RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Synthesis of piperazine tethered 4-aminoquinoline-pyrimidine hybrids as potent antimalarial agents

Anuj Thakur,[a] Shabana I. Khan[b] and Diwan S. Rawat[a,*]

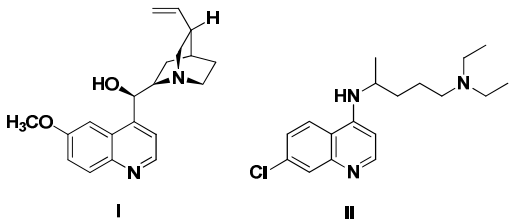
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

A series of 4-aminoquinoline-pyrimidine hybrids linked through piperazine were synthesized and evaluated for their *in vitro* antimalarial activity against chloroquine (CQ)-sensitive and chloroquine (CQ)-resistant strains of *Plasmodium falciparum* and cytotoxicity against mammalian cell line (Vero). Nine compounds (**5e**, **5f**, **5g**, **5h**, **5i**, **5j**, **5k**, **7a**, **7d**) displayed good antimalarial activity against both the strains out of which compound **5j** was the most potent with IC_{50} values in the range of 0.13 – 0.14 µM. The antimalarial activity of **5j** was 2.5 fold stronger than chloroquine in the CQ-resistant strain of *P. falciparum*. None of the compounds were found to be cytotoxic against Vero cells. The X-ray crystal structure of one of the compound was also determined.

Introduction

Malaria is one of the most virulent devastating diseases caused by five species of *Plasmodium viz. P. vivax*, *P. malariae*, *P. ovale*, *P. knowlesi* and *P. falciparum*. Among these, infection caused by *P. falciparum* is the most fatal leading to 60% malaria related deaths.[1] Impact of this disease on the public health can be judged by the fact that it affects 200-500 million people worldwide and causes over 1.2 million deaths annually.[2] Since the development of quinine (I) as an antimalarial drug, quinoline nucleus has been the mainstay of antimalarial therapy.[3] Chloroquine (CQ, II), an aminoquinoline based compound, has been used most widely for the treatment of malaria (figure 1). CQ, once hailed the status of wonder drugs for malarial chemotherapy, lost its efficacy due to the wide spread of CQ-resistant strains of *P. falciparum*.[4] In spite of the problem of drug resistance, the 4-aminoquinoline class of therapeutics remains a frontline pharmacophore for the drug development purposes due to its excellent clinical efficacy, ease of administration, low toxicity and low cost of synthesis.[5] due these features there is a continous interest in further development of this pharmacophore.



I II

Figure 1: Quinine (I) and chloroquine (CQ, II)

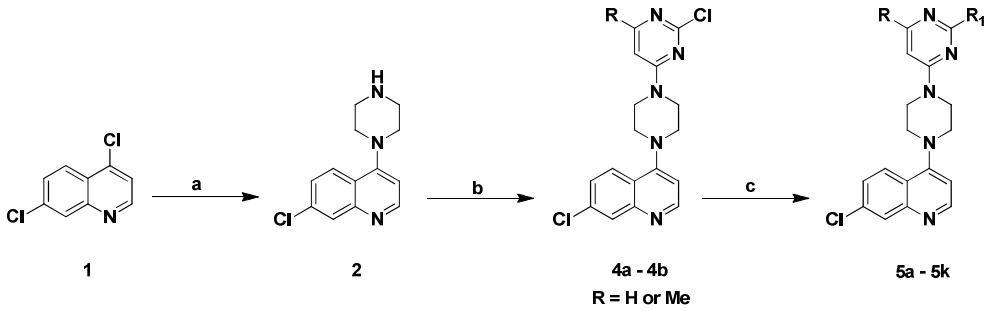
In order to solve the problem of drug resistance, numerous strategies such as modification of existing leads, combination therapy, reversal of drug-resistance by chemo-sensitizers and exploring new chemotherapeutics[6] have been used. More recently, the concept of molecular hybrids[7,8] has been introduced in an anticipation that these kind of molecules may overcome the drug resistance problem to a considerable extent. In this strategy two or more pharmacophore are covalently linked and it is anticipated that these kinds of double sword molecules may act by inhibiting simultaneously two or more conventional targets of antimalarial therapy. This multiple target strategy led to the discovery of various molecular hybrids such as 4-aminoquinoline-cinnamic acid,[9] 4-aminoquinoline-ferrocenophane,[10] quinoline-rhodanine,[11] 4-aminoquinoline-clotrimazole,[12] 4-aminoquinoline-triazine,[13] 4-aminoquinoline-γ-hydroxy-γ-lactam,[14] 4-aminoquinoline-furoxan,[15] and more recently 4-aminoquinoline-pyrimidines.[16,17] It is important to mention here that some of these hybrid compounds have also entered into the clinical trials.[7] Encouraged by these reports and therapeutic advantages of the molecular hybrids in medicinal chemistry, recently we reported *in vitro* and *in vivo* antimalarial activity and cytotoxicity of 4-aminoquinoline-pyrimidine based molecular hybrids.[16] These hybrids exhibited promising antimalarial activity against CQ-sensitive as well as CQ-resistant strain. In order to gain further insight on the effect of the linker on antimalarial activity and in continuation of our ongoing efforts in this area,[18] we decided to connect these two pharmacophores *via* more rigid linker and therefore used piperazine as a scaffold to covalently link these two pharmacophores.

Results and discussion

Chemistry

Synthesis of piperazine linked quinoline-pyrimidine hybrids is depicted in scheme 1 and 2. To start with, aromatic nucleophilic substitution of commercially available 4,7-dichloroquinoline (**1**) with excess of piperazine, afforded intermediate **2**,[19] which was coupled with 2,4-dichloro-pyrimidine (**3a**) or 2,4-dichloro-6-

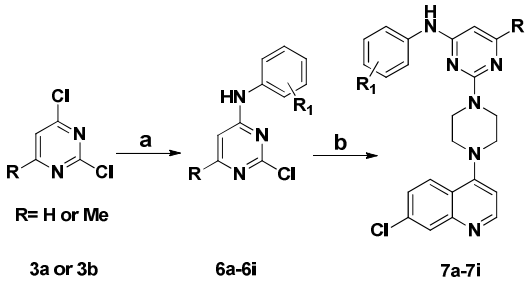
methyl-pyrimidine (**3b**) at 0-15 °C, yielding intermediate (**4a** or **4b**) (scheme 1). Intermediate (**4a** or **4b**) was then reacted with various primary and secondary aliphatic amines at an elevated



Scheme 1: (a) Piperazine, DMF, 90-100 °C, 4-5 h, 75-85%; (b) 2,4-Dichloro-pyrimidine (**3a**) or 2,4-dichloro-6-methyl-pyrimidine (**3b**), *N,N*-diisopropylethylamine, THF, 0-15 °C, overnight, 80-90%; (c) R_1 = aliphatic amines, DMF, 110-120 °C, 8-10 h, 62-90%.

temperature (110-120 °C) in DMF leading to the desired 4-aminoquinoline-pyrimidine hybrids (**5a-5k**) in good to excellent yields.

The reaction of substituted anilines with **4a** or **4b** led to the formation of the desired product in very poor yield. To circumvent these problems, the second approach (scheme 2) was adopted. Initially dichloropyrimidines (**3a or 3b**) were subjected to nucleophilic substitution with different substituted anilines yielding intermediates **6a-6i** in 95% yield, followed by reaction with intermediate (**2**) to yield final products (**7a-7i**). All the compounds were purified by column chromatography over silica gel and structures were established on the basis of various spectroscopic techniques and elemental analysis. Crystal structure of compound **4a** was also determined (figure 2).



