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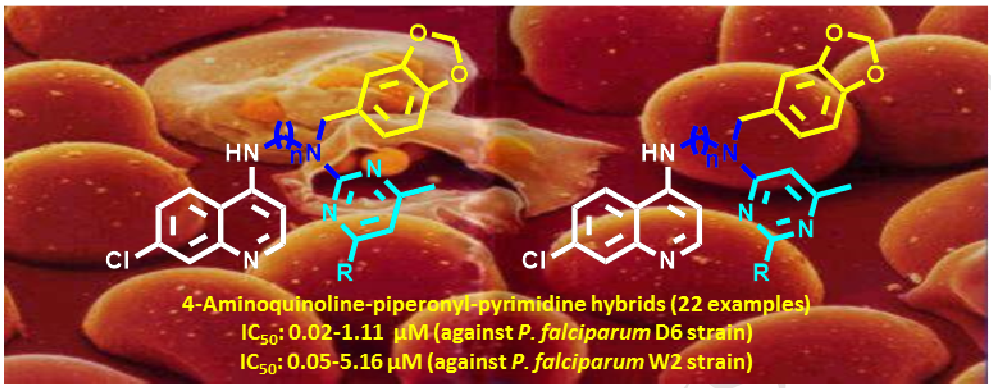

N-Piperonyl Substitution on Aminoquinoline-Pyrimidine Hybrids: Effect on the Antiplasmodial Potency

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Abstract: A series of 4-aminoquinoline-piperonyl-pyrimidine hybrids were synthesized with the aim of identifying compounds with enhanced antimalarial activity. All the synthesized molecules were evaluated *in vitro* against cultured *Plasmodium falciparum* W2 and D6 strains and exhibited potent antiplasmodial activities with IC_{50} values in the range of 0.02-5.16 µM. Out of the 22 synthesised hybrids, 12 were found to be better (up to eight-fold more active) than chloroquine (CQ), particularly against the CQ-resistant W2 strain of *P. falciparum* with no significant cytotoxicity towards the mammalian cells. Mechanistic studies reveal that these compounds bind with heme and computational docking studies showed good docking interactions within the active site of *Pf*-DHFR.

Key words: Antimalarial, aminoquinoline, pyrimidine, piperonyl, *Plasmodium falciparum*, hybrids.

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1. Introduction

Malaria is still a serious threat to humans all around the world despite a notable decrease in mortality rates during the last decade. According to the WHO World Malaria Report 2015, an estimated 438000 people died in 2014 and 214 million new clinical cases of malaria were reported with Sub-Saharan Africa being the most affected [1]. Malaria is caused by any of the five species of *Plasmodium viz., P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi;* among these *P. falciparum* is the most fatal and responsible for most of the malaria related deaths [2,3]. The traditional first line antimalarial drugs belonging to the 4-aminoquinoline class, such as chloroquine, amodiaquine and mefloquine (Figure 1) have been used for the treatment of malaria for a long time, but *P. falciparum* has developed resistance against these compounds. Chloroquine (CQ), one of the most widely used antimalarial agents discovered during World War II, was once hailed as a wonder antimalarial drug [4,5]. However, multi-drug resistant plasmodial strains have severely limited its clinical value as an antimalarial medication [6-8]. Amodiaquine (AQ) and mefloquine (MQ) are more potent than CQ, but toxicity (hepatotoxicity in AQ and CNS-toxicity in MQ), high cost and development of parasitic-resistance have greatly affected their use as antimalarials [9-11].

Currently, artemisinin-based combination therapy (ACT) is the best available treatment for the malaria caused by *P. falciparum* [12]. In ACT, artemisinin and its derivatives are combined with companion drugs such as AQ, MQ, lumefantrine, sulfadoxine/pyrimethamine, chlorproguanil/ dapsone and piperaquine (Figure 1) in an effort to contain the development of parasitic resistance towards rapid-acting artemisinin derivatives [13]. But recent reports of artemisinin resistance in five South-east Asian countries namely, Cambodia, the Lao People's Democratic Republic, Myanmar, Thailand and Vietnam, with emergence of multi-drug resistant *P. falciparum* strains in Cambodia-Thailand border [14-16] has worsened the situation, limiting the choice of drugs for treating severe malaria. This alarming situation calls for the development of novel antimalarials effective against resistant plasmodial strains, in a short time.

<space for figure 1>

To overcome the problem of drug-resistance, several strategies have been previously adopted including the use of combination therapy, development of analogs of the existing drugs as well as incorporation of drug resistance reversers [17-20]. The concept of 'hybrid' drug was put forward in which two or more pharmacophores are covalently hybridized in an anticipation that such molecules will concurrently act on the parent targets and show better efficacy and pharmacokinetics than the combination of drugs [21,22]. Our research group has successfully implemented this concept and developed rational molecular hybrids as anticancer, antimicrobial and antimalarial agents and demonstrated that the molecular hybridization approach can lead to the identification of highly potent molecules [23-30].

In order to study the effect of structural modifications at the linker chain of the 4-aminoquinoline-pyrimidine hybrids on their pharmacokinetic behavior and antimalarial activity, we thought to substitute the terminal free -NH of diamine linker with appropriate aryl/hetero-aryl functionality through a simple reductive amination strategy with an appropriate aldehyde. On literature survey, it was found that the 1,3-benzodioxole moiety is a part of various antimalarial agents (Figure 2) [31-34] and this scaffold was also shown to enhance the antimalarial activity and pharmacokinetic behaviour, when present in conjunction with 4-aminoquinolines through appropriate spacers [31-36]. Furthermore, assimilation of an intramolecular hydrogen-bonding moiety in the side chain of 4-aminoquinolines has been proposed to improve the antimalarial activity [37]. Hence, with these perspectives in our mind, we introduced the 1,3-benzodioxole moiety in form of the piperonyl group to our previously reported aminoquinoline-pyrimidine hybrids (Figure 3).

<space for figure 2>

<space for figure 3>

In the present study, we report the synthesis, antimalarial activity and cytotoxicity of a new series of 4-aminoquinoline-piperonyl-pyrimidine hybrids. In addition, heme binding studies and molecular docking with the reported crystal structure of *Pf*-DHFR-TS were performed to determine their possible mode of action. Also, ADME properties of the best active compounds were predicted to assess the pharmacokinetic behavior of the synthesized hybrids.

2. Results and Discussion

2.1 Synthesis

Scheme 1 shows the synthetic pathway for the synthesis of designed hybrids. Intermediates **2a** and **2b** were synthesized by the reaction of 4,7-dichloroquinoline (**1**) with diaminoalkanes (ethane-1,2-diamine and propane-1,3-diamine) under neat conditions at 110 ^{o}C [38]. Reaction of these intermediates (**2a** and **2b**) with piperonal (1,3-Benzodioxole-5-carboxaldehyde) at room temperature in ethanol resulted in the formation of Schiff bases **3a** and **3b**, respectively. Reduction of these intermediates with $NaBH_4$ in methanol yielded the corresponding aminoquinoline-piperonyl conjugates **4a** and **4b**. Reaction of 2,4-dichloro-6-methylpyrimidine with **4a** or **4b** at 60 ^{o}C in presence of trimethylamine (base) in THF yielded two sets of regioisomers (**5a** and **6a** from **4a**; **5b** and **6b** from **4b**). These regioisomers were then reacted with various secondary amines at elevated temperatures (110-120 ^{o}C) in DMF and K_2CO_3 as a base to give the final products (**7a-h** and **8a-h**) in 77-95% isolated yields.

<space for scheme 1>

2.2 Antimalarial activity and structure-activity relationship

All the synthesized compounds were screened for *in vitro* antimalarial potential against both CQ-resistant (W2) and CQ-sensitive (D6) strains of *P. falciparum via* the calculation of plasmodial LDH activity as previously reported [23,39-40]. The results of antimalarial activity (Table 1) indicated that all of the 22 synthesized hybrids showed moderate to excellent *in vitro* potency against both of the strains (W2 and D6) of *P. falciparum*. Ten compounds (**4a-b, 6b, 7a-d, 7f, 7h** and **8d**) showed comparable or better antimalarial activity than CQ against the sensitive strain while 12 hybrids (**4a-b, 7a-d, 7f, 7h, 8a, 8d-e** and **8h**) were more efficacious than CQ against the resistant W2-strain, with 2 compounds *viz.*, **4a** and **7d** having eight-fold better potency than CQ (both having IC_{50} value of 0.05 μM, compared to IC_{50} of 0.43 for CQ). Also, compounds **4b**, **7c**, **7f**, **7h**, **8d** and **8h** were found to be 2-5 fold more active (IC_{50} = 0.09-0.22 μM) than CQ against the CQ-resistant (W2) strain.

<space for Table 1>

The pyrimidine ring was introduced in conjunction with the piperonyl moiety in the 4-aminoquinoline-based hybrids with an anticipation of increased antimalarial potency. However, the antimalarial efficacy of the sets of pyrimidine regio-isomers **5a-b** and **6a-b** was lower in comparison with the aminoquinoline-piperonyl conjugates (**4a-b**). Interestingly, the antimalarial activity of the 4-aminoquinoline-piperonyl- pyrimidine hybrids (**7a-h** and **8a-h**) was regained with the substitution of the -Cl group of compounds **5a-b**, **6a-b** with various secondary carbocyclic amines. In general, it was observed that compounds in which -Cl group of pyrimidine was replaced with 4-ethyl piperazine (**7d, 7h, 8d** and **8h**) were found to be more active compared to other amino substituted compounds (**7a-c**, **7e-g**, **8a-c** and **8e-g**).

On comparing the antiplasmodial activity of the two sets of regioisomers (compounds **5a-b, 8a-h***vs* **6a-b, 7a-h**), differing in the point of attachment of the pyrimidine ring to the 4-aminoquinoline-piperonyl intermediates **4a-b**, it was found that hybrids in which pyrimidine ring is attached from its 4^{th}-position (compounds **5a-b** and **8a-h**) show better antiplasmodial activity than the compounds **6a-b** and **7a-h** in which the point of attachment of the pyrimidine ring to the 4-aminoquinoline-piperonyl group is 2^{nd}-position of the pyrimidine ring.

In general, it was also observed that increasing the length of the diamine spacer from n = 2 (compounds **4a, 5a, 6a, 7a-d** and **8a-d**) to n=3 (**4b, 5b, 6b, 7e-h** and **8e-h**) led to a decrease in the antimalarial potency of the synthesized hybrids. Overall, it was found that compounds (**7a-d**) having ethylene diamine linkers were most potent against both the strains of *P. falciparum* (IC_{50} values in the range of 0.02-0.05 µM against sensitive strain and 0.05-0.29 µM against resistant strain).

