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ARTICLE TYPE

Synthesis, antimalarial activity, heme binding and docking studies of 4-aminoquinoline-pyrimidine based molecular hybrids

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A series of novel 4-aminoquinoline-pyrimidine hybrids was synthesized and evaluated for their antimalarial activity. Several compounds showed potent antimalarial activity against both CQ-sensitive and CQ-resistant strains of *P. falciparum* with no cytotoxicity against Vero cell lines. Selected compound **7f**, when evaluated for *in vivo* activity showed mild suppression of parasite in *P. berghei*-mouse malaria model. The heme binding studies were conducted to determine the probable mode of action of these hybrids. Compound **8d** formed a stable 1:1 complex with hematin suggesting that these hybrids act on a heme polymerization target. The binding of most active hybrids were studied by molecular docking analysis in the active site of Pf-DHFR-TS. The top scored compounds having low binding energy interact in the active site of Pf-DHFR-TS similar to the protein natural substrate dihydrofolate. The pharmacokinetics properties of best active compounds were also assessed using ADMET prediction.

Introduction

Despite the long efforts in eradicating the malaria, the disease continues to present a major public health challenge especially in tropical and subtropical areas affecting millions of people.¹ According to 2013 WHO report, there were 207 million cases and an estimated 627000 deaths due to malaria worldwide.² The African subcontinent has the highest disease burden with more than 75% related deaths in pregnant women and children under the age of five.³ The disease is transmitted by female mosquitoes and is caused by five different species of the protozoan *Plasmodium* parasite, namely, *falciparum*, *vivax*, *malariae*, *ovale* and *knowlesi*.⁴ Amongst these *P. falciparum* is the most prevalent species and accounts for more than 95% of clinical cases and deaths due to malaria. Malaria in most instances is a curable disease if it is diagnosed in time and treated with proper medicines. However, the rapid development of drug resistance has compromised the use of conventional antimalarial drugs such as chloroquine, amodiaquine, pamaquine, mefloquine and pyrimethamine (Fig. 1).⁵ Moreover, there is no commercially available malaria vaccine developed despite the intensive efforts of scientist working in this field. Though, recently a vaccine candidate, RTS, S/AS01, developed by GlaxoSmithKline pharmaceuticals is being evaluated in Phase III clinical trials providing only modest protection against both clinical and severe malaria in young infant.^{6,7} Therefore chemotherapy remains the mainstay for dealing with this enormous problem. The growing resistance and the lack of an effective antimalarial vaccine emphasize the need to develop a novel, safe, affordable antimalarial drug effective against multi drug-resistant malaria. Currently, the best option for the treatment of uncomplicated malaria is artemisinin-based combination therapies (ACTs) which includes rapidly acting artemisinin or its analogues such as

dihydroartemisinin, artemether, artesunate along with a partner drug such as lumefantrine, mefloquine, amodiaquine, piperazine, sulfadoxine, pyrimethamine, dapsone etc.² Although ACTs are fast acting, highly effective and reduce the chances of resistance development,⁸ few cases of artemisinin resistance have recently been reported in South-East Asia.^{9,10} Hence the development of new antimalarial agents is urgently needed to counter the ever-increasing spread of drug resistant malaria.

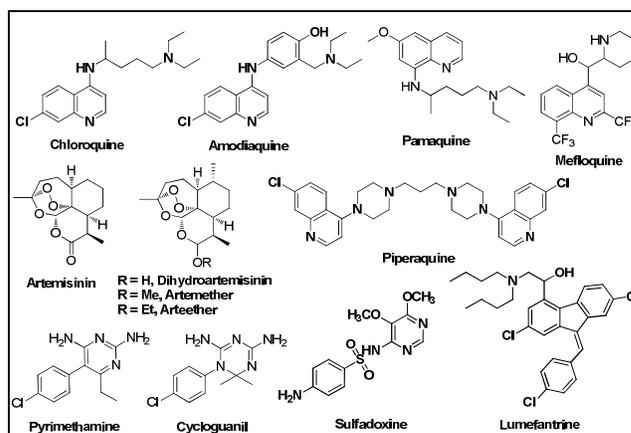


Fig. 1 Antimalarial drugs

In order to search a new drug, various approaches are being engaged of which the molecular hybridization is quite an attractive strategy which involves designing of new chemical entities by covalent linking of two pharmacophore units derived from the known biologically active molecules with complimentary activities and multiple pharmacological targets.¹¹⁻¹³ Various research groups across the world have successfully employed this

hybridization approach towards the synthesis of novel hybrids which have shown potent antimalarial activity against sensitive and resistant strain of *P. falciparum*.^{1,14-22} Heme and *Plasmodium falciparum* dihydrofolate reductase (Pf-DHFR) are among the most important targets for antimalarial drug discovery. Quinoline based drugs such as chloroquine and its derivatives are known to affect the parasite metabolism and causes death of the parasite by blocking the polymerization of toxic heme into an insoluble and non-toxic pigment, hemozoin, resulting in cell lysis.²³ On the other hand, triazine and pyrimidine-based compounds such as cycloguanil and pyrimethamine exhibit the antimalarial activity due to their ability to inhibit dihydrofolate reductase enzyme.²⁴ Thus, linking of the quinoline unit with triazine or pyrimidine nucleus can deliver hybrids that might show potent antimalarial activity than each of the parent molecules. Several research groups have synthesized such kind of hybrid molecules²⁵⁻³³ and many of these derivatives have shown excellent *in vitro* and *in vivo* activity against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*. Our lab has also successfully adopted the hybrid concept to generate new antimalarial agents in which 4-aminoquinoline moiety was covalently attached to different pharmacophores present in other antimalarial agents.³⁴⁻³⁸ Recently, a series of

novel hybrid molecules was synthesized in which 4-aminoquinoline moiety was covalently attached to pyrimidine ring present in antifolate class of antimalarial drugs such as cycloguanil/pyrimethamine.^{39,40} All these compounds displayed potent *in-vitro* antimalarial activity ($IC_{50} = 0.005-0.44 \mu\text{M}$) against both CQ-sensitive (D6 clone) and CQ-resistant (W2 clone) of *P. falciparum* with no cytotoxicity against VERO cells (Fig. 2). The hybrids also possessed excellent *in-vivo* antimalarial activity without any apparent toxicity when tested in *P. berghei* infected mouse malaria model. It was found that the activity of these hybrids was dependent upon the substituents on the pyrimidine nucleus as well as the linker connecting the two pharmacophores.

In continuation of our efforts towards the development of novel antimalarial agents and in order to gain complete structural information to establish structure activity relationship, herein we present the synthesis and antimalarial activity of a new series of 4-aminoquinoline-pyrimidine (7a-7g, 8a-8g, 9a-9g) hybrids in which the secondary cyclic amines were replaced by substituted anilines at the pyrimidine nucleus (Fig. 2). The docking studies were also performed in the binding site of *P. falciparum* dihydrofolate reductase (Pf-DHFR) to investigate the interaction of these hybrids in Pf-DHFR protein.

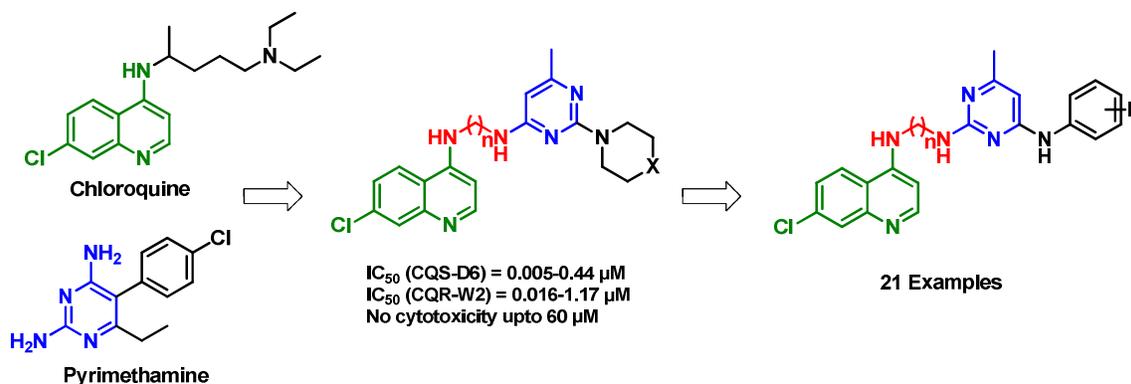
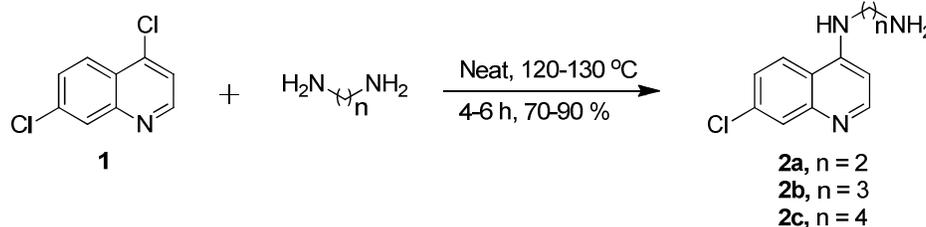


Fig. 2 Design strategy for the synthesis of novel 4-aminoquinoline-pyrimidine hybrids

Chemistry

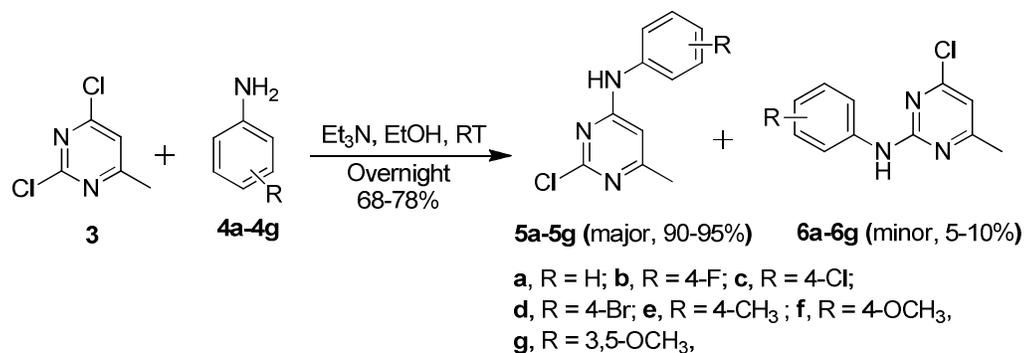
Synthesis of aminoquinoline-pyrimidine conjugates was carried out as outlined in schemes 1-3. Firstly, *N*¹-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**2a**), *N*¹-(7-chloroquinolin-4-yl)propane-1,3-diamine (**2b**) and *N*¹-(7-chloroquinolin-4-yl)butane-1,4-diamine (**2c**) were synthesized by the reaction of 4,7-dichloroquinoline (**1**) with the excess of ethane-1,2-diamine, propane-1,3-diamine and butane-1,4-diamine, respectively under neat condition at 120 °C (scheme 1).⁴¹ The substituted pyrimidines were synthesized by the reaction between commercially available 2,4-dichloro-6-methylpyrimidine (**3**) with

different substituted anilines at 0 °C to room temperature in the presence of triethylamine using THF as a solvent (scheme 2).^{42,43} The reaction of pyrimidine with substituted anilines yielded two regio-isomers **5a-5g** as a major and **6a-6g** as minor isomers. The major products were separated by column chromatography. Finally the 4-aminoquinolines (**2a-2c**) with free NH₂ group were coupled with aniline substituted pyrimidines (**5a-5g**) in the presence of K₂CO₃ and using *N*-methyl pyrrolidone (NMP) as solvent at reflux condition to give 4-aminoquinoline-pyrimidine hybrids (**7-9**) (scheme 3).

