Biomedical Communication

In-silico Identification of Triclosan Analogs as Novel Inhibitors of Enoyl-ACP Reductase from *Plasmodium falciparum*

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ABSTRACT

Malaria parasite resistance to currently available drugs has emerged as a major global health problem. As a result, new approaches to developing more target-specific antimalarial drugs are urgently needed. In apicoplast, the fatty acid biosynthesis enzyme enoyl-acyl carrier protein reductase (FabI) plays a vital role in the growth of malaria parasite *Plasmodium falciparum* in the liver-stage; hence it is an excellent target for the development of novel antimalarials drugs. In order to identify novel inhibitors of the FabI enzyme, a computational investigation of the fatty acid production pathway in *Plasmodium falciparum* is presented. Latest research findings has shown that antimicrobial agent triclosan inhibits the growth of *Plasmodium falciparum* by inhibiting the enzyme Fab I. Virtual screening focused on ligand was used to identify triclosan analogs against drug libraries, bioactive, commercial and virtual compounds for inhibiting PfFabI with Swiss similarity tool. Using SwissADME tool, screened compounds were subjected to physicochemical, pharmacokinetic, and toxicological analysis. Compound fulfilling ADME/Tox parameters is docked using MTiAutoDock server with binding pocket of FabI enzyme. The docked complexes were validated and enumerated based on the AutoDock scoring function to pick the best conformation. Triclosan analogs Pubchem CID 448623 and 71579715 having lowest binding energy with FabI enzyme even lower than known antimicrobial agent triclosan were predicted as novel inhibitors of PfFabI. To maximize accuracy of result, best predicted compounds again docked with FabI enzyme with different algorithm using PatchDock server. Our research has led to the discovery of two novel FabI inhibitors, which may be confirmed by laboratory experiments.

KEY WORDS: ENOYL-ACP REDUCTASE, FABI, MALARIA, PLASMODIUM FALCIPARUM, TRICLOSAN.

INTRODUCTION

Malaria is a major public health issue that has become increasingly critical and continues to promote study, impacting the world's main tropical and subtropical areas. 228 million malaria cases occurred worldwide in 2018 compared to 231 million in 2017 (WHO 2019). India and 19 countries in sub-Saharan Africa accounted for approximately 85% of the global malaria burden. An estimated 405 000 deaths from malaria were reported globally in 2018, compared with an estimated 416 000 deaths in 2017. *Plasmodium falciparum* has to be the most dominant malaria parasite in the South East Asia region of the WHO, accounting for 50% of confirmed cases of malaria in 2018 (WHO 2019).

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Anti-malarial drug formulation; vaccination and vector control are the main methods to control this parasitic disease. Parasitic drugs are between these the first line of defence. The P. falciparum 'chloroquine resistance transporter' (PfCRT) was first identified as a main determinant of chloroquine (CQ) resistance, but mutations in this protein are now known to affect the parasite's susceptibility to a variety of current and potential antimalarials (Martin, 2020). Thus, developing a new generation of anti-malarial drugs will represent a significant advance to consider. Apicoplast in malaria parasites has important consequences in combating their anti-malarial drug resistance (Gleeson, 2000; Liting and Geoffrey 2010).

Apicoplast metabolic pathways of isoprenoid precursor synthesis, fatty acid synthesis, heme synthesis and biogenesis of the iron sulfur cluster acting role in genome replication, transcription, translation, post-translational

modification and protein turnover (Ralph et al. 2004; Liting and Geoffrey 2010). The apicoplast is a remnant of an ancient cyanobacterium that incorporates enzymes and biosynthetic processes that are more bacterial than eukaryotic in nature, allowing antimalarial agents to be designed that are selective against apicoplast targets (Ralph et al. 2004; Liting and Geoffrey 2010).

Apicoplast pathways fatty acid and isoprenoid precursor synthesis are clearly bacterial in origin and are potential targets for antibiotic drugs because they differ from the eukaryotic host processes.The *Plasmodium* FAS-II pathway was of particular therapeutic significance as it is distinct from the mammalian type I (FAS-I) pathway. The fatty acid biosynthesis enzyme enoyl-ACP reductase (FabI) plays a vital role in the growth of parasites in the liver-stage (Yu et al. 2008). Among the many known PfFabI inhibitors studied so far, triclosan remains the most active with an IC50 = 14 ng / ml (50 nM), but due to human health and environmental concerns, the compound is not appropriate for medicinal application (Kapoor et al. 2004). In this study, we have incorporated ligand based virtual screening method to search out the potential triclosan analogs for the treatment against maleria targeting the *Plasmodium falciparum* enoyl-acyl-carrier-protein reductase, which were not reported previously.

