NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

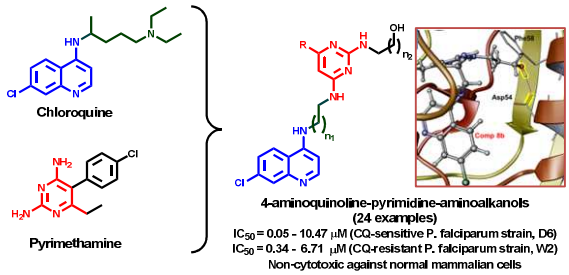
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

4-Aminoquinoline-pyrimidine-aminoalkanols: Synthesis, *in vitro* antimalarial activity, docking studies and ADME predictions

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

4-Aminoquinoline-pyrimidine-aminoalkanols displaying good *in vitro* antimalarial activities against both CQ-sensitive and resistant strains of *P. falciparum* together with favourable resistance-indices and predicted ADME properties are reported.



Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

4-Aminoquinoline-pyrimidine-aminoalkanols: Synthesis, *in vitro* antimalarial activity, docking studies and ADME predictions+

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

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

DOI: 10.1039/b000000x

Twenty-four new 4-aminoquinoline-pyrimidine hybrids containing a terminal aliphatic amino-alcohol chain were synthesized and assessed for their antimalarial activity against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. All compounds displayed potent antiplasmodial activities (IC_{50} values in the range of 0.05-10.47 μM) with no appreciable cytotoxicity towards mammalian cells up to the highest tested concentration of 12 μM. Molecular docking studies of the most active compounds (**8b-8f, 8u** and **8v**) with both wild type and quadruple mutant Pf-DHFR-TS were performed, which exhibited comparable interactions as the conventional folate inhibitors. ADME predictions also revealed favourable pharmacokinetic parameters for the synthesized hybrids which warrant their suitability for development as potent antimalarials.

Introduction

Drug-resistance is not only a major public health concern but also rattles the scientific community involved in drug research. This problem is rising in epic proportions and mounting an enormous pressure on the governments and researchers worldwide. The rational strategy to counter drug-resistant diseases is to combine two or more active compounds with independent mode of action (combination therapy) to prevent the advent of resistance. This strategy was first formulated for anti-tuberculosis chemotherapy,[1,2] followed by cancer chemotherapy,[3] and lately for the treatment of malaria[4] and AIDS[5]. Malaria, transmitted in humans through the bite of female *Anopheles* mosquito is caused by the infection with any of the five species of *Plasmodium* of which *P. falciparum* is the most lethal. According to the WHO World Malaria Report 2013, 97 countries had ongoing malaria transmission in 2013 with an estimated 3.4 billion people at risk of malaria, of which 1.2 billion are at a high risk.[6]

Development and use of anti-malarial medications such as chloroquine (CQ, a 4-aminoquinoline), folate inhibitors (pyrimethamine, cycloguanil etc.) and artemisinins remained a mainstay towards the control and treatment of malaria (Figure 1). However, with the spate of reports on drug-resistant parasite strains including the recent findings of artemisinin-resistance in four south-east Asian countries of the Greater Mekong sub-region,[6-9] there is a dire need to develop cost-effective anti-malarials. Currently, WHO recommends artemisinin-based combination therapy (ACT) with a conventional anti-malarial agent, particularly for the infection caused by *P. falciparum*.[6]

Several strategies have been explored towards the development of new antimalarial agents to address the problem of drug-resistance. The concept of molecular hybrids has shown promising results and may hold a key *en route* in solving this

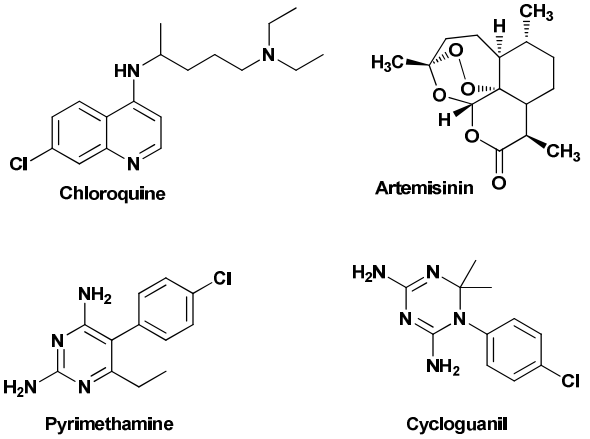


Fig. 1 Conventional antimalarial drugs

menace. In hybrid antimalarials, two (or more) discrete antimalarial scaffolds are linked covalently, through a spacer, in an anticipation that these compounds may act by inhibiting concurrently two or more traditional targets.[10] These multi-targeted hybrids not only counter the threat of drug-resistance against the parent pharmacophore(s), but also overcome most of the problems associated with the combination therapies by ensuring more predictable pharmacokinetic profiles, reduced risk of drug-drug interactions and most significantly, the patient-compliance. Lately, numerous hybrids for the treatment of malaria have been reported[11,12] and 4-aminoquinoline based hybrids have exhibited the most encouraging results.[12-17] Some of them are currently undergoing pre-clinical studies.[13] 4-Aminoquinolines disrupt the parasite's detoxification pathway for conversion of the toxic-heme to non-toxic hemozoin[18] within its digestive vacuole. This target is ideal as it is host-derived and inherently not under the control of the parasite.[19] Thus, long-

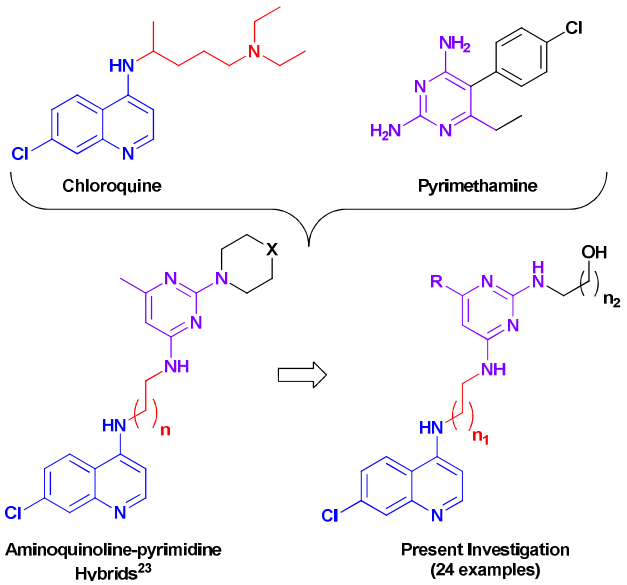


Fig. 2 Design of 4-aminoquinoline-pyrimidine hybrids

acting 4- aminoquinolines, with the added advantages of easy and cheap synthesis and excellent clinical efficacy, remain crucial for the development of antimalarial molecular hybrids. Towards this end, we had previously reported 4-aminoquinoline based hybrids with folate inhibitor triazine[20-22] and pyrimidine[23-26] pharmacophores.

