Docking studies of Diphenylether Derivatives with Enoyl-ACP-Reductase of *Plasmodium falciparum*

A thesis submitted in partial fulfilments of requirements for the degree of

Master of Science in Biochemistry

Supervisor

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Ravinder Kaur

Patiala
Date: 30th June 2018

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Candidate's Declaration

I hereby declare that work being presented in this dissertation entitled "**Docking studies of Diphenylether Derivatives with Enoyl-ACP-Reductase of *Plasmodium falciparum***" in partial fulfilment of the requirement for the award of **Master of Science in Biochemistry** carried out at School of Chemistry and Biochemistry, Thapar Institute of Engineering and Technology, Patiala is my own carried out under the supervision of **Dr. Manmohan Chhibber** TIET, Patiala during the period **January to June 2018**.

Ravinder Kaur

Patiala
Date: 30th June 2018

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This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

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Contents

Abstract

Malaria is one of the leading causes of death among infectious diseases. Among five species of the disease causing organisms, namely *Plasmodium*, two P. falciparum and *P. Vivax* are noxious. Diphenyl ethers are known to inhibit fatty acid biosynthesis in most organisms including *Plasmodium*. In this study, selected diphenyl ether molecules were docked against enoyl-Acyl Carrier Protein- reductase of *P. falciparum*. Using Triclosan, a known inhibitor of the enzyme, as standard fourteen molecules were docked using Autodock4.0 and various interaction and binding energies were observed. Seven of the fourteen compounds were nitro while rest others were containing amino group at ortho position of the ether linkage. **Compound 9** showed the least binding energy of -9.7 Kcal/mol as compared to -9.8 Kcal/mol for the standard triclosan.

Compound 5 having nitro group displayed maximum binding energy -6.9 Kcal/mol and exhibits minimum affinity towards enoyl-Acyl Carrier Protein- reductase of *P. falciparum.*

Malaria is one of the leading causes of death among infectious diseases. As per WHO in the years 2015 and 2016 an estimated 211 and 216 million cases of malaria, respectively, were reported worldwide. The deaths due to the disease were 446000 in 2015 and 445000 in 2016. Among five species of the disease causing organisms, namely *Plasmodium*, two *P. falciparum* and *P. Vivax* are noxious. While former is responsible for malaria related deaths globally the later is dominant in countries outside sub-saharan african region [1, 2].

Plasmodium falciparum, a protozoan, is dispersed through the bite of infected female Anopheles mosquito. Mosquito's bite introduces parasite into human's blood, which multiply in host's liver and destroys the red blood cells. Symptoms of the infection include fever, vomiting, headache, tiredness and in some cases yellow skin, coma leading to death [3].

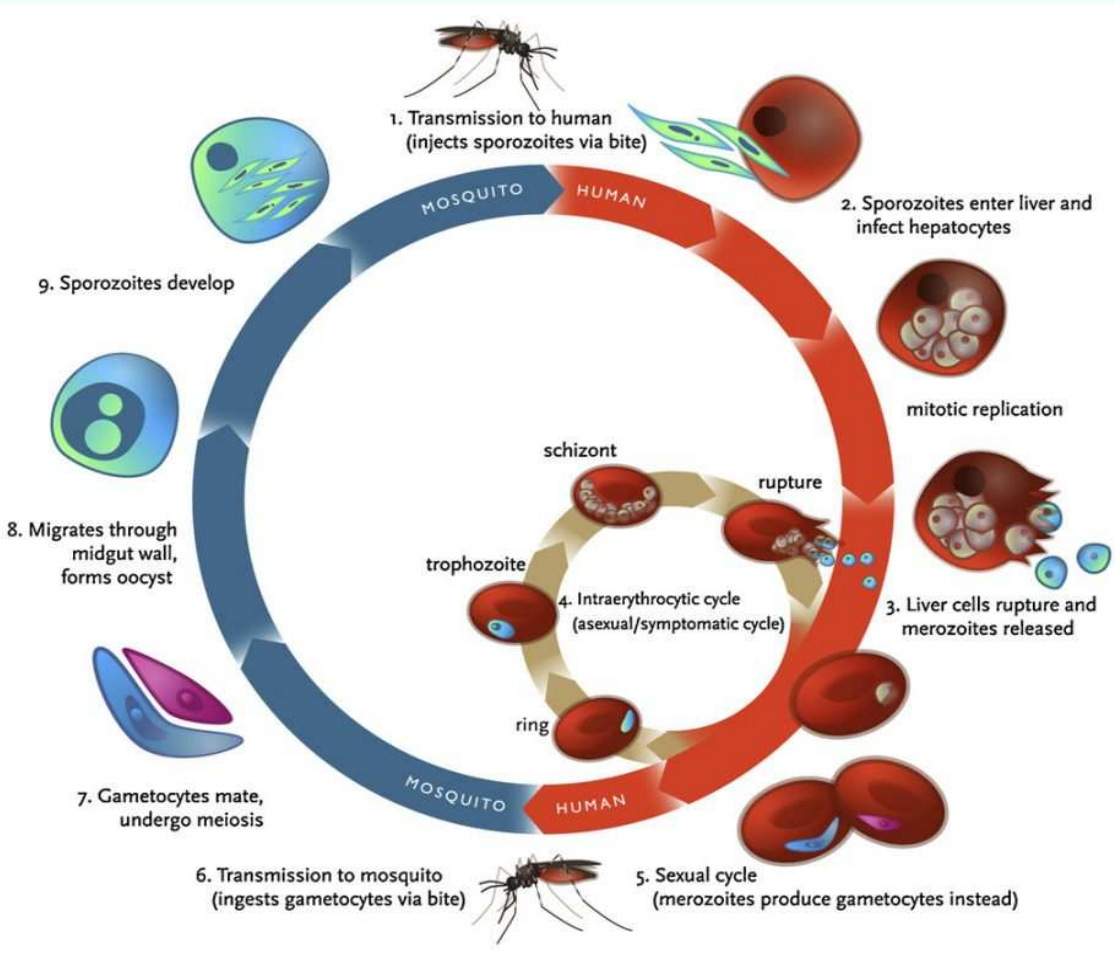


Figure 1: Life cycle of Malaria Parasite [2]

