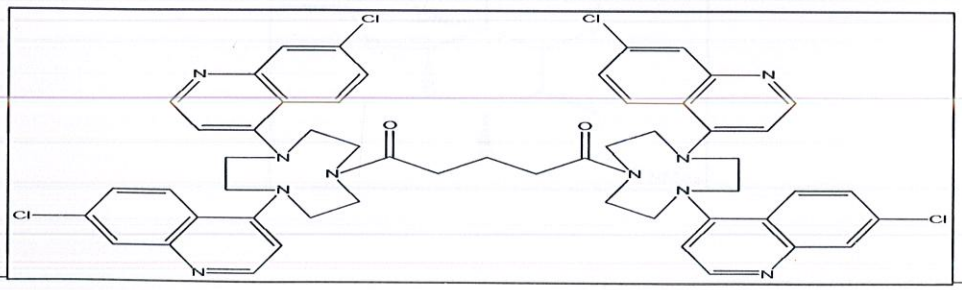
8, 903	
6, 391	
5, 1913	
5, 1927,1(51)	
4, 1663,1(53)	
3, 1707,1(54)	
2, 623,1(55)	
2, 497,1(56)	
1, 497,1(57)	

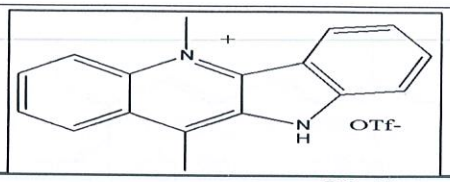
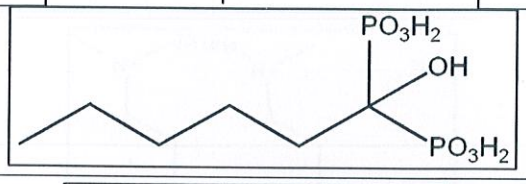
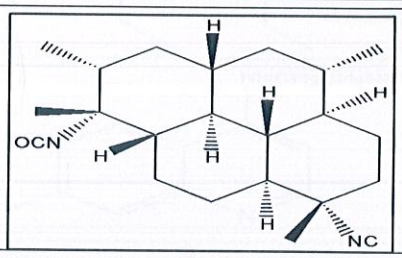
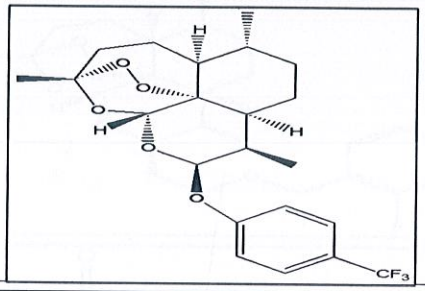
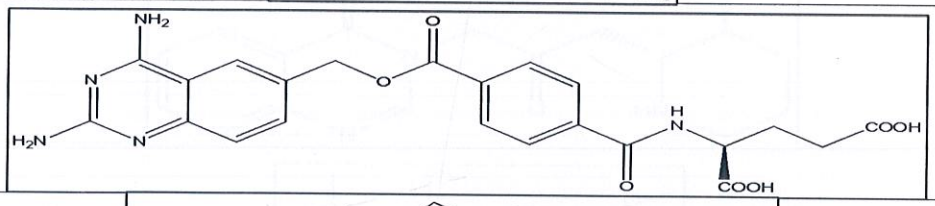
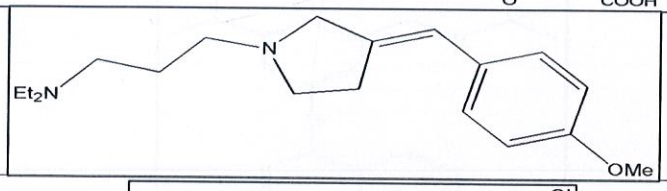
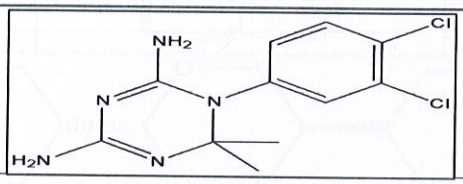
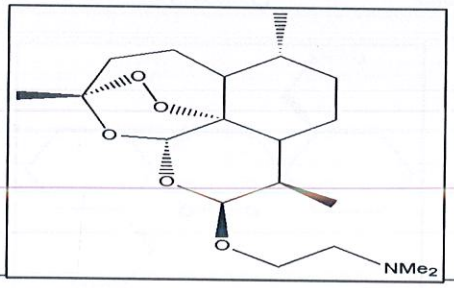
0b	
4	
6	
13	
17	

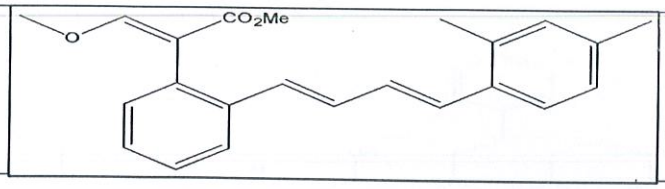
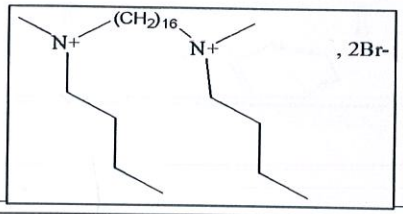
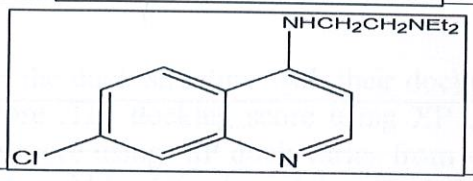
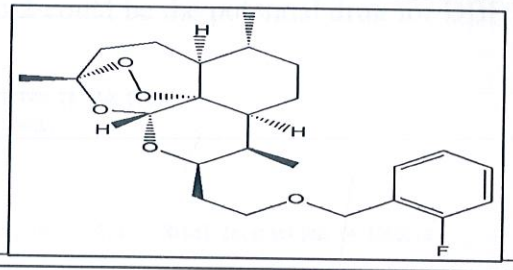
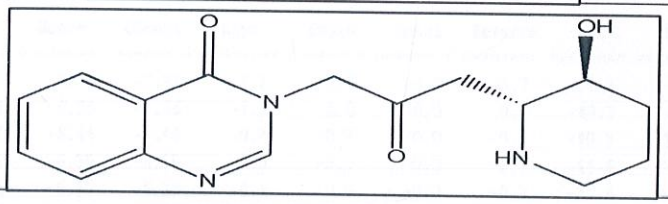
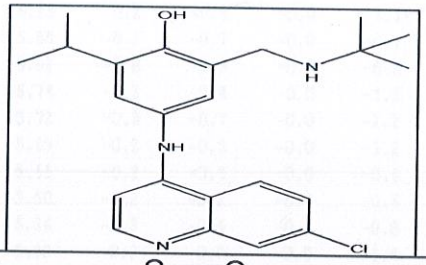
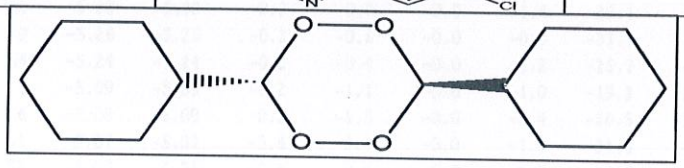
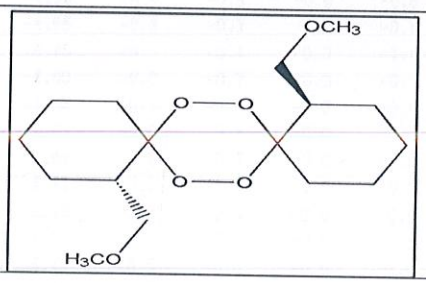
3(11)	
3(15)	
3(16)	
3(19)	