It was envisaged that hybridization of such DHFR-inhibitors with 4-aminoquinolines may result in more potent molecules that may act by simultaneously disrupting the heme-detoxification pathway and halting the tetrahydrofolate synthesis of the malarial parasite. In order to study the effect of structural modifications at the pyrimidine core of the 4-aminoquinoline-pyrimidine conjugates on their antimalarial potency and pharmacokinetic behaviour, and in the larger interest of deducing the structure-activity relationships, we decided to substitute the cyclic secondary amines attached to the pyrimidine nucleus with various open chain aliphatic amino-alcohols (Figure 2). Also, analogous conjugates *sans* a methyl group at the 6th position of pyrimidine ring were prepared. In addition, molecular docking with Pf-DHFR-TS and prediction of ADME properties for the active compounds were performed to perceive their possible mode of action and their aptness towards further development into a drug, respectively.

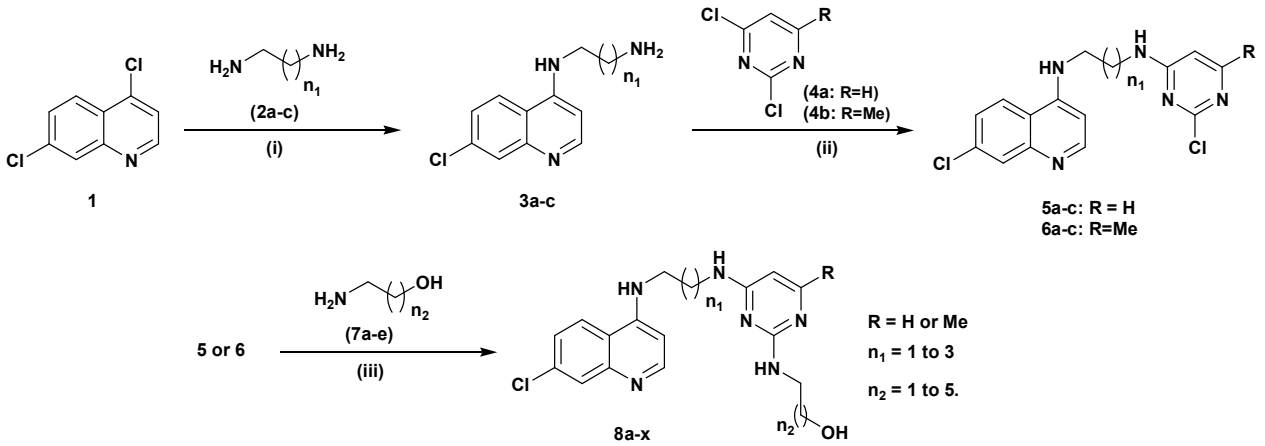
Results and discussion

Chemistry

Scheme-1 depicts the synthetic route to the present hybrids. Commercially available 4,7-dichloroquinoline (**1**) was selected as the starting material to which were appended various diaminoalkanes (**2a-c**) *via* an S_NAr reaction by heating under neat conditions to give intermediates **3a-c**. These intermediates were then reacted with either 2,4-dichloropyrimidine (**4a**) or 2,4-dichloro-6-methylpyrimidine (**4b**) to yield respectively **5a-c** or **6a-c** intermediates. Intermediates (**5** or **6**) were then reacted with various aliphatic linear chain amino-alcohols (**7a-e**) at an elevated temperature (120 °C) in presence of K_2CO_3 and DMF (N,N-dimethylformamide) as a solvent, leading to the desired 4-aminoquinoline-pyrimidine-aminoalkanols (**8a-x**) in good yields. During the course of the synthesis, it was observed that solubility of 6-methyl pyrimidine hybrids was better than their non-methyl counterparts in organic solvents. Additionally, a steady increase in the lipophilicity of the hybrids was observed with the increasing chain length of amino alcohols.

Biological Activities

The antimalarial potential of all the synthesised hybrids against both CQ-sensitive (D6) and CQ-resistant (W2) strains of *P. falciparum* was assessed *via* previously reported procedure based on the calculation of plasmodial LDH activity.[27,28] All the 24 compounds showed potent antimalarial activity, with seven compounds (**8b-8e** and **8t-8v**) displaying sub-micromolar IC_{50} values against both the *P. falciparum* strains (Table-1). Compound **8e** exhibited comparable activity (IC_{50} = 0.05 µM) to CQ against CQ-sensitive (D6) strain whereas hybrids **8b** (0.47 µM), **8c** (0.34 µM) and **8d** (0.41 µM) displayed antimalarial activities akin to the reference drug against the CQ-resistant strain.



Scheme 1 (i) Neat, 120-130 °C, 6 h, 70-90 %; (ii) N,N-diisopropylethylamine, THF, 0 °C –RT, 14 h, 80-85%; (iii) K_2CO_3, DMF, 120 °C, 10h, 70-90%

As mentioned earlier, the lipophilicities of 6-methyl pyrimidine analogues were found to be better in comparison to the non-methyl ones and a gradual rise in their lipophilicity was observed on increasing the chain length of amino-alcohols attached to the pyrimidine nucleus. The cumulative effects of both these structural features on the lipophilicity of the hybrids were also correspondingly observed on their antimalarial activities as well, and the same can be observed in Table-1, especially on comparing series **8o-s** (having no methyl group on pyrimidine) with **8t-x** series (having a methyl group at 6th position of pyrimidine) of compounds. These results are in concordance with the earlier reported observations on relation of higher antiplasmodial activity with better lipophilicity of quinoline antimalarials.[29,30] The resistance-index (RI) of these molecules, which is the ratio of IC_{50} value against the CQ-resistant strain to the IC_{50} value against the CQ-sensitive strain, were significantly lower (up to 0.57) than CQ (RI = 10.75), indicating that the synthesized hybrids were potent against both the sensitive and resistant *P. falciparum* strains. A lower RI is suggestive of a promising lead antimalarial as this exemplifies its equipotent nature irrespective of the vulnerability of the strain which further signifies its utility in solving the drug resistance nuisance.

All the synthesized hybrids were also assessed for mammalian cytotoxicity by testing against VERO cells. None of them exhibited any cytotoxicity up to the highest tested concentration of 12 μM which, in turn, signifies their safety towards mammalian cells and correspondingly indicative of a higher therapeutic index. Accordingly, the selectivity indices (SI) for these hybrids (defined as the ratio of IC_{50} value for cytotoxicity to Vero cells and IC_{50} value for antimalarial activity) were high.

On comparing the presently synthesized molecules with the previously reported lead compounds,[23-26] it was found that substituting the carbocyclic/aromatic amines attached to the pyrimidine nucleus with open chain amino alcohols brings about a slight decrease in their antimalarial activities. However, the present analogues had a significantly lower Resistance-Index implying that these hybrids are more compliant to solve the plasmodial drug resistance issue. Moreover, this structural modification wiped out any inherent mammalian cytotoxicity associated with previously reported molecules.[23-26] Overall, derivatives containing 6-methyl pyrimidine (**8a-e**, **8k-n** and **8t-x**) were more active than their corresponding non-methyl pyrimidine derivatives and analogues having ethylene diamine linker (n_1 =1, **8a-e**) and butane-diamine linker (n_1 = 3, **8t-x**) were found to be the most potent against both the plasmodial strains.