Cytotoxicity of these compounds was determined against VERO cells and almost all the synthesized compounds were found non-cytotoxic up to a high concentration of 9 µM (except compound **8c** and **8g** with cytotoxicity IC_{50} values of 6.86 and 8.68 µM, respectively). The selectivity index (SI) of antiplasmodial activity (ratio of IC_{50} for cytotoxicity to VERO cells and IC_{50} for antiplasmodial activity) for all the active compounds was found better than CQ against W2 strain while comparable to CQ against D6 strain.

2.3 Heme binding studies

Chloroquine (CQ) and other 4-aminoquinoline antimalarial compounds are believed to bind with ferriprotoporphyrin-IX (FPIX) inside the food vacuole of parasite via π-π stacking interaction of the quinoline ring with the porphyrin ring of heme, thus inhibiting the transformation of toxic heme to non-toxic hemozoin [41]. Malarial parasite *P. faciparum* in its intra-erythrocytic stages inside the human body degrades the red blood cells (RBCs) and feeds on the globin (protein part) of hemoglobin and converts the toxic heme by-product (Fe(III)-protoporphyrin-IX) to the non-toxic hemozoin. CQ binds with the heme and inhibits the formation of hemozoin (non-toxic to parasite) and the accumulation of toxic heme in the parasitic food vacuole leads to the death of parasite [42].

To understand the possible mode of action of the synthesized hybrids, we decided to study the binding interactions of **7d,** one of the most potent compounds of the series, with monomeric as well as dimeric heme, including the determination of binding stoichiometry as per standard methods reported in literature [43-45]. In brief, the decrease in the intensity of the Soret band at 402 nm of a solution of hemin in 40% DMSO with increasing concentrations of the drug, indicates the drug-heme binding with monomeric heme. Spectrophotometric titrations of compound **7d** with the monomeric heme solutions at two different pH (using 0.02 M HEPES buffer, pH 7.4 and 0.02 M MES buffer, pH 5.4) corresponding to the physiological pH and that of parasite's acidic digestive vacuole, showed a significant decrease in the intensity of the Soret band of monomeric heme (Figure 3). A continuous-variation method (Job's plot) was used to determine the binding stochiometry of **7d** with heme, keeping the total concentration of the solution constant at 10 μM. The Job's plot showed maxima at 0.5 molefraction of **7d** indicating a 1:1 binding stoichiometry with monomeric heme at both pH 7.4 (Figure 4) and 5.4 (Figure 5).

<space for figure 4>

<space for figure 5>

Similar effects were also observed for dimeric heme binding where the changes in absorbance band intensity at 362 nm (10 μM solution in 20mM pH 5.8 phosphate buffer)

were examined as a function of mole-fraction of compound **7d**, to get a Job's plot showing 1:1 binding stoichiometry (Figure 6).

<space for figure 6>

2.4 Binding mode analysis with Pf-DHFR

Antifolate antimalarials such as proguanil (prodrug of active agent cycloguanil) and pyrimethamine, which are inhibitors of the dihydrofolate reductase (DHFR) enzyme, comprise an important family of antimalarial drugs. DHFR is a well-known drug target as it catalyses the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate in the final step of the tetrahydrofolate biosynthesis. Inhibition of this enzyme would lead to halting of the folate production deemed essential for the parasite's survival. Effectiveness of antifolate antimalarials is failing due to the emergence of resistance attributed to the mutations in the active site amino acid residues of plasmodial DHFR (existing in *P. falciparum* as the bifunctional enzyme dihydrofolate reductase-thymidylate synthase, *Pf*-DHFR-TS) [46]. These mutations hinder the efficient binding of the conventional antifolate drugs in the binding site of *Pf*-DHFR. The double mutant (C59R, S108N) and quadruple mutant (N51I, C59R, S108N, I164L) *Pf*-DHFR show high resistance to pyrimethamine and cycloguanil but are still sensitive to WR99210, an experimental antimalarial agent [47]. The aim of our present study was to investigate the interactions of novel 4-aminoquinoline-piperonyl-pyrimidine hybrids in the active site of wild-type and quadruple-mutant *Pf*-DHFR-TS. The molecular docking studies of the most active compounds from the *in vitro* assay (**4a-b, 7a-b, 7d, 7f, 7h, 8d-e and 8h**), were performed in the binding pocket of both the wild type *Pf*-DHFR-TS (PDB ID:3QGT) and quadruple mutant *Pf*-DHFR-TS (PDB ID:3QG2) crystal structures. The results of docking studies along with the corresponding Glide Scores (GScore) for the binding poses of the compounds are shown in table 2. The results clearly indicate that the most active compounds in the study show substantial Glide energies on docking with the wild (Glide energy range -58.27 kcal mol^{-1} to -45.03 kcal mol^{-1}) as well as quadruple mutant (Glide energy range -57.98 kcal mol^{-1} to -33.09 kcal mol^{-1}) *Pf*-DHFR-TS.

<space for table 2>

Figure 7 and 8 show 3-dimensional binding poses of the two most active compounds (**7d** and **4a**) with wild type and mutant *Pf*-DHFR-TS. The 1,3-benzodioxole moiety of compound **4a** was found to show π-π interactions with Phe58 in case of mutant *Pf*-DHFR and with Phe116 in case of wild *Pf*-DHFR, respectively. In case of **7d**, the protonated nitrogen of piperazine was observed to form H-bond interactions with the main chain of Asp54 in the wild as well as the mutant *Pf*-DHFR. Interaction with Asp54 is of great importance for the binding substrate in the active site of *Pf*-DHFR enzyme, and its interaction with compound **7d** validates its binding with *Pf*-DHFR. Furthermore, π-π interactions could be present between the 1,3-benzodioxole moiety of **7d** with Phe116 in case of mutant *Pf*-DHFR and with Phe58 in case of wild *Pf*-DHFR as observed in Glide results. Phe58 is an active site residue of *Pf*-DHFR, and hence the plausible interaction with Phe58 is significant and justifies the incorporation of the piperonyl group in the aminoquinoline-pyrimidine hybrids. The docking results also correspond well to the experimental *in vitro* antiplasmodial activity and justify why there was no substantial increase in the antimalarial potency when moving from **4a** (aminoquinoline-piperonyl) to **7d** (aminoquinoline-piperonyl-pyrimidine), as the 1,3-benzodioxole moiety of **4a** shows good binding interactions within the enzyme's binding site in both wild and quadruple mutant DHFR. Also, compound **7d** was found to show the best activity in comparison to the other analogues (differing in substitution of secondary cyclic amines at pyrimidine ring) and this can be attributed to the interaction of piperazine's nitrogen with the Asp54 residue in the *Pf*-DHFRs binding site.

<space for figure 7>

<space for figure 8>

2.5 Prediction of pharmacokinetic properties

The pharmacokinetic analysis of the compounds under study was done by ADME predictions for the best active compounds using Qikprop v3.5 [48]. The most important of these parameters together with their permissible ranges are given in Tables 3 and 4. The compounds were examined for violations of the Lipinski's rule of five, which has a profound impact in determining the drug-likeness of compounds. An orally active compound should not have more than 4 violations of Lipinski's rule. It was found that

barring **4a** and **4b**, rest of the compounds showed two violations of the Lipinski's rule, owing to their high molecular weights (MW being greater than 500) and higher $logP_o/P_w$ predicted values. However, a higher MW is a characteristic feature for hybrid compounds as two or more pharmacophores are covalently linked together and this violation does not hold much significance as several high MW drugs are currently in clinical use. Furthermore, as can be seen from the predicted QPlogPo/w values, these hybrids are highly lipophilic and their antimalarial activity may be attributed to the latter physicochemical feature. However, literature reports suggest that although the antiplasmodial potencies of aminoquinolines seem to correlate with their lipophilicities, it is the drug accumulation which actually accounts for the observed activity; the latter not having a clear relationship with the observed lipophilicity [49]. Hence, increased activity of the present hybrids should not be solely attributed to their greater lipophilicities as drug accumulation at the acidic digestive vacuole of the parasite is related to the basicity of the molecule (presence of H-bond acceptors), while permeation inside the cytoplasm is related to its lipophilicity [50]. Furthermore, although the calculated logP values are greater than five, there have been reports of FDA approved drugs such as atorvastatin, montelukast, telmisartan, tacrolimus, olmesartan etc. having logP value >5 [51].

<space for table 3>

Prediction of oral drug absorption (PercentHumanOralAbsorption) was good enough for all the test set compounds with values better than the reference molecules pyrimethamine and cycloguanil. Studies have shown that the extent to which a compound is flexible also contributes to oral drug absorption to some extent, which in turn can be measured by the number of rotatable bonds (<15) and polar surface area (70$Å^2$–200 $Å^2$) [52]. All the compounds under study displayed less than 15 rotatable bonds and the polar surface area in the permissible range (Table 4). Other properties like Caco-2 cells permeability (QPPCaco) a measure of intestinal drug absorption also showed excellent results. QPPCaco predictions for compounds **7a, 7b, 7f** and **8e** showed exceptionally high values. QPlogKhsa, the prediction for human serum albumin binding also lied in the permissible range for all the active molecules. Further brain/blood partition coefficient

(QPlogBB) and the blood-brain barrier mimic MDCK cell permeability (QPPMDCK) showed satisfactory results.

3. Conclusions

The present work describes the synthesis of twenty-two 4-aminoquinoline-piperonyl-pyrimidine hybrids and the evaluation of their antimalarial activity against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *P. falciparum* and nine hybrids **4a-4b, 7a-7d, 7f, 7h** and **8d** were found to possess comparable to better antimalarial activity against both the strains (W2 and W6) of *P. falciparum.* Heme binding interactions of the most active compound **7d** were evaluated using standard spectrophotometric methods and strong binding interactions with monomeric as well as dimeric heme with a 1:1 binding stoichiometry, similar to the reference drug, CQ were observed. Almost all the compounds were found to possess no cytotoxicity against mammalian cells (VERO) up to a highest tested concentration of 9 μM. Molecular docking analysis of the most potent compounds were performed with both wild and mutant type *Pf*-DHFR-TS and the results corresponded well to the observed *in vitro* activities. The piperonyl group was found to show good π-π interactions with the binding site residues and flexibility of compound **4a** (aminoquinoline-piperonyl) over **7d** (aminoquinoline-piperonyl-pyrimidine) led to no significant increase in antimalarial activity. ADME predictions for the most active compounds were also made and the predicted pharmacokinetic factors were in permissible ranges and the present hybrids were estimated to have good oral bio-availabilities.

