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Pharmacological analysis for prepared novel Leads of Thiolactomycin standard targeting regulation of FabB enzyme in P. *falciparum:* An *In-silico* study

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ABSTRACT

The biosynthesis of Fatty acid enzymes (Fab enzyme) are universal in nature and remains an useful existenceas important target to develop the antimalarial drug.It has been postulated that it raised throughout the erythrocytic phase of parasite life cycle. The present study predictsthe screening methods for toxicity level of designed novel analogues which repress FabB enzyme regulation, a protein which showsmultifunctional property in nature, throughcomputational approach. The Chem Draw Ultra 10 Software has been used to prepare novel analogues which further converted into 3D PDB structure. The FabB proteinhas retrieved from RCSB PDB tool. Then ADMET profiling for prediction of toxicityandLipinski's rule of 5has beenexecuted on designed analogues .The result declares that TH-21, may play an potentlead which candemonstrate thetopological potential towardsprohibition at active site of FabB enzyme and thus promotes regarding less toxicity outlining.

Keywords: Lipinski's rule, Chew Draw, FabB, Thiolactomycin, analogues.

INTRODUCTION

The most remarkable tropical parasitic diseasesis malaria[1]. The World Health Organization (WHO)admits that around 300–500 million susceptible clinical malarial cases every year and around 1 million deaths to be found every year [1]. The poorest populations are more prone to malaria in the World and it is expanded over in Africa, Asia, and in several South American countries. The four types of Plasmodium species are responsible for malaria, but Plasmodium *falciparum* isoverallsubstantial for the most serious and deadly form of the disease and is significant for malaria-related deathsupto90% in Africa [1]. Malarial assistance is most and widepreference to the National Institutes of Health (NIH) and the implicationways to the calls of complicacy for multiple steps to hook this world-wide problem. At present the medicinaldiagnose procedure are concentrating in three main areas: (a) vaccine development, (b) drug development, and (c) pathogenesis. There is a constant need to establish new drugs within drug development to pacifyover existing ones for the evaluation of malarial infections due to the severe problem of the growing resistance to certain and present drugs. There is urgent need of identification and characterization of the absolute parasitival biochemical pathways which may provide as targets for the development of new drugs whichregulate the mode of action of existing and potential new drugs, and to incinerate possible operations of resistance to existing drugs.

The available present drugs respond to three classes of compounds: (1) aryl amino alcohol compounds e.g. :quinine (2) antifolates–dihydrofolatereductase inhibitors,eg.:pyrimethamine, and (3) Derivatives of artemisinin. Chinese scientists

had first isolatedartemisinin in 1970 from Artemisia annua[2,3]. However, assistance of only one drug is uncommon and now there is a general compliance between scientists that collegialimpact of two drugs probably throes to the best option for assistancewhichconstrict the risk of resistance. Examples of drug collegial effects are the artemisinin–amodiaquine pair and the artemether–lumefantine[4].

There are wide range of Fatty acids in nature but marine organisms, such as particular sponges, have mangea stage for some of the most immersing varieties based on structure. Among themmost ofthese marine fatty acids drops-in from unusual biosynthetic artery and excellent evaluation have listed in recent years as the fatty acidmiscellaneous structure types present in these organisms, their main role in membranes, and their biogenesis proceedings[5-8]. However, it has been admitted and very short known that biomedical potential of these exceptional sponge fatty acids;in particular as to what divergencespresent in their bioavailability in association to reported for the more common fatty acids. Due to recent activity in research, we are now able in makingscaffold toinitiate and take a step towards potentiality of these marine compounds to scrap of these infectious diseases such as malaria, tuberculosis, and fungal infections.Due to the limited number of drugs presently available malarial chemotherapy is an area that is in continuous growth and revision, the available drugs shows severe side effects,and the prolonged development of combat developed by the parasite to some of these drugs [9].The most deleterious malarial parasite of the phylum Apicomplexa is P. *falciparum,* which comprise an apicoplast, an organelle which generally developed from a cyanobacterium through a channels of secondary endosymbiotic, which direct towards formation of two membranes [10]. The several vital metabolic processes occurs at the apicoplast containing site of malarial parasite.

The main processes among them are isoprene biosynthesis, haem biosynthesis, and fatty acid biosynthesis take place. A type I fatty acid synthesis (FASI) system used by higher eukaryotes, wherea single protein with multiple domains catalyzes the each fatty acid biosynthetic pathway. In comparison to type I fatty acid synthesis, in the apicoplast a type II fatty acid synthase (FASII) system is functional, where each fatty acid biosynthetic pathway is leads towards a different enzyme encoded by different gene[11].