Table 1 *In vitro* antimalarial activity and cytotoxicity of 4-aminoquinoline-pyrimidine-aminoalcohols.

Compound	R	n_1	n_2	*P. falciparum* (D6 clone)		*P. falciparum* (W2 clone)		Resistance Index (RI)	Cytotoxicity (VERO cells), IC_{50} (μM)
				IC_{50} (μM)	SI	IC_{50} (μM)	SI		
8a	Me	1	1	3.37	>3.8	2.22	>5.7	0.65	NC
8b	Me	1	2	0.13	>94	0.47	>26.2	3.61	NC
8c	Me	1	3	0.12	>99.3	0.34	>34.8	2.83	NC
8d	Me	1	4	0.12	>92.5	0.41	>27.9	3.42	NC
8e	Me	1	5	0.05	>216.4	0.63	>17.5	12.6	NC
8f	H	2	1	3.95	>3.2	4.15	>3.1	1.05	NC
8g	H	2	2	1.87	>6.6	2.6	>4.7	1.39	NC
8h	H	2	3	10.47	>1.1	6.01	>2	0.57	NC
8i	H	2	4	3.87	>3	4.02	>2.9	1.04	NC
8j	H	2	5	3.35	>3.3	2.62	>4.2	0.78	NC
8k	Me	2	1	3.72	>3.3	2.71	>4.5	0.73	NC
8l	Me	2	2	3.23	>3.7	3.46	>3.4	1.07	NC
8m	Me	2	3	3.08	>3.7	1.9	>6	0.62	NC
8n	Me	2	4	2.64	>4.2	2.07	>5.4	0.78	NC
8o	H	3	1	1.66	>7.4	1.92	>6.4	1.15	NC
8p	H	3	2	2.57	>4.6	2.13	>5.6	0.83	NC
8q	H	3	3	2.74	>4.2	2.14	>5.4	0.78	NC
8r	H	3	4	3.05	>3.6	2.62	>4.2	0.86	NC
8s	H	3	5	3.17	>3.4	2.53	>4.2	0.80	NC
8t	Me	3	1	0.15	>76.7	0.91	>13	6.07	NC
8u	Me	3	2	0.13	>85.2	0.89	>12.9	6.85	NC
8v	Me	3	3	0.13	>82	0.63	>17.6	4.85	NC
8w	Me	3	4	1.54	>7	1.54	>7	1.0	NC
8x	Me	3	5	1.43	>7.3	1.19	>8.7	0.83	NC
CQ	-	-	-	0.04	>372	0.43	>34.6	10.75	NC
Pyrimethamine	-	-	-	0.01	-	NA	-	-	NT

SI, Selectivity Index = (IC_{50} for cytotoxicity to Vero cells)/(IC_{50} for antimalarial activity); RI, Resistance index = IC_{50} (W2 strain)/IC_{50} (D6 strain); NC: No cytotoxicity up to 12 μM; Vero: monkey kidney fibroblasts; NA: Not active up to 19 μM; NT: Not tested.

Computational Studies

To gain a better understanding of the molecular basis through which these aminoquinoline–pyrimidines work, molecular docking studies of best active compounds (**8b-8f**, **8u** and **8v**) were performed in the active sites of both the wild type and quadruple mutant Pf-DHFR-TS (PDB ID: 3QGT and 3QG2, respectively) structures using Glide v5.8[31] and the results are summarized in Table 2. The docked conformations of best scored ligands (**8b** and **8c**) in the binding pocket of wild type and quadruple mutant Pf-DHFR-TS are illustrated in Figure 3.

Table 2 Glide docking energies and docking scores of aminoquinoline-pyrimidine hybrids **8b-8f**, **8u** and **8v** along with the reference compounds in wild type and quadruple mutant pf-DHFR-TS

Compounds	Docking results with Wild type Pf-DHFR-TS		Docking results with Quadruple mutant Pf-DHFR-TS	
	Glide Energy	XP GScore	Glide Energy	XP GScore
8b	-55.27	-8.60	-62.49	-6.88
8c	-52.55	-8.55	-49.94	-6.81
8d	-41.48	-7.70	-47.08	-6.51
8e	-47.33	-6.95	-47.24	-6.41
8f	-48.96	-7.20	-46.45	-5.76
8u	-37.77	-6.68	-42.01	-5.37
8v	-39.04	-6.95	-44.56	-5.53
Dihydrofolate	-65.11	-9.34	-63.69	-10.59
Pyrimethamine	-44.55	-9.08	-46.48	-9.39
WR99210	-38.04	-5.61	-40.07	-5.93
Cycloguanil	-37.97	-8.83	-59.48	-8.68

The best active compounds in the study displayed excellent binding affinities towards both the quadruple mutant (–62.49 kcal mol^{-1} to –42.01 kcal mol^{-1}) and wild type (–55.27 kcal mol^{-1} to –37.77 kcal mol^{-1}) Pf-DHFR-TS structures which are comparable to those of the native substrate dihydrofolate and standard Pf-DHFR inhibitors (pyrimethamine, WR99210 and cycloguanil; Table 2). It was observed that compounds **8b-f** (with ethylene diamine linker between 4-aminoquinoline and pyrimidine rings) had better Glide energy scores than the corresponding analogues having a butane-1,4- diamine linker (**8u** and **8v**).

Figure 3 represents the docking conformations of the highest scored compounds **8b** and **8c** for both the wild type and mutant Pf-DHFR-TS structures, respectively and elucidates the hydrogen-bonding pattern together with any π–π interactions and van der Waals interactions in their predicted binding poses with the binding pocket residues. Compound **8b**, with lowest binding energy (–62.49 kcal mol^{-1}) and significantly good XP score (–6.88 kcal mol^{-1}) for quadruple mutant Pf-DHFR, shows formation of a H-bond between its terminal chain –OH group and the carboxylic side chain of Asp54 in both the types of Pf-DHFR protein. Another hydrogen bonding interaction is observed between pyrimidine's terminal –NH group and Ile164 main chain oxygen atom within wild type Pf-DHFR. Further, pyrimidine ring of compound **8b** shows a π–π interaction with the aromatic ring of Phe58 in the mutant protein's active site.

Compound **8c** was another molecule estimated to display a low binding energy (–49.94 kcal mol^{-1}) and a correspondingly high glide score (–6.81) against the quadruple mutant Pf-DHFR-TS protein and exhibits a similar hydrogen-bonding pattern between terminal –OH group of the molecule and carboxylic side chain of Asp54. Compound **8c** also forms H–bond interaction between terminal –NH atom and Ile164 main chain oxygen atom in wild type Pf-DHFR's active site, and with Leu164 in the mutant Pf-DHFR, akin to that observed with compound **8b**.