Scheme 2: (a) Substituted anilines, *N,N*-diisopropylethylamine, *tert*-BuOH, 50-60 °C, 7-9 h, 85-95%; (b) **2**, K_2CO_3, DMF, 110-120 °C, 8-10 h, 80-90%.

Antimalarial activity and cytotoxicity

The present work was designed to understand the role of linker between aminoquinoline and pyrimidine scaffolds. Therefore, we decided to introduce some rigidity yet maintaining the basic nature of the linker. Piperazine met all the criteria and was used as a linker between aminoquinoline and pyrimidine in the present study. All the synthesized compounds **4a**-**4b**, **5a**-**5k** and **7a**-**7i** were evaluated for their *in vitro* antimalarial activity against CQ-sensitive (D6) strain and CQ-resistant (W2) strain of *P. falciparum* (Table 1) using procedure as described earlier.[20]

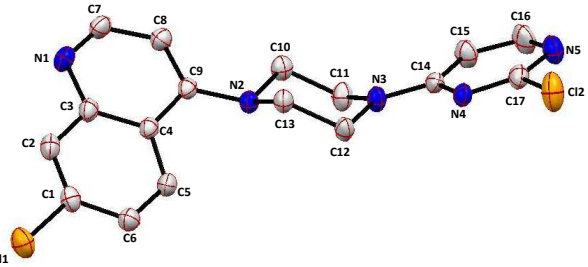


Figure 2: Molecular structure of compound **4a**. Thermal ellipsoidal is drawn at the 30 % level; hydrogen atoms have been omitted for clarity.

Among 22 compounds, several compounds showed antimalarial activity with IC_{50} values below 1 µM for both the strains. However, compounds **5j** was the most potent with IC_{50} values of 0.13 µM and 0.14 µM for D6 and W2 strains, respectively and a high selectivity index (> 80). The activity of **5j** was 2.5 times higher than CQ against CQ-resistant strain (W2) as shown in table 1. Seven compounds (**5e – 5i, 5k** and **7d**) have displayed activity in the IC_{50} range of 0.27 – 0.44 µM for D6 strain and 0.30 – 0.77 µM for W2 strain. Structure activity relationship indicates that substitution of Cl at 4th position of pyrimidine ring of the hybrids with different aliphatic amines increases activity. Piperidine and morpholine substituted compounds (**5a**-**5d**) have shown a similar activity profile. On decreasing ring size from piperidine to pyrrolidine (**5c-5d** vs **5e-5f**), three to four fold improvement in activity was observed (IC_{50} 1.21-1.50 µM vs 0.38-0.60 µM). Substitution of secondary cyclic amines with primary amines in the pyrimidine ring of the hybrids (**5g**-**5k**) led to the improvement of activity with IC_{50} values ranging between 0.13-0.39 µM against D6 strain while some of these compounds were more active than CQ against resistant strains (IC_{50} = 0.14-0.48 µM). In order to understand the role of amine functionality at 2nd position of the pyrimidine ring of the hybrids, we replaced primary and secondary amines with substituted anilines which led to a decrease in activity with exception of compounds **7a** and **7d**.

Cytotoxicity of all the synthesized hybrids (**4a**-**4b**, **5a**-**5k and 7a**-**7i**) was determined against Vero cells. All the compounds were non-cytotoxic up to the highest tested concentration of 4.76 µg/mL, demonstrating safety towards mammalian cells.

ADME properties of the most potent hybrid (**5j**) were evaluated using Quikprop (Schrodinger software) [21] as shown in table 2. Compound **5j** satisfies the Lipinski rule of five (MW <500, logPo/w < 5, donor HB ≤ 5, accpt HB ≤ 10) which demonstrates its drug-likeness. For a drug molecule to pass through biological membranes and eventually enter the systemic circulation, an optimal balance in its lipophilic and hydrophilic properties is required.[22] This, in turn, can be predicted by its octanol/water partition coefficient (logPo/w) and aqueous solubility (logS). Compound **5j** shows an optimal logPo/w and logS value of 3.90 and -5.98 respectively. From the predicted cell permeability values (PCaco2, PMDCK), it is clear that after the absorption process, distribution, metabolism and excretion parameters are within acceptable range. The predicted HERG inhibition potential of compound 5j is slightly on the higher side but comparable to CQ (predicted = -6.29, experimental = -5.60[23]). Predicted solvent accessible surface area with its hydrophobic and hydrophilic components of the compounds determines that they have 'transfer free energy' to move from the aqueous environment to non-polar environment. Almost all the predicted properties were found to be within the acceptable range.

Table 1: *In vitro* antimalarial activity and cytotoxicity of the title and reference compound.

Entry	R	R_1	*P. falciparum* (D6 Clone)		*P. falciparum* (W2 Clone)		Vero cell cytotoxicity
			IC_{50} (µM)	S.I	IC_{50} (µM)	S.I	
4a	H	Cl	2.65	>4.9	4.77	>2.7	NC
4b	Me	Cl	2.45	>5.1	3.33	>3.8	NC
5a	H	Morpholine	1.45	>7.9	2.28	>5.07	NC
5b	Me	Morpholine	1.26	>8.8	2.06	>5.43	NC
5c	H	Piperidine	1.33	>8.7	1.50	>7.72	NC
5d	Me	Piperidine	1.21	>8.7	1.46	>7.70	NC
5e	H	Pyrrolidine	0.44	>26.8	0.60	>19.9	NC
5f	Me	Pyrrolidine	0.38	>30.2	0.47	>24.6	NC
5g	H	Cyclohexylamine	0.29	>37.9	0.43	>25.9	NC
5h	Me	Cyclohexylamine	0.27	>38.9	0.30	>36.1	NC
5i	H	Ethanolamine	0.39	>31.4	0.48	>25.6	NC
5j	Me	Ethanolamine	0.13	>88.4	0.14	>83.5	NC
5k	H	Propanolamine	0.30	>38.9	0.40	>31.3	NC
7a	H	3,5-Dimethoxy	0.48	>20.5	1.68	>5.9	NC
7b	Me	3,5-Dimethoxy	1.35	>7.2	1.16	>8.3	NC
7c	H	4-Methoxy	1.2	>8.7	1.86	>5.7	NC
7d	Me	4-Methoxy	0.44	>23.3	0.77	>13.4	NC
7e	H	H	2.35	>4.8	2.96	>3.9	NC
7f	H	4-Fluro	2.83	>3.9	2.04	>5.4	NC
7g	Me	4-Fluro	3.98	>2.7	3.43	>3.1	NC
7h	H	4-Chloro	1.19	>8.8	1.88	>5.6	NC
7i	H	4-Bromo	1.10	>8.7	1.25	>7.7	NC
	Chloroquine		0.03	>300	0.34	>26.5	NC

NC: non cytotoxic; Selectivity index (SI): IC_{50} for cytotoxicity towards VERO cells/IC_{50} for antimalarial activity;

Table 2: Predicted ADME properties of compound **5j**.