As we are eukaryotic in nature so type II FAS system is absent in human but is common in bacteria and algae [12]. After invasion to host cell, a parasite creates a parasitophorous vacuole to hide itself, which protect it against the hostimmune system. The parasite urges to make its own fatty acids in this procedure for de novo so as to form its membrane expanded. The principal membrane fatty acids in P. *falciparum* are decanoic acid (10:0), lauric acid (12:0), and myristic acid (14:0). The fatty acidsbiosynthesis pathwayinP. *falciparum*is encoded by several enzymes. Some drugs can suppressthe incorporation of biosynthetic pathway of several enzymes. Some remarkable drug eg.are, Thiolactomycin and derivatives (which inhibit FabB and FabH),Isoniazid (which inhibits FabI)[13,14].

The effect of antimalarialon fatty acids hadmoved towards consideration in the past but the attentivenessfor fatty acids that it themselves might inhibit the fatty acid biosynthetic machinery of the parasite P.*falciparum* has only been presentlyreviewed to makea procedure towardscombatingof parasite. The work of Kumaratilake et al. which was carried out in 1992 affirm that n3 and n6 fatty acids which had antimalarial properties were polyunsaturated in nature and postulate the in-vitro invasion of intraerythrocytic forms of P. *falciparum*[15]. The fatty acids which consists of methyl esters wereannounced to be as potent as the free acids in killing the parasite. The bindingproperties of the fatty acids to albumin in-vivo was also consider as unlikely to suppress the antimalarial effect of the polyunsaturated fatty acids [15].

METHODOLOGY

Protein structures retrieval:
TheProtein Data Bank has been used to retrieve the structures of enzymes (FabB) involved in regulation of Plasmodium*falciparum*[19].

Active sites prediction of Protein:
The Castp Findertool were used for several separate procedures to perform active/ binding site prediction. To minimization process of the box (pocket) which encloses the protein is carried out by generating their coordinates. According to their spatial proximity, and the full interaction to their energies, every probe coordinates are then clustered. This further leads to joins all adjacent sites but not on the diagonals of the cube. According to their total interaction energies, with the most remarkable being identified as the first predicted binding site the probe clusters werethan ranked. Themeta pocket predict thevariables for estimation of site volume and identification of proteins / enzymes.

Preparation of Compounds/Analogues:
The ACD lab software extension ChemDraw in MDL .mol format has been used to preparethe 2D-structure of Thiolactomycin and its 21 analogues which further carried out to Discovery Studio 2.5 window togenerate the 3D-structure (Table 1).

Prediction of Drug-likeliness for prepared novel Thiolactomycin Analogues:
The estimation of solubility and permeability forprepare compounds has been carried out through computational approaches by using, 'the Lipinski rule of 5's. This rule predicts the pharmacological, biological and ADME(absorption, distribution, metabolism and excretion) exercise of the prepared compound and also its potentialityto an orally active drug in humans [16].

The 'rule of 5' states that: poor absorption or permeation is more likely when:
a) The compounds/ analogues shows more than 5 H-bond donors [expressed as the sum of OHs and NHs).
b) The molecular weight of compounds/ analogues is over 500.
c) The Log P [octanol-water partition coefficient) of compounds/ analogues is over 5.
d) There are more than 10 H-bond acceptors in compounds/ analogues [expressed as the sum of Ns and Os).
e) compounds/ analogues classes that are substrates for biological transporters are exceptions to the rule.

The prediction ofADMET forprepared novel Thiolactomycin Analogues:
The pharmacokinetics criteria like Adsorption, Distribution, Metabolism, Excretion and Toxicology (ADME/T) was performed through Pre ADMET online server (http://preadmet.bmdrc.org).The property like Human Intestinal Absorption (% HIA), Caco-2 permeability, MDCK cell Permeability, Skin Permeability, Blood Brain Barrier Penetration and Carcinogenicity all theseparameters weredeliberated.

RESULTS

The potential binding sites/active site in FabB(2PWP) by Castp:
The different amino acids residues of the enzymes under study was found out but the residues which shows the potential/active binding sites, on the basis of Castp finder tool result are the following:

In FabB (2PWP) :-TYR26,THR28,LYS29,SER30,LYS31,TYR32,ILE109,ASP110,GLU111,THR112, GLU115, PHE136,ILE137,GLU138,ASP139,LYS142.

Table 1: DifferentLeads/Analogues designed/constructed by taking Thiolactomycin as a standard.