4. Experimental Section

All of the starting materials and reagents used in this study were purchased from Sigma Aldrich, Alfa Aesar and TCI. Analytical grade solvents were used without further purification for the chemical synthesis. The progress of the reaction was monitored by using TLC (Merck Kieselgel 60 F254, 0.2 mm thickness) and visualization was accomplished using iodine and UV light. All of the reaction intermediates and final compounds were purified over silica gel column chromatography (60-120 mesh silica; elution with 0–10% methanol–chloroform). ^{1}H and ^{13}C NMR spectra were recorded using Jeol Spectrospin spectrometer at 400 MHz and 100 MHz, respectively, and the values of

chemical shift are given in parts per million (ppm) on the delta scale (d). Tetramethylsilane (TMS) was used as an internal reference and $CDCl_3$ or DMSO-d_6 was used as solvent. Perkin-Elmer FT-IR spectrophotometer was used to record IR spectra, using KBr pellets and the values were expressed in cm^{-1}. Elemental analysis of the compounds was done on Elementar analysensysteme vario micro cube analyzer. Agilent Accurate Mass Q-TOF MS system was used to record mass spectra. Melting points of all the compounds were recorded using EZ-Melt automated melting point apparatus, Stanford Research Systems and are uncorrected. All compounds were sufficiently pure (>95%) for biological studies as per their analytical analysis, NMR and HRMS spectrums. Biological evaluation of the synthesized compounds for their antimalarial and mammalian cytotoxic activities have been done using known procedure described earlier [36,37].

*4.1 General procedure for the preparation of intermediates **2a-2b***

A mixture of 4,7-dichloroquinoline (1, 10.0 g, 50.49 mmol) and ethane-1,2-diamine (10.2 mL, 151.47 mmol) was stirred for 5h at 110-120 ^oC in neat condition (Scheme 1) [31]. Progress of the reaction was monitored by TLC and on completion, the reaction mixture was cooled to room temperature and ice-cold water was added. The solid product thus obtained was filtered and washed with excess water. Similarly, intermediate **2b** was prepared by employing propane-1,3-diamine. The crude-products thus obtained were crystallized by using ethanol and all the intermediates were characterized and matched with previously reported data [53].

*4.2 General procedure for the preparation of intermediates **3a-3b***

Intermediate **2a** (5.0 g, 22.65 mmol) was dissolved in ethanol at room temprature, then piperonal (3.4 g, 22.65 mmol) was added to it and the reaction mixture was stirred at room temperature for 12-16 h. The product **3a** was precipitated out as a white solid. After completion of the reaction, as monitored by TLC, the reaction mixture was filtered and solid product was used for the next step, without further purification. Similarly, intermediate **3b** was obtained and used for next step.

*4.3 General procedure for the preparation of intermediates **4a-4b***

Intermediate **3a** (7.8 g, 21.97 mmol) was dissolved in methanol and cooled to 0 ^{o}C then $NaBH_4$ (2.45g, 65.91 mmol) was added to it in fractions and reaction mixture was allowed to stirr at room temperature for 5-6 h. After completion of reaction as monitored by TLC, the excess methanol was evaporated *in vacuo* and ice-cold water was added. The solid product thus obtained was filtered and washed with excess of water. Similarly, intermediate **4b** was obtained.

*4.3.1 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**4a**)*: Pale yellow solid; yield: 93%; mp 96–98 ^{o}C; IR (KBr pellet): 3236, 2883, 2825, 1580, 1548, 1489, 1442, 1377, 1331, 1281, 1251, 1201, 1140, 1103, 1035, 929, 897, 865, 847, 807, 763, 636 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.49 (d, *J* = 5.3 Hz, 1H), 7.94 (d, *J* = 1.5 Hz, 1H), 7.68 (d, *J* = 9.1 Hz, 1H), 7.38-7.35 (m, 1H), 6.84 (s, 1H), 6.75 (s, 2H), 6.35 (d, *J* = 5.3 Hz, 1H), 5.93 (s, 2H), 5.85 (br s, 1H), 3.75 (s, 2H), 3.31 (m, 2H), 3.02 (t, *J* = 5.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 151.93, 149.80, 148.88, 147.75, 146.63, 134.69, 133.73, 128.39, 125.02, 121.25, 117.24, 108.50, 108.03, 100.91, 98.95, 53.00, 46.42, 41.93; ESI-HRMS (m/z) calcd for $C_{19}H_{18}ClN_3O_2$: 355.1088 (M^+), found: 356.1145 $(M + H)^+$, 358.1123 $(MH + 2)^+$; Anal. calcd. for $C_{19}H_{18}ClN_3O_2$: C, 64.13; H, 5.10; N, 11.81, found: C, 64.22; H, 5.28; N, 11.69.

*4.3.2 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)propane-1,3-diamine (**4b**)*: Off white solid; yield: 91%; mp 105–107 ^{o}C; IR (KBr pellet): 3240, 2888, 1580, 1547, 1501, 1486, 1449, 1366, 1256, 1227, 1139, 1037, 933, 899, 864, 840, 808, 765, 639 cm^{-1}; ^{1}H NMR (400 MHz; DMSO-d_6) δ (ppm): 8.23 (d, *J* = 5.3 Hz, 1H), 8.01 (d, *J* = 9.1 Hz, 1H), 7.63 (d, *J* = 2.2 Hz, 1H), 7.38 (br s, 1H), 7.23 (dd, *J* = 8.3, 2.2 Hz, 1H), 6.78 (s, 1H), 6.68-6.62 (m, 2H), 6.31 (d, *J* = 5.3 Hz, 1H), 5.82 (s, 2H), 3.20-3.15 (m, 4H), 2.47 (t, *J* = 6.1 Hz, 2H), 1.70-1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 151.91, 150.10, 149.06, 147.15, 145.81, 134.72, 133.30, 127.49, 123.96, 123.92, 120.99, 117.43, 108.41, 107.78, 100.65, 98.53, 52.74, 46.47, 41.13, 40.12, 39.91, 39.70, 39.50, 39.29, 39.08, 38.87, 27.75; ESI-HRMS (m/z) calcd for $C_{20}H_{20}ClN_3O_2$: 369.1244 (M^+), found: 370.1299 $(M + H)^+$, 372.1277 $(MH + 2)^+$; Anal. calcd. for $C_{20}H_{20}ClN_3O_2$: C, 64.95; H, 5.45; N, 11.36, found: C, 64.82; H, 5.58; N, 11.44.

*4.4 General procedure for the preparation of compounds **5a-5b** and **6a-6b***:

Intermediate **(4a)** (7g, 19.67 mmol) was dissolved in THF (70 ml) and triethyl amine (8.22 ml, 59.01 mmol) was added followed by the addition of 2, 4-dichloro-6-methyl-pyrimidine (3.2g, 19.67 mmol) and reaction mixture was allowed to stirr at 60 ^{o}C for 12 h. The progress of reaction was monitored by TLC, it resulted in the formation of two regioisomers **5a** and **6a** (**5a** as minor and **6a** as major). On the completion of reaction, the excess THF was evaporated and the residue was diluted with water and extracted using ethyl acetate (3 x 500 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude product thus obtained was purified by SiO_2 column chromatography using 5% MeOH–$CHCl_3$ as the eluent to give respective compounds **5a-b** and **6a-6b**.

*4.4.1 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N1-(4-chloro-6-methyl pyrimidin-2-yl)-N2-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**5a**)*: Off white solid; yield: 29%; mp 193–195 ^{o}C; IR (KBr pellet): 3284, 2935, 1584, 1543, 1491, 1434, 1366, 1334, 1292, 1248, 1202, 1121, 1095, 1044, 936, 866, 849, 809, 673 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.49 (d, *J* = 4.8 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 7.72-7.54 (m, 1H), 7.30 (d, *J* = 9.1 Hz, 1H), 6.77 (s, 1H), 6.73 (s, 2H), 6.57 (s, 1H), 6.46 (br s, 1H), 6.34 (d, *J* = 4.8 Hz, 1H), 5.91 (s, 2H), 4.85 (s, 2H), 3.98 (t, *J* = 5.4 Hz, 2H), 3.47-3.43 (m, 2H), 2.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.56, 151.81, 150.01, 148.75, 147.98, 147.06, 134.80, 131.49, 128.23, 124.97, 121.10, 117.00, 109.47, 108.15, 101.05, 98.39, 50.57, 45.19, 43.01, 29.47, 24.16; ESI-HRMS (m/z) calcd for $C_{24}H_{21}Cl_2N_5O_2$: 481.1072 (M^+), found: 482.1121 $(M + H)^+$, 484.1098 $(MH + 2)^+$; Anal. calcd. for $C_{24}H_{21}Cl_2N_5O_2$: C, 59.76; H, 4.39; N, 14.52, found: C, 59.68; H, 4.48; N, 14.63.

*4.4.2 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N1-(4-chloro-6-methyl pyrimidin-2-yl)-N3-(7-chloroquinolin-4-yl)propane-1, 3-diamine (**5b**)*: White solid; yield: 32%; mp 182–184 ^{o}C; IR (KBr pellet): 3241, 2925, 1590, 1579, 1539, 1502, 1488, 1446, 1370, 1291, 1244, 1139, 1099, 1044, 930, 866, 847, 813, 662 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.49 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 7.92-7.89 (m, 1H), 7.35 (dd, *J* = 9.1, 1.8 Hz, 1H), 6.78 (s, 1H), 6.72 (s, 2H), 6.48 (s, 1H), 6.37 (d, *J* = 4.8 Hz, 1H), 5.92 (s, 2H), 5.83 (br s, 1H), 4.81 (s, 2H), 3.69 (t, *J* = 6.4 Hz, 2H), 3.35-3.30 (m, 2H), 2.33 (s, 3H), 1.91 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 169.53, 169.03, 160.99, 151.89, 149.74, 149.16, 147.87, 146.91, 134.85, 131.86, 128.58, 125.02, 121.66, 121.11,

117.40, 108.72, 108.26, 108.11, 100.99, 98.87, 50.10, 43.36, 39.70, 25.99, 24.17; ESI-HRMS (m/z) calcd for $C_{25}H_{23}Cl_2N_5O_2$: 495.1229 (M^+), found: 496.1259 (M + H)$^+$, 498.1200 (MH + 2)$^+$; Anal. calcd. for $C_{25}H_{23}Cl_2N_5O_2$: C, 60.49; H, 4.67; N, 14.11, found: C, 60.52; H, 4.78; N, 14.29.

*4.4.3 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N1-(2-chloro-6-methyl pyrimidin-4-yl)-N2-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**6a**)*: White solid; yield: 63%; mp 239–241 ^{o}C; IR (KBr pellet): 3243, 3067, 2958, 1589, 1546, 1501, 1490, 1455, 1433, 1373, 1278, 1248, 1192, 1170, 1038, 970, 868, 761, 642 cm^{-1};^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.51 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 7.79 (d, *J* = 9.1 Hz, 1H), 7.39 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.60-6.59 (m, 2H), 6.50 (br s, 1H), 6.34 (d, *J* = 4.8 Hz, 1H), 6.19 (s, 1H), 5.95 (s, 2H), 4.56 (s, 2H), 4.11-4.08 (m, 2H), 3.53-3.49 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 167.25, 163.24, 159.06, 151.85, 149.88, 149.06, 147.46, 146.52, 133.46, 127.50, 124.22, 123.79, 119.68, 117.51, 108.26, 100.85, 98.90, 45.61, 23.09; ESI-HRMS (m/z) calcd for $C_{24}H_{21}Cl_2N_5O_2$: 481.1072 (M^+), found: 482.1101 (M + H)$^+$, 484.1079 (MH + 2)$^+$; Anal. calcd. for $C_{24}H_{21}Cl_2N_5O_2$: C, 59.76; H, 4.39; N, 14.52, found: C, 59.62; H, 4.51; N, 14.64.