Table 1. The pharmacokinetics and drug likeliness properties of triclosan analogs.

Sl. No.	Pubchem CID	BBB	GI	CYP1A2 inhibitor	CYP2D6 inhibitor	LogKp (Cm/s)	Bioavailab-ility Score	Lipinski	Ghose	Veber	Egan	Muegge
1	5564 (Triclosan)	High	Yes	Yes	No	-4.69	0.56	Yes	Yes	Yes	Yes	Yes
2	7638	High	Yes	Yes	Yes	-5.09	0.55	Yes	Yes	Yes	Yes	Yes
3	18807	High	Yes	Yes	No	-4.63	0.56	Yes	Yes	Yes	Yes	Yes
4	44129612	High	Yes	Yes	No	-5.58	0.55	Yes	Yes	Yes	Yes	Yes
5	448978	High	Yes	Yes	No	-5.04	0.56	Yes	Yes	Yes	Yes	Yes
6	447966	High	Yes	Yes	Yes	-6.02	0.55	Yes	Yes	Yes	Yes	Yes
7	2255489	High	No	Yes	No	-6.74	0.55	Yes	Yes	Yes	Yes	Yes
8	448623	High	Yes	Yes	Yes	-5.47	0.55	Yes	Yes	Yes	Yes	Yes
9	69545180	High	Yes	Yes	Yes	-6.35	0.55	Yes	Yes	Yes	Yes	Yes
10	15624	High	Yes	Yes	No	-5.17	0.56	Yes	No	Yes	Yes	No
11	16122582	High	No	No	No	-6.95	0.55	Yes	Yes	Yes	Yes	Yes
12	16220130	High	Yes	Yes	Yes	-4.69	0.55	Yes	Yes	Yes	Yes	No
13	3758	High	No	No	No	-6.62	0.55	Yes	Yes	Yes	Yes	Yes
14	5564	High	Yes	Yes	No	-4.69	0.55	Yes	Yes	Yes	Yes	Yes
15	18807	High	Yes	Yes	No	-4.63	0.55	Yes	Yes	Yes	Yes	Yes
16	5271320	High	Yes	Yes	No	-4.84	0.55	Yes	Yes	Yes	Yes	Yes
17	23656591	High	Yes	Yes	Yes	-4.94	0.55	Yes	Yes	Yes	Yes	Yes
18	852148	High	Yes	Yes	No	-5.50	0.55	Yes	Yes	Yes	Yes	Yes
19	45490026	High	Yes	No	No	-5.50	0.55	Yes	Yes	Yes	Yes	Yes
20	22947105	High	Yes	Yes	No	-4.75	0.55	Yes	Yes	Yes	Yes	Yes
21	71718966	High	Yes	Yes	Yes	-4.42	0.55	Yes	Yes	Yes	Yes	Yes
22	44410251	High	Yes	Yes	No	-5.74	0.55	Yes	Yes	Yes	Yes	Yes
23	25023958	High	Yes	Yes	Yes	-4.53	0.55	Yes	Yes	Yes	Yes	Yes
24	44405311	High	Yes	Yes	No	-5.40	0.55	Yes	Yes	Yes	Yes	Yes
25	21272512	High	Yes	Yes	No	-5.27	0.55	Yes	Yes	Yes	Yes	Yes
26	11659169	High	Yes	Yes	No	-5.28	0.55	Yes	Yes	Yes	Yes	Yes
27	6852148	High	Yes	Yes	No	-5.50	0.55	Yes	Yes	Yes	Yes	Yes
28	25023955	High	Yes	Yes	No	-5.28	0.55	Yes	Yes	Yes	Yes	Yes
29	71579715	High	Yes	Yes	Yes	-5.10	0.55	Yes	Yes	Yes	Yes	Yes
30	25023959	High	Yes	Yes	Yes	-4.23	0.56	Yes	Yes	Yes	Yes	No
31	5564	High	Yes	Yes	No	-4.69	0.55	Yes	Yes	Yes	Yes	Yes
32	94509	High	Yes	Yes	No	-4.63	0.55	Yes	Yes	Yes	Yes	Yes
33	12774295	High	Yes	Yes	No	-4.70	0.55	Yes	Yes	Yes	Yes	Yes
34	10846712	High	Yes	Yes	No	-4.97	0.55	Yes	Yes	Yes	Yes	Yes

