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A REVIEW ON VARIOUS SCAFFOLDS ACTIVE AGAINST *Pf*PFT TARGET

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ABSTRACT

Protein Farnesyltransferase(FTase) catalyzes an essential posttranslational lipid modification of more than 60 proteins involved in intracellular signal transduction networks. FTase inhibitors have emerged as a significant target for development of anticancer therapeutics and, more recently, for the treatment of parasitic diseases caused by protozoan pathogens, including malaria (*Plasmodium falciparum*). In this review we reported various scaffolds i.e. Peptidomimetics, Tetrahydroquinolines(THQs), Oxy-THQs, Benzophenone, Benzamide, Naphthyridine and Ethylenediamine which are acted against *Pf*PFT Target and proved as an effective antimalarial agents.

KEYWORDS: *Plasmodium falciparum* Protein Farnesyltransferase, malaria, scaffolds, Farnesyltransferase, Docking.

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INTRODUCTION:

Parasitic diseases continue to have a major impact on the morbidity and mortality in tropical and subtropical regions. Among these, malaria causes about 300 million infections annually, with 1-3 million deaths occurring in Africa [1]. Acquisition and spread of drug resistance to existing antimalarials is largely responsible for the recent increase in malaria -related mortality. This increased disease burden has led investigators to seek out new chemotherapeutics [2,3]. Studies have identified an essential post -translational modification of proteins by isoprenyl lipids, C15 farnesyl and C20 geranylgeranyl, using precursors of the isoprenoid biosynthesis pathway in these parasites. The isoprenyl lipid modification of proteins has been shown to be critical for various cellular activities in mammals and yeast including proliferation and apoptosis. Growth of the protozoan parasites has been shown to be severely impaired by inhibition of protein farnesylation compared to mammalian cells, suggesting high potential of the enzyme Protein Farnesyltransferase(PFT) to be utilized as an anti-parasitic drug target [4].

Protein Farnesyltransferase

PFT, a member of the prenyltransferase family, is one of three closely related heterodimeric zinc metalloenzymes(the others being the protein geranylgeranyltransferases I and II, PGGT-I and PGGT-II, respectively) that are important post- translational modification enzymes, catalyzing protein prenylation and subsequent membrane association [5].PFT catalyzes the transfer of a C15 isoprenoid(farnesyl) unit from farnesylpyrophosphate(FPP) to the free thiol of a cysteine residue within a specific CaaX tetrapeptide sequence, located at the C-terminus of the substrate protein(e.g., RasGTPase), where an aliphatic amino acid and X(which contributes to substrate specificity)=M,S,A,orQ. *Chakrabarti et.al.* have identified prenylated proteins and associated prenyltransferase activity in *P. falciparum* and confirmed the viability of *P . falciparum* Protein Farnesyltransferase(*Pf*PFT) as a new antimalarial target [6].

There are currently >2000 primary publications on PFT inhibitors and >300 patents worldwide. Four companies have initiated clinical trials for the development of PFTIs as a cancer chemotherapeutic: Janssen/Johnson and Johnson, Schering -Plough, Merck and Brystol Myers Squibb [7, 8, 9, 10, 11, 12]. Janssen/Johnson and Johnson and Schering- Plough are advancing with late clinical trials for use of PFTIs in the treatment of certain leukemias [13]. Because of strong interest in the development of PFTIs for the treatment of cancer, there is a wealth of pharmacologic information about PFTIs. This research knowledge along with small- molecule chemical libraries provide an excellent opportunity for the investigation of using PFTIs for the treatment of tropical diseases such as malaria.

