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Implication of serine-decarboxylase-phosphoethanolamine-methyltransferase Pathway and phosphoethanolamine-methyltransferase in Malaria abolition

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Abstract
The growing resistance to current antimalarial drugs is a major concern for global public health, demands the novel essential regulatory pathway of plasmodium to be targeted for new potent antimalarial development. The biological processes that regulate both growth and gametocytogenesis of plasmodium still remain unknown. Identification and thorough understanding of such pathways is crucial to the development of a new generation of dual activity antimalarials that can inhibit both infection and transmission. There one pathway responsible for phosphatidylcholine (PC) a phospholipid biosynthesis in Bacteria, eukaryotes and plants through different routes, has also been elucidated in plasmodium. Plasmodium normally synthesizes PC for membrane development from choline through CDP-choline route but at infection stage in host it needs faster development of PC abundantly for membrane development from serine of the host blood through plant like serine-decarboxylase-phosphoethanolamine-methyltransferase (SDPM) alternate pathway absent in human host. Phosphoethanolamine methyltransferase (PMT) an enzyme crucial for catalyzing trimethylation, a rate limiting step to synthesize PC is a promising target for research on a novel class of antimalarials. In this review, we have emphasized development of PC biosynthesis through SDPM pathway and the significance of PC in Plasmodium to provide novel directions in which research might be most beneficial. We also focused on important perspectives and applicability of PMTs as therapeutic target for structure-based drug designing for PMT inhibitor discovery as antimalarials.

Keywords: SAM**,** phosphocholine, phosphatidylcholine, SDPM**,** PMT, CDP-choline

Introduction

Malaria is a mosquito borne disease in humans caused by unicellular microorganism called parasitic *Plasmodium protozoa* [1]. According to the fact sheet 2015, cases of malaria (88%), were estimated African Region, 10% in South-East Asia Region and the 2% in Eastern Mediterranean Region. 438 000 peoples were killed by malaria in 2021 among these were 306 000 children and the malaria was found the fourth highest cause of death [1, 2]. Antimalarial drug resistance is greatest challenge for malaria control today responsible for the malaria in new areas and re-emergence and the war against resistance problem led WHO to establish strategies against drug resistance [3, 4,]. The plasmodium spps (*P. falci and vivax*) has been reported resistant against antimalarial drugs where *P. falciparum* has been reported resistant against nearly all the available antimalarial drugs [5, 6]. WHO recommends artemisinin and its derivatives as first choice of drug in combination of other antimalarial, Artemisinin combination therapy (ACT) [7]**.**

The artemisinin drug resistance was reported in Peru 2008 against *plasmodium falciparum* [8] but still ACTs is first choice of treatment for uncomplicated malaria [9]. Chloroquine which prevents hemozoin formation in food vacuoles within the parasite has been reported to be expelled out at too large amount to reach level of its action [10, 11]. *Pf*crt which is a drug exporter gene has found to be responsible for chloroquine resistance [12, 13]. Sulfadoxine and pyrimethamine antifolate combination drugs inhibit folate synthesis by inhibiting dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) respectively have shown resistance due to mutations DHFR and DHPS and their alleles were present in resistant malaria parasite [14, 15].

Since, plasmodium has shown resistance against most commonly used antimalarial. Hence, there is need of novel and unique pathway to find new potent antimalarial drug. It is very clear that in all unicellular and multicellular membrane systems, phospholipid bilayers are very important for formation and maintenance of the structure and functionality of the system. Among the important phospholipids phosphatidylcholine (PC) is the most abundant phospholipid found in membranes of bacteria, plants, eukaryotes and even in plasmodium. The aim of review is to focus on importance of SDPM pathway and phosphoethanolamine methyltransferase enzyme for the synthesis of PC for development of membrane throughout the plasmodium life cycle within the host for survival and transmission. The phosphoethanolamine methyltransferase absent in human, catalyzes the trimethylation of phosphoethanolamine a rate limiting step makes it a novel potential drug target to overcome the problem of drug resistance [16, 17].

Significance of PC for Growth and Development

PC is most frequently needed and also an essential eukryotic membrane phospholipid for survival of many organisms such as *Leishamnia major*, bacteria and eukaryotes [18, 19]. PC and phosphatidylethanolamine synthesis occurs mainly by three routes, *de novo* (CDP-choline) and CDP- ethanolamine (kennedy pathway) and CDP-diacylglycrol pathway where CDP-diacylglycerol route is primary route for PC synthesis in

yeast and mammalian hepatocytes [20, 21, 22]. In *de novo* CDP-choline route the choline is the precursor for the synthesis of PC via CDP-choline route. In the CDP-ethanolamine (kennedy pathway), PC and PE both get synthesized from choline and ethanolamine respectively though both CDP-choline and CDP-ethanolamine routes. In CDP-ethanolamine route phosphatidylethanolamine is formed gets trimethylated by phosphatidylethanolamine methytransferase to synthesize the phosphatidylcholine [23].