*4.4.4 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N1-(2-chloro-6-methylpyrimidin-4-yl)-N3-(7-chloroquinolin-4-yl)propane-1,3-diamine (**6b**)*: White solid; yield: 57%; mp 160–162 ^{o}C; IR (KBr pellet): 3219, 2921, 1591, 1502, 1431, 1368, 1321, 1249, 1212, 1157, 1135, 1039, 970, 922, 845, 804, 769 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.50 (d, *J* = 5.4 Hz, 1H), 8.09-8.00 (m, 1H), 7.96-7.95 (m, 1H), 7.40 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.76 (d, *J* = 7.3, 1H), 6.61-6.59 (m, 2H), 6.39 (d, *J* = 5.4 Hz, 1H), 6.17 (s, 1H), 6.04 (br s, 1H), 5.95 (s, 2H), 4.53 (s, 2H), 3.76 (s, 2H), 3.41-3.37 (m, 2H), 2.30 (s, 3H), 1.95 (m, 2H)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 168.00, 163.96, 160.05, 151.58, 149.72, 148.92, 148.35, 147.27, 135.06, 128.31, 125.25, 121.86, 119.52, 117.35, 108.62, 106.69, 101.25, 100.25, 100.10, 98.78, 51.26, 44.89, 39.55, 26.02, 24.01; ESI-HRMS (m/z) calcd for $C_{25}H_{23}Cl_2N_5O_2$: 495.1229 (M^+), found: 496.1310 (M + H)$^+$, 498.1287 (MH + 2)$^+$; Anal. calcd. for $C_{25}H_{23}Cl_2N_5O_2$: C, 60.49; H, 4.67; N, 14.11, found: C, 60.37; H, 4.76; N, 14.26.

*4.5 General Procedure for the synthesis of compounds **7a-h** and **8a-h***

In a 100 ml round bottom flask, compound **5a-b** and **6a-d** (1 mmol) was taken and dissolved in 10 ml of DMF then K_2CO_3 (3 eq.) was added to it. To this, a solution of respective amine (3 eq.) in DMF (5 ml) was added dropwise. Reaction mixture was allowed to stir at 100-120 ^oC for 10 hours monitored by TLC. After completion, water (50 ml) was added to reaction mixture and it was extracted with EtOAc (2 × 25 ml). Organic layer was then collected, washed with water (2 × 100 ml) and brine, dried over Na_2SO_4 and finally excess of solvent was evaporated under vacuum. The crude product thus obtained was purified by SiO_2 column chromatography using 0-5% MeOH–$CHCl_3$ as the eluent to yield respective compounds **7a-h** and **8a-h**.

*4.5.1 N^1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N^2-(7-chloroquinolin -4-yl)-N^1-(6-methyl-2-(piperidin-1-yl)pyrimidin-4-yl)ethane-1,2-diamine (**7a**)*: Off white solid; yield: 90%; mp 175–177 ^oC; IR (KBr pellet): 3320, 2921, 2849, 1582, 1487, 1447, 1416, 1370, 1335, 1311, 1279, 1242, 1193, 1135, 1080, 1036, 993, 937, 921, 872, 854, 793 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.48 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.38 (d, *J* = 9.1 Hz, 1H), 7.25-7.22 (m, 1H), 7.13 (br s, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.64-6.62 (m, 2H), 6.24 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.68 (s, 1H), 4.51 (s, 2H), 4.09 (t, *J* = 4.5 Hz, 2H), 3.86-3.83 (m, 4H), 3.46-3.43 (m, 2H), 2.19 (s,3H), 1.66-1.58 (m, 6H)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.29, 164.19, 161.60, 151.77, 150.29, 148.84, 148.19, 146.92, 134.77, 130.66, 128.24, 125.13, 122.07, 119.45, 117.06, 108.44, 106.78, 101.10, 98.02, 91.17, 50.91, 45.26, 43.68, 29.66, 25.85, 24.77, 24.66; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_2$: 530.2197 (M^+), found: 531.2292 $(M+H)^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_2$: C, 65.59; H, 5.88; N, 15.83, found: C, 65.71; H, 5.99; N, 15.91.

*4.5.2 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yl)ethane-1,2-diamine (**7b**)*: White solid; yield: 95%; mp 179–181 ^oC; IR (KBr pellet): 3301, 2967, 2864, 1571, 1490, 1472, 1456, 1414, 1378, 1336, 1243, 1137, 1033, 921, 790 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.47 (d, *J* = 4.8 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.35-7.33 (m, 2H), 7.18 (dd, *J* = 9.1, 1.8 Hz, 1H), 6.72 (d, *J* = 7.3 Hz, 1H), 6.65-6.63 (m, 2H), 6.24 (d, *J* = 4.8 Hz, 1H), 5.91 (s, 2H), 5.70 (s, 1H), 4.52 (s, 2H), 4.12 (t, *J* = 4.8 Hz, 2H), 3.70-3.63 (m, 4H), 3.46-3.42 (m, 2H), 2.22 (s, 3H), 1.98-1.91 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.19, 163.96, 160.31, 151.90, 150.15, 149.00, 148.12, 146.85, 134.56, 130.65, 128.36, 124.68, 121.89, 119.38,

117.11, 108.37, 106.72, 101.04, 97.94, 90.94, 77.351, 77.00, 76.67, 50.84, 46.88, 45.17, 43.62, 25.47, 24.61; ESI-HRMS (m/z) calcd for $C_{28}H_{29}ClN_6O_2$: 516.2041 (M^+), found: 517.2125 $(M + H)^+$, 519.2108 $(MH + 2)^+$; Anal. calcd. for $C_{28}H_{29}ClN_6O_2$: C, 65.05; H, 5.65; N, 16.25, found: C, 65.11; H, 5.54; N, 16.36.

*4.5.3 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(6-methyl-2-morpholinopyrimidin-4-yl)ethane-1,2-diamine (**7c**)*: White solid; yield: 92%; mp 209–211 ^oC; IR (KBr pellet): 2957, 2919, 1576, 1481, 1444, 1421, 1369, 1246, 1183, 1136, 1111, 1038, 933, 861, 795, 643 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.49 (d, *J* = 4.8 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 7.36 (d, *J* = 9.1 Hz, 1H), 7.28 (d, *J* = 2.4 Hz, 1H), 6.83 (br s, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.64-6.62 (m, 2H), 6.26 (d, *J* = 5.4 Hz, 1H), 5.92 (s, 2H), 5.76 (s, 1H), 4.54 (s, 1H), 4.08 (t, *J* = 4.8 Hz, 2H), 3.86-3.82 (m, 4H), 3.78-3.75 (m, 4H), 3.48-3.44 (m, 2H), 2.21 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.31, 164.10, 161.62, 151.94, 149.98, 148.96, 148.20, 146.96, 134.74, 130.30, 128.52, 125.29, 121.64, 119.40, 116.99, 108.47, 106.70, 101.14, 98.11, 92.30, 77.31, 77.00, 76.68, 66.85, 50.92, 45.32, 44.74, 43.43, 24.56; ESI-HRMS (m/z) calcd for $C_{28}H_{29}ClN_6O_3$: 532.1990 (M^+), found: 533.2064 $(M + H)^+$, 535.2045 $(MH + 2)^+$; Anal. calcd. for $C_{28}H_{29}ClN_6O_3$: C, 63.09; H, 5.48; N, 15.77, found: C, 63.23; H, 5.56; N, 15.89.

*4.5.4 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(2-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-4-yl)ethane-1,2-diamine (**7d**)*: Pale yellow solid; yield: 84%; mp 163–165 ^oC; IR (KBr pellet): 3287, 2928, 1577, 1488, 1442, 1418, 1374, 1311, 1242, 1193, 1133, 1030, 921, 796 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.48 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.37 (d, *J* = 8.5 Hz, 1H), 7.27-7.24 (m, 1H), 6.96 (br s, 1H), 6.72 (d, *J* = 7.9 Hz, 1H), 6.64-6.62 (m, 2H), 6.25 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.72 (s, 1H), 4.52 (s, 2H), 4.09 (t, *J* = 4.8 Hz, 2H), 3.90 (t, *J* = 5.1 Hz, 4H), 3.47-3.43 (m, 2H), 2.52-2.41 (m, 6H), 2.50 (s, 3H), 1.09 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.25, 164.10, 161.46, 151.98, 150.04, 148.97, 148.16, 146.90, 134.64, 130.44, 128.38, 125.53, 121.79, 119.40, 117.02, 108.44, 106.70, 101.08, 98.12, 91.83, 52.73, 52.38, 50.87, 45.25, 44.18, 43.49, 24.57, 11.84; ESI-HRMS (m/z) calcd for $C_{30}H_{34}ClN_7O_2$: 559.2463 (M^+), found: 560.2540 $(M + H)^+$, 562.2516 $(MH + 2)^+$; Anal. calcd. for $C_{30}H_{34}ClN_7O_2$: C, 64.33; H, 6.12; N, 17.51, found: C, 64.44; H, 6.25; N, 17.39.

*4.5.5 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(6-methyl-2-(piperidin-1-yl)pyrimidin-4-yl)propane-1,3-diamine (**7e**)*: Off white solid; yield: 94%; mp 166–168 ^{o}C; IR (KBr pellet): 3228, 3064, 2926, 1582, 1501, 1487, 1444, 1419, 1369, 1329, 1282, 1245, 1187, 1137, 1040, 939, 846, 802, 785, 766 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.51 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 2.4 Hz, 1H), 7.41 (d, *J* = 9.1 Hz, 1H), 7.30 (dd, *J* = 9.1, 1.8 Hz,1H), 6.70-6.68 (m, 2H), 6.63-6.61 (m, 1H), 6.34 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.62 (s, 1H), 5.17-5.10 (m, 1H), 4.52 (s, 2H), 3.74 (t, *J* = 5.4 Hz, 4H), 3.67 (t, *J* = 6.7 Hz, 2H), 3.38-3.34 (m, 2H), 2.17 (s, 3H), 2.02 (m, 2H), 1.61-1.60 (m, 2H), 1.55-1.53 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.35, 162.80, 161.61, 151.74, 149.61, 148.91, 147.92, 146.69, 134.81, 131.79, 128.45, 125.13, 121.04, 120.06, 117.11, 108.14, 107.44, 100.96, 98.58, 90.72, 77.32, 77.00, 76.69, 50.57, 45.12, 44.78, 41.20, 26.46, 25.75, 24.90, 24.57; ESI-HRMS (m/z) calcd for $C_{30}H_{33}ClN_6O_2$: 544.2354 (M^+), found: 545.2443 $(M + H)^+$, 547.2431 $(MH + 2)^+$; Anal. calcd. for $C_{30}H_{33}ClN_6O_2$: C, 66.10; H, 6.10; N, 15.42, found: C, 66.28; H, 6.19; N, 15.51.