The parasite **(Figure-1)**, is introduced into human host's body by mosquito's bite in the form of sporozoites. Sporozoites then move into the liver through blood circulation,

where they reproduce by mitotic division and are released as infective merozoites when hepatocytes of liver rupture. Merozoites then infect red blood cells, and these infected red-blood cells when multiply leads to parasite replication [4]. In the infected red blood cell parasites multiply asexually in the form of schizonts, trophozites and rings and sexually in the form of gametocytes. These gametocytes are taken with blood meal when uninfected mosquito bites infected person. The parasite then grows in the gut of mosquito. Gametocytes fuses with each other and form ookinete, which further develops into new sporozoites, then being transmitted to uninfected human hosts in its life cycle [5].

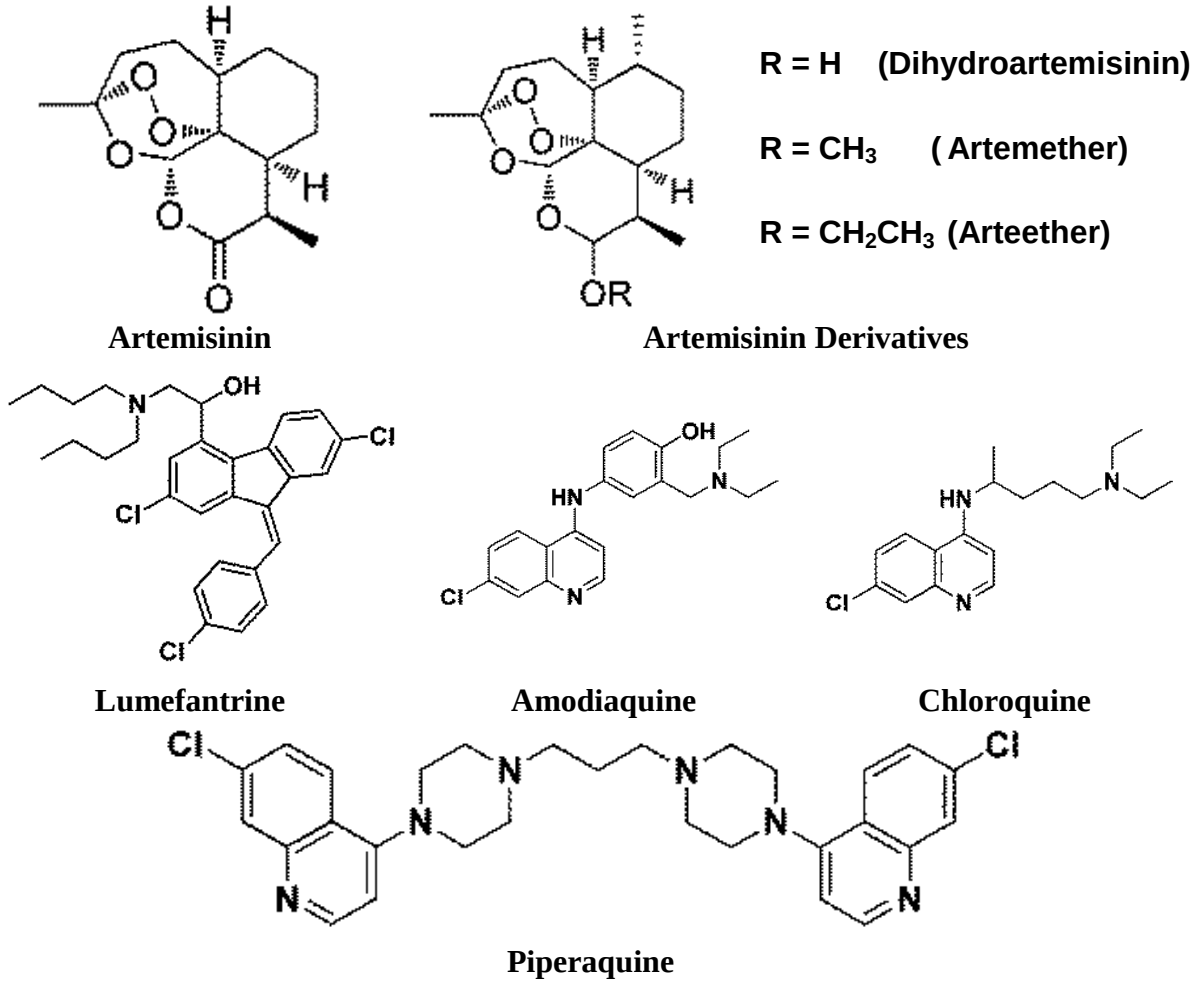


Figure-2: Drug molecules used in artemisinin-based combination therapy (ACTs)

In recent years, artemisinin-based combination therapy (ACTs) has proved successful for global malaria control [6]. Artemisinin **(Figure-2)**, a terpene-based molecule traditionally used in Chinese medicine, in combination with various other drugs like lumefantrine, amodiaquine, piperaquine constitute ACT. The mode of action for this therapy based on the fact that artemisinin or its derivatives quickly reduce parasite population in the infected

person while the other drug eliminates its remaining population to cure the disease [2]. Chloroquine, however, still remains first-line of treatment against *P. vivax* in many nations.