In bacteria, PC a typical eukaryotic membrane phospholipid is present in only about 10% of all bacterial species in particular in pathogenic bacteria, revealed that PC plays a fundamental role in symbiotic and pathogenic microbe and host interactions [24, 25]. Bacteria, synthesizes PC either through methylation pathway or the phosphatidylcholine synthase pathway or through both the pathways [26, 27]. The methylation pathway involves a three-step methylation of phosphatidylethanolamine by phospholipid *N*-methyltransferase. These biosynthetic pathways have significance on plant–microbe interactions [28, 29]. In Plants, Phosphatidyl-N-methylethanolamine N-methyltransferase (PLMT) enzymes use phosphatidyl mono methyl ethanolamine (PtdMME) and phosphatidyl dimethyl ethanolamine (PtdDME) and are homologous of the rat PLMT [30]. Presence of two different N-methyltransferases was confirmed in spinach for catalyzing methyl transfer to p-ethanolamine to form p-choline [31, 32]. The inhibition of enzymes diminished the growth of *Haemonchus contortus a*nd the gene silencing of HcPMT1 and HcPMT2 implied the essentiality of HcPMT1 and HcPMT2 for the survival of H. contortus [33, 34].

Eukaryotes synthesize PC through two major biosynthetic pathways, phospholipid N-methylation pathway and through CDP-choline pathway and their relative's requirement diverges from organism to organism and even from tissue to tissue within the same organism [35]. The enzymes involved in the CDP-choline biosynthesis in euckryotic and *P. falciparum* are different. Choline, an important nutrient primarily provided from diet is regulated by both the CDP-choline biosynthetic pathway and choline metabolism [35, 36]. Choline is phosphorylated into phosphocholine by choine kinase (CK) which finally gets coverted into PC via CDP–choline [37]. Phosphatidylcholine formed is converted into phosphatidylserine by enzyme phosphatidylserine synthase1 which further gets converted into phosphatidylethanolamine by phosphatidylserine decarboxylase enzyme but the enzyme phosphatidylserine decarboxylase is absent in humans and plants [38, 39].

Plasmodium parasite also requires PC for rapid membrane development important for the survival and multiplication of the parasite within the host [40]. So, the serine-decarboxylase-phosphoethanolamine-methyltransferase (SDPM) unique pathway absent in humans becomes the alternative pathway in *Plasmodium falciparum* for PC biosynthesis. In the SDPM pathway *P. falciparum* synthesizes phosphatidylcholine using host serine [41, 42].

Sdpm Pathway (Kennedy Pathway)

Normally, plasmodium parasite synthesizes PC from choline through CDP-choline route but when the parasite infects host and need to develop membrane at faster rate for growth and development. Availability of PC phospholipid abundantly is must for faster membrane development. Parasite use alternative pathway SDPM to meet. This pathway is unique in the *plasmodium falciparum* which makes it as essential target. In SDPM pathway serine from host blood is utilized for PC synthesis. The *P. falciparum* biosynthesizes PC from serine via CDP-Choline route as demonstrated in **Figure 1**.The serine of host and get converted into ethanolamine with the help of serine decarboxylase (SD) enzyme which is then converted into phosphoethanolamine using ethanolamine kinase (EK). The role of phosphoethanolamine methyltransferase (*Pf*PMT) absent in human host becomes essential since, itcatalyzes the slowest step of the pathway, i.e., the conversion of phosphoethanolamine into phosphocholine through triple methylation of phosphoethanolamine. Once the phosphocholine is formed it is used as substrate for CDP-choline formation. The (CPT) enzyme completes the last step of the pathway by converting the CDP-choline in the phosphatidycholine [43]. The key enzyme of the SDPM pathway found is *Pf*PMT for triple methylation. When the *Pf*PMT was inhibited or knocked out, the fatal growth of *P. falciparum* was observed and also greatly affected the gametocytes formation [44].