*4.5.6 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yl)propane-1,3-diamine (**7f**)*: White solid; yield: 94%; mp 207–209 ^{o}C; IR (KBr pellet): 3230, 2956, 2871, 1584, 1563, 1503, 1473, 1455, 1420, 1371, 1332, 1280, 1247, 1203, 1137, 1041, 926, 862, 846, 804, 785, 593 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm):8.50 (d, *J* = 5.4 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 7.41 (d, *J* = 9.1 Hz, 1H), 7.29 (dd, *J* = 9.1, 1.8 Hz, 1H), 6.70-6.68 (m, 2H), 6.63-6.61 (m, 1H), 6.35 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.64 (s, 1H), 5.19 (br s, 1H), 4.52 (s, 2H), 3.69 (t, *J* = 6.1 Hz, 2H), 3.52 (t, *J* = 6.7 Hz, 4H), 3.39-3.35 (m, 2H), 2.19 (s, 3H), 2.06-1.99 (m, 2H), 1.89-1.86 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.27, 162.68, 160.39, 151.87, 149.54, 149.05, 147.91, 146.67, 134.74, 131.79, 128.58, 125.06, 121.00, 120.03, 117.14, 108.15, 107.43, 100.96, 98.88, 90.57, 50.45, 46.46, 45.02, 41.18, 26.43, 25.38, 24.55; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_2$: 530.2197 (M^+), found: 531.2238 $(M + H)^+$, 533.2232 $(MH + 2)^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_2$: C, 65.59; H, 5.88; N, 15.83, found: C, 65.71; H, 5.99; N, 15.93.

*4.5.7 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(6-methyl-2-morpholinopyrimidin-4-yl)propane-1,3-diamine (**7g**)*: Off white solid; yield: 93%; mp 182–184 ^{o}C; IR (KBr pellet): 3225, 3064, 2954, 1583, 1501, 1487, 1443, 1421, 1367,

1329, 1280, 1243, 1184, 1138, 1116, 1039, 997, 964, 937, 903, 860, 845, 799, 784, 766, 639, 459 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.51 (d, *J* = 5.4 Hz, 1H), 7.96 (d, *J* = 2.4 Hz, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 9.1, 1.8 Hz, 1H), 6.71-6.67 (m, 2H), 6.62 (d, *J* = 7.9 Hz, 1H), 6.34 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.70 (s, 1H), 5.05-5.01 (m, 1H), 4.55 (s, 2H), 3.73-3.63 (m, 10H), 3.37-3.32 (m, 2H), 2.17 (s, 3H), 2.06-1.99 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.40, 162.75, 161.66, 151.84, 149.45, 149.01, 147.97, 146.78, 134.84, 131.58, 128.69, 125.19, 120.79, 120.07, 117.06, 108.20, 107.39, 101.01, 98.90, 91.75, 66.82, 50.63, 45.11, 44.33, 41.09, 26.53, 24.47; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_3$: 546.2146 (M^+), found: 547.2255 $(M + H)^+$, 549.2239 $(MH + 2)^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_3$: C, 63.67; H, 5.71; N, 15.36, found: C, 63.88; H, 5.89; N, 15.48.

*4.5.8 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(2-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-4-yl)propane-1,3-diamine (**7h**)*: Off white solid; yield: 88%; mp 170–172 °C; IR (KBr pellet): 3213, 2967, 1581, 1557, 1489, 1474, 1445, 1417, 1368, 1332, 1277, 1238, 1169, 1141, 1083, 1038, 996, 966, 937, 839, 790, 530 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.51 (d, *J* = 4.8 Hz, 1H), 7.94 (d, *J* = 1.8 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.31 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.69 (t, *J* = 8.5 Hz, 2H), 6.61 (d, *J* = 7.9 Hz, 1H), 6.34 (d, *J* = 5.4 Hz, 1H), 5.91 (s, 2H), 5.65 (s, 1H), 5.11 (br s, 1H), 4.53 (s, 2H), 3.77 (t, *J* = 4.8 Hz, 4H), 3.69-3.62 (m, 2H), 3.37-3.33 (m, 2H), 2.43-2.38 (m, 6H), 2.16 (s, 3H), 2.05-1.98 (m, 2H), 1.09 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.40, 162.78, 161.57, 151.92, 149.45, 149.07, 147.97, 146.75, 134.81, 128.72, 125.23, 120.82, 120.05, 117.10, 108.19, 107.41, 100.99, 98.93, 91.34, 52.72, 52.41, 50.63, 45.13, 43.75, 41.17, 26.55, 24.53, 11.88; ESI-HRMS (m/z) calcd for $C_{31}H_{36}ClN_7O_2$: 573.2619 (M^+), found: 574.2691 $(M + H)^+$, 576.2670 $(MH + 2)^+$; Anal. calcd. for $C_{31}H_{36}ClN_7O_2$: C, 64.85; H, 6.32; N, 17.08, found: C, 64.93; H, 6.39; N, 17.24.

*4.5.9 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-(piperidin-1-yl)pyrimidin-2-yl)ethane-1,2-diamine (**8a**)*: White solid; yield: 76%; mp 151–153 °C; IR (KBr pellet): 3295, 2932, 1583, 1553, 1479, 1425, 1368, 1329, 1245, 1224, 1132, 1040, 979, 938, 872, 802 cm^{-1}; ^1H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.46 (d, *J* = 4.8 Hz, 1H), 7.88 (d, *J* = 1.8 Hz, 1H), 7.50 (br s, 1H), 7.21 (d, *J* = 7.3 Hz,1H), 6.82 (s, 1H), 6.75-6.69 (m, 2H), 6.22 (d, *J* = 4.8 Hz, 1H), 5.89 (d, *J* = 7.3 Hz, 3H), 5.90-5.88 (m,

3H), 4.81 (s, 2H), 3.99 (t, *J* = 4.8 Hz, 2H), 3.59-3.54 (m, 4H), 3.39-3.36 (m, 2H), 1.64-1.63 (m, 3H), 1.57-1.55 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.84, 162.65, 152.03, 150.42, 149.05, 147.76, 146.56, 134.40, 133.22, 128.35, 124.38, 122.23, 120.75, 117.24, 108.13, 107.86, 100.83, 97.97, 92.17, 49.99, 45.15, 44.45, 43.92, 25.46, 24.66, 24.55; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_2$: 530.2197 (M^+), found: 531.2265 (M + H)$^+$, 533.2251 (MH + 2)$^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_2$: C, 65.59; H, 5.88; N, 15.83, found: C, 65.46; H, 5.77; N, 15.95.

*4.5.10 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-(pyrrolidin-1-yl)pyrimidin-2-yl)ethane-1,2-diamine (**8b**)*: White solid; yield: 79%; mp 149–151 ^{o}C; IR (KBr pellet): 3293,2922, 2856, 1583, 1477, 1411, 1375, 1243, 1143, 1039, 937, 840, 791 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.46 (d, *J* = 5.4 Hz, 1H), 7.88 (d, *J* = 2.4 Hz, 1H), 7.53-7.43 (m, 2H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.84 (s, 1H), 6.75 (dd, *J* = 7.9, 1.2 Hz, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 6.22 (d, *J* = 5.4 Hz, 1H), 5.88 (s, 2H), 5.70 (s, 1H), 4.84 (s, 2H), 4.01 (t, 2H), 3.44-3.35 (m, 6H), 2.37 (s, 3H), 2.03-1.87 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.76, 161.02, 151.76, 150.64, 148.75, 147.75, 146.57, 134.57, 133.36, 128.11, 124.41, 122.21, 120.81, 117.23, 108.20, 107.87, 100.85, 97.94, 93.04, 49.88, 46.24, 44.33, 43.99, 24.37; ESI-HRMS (m/z) calcd for $C_{28}H_{29}ClN_6O_2$: 516.2041 (M^+), found: 517.2105 (M + H)$^+$, 519.2087 (MH + 2)$^+$; Anal. calcd. for $C_{28}H_{29}ClN_6O_2$: C, 65.05; H, 5.65; N, 16.25, found: C, 64.97; H, 5.76; N, 16.33.

*4.5.11 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-morpholinopyrimidin-2-yl)ethane-1,2-diamine (**8c**)*: White solid; yield: 87%; mp 123–125 ^{o}C; IR (KBr pellet): 3310, 2918, 2857, 1584, 1562, 1470, 1448, 1419, 1377, 1248, 1118, 1036, 927, 787, 642 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.47 (d, *J* = 5.4 Hz, 1H), 7.90 (d, *J* = 1.8 Hz, 1H), 7.50 (br s, 1H), 7.23 (dd, *J* = 9.1, 1.8 Hz, 2H), 6.78 (s, 1H), 6.71 (t, *J* = 9.1 Hz, 2H), 6.25 (d, *J* = 5.4 Hz, 1H), 5.89 (s, 3H), 4.81 (s, 2H), 4.01 (t, *J* = 4.8 Hz, 2H), 3.72 (t, *J* = 4.2 Hz, 4H), 3.56 (t, *J* = 4.2 Hz, 4H),3.41-3.37 (m, 2H), 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.68, 151.90, 150.35, 148.89, 147.81, 146.63, 134.52, 132.86, 128.28, 124.53, 121.98, 120.63, 117.15, 107.93, 100.88, 98.05, 92.43, 92.16, 66.47, 50.00, 49.17, 44.55, 44.25, 43.76, 41.70, 24.55; ESI-HRMS (m/z) calcd for $C_{28}H_{29}ClN_6O_3$: 532.1990 (M^+), found: 533.2086 (M + H)$^+$, 535.2068 (MH + 2)$^+$; Anal. calcd. for $C_{28}H_{29}ClN_6O_3$: C, 63.09; H, 5.48; N, 15.77, found: C, 62.95; H, 5.59; N, 15.85.