Compound	**5j**
Molecular weight[a]	398.89
LogPo/w[b]	3.90
LogS[c]	-5.98
PCaco2[d]	1052.75
PMDCK[e]	1290.08
logHERG[f]	-5.908
No. of primary metabolites[g]	3
SASA[h]	701.84
FOSA[i]	311.51
FISA[j]	102.66
Percent Human-Oral Absorption	100
Donor HB[k]	2
Accpt HB[l]	6.7

[a]Range 95% of drugs (130/725); [b]Log of the octanol/water partition coefficient, range 95% of drugs (-2/6.5); [c]Log of aqueous solubility S (mol/L), range 95% of drugs (-6.5/0.5); [d]Caco2 cell permeability in nm/s, range 95% of drugs (<25 poor, >500 high); [e]MDCK cell permeability in nm/sec, range 95% of drugs (<25 poor, >500 high); [f] predicted IC_{50} value for the blockage of HERG K+ channels, range 95% of drugs (concern below -5); [g]Range 95% of drugs (1/8); [h]Solvent-accessible surface area (SASA), range 95% of drugs (300.0 /1000.0); [i]Hydrophobic SASA, range 95% of drugs (0.0 / 750.0); [j]Hydrophilic SASA, range 95% of drugs (7.0 / 330.0); [k]Donor - Hydrogen Bonds, range 95% of drugs (0.0 / 6.0); [l]Acceptor - Hydrogen Bonds, range 95% of drugs (2.0 / 20.0).

Experimental Section

All the starting materials were purchased from Sigma Aldrich and were used without further purification. Progress of the reaction was monitored by TLC (E. Merck Kieselgel 60 F254) and visualization was accomplished using UV light. All the intermediates and final compounds were purified using silica gel column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Jeol Spectrospin spectrometer at 400 MHz and 100 MHz respectively, and the chemical shifts are given in parts per million (ppm) on the delta scale (δ) and are referenced to tetramethylsilane. Perkin-Elmer FT-IR spectrophotometer was used for recording IR spectra and the values are expressed as λ_{max} per centimeter. Mass spectra were recorded on Jeol-AccuTOF JMS-T100LC and micromass LCT Mass Spectrometer/Data system. Melting points were recorded on an ERS automated melting point apparatus and are uncorrected.

General procedure for the synthesis of compound 4a and 4b. Compound **2** (5 g, 1 mmol) and *N,N*-diisopropylethylamine (4.5 mL, 1.3 mmol) were mixed in 50 mL of THF at 0 °C for 20 minutes. To this, 2,4-dichloro-pyrimidine (**3a**) or 2,4-dichloro-6-methyl-pyrimidine (**3b**) (3 g, 1 mmol) was added and the resulting reaction mixture was allowed to stir overnight at 10-15 °C. After complete consumption of starting material as evidenced by the TLC, THF was evaporated under reduced pressure. The residue was treated with water and extracted with EtOAc (3 x 50 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by column chromatography to afford **4a** or **4b**.

7-Chloro-4-[4-(2-chloro-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (4a). Yield: 86%; mp 184-186 °C; IR (cm^{-1}, Film): 2980, 2895, 1578, 1534, 1495, 1449, 1424, 1356, 1249, 1173, 979, 870, 754; ^{1}H NMR (400 MHz, $CDCl_3$): 3.28-3.31 (m, 4H), 3.89-4.01 (m, 4H), 6.49 (d, 1H, *J* = 5.95 Hz), 6.87 (d, 1H, *J* = 5.04 Hz), 7.48 (dd, 1H, *J* = 9.16 Hz, *J* = 1.83 Hz), 7.98 (d, 1H, *J* = 8.70 Hz), 8.08 (d, 1H, *J* = 1.83 Hz), 8.12 (d, 1H, *J* = 5.95 Hz), 8.76 (d, 1H, *J* = 5.04 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 43.96, 51.62, 101.31, 109.26, 121.72, 124.63, 126.64, 129.03, 135.18, 150.10, 151.92, 156.15, 157.65, 160.80, 162.76; ESI-MS (m/z): 360.09 $(M+H)^+$, 362.07 $(M+2)^+$; Anal. Calcd for $C_{17}H_{15}Cl_2N_5$: C, 56.68; H, 4.20; N, 19.44; Found: C, 56.73; H, 4.29; N, 19.49.

7-Chloro-4-[4-(2-chloro-6-methyl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (4b). Yield: 80%; mp 118-120 °C; IR (cm^{-1}, Film): 2964, 2851, 1593, 1496, 1424, 1380, 1296, 1214, 1194, 1073, 985, 871, 755; ^{1}H NMR (400 MHz, $CDCl_3$): 2.31 (s, 3H), 3.21-3.23 (m, 4H), 3.82-3.91 (m, 4H), 6.29 (s, 1H), 6.80 (d, 1H, *J* = 5.13 Hz), 7.41 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 7.92 (d, 1H, *J* = 8.79 Hz), 8.00 (d, 1H, *J* = 2.20 Hz), 8.69 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 374.06 $(M + H)^+$, 376.09 $(M + 2)^+$; Anal. Calcd for $C_{18}H_{17}Cl_2N_5$: C, 57.76; H, 4.58; N, 18.71; Found: C, 57.81; H, 4.60; N, 18.76.

General procedure for the synthesis of compounds (5a-5k). To a solution of **4a** or **4b** (0.2 g, 2 mmol) in DMF (7 mL), respective amine (3 eq.) was added dropwise and the reaction mixture was allowed to stir at 110-120 °C for 8-10 h. Upon the completion of reaction (TLC), water (15 mL) was added to the reaction mixture and it was extracted with EtOAc (3 x 30 mL). The organic extract was dried over Na_2SO_4 and concentrated in vacco. The crude residue thus obtained was purified by column chromatography using MeOH/$CHCl_3$ as eluent to afford respective compounds **5a-5k** in excellent yield.

7-Chloro-4-[4-(2-morpholin-4-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5a). Yield: 85%; mp 166-168 °C; IR (cm^{-1}, Film): 2966, 2859, 1576, 1474, 1446, 1380, 1236, 1112, 1070, 999, 944, 865, 789, 712, 630; ^{1}H NMR (400 MHz, $CDCl_3$): 3.26-3.29 (m, 4H), 3.75-3.77 (m, 8H), 3.86-3.88 (m, 4H), 5.97 (d, 1H, *J* = 6.10 Hz), 6.86 (d, 1H, *J* = 4.88 Hz), 7.46 (dd, 1H, *J* = 8.54 Hz, *J* = 2.44 Hz), 7.99-8.05 (m, 2H), 8.07 (d, 1H, *J* = 2.44 Hz), 8.75 (d, 1H, *J* = 5.49 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 43.75, 44.24, 51.84, 66.86, 93.25, 109.10, 121.79, 124.85, 126.41, 128.93, 135.05, 150.06, 151.90, 156.57, 156.89, 161.53, 162.45; ESI-MS (m/z): 411.19 $(M+H)^+$, 413.16 $(M+2)^+$; Anal. Calcd for $C_{21}H_{23}ClN_6O$: C, 61.38; H, 5.64; N, 20.45; Found: C, 61.44; H, 5.70; N, 20.49.

7-Chloro-4-[4-(6-methyl-2-morpholin-4-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5b). Yield: 83%; mp 188-190 °C; IR (cm^{-1}, Film): 2960, 2849, 1575, 1420, 1380, 1265, 1245, 1193, 1116, 1013, 991, 870, 753; ^{1}H NMR (400 MHz, $CDCl_3$): 2.27 (s, 3H), 3.26-3.28 (m, 4H), 3.73-3.79 (m, 8H), 3.84-3.87 (m, 4H), 5.86 (s, 1H), 6.85 (d, 1H, *J* = 5.13 Hz), 7.46 (dd, 1H, *J* = 8.79 Hz,

J = 2.20 Hz), 8.00 (d, 1H, *J* = 9.52 Hz), 8.06 (d, 1H, *J* = 1.46 Hz), 8.74 (d, 1H, *J* = 4.39 Hz); ESI-MS (m/z): 425.16 $(M+H)^+$, 427.19 $(M+2)^+$; Anal. Calcd for $C_{22}H_{25}ClN_6O$: C, 62.18; H, 5.93; N, 19.78; Found: C, 62.22; H, 5.95; N, 19.76.