Sr.No.	Name/Symbol	CanonicalSMILE Version
1.	Thiolactomycin (Standard)	CC1=C(C(SC1=O)(C)C=C(C)C=C)O
2.	TH1	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(O)[H]
3.	TH2	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Cl)[H]
4.	TH3	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Br)[H]
5.	TH4	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(F)[H]
6.	TH5	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(I)[H]
7.	TH6	C1(C([H])([H])[H])=C(I)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(I)[H]
8.	TH7	C1(C([H])([H])[H])=C(Br)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Br)[H]
9.	TH8	C1(C([H])([H])[H])=C(Cl)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Cl)[H]
10.	TH9	C1(C([H])([H])[H])=C(F)SC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(F)[H]
11.	TH10	C1(C([H])([H])[H])=C(F)OC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(F)[H]
12.	TH11	C1(C([H])([H])[H])=C(Cl)OC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Cl)[H]
13.	TH12	C1(C([H])([H])[H])=C(Br)OC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(Br)[H]
14.	TH13	C1(C([H])([H])[H])=C(I)OC(C(=C([H])C([H])=C([H])[H])[H])([H])C1(I)[H]
15.	TH14	C1(C([H])([H])[H])=C(O)C(=O)C(C(=C([H])C([H])=C([H])[H])[H])([H])C1=O
16.	TH15	C1(C(O)(O)O)=C(O)C(=O)C(C(=C([H])C([H])=C([H])[H])[H])([H])C1=O
17.	TH16	C1(C([H])([H])[H])=C(O)C(=O)C(C(=C([H])C([H])=C(O)O)[H])([H])C1=O
18.	TH17	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])S1
19.	TH18	C1(C(O)(O)O)=C(O)SC(C(=C([H])C([H])=C([H])[H])[H])([H])S1

20.	TH19	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C([H])=C(O)O)[H])([H])S1
21.	TH20	C1(C([H])([H])[H])=C(O)SC(C(=C([H])C(O)=C(O)O)[H])([H])S1
22.	TH21	CC1=C(C(SC1=O)(C)CCC(C)C)O

Table 2:Pharmacokineticstudies to measure the drug concentrations in blood or plasma.

Sr.No.	ADME Properties	Activity Range
1.	Human intestinal absorption (HIA)	Poorly- 0~20% Moderate- 20~70% High- 70~100%
2.	Blood brain barrier (BBB)	CNS active compounds (+); >1 CNS inactive compounds (-); < 1
3.	Madin-Darby canine kidney (MDCK) cell permeability	Lower- < 25 Moderate- 25~500 Higher- > 500
4.	Heterogenous human epithelial colorectal adenocarcinoma (Caco2) cell permeability	Lower- < 4 Moderate- 4~70 Higher- < 70
5.	Plasma protein Binding (% PBP)	Chemicals strongly bound >90% Chemicals weakly bound < 90%

Table 3: ADMET prediction of novel designed Analogues with compare to parent compound.

Sr.No.	Lead Name/Symbol	ADME Properties					
		Caco2	MDCK	Skin Permeability	HIA	BBB	Toxicity(M/C)
1.	Thiolactomycin(Standard)	**21.52**	**42.775**	**-1.781**	**95.74**	**0.79**	-/-
2.	TH1	13.40	63.283	-2.737	91.64	1.43	**+/-**
3.	TH2	41.56	51.093	-1.463	95.34	2.92	+/-
4.	TH3	53.28	1.220	-1.456	95.66	3.24	+/+
5.	TH4	23.29	35.781	-1.584	94.95	2.37	+/+
6.	TH5	51.60	0.321	-1.333	96.86	4.51	+/+
7.	TH6	58.03	0.518	-1.092	100.00	3.94	+/+
8.	TH7	58.05	0.261	-1.254	100.00	2.33	+/+
9.	TH8	57.46	9.659	-1.182	100.00	1.88	+/-
10.	TH9	25.86	23.084	-1.383	100.00	1.22	+/+
11.	TH10	57.92	52.634	-1.662	100.00	2.02	+/+
12.	TH11	57.98	92.243	-1.342	100.00	3.30	+/-
13.	TH12	57.98	0.304	-1.119	100.00	4.08	+/+
14.	TH13	57.98	0.518	-1.001	100.00	6.50	+/+
15.	TH14	18.44	17.994	-2.016	93.44	0.46	+/+
16.	TH15	0.27	1.342	-3.627	48.01	0.13	+/-
17.	TH16	20.94	5.173	-3.84	70.41	0.29	-/+
18.	TH17	28.58	42.491	-2.05	96.25	0.78	-/-
19.	TH18	2.27	28.630	-1.73	75.68	0.37	-/-
20	TH19	0.80	37.455	-3.588	87.32	0.83	+/-
21.	TH20	0.95	191.428	-4.011	75.21	0.37	+/-
22.	TH21	**21.56**	**26.33**	**-2.33**	**95.24**	**1.10**	-/-

Abbreviations: Caco-2- heterogenous human epithelial colorectal adenocarcinoma; **M**- mutagen; **C**-carcinogen(rat, mouse), **BBB** - Blood brain barrier; **HIA**-Human intestinal absorption; **SP**-Skin permeability; **MDCK**- Madin-Darby canine kidney.

