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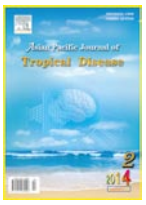

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Effect of degree of unsaturation of fatty acids on the activity of FabI (enoyl-acyl carrier protein reductase) enzyme from *Plasmodium falciparum*: an enzoinformatics study

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ABSTRACT

Objective: To elucidate molecular interactions of enoyl-acyl carrier protein reductase (FabI) with unsaturated fatty acids such as docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, octadecatrienoic acid, stearic acid and arachic acid to investigate the inhibitory activities of degree of unsaturation.
Methods: Docking between these ligands and enzymes were performed using Autodock4.2.
Results: Docosahexaenoic acid (a polyunsaturated fatty acid) is more efficient inhibitor of enoyl-acyl carrier protein reductase (FabI) compared to other unsaturated fatty acids with lesser double bonds and saturated fatty acid with reference to ΔG and Ki values. Hydrophobic interactions play an important role in the correct positioning of these fatty acids within the catalytic site of FabI enzyme to permit docking.
Conclusions: It has been also observed that not only the degree of unsaturation affects the antiplasmodial activity, but the length of carbon chain also plays an important role in their inhibitory activity. Such information may aid in the design of versatile FabI-inhibitors.

1. Introduction

Over three billion people live under the threat of malaria across the world and it kills over a million each year, mostly children[1]. Of the four species of *Plasmodium* that cause human malaria, *Plasmodium falciparum* (*P. falciparum*) accounts for the most severe and fatal form of the disease, cerebral malaria. Malaria has primarily been treated with chloroquine or pyrimethamine sulfadoxine. Emergence of strains of the pathogen resistant to these drugs has made the situation worse[2]. Hence, finding novel pathways unique to the malaria parasite and identifying lead compounds against these pathways becomes necessary[3].

In recent years, there has been an increase in the role of specific dietary fatty acids and their medicinal properties. Recently, there has been a growing scientific interest in exploring potential health-related benefits of polyunsaturated fatty acids (PUFA) such as improving heart disease related outcomes, decreasing tumor growth and metastasis, and regulation of insulin sensitivity[4]. Moreover, there is a growing realization that fatty acids have the potential to inhibit the fatty acid biosynthetic machinery of *P. falciparum* parasite. Fatty acids have shown antimalarial, antimycobacterial and antifungal properties[5].

Fatty acids have been reported to display antimalarial activity[4]. However, there is a paucity of literature reports regarding the same. Also, there is no consensus as to what

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structural characteristics (*i.e.*, unsaturation level, position or chain length) favor the best antimalarial fatty acids[6].

PUFA, especially the essential fatty acids have medicinal properties. PUFA are hydrocarbon chains with ≥2 double bonds. These hydrocarbons can be classified as n-6 or n-3 depending upon the location of the first double bond relative to the methyl terminus. Essential fatty acids are a group of unsaturated fatty acids which are not produced in humans, can be obtained in diet and are necessary for the proper functioning of the human body[7].

Enoyl-acyl carrier protein reductase (FabI) is a key enzyme of the type II fatty acid synthesis system. FabI is an attractive target for narrow-spectrum antibacterial drug discovery because of its essential role in metabolism and its sequence conservation across many bacterial species. It catalyses the nicotinamide adenine dinucleotide-specific reduction of a trans carbon-carbon double bond to produce saturated acyl carrier protein[8].

The medicinal properties of essential fatty acids have recently drawn attention. Moreover, the antiplasmodial properties of unsaturated fatty acids and effect of degree of unsaturation are yet to be fully explored. Therefore, the aim of the present study was to investigate the inhibitory activities of degree of unsaturation of fatty acids using molecular docking approach. To the best of our knowledge, there is no study reporting binding interactions of the fatty acids described herein with FabI enzyme in the scientific literature as yet.

