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Natural Compounds as Inhibitors of *Plasmodium Falciparum* Enoyl-acyl Carrier Protein Reductase (PfENR): An *In silico* Study

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Abstract

Demand for a new anti-malarial drug has been dramatically increasing in the recent years. *Plasmodium falciparum* enoyl-acyl carrier protein reductase (PfENR) plays a vital role in fatty acid elongation process, which now emerged as a new important target for the development of anti-microbial and anti-parasitic molecules. In the present study, 19 compounds namely alginic acid, atropine, chlorogenic acid, chrotacumine A & B, coenzyme Q_1, 4-coumaric acid, curcumin, ellagic acid, embelin, 5-*O*-methyl embelin, eugenyl glucoside, glabridin, hyoscyamine, nordihydroguaiaretic acid, rohitukine, scopolamine, tlatlancuayin and ursolic acid were evaluated on their docking behaviour on *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) using Auto dock 4.2. The docking studies and binding free energy calculations exhibited that glabridin gave the highest binding energy (-8.07 kcal/mol) and 4-coumaric acid in contrast showed the least binding energy (-4.83 kcal/mol). All ligands except alginic acid, ellagic acid, hyoscyamine and glabridin interacted with Gln409 amino acid residue. Interestingly four ligands namely coenzyme Q_1, 4-coumaric acid, embelin and 5-*O*-methyl embelin interacted with Gln409 amino acid residue present in both chains (A & B) of PfENR protein. Thus, the results of this present study exhibited the potential of these 19 ligands as *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) inhibitory agents and also as anti-malarial agents.

Keywords: *Plasmodium Falciparum* Enoyl-acyl Carrier Protein Reductase (PfENR), Ellagic Acid, Glabridin, Eugenyl Glucoside, Rohitukine, Tlatlancuayin

Introduction

Malaria is one of the major health problems in which more than three billion people, mostly in Africa and Southeast Asia, are at risk for malaria infection. In Malaysia, malaria is still a big threat to public especially in East Malaysia (Sabah and Sarawak) as well as in interior central areas of Peninsular Malaysia (Perak, Pahang and Kelantan). Malaria is caused by protozoan parasite of the genus *Plasmodium*[1]. In Malaysia, *Plasmodium knowlesi* followed by *Plasmodium falciparum* cause the most severe form of malaria disease[2]. Clinical manifestations of malaria are fever, chills, prostration and anaemia, in severe condition leading to delirium, metabolic acidosis, cerebral malaria, multi-organ system failure, coma and even death. Chloroquine (CQ) is the most widely used anti-malarial drug, however recent emergence of multidrug-resistant parasites became the major concern for malaria control and eradication. New drugs that meet the requirements of rapid efficacy, safe and affordable cost are desperately needed to combat malaria. Therefore, new chemotherapeutic targets are required[3]. Fatty acids play a vital role in cells as metabolic precursors for biological membranes. Inhibition of fatty acid biosynthesis has been validated as an excellent target in anti-microbial and anti-parasitic drug discovery[4].

P. falciparum enoyl-acyl carrier protein reductase (PfENR) reduces the trans-2-enoyl bond of enoyl- acyl carrier protein (ACP) substrate to saturated acyl-ACPs and it plays a vital role in completing the successive rounds of fatty acid elongation process. PfENR is an emerging new important target for the development of anti-microbial and anti-parasitic molecules[5]. In the present study, a selected 19 compounds namely alginic acid, atropine, chlorogenic acid, chrotacumine A & B,

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coenzyme Q_1, 4-coumaric acid, curcumin, ellagic acid, embelin, 5-*O*-methyl embelin, eugenyl glucoside, glabridin, hyoscyamine, nordihydroguaiaretic acid, rohitukine, scopolamine, tlatlancuayin and ursolic acid. These compounds were evaluated on the docking behaviour of *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) using Auto dock 4.2, whereby the results have given useful information for the future design of a potent anti-malarial agent.