Diphenyl ethers represent another class of molecules that are known to inhibit fatty acid biosynthesis in most organisms including *Plasmodium* [7]. The work in the thesis describes docking studies with selected diphenyl ether molecules against enoyl-Acyl Carrier Protein- reductase of *P. falciparum* enzyme. Antibacterial and docking studies done with the same compounds against *E. coli* have shown encouraging results [8].

Proteins are large macromolecule, also known as building block of life, are made up of amino acids linked by peptide bonds. The functions performed by different proteins are humongous. Some of them are disease protectors, transporters, catalysts, growth controllers, storage houses, messengers, mechanical supporters and many more.

Most of the proteins exist in the folded three dimensional structures. The shape that any protein molecule naturally folds into is called its native conformation. The native conformation has minimum energy conformation of amino acids and maximum intermolecular interactions between them. **Figure-3** below shows various levels of folding in any protein before it acquires its native conformation. The last level of folding, called quaternary structure that results due to interactions among more than one polypeptide chains is optional. Although many proteins are able to perform their functions after acquiring their quaternary structure, many others can perform their usual task in tertiary structure only. [9].

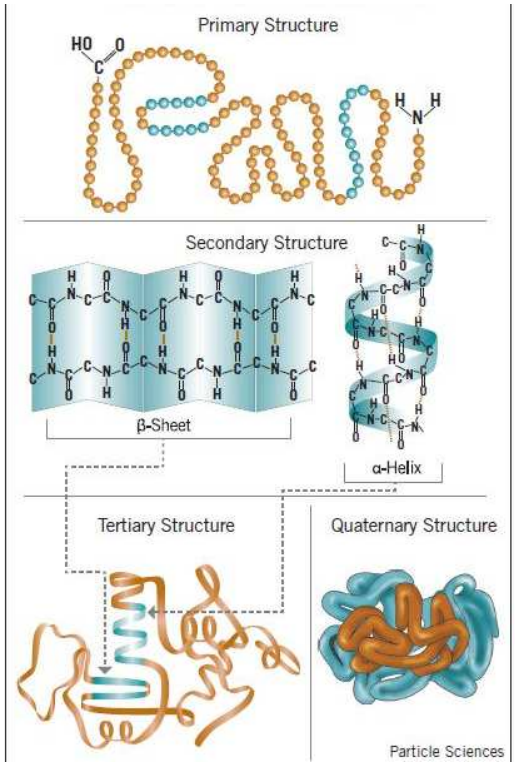


Figure 2: shows the different level of protein structure

Stability and complexity of protein's folded structure can be determine by presence of various interaction between amino acid of protein chain [10]. Protein interactions can form by the complex formation, either long lasting or transient, which occur because of balance

between different properties: shape, sequence, dynamic, entropy etc [11]. Various type of interaction are :

(a) Hydrogen bonding- when an electronegative atom having lone pair of electron attracts the hydrogen atom which bonded electronegative atom. Hydrogen bond contribute to stability and integrity of protein structure . Some amino acids which shows this type of interaction are SER, THR, LYS, ASN etc [11,12].

(b) Disulfide bond-where S-S bond formed by two cysteine residues and these bonds are formed between two thiol group to cyclise the peptide. These bonds stabilise the tertiary structure of protein and influence the quaternary structure [13].

(c) Hydrophobic interaction- these interaction also help in stabilisation of protein tertiary structure. In an aqueous medium polar group is repelled by non polar group, cluster is formed which known as hydrophobic interaction. ALA,VAL interact with aromatic ring of TYR,PHE these are the amino acids which show such type of interaction [14]

(d) Salt bridge- this is a combination of two non covalent interaction that are hydrogen bonding and ionic bonding. The bridge is form between anionic carboxylate (ASP, GLU) and cationic ammonium(LYS, ARG).[15]