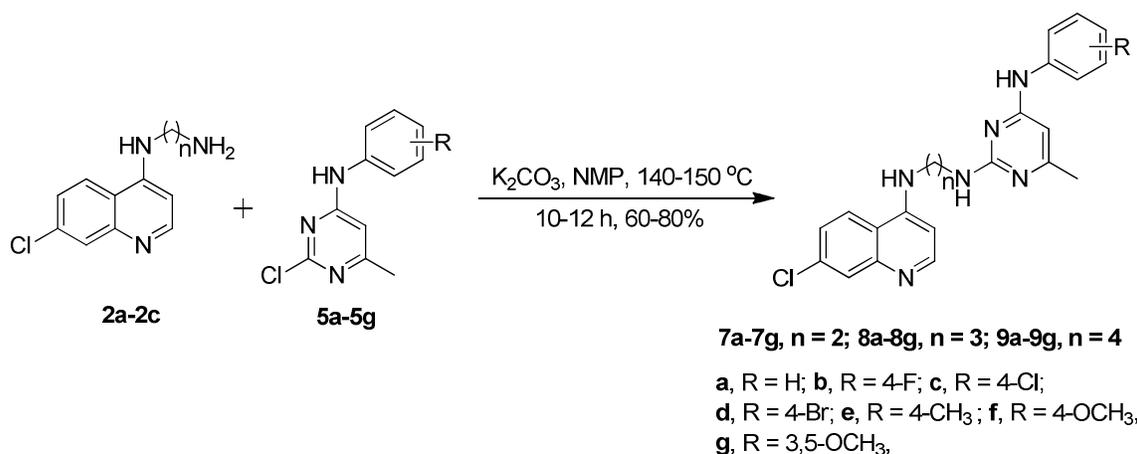


Scheme 1

70



Scheme 2



Scheme 3

Biological activity

In Vitro antimalarial activity

The antimalarial activity was determined by measuring plasmodial LDH activity as described in the literature.⁴⁴ A suspension of red blood cells infected with D6 or W2 strain of *P. falciparum* (200 μ L, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μ g/mL amikacin) was added to the wells of a 96-well plate containing 10 μ L of serially diluted test samples. The plate was flushed with a gas mixture of 90% N₂, 5% O₂, and 5% CO₂ and incubated at 37 °C, for 72 h in a modular incubation chamber (Billups-Rothenberg, CA). Parasitic LDH activity was determined according to the procedure of Makler and Hinrichs.⁴⁵ Briefly, 20 μ L of the incubation mixture was mixed with 100 μ L of the MalstatTM reagent (Flow Inc., Portland, OR) and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was then added and the plate is further incubated in the dark for 1 h. The reaction was then stopped by the addition of 100 μ L of a 5% acetic acid solution. The plate was read at 650 nm. Chloroquine and pyrimethamine were included in each assay as antimalarial drug controls. IC₅₀ values were computed from the dose response curves. To determine the selectivity index of antimalarial activity of compounds, *in vitro* cytotoxicity of these compounds against

mammalian cells was also determined. The assay was performed in 96-well tissue culture-treated plates as described earlier.⁴⁶ Vero cells (monkey kidney fibroblasts) were seeded to the wells of 96-well plate at a density of 25,000 cells/well and incubated for 24 h. Compounds at different concentrations were added and plates were again incubated for 48 h. The number of viable cells was determined by Neutral Red assay. The IC₅₀ values were obtained from dose response curves.

Heme binding studies

Chloroquine and other 4-aminoquinoline are believed to exert their antimalarial activity by binding with heme and thereby interfering with the formation of hemozoin through the π - π stacking interaction of the quinoline ring with the porphyrin ring.^{47,48} This can be demonstrated *in vitro* by showing the capability of chloroquine to inhibit the formation of β -hematin, a process that is similar to the hemozoin synthesis within the parasite food vacuole.⁴⁹ Cohen *et al* were the first to show that CQ forms a complex with ferriprotoporphyrin IX (FPIX) in the aqueous solution, which was demonstrated on the basis of red shifts observed in the heme absorbance data in the presence of a drug.⁵⁰ Since then several studies have explained the quinoline-FPIX complex formation by computational methods as well as the spectroscopic methods.⁵¹⁻⁵³ The FPIX exists either as a monomer, μ -oxo-dimer or an aggregate of μ -oxo-dimers, and the

relative proportions of these species are pH-dependent.^{54,55} Recently it has been determined that CQ forms complexes with both monomeric and μ -oxo-dimeric FPIX.^{29,56,57} Therefore, we decided to evaluate the binding of the most potent compound **8d** from the series with heme.

Docking Studies

The Pf-DHFR has been considered as an important target for the antimalarial drug discovery. Several antimalarial drugs such as pyrimethamine and cycloguanil exhibit antimalarial activity due to their ability to inhibit dihydrofolate reductase enzyme. In the present manuscript, we have reported molecular docking studies of the most active hybrids using wild type and quadruple mutant Pf-DHFR-TS (N51I, C59R, S108N, I164L) to study the inhibitory effect of these compounds on wild and mutant type Pf-DHFR. Clinical isolates of *P. falciparum* resistant to antifolates such as pyrimethamine and cycloguanil is caused by various combinations of four point mutations in the active site of DHFR domain of Pf-DHFR-TS. In the quadruple mutant protein, the first mutation occur at codon 108 (S108N), followed by codon 59 (C59R) and codon 51 (N51I), finally at codon 164 (I164L). The effect of quadruple mutations (N51I, C59R, S108 N, I164L) is ascribed to the movement of residues in the active site of DHFR and interferes in the inhibitor binding.⁵⁸ For docking, molecular structures of all the compounds were drawn using ChemBioDraw Ultra 12.0 (www.cambridgesoft.com). These structures were then imported into Maestro implemented in Schrödinger and Ligprep module was used to generate energy minimized 3D structures. The possible Lewis structure, tautomers and ionization states (pH 7.0 +/- 2.0) for each of these compounds were generated and optimized with default settings provided in the LigPrep module (Ligprep 2.5, Schrödinger, LLC, New York, NY, 2012). Partial atomic charges were computed using the OPLS_2005 force field. The crystal structures of wild type Pf-DHFR-TS (PDB ID: 3QGT; resolution 2.30 Å) and quadruple mutant (N51I+C59R+S108N+I164L) PfDHFR-TS (PDB ID: 3QG2; resolution: 2.30 Å) complexed with pyrimethamine was extracted from protein data bank (www.rcsb.org). Protein Preparation Wizard (Maestro 10.0 Schrödinger, LLC, New York, NY, 2012) was used to prepare proteins for docking. Water molecules within 5 Å of the protein structures were considered. Bond order and formal charges were assigned and hydrogen atoms were added to the crystal structure. Further to refine the structure OPLS-2005 force field parameter was used to remove the steric clashes and the minimization was terminated when RMSD reached maximum cut off value of 0.30 Å.

In silico ADMET prediction

The pharmacokinetic profile of the test compounds showing good antimalarial activity were predicted by using programs Qikprop v3.5 (Schrödinger, Inc., New York, NY, 2012). All the compounds prepared by LigPrep were used for the calculation of pharmacokinetic properties by QikProp. The program QikProp, utilizes the method of Jorgensen⁵⁹ to compute pharmacokinetic properties and descriptors such as octanol/water partitioning coefficient, aqueous solubility, brain/blood partition coefficient, intestinal wall permeability, plasma protein binding and others.

Results and Discussion

The aminoquinoline-pyrimidine hybrids were evaluated for their *in vitro* antimalarial activity against both CQ-sensitive (D6 clone) and CQ-resistant (W2 clone) strains of *P. falciparum* using chloroquine and pyrimethamine as reference drugs (Table 1). Cytotoxicity was also determined against Vero cell lines (Table 1). Most of the compounds showed potent antimalarial activity. Eight compounds (**7a**, **7f**, **7g**, **8d**, **8f**, **8g**, **9d** and **9b**) exhibited antimalarial activity with $IC_{50} < 0.05 \mu M$, whereas one compound **8f** was found to be equally potent to chloroquine against CQ-sensitive strain. All the compounds except compounds **9c** and **7e** showed better activity than chloroquine against CQ-resistant strain (W2 clone). Compounds which showed better activity against CQ-sensitive strain also found to possess good activity against CQ-resistant strain.

Table 1: *In-vitro* antimalarial activity and cytotoxicity of aminoquinoline-pyrimidine conjugates

Com	<i>P. falciparum</i> (D6 Clone)		<i>P. falciparum</i> (W2 Clone)		Cytotoxicity (Vero cells)
	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)
7a	0.049	>239.79	0.142	>82.74	>11.75
7b	0.052	>216.34	0.046	>244.56	>11.25
7c	0.050	>216.60	0.056	>193.39	>10.83
7d	0.056	>175.53	0.183	>53.71	>9.83
7e	0.360	>31.55	0.403	>28.18	>11.36
7f	0.041	>266.82	0.051	>214.50	>10.94
7g	0.045	>227.33	0.050	>204.60	>10.23
8a	0.051	>222.74	0.075	>151.46	>11.36
8b	0.058	>187.75	0.107	>101.77	>10.89
8c	0.060	>174.83	0.094	>111.59	>10.49
8d	0.043	>222.32	0.048	>199.16	>9.56
8e	0.350	29.85	0.349	29.94	10.45
8f	0.034	>311.76	0.060	>176.66	>10.60
8g	0.046	209.13	0.079	121.77	9.62
9a	0.074	>148.51	0.070	>157.0	>10.99
9b	0.047	>224.46	0.047	>224.46	>10.55
9c	4.589	>2.22	6.502	>1.56	>10.18
9d	0.045	>206.44	0.126	>73.73	>9.29
9e	0.328	27.71	0.267	34.04	9.09
9f	0.054	>190.37	0.063	>163.17	>10.28
9g	0.076	110.78	0.082	102.68	8.42
CQ	0.035	>212.57	0.367	>20.27	>7.440
Pyr	0.01	–	NA	–	NT

^a IC_{50} the concentration that causes 50% growth inhibition; S.I. selectivity index (IC_{50} for cytotoxicity to Vero cells/ IC_{50} for antimalarial activity); NA: Not active up to 19 μM ; NT: Not tested; Pyr-pyrimethamine.

For particular aniline substituted 4-aminoquinoline-pyrimidine conjugates (**7a-9a** or **7b-9b** or **7c-9c** or **7d-9d** or **7e-9e** or **7f-9f** or **7g-9g**), no obvious trend of activity was observed with increasing or decreasing the length of carbon chain linker. Compound having phenyl ring at pyrimidine nucleus (**7a**, **8a**, **9a**) showed antimalarial activity in the range of 0.049-0.074 μM (D6) and 0.075-0.142 μM (W2) with high selectivity index and compound with ethylene linker (**7a**) was found to be most active against CQ-sensitive strain while compound with butylene linker (**9a**) was the most active against CQ-resistant strain. When phenyl ring was substituted with halogen groups (**7b-7d**, **8b-8d**, **9b-9d**), most of the compounds showed potent antimalarial activity against both the strains with exception of compound **9c** in which chloro group is present at *para* position of the phenylene ring attached to pyrimidine ring and spacer is butylene but same compound with ethylene linker (**7c**) showed improved antimalarial activity against both the strains. Compounds with methyl group at *para* position of the phenyl ring (**7e**, **8e**, **9e**) led to partial decrease in the antimalarial activity against both the strains, while methoxy substitution at 4-position (**7f**, **8f**, **9f**) and 3,5-positions (**7g**, **8g**, **9g**) of the phenyl ring led to increase in the antimalarial activity with all the compounds more active against CQ-sensitive strain of *P. falciparum*. Compound **8f** having 4-OCH₃ group at phenyl ring with propylene linker was found to be the most potent against CQ-sensitive strain with IC₅₀ value of 0.034 μM . While compound **7b** with 4-F group and ethylene linker displayed the most potent activity against CQ-resistant strain with IC₅₀ value of 0.046 μM . Cytotoxicity was also determined against Vero cell lines (Table 1). All the compounds showed toxicity at very high concentration as compared to their concentrations (IC₅₀) responsible for their antimalarial activity. (Table 1).