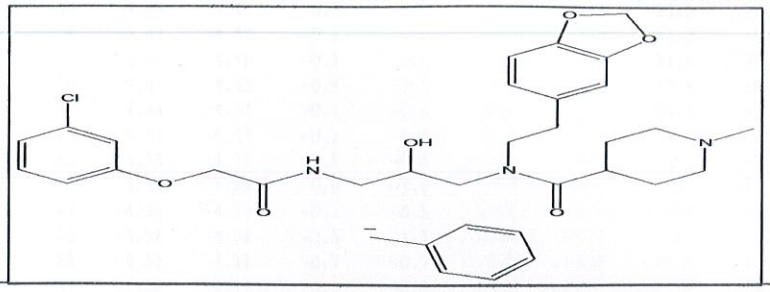
3(21)	
3(22)	
3(23)	
3(24)	
3(25)	
3(31)	
3(35)	
3(36)	
3(37)	

3(38)	
3(39)	
3(40)	
3(41)	
3(42)	
3(43)	
3(44)	
3(45)	
3(46)	

3(47)	
3(49)	
3(50)	
3(52)	
3(53)	
3(56)	
3(58)	

3(59)	
3(60)	
3(61)	
3(62)	
3(63)	
3(64)	
3(68)	
3(69)	

3(71)	
3(72)	
3(73)	
3(74)	
3(76)	
3(77)	
3(78)	
3(79)	

3(80)	

iv). Docking using schrodinger

By the XP and SP dock result we get the dock structure with their docking score ..The ranking of ligands was done based on Glide Score .The docking score using XP dock varies from -1.54 to maximum of -7.93 , and The docking score using SP dock varies from -1.07 a maximum of -5.33 This proves that ligand id 133647.mol2 could be the potential drug for DBL domain of Pf332.

```
The receptor and sorted ligand structures were written to the file
avin22Aprglide_dock_pv.mae  for use in the Pose Viewer.

Final rankings based on GlideScore
0 poses were rejected by the energy filters,
    Coul+vdw Energy <=      0.0     Hbond Interaction <=      0.0     Metal Interaction <= 10000.0
```

Rank	Title	Lig#	Score	GScore	Lipo	HBond	Metal	Rewards	vdW	Coul	RotB	Site
1	13,3447	5	-7.93	-7.93	-2.2	-0.5	-0.0	-0.7	-34.3	-3.5	0.8	0.1
2	3(68)	40	-6.56	-6.56	-1.2	0.0	-0.0	0.0	-40.7	-0.3	0.3	0.0
3	3(58)	20	-6.44	-6.44	-0.4	-0.5	-0.0	-0.0	-40.3	-10.5	0.5	0.5
4	1(12)	32	-6.39	-6.39	-0.0	-0.4	-0.0	-0.7	-44.6	-6.4	0.7	0.5
5	1(22)	43	-6.18	-6.18	-0.1	0.0	-0.0	-0.7	-37.6	-2.8	0.1	0.0
6	3(39)	14	-6.15	-6.15	-0.2	-0.4	-0.0	-1.1	-32.5	-5.7	0.3	0.0
7	1(16)	27	-5.88	-5.88	-0.2	-0.7	-0.0	-0.1	-42.0	-7.3	0.2	0.8
8	3(45)	62	-5.82	-5.82	-0.8	-0.5	-0.0	-0.5	-32.4	-4.4	1.1	0.2
9	1(51)	2	-5.74	-5.74	-0.3	-0.4	-0.0	-1.2	-23.7	-19.5	0.0	0.3
10	12,1701	3	-5.72	-5.72	-0.9	-0.7	-0.0	-2.2	-19.6	-6.1	0.5	0.2
11	15, 4484	17	-5.69	-5.69	-0.2	-0.5	-0.0	-1.2	-27.9	-8.2	0.5	0.1
12	1(8)	26	-5.65	-5.65	-0.2	-0.4	-0.0	-0.6	-35.4	-8.4	0.3	0.0
13	3(64)	55	-5.50	-5.50	-0.2	-0.2	-0.0	-0.5	-35.2	-6.6	0.2	0.5
14	1(4)	50	-5.36	-5.36	-0.3	-0.4	-0.0	-0.8	-29.2	-4.0	0.4	0.3
15	3(46)	48	-5.32	-5.32	-0.2	0.0	-0.0	-1.4	-29.1	-3.9	0.3	0.1
16	3(35)	29	-5.26	-5.26	-0.2	-0.6	-0.0	-0.3	-33.1	-9.3	0.3	0.2
17	3(53)	54	-5.24	-5.24	-0.2	-0.4	-0.0	-1.2	-25.7	-2.8	0.5	0.1
18	1(33)	7	-5.09	-5.09	-0.2	-1.1	-0.0	-1.0	-19.3	-11.1	0.0	0.1
19	1(6)	16	-5.08	-5.08	-0.2	-1.5	-0.0	-0.4	-28.5	-11.6	0.7	0.7
20	1(23)	31	-5.07	-5.07	-0.4	-0.4	-0.0	-1.0	-31.6	-4.7	0.4	0.2
21	1(56)	52	-4.96	-4.96	-0.2	-0.4	-0.0	-0.8	-22.5	-4.7	0.3	0.0
22	1(7)	28	-4.95	-4.95	-0.3	-0.3	-0.0	-0.6	-31.0	-6.2	0.0	0.1
23	3(41)	45	-4.95	-4.95	-0.3	-0.1	-0.0	-1.4	-24.5	-1.4	0.0	0.2
24	1(18)	30	-4.80	-4.80	-0.0	-0.7	-0.0	-0.1	-41.8	-10.3	0.6	0.9
25	2(6)	35	-4.73	-4.73	-0.6	-0.3	-0.0	-0.8	-22.7	-5.7	0.2	0.2
26	3(43)	70	-4.64	-4.64	-0.0	-0.3	-0.0	0.1	-28.8	-7.7	0.1	0.4
27	3(23)	13	-4.64	-4.64	0.0	-0.7	-0.0	-1.2	-21.1	-20.1	0.3	0.9
28	11,761	18	-4.62	-4.62	-0.2	-1.0	-0.0	-0.3	-42.4	-7.0	0.8	0.6
29	1(3)	61	-4.57	-4.57	-0.3	0.0	-0.0	-0.9	-32.1	0.3	0.4	0.1
30	3(78)	22	-4.57	-4.57	0.0	-0.7	-0.0	-1.2	-29.8	-11.2	0.5	0.6
31	1(55)	12	-4.49	-4.49	-0.5	-0.8	-0.0	-2.0	-18.0	-5.7	0.6	0.1
32	13,3783	76	-4.47	-4.47	-0.7	-0.3	-0.0	-0.2	-29.0	-1.4	0.8	0.1
33	2(17)	10	-4.47	-4.47	-2.2	-0.2	-0.0	-0.7	-35.4	-6.7	1.4	0.3