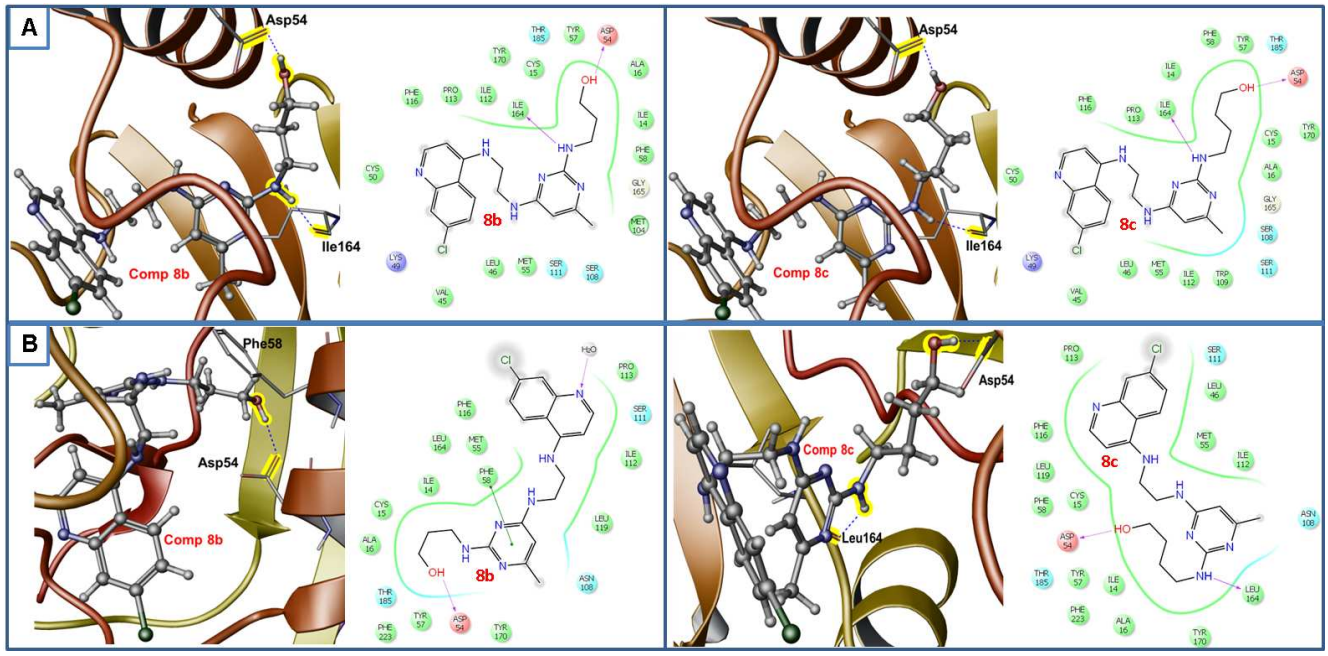


Fig. 3 3D and 2D docking pose showing interaction for compound **8b** and **8c** in the binding site of A) wild type Pf-DHFR-TS (PDB ID: 3QGT) and B) quadruple mutant Pf-DHFR-TS (PDB ID: 3QG2)

Resistance against conventional Pf-DHFR inhibitors is attributed to the quadruple mutations (N51I, C59R, S108N and I164L) in the binding site of Pf-DHFR which, in turn, impedes an efficient binding of the folate inhibitors with the enzyme. While N51I and C59R mutations do not stimulate any major structural changes in the protein's binding site, I164L mutation leads to a spatial shifting of the residues 164–167 and influences the cavity of the binding site in such a way that it leads to steric clashing of Phe58 with rigid inhibitors such as pyrimethamine and cycloguanil.[32] In the present docking study, we have observed H–bond interactions of test compounds with Asp54 (Figure 3), which is noteworthy, as this active site residue is crucial for ligand binding in DHFR and this interaction is also observed in DHFR native substrate *viz.* dihydrofolate. Moreover, the highest scored ligand **8b** is at a convenient distance from Phe58, thus evading steric interaction with the Phe58's aromatic side-chain while at the same time, forming a π–π interaction with it.

The S108N mutation modifies the active site of Pf-DHFR and induces a steric confrontation between the side chain of Asn108 and the rigid *p*-chlorophenyl moiety in pyrimethamine and cycloguanil in the binding domain of the protein.[32] However, if the inhibitor molecule is so designed as to dwell within the surface volume of the native substrate, there is a more likelihood of it being less prone to resistance occurring due to steric interactions in the mutant protein's binding site.[33,34] To address this possibility, the docking poses of the best active compounds in the present study, along with those of the reference molecules were superimposed on the dihydrofolate surface envelope (Figure 4) and which clearly demonstrates that the test compounds possessing flexible aliphatic linker chains occupy a similar volume as that of the native substrate while, at the same time, evade any steric confrontation(s) with Asn108.

To further check the drugability of the synthesized aminoquinoline-pyrimidine hybrids, ADMET predictions for the best active compounds (**8b**-**8f**, **8u** and **8v**) by Qikprop v3.5[35] were performed and the results are presented in Table 3. Lipinski's rule of 5, which ascribes a primary criterion for drug-likeness of a molecule,[36] was calculated for the test compounds and the Qikprop results for various parameters are shown in Table 3.

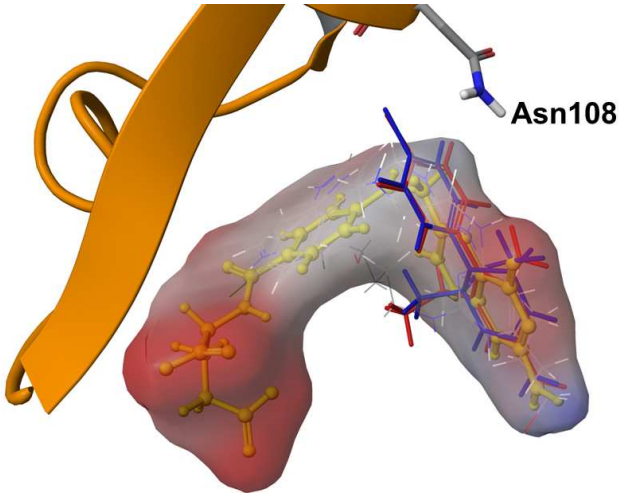


Fig. 4 Molecular overlay of the docking poses of best active test compounds (grey sticks), cycloguanil (blue sticks), pyrimethamine (red sticks) and the Pf-DHFR substrate dihydrofolate (yellow balls and sticks) at the binding site of quadruple mutant Pf-DHFR–TS (PDB ID: 3QG2)

The test compounds displayed zero violation for Lipinski's rule of 5 signifying that these compounds are anticipated to possess drug-like properties. Further, this prediction also implies that the test compounds can be used as a prospective orally-active antimalarial. This notion is further supported by the prediction of the oral drug absorption (PercentHumanOralAbsorption), which was highly encouraging for all the test compounds. QPPCaco i.e. Caco–2 cells permeability estimations for all the test compounds exhibited favourable values comparable to those of standard Pf-DHFR-inhibitors, and suggestive of their good intestinal absorption/permeation. Moreover, the polar surface area of the active test compounds falls adequately in the permissible range and the rotatable bonds are <15 (Table 3). The latter descriptors are again important for predictions of oral bioavailability of a compound as studies have revealed that oral bioavailability is affected by molecular flexibility which in turn, can be predicted by the polar surface area (7 Å^2–200 Å^2) and number of rotatable bonds (<15).