Compound **7f** with significant activity *in vitro* was selected for further *in vivo* evaluation. *In vivo* antimalarial activity was determined through oral route of administration in *P. berghei*-mouse malaria model. The compounds were administered to the *P. berghei* infected mice, through oral gavage, once daily on days 0, 1 and 2 post infection and monitored for apparent signs of toxicity, parasitemia and survival till day 28 post infection (Table 2). It was found that the compound **7f** causes 17.85, 37.62 and 96.42% parasite suppression at 11.1, 33.3 and 100 mg/kg doses on day 5 as compared to 100% suppression displayed by CQ. On day 7, the effect almost disappeared at the highest dose level and the mean survival time was only 12.2 days as compared to 26.2

days for chloroquine treated animals.

Table 2: *In Vivo* antimalarial activity of compound **7f** in *P. berghei*-mouse malaria model

Treatment (PO)	Dose (mg/kg × no. of days post-infection)	% Parasitemia suppression ^a		MST ^b	Toxicity
		Day 5	Day 7		
Vehicle	NA × 3	-	-	7.6	NA
CQ	100 × 3	100	100	26.2	NC
Comp 7f	11.1 × 3	17.85	11.07	7.6	NC
	33.3 × 3	37.62	12.79	7	NC
	100 × 3	96.42	2.68	12.2	NC

^a % suppression in parasitemia is calculated by considering the mean parasitemia in the vehicle control as 100%; ^bMST - mean survival time (days); NA-Not active; NC- No cytotoxicity up to highest concentration tested

In order to understand the primary mode of action of these hybrids, heme binding studies were performed using compound **8d**. A solution of hematin in 40% DMSO/water shows an absorption band at 402 nm, indicating the presence of monomeric heme, Fe(III) PPIX, under the conditions used (0.02 M HEPES buffer, pH 7.4 and 0.02 M MES buffer, pH 5.6). When compound **8d** was added into a constant concentration of monomeric heme (5.0 μM), a substantial decrease in the intensity of the absorption band at 402 nm was observed with no shift in the absorption maxima. This indicates the association of compounds with hematin. The stoichiometry ratio of the most stable complexes of compound **8d** with monomeric heme at pH 7.4 and 5.6 was inferred from the Job's plot. The absorbance at 402 nm got maximum value when mole fraction of compound was approximately 0.5, which confirms the association of compound with heme in 1:1 ratio at both the pH values (Figure 4).

As discussed earlier that CQ and its derivatives also bind to heme dimer (μ -oxo-heme). Therefore, the binding of compound **8d** was also studied with μ -oxo dimer of heme at pH 5.8. In aqueous NaOH solution heme exists as μ -oxo-dimer and shows an absorption band at 362 nm. Addition of compound **8d** (0–20 μM) to a solution of μ -oxo dimer (10 μM) in 20 mM phosphate buffer at pH 5.8 resulted in a decrease in intensity of absorbance at 362 nm (Figure 5), which shows the interaction between heme and compound **8d**. The Job's plot indicated a 1:1 stoichiometry for the most stable complex formed between μ -oxo heme and compound **8d** (Figure 5).

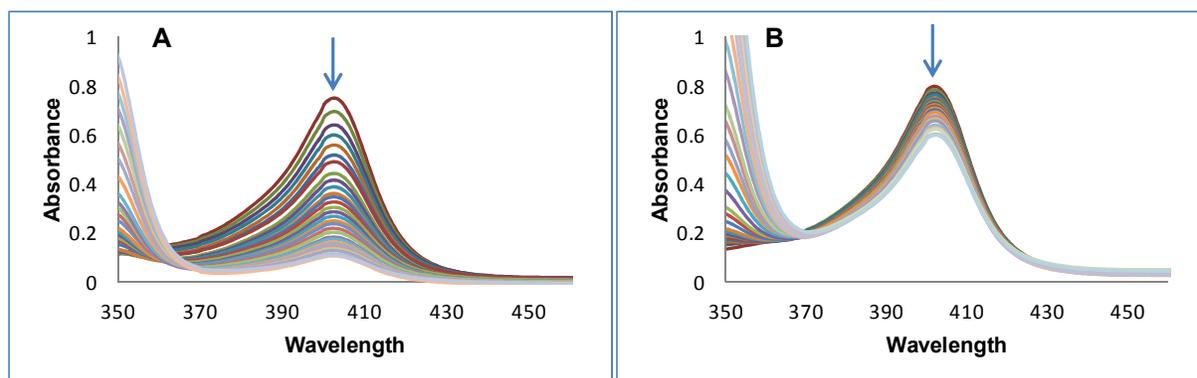


Fig. 3 A) Titration of compound **8d** with monomeric heme at pH 7.4; B) Titration of compound **8d** with monomeric heme at pH 5.6

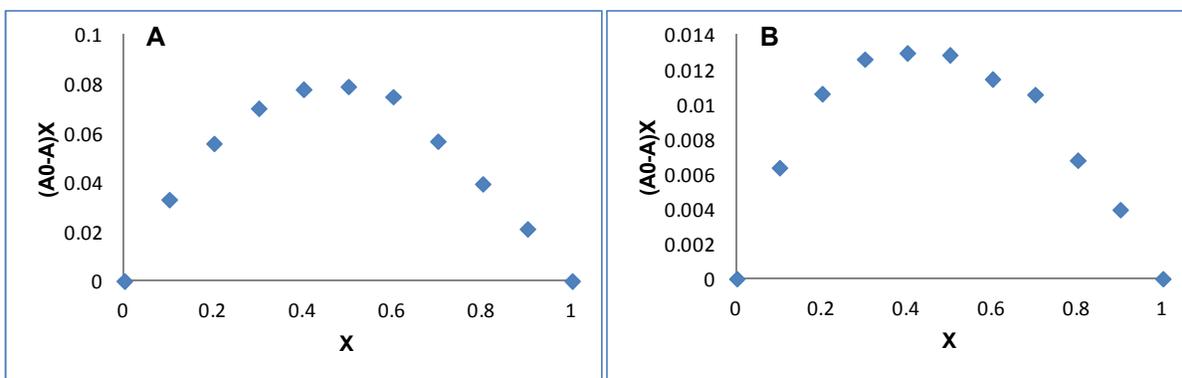


Fig. 4 Job's plot of monomeric heme complex formation with compound **8d**; **A**) at pH 7.4; **B**) at pH 5.6; X (mole fraction of compound **8d**) = [compd **8d**]/[compd **8d**]+[heme]; A0 is the absorbance, when x = 1 and A is the absorbance at respective values of x

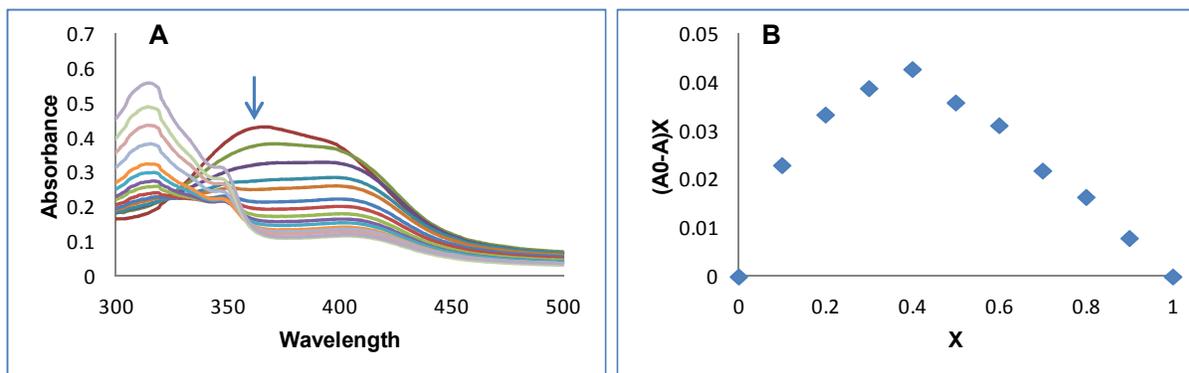


Fig. 5 **A**) Titration of compound **8d** with μ -oxodimeric heme at pH 5.8; **B**) Job's plot of μ -oxodimeric heme complex formation with compound **8d** at pH 5.8

The association constants for the complexes formed between monomeric Fe(III) PPIX and compound **8d** at pH 7.4 and 5.6 were calculated by the analysis of titration data and the results are shown in Table 3. The association constant at pH 7.4 (log K 5.048) was comparable to that of the standard antimalarial drug CQ (log K 5.15). Furthermore, decreasing the pH from 7.4 to 5.6 (food vacuole pH), compound displayed improved binding constant (log K 5.272) indicating that binding is stronger even at acidic pH of food vacuole. Interestingly, at pH 5.6, compound showed large value of binding constant than the standard drug chloroquine. The association constants for the binding with μ -oxo-heme at pH 5.8 (log K 5.171) was found to be even greater than that of monomeric heme complexes at pH 7.4. From the data shown in table 3, it is clear that the compound **8d** binds strongly with monomeric heme (log K 5.272) as well as μ -oxo-heme (log K 5.171) and the observed results are comparable to the standard CQ (log K 5.58). Thus the formation of complex between heme and compound **8d** suggests the inhibition of formation of β -hematin, which could be correlated to the observed antimalarial activity of these compounds.

The mode of action of these 4-aminoquinoline-pyrimidine hybrids were further substantiated by molecular docking studies into the binding pocket of wild type Pf-DHFR-TS and quadruple mutant Pf-DHFR-TS (N511, C59R, S108 N, I164L). The results of molecular docking of active compounds in the binding site of both wild type and mutant Pf-DHFR-TS are shown in Table 4. The Glide XP Gscores and glide energies clearly indicate that the

most active compounds in the study exhibited significant binding affinities towards the wild (Glide energy range -65.11 kcalmol $^{-1}$ to -37.00 kcalmol $^{-1}$) and quadruple mutant (Glide energy range -72.10 kcalmol $^{-1}$ to -36.43 kcalmol $^{-1}$) Pf-DHFR-TS structures and the energy ranges are comparable to that of reference compounds (pyrimethamine, cycloguanil and WR99210) and the native substrate of DHFR dihydrofolate (Table 4).

Table 3 Binding constants for compound **8d** and chloroquine with heme

Comp	Monomeric heme	Monomeric heme	μ -oxo-heme
	log K (pH = 5.6)	log K (pH = 7.4)	log K (pH = 5.8)
8d	5.272	5.048	5.171
CQ	4.65 ^a	5.15 ^a	5.58 ^a
Stoichiometry	1:1	1:1	1:1

^a See text reference 29

Figure 6 and 7 represents the binding mode of the two best selected active compounds **7f** and **8d** having high Glide XP scores and low Glide binding energies. These compounds occupy similar binding pocket as the native Pf-DHFR substrate dihydrofolate. Compound **7f** showed lowest binding energy (-65.11 kcalmol $^{-1}$) and considerably high Glide XP score (-8.91 kcalmol $^{-1}$) for wild type Pf-DHFR and -72.10 and -8.59 respectively for mutant type Pf-DHFR. A hydrogen bond interaction was observed between NH group of ethylene linker

attached to pyrimidine nucleus and oxygen side chain of Asp54 of both wild and mutant Pf-DHFR. An additional H-bond interaction was also observed between the NH group attached between pyrimidine and methoxy substituted phenyl ring, and main chain oxygen of Ile164 in wild type PfDHFR. (Fig. 6). Further, compound **7f** showed two π - π interactions in case of wild

type, one between the aromatic ring of Phe116 and pyridine ring of quinoline nucleus and the other between the aromatic ring of Phe58 and the pyrimidine aromatic ring. In mutant type Pf-DHFR, a π - π interaction was observed between the aromatic ring of Phe58 and the methoxy substituted phenyl ring.