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RECENT WORK ON *Pf*PFT

Usually up to till date mainly 7 scaffolds have been acted on *Pf*PFT target these were: Peptidomimetics scaffold, Tetrahydroquinoline scaffold(THQ), Oxytetrahydroquinoline scaffold(OxyTHQ), Naphthyridine scaffold, Benzamide scaffold, Benzophenone scaffold & Ethylenediamine scaffold. Earlier in 1998, *Charles M. allen et. al*. gives Protein prenyltransferase activities of *plasmodium falciparum* [14]. As it has been analysed in literature review that very less work has been done on *Pf*PFT as antimalarial activity.
First scaffold which has been introduced against *Pf*PFT target was Peptidomimetics in 2001 by *Andrew D. Hamilton et. al*. that are potent inhibitors of the growth of *P. falciparum*. He developed a series of Non-Thiol FTase inhibitors which mimics the design of Caax tetrapeptide in which the central aliphatic dipeptide is replaced by a hydrophobic and rigid spacer such as a substituted 4-aminobenzoyl group [15]. This led to a family of highly potent inhibitors of FTase such as, FTI-276, which has IC_{50} against the mammalian enzyme of 0.5nm but this compound1(FTI-276) did not show any apparent inhibition of parasite growth, whereas all of the imidazole containing derivatives inhibited parasite growth at concentration below 30(with exception 4 and 12) as shown in **Figure 1**.

However, In 2004 *Jochen Wiesner et. al.* suggests that Benzophenone scaffold which shows highest In- vitro activity but poor In-vivo activity. Earlier studies reveal that the development of Farnesyltransferase inhibitors as antimalarial drugs is hampered by the fact that the heterologous expression of the Farnesyltransferase gene from *P. falciparum* has not been achieved so far, and, therefore, no recombinant enzyme is available for routine screening. Only the native protein has been purified from in vitro parasite cultures and used to demonstrate its inhibition by different established Farnesyltransferase inhibitors. However, for the development of new chemotherapeutics growth inhibition data obtained with cultured blood stages of the parasite seem to be more significant. *Gelb et. al.* have tested a series of Farnesyltransferase inhibitors derived from the group of Sebti and Hamilton against such blood cultures and reported activities in the micromolar range. A new class of Farnesyltransferase Inhibitors based on a Benzophenone scaffold was developed which suppress the growth of the multiresistant *P. falciparum* strain Dd2 with IC50 values in the nanomolar range and are thus one to two orders of magnitude more active than previously described Farnesyltransferase inhibitors. However, the inhibitors with the highest in vitro potency, such as compounds 1 and 3, turned out to be inactive in a murine malaria model, presumably due to insufficient solubility in water as shown in **Figure 2** [16]. *Jochen Wiesner et. al.* now tried to achieve this required In-vivo activity by introducing a methylpiperazinyl residue to the α-position of the phenyl acetyl moiety of the 2-amino group of the Benzophenone scaffold. This modification resulted in significantly improved solubility in water **Table 1**.

In 2004, *Dora Carrico et. al*. evaluate the effects of ester structure modification on antimalarial activity and for further development of a Farnesyltransferase inhibitor with In-vivo activity [17]. Evaluation against *P. falciparum* in red blood cells showed that

all the investigated esters exhibited significant antimalarial activity, with the benzyl ester compound 16 showing the best inhibition(ED50=150nM) as shown in **Figure 3**. Additionally, compound 16 displayed In-vivo activity and was found to suppress parasitemia by 46.1% at a dose of 50mgkg 1day 1 against *Plasmodium berghei* in mice. The enhanced inhibition potency of the esters is consistent with improved cell membrane permeability compared to that of the free acid. The results of this study suggest that Protein Farnesyltransferase is a valid antimalarial drug target and that the antimalarial activity of these compounds derives from a balance between the hydrophobic character and the size and conformation of the ester moiety.