Table 3 Prediction of Lipinski's 'Rule of 5' for most active aminoquinoline-pyrimidine hybrids (**8b-8f**, **8u** and **8v**) and other ADMET properties

Compound	**8b**	**8c**	**8d**	**8e**	**8f**	**8u**	**8v**	**Pyr**	**Cyg**
Lipinski's 'Rule of 5'[a]									
mol_MW	386.88	400.91	414.94	428.96	400.91	414.94	428.96	248.71	253.73
donorHB	4	4	4	4	4	4	4	4	5
accptHB	7	7	7	7	7	7	7	3	3
QPlogPo/w	2.77	3.16	3.51	3.87	3.56	3.93	4.4	1.81	0.89
RuleOfFive	0	0	0	0	0	0	0	0	0
ADMET properties									
PercentHumanOralAbsorption[a] (>80%-high,<25% poor)	87.91	89.90	91.96	94.07	92.32	94.56	100	84.35	68.81
QPPCaco (nms) (<25 poor, >500 great)[a]	315.57	305.31	304.6	304.96	307.37	310.69	311.19	412.29	111.85
QplogBB (-3.0 - 1.2)[a]	-1.36	-1.532	-1.64	-1.75	-1.73	-1.83	-1.99	-0.78	-0.17
QPPMDCK (<25 poor, >500 great)[a]	322.82	306.63	305.78	306.20	341.05	345.04	345.64	468.85	126.60
QplogKhsa (-1.5 to 1.5)[a]	-0.03	0.07	0.16	0.26	0.17	0.27	0.41	-0.24	-0.31
QPlogHERG (concern below -5)[a]	-4.95	-5.40	-5.61	-5.79	-6.39	-6.55	-6.91	-4.32	-4.58
PSA (7.0 – 200.0)[a]	95.33	95.31	95.32	95.32	95.12	95.18	95.18	73.73	76.26
#rotor (0 – 15)[a]	10	11	12	13	11	12	13	4	2

[a]Calculated using QikProp v 3.5. Range/recommended values calculated for 95% known drugs; Pyr = Pyrimethamine; Cyg = Cycloguanil

Furthermore, all inhibitors were predicted to lie within the acceptable ranges for QPlogKhsa (human serum albumin binding prediction; acceptable range: –1.5 to 1.5). Likewise, QPlogBB (blood/brain partition coefficient) and QPPMDCK (estimation of MDCK cell permeability; a model for blood/brain barrier) show satisfactory values for all the test compounds. Additionally, QPlogHERG descriptor (prediction of hERG potassium channel blocking potency) for compound **8b** was predicted to have values in permissible range as good as the reference compounds. Thus, in totality, the most active compounds were shown to possess excellent predicted absorption, distribution, metabolism and excretion parameters, suggestive for their development as orally-active lead molecules.

Conclusions

The present investigation describes the synthesis of twenty-four 4-aminoquinoline-pyrimidine-alkanols and evaluation of their antimalarial potential against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *Plasmodium falciparum*. Seven hybrids **8b-8e** and **8t-8v** were found to possess sub-micromolar IC_{50} values against both the plasmodial strains. The present hybrids were slightly less active than previously described lead aminoquinoline-pyrimidine conjugates but were found to possess better resistance-indices without any cytotoxicity against mammalian cells (VERO). Docking studies of the best active compounds **8b-8e**, **8u** and **8v** with both wild type and mutant Pf-DHFR-TS were performed which displayed strong interactions between the ligand and binding-site residues analogous to known DHFR-inhibitors and the native substrate. The presence of a terminal amino-alcohol chain at the pyrimidine core was found to be imperative for binding within the DHFR active site. Furthermore, the flexibility of the synthesized hybrids was shown to facilitate the evasion of any steric confrontation(s) resulting from the amino acid mutations in the mutant Pf-DHFR protein. The most active compounds were predicted to be endowed with favourable pharmacokinetic properties suggestive of their further refinement into orally-active anti-malarial drug molecules.

Experimental

All the reagents and starting materials were purchased from Sigma Aldrich and were used as supplied. Progress of the reaction was monitored by TLC (E. Merck Kieselgel 60 F254) and visualization was accomplished using UV light and iodine. All the intermediates and final compounds were purified using silica gel column chromatography (100-200 mesh silica; elution with 0-15% Methanol/Chloroform). ^{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 shift values are given in parts per million (ppm) on the delta scale (δ) and are referenced to tetramethylsilane (TMS) used as an internal standard. IR spectra were recorded on a Perkin-Elmer FT-IR spectrophotometer using either KBr pellets or as a film in chloroform and the values are expressed in cm^{-1}. Mass spectra were recorded on Agilent Accurate Mass Q-TOF MS system or micromass LCT Mass Spectrometer/Data system. Melting points were recorded on EZ-Melt automated melting point apparatus, Stanford Research Systems and are uncorrected. Procedures used for the biological evaluation of the synthesized compounds for antimalarial and mammalian cytotoxic activities have been previously described.[27,28] The detailed methodology for computational studies of the active compounds has been elucidated in an earlier report.[37]

Typical procedure for the synthesis of intermediate 3a and related compounds (3b-3c)

A mixture of 4,7-dichloroquinoline (**1**, 10.0 g, 50.49 mmol) and ethane-1,2-diamine (**2a**, 16.9 mL, 252.45 mmol) was stirred at 120 °C for 6 h (Scheme 1). The reaction mixture was then cooled down to room temperature and ice-cold water was added to it. The solid thus obtained was filtered and washed with excess water Similarly, intermediates **3b** and **3c** were obtained by reacting **1** with propane-1,3-diamine (**2b**) and butane-1,4-diamine (**2c**) respectively. The crude-products were crystallized by using ethanol and the data corresponds to that reported in the literature.[38]

Typical procedure for the synthesis of intermediate 5a and related compounds (5b-c) and (6a-c)