Table 4:prediction o fDrug-Likeliness for designed analogues along with the standard drug.

Sr. No.	Analogues	MW	HBA	HBD	Log P
1.	Thiolactomycin (Standard)	**210.07**	**3**	**1**	**2.54**
2.	TH1	184.06	3	2	1.67
3.	TH2	202.02	2	1	3.13
4.	TH3	245.7	2	1	3.29
5.	TH4	186.05	2	1	2.81
6.	TH5	23.6	2	1	3.18
7.	TH6	403.86	1	0	4.28
8.	TH7	307.8	1	0	4.16
9.	TH8	219.99	1	0	4.03
10.	TH9	188.05	1	0	3.51
11.	TH10	172.07	1	0	3.00
12.	TH11	204.01	1	0	3.52
13.	TH12	21.1	1	0	3.65
14.	TH13	387.88	1	0	3.77
15.	TH14	178.06	3	1	1.11
16.	TH15	226.05	6	4	-0.40
17.	TH16	210.05	5	3	0.18
18.	TH17	186.02	3	1	2.39
19.	TH18	234.00	6	4	0.87
20	TH19	218.01	5	3	1.46
21.	TH20	234.00	6	4	1.52
22.	TH21	**214.10**	**3**	**1**	**3.18**

Abbreviations:MW- molecular weight; HBA-hydrogen bond acceptors; Log P- partition coefficient; HBD- hydrogen bond donor.

DISCUSSION

The filtration of “drug-like” compounds from non-drug like compound is a key area on present research in computer aided drug designing field.The above study shows that Thiolactomycin drug inhibit the FabB enzyme regulation during the erythrocytic phase of parasite incubation. Thus different potential and active residues of the enzyme were obtained by using Castp finder tool. The prediction of ADME as well as Drug-likelinessstudies were carried out for prepared novel leads/analogues. The filtered andpotent compounds among them have further examined for their pharmacokinetics properties , metabolism and potential toxicity. This way thehigh throughput ADME screening and combinatorial chemistry was completed. The PreADMET on line tool has been used to perform the ADME prediction of prepared novel leads/analogues[20] as given in (Table 2 and 3). All the 21 designedleads/analogues were screened through different criteria of Drug-likeliness predictionand also assessed by using the Lipinski’s rule of 5[17,18] followed by ADMET evaluation(Table4).The pharmacological analysis of above study reveals that TH-21 analogue have the best binding features and less toxicity profiling with FabB enzyme for further *in vivo* and *in vitro* consideration.

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REFERENCES

[1]. Thayer AM, ChemEng News 2005 83:69.
[2]. Klayman DL, Science 1985 228:1049.
[3]. Acton N et al. Planta Med 1985 47:442.
[4]. Piola P et al. Lancet 2005 365:1467.
[5]. Djerassi C et al. AccChem Res 1991 24: 69.
[6]. Dembitsky VM et al. ChemPhys Lipid 2003 123:117.
[7]. Carballeira NM et al. Prog Lipid Res 2002 41:437.
[8]. Berge JP et al. AdvBiochemEngBiotechnol 2005 96:49.

[9]. Thayer AM, Preventing Malaria. ChemEngNews 2005 83:85.
[10]. McFadden GI et al. Nature 1996 381:482.
[11]. Waller RF et al. ProcNatlAcadSci USA 1998 95:12352.
[12]. Tasdemir D, Phytochem Rev 2006 5: 99.
[13]. Heath RJ et al. Prog Lipid Res 2001 40:467.
[14]. Jones SM et al. J Med Chem 2005 48:5932.
[15]. Kumaratilake LM et al. J Clin Invest 1992 89:961.
[16]. Lipinski CA et al. Adv. Drug Deliv. Rev. 1997 23:3.
[17]. Singh S et al. Res. J. Pharm. Biol. Chem. Sci. 2013 4:882.
[18]. Bonate PL et al. Pharmacokinetics in Drug Development: Regulatory and Development Paradigms. 2004 1:32 [PMID: 16353932].
[19]. http://www.rcsb.org/
[20]. http://preadmet.bmdrc.org/