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Docking studies and development of novel 5-heteroarylamino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines as potential antimalarials

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Abstract—MOE-Dock (Docking software) was used to predict the binding modes of 10 novel and potent 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines (compounds **I–X**) as part of our antimalarial drug development programme. This was done by analyzing the interaction of these compounds with the active sites of 11 enzymes present in *Plasmodium falciparum* and based on this, effective binding was observed to enzyme *P. falciparum* glutathione reductase (*Pf*GR). The binding scores for compounds **I–X** with *Pf*GR were also congruent with their antimalarial activity. Three additional analogs were then designed and synthesized based on the above docking study and the pharmacophoric requirements for this class.

Malaria is a serious health problem and according to the recent report of WHO, there are one million deaths and 500 million new cases due to malaria annually, predominantly attributed to *Plasmodium falciparum*.[1] Moreover, drug resistance is a serious problem in malaria and it can be attributed to the use of single drug (monotherapy) for treatment and to the adaptation of the malarial parasite by developing alternate pathways for survival. Hence, the present strategy for new drug development is directed towards identifying essential enzyme systems in the parasite and developing potent molecules to inhibit them.

In our previous publication,[2] we had presented the development of a novel series of 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines based on pharmacophoric studies and six out of ten compounds were found to be active when evaluated in vivo in mice by Rane's test (Table 1).

In this report, we present the extension of the work on the above series of compounds wherein we carried out docking studies with 11 enzymes reported to be present in *P. falciparum*. Based on the results of this study we developed additional three novel compounds.

The crystal structures of 11 enzymes from *P. falciparum* were obtained from the Protein Data Bank.[3] The enzymes were Dihydrofolate reductase-thymidylate synthase (DH-TS) [1J3I], *P. falciparum* antioxidant protein (*Pf*AOP) [1XIY], glutathione reductase (*Pf*GR) [1ONF], lactate dehydrogenase (LDH) [1LDG], *S*-adenosyl-L-homocysteine hydrolase (*Pf*SAHH) [1V8B], malarial purine phosphoribosyltransferase (HGPRT) [1CJB], plasmepsin II (PlasII) [1SME], proplasmepsin II (ProplasII) [1PFZ], *P. falciparum* protein kinase 5 (*Pf*PK5) [1OB3], glutamate dehydrogenase (GDH) [2BMA] and *Plasmodium falciparum* peptide deformylase (*Pf*PDF) [1RQC]. The PDB code of each is mentioned in square brackets. All the enzyme structures were checked for missing atoms, bonds and contacts. Hydrogens were added to the enzyme structures. Water molecules and bound ligands were manually deleted. All the computations were carried out on a Pentium 1.6 GHz workstation, 512 MB memory with Windows operating system and Molecular Operating Environment (MOE 2003.02)[4] as the computational software.

The above operation was repeated for each identified enzyme and then the active site was generated

Keywords: MOE-Dock; Pyrimidoquinolines; *P. falciparum* glutathione reductase.
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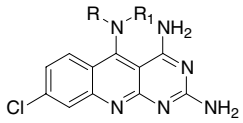
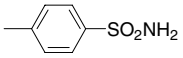
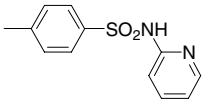
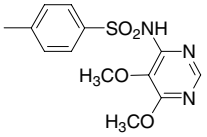
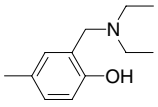
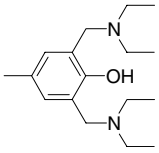
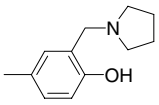
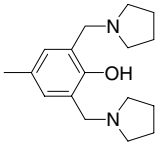
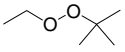
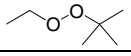
Table 1. Activity profile of the novel substituted pyrimidoquinolines