To a solution of 2,4-dichloropyrimidine (**4a**, 5.0 g, 33.56 mmol) in THF at 0 °C , intermediate **3a** (7.4 g, 33.56 mol) was added, followed by the drop-wise addition of N,N-diisopropylethylamine (11.7 mL, 67.12 mmol). The reaction mixture was then stirred overnight (14h) at the ambient temperature. After completion of the reaction as observed by TLC, excess THF was evaporated and the residue was diluted with water and extracted using ethyl acetate (3 x 100 mL). The combined organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography using 5% MeOH/$CHCl_3$ as the eluent to afford pure compound **5a**. In a similar manner, intermediates **5b** and **5c** were obtained by the reaction of **4a** with intermediates **3b** and **3c** respectively. The corresponding intermediates **6a-c** were obtained by reacting 2,4-dichloro-6-methylpyrimidine (**4b**) instead of 2,4-dichloro pyrimidine (**4a**) with the intermediates **3a-c**, respectively. The characterization data for all the synthesised intermediates corresponds to that reported previously in literature.[23-26]

General procedure for the synthesis of 4-aminoquinoline-pyrimidine hybrids (8a–x)

To a solution of **5** or **6** (2 mmol) in DMF (10 mL), respective amino alcohol (**7a-e**, 3 eq.) was added and the reaction mixture was allowed to stir at 120 °C for 10 h. Upon the completion of reaction (as inferred through TLC), the reaction mixture was allowed to cool down to the ambient temperature and was diluted with ice-cold water (15 mL). It was then extracted with 10% isopropanol in chloroform (3 x 50 mL) and the combined organic extract was dried over Na_2SO_4 and concentrated *in vacuo*. The crude residue thus obtained was purified by column chromatography using 0-15% MeOH/$CHCl_3$ as eluent to afford the respective compounds **8a–x** in good yields. The

characterization data for all the final compounds is detailed as follows:

2-((4-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)-6-methyl pyrimidin-2-yl)amino)ethanol (**8a**): White solid; yield: 86%; mp 160-162 °C; IR (ν_{max}/cm^{-1}, KBr): 3317, 2926, 1583, 1430, 1380, 1233, 1139, 1066, 801; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.01 (m, 3H), 3.33-3.35 (m, 2H), 3.41-3.42 (m, 2H), 3.49-3.52 (m, 4H), 3.83 (br s, 1H), 5.61 (s, 1H), 6.30 (br s, 1H), 6.57 (d, 1H, J = 5.86 Hz), 7.05 (br s, 1H), 7.43 (dd, 2H, J = 2.20 Hz, 8.79 Hz), 7.78 (d, 1H, J = 2.20 Hz), 8.19 (d, 1H, J = 8.79 Hz), 8.41 (d, 1H, J = 5.86 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 23.34, 38.06, 42.51, 43.53, 60.61, 98.68, 117.38, 124.03, 124.09, 127.46, 133.42, 149.01, 150.09, 151.95, 162.01, 163.36; ESI-HRMS (m/z) calcd for $C_{18}H_{21}ClN_6O$: 372.1465 (M^+), found: 373.1535 $(M + H)^+$, 375.1521 $(MH + 2)^+$.

3-((4-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)-6-methyl pyrimidin-2-yl)amino)propan-1-ol (**8b**): Off-white solid; yield: 81%; mp 144-146 °C ; IR (ν_{max}/cm^{-1}, KBr): 3312, 2928, 2852, 1582, 1425, 1230, 1141, 1076, 807; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.61-1.67 (m, 2H), 2.02 (s, 3H), 3.28-3.33 (m, 2H), 3.43-3.46 (m, 4H), 3.54 (br s, 3H), 5.63 (s, 1H), 6.58 (d, 2H, J = 5.13 Hz), 7.44 (d, 2H, J = 8.79 Hz), 7.54 (br s, 1H), 7.79 (d, 1H, J = 2.20 Hz), 8.21 (d, 1H, J = 8.79 Hz), 8.41 (d, 1H, J = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 21.19, 32.53, 37.83, 41.14, 42.35, 58.61, 98.67, 103.45, 114.20, 117.32, 124.10, 124.17, 127.13, 133.59, 148.60, 150.29, 151.55, 163.27; ESI-HRMS (m/z) calcd for $C_{19}H_{23}ClN_6O$: 386.1622 (M^+), found: 387.1688 $(M + H)^+$, 389.1676 $(MH + 2)^+$.

4-((4-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)-6-methyl pyrimidin-2-yl)amino)butan-1-ol (**8c**): White-solid; yield: 78%; mp 174-176 °C; IR (ν_{max}/cm^{-1}, KBr): 3265, 2925, 2856, 1588, 1356, 1138, 796; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.42-1.53 (m, 4H), 2.00 (s, 3H), 3.22-3.26 (m, 2H), 3.34-3.42 (m, 4H), 3.52 (br s, 2H), 4.38 (br s, 1H), 5.57 (s, 1H), 6.36 (br s, 1H), 6.56 (d, 1H, J = 5.13 Hz), 6.92 (br s, 1H), 7.41-7.44 (m, 2H), 7.78 (d, 1H, J = 2.20 Hz), 8.17 (d, 1H, J = 9.52 Hz), 8.39 (d, 1H, J = 5.13 Hz); Anal. calcd for $C_{20}H_{25}ClN_6O$: C, 59.92; H, 6.29; N, 20.96; found: C, 59.99; H, 6.35; N, 21.01; ESI-MS (m/z): 401.20 $(M + H)^+$, 403.19 $(MH + 2)^+$.

5-((4-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)-6-methyl pyrimidin-2-yl)amino)pentan-1-ol (**8d**): Off-white-solid; yield: 83%; mp 144-146 °C; IR (ν_{max}/cm^{-1}, KBr): 3293, 2928, 2861, 1586, 1507, 1341, 1203, 1137, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.29-1.33 (m, 2H), 1.38-1.43 (m, 2H), 1.45-1.52 (m, 2H), 2.00 (s, 3H), 3.20-3.25 (m, 2H), 3.34-3.52 (m, 6H), 4.34 (br s, 1H), 5.57 (s, 1H), 6.34 (br s, 1H), 6.55 (d, 1H, J = 5.13 Hz), 6.95 (br s, 1H), 7.41-7.43 (m, 2H), 7.78 (d, 1H, J = 2.20 Hz), 8.18 (d, 1H, J = 9.52 Hz), 8.39 (d, 1H, J = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 23.08, 23.41, 29.29, 32.38, 38.16, 40.63, 42.56, 60.69, 98.65, 117.40, 124.04, 127.46, 133.42, 149.02, 150.12, 151.87, 161.98, 163.35; Anal. calcd for $C_{21}H_{27}ClN_6O$: C, 60.79; H, 6.56; N, 20.25; found: C, 60.88; H, 6.59; N, 20.29; ESI-MS (m/z): 415.20 $(M + H)^+$, 417.21 $(MH + 2)^+$.