2. Materials and methods

The 3-dimensional structure of FabI used for the docking study was retrieved from Protein Data Bank (ID: 1NHG). The structure of ligands namely docosahexaenoic acid (CID: 445580), eicosapentaenoic acid (CID: 446284), arachidonic acid (CID: 466880), octadecatrienoic acid (CID: 929600), stearic acid (CID: 5281) and arachic acid (CID: 10467) were retrieved from 'Pubchem Compound'. For energy minimization of the ligand molecule, MMFF94 force field was used. Gasteiger partial charges were added to the ligand atoms, non-polar hydrogen atoms were merged, and rotatable bonds were defined and docking calculations were accomplished on the protein model. Using the AutoDock tools essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added. Affinity (grid) maps of 40 Å×40 Å×40 Å grid points and 0.375 Å spacing were generated using the Autogrid program aimed to target grid coordinates in proximity with the active site of FabI enzyme. Accordingly, the values of x, y and z coordinates used for targeting the FabI active site were 12.79, 101.56 and 25.64, respectively. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm and the Solis-Wets local search method. Initial position, orientation and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250 000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

3. Results

Unsaturated fatty acids themselves can inhibit the fatty acid biosynthetic pathways of the parasite *P. falciparum*. In order to observe effect of degree of unsaturation on inhibition efficacy of fatty acids against fatty acid biosynthetic pathways of the parasite *P. falciparum*; docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, octadecatrienoic acid, stearic acid and arachic acid were docked to enoyl-acyl carrier protein reductase (FabI). It was found that the catalytic domain of enoyl-acyl carrier protein reductase (FabI) interacted with docosahexaenoic acid through 17 amino acid residues, namely Y111, L216, A217, L265, T266, Y267, Y277, M281, K285, A312, G313, P314, S317, L315, A319, A320 and I369 (Figure 1, Table 1). The free energy of binding and estimated inhibition constant (Ki) for the docosahexaenoic acids-enoyl-acyl carrier protein reductase (FabI) catalytic domain-interaction were determined to be -6.37 kcal/mol and 21.25 μmol/L, respectively. Here, nine carbon atoms of docosahexaenoic acid, namely, C4, C6, C8, C9, C13, C14, C15, C16 and C22 were predicted to be involved in hydrophobic interactions with amino acid residues Y111, A217, L265, Y267, Y277 and A319 of the enzyme. Total intermolecular energy of docking for docosahexaenoic acid-FabI catalytic domain-interaction was found to be -7.96 kcal/mol. Van der Waals, hydrogen bond and desolvation energy components together contributed -7.97 kcal/mol, while the Electrostatic energy components was found to be +0.01 kcal/mol. Total interacting surface area for docosahexaenoic acids-FabI catalytic domain-interaction was found to be 938.591 $Å^2$.

Table 1

Comparative analysis of FabI (enyol-acyl carrier protein reductase) inhibitory activity of fatty acids and interacting amino acids between enzyme and ligands.

Fatty acids	Target	Binding energy (kcal/mol)	Inhibition constant (μmol/L)	Interacting amino acid
Docosahexaenoic acids cis,cis,cis,cis,cis,cis-Δ4,Δ7,Δ10,Δ13,Δ16,Δ19 (Six double bonds)	FabI	-6.37	21.25	Y111, L216, A217, L265, T266, Y267, Y277, M281, K285, A312, G313, P314, S317, L315, A319, A320, I369
Eicosapentaenoic acids cis,cis,cis,cis,cis-Δ5,Δ8,Δ11,Δ14,Δ17 (Five double bonds)	FabI	-5.92	45.64	Y111, L216, A217, L265, T266, Y267, Y277, K285, A312, G313, P314, L315, S317, A319, A320, I323, I369
Arachidonic acids cis,cis,cis,cis-Δ5Δ8,Δ11,Δ14 (Four double bonds)	FabI	-5.77	58.69	Y111, N218, A217, A219, V222, L265, T266, Y267, Y277, M281, K285, C313, L315, S317, I323
Octadecatrienoic acids cis,cis,cis-Δ9,Δ12,Δ15 (Three double bonds)	FabI	-5.46	87.52	Y111, S215, L216, A217, N218, L265, T266, Y267, G313, A312, P314, L315, S317, F368, I369, A372
Stearic acids (No double bond)	FabI	-3.99	1.91	Y111, A217, N218, A219, K220, V222, L265, T266, Y267, K285, A312, G313, P314, L315, S317, A319, I369
Arachic acids (No double bond)	FabI	-1.81	46.82	G104, W131, S215, L216, A217, T266, Y267, Y277, G313, P314, L315, S317, A317, R318, A319, F368, I369, A372