Then in 2005, *Jean-Pierre Henichart et. al*. suggests that Novel *N*-(4-Piperidinyl) benzamide Antimalarials also exhibits potent mammalian Protein Farnesyltransferase inhibitor activity. This work was focused on Peptidomimetic inhibitors based on the Ca_1a_2X tetrapeptide, known to be responsible for interaction with the mammalian FTase, where the a_1a_2 peptide is replaced by the structurally restricted *N*-(4-piperidinyl)benzamide scaffold. Initial studies reveal compound 1 which possessed an IC_{50}(isolated enzyme FTase) as low as 22.8nM, but did not inhibit the proliferation of tumor cells in culture. More recently, a series of derivatives of compound 1, of general structure 2 was synthesized as shown in **Figure 4** which was the outcome of three structural modifications: (i) replacement of methioninate by phenylalaninate or isoleucinate(R_1) with the aim of increasing selectivity *versus* geranylgeranyl transferase, based on our previous result (ii) replacement of the cysteinyl moiety by a known metal chelator, *i.e.*(1-benzylimidazol-5-yl)methyl substituted in *para* position(R_2) of benzyl in order to increase cellular uptake (iii) reduction of the benzoyl group into benzyl(R_3) in order to introduce flexibility into this region of the molecule [18].

Again in this year 2005, *Laxman Nallan et. al.* suggests that Low nanomolar concentrations of Tetrahydroquinoline(THQ) based PFTIs inhibit *P. falciparum* PFT and are cytotoxic to cultured parasites. Biochemical studies suggest inhibition of parasite PFT as the mode of THQ cytotoxicity. Studies with malaria infected mice show that THQ PFTIs dramatically reduce parasitemia and lead to parasite eradication in the majority of animals. These studies validate *P. falciparum* PFT as a target for the development of antimalarials and describe a potent new class of THQ PFTIs with antimalarial activity. Structure of THQ as shown in **Figure 5** [19].

As *Wiesner et. al.* recently developed a novel class of antimalarials derived from Farnesyltransferase inhibitors based on a 2,5-diaminobenzophenone scaffold. However In 2006, *Aihua Xie et. al.* investigate quantitatively the local physicochemical properties involved in the interaction between drug and biotarget, by using 3D-QSAR methods CoMFA and CoMSIA to study some of the screened lead compound and found that steric, electrostatic, and hydrophobic properties of substituent groups play key roles in the bioactivity of the series of compounds [20].

Then again in 2006, *Matthew P. Glenn et. al.* report a new class of antimalarial Protein Farnesyltransferase(PFT) inhibitors Ethylenediamine [21], designed with specific emphasis on simple molecular architecture, to facilitate easy access to therapies based on this recently validated antimalarial target. This novel series of compounds represents the first *Plasmodium falciparum* selective PFT inhibitors reported(up to 145-fold selectivity), with lead inhibitors displaying excellent In-vitro activity(IC50 < 1nM) and toxicity to cultured parasites at low concentrations(ED50 <100nM). Initial studies of absorption, metabolism, and oral bioavailability are reported.

A homology model of the active site of *P. falciparum* was generated at the University of Washington by using the sequence alignment of *Pf*PFT on the template crystal structure of rat PFT complexed with the nonsubstrate tetrapeptide inhibitor CVFM and Farnesyl- pyrophosphate(FPP) [22]. The homology model **Figure 6** indicates a large, open, and predominantly hydrophobic cavity for the active site(~20 × 20 × 20 Å3), with the phospholipid binding partner(FPP) extending across the cavity base. The Zn ion coordinates to three residues (Cys 661, Asp 659, and His 838), with a water molecule hydrogen- bonded between the terminal phosphate of FPP and Asp 659 defining the limit of the Zn binding domain. The remainder of the active site cavity includes two well –defined hydrophobic pockets(Lys 149, Asn 317, Ser 150, Phe 151; Trp 456, Trp 452, Tyr 837) and a larger hydrophilic domain formed by Arg 564 and three water molecules participating in a hydrogen -bonded network between Ser 449 and Gln 152. *Matthew P. Glenn et.al.* envisaged accessing these four pockets from a simple aliphatic tether. Application of a flexible scaffold offers several advantages to the design of a new series of PFT inhibitors. A simple acyclic scaffold may be obtained through a short series of straightforward chemical transformations and may be refractory to resistance arising from mutation of *Pf*PFT. One of the simplest of scaffolds conceivable, Ethylenediamine affords an inexpensive, 4-fold substitutable flexible tether of suitable size to project the appended diversity into the active site pockets. Imidazole provides a convenient zinc binding group, which has been consistently demonstrated to confer activity in other series of inhibitors [23]. Flexible ligand docking studies (GOLD) [24] of a series of compounds incorporating this basic design demonstrate complementarily to the active site of the homology model.