6-((4-((2-((7-chloroquinolin-4-yl)amino)ethyl)amino)-6-methyl pyrimidin-2-yl)amino)hexan-1-ol (**8e**): Yellow solid; yield: 75%; mp 134-136 °C ; IR (ν_{max}/cm^{-1}, Film): 3296, 2929, 2585, 1578, 1330, 1213, 1138, 1079, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.22-1.27 (m, 4H), 1.35-1.38 (m, 2H), 1.46-1.48 (m, 2H), 2.00 (s, 3H), 3.20-3.25 (m, 2H), 3.34-3.52 (m, 6H), 4.34 (br s, 1H), 5.58 (s, 1H), 6.34 (br s, 1H), 6.55 (d, 1H, J = 5.13 Hz), 7.06 (br s, 1H), 7.41-7.46 (m, 2H), 7.78 (d, 1H, J = 1.46 Hz), 8.20 (d, 1H, J = 8.79 Hz), 8.39 (d, 1H, J = 5.86 Hz) ESI-HRMS (m/z) calcd for $C_{22}H_{29}ClN_6O$: 428.2091 (M^+), found: 429.2165 $(M + H)^+$, 431.2147 $(MH + 2)^+$.

2-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)pyrimidin-2-yl) amino) ethanol (**8f**): Pale-yellow solid; yield: 86%; mp 172-174 °C; IR (ν_{max}/cm^{-1}, KBr): 3240, 2926, 2850, 1582, 1079, 795; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.87-1.94 (m, 2H), 3.23-3.27 (m, 2H), 3.31-3.34 (m, 4H), 3.44-3.47 (m, 2H), 4.68 (br s, 1H), 5.73 (d, 1H, J = 5.49 Hz), 6.21 (br s, 1H), 6.47 (d, 1H, J = 5.49 Hz), 6.99 (br s, 1H), 7.30-7.33 (m, 1H), 7.44 (dd, 1H, J = 2.44 Hz, 9.16 Hz), 7.62 (br s, 1H), 7.78 (d, 1H, J = 1.83 Hz), 8.26 (d, 1H, J = 9.16 Hz), 8.38 (d, 1H. J = 5.49 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 23.72, 33.74, 36.34, 39.50, 56.50, 94.75, 113.53, 120.10, 120.16, 123.47, 129.47, 145.04, 146.16, 147.87, 158.05, 158.71; ESI-HRMS (m/z) calcd for $C_{18}H_{21}ClN_6O$: 372.1465 (M^+), found: 373.1542 $(M + H)^+$, 375.1528 $(MH + 2)^+$.

3-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)pyrimidin-2-yl) amino) propan-1-ol (**8g**): Yellow solid; yield: 82%; mp 118-120 °C; IR (ν_{max}/cm^{-1}, KBr): 3256, 2926, 2854, 1584, 1329, 1234, 1135, 1078, 794; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.56-1.62 (m, 2H), 1.86-1.93 (m, 2H), 3.17-3.24 (m, 2H), 3.35-3.42 (m, 6H), 4.11 (br s, 1H), 5.70 (d, 1H, J = 5.86 Hz), 6.28 (br s, 1H), 6.47 (d, 1H, J = 5.13 Hz), 6.95 (br s, 1H), 7.31 (m, 1H), 7.44 (dd, 1H, J = 2.20 Hz, 8.79 Hz), 7.61 (br s, 1H), 7.77 (d, 1H, J = 2.20 Hz), 8.26 (d, 1H, J = 8.79 Hz), 8.38 (d, 1H, J = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 29.41, 34.31, 39.50, 42.00, 60.43, 100.38, 119.18, 125.74, 125.79, 129.12, 135.11, 150.71, 151.81, 153.54, 163.73, 164.36; ESI-HRMS (m/z) calcd for $C_{19}H_{23}ClN_6O$: 386.1622 (M^+), found: 387.1696 $(M + H)^+$, 389.1681 $(MH + 2)^+$.

4-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)pyrimidin-2-yl) amino) butan-1-ol (**8h**): Pale-yellow solid; yield: 83%; mp 122-124 °C; IR (ν_{max}/cm^{-1}, KBr): 3276, 2926, 2856, 2345, 1581, 1364, 1235, 1136, 1079, 974, 851, 794; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.36-1.41 (m, 4H), 1.87-1.90 (m, 2H), 3.90-3.12 (m, 2H), 3.33-3.38 (m, 6H), 4.22 (br s, 1H), 5.72 (d, 1H, J = 6.59 Hz), 6.31 (br s, 1H), 6.47 (d, 1H, J = 5.86 Hz), 7.01 (br s, 1H), 7.44 (m, 2H), 7.57 (br s, 1H), 7.77 (d, 1H, J = 2.20 Hz), 8.23 (d, 1H, J = 8.79 Hz), 8.35 (d, 1H, J = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.92, 27.61, 30.05, 40.24, 40.40, 60.62, 98.67, 117.42, 124.11, 124.16, 127.21, 133.51, 148.75, 150.23, 151.62, 162.60; ESI-HRMS (m/z) calcd for $C_{20}H_{25}ClN_6O$: 400.1778 (M^+), found: 401.1858 $(M + H)^+$, 403.1832 $(MH + 2)^+$.

5-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)pyrimidin-2-yl)amino) pentan-1-ol (**8i**): Off-white solid; yield: 78%; mp 124-126 °C; IR (ν_{max}/cm^{-1}, KBr): 3416, 3254, 2915, 2870, 1587, 1534, 1408, 1358, 1227, 1138, 1066, 974, 852, 800, 714; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.22-1.25 (m, 2H), 1.35-1.43 (m, 4H), 1.87-1.93 (m, 2H), 3.11-3.13 (m, 2H), 3.30-3.39 (m, 6H), 4.39 (br s. 1H), 5.71 (d, 1H, J = 5.34 Hz), 6.38 (br s, 1H), 6.47 (d, 1H, J = 5.34 Hz), 7.04 (br s, 1H), 7.35-7.37 (m, 1H), 7.44

(dd, 1H, J = 2.29 Hz, 9.16 Hz), 7.60 (br s, 1H), 7.77 (d. 1H, J = 1.53 Hz), 8.27 (d, 1H, J = 9.16 Hz), 8.38 (d, 1H, J = 5.39 Hz); ESI-HRMS (m/z) calcd for $C_{21}H_{27}ClN_6O$: 414.1935 (M^+), found: 415.2012 $(M + H)^+$, 417.1991 $(MH + 2)^+$.

6-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)pyrimidin-2-yl)amino)hexan-1-ol (**8j**): Off-white solid; yield: 86%; mp 118-120 °C; IR (ν_{max}/cm^{-1}, KBr): 3422, 2927, 2855, 1591, 1406, 1237, 1136, 1078, 899, 851, 794; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.20-1.24 (m, 4H), 1.35-1.40 (m, 4H), 1.90-1.96 (m, 2H), 3.10-3.15 (m, 2H), 3.33-3.39 (m, 6H), 4.30 (br s, 1H), 5.77 (d, 1H, J = 6.10 Hz), 6.47 (d, 1H, J = 5.19 Hz), 6.61 (br s, 1H), 7.25 (br s, 1H), 7.43-7.47 (m, 2H), 7.61 (d, 1H, J = 5.49 Hz), 7.78 (d, 1H, J = 1.83 Hz), 8.31 (d, 1H, J = 9.16 Hz), 8.37 (d, 1H, J = 4.88 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 21.11, 25.29, 26.37, 27.50, 29.18, 32.49, 37.65, 40.40, 60.62, 98.59, 117.34, 124.10, 124.21, 126.93, 133.59, 148.43, 150.33, 151.28, 162.52; ESI-HRMS (m/z) calcd for $C_{22}H_{29}ClN_6O$: 428.2091 (M^+), found: 429.2168 $(M + H)^+$ 431.2149 $(MH + 2)^+$.