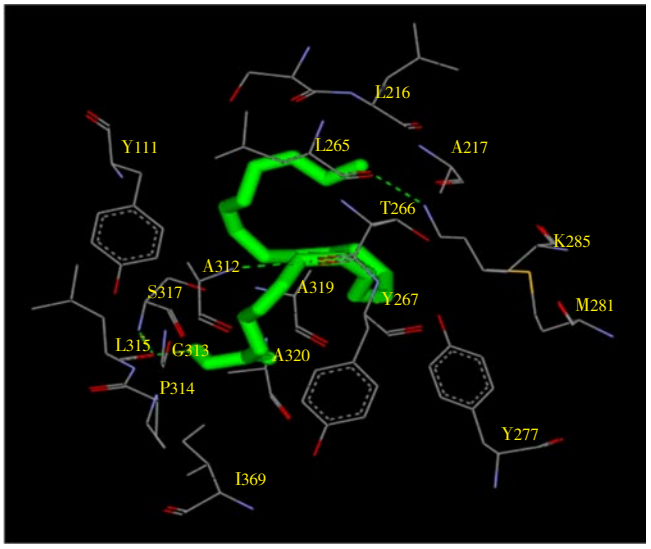

Figure 1. Interaction of docosahexaenoic acid docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand docosahexaenoic acid is shown in 'stick' representation.

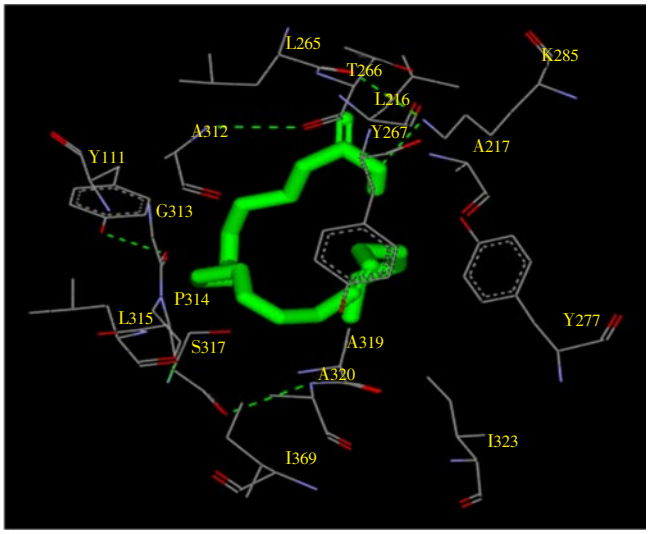

Figure 2. Interaction of eicosapentaenoic acids docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand eicosapentaenoic acid is shown in 'stick' representation.

On the other hand, catalytic domain of FabI was determined to interact with eicosapentaenoic acid through 17 amino acid residues, namely Y111, L216, A217, L265, T266, Y267, Y277, K285, A312, G313, P314, L315, S317, A319, A320, I323 and I369 (Figure 2, Table 1). However, the free energy of binding and estimated inhibition constant (Ki) for eicosapentaenoic acid-FabI catalytic domain-interaction were found to be -5.92 kcal/mol and 45.64 μmol/L, respectively. While, seven carbon atoms of eicosapentaenoic acid, namely C1, C2, C3, C4, C6, C17 and C19 were observed to make hydrophobic interactions with amino acid residues Y111, W131 and L216 of the enzyme. Total intermolecular energy of docking for eicosapentaenoic-FabI catalytic domain-interaction was found to be -6.41 kcal/mol. Van der Waals, hydrogen bond and desolvation energy components together contributed -6.43 kcal/mol, while the electrostatic energy components was found to be +0.02 kcal/mol. Total interacting surface area for docosahexaenoic acids-FabI catalytic domain-interaction was found to be 846.69 $Å^2$.

Arachidonic acid was found to interact with the catalytic domain of FabI through 15 amino acid residues namely, Y111, N218, A217, A219, V222, L265, T266, Y267, Y277, M281, K285, C313, L315, S317, I323 (Figure 3, Table 1). However, octadecatrienoic acid interacted with the catalytic domain of FabI through 16 amino acid residues namely, Y111, S215, L216, A217, N218, L265, T266, Y267, G313, A312, P314, L315, S317, F368, I369 and A372 (Figure 4, Table 1). The free energy of binding and estimated inhibition constant (Ki) for arachidonic acid-FabI catalytic domain-interaction were found to be -5.77 kcal/mol and 58.69 μmol/L, respectively, while for octadecatrienoic acid-FabI catalytic domain-interaction the same were -5.54 kcal/mol and Ki of 87.52 μmol/L, respectively. Two carbon atoms of arachidonic acid, namely, C16 and C19 were found to be involved in hydrophobic interactions with amino acid residue A296 of the enzyme. One oxygen atom of octadecatrienoic acid, namely, O_2 was found to be involved in polar interaction with amino acid residue A312 of the enzyme, while five