In 2007, *Pravin Bendale et. al.* prepared 266 THQ-based PFTIs and discovered several compounds that inhibit the malarial enzyme in the sub- to low- nanomolar range and that block the growth of the parasite(*P. falciparum*) in the lownanomolar range. This body of structure -activity data can be rationalized in most cases by consideration of the X-ray structure of one of the THQs bound to mammalian PFT together with a homology structural model of the malarial enzyme. The results of this study provide the basis for selection of antimalarial PFTIs for further evaluation in preclinical drug discovery assays [25]. In this study they showed that THQ-based PFTIs are potent inhibitors of *Pf*-PFT activity and of erythrocytic stage of *P. falciparum* growth. Several compounds were found with growth inhibition potency down in the low nanomolar range, with several

compounds blocking parasite growth at concentrations <5nM. A good deal of the structure -activity data for the inhibition of *Pf*-PFT can be accounted for based on the structural consideration of one of the compounds, 162 as shown in **Figure 7** bound to mammalian PFT. They also carried out detailed preclinical pharmacokinetic studies of most potent *Pf*-PFT inhibitors. Together, these two studies provide the basis for further development of *Pf*-PFT inhibitors as novel antimalarial drugs.

Then in 2008, *Michael H. Gelb et. al.* reported a series of novel inhibitors of Protein Farnesyltransferase based on the 2-Oxotetrahydroquinoline scaffold as shown in **Figure 8**. These compounds show selective inhibition of the malaria versus human Farnesyltransferase and inhibit the growth of the malaria parasite in the low nanomolar range. Some of the compounds are at least an order of magnitude more stable to metabolic degradation than the corresponding Tetrahydroquinolines [26].

In this year 2008, *Srinivas Olepu et. al*. reported a new class of 2-Oxo-tetrahydro-1,8 naphthyridine as shown in **Figure 9** based Protein Farnesyltransferase inhibitors that are found to inhibit Protein Farnesyltransferase from the malaria parasite with potencies in the low nanomolar range. These compounds were much less potent on mammalian Protein prenyltransferases. Two of the compounds block the growth of malaria growth in culture with potencies in the sub-micromolar range. Some of the compounds also were found to be much more metabolically stable than previously described Tetrahydroquinoline based Protein Farnesyltransferase inhibitors. Studies suggests that Naphthyridine nucleus shows less activity as compared to THQ Analogs due to the presence of pyridine moiety which decrease the activity [27].

In 2008, *Martin Schlitzer et. al.,* shows that piperazinyl moiety was replaced by N,N,N,' trimethylethylenediamine moiety resulted in highly selective In-vivo and In-vitro antimalarial activity [28]. Starting from Benzophenone -based Farnesyltransferase inhibitors as shown in **Figure 10** with high In-vitro antimalarial activity but no In-vivo activity, the first Farnesyltransferase inhibitors with In-vivo antimalarial activity were obtained through the introduction of a methylpiperazinyl moiety into the α-position of the phenylacetic acid substructure [29]. There homology modeling revealed some differences between the active sites of rat/human and *P. falciparum* Farnesyltransferases, but the main clues derived from docking studies with the rat enzyme remain valid. Based on these docking results, in the following design cycle the piperazinyl moiety of Benzophenone -based inhibitors was replaced by a N,N,N'-trimethylethylenediamine moiety. This resulted in an inhibitor with significantly improved In-vitro and In-vivo antimalarial activity. Furthermore, this inhibitor displayed notable selectivity towards malaria parasites in comparison to human cells. This is a particularly important result for the development of specific antimalarial Farnesyltransferase inhibitors.