7-Chloro-4-[4-(2-piperidin-1-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5c). Yield: 88%; mp 126-128 °C; IR (cm^{-1}, Film): 2935, 2851, 1577, 1444, 1377, 1231, 1204, 1122, 991, 869, 788; ^1H NMR (400 MHz, $CDCl_3$): 1.59-1.66 (m, 6H), 3.26-3.28 (m, 4H), 3.73-3.76 (m, 4H), 3.85-3.87 (m, 4H), 5.89 (d, 1H, *J* = 6.59 Hz), 6.85 (d, 1H, *J* = 5.13 Hz), 7.45 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 7.99 (s, 1H), 8.00-8.01 (m, 1H), 8.06 (d, 1H, *J* = 2.20 Hz), 8.74 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 409.17 $(M+H)^+$, 411.21 $(M+2)^+$; Anal. Calcd for $C_{22}H_{25}ClN_6$: C, 64.62; H, 6.16; N, 20.55; Found: C, 64.65; H, 6.19; N, 20.51.

7-Chloro-4-[4-(6-methyl-2-piperidin-1-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5d). Yield: 83%; mp 158-160 °C; IR (cm^{-1}, Film): 2932, 2848, 1571, 1444, 1418, 1295, 1244, 1196, 101, 1011, 990, 869, 754; ^1H NMR (400 MHz, $CDCl_3$): 1.59-1.64 (m, 4H), 2.25 (s, 3H), 3.25-3.28 (m, 4H), 3.74-3.77 (m, 4H), 3.83-3.86 (m, 4H), 5.79 (s, 1H), 6.85 (d, 1H, *J* = 5.13 Hz), 7.45 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.00 (d, 1H, *J* = 8.79 Hz), 8.06 (d, 1H, *J* = 2.20 Hz), 8.74 (d, 1H, *J* = 5.13 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 24.64, 24.96, 25.84, 43.91, 44.73, 51.95, 90.99, 109.06, 121.86, 124.97, 126.32, 128.94, 134.99, 150.13, 151.93, 156.75, 161.69, 163.29, 166.67; ESI-MS (m/z): 423.24 $(M+H)^+$, 425.22 $(M+2)^+$; Anal. Calcd for $C_{23}H_{27}ClN_6$: C, 65.31; H, 6.43; N, 19.87; Found: C, 65.34; H, 6.44; N, 19.85.

7-Chloro-4-[4-(2-pyrrolidin-1-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5e). Yield: 87%; mp 152-154 °C; IR (cm^{-1}, Film): 2969, 2856, 1578, 1554, 1471, 1424, 1381, 1322, 1242, 1190, 1125, 1071, 1014, 972, 870, 752; ^1H NMR (400 MHz, $CDCl_3$): 1.94-1.98 (m, 4H), 3.26-3.28 (m, 4H), 3.52-3.58 (m, 4H), 3.87-3.91 (m, 4H), 5.90 (d, 1H, *J* = 6.10 Hz), 6.86 (d, 1H, *J* = 4.88 Hz), 7.44-7.47 (m, 1H), 7.98-8.01 (m, 2H), 8.05-8.07 (m, 1H), 8.74 (d, 1H, *J* = 4.88 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 25.48, 43.69, 46.34, 51.89, 91.84, 109.07, 121.82, 124.93, 126.36, 128.88, 135.00, 150.05, 151.89, 156.69, 156.89, 160.09, 162.37; ESI-MS (m/z): 395.18 $(M+H)^+$, 397.19 $(M+2)^+$; Anal. Calcd for $C_{21}H_{23}ClN_6$: C, 63.87; H, 5.87; N, 21.28; Found: C, 63.91; H, 5.93; N, 21.34.

7-Chloro-4-[4-(6-methyl-2-pyrrolidin-1-yl-pyrimidin-4-yl)-piperazin-1-yl]-quinoline (5f). Yield: 85%; mp 120-122 °C; IR (cm^{-1}, Film): 2966, 2852, 1573, 1514, 1455, 1417, 1380, 1343, 1273, 1246, 1187, 1071, 992, 963, 871, 752, 662; ^1H NMR (400 MHz, $CDCl_3$): 1.92-1.95 (m, 4H), 2.27 (s, 3H), 3.25-3.28 (m, 4H), 3.55-3.58 (m, 4H), 3.85-3.88 (m, 4H), 5.80 (s, 1H), 6.85 (d, 1H, *J* = 5.13 Hz), 7.45 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.00 (d, 1H, *J* = 8.79 Hz), 8.06 (d, 1H, *J* = 2.20 Hz), 8.73 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 409.22 $(M+H)^+$, 411.17 $(M+2)^+$; Anal. Calcd for $C_{22}H_{25}ClN_6$: C, 64.62; H, 6.16; N, 20.55; Found: C, 64.66; H, 6.18; N, 20.57.

{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-2-yl}-cyclohexyl-amine (5g). Yield: 62%; mp 180-180 °C; IR (cm^{-1}, Film): 3256, 2928, 2853, 1577, 1449, 1423, 1380, 1294, 1246, 1159, 1012, 1012, 977, 865, 791, 755; ^1H NMR (400 MHz, $CDCl_3$): 1.17-1.25 (m, 4H), 1.33-1.40 (m, 2H), 1.59-1.62 (m, 1H), 1.70-1.74 (m, 2H), 2.00-2.04 (m, 2H), 3.23-3.26 (m, 4H), 3.83-3.85 (m, 4H), 4.91 (br s, 1H), 5.90 (d, 1H, *J* = 6.10 Hz), 6.84 (d, 1H, *J* = 4.88 Hz), 7.45 (dd, 1H, *J* = 9.16 Hz, *J* = 2.44 Hz), 7.91 (d, 1H, *J* = 6.10 Hz), 7.97-7.99 (m, 1H), 8.05 (d, 1H, *J* = 1.83 Hz), 8.72 (d, 1H, *J* = 4.88 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 24.87, 25.73, 33.26, 43.66, 49.51, 51.84, 92.91, 109.07, 121.78, 124.88, 126.37, 128.90, 134.98, 150.07, 151.87, 156.57, 156.74, 161.22, 162.63; ESI-MS (m/z): 423.22 $(M+H)^+$, 425.17 $(M+2)^+$; Anal. Calcd for $C_{23}H_{27}ClN_6$: C, 65.31; H, 6.43; N, 19.87; Found: C, 65.34; H, 6.42; N, 19.89.

{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-6-methyl-pyrimidin-2-yl}-cyclohexyl-amine (5h). Yield: 66%; mp 106-108 °C; IR (cm^{-1}, Film): 3393, 2924, 2852, 1577, 1420, 1379, 1294, 1248, 1199, 1072, 1011, 928, 872, 790; ^1H NMR (400 MHz, $CDCl_3$): 1.22-1.27 (m, 4H), 1.33-1.42 (m, 2H), 1.59-1.62 (m, 1H), 1.72-1.75 (m, 2H), 2.00-2.02 (m, 2H), 2.23 (s, 3H), 3.26-3.29 (m, 4H), 3.84-3.91 (m, 4H), 5.81 (s, 1H), 6.86 (d, 1H, *J* = 4.88 Hz), 7.46 (dd, 1H, *J* = 9.16 Hz, *J* = 1.83 Hz), 8.00 (d, 1H, *J* = 9.16 Hz), 8.06-8.07 (m, 1H), 8.73 (d, 1H, *J* = 4.88 Hz); ESI-MS (m/z): 437.25 $(M+H)^+$, 439.22 $(M+2)^+$; Anal. Calcd for $C_{24}H_{29}ClN_6$: C, 65.97; H, 6.69; N, 19.23; Found: C, 65.95; H, 6.72; N, 19.22.