2. Materials and Methods

2.1. Ligand Preparation

Chemical structures of the ligands namely i) alginic acid [ID15971]; ii) atropine [ID10194105]; iii) chlorogenic acid [ID1405788]; iv) chrotacumine A [ID 24678919]; v) chrotacumine B [ID24657802]; vi) coenzyme Q_1 [CID4462]; vii) 4-coumaric acid [ID553148]; viii) curcumin [CID2889]; ix) ellagic acid [CID 5281855]; x) embelin [CID3218]; xi) 5-O-methyl embelin [CID171489]; xii) eugenyl glucoside [CID 3084296]; xiii) glabridin [CID124052]; xiv) hyoscyamine [ID10246417]; xv) nordihydroguaiaretic acid [CID 4534]; xvi) rohitukine [ID4533914]; xvii) scopolamine [ID10194106]; xviii) tlatlancuayin [CID12444409] and xix) ursolic acid [ID58472] were retrieved from Chemspider (www.chemspider.com), Guide Chem (www. guidechem.com) and PubMed (www. pubmed.com).

2.2. Target protein identification and preparation

The three dimensional structure of the *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) (PDB ID: 3LTO with resolution of 1.96 Å) was obtained from the Research Collaborator for Structural Bioinformatics (RCSB) Protein data bank (Anonymous, www. rcsb.org). The protein was pre-processed separately by deleting ligand, as well as the crystallographically observed water molecules (water without hydrogen bonds).

2.3. Docking studies

Docking was performed using Autodock 4.2. which combined energy evaluation through precalculated grids of affinity potential employing various search algorithms to find the suitable binding position for a ligand on a given protein (PfENR). All rotatable bonds in the ligands were kept free to allow flexible docking. Grid size was set to 60×60×60 grid points (x, y and z), with spacing between grid points kept at 0.375 Å. The Lamarckian genetic algorithm was chosen to search for the best conformers. Standard docking protocol was applied. One hundred independent docking runs for each ligand was generated by using genetic algorithm search[6].

3. Results and Discussion

Current understanding of the malarial parasites biochemistry has paved way to identify many potential targets for new drugs, as well as helped to study the mode of action of the older drugs [3]. Recently, identified potential selective target for anti-malarial drugs include type II fatty acid biosynthesis (FAS-II) system in *Plasmodium falciparum*. In the present study, 19 ligands were selected in which all are naturally occurring compounds except coenzyme Q_1 (COQ_1) and they were evaluated on the docking behaviour of *P. falciparum* enoyl-acyl carrier protein reductase (PfENR). Table 1 depicts the botanical sources for 17 ligands except for alginic acid and coenzyme Q_1. Six of them are alkaloids in which atropine, hyoscyamine and scopolamine belong to tropane alkaloid, whereas chrotacumine A, B and rohitukine belong to chromone alkaloid group. Chlorogenic acid, 4-coumaric acid, ellagic acid and nordihydroguaiaretic acid (lignan) are phenolic compounds. Alginic acid is a naturally occurring polysaccharide generally obtained from brown seaweeds. The docking studies and binding free energy calculations as in Table 2 show glabridin with the highest binding energy (-8.07 kcal/mol) while 4-coumaric acid gave the least binding energy (-4.83 kcal/mol). The binding free energy calculation showed the following order of glabridin, chrotacumine A < tlatlancuayin, < chrotacumine B, < chlorogenic acid, < ellagic acid, < rohitukine, < alginic acid, eugenyl glucoside, < curcumin, ursolic acid, < nordihydroguaiaretic acid, atropine, < hyoscyamine, embelin, < scopolamine, < coenzyme Q_1, 5-*O*-methyl embelin and < 4-coumaric acid. All ligands except for alginic acid, ellagic acid, hyoscyamine and glabridin interacted with Gln409 amino acid residue. Four ligands which are coenzyme Q_1, 4-coumaric acid, embelin and 5-*O*-methyl embelin interacted with Gln409 amino acid residue that was present in both chains (A & B) of PfENR protein.