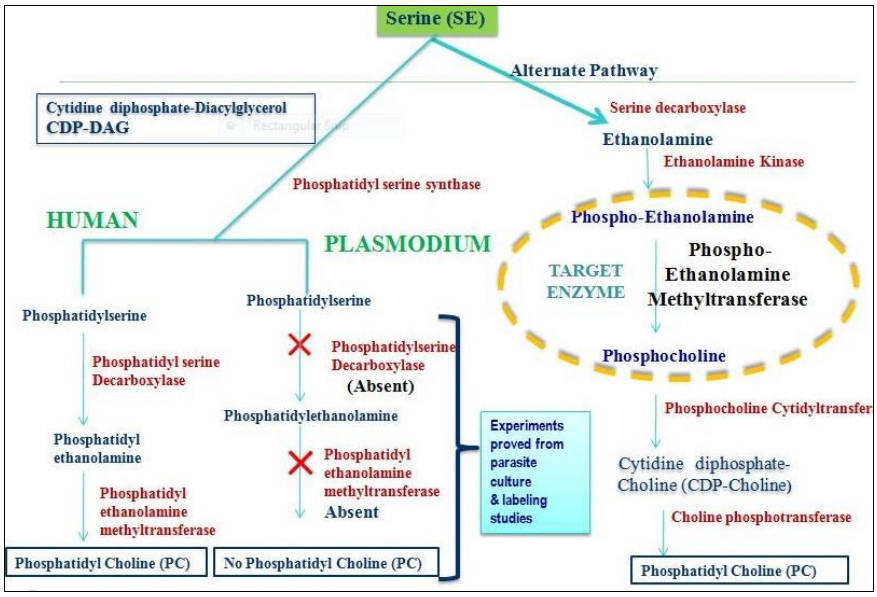


Fig 1: SDPM pathway from serine, different route for phosphatidylcholine biosynthesis in plasmodium via CDP-choline.

Significance of PFPMT in SDPM Pathway PC Synthesis

For the rapid multiplication of the parasite within human erythrocytes it requires an active production of new membranes at very fast rate [44]. PC an important component of membrane is synthesized abundantly from serine decarboxylation pathway during not only intraerythrocytic cycle (with maximum expression at trophozoite stage) but also during gametocyte development cycles of *Plasmodium falciparum.* Hence, the gene is crucial for intraerythrocytic growth as well as malaria transmission. So, the work done on the *Pf*PMT gene has demonstrated the essential role of this gene in the survival and transmission of *P. falciparum* parasite [44]. The knock out study also revealed that when the *Pf*PMT was disrupted the PC biosynthesis from serine was completely abrogated and caused serious defects in survival and multiplication of *plasmodium falciparum.* Loss of *Pf*PMT severely distorted the asexual replication and gametocyte development which revealed that *Pf*PMT plays crucial role in intraerythrocytic asexual replication and gametocyte development. Gametocytes were not produced even when the choline was present but when *Pf*PMT was reintroduced in knockout, *gametocyte* differentiation was again restored to wild type level. The expression of *Pf*PMT throughout the intraerythrocytic cycle as well as gametocyte development is very important for survival and transmission of parasite that makes it an excellent target for antimalarial drug designing and moreover absence of *phosphoethanolamine methyltransferse* in human makes this enzyme an excellent drug target for development of new antimalarial [45, 46, 47].

Phosphatidylserine decarboxyase enzyme is absent in both the *P. falciparum and* plants. *Pf*PMT and plant methyltransferase *(*PEAMT) are homologues but plants PEAMT have two catalytic domains [43, 48]. *Pf*PMT has one catalytic domain for three methylation steps this implies that same catalytic domain is used for all three methylations. Hence, the inhibition of catalytic domain of *Pf*PMT can lead to complete inhibition of SDPM pathway. Therefore, *Pf*PMT is an essential enzyme for survival of *Plasmodium falciparum* moreover absence of phosphoethanolamine methyltransferase in human makes this enzyme an excellent drug target for development of new antimalarial. Since, *Pf*PMT is the only enzyme in SDPM pathway which catalyzes the triple methylation of phosphoethanolamine. Hence it represents a potential target for the rational design of chemotherapeutic agents to treat malaria.

Enzyme Kinetics of *PF*PMT

An SAM-dependent triple Methylation coupling assay system for *Pf*PMT activity using of two enzymes SAH nucleosidase (SAHN) and adenine deaminase (BsAda) was developed. As SAH and phosphocholine are products of *Pf*PMT mediated activity using phosphoethanolamine and SAM as substrate and methyl donor respectively. So, enzyme SAHN added is responsible for the conversion of SAH into adenine and S-ribosylhomocysteine. Where, BsAda converts adenine into hypoxanthine showed absorption at 265 nm decreases [49].