34 3(31)	44	-4.46	-4.46	-0.1	-0.4	-0.0	-1.4	-25.8	-0.4	0.2	0.0
35 1(53)	9	-4.45	-4.45	-0.1	-0.3	-0.0	-1.9	-31.2	-3.4	0.0	0.1
36 13,2013	24	-4.45	-4.45	-0.2	0.0	-0.0	-1.6	-21.3	-6.1	0.0	0.1
37 2(12)	19	-4.45	-4.45	-0.6	-0.8	-0.0	-2.0	-27.4	-4.6	1.2	0.3
38 3(80)	37	-4.44	-4.44	-0.1	-0.6	-0.0	-1.5	-30.1	-2.3	0.5	0.4
39 3(76)	64	-4.44	-4.44	-0.3	-0.4	-0.0	-0.4	-30.4	-4.1	0.4	0.2
40 8903	41	-4.39	-4.39	-0.2	-0.2	-0.0	-0.3	-34.1	-9.1	0.5	0.0
41 3(24)	8	-4.39	-4.39	0.0	-1.2	-0.0	-1.1	-24.7	-14.7	0.6	0.3
42 1(11)	47	-4.38	-4.38	-0.1	-0.3	-0.0	-2.0	-12.9	-1.6	0.0	0.0
43 11,1851	25	-4.32	-4.32	-0.5	-1.1	-0.0	0.0	-31.0	-9.8	0.3	0.4
44 3(62)	59	-4.32	-4.32	-0.7	-0.7	-0.0	-1.5	-22.9	-15.5	3.1	0.4
45 3(25)	56	-4.30	-4.30	-0.1	-0.4	-0.0	-0.7	-27.0	-4.4	0.3	0.2
46 12,2595	68	-4.18	-4.18	-0.5	-0.2	-0.0	0.0	-34.2	-12.5	0.5	0.6
47 3(71)	33	-4.04	-4.04	-0.3	-1.0	-0.0	-0.9	-23.8	-5.9	0.4	0.3
48 3(72)	66	-4.03	-4.03	-0.1	-0.1	-0.0	-1.3	-20.6	-3.7	0.0	0.6
49 3(13)	36	-4.02	-4.02	0.0	-0.3	-0.0	-1.4	-29.6	-3.9	0.4	0.0
50 1(10)	42	-4.00	-4.00	-0.1	-0.4	-0.0	-1.7	-23.0	-6.8	0.5	0.5
51 9731	4	-3.92	-3.92	-1.3	-0.3	-0.0	-2.2	-19.7	-3.1	0.5	0.0
52 1(13)	74	-3.91	-3.91	-0.1	-0.3	-0.0	-1.3	-17.5	-1.8	0.4	0.1
53 3(40)	6	-3.87	-3.87	-0.0	-0.9	-0.0	-1.3	-39.4	-12.4	0.7	0.5
54 1(57)	58	-3.87	-3.87	0.0	-0.9	-0.0	-1.0	-26.8	-13.1	0.4	1.6
55 51913	53	-3.83	-3.83	-0.4	-0.1	-0.0	-1.5	-22.4	-2.3	0.5	0.2
56 1(9)	49	-3.82	-3.82	-0.2	-0.4	-0.0	-1.7	-19.9	-4.2	0.6	0.2
57 1(29)	38	-3.70	-3.70	-0.0	-0.2	-0.0	-1.6	-18.2	-5.1	0.0	0.2
58 3(69)	63	-3.59	-3.59	0.0	0.0	-0.0	-1.3	-29.0	-1.9	0.0	0.3
59 3(63)	69	-3.58	-3.58	-0.1	0.0	-0.0	-1.0	-28.1	-0.8	0.1	0.1
60 3(21)	57	-3.57	-3.57	0.0	-0.3	-0.0	-1.1	-22.9	-15.7	1.4	0.1
61 3(52)	75	-3.40	-3.40	-0.4	-0.4	-0.0	0.0	-31.8	-1.7	0.2	0.3
62 3(15)	65	-3.39	-3.39	-0.2	-0.1	-0.0	-0.1	-31.6	-7.8	0.1	0.1
63 1(54)	46	-3.36	-3.36	-0.0	-0.3	-0.0	-2.0	-12.3	-5.1	0.5	0.2
64 3(11)	79	-3.16	-3.16	-0.2	-0.0	-0.0	-0.8	-22.4	-3.1	0.0	1.2
65 12,539	39	-3.05	-3.05	-0.3	-0.5	-0.0	-0.0	-34.9	-9.4	0.3	0.0
66 1(20)	11	-2.94	-2.94	-0.0	-1.0	-0.0	-1.4	-22.8	-13.6	0.7	0.2
67 2(4)	21	-2.64	-2.64	-0.2	-1.1	-0.0	-0.0	-38.4	-9.8	0.3	0.5
68 3(44)	60	-2.62	-2.62	0.0	-0.1	-0.0	-1.1	-25.9	-2.9	0.3	0.0
69 3(50)	73	-2.43	-2.43	-0.2	0.0	-0.0	-0.5	-31.5	-2.9	0.1	0.6
70 1(17)	82	-2.43	-2.43	-0.5	0.0	-0.0	0.0	-14.6	-9.1	0.7	0.8
71 3(37)	15	-2.43	-2.43	-0.1	-1.0	-0.0	-0.4	-26.3	-14.3	0.5	0.0