Table 1. Depicts the botanical source of the seventeen ligands

Ligand name	Nature of chemical class	Botanical source
Atropine	Alkaloid	*Atropa belladonna* L., *Datura alba* Nees and *D. inoxia.*
Chrotacumine A & B	Alkaloid	*Dysoxylum acutangulum*, *D. binectariferum*, *D. beddomei* and *D. malabaricum.*
Chlorogenic acid	Phenol	*Phyllostachys edulis*, *Calluna vulgaris* (L.) Hull and *Eucommia ulmoides* Oliv.
4-coumaric acid	Phenol	*Arachis hypogaea* L., *Phaseolus vulgaris* L., *Allium sativum* L., *Solanum lycopersicum* L., *Coffea arabic*a L and *Hordeum vulgare* L.
Curcumin	Diarylheptanoid	*Curcuma longa* L., *C. zedoria* Roscoe and *C. aromatic* Salisb.
Ellagic acid	Phenol	*Quercus alba* L., *Q. robur* L., *Punica granatum* L., *Vitis vinifera* L., *Vaccinium oxycoccos* L., *Juglans regia* L and *Rubus laciniatus* Willd.
Embelin	Quinone	*Embelia ribes* Burm.f., *E. robusta* Roxb., *E. tsjersium-cottam* A. DC., *Myrsine africana* L and *Ardisia humilis* Vahl
5-O-methyl embelin	Quinone	*Embelia ribes* Burm.f and *Lysimachia punctata* L.
Eugenyl glucoside	Glycoside	*Salvia officinalis* L.
Glabridin	Isoflavone	Glycyrrhiza glabra Linn
Hyoscyamine	Alkaloid	*Hyoscyamus niger* Linn, *Atropa belladonna* L., *Datura alba* Nees, *D. inoxia* and *Mandragora officinarum* L.
Nordihydroguaiaretic acid (NDGA)	Phenol	*Larrea tridentate* (DC.) Coville.
Rohitukine	Alkaloid	*Amoora rohituka* Wall, *Dysoxylum acutangulum*, *D. binectariferum*, *D. beddomei* and *D. malabaricum.*
Scopolamine	Alkaloid	*Hyoscyamus niger* Linn, *Datura alba* Nees and *D. inoxia.*
Tlatlancuayin	Isoflavone	*Celosia argentea* Linn, *C. cristata* L and *Iresine celosia* L.
Ursolic acid	Triterpene	*Ocimum sanctum* L., *Origanum vulgare* L., *Thymus vulgaris* L., *Mentha piperita* L and *Vaccinum myrtillus* L.

Whereas, interaction with Thr410 amino acid residue was shown by ten other ligands namely alginic acid, atropine, chlorogenic acid, curcumin, eugenyl glucoside, glabridin, nordihydroguaiaretic acid, rohitukine, scopolamine and tlatlancuayin. Only scopolamine interacted with Thr410 amino acid residue present in both chains (A & B) of PfENR protein. Six ligands which are chlorogenic acid, chrotacumine A & B, 4-coumaric acid, glabridin and rohitukine were shown to interact with Asn418 amino acid residue. Some of these (studied) compounds have been reported to have anti-malarial activity; especially curcumin has been reported several times to be potential as anti-malarial agent. Cui and co-workers[7] have reported that curcumin has shown potent anti-malarial activity against both chloroquine (CQ) sensitive and resistant *P. falciparum* strains. Curcumin in combination with artemisinin and piperine has shown synergistic and positive interaction effect on anti-plasmodial activity[8,9]. In 2011, Mimche and co-workers[10] also reported that curcumin as a potent candidate for the development of adjunctive therapy for cerebral malaria. Apart from this, Chakrabarti and co-workers[11] have reported that curcumin has shown *P. falciparum* microtubules disruption activity. Ursolic acid is another compound that has shown potent anti-malarial activity against both chloroquine (CQ) sensitive (T9-96) and resistant *P. falciparum* (K1) strains[12]. In addition to this, van Baren and co-workers[13] reported that ursolic acid from *Satureja parvifolia* Phil has shown potent anti-malarial activity against both chloroquine (CQ) sensitive (3D7) and resistant *P. falciparum* (K1) strains. Nordihydroguaiaretic acid (NDGA) was reported by Mahmoudi and co-workers[14] to have activity against the hepatic stage of malaria parasite using molecular topological study. Soh and co-workers [15] reported that ellagic acid has shown anti-malarial