Activity assay was done spectrophotometrically by coupling the formation of S-adenosyl-L-homocysteine to the reaction catalyzed by *Pv*PMTand S-adenosyl L-homomethionine in the presence of phosphoethanolamine. Assay was performed in 96-well UV- transparent plates (acrylic, non-sterile, costar) at 37 ^{0}C. Reaction mixtures were prepared by adding 1 nM $MnSO_4$, 1X HEPES assay buffer (100mM HEPES-KOH, pH 7.5), 200 µM SAM, 200 µM phosphoethanolamine, 0.5 µM BsAda, 4.72 µM SAHN. And mixture was first incubated at 37°C for 10 min and then reaction was started by addition of 2.5 µM *Pf*PMT in a total volume of 200 µl. Absorbance at 265 nm was continuously recorded. Activity was determined from the slope of the linear part of the curve obtained by plotting decrements in absorbance at 265 nm with time (d*A*/d*t*) [49].

When concentrations of the P-EA and SAM were increased *Pf*PMT activity was increased proportionally and activity was optimal at 200 µM. Concentration more than 200 µM, activity of *Pf*PMT was slightly affected by increasing conc. of P-EA whereas activity was inhibited on increasing conc. more than 200 µM. There a proportional increase in activity was noticed on increasing the concentration of *Pf*PMT and decrease in absorbance over time was also reproduced. Above a particular range, the activity of *Pf*PMT enzyme was noticed to deviate from Linearity. The little effect of different buffers at pH 7, 7.5 and 8 on *Pf*PMT activity was found over time [49].

Structural Aspects and Inhibitor Study of *PF*PMT

According to the structural comparison and binding mode, no variation found in position of amino acids interacts with AdoMet, AdoCys and AdoMet analog sinefugin within the *Pf*PMT-AdoMet binding site as shown in **Figure 2**. Critical interaction of AdoMet with Asp-85 and Ile-86 residues within the AdoMet site since site directed mutation of either residue (D85N/D85A or I86F/I86A) greatly affected the binding of AdoMet and abrogated activity [50].

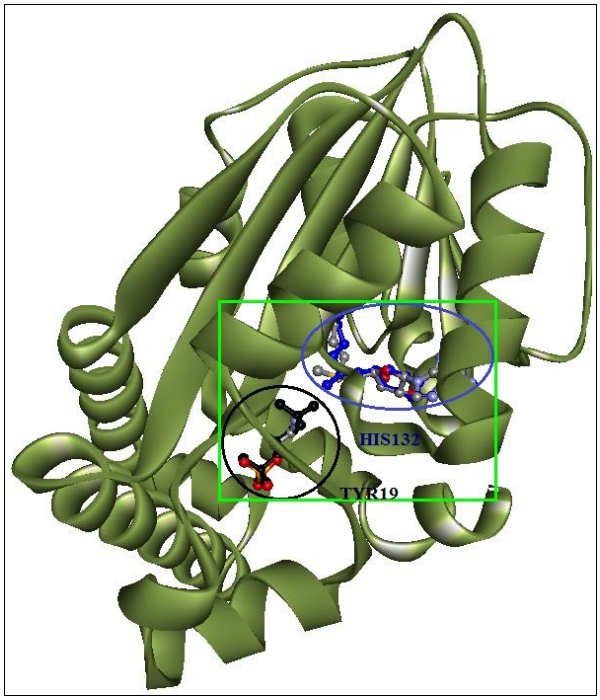


Fig 2: Model showing active pocket of *Pf*PMT protein for conversion of phosphoethanolamine into phosphocholine. Binding of SAM (Element color) and sinefugin (Blue color) in ball and stick view within the binding pocket of crystal structure. Binding mode of Phosphoethanolamine (element color) Phosphocholine (black color) and within the crystal structures

Since Crystal structures of *Pf*PMT have been solved in complex with phosphoethanolamine, phosphocholine, AdoMet, and AdoCys_pEA and are consistent with this kinetic mechanism explains the way how *Pf*PMT can be binding with substrate as well as inhibitors. Phosphocholine and Phosphoethanolamine bind in a similar manner as shown in Figure 3. According to the functional characterization of site- directed mutant studies, the conformations of active site of *Pf*PMTchange as the conformations of Tyr-27, Phe-31, and Arg-179 change for

catalysis. Tyr-19 and His-132 are positioned between the AdoMet and phosphobase and works as catalytic dyad in the transfer of methyl group. But the major conformational change occurs in Arg-179 in active site on binding of either phosphoethanolamine or Phosphocholine. In case of PO_4^{-2}binding, this residue found to get shift away from the active site [50].