Bottom 10 results

72 3(38)	78	-2.42	-2.42	-0.3	0.0	-0.0	-0.4	-28.9	-2.5	0.4	0.7
73 10,2159	67	-2.23	-2.23	-0.4	-0.3	-0.0	-0.0	-40.4	-0.4	0.5	0.0
74 11,2269	81	-2.14	-2.14	-0.3	-1.0	-0.0	-2.0	-23.7	-10.8	4.6	0.0
75 1(28)	71	-2.10	-2.10	-1.4	0.0	-0.0	0.0	-22.2	-5.1	0.9	0.1
76 1(5)	23	-1.99	-1.99	-0.2	-0.8	-0.0	-0.7	-27.3	-11.4	0.8	0.1
77 9,2969	72	-1.77	-1.77	-0.3	-0.3	-0.0	-0.7	-24.4	-6.9	0.6	0.5
78 3(49)	77	-1.60	-1.60	-0.1	-0.4	-0.0	-0.1	-31.4	-4.6	0.3	0.3
79 3(59)	51	-1.08	-1.08	-0.0	-0.1	-0.0	-1.4	-26.4	-2.6	0.0	0.1
80 1(25)	80	-1.07	-1.07	-0.1	-0.4	-0.0	-1.3	-30.7	-6.6	2.6	0.6
81 1(19)	34	-0.52	-0.52	-0.2	-0.4	-0.0	-0.4	-38.1	-10.2	0.2	0.7

GlideScore (GScore) is given by:

GScore = a * vdW + b * Coul + Lipo + HBond + Metal + Rewards + RotB + Site,

where

vdW	= van der Waals interaction energy
Coul	= Coulomb interaction energy
Lipo	= Lipophilic-contact plus phobic-attractive term
HBond	= Hydrogen-bonding term
Metal	= Metal-binding term (usually a reward)
Rewards	= Various reward or penalty terms
RotB	= Penalty for freezing rotatable bonds
Site	= Polar interactions in the active site,

and the coefficients of vdW and Coul are:

a = 0.063, b = 0.120 for Standard Precision (SP) Glide 4.5
(note that the Coulomb contribution is capped at -4 kcal/mol)
a = 0.080, b = 0.100 for Standard Precision (SP) Glide 4.0
a = 0.065, b = 0.130 for Standard Precision (SP) Glide 3.5

MMGBSA and binding free energy

It is used to calculate the free energy of binding

FEB = PMCE-PMLE-PMRE

It tells us about the feasibility of the structure.

Table 3: Calculated free energy values

Ligand_title	PMCE	PMLE	PMRE	PMDB
133447	-13686.36	4.31	-13723.9	33.23
3(68)	-13716.87	10.78	-13723.9	-3.75
3(58)	-13705.03	13.4	-13723.9	5.47
1(12)	-13928.75	-215.67	-13723.9	10.82
1(22)	-13695.42	23.97	-13723.9	4.51
3(39)	-13716.98	3.15	-13723.9	3.77
1(16)	-13959.8	-253.26	-13723.9	17.36
3(45)	-13638.91	12.55	-13723.9	72.44
1(51)	-13787.29	-47.43	-13723.9	-15.96
121701	-13746.9	-14.13	-13723.9	-8.87
154484	-13722.51	20.83	-13723.9	-19.44
1(8)	-13894.7	-165.93	-13723.9	-4.87
3(64)	-13718.11	2.82	-13723.9	2.97
1(4)	-13808.78	-80.21	-13723.9	-4.67
3(46)	-13769.55	-48.41	-13723.9	2.76
3(35)	-13689.49	43.57	-13723.9	-9.16
3(53)	-13703.25	19.92	-13723.9	0.73
1(33)	-13665.28	70.46	-13723.9	-11.84
1(6)	-13758.01	-36.7	-13723.9	2.59
1(23)	-13693.57	41.8	-13723.9	-11.47
1(56)	-13665.39	48.74	-13723.9	9.77
1(7)	-13691.33	18.2	-13723.9	14.37
3(41)	-13690.13	31.59	-13723.9	2.18
1(18)	-13787.49	-72.94	-13723.9	9.35
2(6)	-13663.2	59.37	-13723.9	1.33
3(43)	-13604.85	65.48	-13723.9	53.57
3(23)	-13844.32	-139.53	-13723.9	19.11
11761	-13707.59	-17.13	-13723.9	33.44
1(3)	-13837.12	-164.03	-13723.9	50.81
3(78)	-13767.98	-60.66	-13723.9	16.58
1(55)	-13731.07	6.74	-13723.9	-13.91
133783	-13691.42	33.48	-13723.9	-1
2(17)	-13670.03	1.46	-13723.9	52.41
3(31)	-13629.15	83.32	-13723.9	11.43
1(53)	-13723.59	-22.83	-13723.9	23.14
132013	-13731.46	-5.21	-13723.9	-2.35
2(12)	-13758.72	-28.91	-13723.9	-5.91