Compound	R	R_1	Remarks
I	H	H	Active (at 160 mg/kg)
II		H	Inactive
III		H	Inactive
IV		H	Curative (at 20 mg/kg and above)
V		H	Inactive
VI		H	Curative (at 20 mg/kg and above)
VII		H	Active (at 80 mg/kg and above)
VIII		H	Curative (at 20 mg/kg and above)
IX	$-CH_3$		Active (at 20 and 40 mg/kg)
X	$-C_2H_5$		Inactive

for each enzyme using MOE-Alpha Site Finder. MOE-Dock Box was generated around the active site of each enzyme. The dimensions of the docking box were manipulated so as to accommodate all the amino acid residues present in the active site. After construction of a docking box, the least energy conformers of each of compounds **I**–**X** were docked in the active site. The energy scores of enzyme–ligand complexes (U_{total} in kcal/mol) were recorded. Along with the U_{total} scores, two additional parameters (Predicted pK_i and Andrews pK_i) were measured. Predicted pK_i measures how efficiently and completely the ligand utilizes the space provided to it for binding, while Andrews pK_i is a measure of the total space provided by the protein for ligand binding.[5] Additionally, the ability of the ligand to bind to the active site at 100 nM concentration was measured.[6]

The docking energy scores (U_{total}) indicated better interaction between compounds **I**–**X** and enzymes DH-TS, *Pf*GR and HGPRT (low values, shown in bold face) as compared to binding with other enzymes (high values) (Table 2).

Comparison of predicted pK_i values with Andrews pK_i values (Table 3) showed enhanced occupancy of binding space with *Pf*GR enzyme as compared to DH-TS and HGPRT. This was also evident from the higher pK_i H-bond values obtained with *Pf*GR enzyme as compared to DH-TS and HGPRT (Table 4). Further, the more potent compounds **IV**, **VI** and **VIII** returned a

Table 2. Docking energies (U_{total}) of various enzyme–ligand complexes

Enzyme	Total MOE-Dock energy (U_{total} in kcal/mol) for compounds **I–X**									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
DH-TS	**178.5**	**305.8**	**300.4**	**47.1**	**150.6**	**75.9**	**86.1**	**79.6**	**210.3**	**248.3**
*Pf*AOP	522.1	418.6	1523.1	2556.6	1819.2	56665.2	2653.8	60124.4	4191.2	4653.2
***Pf*GR**	**162.2**	**205.9**	**355.9**	**38.1**	**270.8**	**55.7**	**196.3**	**43.2**	**310.6**	**360.2**
LDH	1123.2	512.2	1258.3	1026.5	1630.1	1010.3	1475.3	1089.3	1256.3	1289.9
*Pf*SAHH	987.6	845.2	965.2	860.5	1013.6	842.3	956.5	812.2	1320.1	1428.3
HGPRT	**89.6**	**85.4**	**98.6**	**84.3**	**91.5**	**115.0**	**100.6**	**80.6**	**81.2**	**82.1**
PlasII	175.6	182.1	560.5	485.2	456.8	491.6	302.5	466.9	261.5	298.9
ProplasII	121.9	125.6	177.2	101.9	151.8	160.8	152.0	125.1	140.2	159.2
*Pf*PK5	291.7	260.6	325.2	320.7	347.4	300.9	354.8	295.4	292.5	308.2
GDH	546.3	718.6	830.2	856.4	811.8	902.6	867.8	891.4	720.5	792.6
*Pf*PDF	1123.2	1020.4	1167.6	1424.3	1323.6	1265.9	1416.2	1532.1	1019.6	1086.2

Table 3. Predicted pK_i versus Andrews pK_i of compounds **I–X**

Enzyme	Predicted pK_i versus Andrews pK_i of test compounds **I–X**									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
DH-TS	4.0/7.6	7.2/7.6	6.4/7.3	**5.0/7.6**	6.4/7.6	**5.9/7.0**	7.7/8.3	**6.0/7.7**	3.9/7.9	3.2/7.9
*Pf*GR	5.6/8.3	4.2/8.0	3.8/6.4	**7.2/8.6**	4.9/8.3	**7.9/8.9**	7.1/8.3	**7.5/8.9**	2.6/8.1	2.1/8.1
HGPRT	2.4/5.4	2.5/5.1	2.3/5.4	**3.3/6.5**	2.9/5.7	**2.5/7.5**	2.3/6.7	**2.2/7.5**	2.5/6.5	2.1/6.5