2-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-2-ylamino}-ethanol (5i). Yield: 82%; mp 214-216 °C; IR (cm^{-1}, Film): 3392, 2919, 2849, 1577, 1420, 1379, 1233, 1070, 1008, 973, 869, 790; ^1H NMR (400 MHz, $CDCl_3$): 3.25-3.29 (m, 4H), 3.52-3.58 (m, 2H), 3.81-3.83 (m, 2H), 3.85-3.88 (m, 4H), 5.28 (br s, 1H), 5.89-6.00 (m, 1H), 6.85-6.87 (m, 1H), 7.45-7.48 (m, 1H), 7.91-7.98 (m, 1H), 8.00-8.02 (m, 1H), 8.07 (d, 1H, *J* = 2.20 Hz), 8.74 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 385.16 $(M+H)^+$, 387.14 $(M+2)^+$; Anal. Calcd for $C_{19}H_{21}ClN_6O$: C, 59.29; H, 5.50; N, 21.84; Found: C, 59.34; H, 5.56; N, 21.88.

2-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-6-methyl-pyrimidin-2-ylamino}-ethanol (5j). Yield: 85%; mp 210-212 °C; IR (cm^{-1}, Film): 3247, 2923, 2852, 2367, 1571, 1422, 1294, 1219, 1071, 991, 928, 871, 722; ^1H NMR (400 MHz, $CDCl_3$): 2.24 (s, 3H), 3.25-3.27 (m, 4H), 3.55-3.57 (m, 2H), 3.80-3.82 (m, 2H), 3.84-3.87 (m, 4H), 5.32 (br s, 1H), 5.88 (s, 1H), 6.85 (d, 1H, *J* = 5.13 Hz), 7.47 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 7.99 (d, 1H, *J* = 8.79 Hz), 8.07 (d, 1H, *J* = 2.20 Hz), 8.74 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 399.18 $(M+H)^+$, 401.15 $(M+2)^+$; Anal. Calcd for $C_{20}H_{23}ClN_6O$: C, 60.22; H, 5.81; N, 21.07; Found: C, 60.21; H, 5.84; N, 21.10.

3-{4-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-2-ylamino}-propan-1-ol (5k). Yield: 87%; mp 190-192 °C; IR (cm^{-1}, Film): 3247, 2922, 2850, 1580, 1520, 1423, 1380, 1244, 1142, 1009, 972, 870, 753; ^1H NMR (400 MHz, $CDCl_3$): 1.73 (s, 2H), 3.23-3.28 (m, 4H), 3.56-3.64 (m, 4H), 3.86 (s, 4H), 4.98 (br s, 1H), 5.97 (d, 1H, *J* = 5.13 Hz), 6.86 (d, 1H, *J* = 5.13 Hz), 7.46 (d, 1H, *J* = 8.79 Hz), 7.91 (d, 1H, *J* = 5.86 Hz), 7.99 (d, 1H, *J* = 8.79 Hz), 8.06 (s, 1H), 8.74 (d, 1H, *J* = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): 32.50, 37.88, 43.18, 51.45, 58.75, 92.23, 109.62, 121.33, 125.86, 126.07, 128.08, 133.64, 149.62, 152.19, 155.90, 56.67, 161.93, 162.18; ESI-MS (m/z): 399.18 $(M+H)^+$, 401.16 $(M+2)^+$; Anal. Calcd for $C_{20}H_{23}ClN_6O$: C, 60.22; H, 5.81; N, 21.07; Found: C, 60.24; H, 5.83; N, 21.06.

General procedure for the synthesis of compounds 6a-6i. To a well stirred solution of substituted aniline (0.5 g, 1 mmol) and *N,N*-diisopropylethylamine (1.3 mmol) in *tert*-BuOH (15 mL) at room temperature, compound **3a** or **3b** (1 mmol) was added. The reaction mixture was allowed to stir at 50-60 ^{o}C for 8-10 h. After completion of reaction as monitored by TLC, *tert*-BuOH was evaporated under reduced pressure and the residue was treated with water. The mixture was extracted with EtOAc (3 x 30 mL) and the combined organic phase was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by column chromatography to give the corresponding intermediates (**6a-6i**).

(2-Chloro-pyrimidin-4-yl)-(3,5-dimethoxy-phenyl)-amine (6a). Yield: 87%; mp 172-174 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 3.80 (s, 6H), 6.33-6.44 (m, 1H), 6.47 (d, 2H, *J* = 2.20 Hz), 6.67 (d, 1H, *J* = 6.59 Hz), 7.00 (br s, 1H), 8.13 (d, 1H, *J* = 5.86 Hz); ESI-MS (m/z): 266.09 $(M+H)^+$, 268.06 $(M+2)^+$; Anal. Calcd for $C_{12}H_{12}ClN_3O_2$: C, 54.25; H, 4.55; N, 15.82; Found: C, 54.57; H, 4.53; N, 15.81.

(2-Chloro-6-methyl-pyrimidin-4-yl)-(3,5-dimethoxy-phenyl)-amine (6b). Yield: 91%; mp 186-188 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 2.35 (s, 3H), 3.81 (s, 6H), 6.32-6.34 (m, 1H), 6.45 (d, 2H, *J* = 2.20 Hz), 6.52 (s, 1H), 6.89 (br s, 1H); ESI-MS (m/z): 280.09 $(M+H)^+$, 282.08 $(M+2)^+$; Anal. Calcd for $C_{13}H_{14}ClN_3O_2$: C, 55.82; H, 5.04; N, 15.02; Found: C, 55.84; H, 5.09; N, 15.06.

(2-Chloro-pyrimidin-4-yl)-(4-methoxy-phenyl)-amine (6c). Yield: 85%; mp 176-178 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H), 6.40 (d, 1H, *J* = 5.13 Hz), 6.93-6.95 (m, 2H), 7.19-7.21 (m, 3H), 8.05 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 236.07 $(M+H)^+$, 238.05 $(M+2)^+$; Anal. Calcd for $C_{11}H_{10}ClN_3O$: C, 56.06; H, 4.28; N, 17.83; Found: C, 56.10; H, 4.32; N, 17.84.

(2-Chloro-6-methyl-pyrimidin-4-yl)-(4-methoxy-phenyl)-amine (6d). Yield: 90%; mp 170-172 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 2.29 (s, 3H), 3.83 (s, 3H), 6.22 (s, 1H), 6.94 (d, 2H, *J* = 8.79 Hz), 7.20 (d, 2H, *J* = 8.79 Hz), 7.28 (br s, 1H); ESI-MS (m/z): 250.10 $(M+H)^+$, 252.09 $(M+2)^+$; Anal. Calcd for $C_{12}H_{12}ClN_3O$: C, 57.72; H, 4.84; N, 16.83; Found: C, 57.71; H, 4.89; N, 16.84.

(2-Chloro-pyrimidin-4-yl)-phenyl-amine (6e). Yield: 92%; mp 184-186 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 6.59 (d, 1H, *J* = 5.86 Hz), 7.23-7.27 (m, 2H), 7.30-7.32 (m, 2H), 7.40-7.44 (m, 2H), 8.12 (d, 1H, *J* = 5.86 Hz); ESI-MS (m/z): 206.06 $(M+H)^+$, 208.05 $(M+2)^+$; Anal. Calcd for $C_{10}H_8ClN_3$: C, 58.41; H, 3.92; N, 20.43; Found: C, 58.44; H, 3.96; N, 20.49.