Again in 2008, *Andrew D. Hamilton et. al.* reported a novel series of antimalarial, Ethylenediamine-based inhibitors of Protein Farnesyltransferase (PFT) [30]. In this current

study, they designed and synthesized a series of second generation inhibitors,as shown in **Figure 11** wherein the core Ethylenediamine scaffold was varied in order to examine both the homology model of *Plasmodium falciparum* PFT(*Pf*PFT) and predicted inhibitor binding mode. They identified several *Pf*PFT inhibitors(*Pf*PFTIs) that are selective for *Pf*PFT versus the mammalian isoform of the enzyme(up to 136-fold selectivity), that inhibit the malarial enzyme with IC50 values down to 1nM, and that block the growth of *P. falciparum* in infected whole cells(erythrocytes) with ED50 values down to 55nM. The structure- activity data for these second generation, Ethylenediamine- inspired PFT inhibitors were rationalized by consideration of the X-ray crystal structure of mammalian PFT and the homology model of the malarial enzyme.

CONCLUSION

Due to the continuing development of resistance of *P. falciparum* to convectional antimalarial drugs, there is a need of new drugs which are active against multi drug resistance Plasmodium strains. An increasing number of efforts to develop antimalarial drugs have been initiated. Thus the present study is to explore various scaffolds which are active against *Plasmodium falciparum* Protein Farnesyltransferase(*Pf*PFT) Target.

ABBREVIATIONS: *Pf*PFT: *Plasmodium falciparum* Protein Farnesyltransferase, PFTIs: Protein Farnesyltransferase inhibitors, FPP: Farnesyl pyrophosphate, THQs: Tetrahydroquinolines, Oxy-THQs: Oxy-tetrahydroquinolines.

Table 1: In vitro activity (determined according to ref. [21]), in vivo activity (in mg kg^{-1} bw), and solubility of compounds **1**–**4**.

Cmpd.	IC_{50} [nM]	ED_{50}	ED_{90}	Solubility [mM] [a]	[b]
1	270 ± 30	–	–	< 0.04	< 0.06
2	270 ± 35	30	40	0.35	> 3.33
3	64 ± 11	–	–	< 0.04	< 0.06
4	210 ± 21	21[c]	25[c]	0.25	1.25

[a] In phosphate buffer pH 7.2. [b] In water. [c] When the experiment was repeated under comparable conditions with compound **4**, an ED_{50} value of 22 mg kg^{-1} bw and an ED_{90} value of 28 mg kg^{-1} bw were determined.