Table 4 Glide docking scores (kcal mol⁻¹) and docking energies of best active molecules along with the reference compounds (pyrimethamine, cycloguanil and WR99210) and dihydrofolate bound to wild and mutant PfDHFR-TS binding site

Compounds	Docking results with wild PfDHFR				Docking results with mutant PfDHFR			
	XP GScore	Van der waals energy	Coulombic Energy	Glide Energy	XP GScore	Van der waals energy	Coulombic Energy	Glide Energy
7f	-8.91	-48.64	-16.47	-65.11	-8.59	-51.62	-16.87	-72.10
8f	-6.94	-44.06	-12.83	-56.90	-7.13	-50.23	-21.86	-61.18
9f	-6.89	-29.25	-15.60	-44.85	-6.89	-32.68	-10.44	-43.12
7g	-6.82	-25.68	-11.92	-37.60	-6.60	-27.99	-8.44	-36.43
8g	-5.88	-27.78	-10.18	-37.97	-5.80	-28.46	-9.13	-37.60
7c	-4.28	-47.79	-14.57	-51.59	-4.20	-46.82	-4.69	-51.51
8d	-7.61	-47.13	-4.45	-62.37	-8.07	-46.99	-17.28	-64.28
9d	-6.21	-23.47	-13.52	-37.00	-4.77	-37.44	-7.69	-45.13
7b	-5.82	-34.43	-7.05	-41.48	-5.32	-48.20	-12.98	-68.49
9b	-5.61	-26.43	-11.34	-37.77	-5.32	-25.06	-12.92	-37.99
Dihydrofolate	-9.33	-52.14	-14.19	-64.84	-11.00	-43.68	-17.61	-61.30
Pyrimethamine	-9.04	-31.70	-15.51	-44.91	-9.39	-33.65	-12.06	-43.55
Cycloguanil	-8.94	-30.12	-10.74	-38.55	-8.95	-34.30	-8.60	-46.60
WR99210	-4.84	-51.18	-6.91	-37.03	-5.48	-27.37	-8.07	-34.30

15

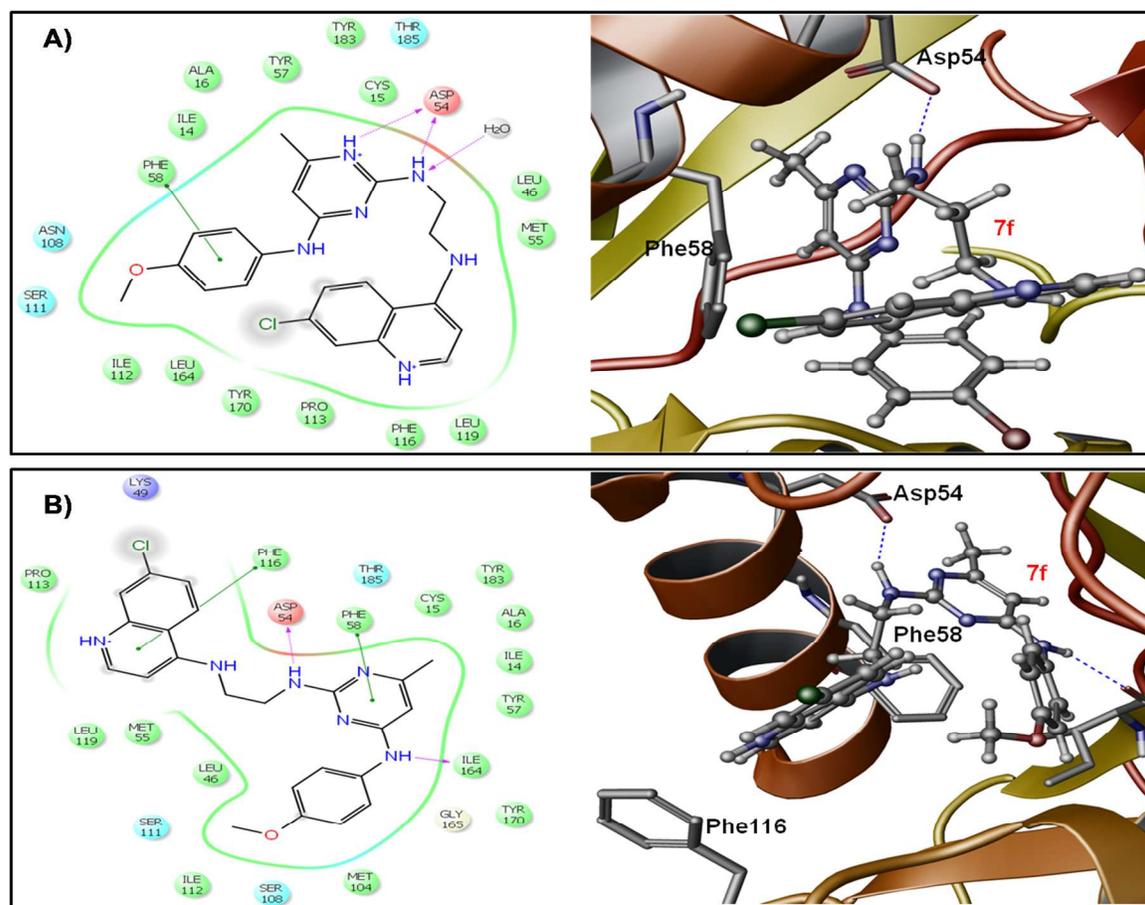


Fig. 6 2D and 3D docking pose showing interaction for compounds **7f** in the binding site of (A) mutant type Pf-DHFR-TS (PDB ID: 3QG2) and (B) wild type Pf-DHFR-TS (PDB ID: 3QGT).

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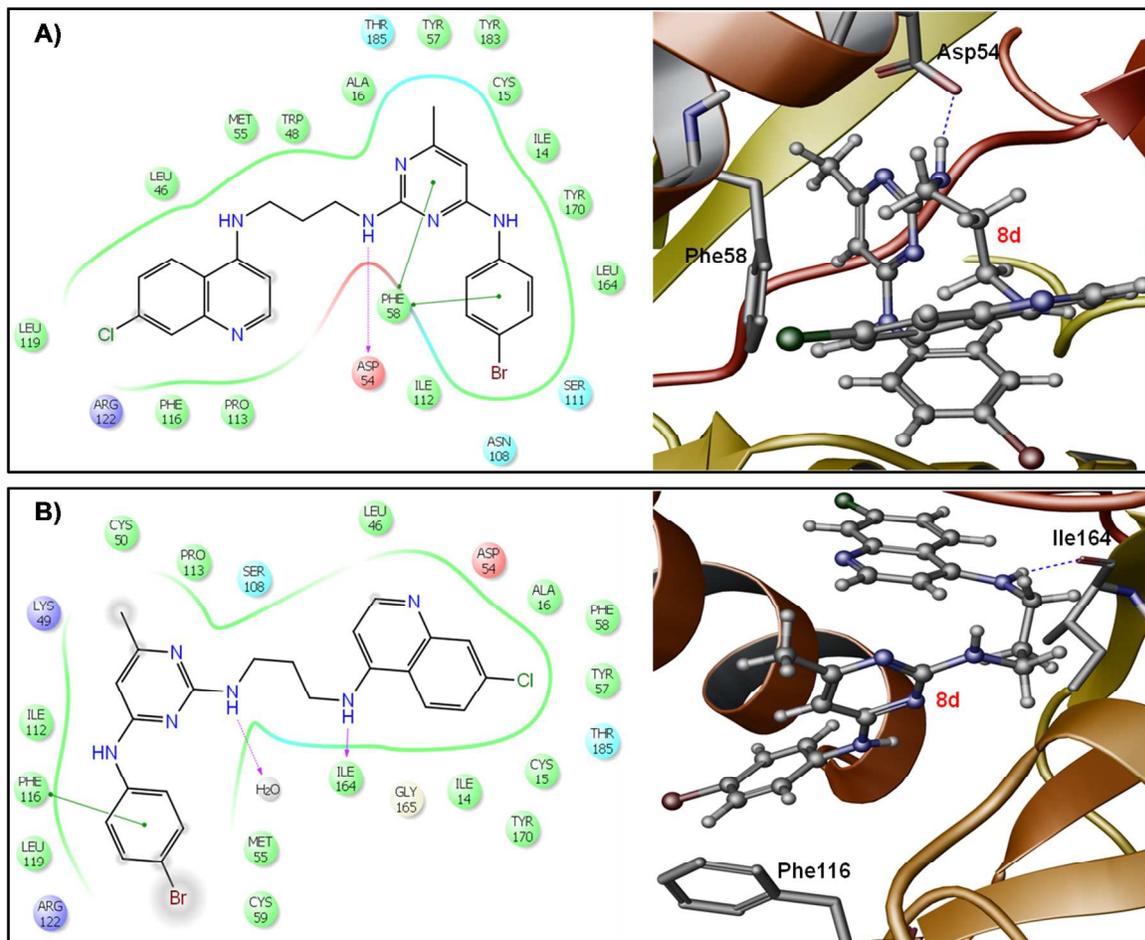


Fig. 7 2D and 3D docking pose showing interaction for compounds **8d** in the binding site of (A) mutant type Pf-DHFR-TS (PDB ID: 3QG2) and (B) wild type Pf-DHFR-TS (PDB ID: 3QGT).

Another compound predicted to have low binding energy (-62.37 kcalmol⁻¹) and high glide score (-7.61) was **8d**, showing similar H-bond pattern between NH group of propylene linker of compound and oxygen side chain of Asp54. The π - π interactions were also observed between the two aromatic rings (4-bromophenyl and pyrimidine ring) of compound **8d** and the aromatic ring of Phe58 of mutant Pf-DHFR. In wild type Pf-DHFR, a π - π interaction was observed between the aromatic ring of Phe116 and 4-bromophenyl ring attached to pyrimidine nucleus. Also, the compound **8d** forms H-bond interaction through its NH group of propylene linker attached to the aminoquinoline ring with the main chain oxygen atom of Ile164 in the wild type protein binding site.

Pharmacokinetic parameters of best active compounds were calculated using ADMET predictions by Qikprop v3.5. The most important of these parameters together with its permissible ranges are listed in the Table 5 and Table S1. Qikprop results for the Lipinski's rule of 5 parameters, a preliminary test of the drug-likeness of the compounds is presented in Table 5. An orally active compound should not have more than 2 violations of these rules.⁶⁰ In the present study, all the active test compounds showed values for Lipinski's rule of 5 violations less than the maximum permissible value of 2, indicating that these active test compounds are having good drug likeness properties.

Table 5 Prediction of Lipinski's 'Rule of 5' for the active test compounds^a

Comp	mol_MW (<500 amu)	donor HB (<5)	acct HB (<10)	QPlogPo/w (<5)	'N' of violations (<2)
7f	434.92	3	6	4.316	0
8f	448.95	3	6	5.288	1
9f	462.98	3	6	5.217	1
7g	464.95	3	6	4.475	0
8g	478.98	3	6	5.445	1
7c	425.32	3	5	4.365	0
8d	497.82	3	5	5.366	1
9d	511.85	3	5	5.436	2
7b	422.89	3	5	4.763	0
9b	450.94	3	5	5.503	1
Pyr	248.71	4	3	1.809	0
Cyg	253.73	5	3	0.888	0

^a All values calculated by QikProp v 3.5 and the explanations of the descriptors are given in the text.

The optimum values of descriptors such as number of rotatable bonds (<15) and polar surface area ($7 \text{ \AA}^2 - 200 \text{ \AA}^2$) can also have significant influence on oral bioavailability of compounds.⁶¹ In the present study, all the test compounds possess a number of rotatable bonds <15 and polar surface area in the permissible range ($7 \text{ \AA} - 200 \text{ \AA}$). Similarly, the test molecules were checked for their intestinal absorption or permeation property, which is confirmed by the predicted Caco-2 cells permeability (QPCCaco),

used as a model for the gut–blood barrier.⁶² QPPCaco predictions for all the test compounds showed values >500. Further, QPlogKhsa, the prediction for human serum albumin binding were calculated and all inhibitors were predicted to lie within the expected range for 95% of known drugs (–1.5 to 1.5). The aqueous solubility (QPlogS) parameters for the test compounds were assessed and all the compounds were predicted to have QPlogS values in the permissible range (–6.5 to 0.5). Furthermore, QPlogHERG descriptor for the prediction of IC₅₀ value of HERG K⁺ channel blockage was predicted for the test compounds. Compounds **7f** and **7g** have been predicted to possess values for in QPlogHERG less than the value of concern (–5), comparable to reference compounds pyrimethamine and cycloguanil (Table S1).