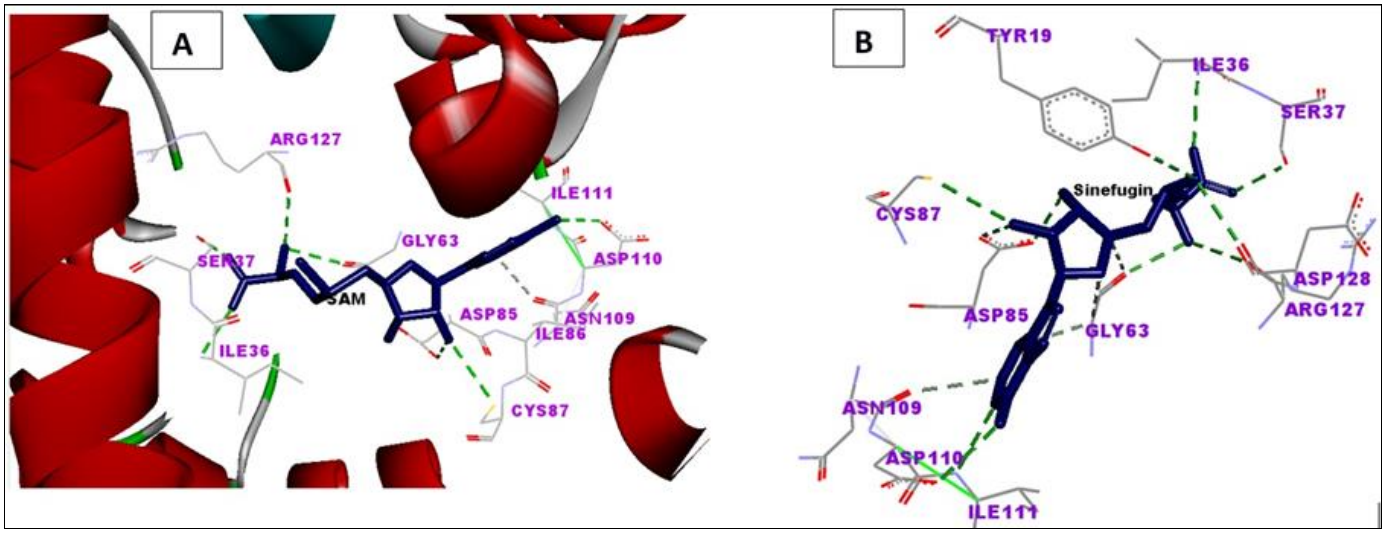


Fig 3: Binding mode of (A) SAM (Blue color) and (B) sinefugin (Blue color) with their interacting amino acids within the same binding pocket of crystal structures PDB: 3UJ6 and 3UJ8 respectively. Hydrogen bonds are in green dashed lines [50]

According to the Mutant studies of residues in the phosphobase site it was found that the Arg-179 and Lys-247 are required for activity as the alanine and methionine mutants of Lys247 were found inactive. But Alanine and lysine mutants of arginine179 showed activity between 1and 43%, respectively. It was also confirmed that each tyrosine (Tyr-27, Tyr-160, Tyr-175, and Tyr-181) residues within the binding site were also crucial for *Pf*PMT activity. As Methylation of pEA was greatly disrupted when either hydroxyl or phenol moieties were removed [50] shown in Figure 4.