*4.5.12 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N2-(7-chloroquinolin-4-yl)-N1-(4-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-2-yl)ethane-1,2-diamine (**8d**)*: White solid; yield: 77%; mp 123–125 ^{o}C; IR (KBr pellet): 2968, 1580, 1485, 1442, 1421, 1375, 1302, 1240, 1214, 1168, 1139, 1095, 1039, 1008, 937, 876, 848, 787, 639 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.47 (d, *J* = 4.8 Hz, 1H), 7.89 (d, *J* = 1.8 Hz, 1H), 7.48 (br s, 1H), 7.40-7.31 (m, 1H), 7.22 (d, *J* = 8.5 Hz, 1H), 6.80 (s, 1H), 6.75-6.69 (m, 2H), 6.23 (d, *J* = 5.4 Hz, 1H), 5.90-5.89 (m, 3H), 4.81 (s, 2H), 4.00 (t, *J* = 4.8 Hz, 2H), 3.66-3.57 (m, 4H), 3.39-3.38 (m, 3H), 2.47-2.38 (m, 8H), 1.08 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 162.93, 162.76, 151.86, 150.46, 148.85, 147.80, 146.62, 134.54, 133.01, 128.22, 124.60, 122.12, 120.72, 117.17, 108.06, 107.92, 100.87, 98.01, 92.25, 77.32, 77.00, 76.68, 52.37, 52.29, 50.01, 44.48, 43.94, 43.86, 29.66, 24.56, 11.85; ESI-HRMS (m/z) calcd for $C_{30}H_{34}ClN_7O_2$: 559.2463 (M^+), found: 560.2503 $(M + H)^+$, 562.2486 $(MH + 2)^+$; Anal. calcd. for $C_{30}H_{34}ClN_7O_2$: C, 64.33; H, 6.12; N, 17.51, found: C, 64.22; H, 6.24; N, 17.65.

*4.5.13 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-(piperidin-1-yl)pyrimidin-2-yl)propane-1,3-diamine (**8e**)*: White solid; yield: 83%; mp 148–150 ^{o}C; IR (KBr pellet): 3231, 3065, 2925, 2853, 1574, 1557, 1500, 1485, 1444, 1417, 1371, 1331, 1320, 1279, 1243, 1207, 1126, 1098, 1079, 1042, 982, 939, 928, 896, 863, 846, 800, 785, 764, 698, 661, 639, 549, 461 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.47 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.29 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.82 (s, 1H), 6.73-6.68 (m, 2H), 6.37 (d, *J* = 5.4 Hz, 1H), 6.30 (br s, 1H), 5.90 (s, 2H), 5.80 (s, 1H), 4.75 (s, 2H), 3.71 (t, *J* = 5.4 Hz, 2H), 3.52 (t, *J* = 5.4 Hz, 4H), 3.36-3.32 (m, 2H), 2.28 (s, 3H), 1.89-1.85 (m, 2H), 1.64-1.63 (m, 2H), 1.54-1.52 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 165.62, 162.61, 162.10, 151.89, 150.11, 149.19, 147.65, 146.39, 134.65, 133.61, 128.41, 124.69, 121.92, 120.71, 117.61, 108.16, 107.84, 100.80, 98.89, 91.67, 49.87, 44.99, 43.11, 39.84, 26.32, 25.43, 24.95, 24.75; ESI-HRMS (m/z) calcd for $C_{30}H_{33}ClN_6O_2$: 544.2354 (M^+), found: 545.2421 $(M + H)^+$, 547.2407 $(MH + 2)^+$; Anal. calcd. for $C_{30}H_{33}ClN_6O_2$: C, 66.10; H, 6.10; N, 15.42, found: C, 66.22; H, 6.27; N, 15.56.

*4.5.14 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-(pyrrolidin-1-yl)pyrimidin-2-yl)propane-1,3-diamine (**8f**)*: White solid; yield: 93%; mp

158–160 ^{o}C; IR (KBr pellet): 3222, 2921, 1583, 1501, 1475, 1419, 1370, 1242, 1141, 1043, 929, 847, 786 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.48 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 2.4 Hz, 1H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.28 (dd, *J* = 9.1, 2.4 Hz, 1H), 6.85-6.84 (m, 1H), 6.74-6.68 (m, 2H), 6.37 (d, *J* = 5.4 Hz, 1H), 6.30 (br s, 1H), 5.90 (s, 2H), 5.58 (s, 1H), 4.78 (s, 2H), 3.72 (t, *J* = 6.1 Hz, 2H), 3.51-3.33 (m, 6H), 2.27 (s, 3H), 1.91-1.86 (m, 6H)); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 164.68, 161.92, 160.94, 151.71, 150.24, 149.00, 147.63, 146.40, 134.75, 133.69, 128.25, 124.71, 121.98, 120.84, 117.58, 108.30, 107.84, 100.80, 98.89, 92.53, 49.70, 46.03, 43.02, 40.00, 29.67, 26.30, 24.71; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_2$: 530.2197 (M^+), found: 531.2276 $(M + H)^+$, 533.2258 $(MH + 2)^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_2$: C, 65.59; H, 5.88; N, 15.83, found: C, 65.47; H, 5.96; N, 15.76.

*4.5.15 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(4-methyl-6-morpholinopyrimidin-2-yl)propane-1,3-diamine (**8g**)*: White solid; yield: 92%; mp 177–179 ^{o}C; IR (KBr pellet): 3229, 2921, 1581, 1500, 1486, 1444, 1417, 1369, 1332, 1281, 1241, 1190, 1138, 1119, 1041, 991, 939, 865, 846, 786, 641 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.49 (d, *J* = 4.8 Hz, 1H), 7.95-7.91 (m, 1H), 7.76 (d, *J* = 9.1 Hz, 1H), 7.32-7.29 (m, 1H), 6.78 (s, 1H), 6.70 (s, 2H), 6.37 (d, *J* = 5.4 Hz, 1H), 6.17 (br s, 1H), 5.90 (s, 2H), 5.77 (s, 1H), 4.75 (s, 2H), 3.73-3.68 (m, 6H), 3.50 (t, *J* = 4.8 Hz, 4H), 3.36-3.68 (m, 2H), 2.29 (s, 3H), 1.90-1.87 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.28, 163.21, 161.96, 151.90, 150.04, 149.15, 147.73, 146.50, 134.75, 133.28, 128.50, 124.80, 121.73, 120.63, 117.55, 108.00, 107.93, 100.88, 98.95, 91.67, 66.52, 49.91, 44.19, 43.17, 39.16, 26.34, 24.96; ESI-HRMS (m/z) calcd for $C_{29}H_{31}ClN_6O_3$: 546.2146 (M^+), found: 547.2227 $(M + H)^+$, 549.2210 $(MH + 2)^+$; Anal. calcd. for $C_{29}H_{31}ClN_6O_3$: C, 63.67; H, 5.71; N, 15.36, found: C, 63.72; H, 5.83; N, 15.19.

*4.5.16 N1-(Benzo[d][1,3]dioxol-5-ylmethyl)-N3-(7-chloroquinolin-4-yl)-N1-(4-(4-ethylpiperazin-1-yl)-6-methylpyrimidin-2-yl)propane-1,3-diamine (**8h**)*: Off White solid; yield: 79%; mp 166–168 ^{o}C; IR (KBr pellet): 3209, 2921, 1579, 1557, 1471, 1443, 1418, 1379, 1330, 1296, 1242, 1214, 1179, 1141, 1127, 1081, 1042, 1011, 990, 870, 836, 787, 649, 529 cm^{-1}; ^{1}H NMR (400 MHz; $CDCl_3$) δ (ppm): 8.48 (d, *J* = 5.4 Hz, 1H), 7.92 (d, *J* = 1.8 Hz, 1H), 7.75 (d, *J* = 9.1 Hz, 1H), 7.30 (dd, *J* = 8.5, 1.8 Hz, 1H), 6.80 (s, 1H), 6.73-6.68 (m, 2H), 6.37 (d, *J* = 5.4 Hz, 1H), 6.17 (br s, 1H), 5.90 (s, 2H), 5.79 (s, 1H), 4.76 (s,

2H), 3.72 (t, *J* = 6.1 Hz, 2H), 3.56 (t, *J* = 4.8 Hz, 4H), 3.36-3.32 (m, 2H), 2.44-2.39 (m, 6H), 2.28 (s, 3H), 1.91-1.85 (m, 2H), 1.09 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 166.00, 162.89, 162.00, 151.80, 150.12, 149.06, 147.70, 146.45, 134.74, 133.42, 128.41, 124.81, 121.80, 120.69, 117.56, 108.12, 107.91, 100.84, 98.92, 91.75, 52.38, 49.92, 43.77, 43.19, 39.95, 29.67, 26.37, 24.93, 11.87; ESI-HRMS (m/z) calcd for $C_{31}H_{36}ClN_7O_2$: 573.2619 (M^+), found: 574.2710 $(M + H)^+$, 576.2693 $(MH + 2)^+$; Anal. calcd. for $C_{31}H_{36}ClN_7O_2$: C, 64.85; H, 6.32; N, 17.08, found: C, 64.99; H, 6.49; N, 17.31.

5. Procedure for heme binding studies

5.1 Monomeric Heme

A 1.0 mM stock solution of heme was prepared by dissolving 6.5 mg hemin in 10 ml HPLC grade DMSO and stored at 4 ^{o}C in the dark until used. A 10 μM working solution of monomeric heme was prepared daily by mixing 100μL of the heme stock solution with 1mL 0.2 M HEPES buffer (pH 7.4) and making it up to 10ml with double distilled deionized water.Stock solution of compound 7d (1.0 mM) was prepared in AR grade DMSO and was used for titration experiment. Heme (10 μM) was titrated with increasing concentrations (0.5-70 μM) of compound 7d. Following each addition sample was mixed & absorbance was recorded at 402 nm. Working solutions of drug and of monomeric heme at pH 5.4 were prepared in a similar manner, employing MES-buffer (pH 5.4) instead of HEPES-buffer and experiment was conducted in the same way as described earlier.

5.2 μ-oxodimeric heme

A stock solution of dimeric heme (1.0 mM) was prepared by dissolving Hemin chloride (6.5 mg) in 10 mL of 0.1 M aq. NaOH and was sonicated for 30 min to ensure complete dissolution. It was then diluted to 10μM in Phosphate buffer (20mM, pH 5.8) to get the working solution of dimeric heme. Titration of this solution with compound **7d** (1.0 mM) were performed by successive addition of aliquots of stock solution of **7d** (0.5-70 μM) and absorbance at 362 nm was recorded.

5.3 Binding Stoichiometry

Job's method of continuous variation was used to determine the binding stoichiometries of drug (**7d**) with monomeric & μ-oxodimeric heme, employing

UV/Visible spectrophotometry. The concentration of **7d** & heme in solution was kept constant and change in absorbance at 402 nm (monomeric)/362 nm (dimeric) was monitored as a function of the mole fraction.