carbon atoms namely, C1, C3, C5, C10 and C11 were found to be involved in hydrophobic interaction with amino acid residues A296 and Y297 of the enzyme. Van der Waals', hydrogen bond and desolvation energy components for arachidonic acid-FabI catalytic domain-interaction together constituted a value of -6.18 kcal/mol. The combined value of the same energy components for the interaction involving octadecatrienoic acid was observed as -5.59 kcal/mol. The electrostatic energy components for the mentioned two interaction were found to be +0.01 and +0.01 kcal/mol, respectively. Total interacting surface area for arachidonic acid-FabI catalytic domain-interaction and octadecatrienoic acid-FabI catalytic domain-interaction complexes were observed to be 539.868 and 606.815 Å^2, respectively.

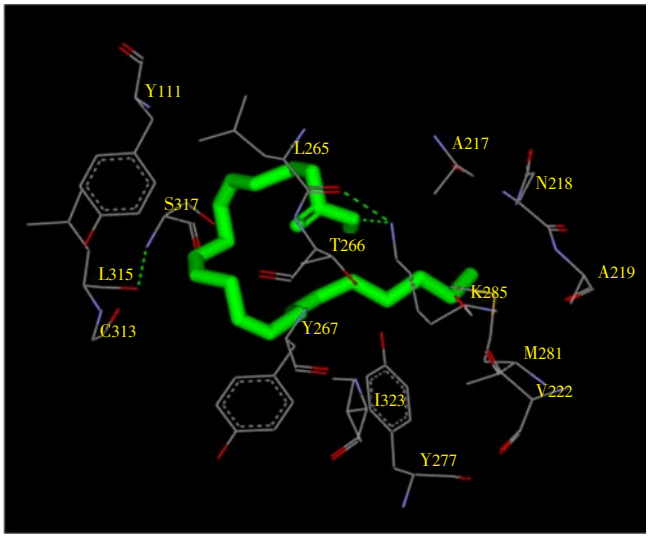

Figure 3. Interaction of arachidonic acid docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand arachidonic acid is shown in 'stick' representation.

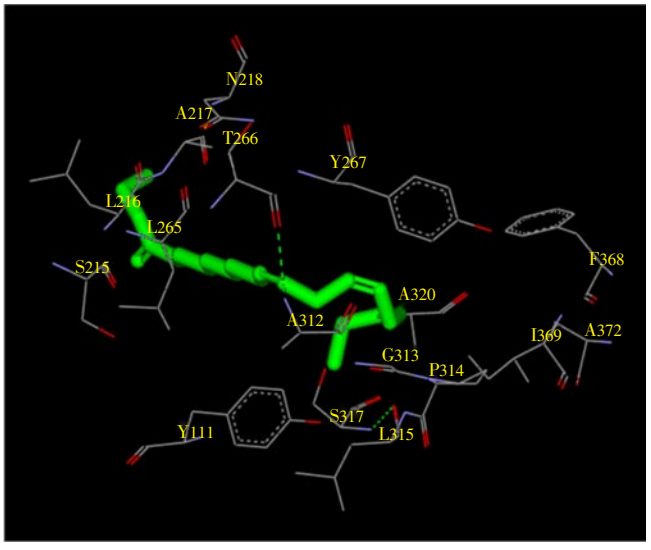

Figure 4. Interaction of octadecatrienoic acid docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand octadecatrienoic acid is shown in 'stick' representation.

The free binding energy (ΔG) and inhibition constant (Ki) were determined to be -3.99 kcal/mol and 1.19 mmol/L, respectively for stearic acid-FabI interaction' while for arachic acid-FabI interaction the same were -1.81 kcal/mol and 46.82 mmol/L, respectively. The catalytic domain FabI was found to interact with stearic acid through 17 amino acid residues, namely Y111, A217, N218, A219, K220, V222, L265, T266, Y267, K285, A312, G313, P314, L315, S317, A319 and I369 (Figure 5, Table 1); and with arachic acid through 18 amino acid residues, namely G104, W131, S215, L216, A217, T266, Y267, Y277, G313, P314, L315, S317, A317, R318, A319, F368, I369 and A372 (Figure 6, Table 1). One oxygen atom of stearic acid namely O_2 was predicted to be involved in polar interaction with amino acid residue ARG308 of the enzyme and five carbon atoms namely C12, C13, C14 and C15 were observed to make hydrophobic interaction through amino acid residue A296 and Y297 of the enzyme. This was in contradiction to arachic acid-FabI interaction where polar interaction was not found, only hydrophobic interactions seemed to play significant role in docking. Van der Waals, hydrogen bond and desolvation energy components for stearic acid and arachic acid with FabI catalytic domain were found to be -4.88 and -5.27 kcal/mol, respectively. Total intermolecular energy of docking and total interacting surface area for stearic acid-FabI catalytic domain-interaction was found to be -4.88 kcal/mol and 494.546 Å^2, respectively, while the same for arachic acid-FabI catalytic domain-interaction was found to be -2.26 kcal/mol and 545.322 Å^2, respectively. Another saturated fatty acid behenic acid did not show any interaction with the catalytic domain of *P. falciparum* FabI.