15 Experimental Section

All the chemicals were purchased from Sigma-Aldrich. Solvents used for the chemical synthesis were of analytical grade and used without further purification. Thin layer chromatography (Merck Kiesel 60 F254, 0.2 mm thickness) was used to monitor the progress of the reactions and the compounds were purified by silica gel (60–120 mesh) column chromatography. IR spectra were recorded on Perkin-elmer FT-IR spectrophotometer using KBr pellets or as film in chloroform and the values were expressed in cm^{–1}. The ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded on Jeol ECX spectrosipin instrument using CDCl₃ or DMSO-*d*₆ as solvent and TMS as internal reference. The chemical shift values were expressed on δ scale and the coupling constant (*J*) in Hz. Melting points were recorded on EZ-Melt automated melting point apparatus, Stanford Research Systems and are uncorrected. Mass data were recorded in Jeol-Accu TOF JMS-T100LC mass spectrometer.

Typical procedure for the synthesis of *N*¹-(7-chloroquinolin-4-yl)ethane-1,2-diamine (2a) and related compounds (2b and 2c): A mixture of 4,7-dichloroquinoline (**1**, 5.0 g, 0.025 mol) and 1,2-ethylene diamine (5.8 g, 0.125 mol) was heated slowly from RT to 120 °C and the reaction mixture was stirred at this temperature for 6 h (scheme 1). Reaction mixture was cooled down to room temperature and ice cold water added to it. The solid thus obtained was filtered and washed with excess of water. The crude product was crystallized by using ethanol and the data corresponds to that reported in the literature.

Typical procedure for the synthesis of 2-chloro-*N*-phenylpyrimidin-4-amine (5a) and related compounds (5b–5g): To a solution of 2,4-dichloropyrimidine (**3**, 2.0 g, 0.013 mol) and triethylamine (1.63 g, 0.016 mol) in ethanol at 0 °C, aniline (**4a**, 1.2 g, 0.013 mol) was added (scheme 2). The reaction mixture was stirred overnight at room temperature. After completion of the reaction as observed by TLC, excess ethanol was evaporated and the reaction mixture was diluted with water. The solid thus obtained was filtered and washed with excess water. The crude product was purified by column chromatography using EtOAc/Hexane as eluent to afford pure compound **5a**.

2-Chloro-6-methyl-*N*-phenylpyrimidin-4-amine (5a): Yield

71%; mp 242–244 °C; IR (Film, cm^{–1}): 3274, 3100, 2921, 1636, 1559, 1525, 1495, 1458, 1236, 1045, 828; ¹H NMR (400 MHz, CDCl₃): δ 2.34 (s, 3H, CH₃), 6.45 (s, 1H, ArH), 6.98 (brs, 1H, NH), 7.28–7.30 (m, 3H, ArH), 7.39–7.43 (m, 2H, ArH); ESI-MS (*m/z*) calculated for C₁₁H₁₀ClN₃: 219.05, found: 220.16 (M + H)⁺.

2-Chloro-*N*-(4-fluorophenyl)-6-methylpyrimidin-4-amine (5b): Yield 68%; mp 147–150 °C; IR (Film, cm^{–1}): 3229, 3081, 3003, 1591, 1507, 1418, 1370, 1276, 1212, 1156, 1033, 974, 914; ¹H NMR (400 MHz, CDCl₃): δ 2.32 (s, 3H, CH₃), 6.29 (s, 1H, ArH), 7.09–7.13 (m, 2H), 7.17 (brs, 1H, NH), 7.27–7.30 (m, 2H, ArH); ¹³C NMR (100 MHz, CDCl₃): δ 23.90, 100.52, 116.39, 126.13, 133.04, 160.18, 160.89, 163.34, 168.87; ESI-MS (*m/z*) calculated for C₁₁H₉ClFN₃: 237.04, found: 238.12 (M + H)⁺.

2-Chloro-*N*-(4-chlorophenyl)-6-methylpyrimidin-4-amine (5c): Yield 75%; mp 129–131 °C; IR (Film, cm^{–1}): 3290, 3208, 3161, 3075, 1606, 1584, 1492, 1412, 1369, 1274, 1240, 1217, 1176, 1090, 1031, 974; ¹H NMR (400 MHz, CDCl₃): δ 2.34 (s, 3H, CH₃), 6.38 (s, 1H, ArH), 7.22 (brs, 1H, NH), 7.27 (d, *J* = 8.79 Hz, 2H, ArH), 7.37 (d, *J* = 8.79 Hz, 2H, ArH); ¹³C NMR (100 MHz, CDCl₃): δ 23.91, 101.04, 124.56, 129.66, 131.05, 135.86, 160.22, 162.70, 168.98; ESI-MS (*m/z*) calculated for C₁₁H₉Cl₂N₃: 253.01, found: 254.13 (M + H)⁺.

***N*-(4-Bromophenyl)-2-chloro-6-methylpyrimidin-4-amine (5d):** Yield 78%; mp 141–144 °C; IR (Film, cm^{–1}): 3291, 3204, 3158, 3078, 2924, 1603, 1580, 1488, 1409, 1368, 1274, 1238, 1216, 1177, 1074, 1031, 974, 915; ¹H NMR (400 MHz, CDCl₃): δ 2.35 (s, 3H, CH₃), 6.39 (s, 1H, ArH), 7.19 (brs, 1H, NH), 7.22 (d, *J* = 8.79 Hz, 2H, ArH), 7.52 (d, *J* = 8.79 Hz, 2H, ArH); ¹³C NMR (100 MHz, CDCl₃): δ 24.03, 95.83, 116.79, 123.54, 132.11, 138.87, 159.37, 161.23, 167.25; ESI-MS (*m/z*) calculated for C₁₁H₉BrClN₃: 296.96, found: 298.04 (M + H)⁺.

2-Chloro-6-methyl-*N*-*p*-tolylpyrimidin-4-amine (5e): Yield 76%; mp 152–154 °C; IR (Film, cm^{–1}): 3289, 3176, 2921, 1653, 1559, 1439, 1358, 1298, 1237, 1175, 1096, 1031, 974; ¹H NMR (400 MHz, CDCl₃): δ 2.31 (s, 3H, CH₃), 2.37 (s, 3H), 6.36 (s, 1H, ArH), 7.05 (brs, 1H, NH), 7.15 (d, *J* = 8.05 Hz, 2H, ArH), 7.21 (d, *J* = 8.05 Hz, 2H, ArH); ESI-MS (*m/z*) calculated for C₁₂H₁₂ClN₃: 233.07, found: 234.21 (M + H)⁺.

2-Chloro-*N*-(4-methoxyphenyl)-6-methylpyrimidin-4-amine (5f): Yield 75%; mp 170–172 °C; IR (Film, cm^{–1}): 3214, 3087, 2924, 1591, 1510, 1420, 1394, 1368, 1276, 1241, 1223, 1173, 1034, 972, 912; ¹H NMR (400 MHz, CDCl₃): δ 2.29 (s, 3H, CH₃), 3.83 (s, 3H, 2×OCH₃), 6.23 (brs, 1H, NH), 6.94 (d, *J* = 8.79 Hz, 2H, ArH), 7.19 (d, *J* = 8.79 Hz, 3H, ArH); ¹³C NMR (100 MHz, CDCl₃): δ 23.78, 55.42, 100.20, 114.73, 126.45, 129.67, 157.98, 159.99, 163.97, 168.38; ESI-MS (*m/z*) calculated for C₁₂H₁₂ClN₃O: 249.06, found: 250.18 (M + H)⁺.

2-Chloro-*N*-(3,5-dimethoxyphenyl)-6-methylpyrimidin-4-amine (5g): Yield 70%; mp 185–188 °C; IR (Film, cm^{–1}): 3293, 3092, 2925, 1599, 1526, 1474, 1339, 1205, 1154, 1063, 829; ¹H NMR (400 MHz, CDCl₃): δ 2.34 (s, 3H, CH₃), 3.80 (s, 6H, 2OCH₃), 6.33 (brs, 1H, NH), 6.46 (d, *J* = 1.46 Hz, 2H), 6.51 (s, 1H), 7.12 (s, 1H); ESI-MS (*m/z*) calculated for C₁₃H₁₄ClN₃O₂: 279.07, found: 280.23 (M + H)⁺.

Typical procedure for the synthesis of N^2 -(2-((7-chloroquinolin-4-yl)amino)ethyl)- N^4 -phenylpyrimidine-2,4-diamine (7a) and related compounds (7b-7g, 8a-8g and 9a-9g): To a stirred solution of compound 2a (400 mg, 1.8 mmol) and compound 5a (372 mg, 1.8 mmol) in *N*-methyl pyrrolidinone (NMP), K_2CO_3 (750 mg, 5.4 mmol) was added. Reaction mixture was stirred at 140 °C for 12 h (scheme 3). After completion of the reaction, water was added to the reaction mixture and the product was extracted with chloroform (3 × 20 mL). The combined organic layer was dried over sodium sulphate and excess solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using MeOH/ $CHCl_3$ as eluent to afford pure compound 7a in quantitative yield.

N^2 -(2-(7-Chloroquinolin-4-ylamino)ethyl)-6-methyl- N^4 -phenyl pyrimidine-2,4-diamine (7a): Yield 75%; mp 214-215° C; IR (KBr, cm^{-1}): 3254, 3061, 2936, 2858, 1578, 1553, 1497, 1443, 1355, 1326, 1248, 1228, 1141, 908, 870; 1H NMR (400 MHz, DMSO- d_6): δ 2.15 (s, 3H, CH_3), 3.42-3.44 (m, 2H, NCH_2), 3.56 (brs, 2H, NCH_2), 5.91 (s, 1H, ArH), 6.54 (brs, 1H, NH), 6.88-6.92 (m, 2H, ArH), 7.22 (t, $J = 7.3$ Hz, 2H, ArH), 7.41 (d, $J = 8.0$ Hz, 2H, ArH), 7.68 (d, $J = 8.0$ Hz, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.16 (d, $J = 9.5$ Hz, 1H, ArH), 8.35 (brs, 1H, NH), 9.06 (s, 1H, NH); ESI-HRMS (m/z) calculated for $C_{22}H_{21}ClN_6$: 404.1516, found: 405.1381 (M + H) $^+$, 407.1700 (M + 2) $^+$; Anal. calcd. for $C_{22}H_{21}ClN_6$: C, 65.26; H, 5.23; Cl, 8.76; N, 20.76, found: C, 65.40; H, 5.20; Cl, 8.65; N, 20.82.

N^2 -(2-(7-Chloroquinolin-4-ylamino)ethyl)- N^4 -(4-fluoro phenyl)-6-methylpyrimidine-2,4-diamine (7b): Yield 55%; mp 216-218 °C; IR (KBr, cm^{-1}): 3299, 3186, 3061, 3006, 1642, 1583, 1504, 1458, 1408, 1368, 1330, 1244, 1216, 1152, 1079, 1013, 906, 872, 821; 1H NMR (400 MHz, DMSO- d_6): δ 2.14 (s, 3H, CH_3), 3.40-3.44 (m, 2H, NCH_2), 3.54 (brs, 2H, NCH_2), 5.87 (s, 1H, ArH), 6.53 (brs, 1H, NH), 6.88 (s, 1H, ArH), 7.05 (d, $J = 8.7$ Hz, 2H, ArH), 7.41 (d, $J = 8.7$ Hz, 2H, ArH), 7.68 (brs, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.16 (d, $J = 9.5$ Hz, 1H, ArH), 8.35 (brs, 1H, NH), 9.08 (brs, 1H, NH); Anal. calcd. for $C_{22}H_{20}ClFN_6$: C, 62.48; H, 4.77; Cl, 8.38; F, 4.49; N, 19.87, found: C, 62.43; H, 4.65; Cl, 8.44; F, 4.51; N, 19.90.