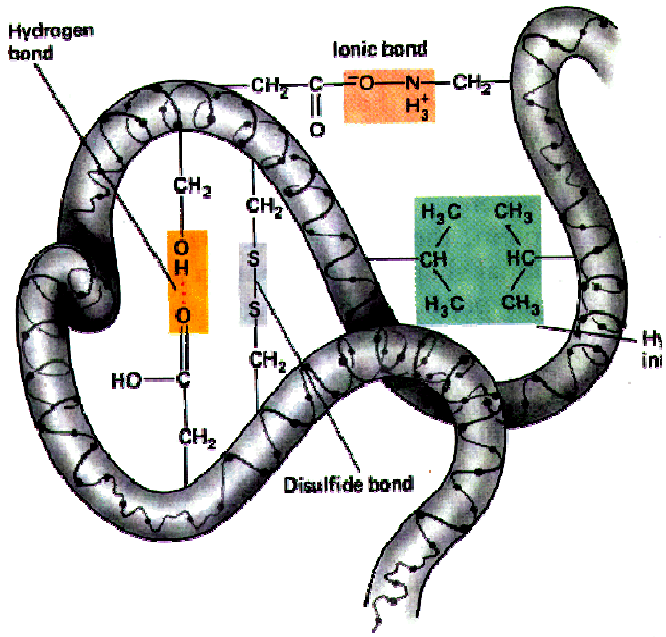
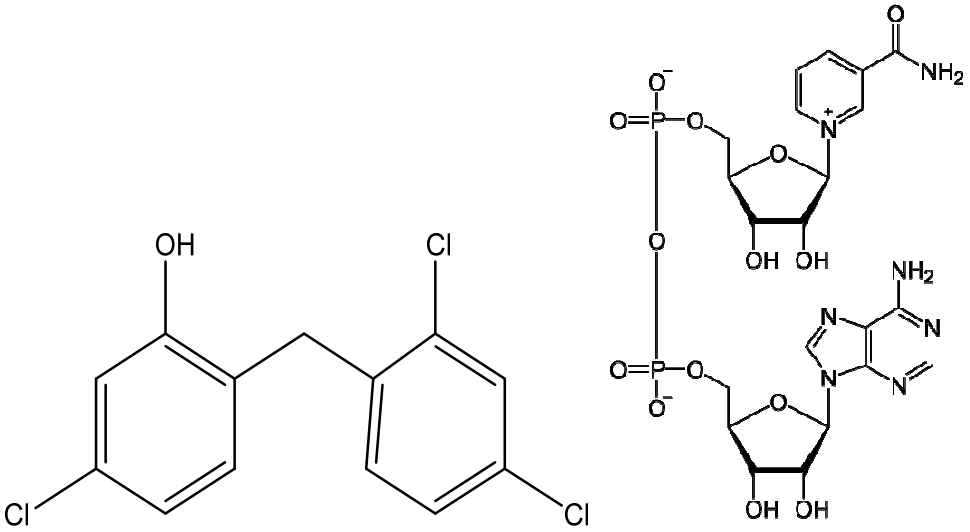


Figure 3: tertiary structure of protein showing various non-covalent interactions.

In fatty acid biosynthesis, Enoyl-acp-reductase is the last step and known as rate limiting enzyme in many bacteria. Triclosan is an antibacterial agent which stops the fatty acid

synthesis by depressing trans-2-enoyl-acp which is bind to FabI. Triclosan is a slow binding inhibitor and dorms the ternary structure FabI-NAD-triclosan. It is used as consumer goods, hands soaps, cutting boards and facial cleanser [16].



(a) (b)

Figure 4: shows the structure of (a) triclosan and (b) NAD+

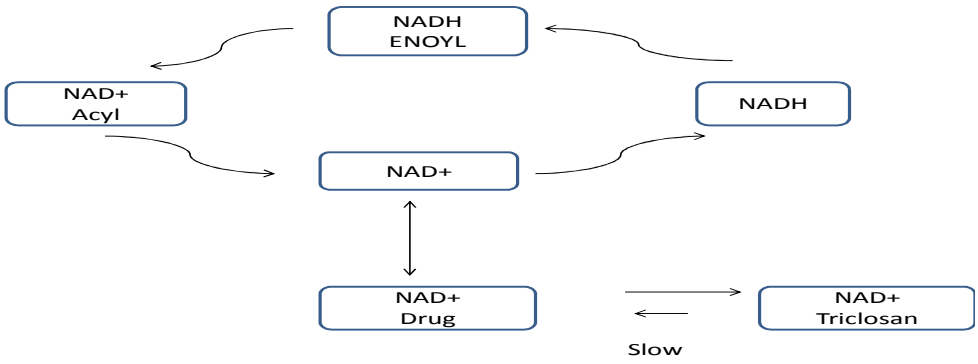


Figure 5: model of FabI inhibition by Triclosan

There are some other inhibitor like triclosan that also inhibit fatty acid biosynthesis and bind with NAD+ and make ternary structure, such as Diazoborenes, Isoniazid and Hexachlorophene. Hexachlorophene is also a antimicrobial compound that is used in surgical

srubs and disinfectants. It is more active for Gram positive bacteria (Staphylococcus) and less active for Gram negative bacteria.

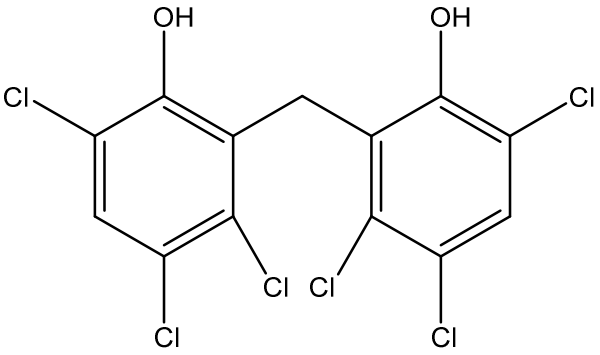


Figure 6: shows the structure of Hexachlorophene

As hexachlorophene and triclosan are mostly similar in structure, one study is done to check whether hexachlorophene is also a FabI inhibitor, where both *E.coli* and *Staphylococcus aureus* FabI are inhibited by both triclosan and hexachlorophene. Observation shows that hexachlorophene makes ternary structure like triclosan make with NAD-FabI and inhibit its synthesis.Diazoborenes in the active site of enzyme, forms the covalent bond with NADH and inhibits the FabI. Isoniazid also a inhibitor of mycobateria FabI by making covalent bonding with with NAD+ in the active site [17,18].

Lead Generation-A set of Diphenylether derivatives were prepared by the assembly of pharmacophoric groups. The 2D structures of the small molecules were drawn and edited using ChemDraw version 15.0. The pdb format for all the compounds was generated using ChemDraw version15.0.