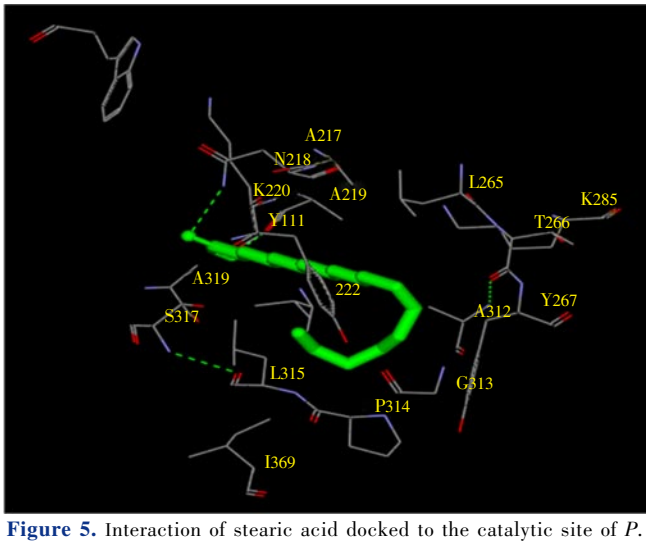

Figure 5. Interaction of stearic acid docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand stearic acid is shown in 'stick' representation.

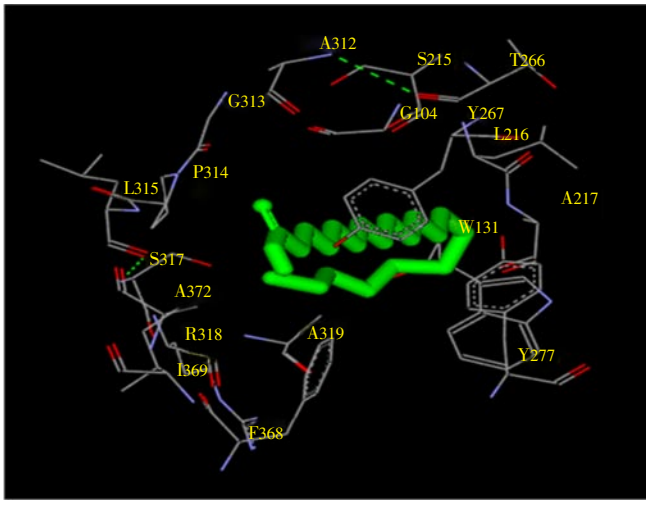

Figure 6. Interaction of arachic acid docked to the catalytic site of *P. falciparum* enyol-acyl carrier protein reductase (FabI).
The ligand arachic acid is shown in 'stick' representation.

4. Discussion

Earlier antimalarial effect of fatty acids has received some contemplation, but inhibition of *P. falciparum* fatty acid biosynthetic machinery by fatty acids themselves has only been recently considered as a likely strategy to combat the parasite[5,9]. In 2012, Melariri *et al.* reported that linolenic acid and linoleic acid both were efficient in inhibiting the growth of *Plasmodium* parasite[4]. These researchers found that the degree of unsaturation was critical for the antiplasmodial effect of the fatty acids towards the parasite. However, to pave the way for the development of more potent lipid inhibitors, enhanced understanding of the mechanism of inhibition as well as the mode of binding of these interesting fatty acid inhibitors are required. Therefore, in the present study, we used six fatty acids (docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid, octadecatrienoic acid, stearic acid and arachic acid) as ligands and observed their interaction to the catalytic site of FabI, an important enzyme of the fatty acids biosynthetic pathway of *P. falciparum*. In addition, FabI has been investigated as an attractive target because it plays an important role in membrane construction and energy production in the parasite, and does not have any human homologues. It was originally identified as a target for *Mycobacterium tuberculosis*[10], and also for *P. falciparum*[11].