35	10711808	High	Yes	Yes	No	-5.45	0.55	Yes	Yes	Yes	Yes	Yes
36	82267072	High	Yes	Yes	No	-5.10	0.55	Yes	Yes	Yes	Yes	Yes
37	21272514	High	Yes	Yes	No	-4.53	0.55	Yes	Yes	Yes	Yes	Yes
38	12346120	High	Yes	Yes	No	-4.61	0.55	Yes	Yes	Yes	Yes	Yes
39	82267104	High	Yes	Yes	Yes	-5.03	0.55	Yes	Yes	Yes	Yes	Yes
40	82053313	High	Yes	Yes	No	-5.39 cm/s	0.55	Yes	Yes	Yes	Yes	Yes
41	14094464	High	Yes	No	No	-5.66	0.55	Yes	Yes	Yes	Yes	Yes
42	45093134	High	Yes	No	No	-5.59	0.55	Yes	Yes	Yes	Yes	Yes
43	59319916	High	Yes	Yes	Yes	-5.04	0.55	Yes	Yes	Yes	Yes	Yes
44	156913	High	No	No	No	-4.22	0.56	Yes	No	Yes	No	No
45	627458	High	Yes	No	No	-4.52	0.55	Yes	Yes	Yes	Yes	Yes

GI-Gastrointestinal absorption, BBB-Blood Brain Barrier penetration,CYP: Cytochrome P450, Log Kp-Skin Permeation Coefficient.

MATERIAL AND METHODS

Ligand based virtual screening of triclosan against databases using Swisssimilarity web tool. Swisssimilarity tool's screenable libraries include drugs, bioactive and commercial compounds and millions of virtual compounds readily synthesizable from commercially available synthetic reagents (Zoete et al. 2016).A drug with good oral absorption must meet the following parameters: molecular weight of less than 500 Da, logP (lipophilicity) of less than five (5); maximum of five (5) groups of hydrogen donors and maximum of ten (10) groups of accepters binding intestinal permeability and consisting of the first steps towards good oral bioavailability. *In-silico* physicochemical, pharmacokinetic and toxicological properties of triclosan analogs were perfomed by SwissADME web tool. The crystal structure of plasmodium falciparum enoyl-acyl-carrier-protein reductase with triclosan (PDB ID: 1NHG) was retrieved from the Protein Data Bank (https://www.rcsb.org/). All the Nicotinamide-adenine-dinucleotide (NAD) and triclosan compound were removed and polar hydrogen added to make the structure of enoyl-acyl-carrier-protein reductase prepared for molecular docking processes (Daina et al. 2017).

inding site residues of FabI enzyme was predicted by CASTp3.0 server (Tian et al. 2018). Computed Atlas of Surface Topography of proteins (CASTp) is a web server that provides online resources to find delineate and quantify the geometric and topological properties of protein structures. The molecular docking method can be used to model the atomic level interaction between a small molecule and a protein, which enables us to describe the actions of small molecules in the target protein binding site as well as to elucidate basic biochemical processes. Screened triclosan analogs were docked with enoyl-ACP reductase using MTiAutoDock (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::MTiAutoDock). The most negative binding affinity score of compounds were chosen as candidate compounds (Daina et al. 2017).

RESULTS AND DISCUSSION

In Silico ADME/Tox Screening of Triclosan Analogs: About half of the drug candidates struggled during development due to ADME/Tox deficiencies. In order to prevent this development failure a series of *In-silico* ADME/Tox screens was introduced with the intention of discarding compounds during the discovery process. The ADME predictions of triclosan analogs for passive human gastrointestinal absorption (GI) and permeation of the blood-brain barrier (BBB) are both based on the BOILED-Egg model were shown in table 1. The properties of human intestinal absorption are determinant of the drug production and are required to be administered orally. The blood-brain barrier (BBB) plays a significant role in drug pharmacology (Ma et al. 2005). Carcinogenicity is a cancer-causing risk in the body. Triclosan analogs were accessed by five different rule-based filters such as Lipinski filter implemented rule-of-five, Ghose, Veber, Egan and Muegge methods, respectively and shown in table 1. The result presented in Table 1 shows that all of the investigated compounds present high gastrointestinal absorption, good skin permeation and inhibit xenobiotic metabolism involving cytochrome CYP1A and CYP2D6. At the end of the entire ADMET evaluation screening, four compounds, CID 15624, 16220130, 25023959 and 156913, failed the drug likeliness test and were deleted for subsequent analysis (Ghose et al. 1999; Egan et al. 2000; Muegge et al. 2001; Veber et al. 2002; Lipinski 2004; Ma et al. 2005).