N^4 -(4-Chlorophenyl)- N^2 -(2-((7-chloroquinolin-4-yl)amino) ethyl)-6-methylpyrimidine-2,4-diamine (7c): Yield 70%; mp 238-242 °C; IR (KBr, cm^{-1}): 3250, 3005, 2881, 1610, 1586, 1521, 1491, 1459, 1402, 1342, 1330, 1248, 1133, 1087, 1010, 906, 870, 818, 804; 1H NMR (400 MHz, DMSO- d_6): δ 2.15 (s, 3H, CH_3), 3.42-3.45 (m, 2H, NCH_2), 3.55 (brs, 2H, NCH_2), 5.89 (s, 1H, ArH), 6.81 (brs, 1H, NH), 6.93 (s, 1H, ArH), 7.25 (d, $J = 9.2$ Hz, 2H, ArH), 7.42 (dd, $J = 2.2, 8.7$ Hz, 2H, ArH), 7.73 (d, $J = 8.0$ Hz, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.16 (d, $J = 9.5$ Hz, 1H, ArH), 8.35 (brs, 1H, NH), 9.20 (brs, 1H, NH); ESI-HRMS (m/z) calculated for $C_{22}H_{20}Cl_2N_6$: 438.1127, found: 439.0452 (M + H) $^+$, 441.0577 (M + 2) $^+$; ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.72, 42.63, 98.83, 117.39, 120.63, 123.87, 124.03, 124.73, 127.51, 128.36, 133.39, 139.74, 149.0, 150.16, 151.83, 160.92, 161.92; Anal. calcd. for $C_{22}H_{20}Cl_2N_6$: C, 60.14; H, 4.59; Cl, 16.14; N, 19.13, found: C, 60.28; H, 4.64; Cl, 16.30; N, 19.33.

N^4 -(4-Bromophenyl)- N^2 -(2-((7-chloroquinolin-4-yl)amino) ethyl)-6-methylpyrimidine-2,4-diamine (7d): Yield 70%; mp

238-242 °C; IR (KBr, cm^{-1}): 3245, 3189, 3066, 2974, 2935, 1610, 1583, 1519, 1488, 1459, 1398, 1341, 1329, 1247, 1074, 1006, 906, 870, 804; 1H NMR (400 MHz, DMSO- d_6): δ 2.15 (s, 3H, CH_3), 3.43-3.44 (m, 2H, NCH_2), 3.55 (brs, 2H, NCH_2), 5.90 (s, 1H, ArH), 6.79 (brs, 1H, NH), 6.94 (s, 1H, ArH), 7.37 (d, $J = 8.7$ Hz, 2H, ArH), 7.42 (dd, $J = 2.2, 8.7$ Hz, 2H, ArH), 7.68 (d, $J = 6.5$ Hz, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.17 (d, $J = 8.7$ Hz, 1H, ArH), 8.36 (brs, 1H, NH), 9.21 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.72, 42.61, 98.85, 112.60, 117.98, 121.04, 123.89, 124.06, 127.47, 131.25, 133.41, 140.16, 148.96, 150.18, 151.81, 160.89, 161.90; ESI-HRMS (m/z) calculated for $C_{22}H_{20}BrClN_6$: 482.0621, found: 482.9981 (M + H) $^+$, 484.9908 (M + 2) $^+$; Anal. calcd. for $C_{22}H_{20}BrClN_6$: C, 54.62; H, 4.17; Br, 16.52; Cl, 7.33; N, 17.37, found: C, 54.71; H, 4.12; Br, 16.49; Cl, 7.19; N, 17.40.

N^2 -(2-(7-Chloroquinolin-4-ylamino)ethyl)-6-methyl- N^4 -*p*-tolyl pyrimidine-2,4-diamine (7e): Yield 80%; mp 214-215 °C; IR (KBr, cm^{-1}): 3253, 3062, 2936, 2859, 1644, 1576, 1508, 1447, 1407, 1355, 1326, 1291, 1247, 1233, 1139, 1084, 1033, 907, 870, 814; 1H NMR (400 MHz, DMSO- d_6): δ 2.13 (s, 3H, CH_3), 2.20 (s, 3H, CH_3), 3.40-3.44 (m, 2H, NCH_2), 3.54 (brs, 2H, NCH_2), 5.87 (s, 1H, ArH), 6.54 (brs, 1H, NH), 6.83 (s, 1H, ArH), 7.02 (d, $J = 7.3$ Hz, 2H, ArH), 7.41 (d, $J = 8.0$ Hz, 2H, ArH), 7.53 (d, $J = 8.0$ Hz, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.15 (d, $J = 8.7$ Hz, 1H, ArH), 8.34 (brs, 1H, NH), 8.94 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 20.34, 23.72, 42.80, 98.82, 117.38, 119.63, 123.85, 123.98, 127.52, 129.0, 130.30, 133.34, 138.04, 149.02, 150.12, 151.89, 161.23, 162.04; ESI-HRMS (m/z) calculated for $C_{23}H_{23}ClN_6$: 418.1673, found: 419.1968 (M + H) $^+$, 421.2253 (M + 2) $^+$; Anal. calcd. for $C_{23}H_{23}ClN_6$: C, 65.94; H, 5.53; Cl, 8.46; N, 20.06, found: C, 66.14; H, 5.70; Cl, 8.41; N, 19.82.

N^2 -(2-(7-Chloroquinolin-4-ylamino)ethyl)- N^4 -(4-methoxy phenyl)-6-methylpyrimidine-2,4-diamine (7f): Yield 65%; mp 200-201 °C; IR (KBr, cm^{-1}): 3248, 3057, 2929, 1582, 1509, 1409, 1354, 1245, 1166, 1139, 1033, 922, 874, 828, 795; 1H NMR (400 MHz, DMSO- d_6): δ 2.11 (s, 3H, CH_3), 3.37-3.43 (m, 2H, NCH_2), 3.53-3.56 (m, 2H, NCH_2), 3.65 (s, 3H, OCH_3), 5.81 (s, 1H, ArH), 6.53 (brs, 1H, NH), 6.79-6.81 (m, 3H, ArH), 7.41 (d, $J = 7.3$ Hz, 2H, ArH), 7.52 (d, $J = 8.7$ Hz, 2H, ArH), 7.76 (d, $J = 2.2$ Hz, 1H, ArH), 8.15 (d, $J = 9.5$ Hz, 1H, ArH), 8.34 (brs, 1H, NH), 8.85 (brs, 1H, NH); ESI-HRMS (m/z) calculated for $C_{23}H_{23}ClN_6O$: 434.1681, found: 435.1748 (M + H) $^+$, 437.1731 (M + 2) $^+$; Anal. calcd. for $C_{23}H_{23}ClN_6O$: C, 63.52; H, 5.33; Cl, 8.15; N, 19.32; O, 3.68, found: C, 63.41; H, 5.46; Cl, 8.22; N, 19.10; O, 3.73.

N^2 -(2-(7-Chloroquinolin-4-ylamino)ethyl)- N^4 -(3,5-dimethoxy phenyl)-6-methylpyrimidine-2,4-diamine (7g): Yield 50%; mp 229-231 °C; 1H NMR (400 MHz, DMSO- d_6): δ 2.13 (s, 3H, CH_3), 3.35 (brs, 2H, NCH_2), 3.57 (brs, 2H, NCH_2), 3.66 (s, 6H, 2× OCH_3), 5.89 (s, 1H, ArH), 6.06 (s, 1H, ArH), 6.49 (brs, 1H, NH), 6.88 (s, 1H, ArH), 6.96 (d, $J = 2.2$ Hz, 2H, ArH), 7.41 (d, $J = 8.7$ Hz, 2H, ArH), 7.77 (d, $J = 2.2$ Hz, 1H, ArH), 8.14 (d, $J = 8.0$ Hz, 1H, ArH), 8.34 (brs, 1H, NH), 9.05 (brs, 1H, NH); Anal. calcd. for $C_{24}H_{25}ClN_6O_2$: C, 62.00; H, 5.42; Cl, 7.63; N, 18.08; O, 6.88, found: C, 62.15; H, 5.38; Cl, 7.50; N, 17.87; O, 7.04.

N^2 -(3-(7-Chloroquinolin-4-ylamino)propyl)-6-methyl- N^4 -

phenylpyrimidine-2,4-diamine (8a): Yield 65%; mp 199-200 °C; IR (KBr, cm⁻¹): 3231, 3052, 2954, 1583, 1526, 1496, 1401, 1365, 1284, 1266, 1130, 877, 855, 801, 754; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.88-1.95 (m, 2H, NCH₂CH₂CH₂N), 2.08 (s, 3H, CH₃), 3.37-3.40 (m, 4H, 2×NCH₂), 5.85 (s, 1H, ArH), 6.44 (d, *J* = 5.8 Hz, 1H, ArH), 6.78 (brs, 1H, NH), 6.88 (t, *J* = 7.3 Hz, 1H, ArH), 7.21 (t, *J* = 7.3 Hz, 2H, ArH), 7.30 (t, *J* = 5.1 Hz, 1H, NH), 7.41 (dd, *J* = 2.2, 9.5 Hz, 1H, ArH), 7.69 (d, *J* = 7.3 Hz, 2H, ArH), 7.76 (d, *J* = 2.2 Hz, 1H, ArH), 8.24 (d, *J* = 8.7 Hz, 1H, ArH), 8.33 (d, *J* = 5.1 Hz, 1H, ArH), 8.99 (brs, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 24.57, 28.74, 39.50, 41.22, 99.53, 118.39, 120.10, 122.07, 124.89, 124.95, 128.40, 129.42, 134.26, 141.76, 150.0, 150.94, 152.78, 162.02, 162.96, 165.88; ESI-HRMS (*m/z*) calculated for C₂₃H₂₃ClN₆: 418.1724, found: 419.1797 (M + H)⁺, 421.1778 (M + 2)⁺; Anal. calcd. for C₂₃H₂₃ClN₆: C, 65.94; H, 5.53; Cl, 8.46; N, 20.06, found: C, 66.04; H, 5.59; Cl, 8.53; N, 19.88.

N²-(3-(7-Chloroquinolin-4-ylamino)propyl)-N⁴-(4-fluoro phenyl)-6-methylpyrimidine-2,4-diamine (8b): Yield 65%; mp 230-232 °C; IR (KBr, cm⁻¹): 3232, 3049, 2954, 2922, 1613, 1583, 1504, 1449, 1407, 1365, 1284, 1207, 1130, 1079, 876; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.87-1.94 (m, 2H, NCH₂CH₂CH₂N), 2.07 (s, 3H, CH₃), 3.30-3.33 (m, 4H, 2×NCH₂), 5.81 (s, 1H, ArH), 6.43 (d, *J* = 5.8 Hz, 1H, ArH), 6.78 (brs, 1H, NH), 7.03 (t, *J* = 8.7 Hz, 2H, ArH), 7.30 (t, *J* = 5.1 Hz, 1H, NH), 7.40 (dd, *J* = 2.2, 8.7 Hz, 1H, ArH), 7.67-7.70 (m, 2H, ArH), 7.76 (d, *J* = 2.2 Hz, 1H, ArH), 8.23 (d, *J* = 9.5 Hz, 1H, ArH), 8.33 (d, *J* = 5.8 Hz, 1H, ArH), 9.02 (brs, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 24.57, 28.75, 39.50, 39.81, 99.55, 115.77, 115.99, 118.40, 121.65, 121.72, 124.89, 124.94, 128.42, 134.28, 138.13, 150.02, 150.96, 152.77, 156.69, 159.06, 161.91, 162.93, 165.92; ESI-HRMS (*m/z*) calculated for C₂₃H₂₂ClFN₆: 436.1579, found: 437.1331 (M + H)⁺, 439.1596 (M + 2)⁺; Anal. calcd. for C₂₃H₂₂ClFN₆: C, 63.23; H, 5.08; Cl, 8.11; F, 4.35; N, 19.24, found: C, 63.40; H, 5.02; Cl, 8.14; F, 4.55; N, 19.38.