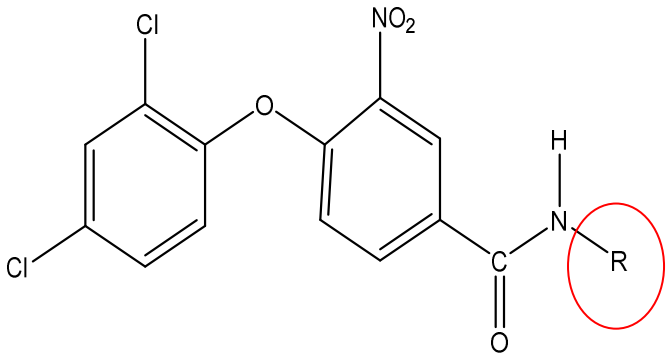


Figure 7: Compund1

Table 1: shows the R groups for compound 1

1	CH_2-CH_2-CH_2-OCH_3
2	CH_2-CH_3
3	CH_2-CH_2-CH_3
4	CH_2-$(CH_2)_2$-CH_3
5	CH_2-$(CH_2)_3$-CH_3
6	CH_2-$(CH_2)_4$-CH_3
7	CH_2-$(CH_2)_5$-CH_3

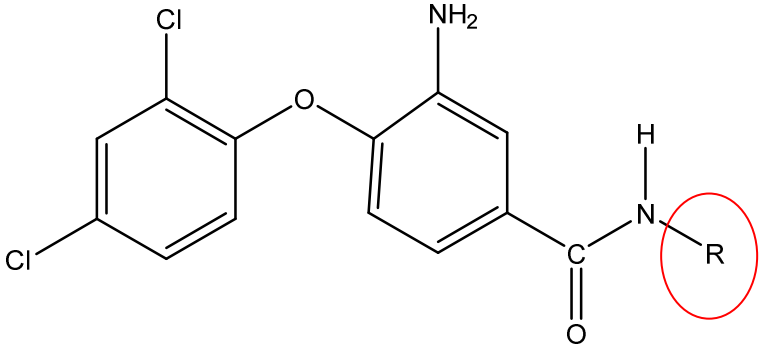


Figure 8: Compund2

Table 2: shows the R groups for compound 2

8	CH_2-CH_2-CH_2-OCH_3
9	CH_2-CH_3
10	CH_2-CH_2-CH_3
11	CH_2-$(CH_2)_2$-CH_3
12	CH_2-$(CH_2)_3$-CH_3
13	CH_2-$(CH_2)_4$-CH_3
14	CH_2-$(CH_2)_5$-CH_3

Protein extraction and molecular docking -

Macromolecule (PDB:1NHG) taken from RCSB PDB in pdb format. As the protein have dimer chain which is identical to each other, we deleted one chain. Protein have triclosan as a ligand and NAD, here binding site of triclosan is considered as standard active site and derivatives are docked to see the interaction by using Autodock4.0 program in MGLtool.