3(80)	-13668.22	39.92	-13723.9	15.76
3(76)	-13670.35	28.28	-13723.9	25.27
8903	-13702.77	21.23	-13723.9	-0.1
3(24)	-13786.41	-75.78	-13723.9	13.27
1(11)	-13730.14	-16.55	-13723.9	10.31
111851	-13678.63	29.2	-13723.9	16.07
3(62)	-13817.2	-72.19	-13723.9	-21.11
3(25)	-13674.82	26.5	-13723.9	22.58
122595	-13726.19	-3.89	-13723.9	1.6
3(71)	-13694.84	26.83	-13723.9	2.23
3(72)	-13686.36	19.07	-13723.9	18.47
3(13)	-13624.35	94.85	-13723.9	4.7
1(10)	-13819.3	-84.93	-13723.9	-10.47
9731	-13722.31	-1.28	-13723.9	2.87
1(13)	-13660.38	51.42	-13723.9	12.1
3(40)	-13814.82	-116.04	-13723.9	25.12
1(57)	-13735.48	-24.73	-13723.9	13.15
51913	-13678.35	29.54	-13723.9	16.01
1(9)	-13704.27	16.92	-13723.9	2.71
1(29)	-13717.68	-0.74	-13723.9	6.96
3(69)	-13660.74	35.49	-13723.9	27.67
3(63)	-13650.54	65.45	-13723.9	7.91
3(21)	-13734.42	-34.59	-13723.9	24.07
3(52)	-13627.56	57.53	-13723.9	38.81
3(15)	-13697.67	20.01	-13723.9	6.22
1(54)	-13729.15	4.29	-13723.9	-9.54
3(11)	-13661.76	32.04	-13723.9	30.1
12539	-13693.77	-0.63	-13723.9	30.76
1(20)	-13824.09	-102.32	-13723.9	2.13
2(4)	-13742.04	-41.1	-13723.9	22.96
3(44)	-13614.57	72.2	-13723.9	37.13
3(50)	-13669.31	25.26	-13723.9	29.33
1(17)	-13705.18	-22.67	-13723.9	41.39
3(37)	-13708.71	16.45	-13723.9	-1.26
3(38)	-13662.3	39.04	-13723.9	22.56
102159	-13677.58	25.92	-13723.9	20.4
112269	-13757.06	-24.07	-13723.9	-9.09
1(28)	-13605.87	23.65	-13723.9	94.38
1(5)	-13713.18	-1.37	-13723.9	12.09
92969	-13678.84	42.8	-13723.9	2.26
3(49)	-13619.72	59.38	-13723.9	44.8
3(59)	-13676.48	23.67	-13723.9	23.75
1(25)	-13721.1	-16.5	-13723.9	19.3
1(19)	-13650.9	49.13	-13723.9	23.87

v). ADME Screening

It also evaluate the acceptability of the analogues based on the Lipinski's Rule of 5 essential for Drug design and here total of 50 compounds showed drug like characteristics based on Lipinski's rule of 5.

Table 3: Screening of ADME properties of Lead Compounds using QikProp simulation

lsbd Title	MW	dipole	SASA	FOSA	volume	glob	Rule of 5	Rule of 3
13;3447	386.53	2.819	670.227	401.934	1257.906	0.840831	0	0
3(68)	571.505	1.295	904.271	272.138	1650.581	0.746949	0	2
3(58)	494.035	1.309	846.05	461.289	1531.606	0.75951	0	1
1(12)	413.522	5.776	770.747	461.358	1352.584	0.767414	0	1
1(22)	396.46	3.559	624.285	140.381	1123.355	0.83713	0	0
3(39)	346.422	3.391	577.864	309.724	1049.665	0.864384	0	0
1(16)	555.998	11.774	697.295	109.072	1355.845	0.849615	3	1
3(45)	421.662	4.66	884.781	800.647	1582.225	0.742178	1	1
1(51)	304.212	5.983	477.852	0	811.248	0.88032	0	1
12;1701	153.227	5.028	409.277	264.806	643.431	0.880675	0	0
15;4484	316.353	6.967	595.748	240.359	1043.001	0.834883	0	0
1(8)	408.456	3.575	724.406	462.745	1294.124	0.792806	0	0
3(64)	430.463	6.847	645.991	343.144	1203.795	0.847175	0	1
1(4)	369.468	2.938	678.019	211.917	1210.693	0.810238	0	0
3(46)	315.371	5.162	574.361	269.682	1002.158	0.843213	0	0
3(35)								
3(53)	296.365	3.598	613.644	330.319	1052.874	0.815642	0	0
1(33)	330.423	5.826	523.958	390.284	985.711	0.914186	0	0
1(6)								
1(23)	618.473	3.878	874.313	121.497	1597.144	0.755778	0	1
1(56)	362.508	1.224	643.483	609.054	1180.453	0.839448	1	0
1(7)								
3(41)	262.31	7.633	509.563	168.424	866.91	0.862879	0	0
1(18)	480.008	9.69	829.774	325.322	1514.835	0.768745	0	1
2(6)	366.843	2.924	623.686	145.598	1113.748	0.83315	0	1
3(43)								
3(23)	306.326	8.868	572.521	0	965.577	0.825211	1	0
11;761	468.613	10.326	820.222	429.275	1520.37	0.77959	0	0
1(3)	359.473	2.987	710.792	379.203	1259.356	0.793453	0	0
3(78)	301.344	5.932	568.632	241.25	979.983	0.839099	0	0
1(55)	148.158	3.331	339.666	243.755	536.329	0.93987	0	0
13;3783	474.131	6.63	889.821	602.878	1628.071	0.752162	0	0
2(17)	431.632	4.225	823.312	500.051	1482.716	0.763787	1	1
3(31)								
1(53)								
13;2013	228.288	6.132	423.83	340.221	749.644	0.941622	0	0