Table 4. pK_i H-bonds of test compounds **I–X** and dock_100 nM values

Enzyme	pK_i H-bonds of test compounds **I–X** and dock_100 nM values									
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
DH-TS	1.6 [0]	1.5 [0]	1.7 [0]	**1.4** [0]	1.5 [0]	—[a]	1.5 [0]	**1.5** [0]	1.2 [0]	0.6 [0]
*Pf*GR	1.4 [0]	2.1 [0]	2.3 [0]	**2.8** [**1**]	2.3 [0]	**2.6** [**1**]	3.0 [0]	**2.7** [**1**]	2.1 [0]	1.5 [0]
HGPRT	0.1 [0]	1.2 [0]	—[a]	**0.6** [0]	1.1 [0]	**0.4** [0]	—[a]	**0.6** [0]	0.5 [0]	0.2 [0]

[a] No interaction through H-bonding. '0' in square brackets indicates binding above 100 nM and '1' indicates binding up to 100 nM.

value of '1' when binding up to 100 nM was considered as compared to '0' obtained with less active/inactive compounds (Table 4). A representative binding mode of compound **IV** with the active site of *Pf*GR is given in Figure 1.

Assuming that the novel 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines act as antimalarials by binding with enzyme *Pf*GR, additional three novel analogues (compounds **XI–XIII**) with heterocyclic substituents on the 5-amino position (Table 5) were designed based on docking studies with *Pf*GR and the pharmacophoric requirements for this class. The data of docking studies for compounds **XI–XIII** with *Pf*GR are reported in Table 6.

Synthesis of compounds **XI–XIII** is outlined in Scheme 1. Reaction of *o*-phenylenediamine **1** with glacial acetic acid in HCl led to the formation of 2-methylbenzimidazole **2** (mp 176 °C), which on bromination with NBS in CCl_4 led to the formation of 2-bromomethylbenzimidazole **3** (mp 191 °C). This was then condensed with 2,4,5-triamino-8-chloropyrimido-[4,5-*b*]quinoline **4** in the presence of anhydrous DMF and potassium carbonate to yield compound **XI** (mp 212 °C). Similarly, reaction of **1** with aqueous sodium nitrite solution in glacial acetic acid led to the synthesis of benzotriazole **5** (mp 99 °C), which was then refluxed with dichloromethane in the presence of sodium hydride and anhydrous DMF to yield N^1-chloromethylbenzotriazole **6** (mp 131–132 °C), which was then condensed with **4** in the presence of anhydrous DMF and potassium carbonate to yield compound **XII** (mp 172 °C). Reaction of thiourea **7** with malononitrile **8** in the presence of sodium ethoxide and anhydrous ethanol gave 4,6-diamino-2-mercaptopyrimidine **9** (mp 310 °C, dec), which on selective methylation at 0 °C for 3 h using methyl iodide in sodium hydroxide solution led to 4,6-diamino-2-methylthiopyrimidine **10** (mp 181 °C). Compound **10** was then condensed with **4** in the presence of anhydrous DMF to yield compound **XIII** (mp 226 °C).

The progress of all the reactions was monitored by thin layer chromatography (TLC). All the compounds were purified by silica gel column chromatography and then recrystallized. The spectral characteristics of the compounds **XI–XIII** are mentioned in Table 5.