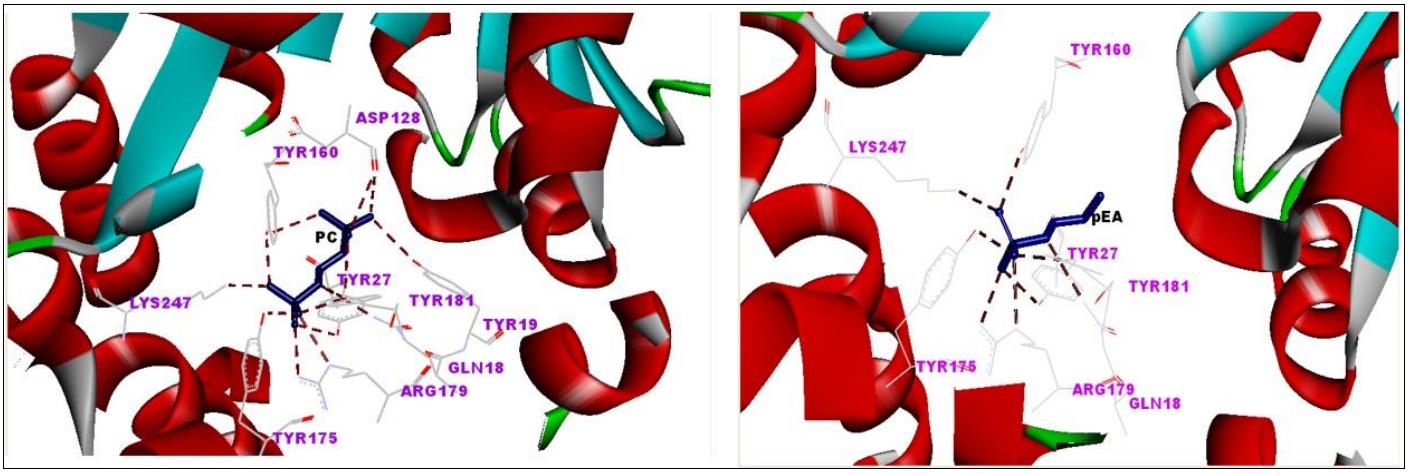


Fig 4: Binding mode of Phosphocholine (PC) and Phosphoethanolamine (pEA) within the crystal structures PDB: 3UJ9 and PDB: 3UJA respectively

Crystal structure of phosphoethanolamine methyltransferase has also been solved in *plasmodium vivax (Pv*PMT) and *knowlsi (Pk*PMT). The general structural folds of *Pv*PMT as well as *Pk*PMT were found very similar to those folds of already solved crystal structure of *Pf*PMT with all atom RMSD 0.452 A˚. Structural comparison *Pv*PMT and *Pk*PMT with *Pf*PMT showed that the secondary structural elements where are almost similar except some local changes. The general structure of *Pk*PMT is almost identical to that of *Pv*PMT and *Pf*PMT with an all-atom alignment RMSD of 0.80 A. The active site residues are well conserved amongst enzymes from *P. falciparum, P. vivax* and *P. knowlesi*. Hence Phosphoethanolamine methyltransferase enzyme may be the important drug target as inhibitor of this enzyme can inhibit PMTs of *P. vivax* and *knowlsi* [51]. Few countable compounds such as sinefugin (AdoMet analog), miltefosine (Choline analog) amodiaquine (4-aminoquinoline) as given in Table.1 had been identified to inhibit the PMTs of Plasmodium and nematode [49, 52].

Crystal structure of phosphoethanolamine methyltransferase has also been solved in *plasmodium vivax (Pv* PMT) and *knowlsi (Pk* PMT). The general structural folds of *Pv*PMT as well as *Pk*PMT were found very similar to those folds of already solved crystal structure of *Pf* PMT with all atom RMSD 0.452 A˚. Structural comparison *Pv* PMT and *Pk*PMT with *Pf* PMT showed that the secondary structural elements where are almost similar except some local changes. The general structure of *Pk*PMT is almost identical to that of *Pv* PMT and *Pf*PMT with an all-atom alignment RMSD of 0.80 A. The active site residues are well conserved amongst enzymes from *P. falciparum, P. vivax* and *P. knowlesi*. Hence Phosphoethanolamine methyltransferase enzyme may be the important drug target as inhibitor of this enzyme can inhibit PMTs of *P. vivax* and

knowlsi. Few countable compounds such as sinefugin (AdoMet analog), miltefosine (Choline analog) amodiaquine (4-aminoquinoline) as given in Table. 1 had been identified to inhibit the PMTs of Plasmodium and nematode [51, 52].

Miltefosine and its analogs had been validated for antitumor and antileishmanial activities. Miltefosine have been previously identified as *Pf*PMT inhibitor in vitro with a radioactivity-based assay. Miltefosine inhibited *Pf*PMT by 60% at 100 uM but at 150 uM the activity of *Pf*PMT was completely lost. Hexadecyltrimethylammonium bromide (HDTA) was also found as an inhibitor of *Pf*PMT. Inhibitory (app. 50% at 100 uM) activity of was found similar to mitefosine and HDTA inhibited the *plasmodium falciparum* parasite in culture with an IC50 of 2.1 μM [53]. Dodecyltrimethylammonium (DDTA) a potent antimalarial activity had shown inhibitory activity for *Pf*PMT [54, 55]. Besides the antimalarial activity, amodiaquine is a potent inhibitor of the human histamine N-methyltransferase (HNMT) [56]. But no other potent HNMT inhibitors such as SKF91488, tacrine diphenhydramine and chlorpromazine [57, 58] were found not to inhibit *Pf*PMT. It was observed that aminoquinolines and amino alcohols such as quinacrine, quinidine and quinine had no *Pf*PMT inhibitory activity at concentrations up to 200 μM. Chloroquine and primaquine were also found to inhibit *Pf*PMT in millimolar.