N⁴-(4-Chlorophenyl)-N²-(3-(7-chloroquinolin-4-ylamino) propyl)-6-methylpyrimidine-2,4-diamine (8c): Yield 68%; mp 235-238 °C; IR (KBr, cm⁻¹): 3234, 3064, 2956, 1610, 1584, 1526, 1490, 1406, 1362, 1305, 1287, 1131, 1089, 878, 855; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.88-1.95 (m, 2H, NCH₂CH₂CH₂N), 2.08 (s, 3H, CH₃), 3.29-3.31 (m, 2H, NCH₂), 3.36-3.39 (m, 2H, NCH₂), 5.84 (s, 1H, ArH), 6.44 (d, *J* = 5.1 Hz, 1H, ArH), 6.84 (brs, 1H, NH), 7.22 (d, *J* = 8.7 Hz, 2H, ArH), 7.31 (t, *J* = 5.1 Hz, 1H, NH), 7.41 (dd, *J* = 2.2, 9.5 Hz, 1H, ArH), 7.73 (d, *J* = 8.7 Hz, 2H, ArH), 7.76 (d, *J* = 2.2 Hz, 1H, ArH), 8.24 (d, *J* = 8.7 Hz, 1H, ArH), 8.33 (d, *J* = 5.8 Hz, 1H, ArH), 9.15 (brs, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 24.51, 28.69, 39.50, 41.15, 99.47, 118.34, 121.33, 124.83, 124.87, 125.41, 128.38, 129.14, 134.24, 140.73, 149.95, 150.90, 152.68, 161.72, 162.85, 166.08; ESI-HRMS (*m/z*) calculated for C₂₃H₂₂Cl₂N₆: 452.1283, found: 453.0463 (M + H)⁺, 455.0528 (M + 2)⁺; Anal. calcd. for C₂₃H₂₂Cl₂N₆: C, 60.93; H, 4.89; Cl, 15.64; N, 18.54, found: C, 61.07; H, 5.00; Cl, 15.51; N, 18.66.

N⁴-(4-Bromophenyl)-N²-(3-(7-chloroquinolin-4-yl)amino) propyl)-6-methylpyrimidine-2,4-diamine (8d): Yield 75%; mp 240-243 °C; IR (KBr, cm⁻¹): 3235, 3163, 3062, 2957, 1612, 1583, 1527, 1487, 1405, 1361, 1304, 1285, 1263, 1135, 1074, 903, 878,

855, 812, 795; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.88-1.95 (m, 2H, NCH₂CH₂CH₂N), 2.08 (s, 3H, CH₃), 3.32-3.39 (m, 4H, 2×NCH₂), 5.83 (s, 1H, ArH), 6.44 (d, *J* = 5.8 Hz, 1H, ArH), 6.84 (brs, 1H, NH), 7.30 (t, *J* = 5.1 Hz, 1H, NH), 7.34 (d, *J* = 8.7 Hz, 2H, ArH), 7.41 (dd, *J* = 2.2, 8.7 Hz, 1H, ArH), 7.68 (d, *J* = 8.7 Hz, 2H, ArH), 7.75 (d, *J* = 2.2 Hz, 1H, ArH), 8.24 (d, *J* = 9.5 Hz, 1H, ArH), 8.33 (d, *J* = 5.1 Hz, 1H, ArH), 9.14 (brs, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 24.52, 28.68, 40.99, 41.14, 99.48, 113.25, 118.33, 121.74, 124.85, 128.38, 132.03, 134.23, 141.15, 149.94, 150.90, 152.70, 161.68, 162.83, 166.10; ESI-HRMS (*m/z*) calculated for C₂₃H₂₂BrClN₆: 496.0788, found: 497.0862 (M + H)⁺, 499.0841 (M + 2)⁺; Anal. calcd. for C₂₃H₂₂BrClN₆: C, 55.49; H, 4.45; Br, 16.05; Cl, 7.12; N, 16.88, found: C, 55.53; H, 4.39; Br, 16.12; Cl, 7.23; N, 16.76.

N²-(3-(7-Chloroquinolin-4-ylamino)propyl)-6-methyl-N⁴-p-tolylpyrimidine-2,4-diamine (8e): Yield 70%; mp 238-241 °C; IR (KBr, cm⁻¹): 3229, 3041, 2955, 1584, 1510, 1450, 1406, 1364, 1286, 1265, 1134, 878, 855; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.87-1.94 (m, 2H, NCH₂CH₂CH₂N), 2.05 (s, 3H, CH₃), 2.17 (s, 3H, CH₃), 3.30-3.32 (m, 2H, NCH₂), 3.35-3.38 (m, 2H, NCH₂), 5.80 (s, 1H, ArH), 6.43 (d, *J* = 5.8 Hz, 1H, ArH), 6.72 (brs, 1H, NH), 6.97 (d, *J* = 7.3 Hz, 2H, ArH), 7.30 (t, *J* = 5.8 Hz, 1H, NH), 7.40 (dd, *J* = 2.2, 8.7 Hz, 1H, ArH), 7.54 (d, *J* = 8.0 Hz, 2H, ArH), 7.75 (d, *J* = 2.2 Hz, 1H, ArH), 8.23 (d, *J* = 9.5 Hz, 1H, ArH), 8.33 (d, *J* = 5.1 Hz, 1H, ArH), 8.87 (brs, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 21.20, 24.54, 28.77, 39.50, 41.22, 99.52, 118.37, 120.25, 124.88, 124.95, 128.37, 129.81, 130.90, 134.25, 139.13, 149.97, 150.95, 152.76, 162.03, 162.96, 165.66; ESI-MS (*m/z*) calculated for C₂₄H₂₅ClN₆: 432.1829, found: 433.1929 (M + H)⁺, 435.2094 (M + 2)⁺; Anal. calcd. for C₂₄H₂₅ClN₆: C, 66.58; H, 5.82; Cl, 8.19; N, 19.41, found: C, 66.52; H, 5.94; Cl, 8.30; N, 19.21.

N²-(3-(7-Chloroquinolin-4-ylamino)propyl)-N⁴-(4-methoxy phenyl)-6-methylpyrimidine-2,4-diamine (8f): Yield 65%; mp 216-219 °C; IR (KBr, cm⁻¹): 3230, 3052, 2931, 1585, 1530, 1508, 1451, 1409, 1368, 1265, 1246, 1208, 1174, 1133, 1040, 877, 854, 797; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.87-1.94 (m, 2H, NCH₂CH₂CH₂N), 2.05 (s, 3H, CH₃), 3.30-3.38 (m, 4H, 2×NCH₂), 3.66 (s, 3H, OCH₃), 5.76 (s, 1H, ArH), 6.44 (d, *J* = 5.1 Hz, 1H, ArH), 6.69 (brs, 1H, NH), 6.80 (d, *J* = 9.5 Hz, 2H, ArH), 7.29 (t, *J* = 5.1 Hz, 1H, NH), 7.41 (dd, *J* = 2.2, 8.7 Hz, 1H, ArH), 7.54 (d, *J* = 8.7 Hz, 2H, ArH), 7.75 (d, *J* = 2.2 Hz, 1H, ArH), 8.24 (d, *J* = 8.7 Hz, 1H, ArH), 8.34 (d, *J* = 5.1 Hz, 1H, ArH), 8.79 (s, 1H, NH); ¹³C NMR (100 MHz, DMSO-*d*₆): δ 24.59, 28.81, 39.50, 41.28, 56.03, 99.59, 114.71, 118.42, 122.14, 124.93, 124.99, 128.42, 134.32, 134.79, 150.02, 151.01, 152.80, 155.13, 162.19, 163.04, 165.59; ESI-MS (*m/z*) calculated for C₂₄H₂₅ClN₆O: 448.1778, found: 449.1529 (M + H)⁺, 451.1860 (M + 2)⁺; Anal. calcd. for C₂₄H₂₅ClN₆O: C, 64.21; H, 5.61; Cl, 7.90; N, 18.72; O, 3.56, found: C, 64.29; H, 5.56; Cl, 7.88; N, 18.70; O, 3.64.

N²-(3-(7-Chloroquinolin-4-ylamino)propyl)-N⁴-(3,5-dimethoxyphenyl)-6-methylpyrimidine-2,4-diamine (8g): Yield 55%; mp 168-170 °C; IR (KBr, cm⁻¹): 3258, 2944, 1583, 1521, 1450, 1400, 1356, 1230, 1203, 1151, 1060, 869, 823; ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.96 (quintet, *J* = 6.5 Hz, 2H, NCH₂CH₂CH₂N), 2.08 (s, 3H, CH₃), 3.40-3.43 (m, 4H, 2×NCH₂), 3.68 (s, 6H, 2×OCH₃), 5.85 (s, 1H, ArH), 6.06 (s, 1H, ArH), 6.56

(d, $J = 5.1$ Hz, 1H, ArH), 6.91 (brs, 1H, NH), 6.98 (s, 2H, ArH), 7.49-7.52 (m, 1H, ArH), 7.81 (d, $J = 1.4$ Hz, 1H, ArH), 8.02 (brs, 1H, NH), 8.33-8.39 (m, 2H, ArH), 9.08 (s, 1H, NH); Anal. calcd. for $C_{25}H_{27}ClN_6O_2$: C, 62.69; H, 5.68; Cl, 7.40; N, 17.55; O, 6.68, found: C, 62.74; H, 5.88; Cl, 7.57; N, 17.50; O, 6.55.

N^2 -(4-(7-Chloroquinolin-4-ylamino)butyl)-6-methyl- N^4 -phenylpyrimidine-2,4-diamine (9a): Yield 65%; mp 210-212 °C; IR (KBr, cm^{-1}): 3249, 3060, 2957, 2934, 2865, 1626, 1581, 1551, 1512, 1436, 1365, 1227, 1204, 1146, 1081, 894, 864, 848, 790, 753; 1H NMR (400 MHz, DMSO- d_6): δ 1.64-1.70 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.06 (s, 3H, CH_3), 3.24-3.31 (m, 4H, $2 \times NCH_2$), 5.83 (s, 1H, ArH), 6.42 (d, $J = 5.8$ Hz, 1H, ArH), 6.71 (brs, 1H, NH), 6.87 (t, $J = 7.3$ Hz, 1H, ArH), 7.21 (t, $J = 7.3$ Hz, 2H, ArH), 7.29 (t, $J = 5.1$ Hz, 1H, NH), 7.40 (dd, $J = 2.2, 8.7$ Hz, 1H, ArH), 7.69 (d, $J = 8.0$ Hz, 2H, ArH), 7.74 (d, $J = 2.2$ Hz, 1H, ArH), 8.24 (d, $J = 8.7$ Hz, 1H, ArH), 8.33 (d, $J = 5.8$ Hz, 1H, ArH), 8.98 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.68, 25.43, 26.96, 40.44, 42.29, 98.63, 117.46, 119.13, 121.13, 123.96, 124.11, 127.46, 128.50, 133.36, 140.92, 149.09, 150.10, 151.87, 161.12, 162.06; ESI-HRMS (m/z) calculated for $C_{24}H_{25}ClN_6$: 432.1829, found: 433.2361 ($M + H$) $^+$, 435.2553 ($M + 2$) $^+$; Anal. calcd. for $C_{24}H_{25}ClN_6$: C, 66.58; H, 5.82; Cl, 8.19; N, 19.41, found: C, 66.64; H, 5.79; Cl, 8.06; N, 19.11.