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Figure 1:-

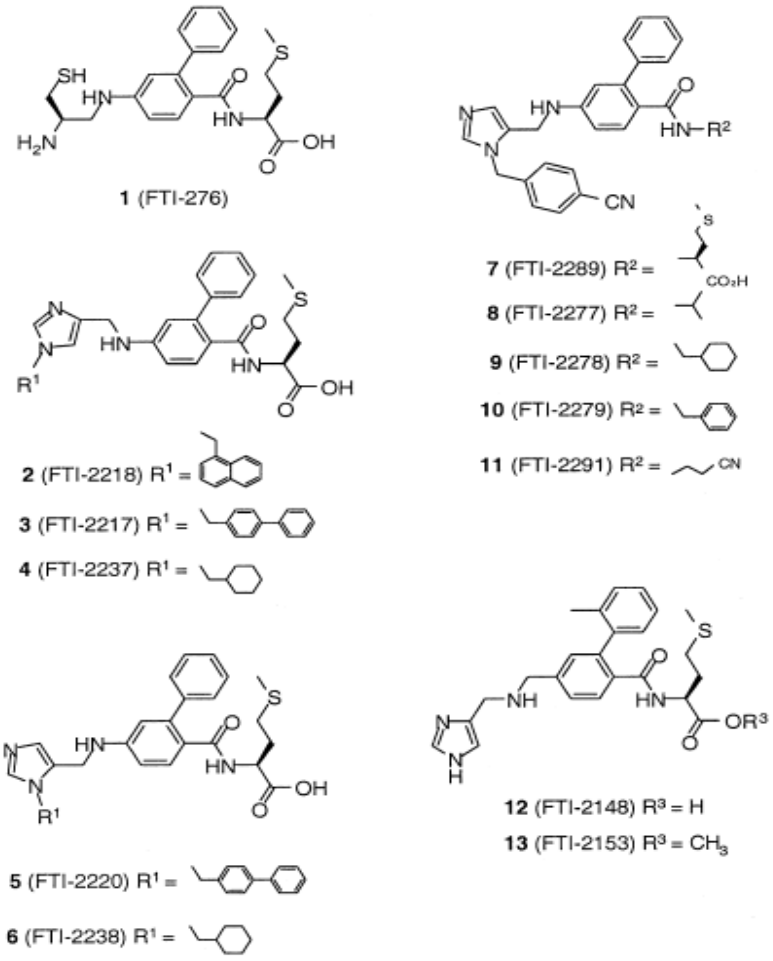


Figure 2:-

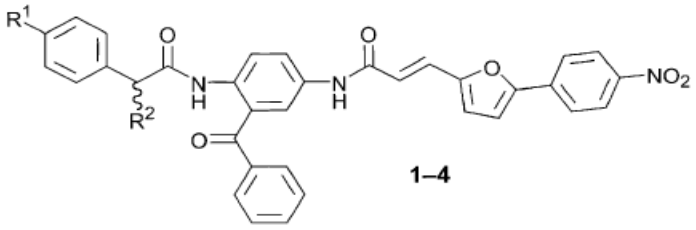
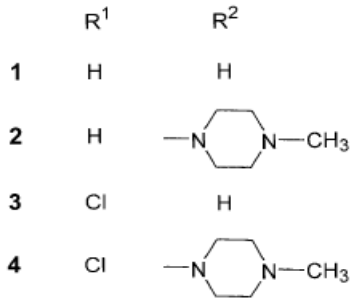


Figure 3:-



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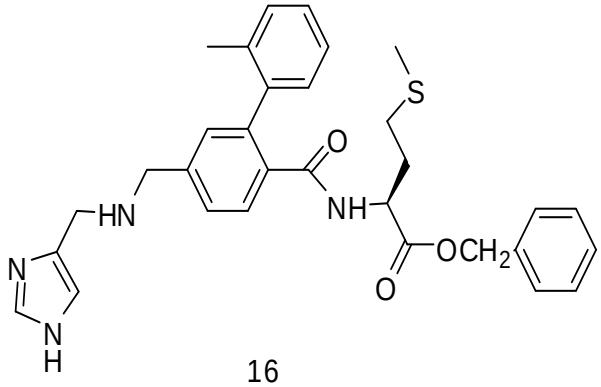

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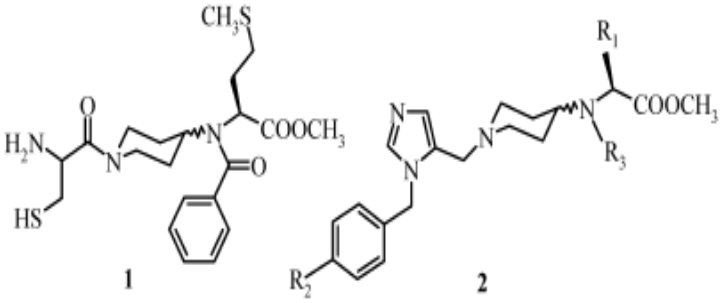
Figure 4:-