Table 1: Compounds tested as inhibitors of *Pf*PMT

S. No.	Inhibitors_*Pf*PMT	Chemical Formula	Smiles notation
1.	Sinefungin(Adomet analog)	$C_{15}H_{23}N_7O_5$	C1=NC2=C(C(=N1)N)N=CN2C3C(C (C(O3)CC(CCC(C(=O)O)N)N)O)O
2.	Miltefosin(pCholine analog)	$C_{21}H_{46}NO_4P$	[O]P(=O)(OCCCCCCCCCCCCCCCC)OCC[N+](C)(C)C
3.	Amodiaquine(4-aminoquinoline)	$C_{20}H_{22}ClN_3O$	CCN(CC)CC1=C(O)C=CC(NC2=C3C=CC(Cl)=CC3=NC=C2)=C1
4.	Primaquine	$C_{15}H_{21}N_3O$	COC1=CC(NC(C)CCCN)=C2N=CC=CC2=C1
5.	Chloroquine	$C_{18}H_{26}ClN_3$	CCN(CC)CCCC(C)NC1=C2C=CC(Cl)=CC2=NC=C1

3161 Compounds from National Cancer Institute Open Chemical Repository were screened based on *Pf*PMT-specific enzyme-coupled spectrophotometric methylation assay. 28 compound inhibitors of *Pf*PMT as given in Table. 2 were identified and out of these 11 compounds (highlighted) inhibited both *Pf*PMT and gametocytostatic activity. Untreated parasites or the artemisinin treated *NF54-Pfs16-GFPLUC* strain which is known to block both infection and transmission was used as control**.** Compounds NSC-641296, NSC-668394, NSC-323241, and NSC-158011 were found to have gametocytocidal activity. Compounds NSC-641296 and NSC-668394 inhibited early and late gametocyte stages. Compounds NSC-323241 and NSC-158011 found effective against early-stage gametocyte development. Drug like properties were found in compound NSC-158011 it was tested for its specificity for *Pf*PMT activity. Based on kinetics studies using a nonlinear fit to the Michaelis–Menten mixed model of inhibition NSC-158011 was found competitive inhibitor of Phosphoethanolamine. The specificity of inhibition of *Pf*PMT was further demonstrated using a yeast mutant, which relies on *Pf*PMT for survival. PMT-like two phosphatidylethanolamine methyltransferases, PEM1 and PEM2 mutant (pem1Δpem2Δ) study resulted in choline auxotrophy. The growth of thepem1Δpem2Δ mutant expressing *Pf*PMT was severely affected in the presence of NSC-158011 compound. Quantitative analyses showed that the in the Mutant strain expressing *Pf*PMT were treated with NSC-158011 decrease in lipid index (PtdCho/PtdIns) by 52% was noticed in compared to the control.

Table 2: Identified inhibitors for *Pf*PMT

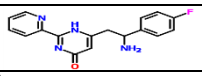
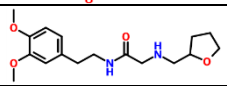
S. No	NSC#	Name/Molecular Farmula	Activity
1.	85459	3,6-Bis(morpholinomethyl)pyrocatechol Pyrocatechol, 3,6-bis(morpholinomethyl)- 1,2-Benzenediol, 3,6-bis (4-morpholinylmethyl)-	Inhibited *Pf*PMT activity
2.	7346	Disodium cupric EDTA	
3.	47924	C18H17NO2	
4.	46613	C13H13N5	
5.	113997	C14H10O4S2	
6.	371777	C15H14N2O4S	
7.	88947	C17H13NO8S2.Na	
8.	57998	C13H19NS.C7H4ClNO3	
9.	37031	C20H14N2O7S2	
10.	88947	C17H13NO8S2.Na	
11.	169942	C16H28Cu2N8O8	
12.	622648	C8H13N2S3.ClO4	
13.	175493	Iron, carbonyl(.eta.(5)-2, 4-cyclopentadien-1-yl) (dimethylcarbamodithioato-S, S')-	
14.	173904	Carbamic acid, [1-[3-chloro-2-oxo-1-(phenylmethyl) propyl]amino]carbonyl]-3-methylbutyl]-, phenylmethyl ester	
15.	638646	4-Piperidinone, 3, 5-bis[(3, 4-dichlorophenyl)methylene]- 1-[3-(4-morpholinyl)-1-oxopropyl]-, monohydrochloride	
16.	24048	C21H18N2O.HI	
17.	632233	C16H15NO2S	
18.	348401	4-(6-thioquanine)-7-nitro-2,1,3-benzoxadiazole	Inhibited *Pf*PMT activity, Gameto cytocidal activity
19.	668394	5,8-Quinolinedione, 7-[[2-(3,5-diobromo-4-hydroxyphenyl)ethyl]amino]-	
20.	310551	Cooper;[(6-methylpyridin-2-yl)methylideneamino]-(methylsulfanyl-sulfoniumylidene-mthyl)azanide	
21.	22225	C9H4CdCl6O4	
22.	39225	C12H20N2O2	
23.	150080	C12H17N3O3	
24.	158011	C18H15NS2 (Competitive inhibitor of p-Eth)	
25.	125034	2, 2'-disulfanediyldiquinolin-8-ol	