2(12)	216.28	0.604	488.418	411.999	819.193	0.86689	0	0
3(80)								
3(76)	436.563	3.205	735.906	521.419	1364.062	0.808287	1	1
8903	448.573	0.487	717.55	338.793	1350.222	0.823347	0	1
3(24)	317.344	6.932	574.52	237.708	1000.941	0.842298	0	0
1(11)	177.633	3.437	380.287	80.765	604.299	0.908981	0	0
11;1851	595.688	6.921	937.775	635.468	1825.184	0.770202	2	1
3(62)	262.136	10.879	472.847	227.279	763.5	0.854379	0	1
3(25)	386.487	6.427	631.164	392.243	1186.183	0.8586	0	0
12;2595	574.783	7.459	902.881	467.728	1776.544	0.785691	1	1
3(71)	355.473	2.128	616.492	581.705	1137.231	0.85468	0	0
3(72)	282.336	7.033	461.383	384.652	847.1	0.938412	0	0
3(13)	266.38	2.998	533.801	510.228	942.847	0.871124	0	0
1(10)	255.682	11.69	487.637	93.646	797.293	0.852734	0	0
9731	161.203	4.593	382.195	104.443	619.703	0.919749	0	0
1(13)	292.417	2.014	565.944	516.641	1030.425	0.871771	0	0
3(40)								
1(57)	351.303	6.257	526.664	86	902.349	0.857461	0	0
51913	246.305	2.594	499.806	256.009	856.705	0.872806	0	0
1(9)	213.279	4.237	439.176	158.143	761.03	0.917897	0	0
1(29)	228.288	3.857	432.869	330.082	747.635	0.920312	0	0
3(69)								
3(63)	354.534	7.214	601.892	518.837	1157.153	0.885606	0	0
3(21)	318.243	11.331	587.853	328.467	1003.907	0.824819	0	1
3(52)	628.845	5.058	1005.862	919.686	1954.591	0.751618	2	1
3(15)	716.844	7.09	1052.184	975.389	2102.305	0.754288	3	1
1(54)								
3(11)	366.377	5.223	533.814	422.948	997.566	0.904488	0	0
12;539	588.573	1.215	839.67	487.202	1671.872	0.811319	2	1
1(20)	296.331	6.582	576.897	0	956.135	0.813604	1	1
2(4)	627.823	14.563	960.525	662.85	1910.584	0.775236	2	2
3(44)								
3(50)								
1(17)	522.476	5.381	886.924	217.579	1595.648	0.744566	2	1
3(37)	432.599	10.319	725.966	434.978	1376.653	0.824388	0	0
3(38)	440.502	6.394	686.281	415.67	1275.661	0.828871	1	1
10;2159	632.795	4.158	1118.175	496.866	2059.955	0.700208	2	1
11;2269								
1(28)	514.152	8.318	954.795	506.295	1733.643	0.730962	2	1
1(5)	392.494	5.485	719.559	366.682	1346.93	0.819714	1	2
9;2969	394.553	3.898	685.985	612.029	1311.389	0.84464	0	0
3(49)	491.069	5.204	779.645	543.032	1462.339	0.799159	1	1
3(59)								
1(25)	287.398	4.03	600.423	537.858	1052.504	0.833406	0	0
1(19)	459.424	13.897	690.325	210.472	1253.76	0.814556	0	2

It gives the values of the various energies calculated which help in predicting the ligand binding.

Table 4: Calculated energies for various compounds

lsbd Title	ETEWC	EVE	EvdWE	EEE	ESE	ECE	EMC
13	3447	19.44	60.45	-156.49	115.48	0	T
3(68)	-124.95	14.87	-111.29	-28.53	0	0	T
3(58)	-116.24	-10.55	-159.86	54.18	0	0	T
1(12)	22.35	72.09	-162.54	112.79	0	0	T
1(22)	-3.32	32.98	-184.68	148.38	0	0	T
3(39)	-119.81	35.12	-112.77	-42.16	0	0	T
1(16)	-166.52	44.87	-148.59	-62.8	0	0	T
3(45)	499.58	23.28	-225.71	702	0	0	T
1(51)	-211.6	21.54	-71.17	-161.97	0	0	T
12	1701	44.39	27.56	-41.45	58.28	0	T
15	4484	121.32	10.69	-143.61	254.24	0	T
1(8)	-94.74	62.58	-200.81	43.48	0	0	T
3(64)	89.04	-22.43	-136.71	248.17	0	0	T
1(4)	167.87	25.31	-145.7	288.26	0	0	T
3(46)	-87.47	9.5	-58.14	-38.83	0	0	T
3(35)	-81.88	-16.53	-119.04	53.69	0	0	T
3(53)	406.16	14.17	-144.41	536.4	0	0	T
1(33)	47.75	21.65	-152.25	178.34	0	0	T
1(6)	14.96	10.22	-100.18	104.91	0	0	T
1(23)	-183.52	15.35	-69.85	-129.02	0	0	T
1(56)	173.71	-23.83	-182.9	380.43	0	0	T
1(7)	-464.31	33.12	-144.79	-352.64	0	0	T
3(41)	147.64	-3.21	-94.14	245	0	0	T
1(18)	-86.84	37.05	-205.68	81.79	0	0	T
2(6)	-162.87	6.83	-72.67	-97.03	0	0	T
3(43)	-91.81	32.94	-190.64	65.89	0	0	T
3(23)	-210.43	27.62	-170.66	-67.39	0	0	T
11	761	49.75	39.62	-219.11	229.24	0	T
1(3)	55.71	103.13	-138.81	91.39	0	0	T
3(78)	-57.8	-0.94	-138.3	81.44	0	0	T
1(55)	107.32	6.09	-69.2	170.43	0	0	T
13	3783	-190.44	15.21	-99.43	-106.22	0	T
2(17)	-13.88	103.01	-136.98	20.09	0	0	T
3(31)	-126.5	5.71	-62.06	-70.16	0	0	T
1(53)	-556.44	82.37	-115.64	-523.18	0	0	T
13	2013	-113.08	32.51	-75.63	-69.96	0	T
2(12)	-158.04	8.01	-80.22	-85.82	0	0	T
3(80)	-8.79	1.13	-134.33	124.4	0	0	T
3(76)	-17.14	4.02	-146.27	125.11	0	0	T
8903	29.15	-11.58	-180.75	221.48	0	0	T
3(24)	-191.11	-46.51	-74.9	-69.7	0	0	T