6. Molecular docking studies

The 2D structures of all the compounds were drawn using ChemBioDraw Ultra 12.0 (www.cambridgesoft.com). Ligprep module of Schrödinger was used to generate the 3D structures with the lowest energy. Partial atomic charges were computed using the OPLS_2005 force field. The correct Lewis structure, tautomers and ionization states (pH 7.0±2.0) for each of the ligands were generated and optimized with default settings (Ligprep 2.5, Schrödinger, LLC, New York, NY, 2012). The 3D crystal structures of wild type *Pf*-DHFR-TS (PDB ID:3QGT; resolution 2.30 Å) and quadruple mutant (N51I+C59R+S108N+I164L) *Pf*-DHFR-TS (PDB ID:3QG2; resolution: 2.30 Å), were retrieved from protein data bank (www.rcsb.org). The proteins were prepared for docking using Protein Preparation Wizard (Maestro 10.0 Schrödinger, LLC, New York, NY, 2012). 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 alleviate steric clashes. The location of co-crystalized ligand pyrimethamine in both wild and mutant protein structures were used to choose the center and size of the receptor grid, which was generated using Glide 5.8 (Schrödinger, LLC, New York, NY, 2012) with default settings for all parameters. The grid size was chosen sufficiently large to include all active site residues involved in substrate binding. The cofactor, NADH in the *Pf*-DHFR-TS wild and mutant structures was also considered as part of the receptor proteins. All ligand conformers were docked to each of the receptor grid files (*Pf*-DHFR-TS wild and mutant structures) using Glide extra precision (XP) mode. Default settings were used for the refinement and scoring.

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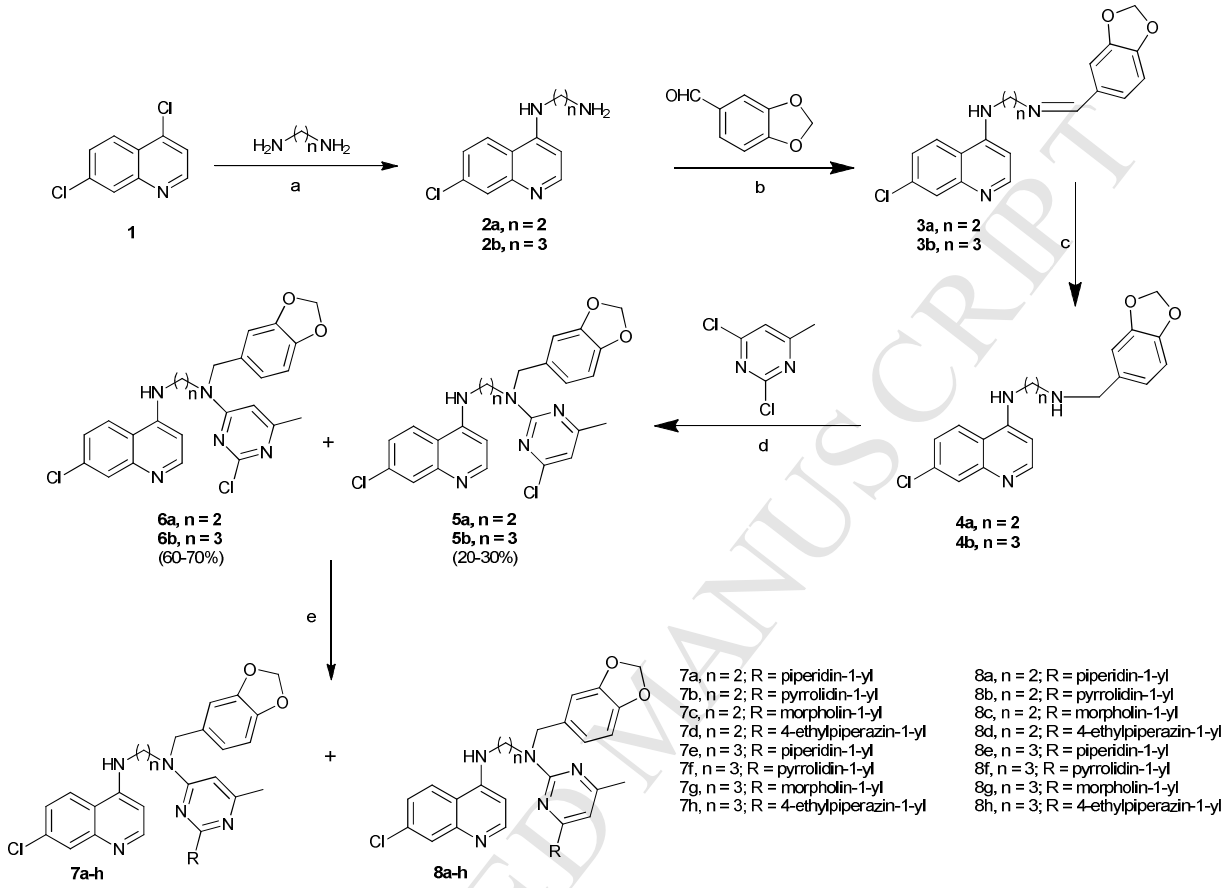
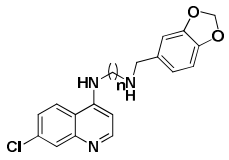
Scheme:



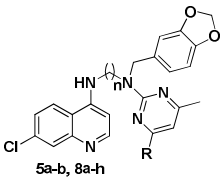
Scheme 1 Reaction conditions: (a) Neat, 110–120 ^{o}C, 5 h, 75–85%; (b) EtOH, RT, 12-16 h, 85-90% (c) MeOH, $NaBH_4$, RT, 5-6 h, 91-93% (d) THF, triethyl amine, 60 ^{o}C, 12 h, 89-92% (e) secondary amines, DMF, K_2CO_3, 120 ^{o}C, 10 h, 77–95%.

Tables:

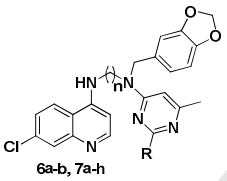
Table 1 *In vitro* antimalarial activity and cytotoxicity of the 4-aminoquinoline-piperonyl-pyrimidines



4a, 4b



5a-b, 8a-h



6a-b, 7a-h

Compound	R	n	*P. falciparum* (D6 clone)		*P. falciparum* (W2 clone)		Cytotoxicity (VERO cells), IC_{50} (µM)
			IC_{50} (µM)	SI	IC_{50} (µM)	SI	
4a	-	2	0.03	>243	0.05	>54.3	NC
4b	-	3	0.04	>243	0.10	>84.5	NC
5a	Cl	2	0.48	>20.5	1.01	>9.8	NC
5b	Cl	3	1.11	>8.6	5.16	>1.9	NC
6a	Cl	2	0.59	>16.8	0.78	>12.3	NC
6b	Cl	3	0.46	>20.9	1.14	>8.4	NC
7a	Piperidin-1-yl	2	0.05	>173.7	0.29	>30.5	NC
7b	Pyrrolidin-1-yl	2	0.04	>243	0.10	>243	NC
7c	Morpholin-1-yl	2	0.05	>243	0.22	>66.8	NC
7d	4-Ethyl-piperazin-1-yl	2	0.02	>243	0.05	>60.4	NC
7e	Piperidin-1-yl	3	0.15	>59.8	0.99	>8.8	NC
7f	Pyrrolidin-1-yl	3	0.05	>192.3	0.09	>96.9	NC
7g	Morpholin-1-yl	3	0.15	>60	0.91	>9.5	NC
7h	4-Ethyl-piperazin-1-yl	3	0.04	>193.6	0.17	>47.6	NC
8a	Piperidin-1-yl	2	0.17	>53.6	0.56	>15.9	NC
8b	Pyrrolidin-1-yl	2	0.15	>59.2	0.93	>9.8	NC
8c	Morpholin-1-yl	2	0.13	51.4	0.81	8.5	6.86
8d	4-Ethyl-piperazin-1-yl	2	0.03	>238.8	0.14	>60.4	NC
8e	Piperidin-1-yl	3	0.15	>57.2	0.36	>24.2	NC
8f	Pyrrolidin-1-yl	3	0.12	>76.7	0.59	>15.1	NC
8g	Morpholin-1-yl	3	0.13	67	0.93	9.4	8.68
8h	4-Ethyl-piperazin-1-yl	3	0.12	>71.1	0.22	>37.9	NC
CQ	-	-	0.04	>372	0.43	>34.6	NC
Pyrimethamine	-	-	0.01	-	NA	-	NT

SI, selectivity index = (IC_{50} for cytotoxicity to VERO cells)/(IC_{50} for antimalarial activity); NC: no cytotoxicity up to 9 µM; VERO: monkey kidney fibroblasts; NT: not tested.

Table 2 Glide docking energies and docking scores for aminoquinoline-piperonyl-pyrimidines, along with the reference compounds, in wild type and quadruple mutant Pf-DHFR-TS

Compounds	Docking results with wild *Pf*-DHFR		Docking results with mutant *Pf*-DHFR	
	XP GScore	Glide Energy	XP GScore	Glide Energy
4a	-8.20	-43.82	-6.7	-42.35
4b	-7.64	-48.51	-7.06	-42.22
7a	-7.92	-50.34	-6.78	-46.73
7b	-8.04	-52.62	-6.22	-33.09
7d	-8.74	-55.86	-7.69	-56.66
7f	-8.55	-58.27	-7.73	-54.53
7h	-7.52	-45.03	-7.68	-41.65
8d	-8.26	-46.48	-7.6	-57.98
8e	-8.22	-57.15	-6.91	-50.81
8h	-7.23	-48.70	-7.25	-50.26
Pyrimethamine	-9.015	-40.37	-8.62	-40.17
Cycloguanil	-9.00	-40.58	-8.66	-39.62
WR99210	-9.37	-52.48	-8.5	-49.44
Dihydrofolate	-10.01	-57.6498	-11.04	-66.36

Table 3 Prediction of Lipinski's 'rule of 5' for the active test compounds.