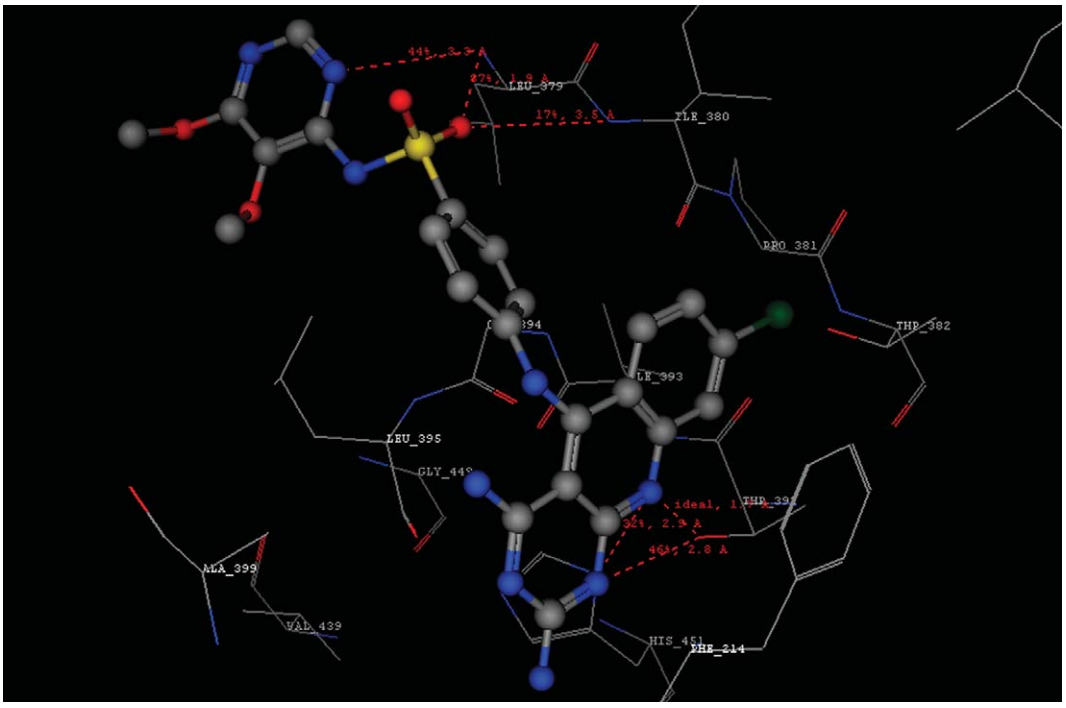


Figure 1. Binding mode of compound **IV** with *Pf*GR.