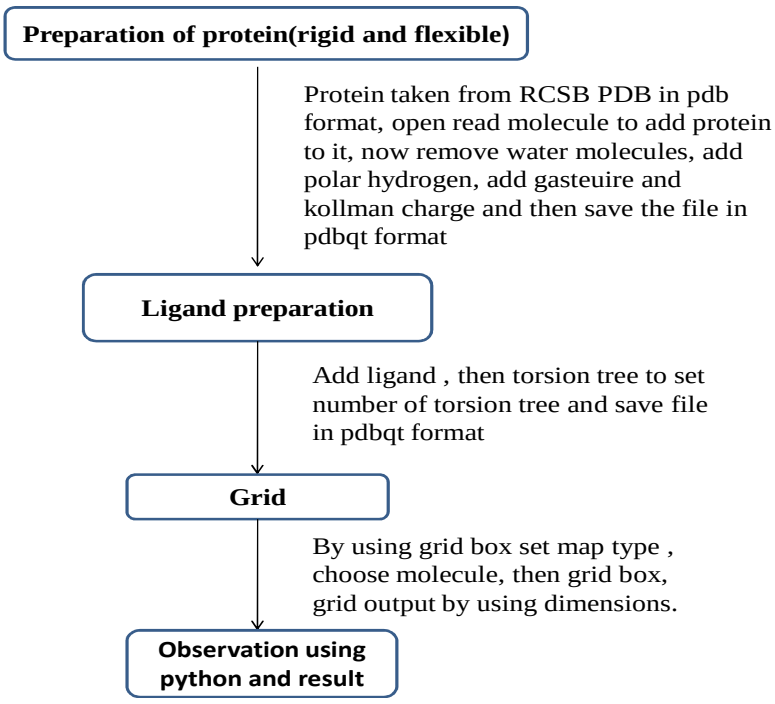


Figure 9: flow chart showing the method for molecular docking using Autodock4.0

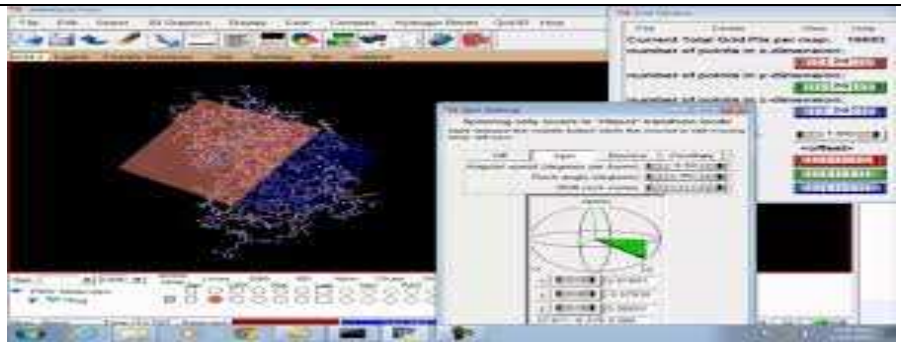
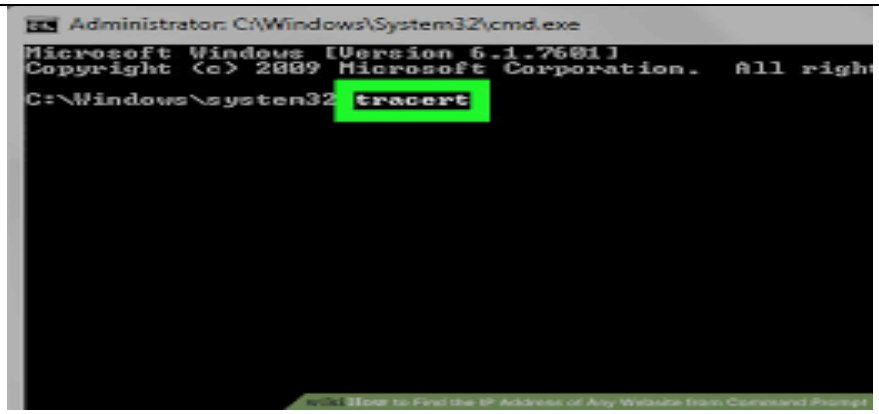
(a)	
(b)	
(c)	

Table 3: shows the Home page of resources used in the experiments (a) autodock vina 4.0 for doing molecular docking (b) command prompt for observation (c) discovery studio to see ineractions.

Virtual Library Screening (VLS) is acquiring importance in comparison to high throughput screening (HTS) due to low cost, less time involved. VLS also facilitates to narrow down experiments on comparatively small set of compounds to give positive results. Malaria caused by *Plasmodium falciparum* is one of the deadliest among the all the four species of the organism and unfortunately the prevalent drugs choloquine and sulfadoxine have been reported to show a failure rate of 10-60% [19].

Fortunately enoyl-acyl carrier protein reductase (ENR) is one of the key malarial enzymes and an important drug target. The enzyme helps in building membrane of the parasite, energy production and does not have any homolog in the human host. This work describes the docking studies with Autodock4.0 for the diphenylether based compounds already synthesized in our research group.

Studies with Enoyl-ACYL- Reductase of *Plasmodium falciparum*

Enoyl-Acyl Carrier Protein- reductase enzyme was taken from the PDB data base having 1NHG as its identity for the *Plasmodium falciparum* (PDB: 1NHG in *Plasmodium falciparum*). *Pf* NHG contains identical dimmer chains (A, B, C, D) and forms a tertiary complex with its inhibitor triclosan and its co-factor nictotinamide-adenine-dinucleotide (NAD^+). As per literature reports all the Enoyl-ACP- reductase enzymes in other bacteria also form similar tertiary complex. Binding site of the enzyme for triclosan and NAD^+ was refined using molecular dynamic stimulation.

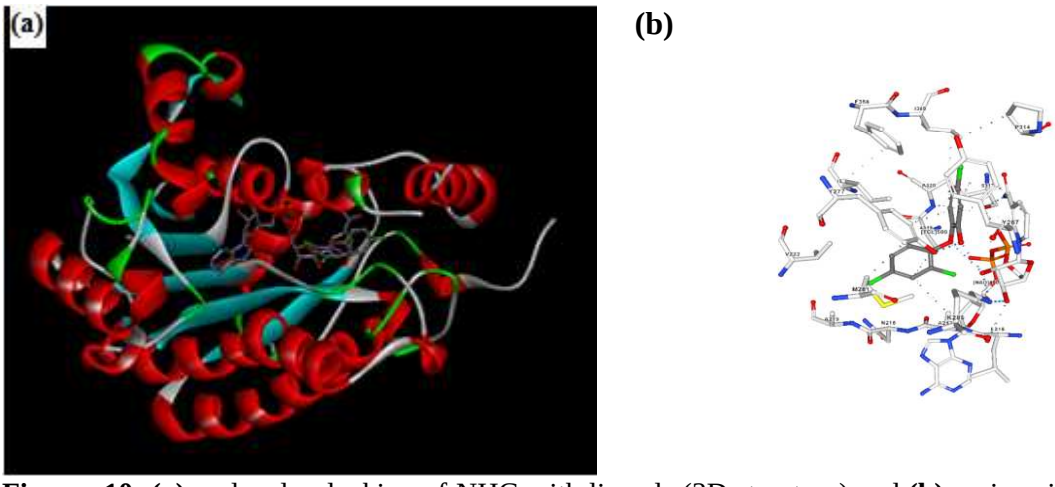
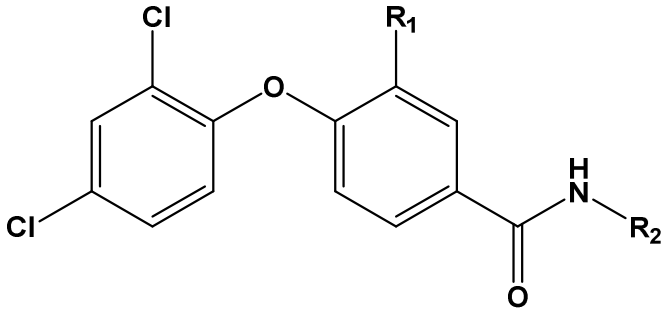


Figure- 10: (a) molecular docking of NHG with ligands (3D structure) and **(b)** various interaction of macromolecule with triclosan and NAD+.