(2-Chloro-pyrimidin-4-yl)-(4-fluoro-phenyl)-amine (6f). Yield: 86%; mp 178-180 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 6.46(d, 1H, *J* = 8.05 Hz), 7.08-7.14 (m, 2H), 7.25 (br s, 1H), 7.28-7.31 (m, 2H), 8.11 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 224.05 $(M+H)^+$; 226.07 $(M+2)^+$; Anal. Calcd for $C_{10}H_7ClFN_3$: C, 53.71; H, 3.15; N, 18.79; Found: C, 53.74; H, 3.21; N, 18.85.

(2-Chloro-6-methyl-pyrimidin-4-yl)-(4-fluoro-phenyl)-amine (6g). Yield: 90%; mp 148-150 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 2.32 (s, 3H), 6.29 (s, 1H), 7.09-7.14 (m, 2H), 7.17 (br s, 1H), 7.27-7.30 (m, 2H); ESI-MS (m/z): 238.06 $(M+H)^+$, 240.05 $(M+2)^+$; Anal. Calcd for $C_{11}H_9ClFN_3$: C, 55.59; H, 3.82; N, 17.68; Found: C, 55.64; H, 3.87; N, 17.74.

(4-Chloro-phenyl)-(2-chloro-pyrimidin-4-yl)-amine (6h). Yield: 94%; mp 200-202 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 6.55 (d, 1H, *J* = 5.13 Hz), 6.97 (br s, 1H), 7.28-7.30 (m, 2H), 7.37-7.39 (m, 2H), 8.15 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 240.05 $(M+H)^+$, 242.01 $(M+2)^+$; Anal. Calcd for $C_{10}H_7Cl_2N_3$: C, 50.03; H, 2.94; N, 17.50; Found: C, 50.10; H, 2.99; N, 17.54.

(4-Bromo-phenyl)-(2-chloro-pyrimidin-4-yl)-amine (6i). Yield: 85%; mp 207-209 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$): 6.56 (d, 1H, *J* = 5.13 Hz), 6.97 (br s, 1H), 7.23-7.25 (m, 2H), 7.51-7.54 (m, 2H), 8.15 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 283.99 $(M+H)^+$, 258.97 $(M+2)^+$; Anal. Calcd for $C_{10}H_7BrClN_3$: C, 42.21; H, 2.48; N, 14.77; Found: C, 42.25; H, 2.59; N, 14.80.

Typical proceudre for the synthesis of {2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(3,5-dimethoxy-phenyl)-amine (7a) and related compounds (7b-7i). To a stirred solution of compound **6a** (300 mg, 1 mmol) and compound **2** (279 mg , 1 mmol) in DMF (7 mL), K_2CO_3 (311 mg, 2 mmol) was added. Reaction mixture was stirred at 110-120 ^{o}C for 8-10 h. After completion of reaction, water was added to the reaction mixture and the product was extracted with EtOAc (3 x 30 mL). The combined organic layer was dried over Na_2SO_4 and solvent was evaporated under reduced pressure. The crude product was purified by column chromatography using EtOAc/hexane as eluent to afford pure compound **7a**.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(3,5-dimethoxy-phenyl)-amine (7a). Yield: 82%; mp 228-230 ^{o}C; IR (cm^{-1}, Film): 3370, 2932, 2849, 1573, 1461, 1423, 1341, 1224, 1152, 1067, 1014, 994, 825, 753; ^{1}H NMR (400 MHz, DMSO-d_6): 3.21-3.24 (m, 4H), 3.72 (s, 6H), 3.99-4.00 (m, 4H), 6.09 (d, 1H, *J* = 5.86 Hz), 6.11-6.12 (m, 1H), 6.93 (d, 2H, *J* = 2.20 Hz), 7.03-7.05 (m, 1H), 7.57 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 7.96-8.00 (m, 2H), 8.13 (d, 1H, *J* = 8.79 Hz), 8.70 (d, 1H, *J* = 5.13 Hz), 9.29 (br s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): 43.48, 51.58, 55.00, 94.36, 96.79, 97.33, 97.41, 109.67, 121.42, 125.92, 126.07, 128.03, 133.62, 141.96, 149.60, 152.21, 156.10, 156.31, 160.42, 161.01; ESI-MS (m/z): 477.19 $(M+H)^+$, 479.17 $(M+2)^+$; Anal. Calcd for $C_{25}H_{25}ClN_6O_2$: C, 62.95; H, 5.28; N, 17.62; Found: C, 62.99; H, 5.33; N, 17.65.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-6-methyl-pyrimidin-4-yl}-(3,5-dimethoxy-phenyl)-amine (7b). Yield: 89%; mp 212-214 ^{o}C; IR (cm^{-1}, Film): 3401, 2958, 2840, 1574, 1479, 1423, 1381, 1250, 1203, 1152, 1070, 927, 875, 754; ^{1}H NMR (400 MHz, $CDCl_3$): 2.26 (s, 3H), 3.25-3.27 (m, 4H), 3.78 (s, 6H), 4.08-4.11 (m, 4H), 5.98 (s, 1H), 6.21-6.24 (m, 1H), 6.52 (br s, 1H), 6.58 (d, 2H, *J* = 2.20 Hz), 6.86 (d, 1H, *J* = 5.13 Hz), 7.46 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.03 (d, 1H, *J* = 8.79 Hz), 8.06 (d, 1H, *J* = 2.20 Hz), 8.73 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 491.22 $(M+H)^+$, 493.17 $(M+2)^+$; Anal. Calcd for $C_{26}H_{27}ClN_6O_2$: C, 63.60; H, 5.54; N, 17.12; Found: C, 63.64; H, 5.56; N, 17.14.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(4-methoxy-phenyl)-amine (7c). Yield: 85%; mp 168-170