Binding site analysis of FabI: CASTp(http://sts.bioe.uic.edu/castp/calculation.html) provides a comprehensive and detailed quantitative characterization of the inner voids and surface pockets on FabI's three-dimensional structure. Identified binding site residues of Fab I on chain A of Plasmodium FalciparumareGLY104, ILE105, GLY106, ASP107, GLY110, TYR111, GLY112, TRP113, GLY129, TRP131, VAL134, PHE167, ASP168, ALA169, SER170, HIS214, SER215, LEU216, ALA217, ASN218, ALA219, VAL222, LYS240, SER241, LEU265, THR266, TYR267, TYR277, MET281, LYS285, LEU288, ALA312, GLY313, PRO314, LEU315, SER317, ARG318, ALA319, ALA320, ALA322, ILE323.

Table 2. Triclosan analogsphysicochemical properties and binding energy with FabI

Sl. No.	Pubchem CID	Molecular weight (g/mol)	No of rotatable bonds	No of H-bond acceptors	No of H-bond donors	TPS (Å²)	Binding energy (kcal/mol)
1	5564(Triclosan)	289.5	2	2	1	29.5	-4.36
2	7638	200.23	3	2	1	29.5	-4.76
3	18807	255.09	2	2	1	29.5	-4.08
4	44129612	384.7	2	3	1	65.8	-4.42
5	448978	327.2	5	4	1	55.8	-4.42
6	447966	272.3	2	3	1	54.5	-4.71
7	2255489	239.3	2	4	2	99.3	-4.86
8	448623	338.17	3	3	2	49.3	-5.10
9	69545180	240.26	1	2	3	65.1	-3.60
10	16122582	257.31	6	5	1	94.8	-3.28
11	3758	222.24	2	3	1	69.3	-3.48
12	5564	289.5	2	2	1	29.5	-4.71
13	18807	255.09	2	2	1	29.5	-4.07
14	5271320	220.65	2	2	1	29.5	-4.14
15	23656591	303.6	3	2	1	29.5	-4.30
16	6852148	270.11	2	3	2	55.5	-4.67
17	45490026	270.11	2	3	2	55.5	-3.34
18	22947105	269.12	2	2	1	29.5	-4.45
19	71718966	270.71	2	2	1	29.5	-4.94
20	44410251	235.66	2	3	2	55.5	-4.45
21	25023958	283.1	3	2	1	29.5	-4.24
22	44405311	294.13	3	3	1	53.2	-4.52
23	21272512	271.09	2	3	2	49.7	-4.49
24	11659169	280.1	2	3	1	53.2	-4.72
25	6852148	270.11	2	3	2	55.5	-4.66
26	25023955	280.1	2	3	1	53.2	-4.66
27	71579715	271.7	2	3	1	42.2	-5.05
28	5564	289.5	2	2	1	29.5	-4.41
29	94509	255.09	2	2	1	29.5	-3.78
30	12774295	220.65	2	2	1	29.5	-4.12
31	10846712	222.67	1	2	1	29.5	-3.34
32	10711808	334.54	1	2	1	29.5	-3.60
33	82267072	221.08	2	2	1	29.5	-3.39
34	21272514	255.09	2	2	1	29.5	-3.61
35	12346120	220.65	2	2	1	29.5	-3.39
36	82267104	221.08	2	2	1	29.5	-4.27
37	82053313	186.63	1	2	1	29.5	-3.68
38	14094464	253.08	3	4	1	47.9	-3.01
39	45093134	209.023	3	3	2	49.7	-3.18
40	59319916	200.66	3	2	1	29.5	-3.39
41	627458	303.6	3	2	0	18.5	-4.20

Analysis of Molecular Docking: Forty oneADME/Tox parameters qualify triclosan were docked in binding site of FabI enzyme using MTiAutoDock server. Binding energy of triclosan analogs were shown in table 2. On analysis of binding energy of analogs, it was found that two analogs, CID 448623 and 71579715 had low binding affinity -5.10 kcal / mol, -5.05 kcal / mol with FabI, respectively, even lower than -4.36 kcal / mol with triclosan, highlighted with bold in table 1.To maximize accuracy of result, best predicted analogs again docked with FabI enzyme from different algorithm using PatchDock server using Patch Dockserver (Duhovny et al. 2002; Schneidman-Duhovny et al. 2005). The clustering RMSD was a default value of 4.0. The top 10 PatchDock solutions produced were refined using Firedock based on their binding energyand the best models had global energy of -39.04 and -43.33kcal/mol for analogs CID 448623 and 71579715 that suggests a good interaction

between ligand and protein (Andrusier et al. 2007; Mashiach et al. 2008).