Binding site of 1NHG in *Plasmodium falciparum* is lined up with amino acid residues like TYR277, ILE323, ALA319, SER317, LYS285, GLY219, LEU315, GLY219, LEU265, LEU216, LEU265, SER317, LYS155, ASP154, ASN144, ASP148, ASN175, ARG301, TYR297, LEU227, GLU179 and many others. However, the active site has important amino acids like TYR277 and ILE 323 that bind to the inhibitor triclosan through H-bonding and hydrophobic interactions. The co-factor NAD binds at the active site through pi-pi interactions with TRP131 and alkyl-alkyl interactions with ILE323. The resides that contribute to hydrogen bonding interactions are ALA319, SER317, LEU315, PRO314, GLY219, LYS285, LEU265, LEU216, SER215 and TRP131. **Figure- 10** above shows molecular docking of NHG with ligands (NAD+ and Triclosan) in three diamensional mode and with various residues.

Table -4: Structure of the molecules that were studied for their interactions with *Pf* NHG

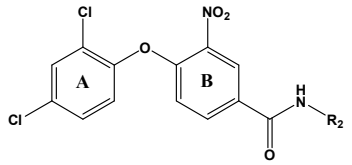
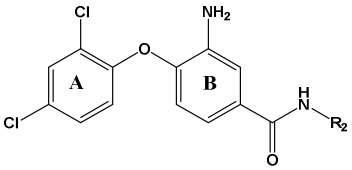


Compound name		
$R_1 = NO_2$	$R_1 = NH_2$	R_2
1	**8**	CH_2-CH_2-CH_2-OCH_3
2	**9**	CH_2-CH_3
3	**10**	CH_2-CH_2-CH_3
4	**11**	CH_2-$(CH_2)_2$-CH_3
5	**12**	CH_2-$(CH_2)_3$-CH_3
6	**13**	CH_2-$(CH_2)_4$-CH_3
7	**14**	CH_2-$(CH_2)_5$-CH_3

For docking studies with diphenyl ether molecules the crystal structure was acquired from PDB database for *Pf* NHG- Tricloan- NAD^+ complex and triclosan deleted. All the molecules as shown in **Table-4** were optimized for their minimum energy configuration before docking with the macromolecule (*Pf* NHG) and the co-factor. Results of blind docking with all the molecules indicated approximately same binding pocket. Also RMSD plot was used to find the stability of the different complexes of the aryl ether derivatives from which the stable complex was used to perform further active pocket site docking.

The co-ordinates obtained from above results were used for fine docking that gave minimum energy of the tertiary complex for all the molecules (**Table -5).**

Table 5: Binding energies of the compound calculated by Auto dock Vina 4.0

Compound Number	Binding energy (Kcal/mol)	Remarks
TCL	**-9.8**	Hydroxy group
1	-8.7	
2	-9.1	
3	-9.5	
4	-8.8	
5	**-6.9**	
6	-8.9	
7	-8.8	
8	-9.3	
9	**-9.7**	
10	-9.2	
11	-7.1	
12	-9.6	
13	-9.4	
14	-9.3	

It can be seen from the above table that for **compound number 1 to 7** the binding energy lies in higher range (-8.8 ± 0.2 Kcal/mol) than for the **compounds 8 to 14** (-9.5 ± 0.3 Kcal/mol). The standard normally used in experimental studies, triclosan, also gave binding energy in lower range as that for later set of compounds. Column -3 of the table above also shows that difference in the structure as far as compounds 1-7 and 8-14 are concerned. Therefore broadly, it can be concluded that amino group being a hydrogen donor behaves in the similar fasion as that of triclosan which has –OH group as a hydrogen donor. Exception to above results was **compound 3 and 2,** which being a hydrohen bond acceptor has exceptionally low binding energy (-9.5 and -9.1 Kcal/mol respectively) as compared to rest of the compounds in the series. This can be attributed to small side chain attached to amide linkage in the molecules. Similarly, among series of compounds with amino group at ortho position (**compounds 8-14**) **compound 9** gave lowest binding energy of -9.7 Kcal/mol which was comparable to triclosan as well. This compound also has a C3 carbon chain attached to the amide group. Thus, it can be concluded that small side chain plays a favourable role for lowering the interactions with the *Pf* NHG.

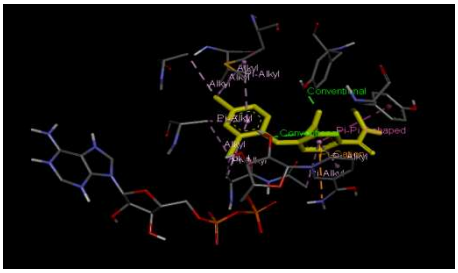
Table -6 shown below lists different interactions between the two series of compounds , amino and nitro, taken for the study compared with triclosan. It is interesting to note that for entire set of amino **compounds 8 to14** ring B shows hydrogen bonding interactions with Tyr 277 which is same as that for triclosan's ring B as well. This is because of hydrogen donor ability of hydroxyl and amino groups to form the hydrogen bond.