^{o}C; IR (cm^{-1}, Film): 3292, 2935, 2835, 1574, 1509, 1463, 1381, 1245, 1124, 1011, 933, 868, 824, 754; ^{1}H NMR (400 MHz, $CDCl_3$): 3.14-3.21 (m, 4H), 3.73 (s, 3H), 3.94-4.00 (m, 4H), 5.84 (d, 1H, *J* = 5.13 Hz), 6.59 (br s, 1H), 6.77 (d, 1H, *J* = 5.13 Hz), 6.82 (d, 2H, *J* = 8.05 Hz), 7.18 (d, 1H, *J* = 8.05 Hz), 7.38 (d, 1H, *J* = 7.32 Hz), 7.89-7.98 (m, 4H), 8.64 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 447.16 $(M+H)^+$, 449.18 $(M+2)^+$; Anal. Calcd for $C_{24}H_{23}ClN_6O$: C, 64.50; H, 5.19; N, 18.80; Found: C, 64.55; H, 5.23; H, 18.84.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-6-methyl-pyrimidin-4-yl}-(4-methoxy-phenyl)-amine (**7d**)**.** Yield: 82%; mp 122-124 ^{o}C; IR (cm^{-1}, Film): 3298, 2955, 2835, 1577, 1508, 1442, 1381, 1244, 1177, 1017, 998, 876, 754; ^{1}H NMR (400 MHz, $CDCl_3$): 2.29 (s, 3H), 3.25-3.27 (m, 4H), 3.82 (s, 3H), 4.06-4.08 (m, 4H), 5.80 (s, 1H), 6.39 (br s, 1H), 6.86 (d, 1H, *J* = 5.13 Hz), 6.91 (d, 2H, *J* = 8.79 Hz), 7.23-7.26 (m, 2H), 7.46 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.02-8.06 (m, 2H), 8.73 (d, 1H, *J* = 5.13 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 24.31, 43.82, 52.27, 55.47, 92.92, 109.05, 114.41, 121.94, 124.83, 125.15, 126.24, 128.83, 131.60, 134.94, 150.10, 151.89, 156.73, 157.10, 161.79, 162.40, 166.83; ESI-MS (m/z): 461.19 $(M+H)^+$, 463.18 $(M+2)^+$; Anal. Calcd for $C_{25}H_{25}ClN_6O$: C, 65.14; H, 5.47; N, 18.23; Found: C, 65.14; H, 5.48; N, 18.23.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-phenyl-amine (**7e**)**.** Yield: 89%; mp 230-232 ^{o}C; IR (cm^{-1}, Film): 3432, 2967, 2843, 1565, 1496, 1476, 1436, 1384, 1296, 1227, 1011, 934, 866, 826, 750, 690; ^{1}H NMR (400 MHz, DMSO-d_6): 3.21-3.24 (m, 4H), 3.96-3.97 (m, 4H), 6.08 (d, 1H, *J* = 5.13 Hz), 6.92-6.98 (m, 1H), 7.04 (d, 1H, *J* = 5.13 Hz), 7.27-7.31 (m, 2H), 7.56 (dd, 1H, *J* = 9.52 Hz, *J* = 2.20 Hz), 7.62 (d, 2H, *J* = 8.79 Hz), 7.96 (d, 1H, *J* = 5.13 Hz), 7.98 (d, 1H, *J* = 2.20 Hz), 8.13 (d, 1H, *J* = 8.79 Hz), 8.70 (d, 1H, *J* = 5.13 Hz), 9.29 (br s, 1H); ESI-MS (m/z): 417.18 $(M+H)^+$, 419.15 $(M+2)^+$; Anal. Calcd for $C_{23}H_{21}ClN_6$: C, 66.26; H, 5.08; N, 20.16; Found: C, 66.30; H, 5.12; N, 20.18.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-(4-fluoro-phenyl)-amine (**7f**)**.** Yield: 81%; mp 226-228 ^{o}C; IR (cm^{-1}, Film): 3275, 2962, 2853, 1577, 1506, 1441, 1350, 1297, 1220, 1125, 1086, 1053, 1011, 979, 933, 870, 834, 731, 655, 517; ^{1}H NMR (400 MHz, DMSO-d_6): 3.20-3.23 (m, 4H), 3.95-3.98 (m, 4H), 6.05 (d, 1H, *J* = 5.86 Hz), 7.02 (d, 1H, *J* = 5.13 Hz), 7.11-7.15 (m, 2H), 7.57 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 7.61 - 7.64 (m, 2H), 7.96 (d, 1H, *J* = 5.86 Hz), 7.98 (d, 1H, *J* = 2.20 Hz), 8.12 (d, 1H, *J* = 8.79 Hz), 8.70 (d, 1H, *J* = 5.13 Hz), 9.31 (br s, 1H); ESI-MS (m/z): 435.17 $(M+H)^+$, 437.14 $(M+2)^+$; Anal. Calcd for $C_{23}H_{20}ClFN_6$: C, 63.52; H, 4.64; N, 19.32; Found: C, 63.58; H, 4.65; N, 19.34.

{2-[4-(7-Chloro-quinolin-4-yl)-piperazin-1-yl]-6-methyl-pyrimidin-4-yl}-(4-fluoro- phenyl)-amine (**7g**)**.** Yield: 86%; mp 236-238 ^{o}C; IR (cm^{-1}, Film): 3308, 2918, 2848, 1577, 1506, 1446, 1361, 1233, 1214, 1176, 1073, 998, 876, 754; ^{1}H NMR (400 MHz, $CDCl_3$): 2.25 (s, 3H), 3.25-3.27 (m, 4H), 4.06-4.08 (m, 4H), 5.84 (s, 1H), 6.48 (br s, 1H), 6.86 (d, 1H, *J* = 5.13 Hz), 7.03-7.07 (m, 2H), 7.30-7.34 (m, 2H), 7.46 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.03 (d, 1H, *J* = 8.79 Hz), 8.06 (d, 1H, *J* = 2.20 Hz), 8.73 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 449.18 $(M+H)^+$, 451.17 $(M+2)^+$; Anal. Calcd for $C_{24}H_{22}ClNFN_6$: C, 64.21; H, 4.94; N, 18.72; Found: C, 64.26; H, 4.97; N, 18.75.

(4-Chloro-phenyl)-{2-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-amine (**7h**)**.** Yield: 88%; mp 198-200 ^{o}C; IR (cm^{-1}, Film): 3314, 3190, 2841, 1609, 1572, 1465, 1380, 1237, 1125, 1087, 1008, 933, 825, 793, 714; ^{1}H NMR (400 MHz, $CDCl_3$): 3.26-3.28 (m, 4H), 4.06-4.08 (m, 4H), 6.01 (d, 1H, *J* = 5.13 Hz), 6.66 (br s, 1H), 6.86 (d, 1H, *J* = 5.13 Hz), 7.30-7.38 (m, 4H), 7.46 (dd, 1H, *J* = 8.79 Hz, *J* = 2.20 Hz), 8.02-8.06 (m, 3H), 8.73 (d, 1H, *J* = 5.13 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 43.82, 52.18, 95.39, 109.12, 121.93, 122.60, 125.09, 126.38, 128.79, 128.83, 129.13, 135.03, 137.42, 150.09, 151.89, 157.00, 157.19, 160.72, 161.61; ESI-MS (m/z): 451.13 $(M+H)^+$, 453.11 $(M+2)^+$; Anal. Calcd for $C_{23}H_{20}Cl_2N_6$: C, 61.20; H, 4.47; N, 18.62; Found: C, 61.26; H, 4.51; N, 18.66.

(4-Bromo-phenyl)-{2-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-pyrimidin-4-yl}-amine (**7i**)**.** Yield: 81%; mp 184-186 ^{o}C; IR (cm^{-1}, Film): 3314, 3098, 2841, 1609, 572, 1488, 1465, 1351, 1295, 1237, 1087, 1008, 933, 867, 825, 793; ^{1}H NMR (400 MHz, $CDCl_3$): 3.26-3.31 (m, 4H), 4.05-4.13 (m, 4H), 6.01 (d, 1H, *J* = 5.13 Hz), 6.60 (brs, 1H), 6.86 (d, 1H, *J* = 5.13 Hz), 7.29-7.33 (m, 2H), 7.44-7.49 (m, 3H), 8.02-8.07 (m, 3H), 8.73 (d, 1H, *J* = 5.13 Hz); ESI-MS (m/z): 495.08 $(M+H)^+$; 497.09 $(M+2)^+$; Anal. Calcd for $C_{23}H_{20}BrClN_6$: C, 55.72; H, 4.07; N, 16.95; Found: C, 55.75; H, 4.06; N, 16.91.

Conclusion

In summary, a series of 4-aminoquinoline-pyrimidine hybrids were designed and their *in vitro* antimalarial activity was evaluated against CQ-sensitive and CQ-resistant strains of *P. falciparum* together with their cytotoxicity against mammalian cells. The most promising compounds **5h** and **5j** exhibited superior potency and selectivity than CQ against CQ-resistant strain and moderate activity against CQ-sensitive strain. Compounds **5e**, **5f**, **5g**, **5i**, **5k** and **7d** have displayed similar efficacy against CQ-resistant strain and moderate efficacy against CQ-sensitive strain when compared to CQ. None of the compounds showed any appreciable toxicity against mammalian cells. However on comparing antimalarial profile of these rigid linked hybrids with previously reported flexible ones,[17] decrease in potency was found which highlights the importance of linker in covalent hybridization. Further structural optimization of these hybrids, especially of **5h** and **5j** may lead to more promising hybrids for malaria chemotherapy.