Table 5. Structures and spectral data of compounds **XI**–**XIII**

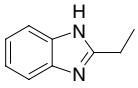
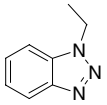
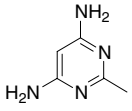
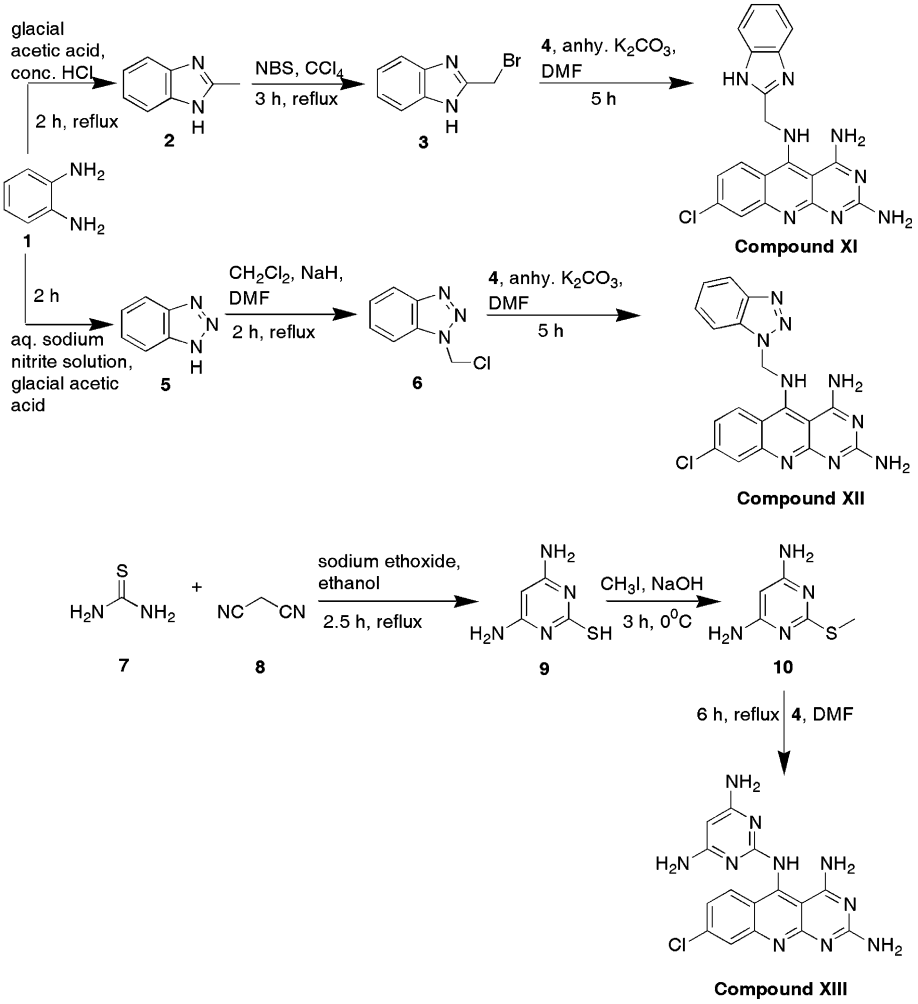
Compound	R	Yield (%)	FT-IR (cm^{-1}) and ^1H NMR (δ in ppm)
XI		63	FT-IR (KBr): 3400 (N–H stretch, amine), 3120 (C–H stretch, aromatic), 2920, 2851 (C–H stretch, alkane), 761 (C–Cl stretch) ^1H NMR (DMSO-d_6): δ 2.5 (s, 2H, –NH), 3.3 (br s, 4H, 2 $-NH_2$), 4.0 (s, 2H, $-CH_2-$), 7.1–7.3 (m, 4H, Ar-H), 7.4–7.6 (m, 3H, Ar-H)
XII		66	FT-IR (KBr): 3321 (N–H stretch, amine), 3003 (C–H stretch, aromatic), 2924 (C–H stretch, alkane), 761 (C–Cl stretch) ^1H NMR (DMSO-d_6): δ 2.5 (s, 1H, –NH), 3.2 (br s, 4H, 2 $-NH_2$), 4.8 (s, 2H, $-CH_2-$), 7.3–7.5 (m, 4H, Ar-H), 7.6–7.8 (m, 3H, Ar-H)
XIII		69	FT-IR (KBr): 3320 (N–H stretch, amine), 3096 (C–H stretch, aromatic), 1645, 1591 (N–H bend, amine), 1261 (C–N vibrations, amine), 763 (C–Cl stretch) ^1H NMR (DMSO-d_6): δ 2.7 (s, 1H, –NH), 3.4 (br s, 8H, 4 $-NH_2$), 7.1–7.3 (m, 3H, Ar-H), 8.0 (s, 1H, Ar-H)

Table 6. Docking studies of compounds **XI**–**XIII** with *Pf*GR

Scores	XI	XII	XIII
Energy, U_{total} (kcal/mol)	39.9	41.9	81
Predicted pK_i versus Andrews pK_i	5.9/6.0	6.1/6.4	5.1/6.5
pK_i H-bonds and dock_100 nM affinity	2.3 [1]	2.6 [1]	3.2 [0]

During the halogenation of **2**, NBS gave better yields as compared to the use of bromine solution or sulfuryl chloride. Moreover, the workup procedure was less tedious and the purification procedure was simple when NBS was used. Longer reaction time was required during the synthesis of **6** when sodium hydroxide or potassium carbonate was used as bases instead of sodium hydride.

In conclusion, it can be stated that docking studies helped in identifying the enzyme (*Pf*GR) as the target for binding for the novel and potent substituted pyrimidoquinolines. This led to the development of three additional novel 5-heteroarylamino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinolines.



Scheme 1. Synthesis of compounds **XI–XIII**.

Acknowledgment

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