N^2 -(4-(7-Chloroquinolin-4-ylamino)butyl)- N^4 -(4-fluoro phenyl)-6-methylpyrimidine-2,4-diamine (9b): Yield 52%; mp 188-200 °C; IR (KBr, cm^{-1}): 3229, 2955, 2868, 1613, 1580, 1505, 1450, 1412, 1366, 1335, 1206, 1137, 1079, 847, 827, 804; 1H NMR (400 MHz, DMSO- d_6): δ 1.63-1.69 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.07 (s, 3H, CH_3), 3.29-3.30 (m, 4H, $2 \times NCH_2$), 5.79 (s, 1H, ArH), 6.44 (d, $J = 5.1$ Hz, 1H, ArH), 6.72 (brs, 1H, NH), 7.05 (d, $J = 8.7$ Hz, 2H, ArH), 7.33 (brs, 1H, NH), 7.42 (d, $J = 8.7$ Hz, 1H, ArH), 7.68-7.69 (m, 2H, ArH), 7.75 (d, $J = 2.2$ Hz, 1H, ArH), 8.25 (d, $J = 9.5$ Hz, 1H, ArH), 8.34 (d, $J = 5.1$ Hz, 1H, ArH), 9.01 (brs, 1H, NH); ESI-HRMS (m/z) calculated for $C_{24}H_{24}ClFN_6$: 450.1735, found: 451.3680 ($M + H$) $^+$, 453.3787 ($M + 2$) $^+$; Anal. calcd. for $C_{24}H_{24}ClFN_6$: C, 63.92; H, 5.36; Cl, 7.86; F, 4.21; N, 18.64, found: C, 64.05; H, 5.29; Cl, 8.00; F, 4.27; N, 18.88.

N^4 -(4-Chlorophenyl)- N^2 -(4-(7-chloroquinolin-4-ylamino) butyl)-6-methylpyrimidine-2,4-diamine (9c): Yield 70%; mp 197-198 °C; IR (KBr, cm^{-1}): 3251, 3070, 2956, 2915, 1582, 1552, 1514, 1491, 1401, 1368, 1332, 1238, 1138, 1081, 908, 856, 823, 801; 1H NMR (400 MHz, DMSO- d_6): δ 1.64-1.71 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.08 (s, 3H, CH_3), 3.24-3.33 (m, 4H, $2 \times NCH_2$), 5.83 (s, 1H, ArH), 6.42 (d, $J = 5.8$ Hz, 1H, ArH), 6.79 (brs, 1H, NH), 7.25 (d, $J = 8.7$ Hz, 2H, ArH), 7.28 (t, $J = 5.1$ Hz, 1H, NH), 7.40 (dd, $J = 2.2, 8.7$ Hz, 1H, ArH), 7.73 (s, 1H, ArH), 7.76 (d, $J = 2.2$ Hz, 2H, ArH), 8.24 (d, $J = 8.7$ Hz, 1H, ArH), 8.34 (d, $J = 5.1$ Hz, 1H, ArH), 9.14 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.66, 25.44, 26.93, 42.29, 98.61, 117.46, 120.43, 123.94, 124.10, 124.51, 127.47, 128.28, 133.36, 139.94, 149.09, 150.10, 151.84, 160.85, 162.0; ESI-HRMS (m/z) calculated for $C_{24}H_{24}Cl_2N_6$: 466.1497, found: 467.1569 ($M + H$) $^+$, 469.1546 ($M + 2$) $^+$; Anal. calcd. for $C_{24}H_{24}Cl_2N_6$: C, 61.67; H, 5.18; Cl, 15.17; N, 17.98, found: C, 61.65; H, 5.23; Cl, 15.20; N, 18.14.

N^4 -(4-Bromophenyl)- N^2 -(4-(7-chloroquinolin-4-yl)amino) butyl)-6-methylpyrimidine-2,4-diamine (9d): Yield 70%; mp

192-194 °C; IR (KBr, cm^{-1}): 3246, 3059, 2957, 2914, 1641, 1583, 1551, 1515, 1488, 1397, 1368, 1237, 1138, 1072, 996, 908, 856, 821, 801; 1H NMR (400 MHz, DMSO- d_6): δ 1.64-1.71 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.08 (s, 3H, CH_3), 3.25-3.33 (m, 4H, $2 \times NCH_2$), 5.83 (s, 1H, ArH), 6.43 (d, $J = 5.8$ Hz, 1H, ArH), 6.80 (brs, 1H, NH), 7.29 (t, $J = 5.1$ Hz, 1H, NH), 7.37 (d, $J = 8.7$ Hz, 2H, ArH), 7.41 (dd, $J = 2.2, 8.7$ Hz, 1H, ArH), 7.70 (d, $J = 8.7$ Hz, 2H, ArH), 7.76 (d, $J = 2.2$ Hz, 1H, ArH), 8.25 (d, $J = 9.5$ Hz, 1H, ArH), 8.34 (d, $J = 5.1$ Hz, 1H, ArH), 9.14 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.66, 25.42, 26.91, 40.42, 42.28, 98.61, 112.35, 117.44, 120.85, 123.96, 124.10, 127.42, 131.17, 133.36, 140.35, 149.03, 150.12, 151.81, 160.81, 161.97; ESI-HRMS (m/z) calculated for $C_{24}H_{24}BrClN_6$: 510.0934, found: 511.0450 ($M + H$) $^+$, 513.0376 ($M + 2$) $^+$; Anal. calcd. for $C_{24}H_{24}BrClN_6$: C, 56.32; H, 4.73; Br, 15.61; Cl, 6.93; N, 16.42, found: C, 56.37; H, 4.84; Br, 15.58; Cl, 7.13; N, 16.31.

N^2 -(4-(7-Chloroquinolin-4-ylamino)butyl)-6-methyl- N^4 -*p*-tolylpyrimidine-2,4-diamine (9e): Yield 70%; mp 175-178 °C; IR (KBr, cm^{-1}): 3250, 3059, 2924, 2856, 1627, 1612, 1581, 1514, 1450, 1422, 1366, 1336, 1315, 1229, 1204, 1134, 1081, 846, 805; 1H NMR (400 MHz, DMSO- d_6): δ 1.64-1.70 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.05 (s, 3H, CH_3), 2.18 (s, 3H, CH_3), 3.25-3.30 (m, 4H, $2 \times NCH_2$), 5.79 (s, 1H, ArH), 6.43 (d, $J = 5.8$ Hz, 1H, ArH), 6.68 (brs, 1H, NH), 7.01 (d, $J = 8.0$ Hz, 2H, ArH), 7.30 (t, $J = 5.1$ Hz, 1H, NH), 7.41 (dd, $J = 2.2, 8.7$ Hz, 1H, ArH), 7.55 (d, $J = 8.0$ Hz, 2H, ArH), 7.75 (d, $J = 2.2$ Hz, 1H, ArH), 8.25 (d, $J = 9.5$ Hz, 1H, ArH), 8.34 (d, $J = 5.1$ Hz, 1H, ArH), 8.87 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 20.31, 23.63, 25.45, 27.02, 40.43, 42.31, 98.61, 117.47, 119.36, 123.95, 124.11, 127.44, 128.91, 130.01, 133.38, 138.30, 149.06, 150.12, 151.82, 161.17, 162.09, 164.75; ESI-MS (m/z) calculated for $C_{25}H_{27}ClN_6$: 446.2049, found: 447.2117 ($M + H$) $^+$, 449.2099 ($M + 2$) $^+$; Anal. calcd. for $C_{25}H_{27}ClN_6$: C, 67.18; H, 6.09; Cl, 7.93; N, 18.80, found: C, 67.30; H, 6.14; Cl, 8.12; N, 18.74.

N^2 -(4-(7-Chloroquinolin-4-ylamino)butyl)- N^4 -(4-methoxy phenyl)-6-methylpyrimidine-2,4-diamine (9f): Yield 62%; mp 134-138 °C; IR (KBr, cm^{-1}): 3281, 3181, 3067, 2945, 1582, 1508, 1445, 1369, 1241, 1138, 1029, 906, 856; 1H NMR (400 MHz, DMSO- d_6): δ 1.63-1.70 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.04 (s, 3H, CH_3), 3.24-3.32 (m, 4H, $2 \times NCH_2$), 3.66 (s, 3H, OCH_3), 5.75 (s, 1H, ArH), 6.43 (d, $J = 5.1$ Hz, 1H, ArH), 6.62 (brs, 1H, NH), 6.81 (d, $J = 8.7$ Hz, 2H, ArH), 7.29 (t, $J = 5.1$ Hz, 1H, NH), 7.41 (dd, $J = 2.2, 8.7$ Hz, 1H, ArH), 7.55 (d, $J = 8.7$ Hz, 2H, ArH), 7.75 (d, $J = 2.2$ Hz, 1H, ArH), 8.25 (d, $J = 8.7$ Hz, 1H, ArH), 8.34 (d, $J = 5.8$ Hz, 1H, ArH), 8.79 (brs, 1H, NH); ESI-HRMS (m/z) calculated for $C_{25}H_{27}ClN_6O$: 462.1935, found: 463.2074 ($M + H$) $^+$, 465.2463 ($M + 2$) $^+$; Anal. calcd. for $C_{25}H_{27}ClN_6O$: C, 64.86; H, 5.88; Cl, 7.66; N, 18.15; O, 3.46, found: C, 64.77; H, 5.92; Cl, 7.71; N, 18.28; O, 3.61.

N^2 -(4-(7-Chloroquinolin-4-ylamino)butyl)- N^4 -(3,5-dimethoxy phenyl)-6-methylpyrimidine-2,4-diamine (9g): Yield 55%; mp 188-190 °C; IR (KBr, cm^{-1}): 3259, 3142, 2932, 1581, 1476, 1452, 1354, 1333, 1248, 1233, 1204, 1155, 1073, 970, 846; 1H NMR (400 MHz, DMSO- d_6): δ 1.64-1.70 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 2.06 (s, 3H, CH_3), 3.27-3.35 (m, 4H, $2 \times NCH_2$), 3.67 (s, 6H, $2 \times OCH_3$), 5.81 (s, 1H, ArH), 6.05 (t, $J = 2.2$ Hz, 1H, ArH), 6.45 (d, $J = 5.8$ Hz, 1H, ArH), 6.77 (brs, 1H, NH), 6.98 (s, 2H, ArH),

7.42–7.45 (m, 2H, NH, ArH), 7.76 (d, $J = 2.2$ Hz, 1H, ArH), 8.26 (d, $J = 8.7$ Hz, 1H, ArH), 8.34 (d, $J = 5.8$ Hz, 1H, ArH), 8.99 (brs, 1H, NH); ^{13}C NMR (100 MHz, DMSO- d_6): δ 23.53, 25.33, 26.86, 40.48, 42.37, 54.93, 93.45, 97.34, 98.60, 117.24, 124.19, 126.66, 133.77, 142.55, 148.07, 150.57, 150.98, 160.42, 161.14, 161.94, 164.84; ESI-HRMS (m/z) calculated for $\text{C}_{26}\text{H}_{29}\text{ClN}_6\text{O}_2$: 492.2110, found: 493.2181 ($\text{M} + \text{H}$) $^+$; Anal. calcd. for $\text{C}_{26}\text{H}_{29}\text{ClN}_6\text{O}_2$: C, 63.34; H, 5.93; Cl, 7.19; N, 17.05; O, 6.49, found: C, 63.44; H, 5.85; Cl, 7.22; N, 17.16; O, 6.53.

10 Conclusions

In summary, we reported the synthesis and antimalarial activity evaluation of a series of 4-aminoquinoline-pyrimidine hybrids. The *in vitro* evaluation of these hybrids against D6 and W2 strains of *P. falciparum* depicted activity in the micromolar range. Also, these hybrids exhibited high selectivity indices and low toxicity against the tested cell lines. Further, the binding capability of compound **8d** was evaluated with heme to find out the probable mode of action of these hybrids. The best active compounds showed good interaction with Pf-DHFR-TS similar to the protein native substrate dihydrofolate. The compounds with best antimalarial activity also showed good ADMET properties. The promising antimalarial activity exhibited by the novel 4-aminoquinoline-pyrimidine conjugates and their good safety profile described in the present study emphasizes their potential for further development as antimalarial drugs.

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Synthesis, antimalarial activity, heme binding and docking studies of 4-aminoquinoline-pyrimidine based molecular hybrids

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Graphical Abstract