1(11)	171.17	33.19	-44.05	182.04	0	0	T
11	1851	-226.4	30.36	-84.53	-172.24	0	T
3(62)	-101.43	108.85	-59.22	-151.06	0	0	T
3(25)	27.66	-8.97	-145.68	182.31	0	0	T
12	2595	-240.56	16.54	-105.53	-151.57	0	T
3(71)	-147.86	2.36	-72.25	-77.97	0	0	T
3(72)	-27.52	11.11	-76.08	37.45	0	0	T
3(13)	68.52	-3.62	-167.02	239.16	0	0	T
1(10)	14.92	10.47	-113.24	117.69	0	0	T
9731	-120.29	19.94	-69.82	-70.41	0	0	T
1(13)	-95.2	1.73	-64.01	-32.92	0	0	T
3(40)	-999.93	-45.8	-185.64	-768.5	0	0	F
1(57)	-114.64	39.82	-167.57	13.1	0	0	T
51913	-134.94	13.29	-68.97	-79.26	0	0	T
1(9)	-144.62	5.5	-57.4	-92.71	0	0	T
1(29)	-150.25	7.33	-55.17	-102.41	0	0	T
3(69)	-18.23	22.23	-108.01	67.55	0	0	T
3(63)	10.6	-0.63	-72.96	84.19	0	0	T
3(21)	-162.85	30.23	-111.77	-81.32	0	0	T
3(52)	-0.46	1.81	-203.07	200.79	0	0	T
3(15)	-152.11	-1.2	-69.34	-81.57	0	0	T
1(54)	-158.58	-29.77	-64.75	-64.06	0	0	T
3(11)	40.75	-5.48	-149.47	195.69	0	0	T
12	539	-44.85	99.27	-231.29	87.17	0	T
1(20)	-234.93	-11.56	-34.86	-188.5	0	0	T
2(4)	-372.74	-14.34	-152.46	-205.94	0	0	T
3(44)	147.2	2.29	-149.04	293.95	0	0	T
3(50)	-526.66	16.93	-178.24	-365.35	0	0	T
1(17)	96.35	50.12	-152.21	198.44	0	0	T
3(37)	-127.01	2.82	-142.57	12.74	0	0	T
3(38)	-16.57	0.51	-159.06	141.98	0	0	T
10	2159	-91.08	6.93	-186.66	88.65	0	T
11	2269	20.8	64.85	-82.79	38.74	0	T
1(28)	-69.21	35.7	-97.34	-7.57	0	0	T
1(5)	-143.72	14.31	-77.87	-80.16	0	0	T
9	2969	-6.91	-20.82	-140.15	154.06	0	T
3(49)	-264.38	21.84	-127.93	-158.3	0	0	T
3(59)	-98.29	-38.39	-102.23	42.32	0	0	T
1(25)	36.77	53.14	-117.28	100.91	0	0	T
1(19)	-298.74	56.22	-122.05	-232.91	0	0	T

Potential lead molecules against DBL domain of pf332:

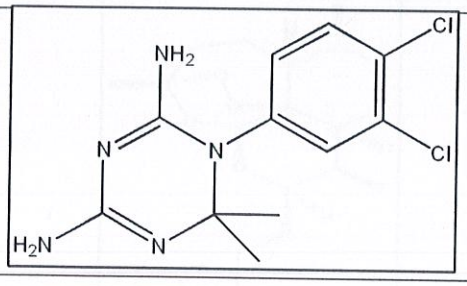
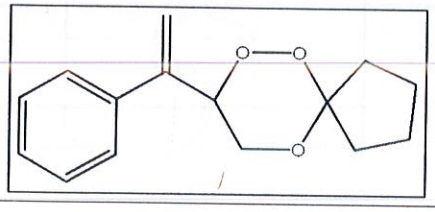
Now we compared the Glide score of Ligands from docking and also Free energy of binding and got set of 18 ligands in which the energy was of negative value, these 18 ligands are the fit for protein with highest negative value for the ligand_id 3(68)

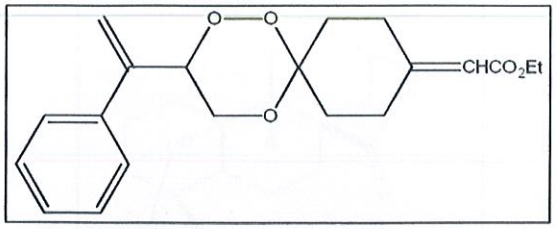
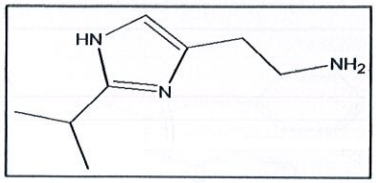
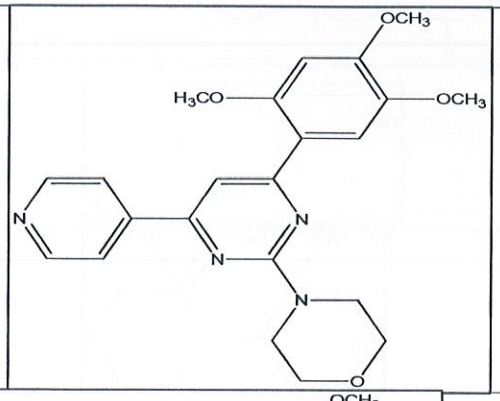
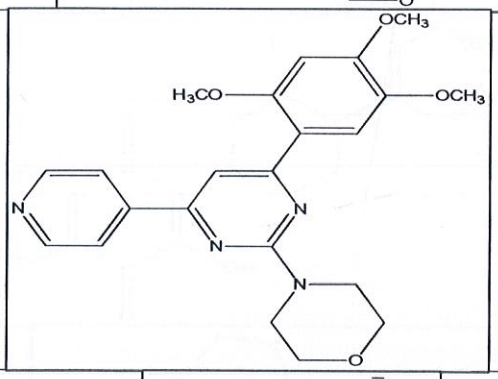
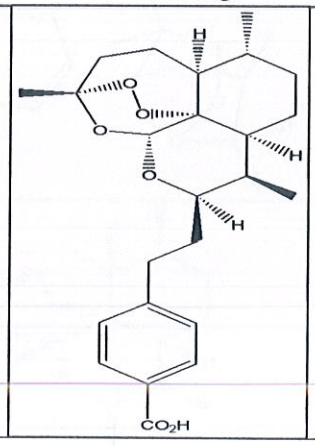
Table 5: Compounds having both glide score and FEB negative.