26.	109268	Copper, di.mu.-chloribis[1-[(1-piperdinyl-.kappa.N)methyl]-2-napthalenolato-.kappa.O]di-	
27.	323241	3-Azabicyclo [3.2.2.]nonane-3-carboselenoic acid, [1-(2-pyridinyl)ethylidene]hydrazide	
26.	641296	Hydrazinecarbothioamide, N,N-dipropyl-2-(2-pyridinemethylene)-, (N, N, S)-copper(II) chloride complex, (SP-4-3)-	

The two compounds ZINC12882412 and ZINC02103914 showed the good schozonticidal activity at µM concentration 3.0 µM and 2.1 µM respectively, that might be reason for the better docking affinity towards *Pf*PMT occupying the crucial catalytic dyad between Tyr19 and His132 to prevent its biological function. These compounds may be used for structure-based drug development against multi drug resistance [59].

The three compounds from Asinex database were also identified through computer aided drug designing approaches as potent inhibitors for the *Pf*PMT with better ADMET and the docking affinity. The identified inhibitors showed the better activity against schizonts and gametocytes of *Plasmodium falciparum*. Two inhibitors ASN.1 and ASN.3 inhibited the *Pf*PMT competitively at IC_{50} 1.49µM and 2.31µM respectively along with the promising reduction of parasite growth in mice (orally and intravenous). These compounds may be used for structure-based drug developmentagainst multi drug resistance [60] Table. 3.

Table 3: *Pf*PMT inhibitors identified based on CADD and *in-vitro* analysis

Comp. Id	Structure	IC_{50}Schizonticidal	IC_{50}Gametocidal	LD_{50}	*Pf*PMT-EC_{50}
ASN.1		1.77µM	3.1µM	191.0 µM	1.49 µM
ASN.3		3.21µM	3.8 µM	176.2 µM	2.31 µM

Future Prospective

The review will emphasize the current scenario of urges the elucidation of novel pathways either absent in human or non-homologous of human. The unique and alternate SDPM pathway alike plants in plasmodium essential for the progression and rapid membrane generation; late stages and gametocyte development in intra erythrocytic forms of malaria offers an attractive molecular drug target for the development of novel inhibitor-based antimalarial therapies. The rate limiting step, conversion of phosphoethanolamine into phosphocholine by plasmodium phospho-ethanolamine methyl transferase (PMT) plays an important role in membrane development for growth and transmission of parasite met the urge of novel drug target for antimalarial development. Since, SDPM and PMT is absent or different from methyltransferases found in humans, a better understanding of molecular and Structural analysis of PMT enzyme in plasmodium may lead to the development of a novel class of antimalarial inhibitor compounds to be used as drugs using rational drug designing. Inhibitor library of compounds designed and identified by *in silico* structural modeling and *in-vitro* studies to inhibit target PMT enzyme may provide novel broad-spectrum antimalarials. The PMTs of plasmodium have conserved domain and shares significant structural identity which suggest that the common PMT inhibitor as antimalarial may be development. In this review the importance of the SDPM pathway in the development and transmission of parasite has been explored along with the inhibition portfolio of *Pf*PMT inhibitors till date will not only advance our knowledge about SDPM and PMT among plasmodium but may also provide the structural lead for the computer aided drug designing.

Conflict of Interest

Author Declare no conflict of interest

Authors' contributions

JS – Developed the manuscript, ASand MK- making and analysis of scientific content, RM and PKA- assisted in making and formatting of manuscript. The final Manuscript was read and approved by all the authors.

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Competing interests

Authors declare no conflicts of interest

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