Table 2. Binding energy analysis of nineteen ligands with that of *Plasmodium falciparum* enoyl-acyl carrier protein reductase (PfENR) using Autodock 4.2

Ligand name	Lowest binding energy (-kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Alginic acid	7.03	GlyB408	2.9
		ThrB410	2.9 & 3.6
		AsnA418	1.9
Atropine	6.41	GlnA409	2.6
		ThrA410	2.0
Chrotacumine A	7.99	ArgA2933	2.7
		GlnB409	2.8
		AsnA418	2.6
Chrotacumine B	7.86	ArgB293	2.6
		GlnA409	2.0 & 2.8
		AsnA418	2.9
Coenzyme Q_1	5.95	GlnA409	3.5
		GlnB409	2.4
Chlorogenic acid	7.63	GlnA409	2.3
		ThrB410	2.6, 3.3 & 3.4
		ThrA410	2.0
		AsnA418	1.8 & 2.1
4-coumaric acid	4.83	ArgB293	3.3
		GlyB408	2.1
		GlnA409	2.0
		GlnB409	3.5
		AsnA418	3.4
Curcumin	6.96	GluA289	1.8
		ArgB293	3.1
		GlnA409	2.2
		ThrA410	2.6
Ellagic acid	7.16	ArgA122	3.1
		ArgB122	3.0
		SerA393	2.5
		SerA396	2.5 & 2.7
		SerB396	2.8
		SerB400	2.4
Embelin	6.25	GlnA409	2.5
		GlnB409	2.6
		TyrA412	3.4
		TyrB412	3.3
5-O-methyl embelin	5.63	GlnA409	2.1 & 3.2
		GlnB409	2.6
		TyrB412	3.5

Table 2. Binding energy analysis of nineteen ligands with that of *Plasmodium falciparum* enoyl-acyl carrier protein reductase (PfENR) using Autodock 4.2

Ligand name	Lowest binding energy (-kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Eugenyl glucoside	7.02	GlnA409	2.5
		GlnB409	2.2
		ThrA410	1.9
		ThrB410	3.4
		TyrA412	3.3
Glabridin	8.07	ArgB293	3.6
		GlyB408	1.7
		ThrA410	2.9
		AsnA418	2.1
Hyoscyamine	6.26	ThrB410	2.9
		TyrA412	2.7
Nordihydroguaiaretic acid (NDGA)	6.41	GlnA409	2.6
		ThrA410	2.0
Rohitukine	7.04	GlnB409	1.9
		ThrB410	2.5
		AsnA418	2.1 , 3.0 & 3.3
Scopolamine	6.06	GlnB409	2.6
		ThrA410	3.0
		ThrB410	2.2, 3.3 & 3.5
Tlatlancuayin	7.95	GlnB409	2.8
		ThrB410	2.9 & 3.4
		TyrA412	3.3
Ursolic acid	6.49	ArgB293	2.7
		GlnA409	1.9

activity against five different *P. falciparum* strains. Anti-malarial activity against chloroquine (CQ) resistant *P. falciparum* (K1) strain was also shown by chlorogenic acid[16]. Embelin isolated from *Embelia ribes* Burm. F., was observed to have potent anti-malarial activity against chloroquine (CQ) sensitive (FCK2) *P. falciparum* strain[17]. Recently, Das and co-workers[18] reported that *p*-coumaric acid from *Carica papaya* L., has shown inhibition against *P. falciparum* dihdropteroate synthase (PfDHPS) using molecular docking study. Another compound, glabridin, was also recently reported by Cheema and co-workers[19] as potent anti-malarial activity against chloroquine (CQ) sensitive (NF54) *P. falciparum* strain. To the best of our knowledge, however, none of these compounds have been reported for *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) inhibition.

4. Conclusion

In the present study, all of the tested ligands have shown to dock with *P. falciparum* enoyl-acyl carrier protein reductase (PfENR). Interestingly, all ligands except for 4-coumaric acid exhibited the lowest binding energy of > -5.0 kcal/mol. Hence, it is strongly suggested that the results of this present study has paved better understanding of these 19 ligands as potential *P. falciparum* enoyl-acyl carrier protein reductase (PfENR) inhibitors and also possible as anti-malarial agents.

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