Compound	mol_MW (<500)	donorHB (<5)	acceptHB (<10)	QPlog Po/w (<5)	Rule of Five (<4)
4a	355.823	2	5	3.589	0
4b	369.85	2	5	3.931	0
7a	531.056	1	6.5	6.534	2
7b	517.029	1	6.5	6.285	2
7d	560.097	1	8.5	5.728	2
7f	531.056	1	6.5	6.617	2
7h	574.124	1	8.5	6.136	2
8d	560.097	1	8.5	5.826	2
8e	545.083	1	6.5	6.896	2
8h	574.124	1	8.5	5.804	2
wr99210	394.69	4	6.45	3.01	0
Pyrimethamine	248.71	4	3	1.82	0
cycloguanil	251.72	4	3.5	1.63	0

Figures:

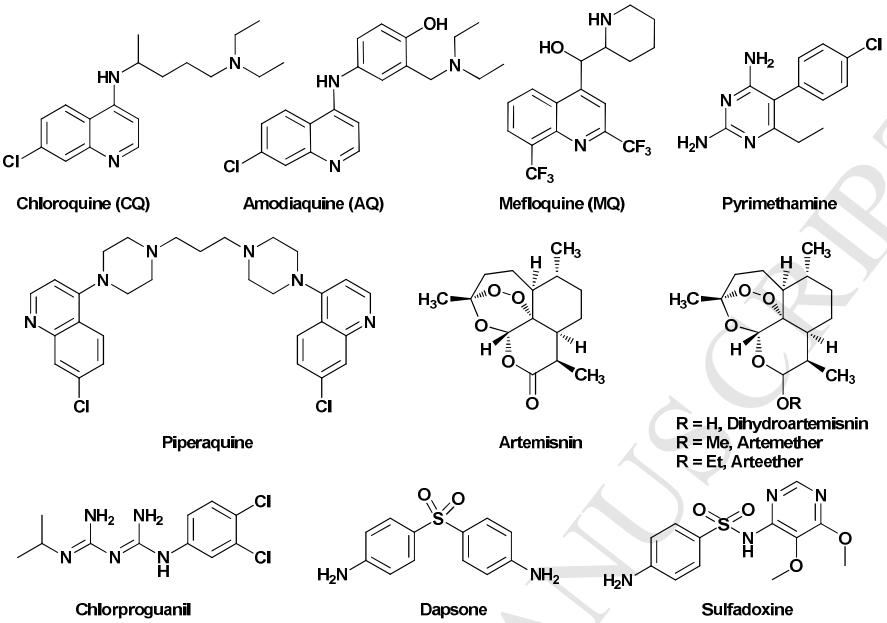


Fig. 1 Conventional antimalarial drugs

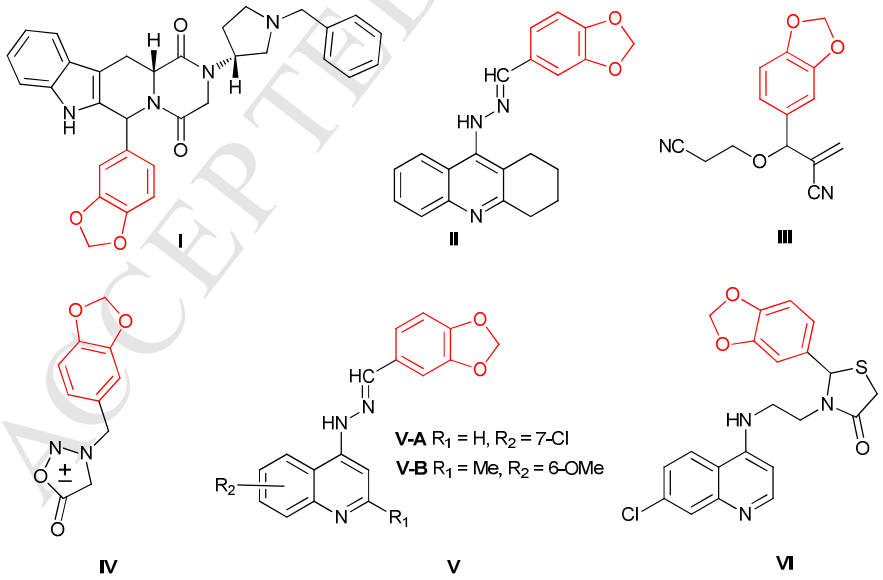


Fig. 2 Antimalarial agents containing the 1,3-benzodioxole scaffold

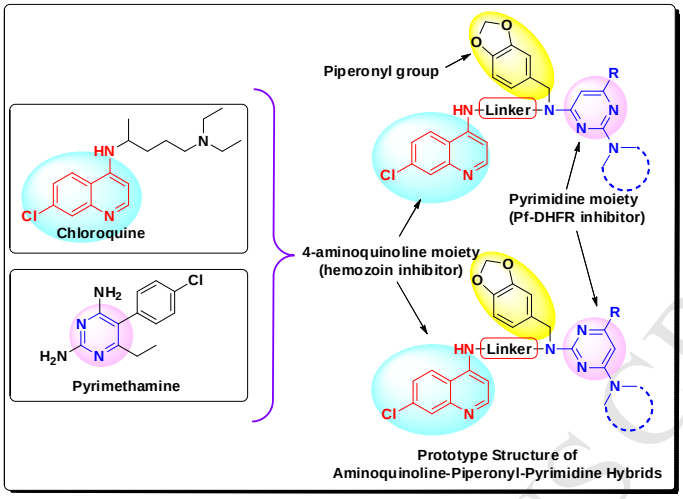


Fig. 3 Design of 4-aminoquinoline-piperonyl-pyrimidine hybrids

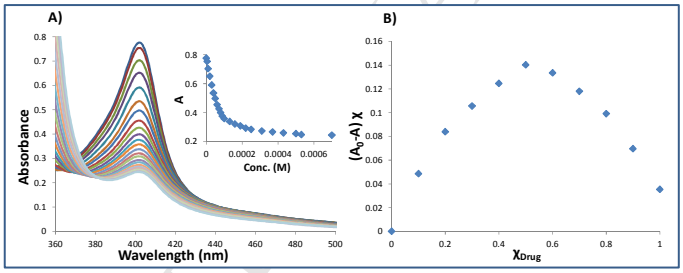


Fig. 4 (A) Titration of compound **7d** at pH 7.4 with monomeric heme; (B) Job's plot of monomeric heme complex formation with compound **7d** at pH 7.4.

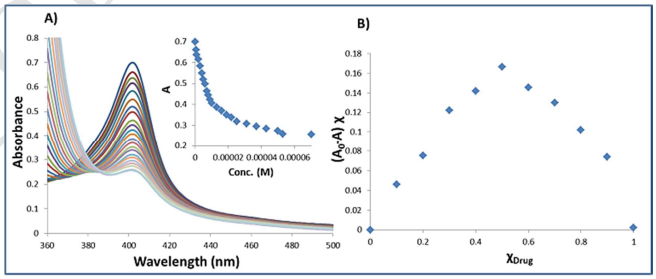


Fig. 5 (A) Titration of compound **7d** at pH 5.6 with monomeric heme; (B) Job's plot of monomeric heme complex formation with compound **7d** at ph 5.6.

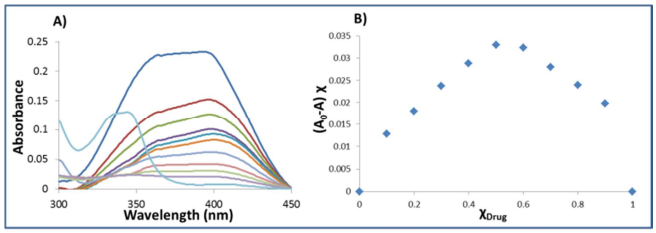


Fig. 6 (A) Titration of compound **7d** at pH 5.8 with μ-oxo-dimeric heme; (B) Job's plot of μ-oxo-dimeric heme complex formation at pH 5.8 with compound **7d**.

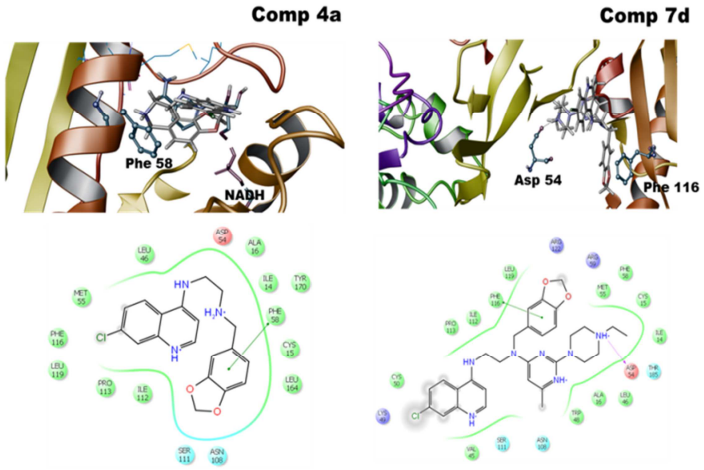


Fig. 7 2D and 3D docking pose showing interaction for compounds **4a** and **7d** in the binding site of mutant type *Pf*-DHFR-TS (PDB ID: 3QG2)

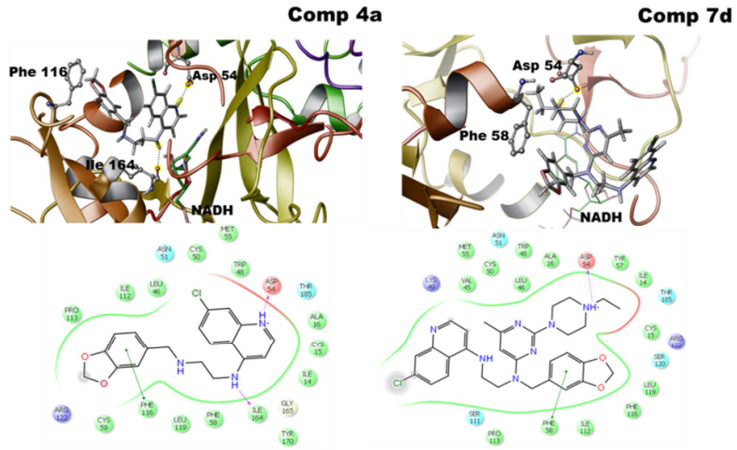


Fig. 8 2D and 3D docking pose showing interaction for compounds **4a** and **7d** in the binding site of wild type *Pf*-DHFR-TS (PDB ID: 3QG2)

Table 4 Calculated ADMET properties

Compound	[a]PercentHumanOralAbsorption (>80%-high,<25% poor)	[a]QPPCaco nms^{-1} (<25 poor, >500 great)	[a]QPlogBB (-3.0-1.2)	[a]QPPMDCK (<25 poor >500 great)	[a]QPlogKhsa (-1.5to1.5)	[a]PSA (7.0– 200.0)	[a]#rotor (0 – 15)
4a	100	806.735	0.271	1070.246	0.286	56.864	6
4b	100	790.593	0.185	1047.135	0.385	56.824	7
7a	100	4108.055	-0.091	5621.798	1.204	64.574	7
7b	100	4910.639	-0.012	6817.873	1.069	63.99	7
7d	87.814	944.02	0.193	1268.924	0.993	70.609	8
7f	100	4316.294	-0.131	5930.631	1.201	63.697	8
7h	92.022	1193.305	0.24	1634.733	1.093	67.892	9
8d	88.213	922.911	0.158	1238.282	1.021	68.76	8
8e	100	3993.167	-0.184	5452.071	1.325	64.982	8
8h	88.636	991.041	0.142	1131.071	1.025	65.755	9
wr99210	91.09	396.41	-0.98	2171.83	-0.07	89.06	8
Pyrimethamine	84.39	412.17	-0.79	468.71	-0.24	73.73	4
Cycloguanil	84.92	507.32	-0.52	586.49	-0.22	73.49	2

Highlights

- A series of 22 new 4-aminoquinoline-piperonyl-pyrimidine hybrids was synthesized.
- 12 hybrids were more active (up to 8-fold) than CQ against W2 *P. falciparum*.
- Heme binding studies revealed a 1:1 binding stoichiometry.
- Good docking interactions within the active site of *Pf*-DHFR.