Acknowledgments:

DSR thanks University Grants Commission [F. No. 41-202/2012(SR)], New Delhi, India and University of Delhi, Delhi, India for financial support. AT is thankful to CSIR for the award of senior research fellowship. Thanks to USIC-CIF, University of Delhi for analytical data. SIK is thankful to United States Department of Agriculture (USDA), Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009 for

partial support of this work. Mr Sumit Srivastava for X-ray crystallography and John Trott for bioassay as also acknowledged.

Notes and references

[a] *Department of Chemistry, University of Delhi, Delhi-110007, India Fax: 91-11-27667501; Tel: 91-11-27662683; *E-mail: dsrawat@chemistry.du.ac.in*
[b] *National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*

1. S. H. Kappe, A. M. Vaughan, J. A. Boddey and A. F. Cowman, *Science*, 2010, **328**, 862; L. Turner, T. Lavstsen, S. S. Berger, C. W. Wang, J. E. V. Petersen, M. Avril, A. J. Brazier, J. Freeth, J. S. Jespersen, M. A. Nielsen, P. Magistrado, J. Lusingu, J. D. Smith, M. K. Higgins and T. G. Theander, *Nature*, 2013, **498**, 502; N. Kumar, R. Singh, and D. S. Rawat, *Med. Res. Rev.*, 2012, **32**, 581; N. Kumar, M. Sharma and D. S. Rawat, *Curr. Med. Chem.*, 2011, **18**, 3889.
2. WHO. *World Malaria Report*; WHO: Geneva, Switzerland, 2013.
3. J. Wiesner, R. Ortmann, H. Jomaa and M. Schlitzer, *Angew. Chem. Int. Ed.*, 2003, **42**, 5274; J. K. Baird, *N. Engl. J. Med.*, 2005, **352**, 1565.
4. A. B. S. Sidhu, D. V. Pinard and D. A. Fidock, *Science*, 2002, **298**, 210; N. J. White, *N. Engl. J. Med*., 2009, **361**, 455.
5. V. V. Kouznetsov and A. G. Barrio, *Eur. J. Med. Chem.*, 2009, **44**, 3091; P. M. O'Neill, S. A. Ward, N. G. Berry, J. P. Jeyadevan, G. A. Biagini, E. Asadollaly, B. K. Park and P. G. Bray, *Curr. Top. Med. Chem.*, 2006, **6**, 479.
6. K. V. Sashidhara, M. Kumar, R. K. Modukuri, R. K. Srivastava, A. Soni, K. Srivastava, S. V. Singh, J. K. Saxena, H. M. Gauniyal and S. K. Puri, *Bioorg. Med. Chem.*, 2012, **20**, 971.
7. B. Meunier, *Acc. Chem. Res.*, 2008, **41**, 69.
8. A. Robert, D. O. Cabaret, J. Cazelles and B. Meunier, *Acc. Chem. Res.*, 2002, **35**, 167.
9. B. Pérez, C. Teixeira, J. Gut, P. J. Rosenthal, J. R. B. Gomes and P. Gomes, *Chem. Med. Chem.,* 2012, **7**, 1537.
10. P. F. Salas, C. Herrmann, J. F. Cawthray, C. Nimphius, A. Kenkel, J. Chen, C. de Kock, P. J. Smith, B. O. Patrick, M. J, Adam and C. Orvig, *J. Med. Chem.*, 2013, **56**, 1596.
11. M. Guo, C. J. Zheng, M. X. Song, Y. Wu, L. P. Sun, Y. J. Li, Y. Liu and H. R. Piao, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 4358.
12. S. Gemma, G. Campiani, S. Butini, B. P. Joshi, G. Kukreja, S. S. Coccone, M. Bernetti, M. Persico, V. Nacci, I. Fiorini, E. Novellino, D. Taramelli, N. Basilico, S. Parapini, V. Yardley, S. Croft, S. K. Maerki, M. Rottmann, R. Brun, M. Coletta, S. Marini, G. Guiso, S. Caccia and C. Fattorusso, *J. Med. Chem.*, 2009, **52**, 502.
13. S. Melato, D. Prosperi, P Coghi, N. Basilico and D. Monti, *Chem. Med. Chem*., 2008, **3**, 873; A. Kumar, K. Srivastava, S. R. Kumar, S. K. Puri and P. M. S. Chauhan, *Bioorg. Med. Chem. Lett*., 2008, **18**, 6530.
14. D. Cornut, H. Lemoine, O. Kanishchev, E. Okada, F. Albrieux, A. H. Beavogui, A. L. Bienvenu, S. Picot, J. P. Bouillon and M. Medebielle, *J. Med. Chem.,* 2013, **56**, 73.
15. B. T. Mott, K. C. C. Cheng, R. Guha, V. P. Kommer, D. L. Williams, J. J. Vermeire, M. Cappello, D. J. Maloney, G. Rai, A. Jadhav, A. Simeonov, J. Inglese, G. H. Posner and C. J. Thomas, *Med. Chem. Commun*., 2012, **3**, 1505.
16. S. Manohar, U. C. Rajesh, S. I. Khan, B. L. Tekwani and D. S. Rawat, *ACS. Med. Chem. Lett.*, 2012, **3**, 555.
17. K. Singh, H. Kaur, P. Smith, C. D. Kock, K. Chibale and J. Balzarini, *J. Med. Chem*., 2014, **57**, 435.
18. S. Manohar, S. I. Khan and D. S. Rawat, *Chem. Biol. Drug Des*., 2013, **81**, 625; N. Kumar, S. I. Khan and D. S. Rawat, *Helv. Chim, Acta*., 2012, **95**, 1181; S. Manohar, S. I. Khan and D. S. Rawat, *Chem. Biol. Drug Des*., 2011, **78**, 124; N. Kumar, S. I. Khan, H. Atheaya, R. Mamgain and D. S. Rawat*, Eur. J. Med. Chem*., 2011, **46**, 2816; S. Manohar, S. I. Khan and D. S. Rawat, *Bioorg. Med. Chem. Lett*., 2010, **20**, 322; N. Kumar, S. I. Khan, Beena, G. Rajalakshmi, P. Kumaradhas, D. S. Rawat, *Bioorg. Med. Chem.,* 2009, **17**, 5632; N. Kumar, S. I. Khan, M. Sharma, H. Atheaya, D. S. Rawat, *Bioorg. Med. Chem. Lett.,* 2009, **19**, 1675;
19. S. I. Pretorius, W. I. Breytenbach, C. D. Kock, P. J. Smith and D. D. N'Da, *Bioorg. Med. Chem*., 2013, **21**, 269.
20. M. Jain, S. I. Khan, B. L. Tekwani, M. R. Jacob, S. Singh, P. P. Singh and R. Jain, *Bioorg. Med. Chem*., 2005, **13**, 4458.
21. MAESTRO 9.3 (Schrodinger,Inc., LLC, NY, USA).
22. G. Caron, F. Reymond, P. A. Carrupt, H. H. Girault and B. Testa, *Pharm. Sci. Technol. Today*, 1999, **2**, 327.
23. M. Traebert, B. Dumotier, L. Meister, P. Hoffmann, M. D. Estevez, W. Suter, *Eur. J. Pharmacol*., 2004, **484**, 41.

Synthesis of Piperazine Tethered 4-Aminoquinoline-Pyrimidine Hybrids as Potent Antimalarial Agents

Anuj Thakur, Shabana I. Khan, Diwan S. Rawat*

Piperazine linked 4-aminoquinoline-pyrimidine hybrids were synthesized and evaluated for *in vitro* antimalarial activity against W2 and D6 strains of *plasmodium falciparum.*