Table -6: List of Different Interactions between the Two Series of Compounds

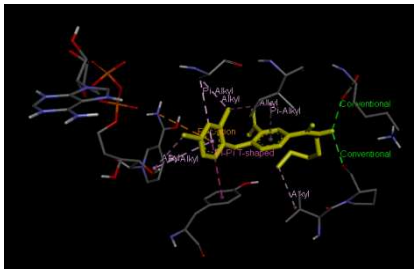
Compd	Hydrogen Bonding	Pi-Alkyl	Alkyl
TCL	TYR277(3.43A˚)ring B	ALA217(3.55A˚)ring B	ALA319(3.46A˚)ring B ALA217(4.40A˚)ring B VAL222(3.48A˚)ring B ALA219(3.68A˚)ring B MET281(4.17A˚)ring B
1	PRO314(3.57A˚)ring A ALA320(3.44A˚)ring A SER317(2.82A˚)ring A	ALA217(4.40A˚)ring B	ALA217 (3.58A˚)ring B ALA219 (4.14A˚)ring B VAL222 (3.62A˚)ring B
2	ALA319(3.43A˚)ring A	TYR267(4.26A˚)ring B MET281(4.43A˚)ring A	ALA319(3.97A˚)ring A ALA320(3.61A˚)ring B
3	PRO314(3.58A˚)ring A SER317(2.77A˚)ring A	ALA217(4.40A˚)ringB	ALA219(4.03A˚)ring B VAL222(3.62A˚)ring B ALA217(3.62A˚)ring B
4	PRO314(3.64A˚)ring A SER317(3.00A˚)ring A	ALA319(4.15A˚)ring B TYR277(3.84A˚)ring A	ALA319(4.26A˚)ring B MET281(3.80A˚)ring B
5	PRO314(2.82A˚)ring A LYS325(2.64A˚)ring A		ALA320(3.35A˚)ring A
6	PRO314(3.50A˚)ring A SER317(2.82A˚)ring A	ALA217(4.41A˚)ring B	VAL222(3.60A˚)ring B ALA219(4.00A˚)ring B
7	ALA319(3.53A˚)ring A	MET281(4.40A˚)ring A TYR267(4.27A˚)ring B	ARG318(4.11A˚)ring B ALA319(3.95A˚)ring A ALA320(3.75A˚)ring B
8	TYR277(2.35A˚)ring B	ALA217(4.39A˚)ring B	VAL222(3.62A˚)ring B MET281(4.08A˚)ring A ALA319(3.45A˚)ring B ALA217(3.59A˚)ring B
9	TYR277(2.35A˚)ring B	ALA217(3.66A˚)ring B	ALA319(3.38A˚)ring B VAL222(3.58A˚)ring B ALA219(3.74A˚)ring B
10	TYR277(2.35A˚)ring B	ALA217(4.43A˚)ring B	VAL222(3.58A˚)ring B MET281(4.09A˚)ring B ALA219(3.70A˚)ring B ALA217(3.55A˚)ring B ALA319(3.50A˚)ring B
11	ALA319(3.59A˚)ring A ASN218(2.44A˚)ring		ARG318(4.42A˚)ring B

	A TYR277(2.35A˚)ring B		
12	TYR277(2.35A˚)ring B	ALA217(4.44A˚)ring B	ALA219(3.74A˚)ring B VAL222(3.55A˚)ring B MET281(4.23A˚)ring B ILE323(4.18A˚)ring A ALA319(3.48A˚)ring B ALA217(3.58A˚)ring B ALA320(3.80A˚)ring A
13	TYR277(2.40A˚)ring B	ALA217(4.43A˚)ring B	ALA217(3.57A˚)ring B ALA320(3.69A˚)ring B ALA319(3.47A˚)ring B ALA219(3.72A˚)ring B VAL222(3.62A˚)ring B MET281(4.18A˚)ring B
14	TYR277(1.91A˚)ring B	ALA217(4.42A˚)ring B	ALA320(3.70A˚)ring A ALA319(3.45A˚)ring B ALA217(3.59A˚)ring B MET281(4.06A˚)ring A ALA219(3.68A˚)ring A VAL222(3.62A˚)ring B

For the set of nitro **compounds 1to7** ring A containing the two chloro groups mostly interacts with Ser317 to form weak hydrogen bond interactions and thus responsible for higher energy as compared to amino groups. . Ala 319 also plays a part in hydrogen bonding interactions with ring A of nitro compounds. Other interactions like pi- pi and pi- alkyl the amino acid residues for both kind of compounds are common. . Ala319 and Ser317 to form comparison of binding energies between compounds and triclosan indicates that **compound 9** shows the least binding energy -9.7, also approx the binding energy of triclosan -9.8 and hence exhibits the maximum affinity towards macromolecule. **Compound 5** shows the maximum binding energy of -6.9 Kcal/mol and exhibits minimum affinity towards macromolecule **(Figure-11)**.



Compound – 9 (-9.7 Kcal/mol)



Compound – 5 (-6.9 Kcal/mol)

Figure-11: Interactions of compound-9 and compound-5 with PfNHG and NAD^+

Conclusion

Over all, following conclusions can be drawn from the above study. One, the presence of hydrogen donating group at ortho to ether linkage is necessary for acquiring minimizing the energy and in case of the absence of such a group, the molecule reorients itself to form hydrogen bonds.

Two, the side chain attached to amide linkage has to be not bigger than 3 carbon atoms. In any case, weather the hydrogen donating groups are present or not lengthening of chain results in increasing the energy of the complex.

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