Predicted Docked complexes were analyzed through Python Molecular Viewer for their interaction study shown in Figure 1 and 2. Triclosan analog was represented in sticks and balls model. Triclosan analog CID 448623 interacted with residues GLY104, LEU216, ASN218, ILE130, GLY129, TRP131, LYS240, SER170, ALA169, ASP168 and PHE167. All the residues interacting with CID 448623 belongs to predicted binding site residues of FabI except ILE130. Triclosan analog CID 71579715 interacted with residues GLY106, ARG316, ILE105, GLY106, VAL134, TRP131, ALA169, and PHE167. All the residues interacting with CID 71579715 also belongs to predicted binding site residues except ARG316. Interacting residues were represented in lines as Figure 1 and 2 (Sanner 1999; Pamudi et al. 2017).

Figure 1: Docking pose of triclosan analog CID448623in binding site of FabI enzyme.

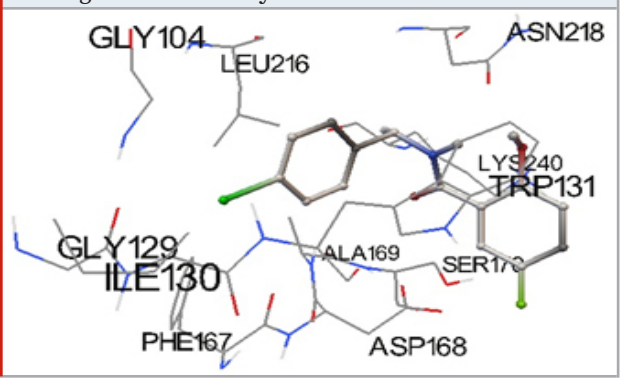
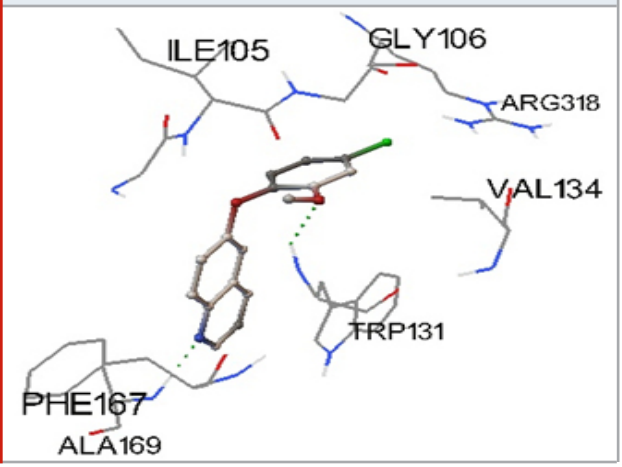


Figure 2: Docking pose of triclosan analog CID 71579715in binding site of FabIenzyme.Two H-bonds were formed between amino acid TRP131 and ALA169 of protein with compound, respectively. Hydrogen bonds are represented with spherical line.



The lack of an effective vaccine and the emergence of *Plasmodium variants* resistant to antimalarial agents emphasize the need for novel chemotherapeutic drugs. In-silico screening, also known as virtual screening, is currently being researched as a strategy for finding antimalarial medicines. Pamudi et al. (2017) explore at the usage of virtual screening to identify Enoyl Acyl Carrier Protein Reductase inhibitors in *Plasmodium falciparum*. The medicinal plants in Indonesia database were used in conjunction with a molecular docking method using GOLD software. They discovered two prospective inhibitor chemicals in tea that have the potential to be developed as antimalarial drugs: kaempferol 3-rhamnosyl-(1-3)-rhamnosyl-(1-6)-glucoside and epigallocatechin 3,5, -di-O-gallate (Pamudi et al. 2017; Dayse et al. 2020).

By using hierarchical virtual screening, Dayse et al. (2020) discovered flavonoids to be inhibitors of *Plasmodium falciparum* enoyl-acyl carrier protein (ACP) reductase (Dayse et al. 2020). A flavonoid library from the ChEMBL database was screened for physico-chemical similarity using the Euclidean distance as a criterion, and then molecular docking was performed using the GridScore scoring tool in the DOCK 6.5 software (Dayse et al. 2020). Using ligands from the Indonesian Medicinal Plants Database, in-silico screening was carried out using the AUTODOCK VINA software to identify potential *Plasmodium falciparum* enoyl-acyl carrier protein (ACP) reductase inhibitor candidates by Malau et al. (2020).

CONCLUSION

The current research utilises computer-based virtual screening to identify Plasmodium falciparum Enoyl-ACP reductase inhibitors that are required for malaria treatment. From several million chemical structures and a sequence of steps of rational refinement, including similarity search, ADME/Tox properties and molecular docking, we identified two triclosan analogs, CID 448623 and 71579715, as good inhibitors for further experimental testing.

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