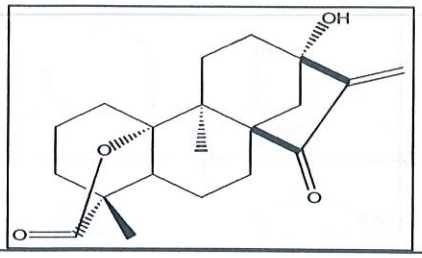
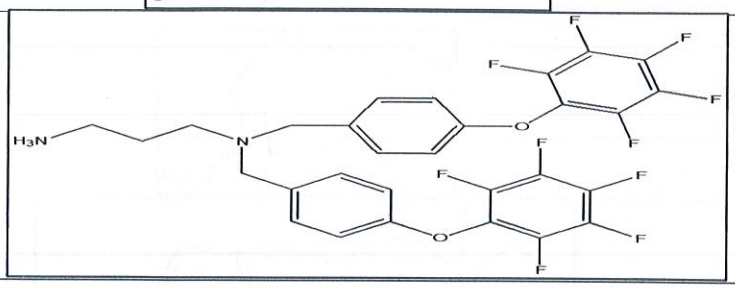
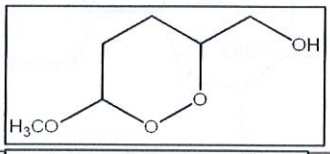
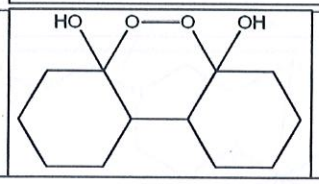
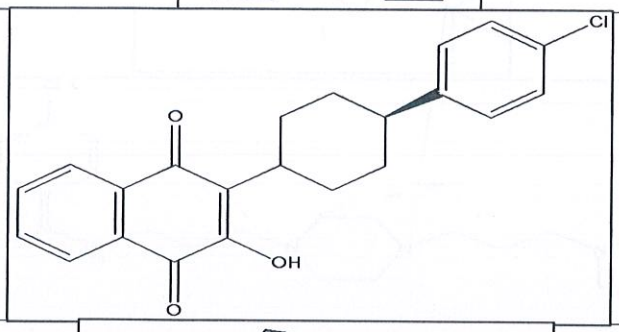
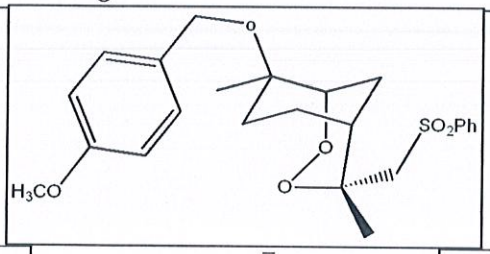
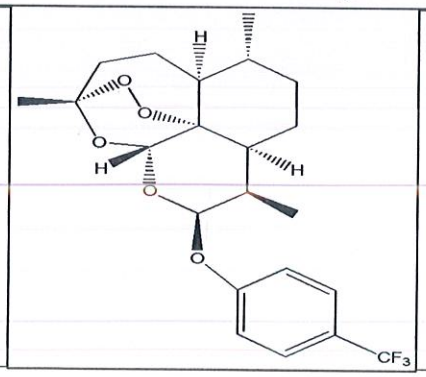
Ligand_title	score	FEB
3(68)	-6.56	-3.75
1(51)	-5.74	-15.96
154484	-5.69	-19.44
121701	-5.72	-8.87
1(8)	-5.65	-4.87
1(4)	-5.36	-4.67
3(35)	-5.26	-9.16
1(33)	-5.09	-11.84
1(23)	-5.07	-11.47
1(55)	-4.49	-13.91
132013	-4.45	-2.35
2(12)	-4.45	-5.91
8903	-4.39	-0.1
3(62)	-4.32	-21.11
1(10)	-4	-10.47
1(54)	-3.36	-9.54
3(37)	-2.43	-1.26
112269	-2.14	-9.09
133783	-4.47	-1

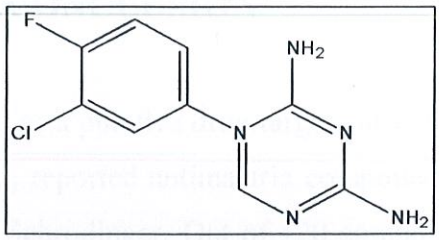
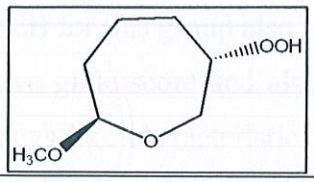
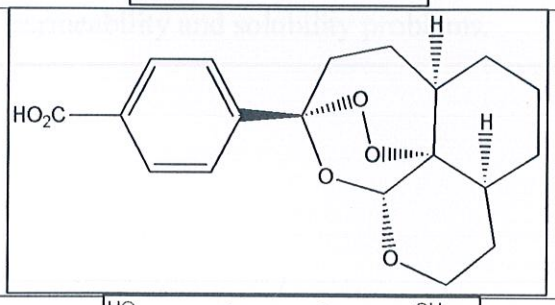
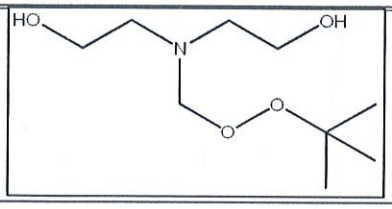
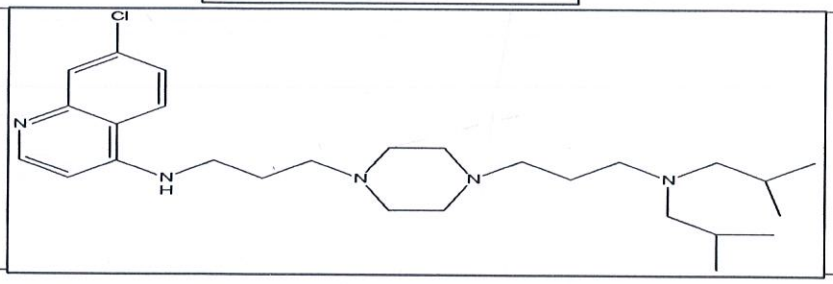
Structures of listed molecules

TABLE-6

Entry	Compound structure
3(68)	
51913	

15, 4484	
121701	
1(8)	
151311	
3(35)	

1(33)	
1(23)	
1(55)	
132013	
2(12)	
8903	
3(62)	

1(10)	
1(54)	
3(37)	
112269	
133783	

CONCLUSION

The DBL domain of Pf332 is considered as a putative drug target since it is conserved in all strains. To design initial set of potential ligands, reported antimalaria compounds whose target not known were docked by SP and XP module of Schrodinger. Out of 120 compounds, 18 have shown good interactions with the target. The calculated FEB for this group also responsibly predicted the activity. Compound with id 3(68) has highest negative glide score and also has negative value for FEB. A total of 14 compounds out of 18 showed drugs like characteristics (based on Lipinski's rule of 5). Thus, these ligands could not have permeability and solubility problems.

FUTURE PROSPECTIVE

Now the binding of these 14 ligands will be studied by molecular dynamics methods. Based on the observations, those above ligands will be modified. New set of ligands will be again docked. This process will be continued till design of optimal ligands. These optimal ligands will be synthesized and tested in wet lab experiments.

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