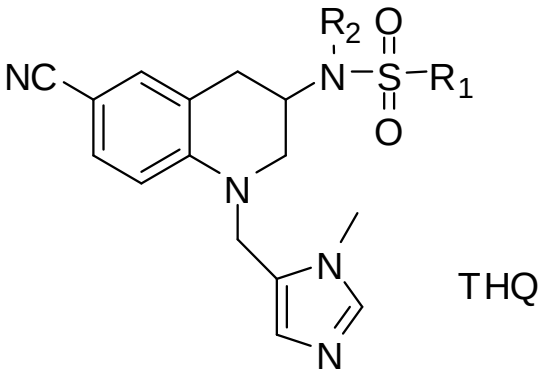


$R_1 = (CH_2)_2SCH_3$, $CH_2C_6H_5$ or $CH(CH_3)CH_2CH_3$

$R_2 = H$, CF_3 or CN

$R_3 = COC_6H_5$ or $CH_2C_6H_5$

Figure 5:-



THQ

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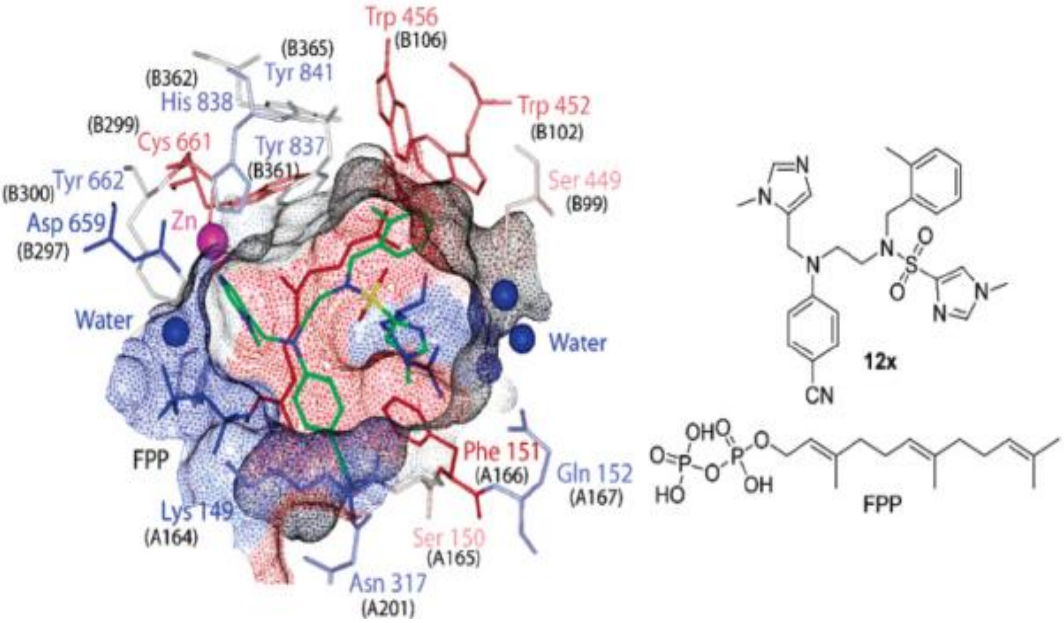
Figure 6 *:-



*** Active site conformation of 12x (colored by atom type), as determined by flexible ligand docking (GOLD), in the homology model of the active site of *Plasmodium* PFT (red hydrophobic to blue hydrophilic). Values in parentheses refer to the corresponding residues of rat FTase (PDB: 1JCR). FPP is shown in red.**