A higher (negative) free energy (ΔG) of binding is an indicator of efficient interaction between an enzyme and inhibitor[12]. In the present study, out of all the fatty acids, docosahexaenoic acid was the most potent inhibitor of FabI enzyme in terms of binding energy and inhibition constant. While six amino acid residues, namely, Y111, A217, L265, Y267, Y277 and A319 of the FabI enzyme were predicted to be involved in hydrophobic interactions with nine carbon atoms of docosahexaenoic acid. In a study, on FabI inhibitors, bromo-benzothiophene carboxamide derivatives were found to occupy the similar hydrophobic pocket composed of Y267, Y277 and A217[13]. In another study triclosan was found to be involved in hydrophobic interactions with FabI enzyme through 8 amino acid residues, namely Y267, Y277, G313, P314, I323, F368, I369, and A372[14]. In present study, amino acid residues Y111, Y277, K285 and Y267 have been proposed to be important to the catalytic mechanism of the enzyme which was consistent with other findings[14,15].

It has been reported that antiplasmodial activity of the unsaturated fatty acid enhances as the degree of unsaturation increases[16]. Similarly, in our study, eicosapentaenoic acid with five double bonds showed more inhibition against FabI enzyme compared to arachidonic acid (four double bonds) and octadecatrienoic acid (three double bonds), while docosahexaenoic acid was the best with six double bonds. In addition, our results showed that not only the degree of unsaturation but chain length of unsaturated fatty acid also play an important role in the antiplasmodial effect of fatty acids.

In an earlier study, it was reported that the neutrophil-mediated killing of the asexual blood forms of *P. falciparum* could be enhanced by fatty acids[17]. Later on, a study was conducted to reveal the mechanism of action of these fatty acids and it was found that C23-C26 Δ5, 9 fatty acids were good inhibitors of the *P. falciparum* enoyl-acyl carrier protein reductase (FabI) enzyme that catalyses the final reduction step of the fatty acid chain elongation cycle in *P. falciparum* and had almost no cytotoxicity on mammalian L6 cells[18]. In another study bromo-benzothiophene carboxamide derivative was observed to make two hydrogen bonds, 28 hydrophobic interactions, 16 aromatic-aromatic interactions, and 12 hydrophobic-hydrophilic interactions with FabI enzyme[13].

Saturated fatty acids, namely, stearic acid and arachic acid showed lesser interaction towards catalytic domain of *P. falciparum* FabI enzyme compared to unsaturated fatty acids. Polar interaction and hydrophobic interaction were observed for stearic acid-FabI interaction. This was in contradiction to arachic acid-FabI interaction where only hydrophobic interactions seemed to play a significant role in docking.

The present study reveals the mode of binding of unsaturated fatty acids to *P. falciparum* FabI enzyme and helps in correlating the degree of unsaturation of fatty acids with their inhibitory effects against *P. falciparum*. Further investigations are needed to better understand the mechanism of inhibition of these interesting fatty acid inhibitors for the development of other more potent lipid inhibitors. However, it can be safely stated that the present study is expected to aid in revealing the mechanism of antiplasmodial action of fatty acids and reflects

a hope for the development of novel agents of biomedical importance. Finally, it seems clear that the very long chains and higher unsaturation levels might be a prerequisite for the good enzyme (*P. falciparum* FabI)-inhibition.

This study explores molecular interactions between *P. falciparum* enoyl-acyl carrier protein reductase (FabI) and the fatty acids. Hydrophobic interactions play an important role in the correct positioning of these fatty acids within the catalytic site of FabI enzyme to permit docking. Such information may aid in the design of versatile FabI-inhibitors. This study predicts that docosahexaenoic acids (a PUFA) is a more efficient inhibitor of *P. falciparum* FabI enzyme compared to other unsaturated fatty acids with lesser double bonds and saturated fatty acid in terms of Ki and ΔG values. Interestingly, it was found that not only the degree of unsaturation affects the antiplasmodial activity of the fatty acids, but the length of carbon chain also plays an important role in the inhibitory activity. It has been observed that in silico results often correlate well with the results obtained in wet lab experiments. Further, *in vitro* and *in vivo* studies are warranted to explore the potential of these interesting lipid inhibitors to validate the findings presented herein.

Conflict of interest statement

We declare that we have no conflict of interest.

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