Figure 7:-

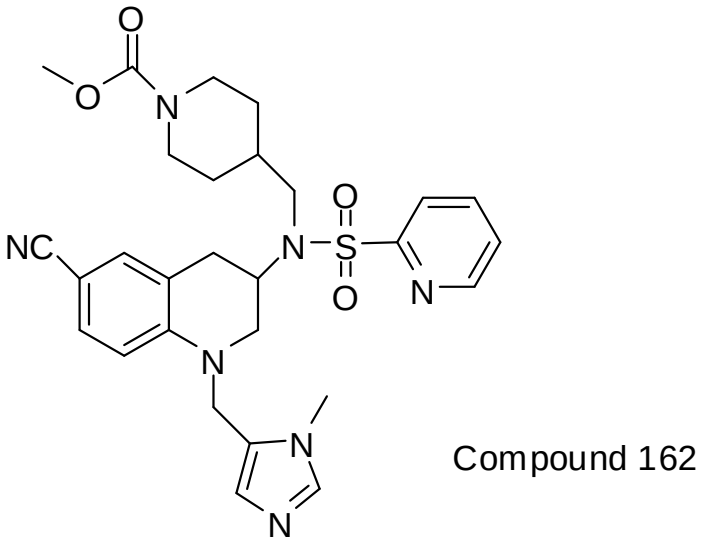


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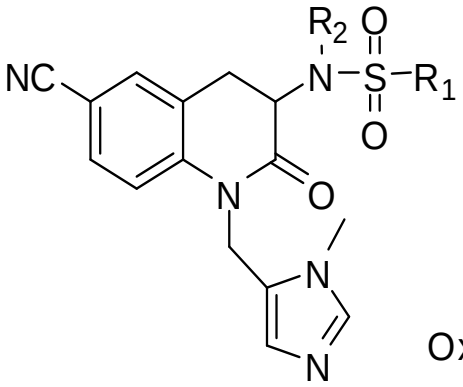
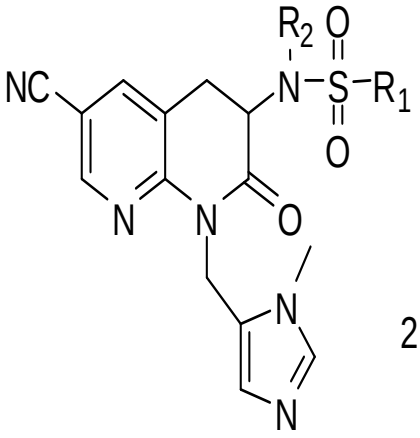
Figure 8:-



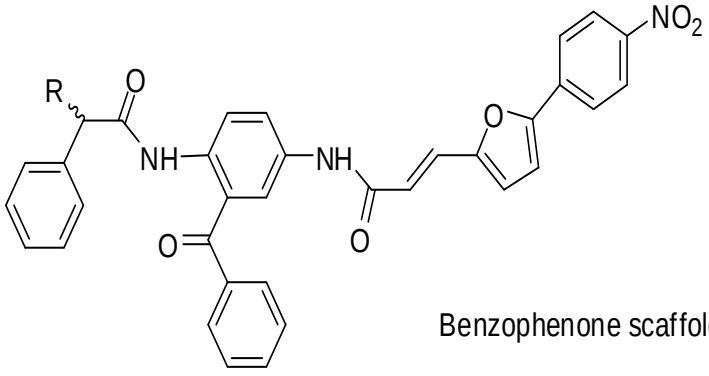
Oxy- THQs

Figure 9:-



2-Oxo-tetrahydro-1,8-naphthridine scaffold.

Figure 10:-



Benzophenone scaffold.

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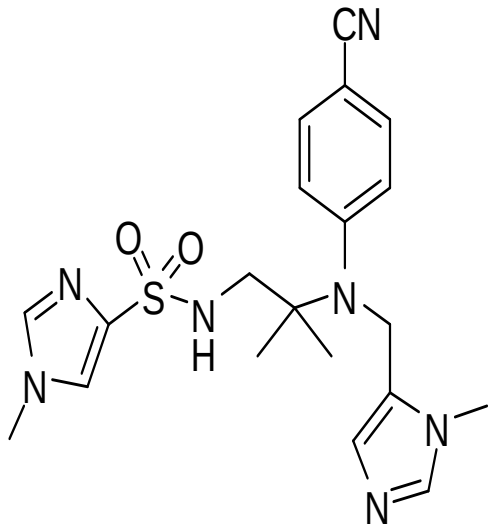
Figure 11:-



Dimethylethylenediamine based scaffold.

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