2-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-6-methyl pyrimidin-2-yl)amino)ethanol (**8k**): Yellow solid; yield: 88%; mp 206-208 °C; IR (ν_{max}/cm^{-1}, KBr): 3299, 2925, 2871, 1583, 1534, 1450, 1367, 1282, 1200, 1140, 1073, 849, 802, 764; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.87-1.90 (m, 2H), 2.01 (s, 3H), 3.16-3.26 (m, 8H), 4.43 (br s, 1H), 5.62 (s, 1H), 6.33 (br s, 1H), 6.48 (d, 1H, J = 5.86 Hz), 7.07 (br s, 1H), 7.38 (br s, 1H), 7.45 (dd, 1H, J = 2.20 Hz, 9.52 Hz), 7.78 (d, 1H, J = 2.20 Hz), 8.27 (d, 1H, J = 8.79 Hz), 8.37 (d, 1H, J = 5.13 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 21.26, 27.57, 40.17, 43.36, 60.40, 62.80, 98.68, 117.40, 124.15, 124.25, 127.07, 133.59, 148.59, 150.30, 151.49, 163.17, 165.17, 165.18, 172.35; ESI-HRMS (m/z) calcd for $C_{19}H_{23}ClN_6O$: 386.1622 (M^+), found: 387.1699 $(M + H)^+$, 389.1671 $(MH + 2)^+$.

3-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-6-methyl pyrimidin-2-yl)amino)propan-1-ol (**8l**): Off-white solid; yield: 90%; mp 108-110 °C; IR (ν_{max}/cm^{-1}, KBr): 3356, 3244, 2904, 2849, 1582, 1429, 1382, 1330, 1238, 1139, 1077, 977, 847, 805, 760; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.55-1.61 (m, 2H), 1.85-1.92 (m, 2H), 1.98 (s, 3H), 3.16-3.24 (m, 4H), 3.40-3.44 (m, 4H), 4.35 (br s, 1H), 5.55 (s, 1H), 6.23 (br s, 1H), 6.46 (d, 1H, J = 5.13 Hz), 6.82 (br s, 1H), 7.30-7.32 (m, 1H), 7.44 (dd, 1H, J = 2.20 Hz, 8.79 Hz), 7.77 (d, 1H, J = 2.20 Hz), 8.26 (d, 1H, J = 8.79 Hz), 8.37 (d, 1H, J = 5.86 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 20.39, 25.14, 29.59, 34.50, 39.50, 42.10, 50.45, 57.89, 60.45, 100.54, 119.30, 125.90, 125.94, 129.25, 135.26, 150.84, 151.95, 153.69, 163.82, 165.82; ESI-HRMS (m/z) calcd for $C_{20}H_{25}ClN_6O$: 400.1778 (M^+), found: 401.1855 $(M + H)^+$, 403.1827 $(MH + 2)^+$.

4-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-6-methyl pyrimidin-2-yl)amino)butan-1-ol (**8m**): Pale-yellow solid; yield: 85%; mp 100-102 °C; IR (ν_{max}/cm^{-1}, KBr): 3313, 2926, 2850, 1586, 1247, 1135, 1051, 799; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.33-1.45 (m, 4H), 1.87-1.93 (m, 2H), 2.01 (s, 3H), 3.14-3.18 (m, 2H), 3.30-3.39 (m, 6H), 3.81(br s, 1H), 5.62 (s, 1H), 6.47 (d, 1H, J = 5.50 Hz), 6.54 (br s, 1H), 7.21 (br s, 1H), 7.42-7.45 (m, 2H), 7.78 (d, 1H, J = 2.29 Hz), 8.30 (d, 1H, J = 9.16 Hz), 8.37 (d, 1H, J = 5.50 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 26.49, 28.22, 30.59, 38.39, 40.95, 61.20, 99.24, 118.00, 124.69, 124.79, 127.72, 134.13, 149.25, 150.85, 152.10, 163.75; ESI-HRMS (m/z) calcd for $C_{21}H_{27}ClN_6O$: 414.1935 (M^+), found: 415.2010 $(M + H)^+$, 417.1989 $(MH + 2)^+$.

5-((4-((3-((7-chloroquinolin-4-yl)amino)propyl)amino)-6-methyl pyrimidin-2-yl)amino)pentan-1-ol (**8n**): Orange solid; yield: 82%; mp 144-146 °C; IR (ν_{max}/cm^{-1}, KBr): 3422, 3245, 2933, 2852, 1578, 1354, 1139, 1071, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.21-1.28 (m, 2H), 1.33-1.45 (m, 4H), 1.86-1.93 (m, 2H), 2.01 (m, 3H), 3.11-3.17 (m, 2H), 3.30-3.36 (m, 6H), 4.07 (br s, 1H), 5.60 (s, 1H), 6.46 (d, 2H, J = 5.50 Hz), 7.08 (br s, 1H), 7.37-7.40 (m, 1H), 7.44 (dd, 1H, J = 2.29 Hz, 9.16 Hz), 7.78 (d, 1H, J = 2.29 Hz), 8.29 (d, 1H, J = 9.16 Hz), 8.37 (d, 1H, J = 5.50 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 22.97, 27.64, 29.07, 32.29, 37.78, 40.44, 60.64, 62.77, 98.61, 117.40, 124.02, 124.12, 127.18, 133.45, 148.74, 150.17, 151.55, 160.89, 163.17; Anal. calcd for $C_{22}H_{29}ClN_6O$: C, 61.60; H, 6.81; N, 19.59; found: C, 61.71; H, 6.88; N, 19.65; ESI-MS (m/z): 429.24 $(M + H)^+$, 431.25 $(MH + 2)^+$.

2-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)pyrimidin-2-yl)amino)ethanol (**8o**): Pale yellow solid; yield: 85%; mp 178-180 °C; IR (ν_{max}/cm^{-1}, KBr): 3318, 3246, 2918, 2854, 1586, 1331, 1135, 1069, 796; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.59-1.73 (m, 4H), 3.23-3.30 (m, 6H), 3.45-3.48 (m, 2H), 4.66 (br s, 1H), 5.70 (d, 1H, J = 5.86 Hz), 6.21 (br s, 1H), 6.46 (d, 1H, J = 5.13 Hz), 6.91 (br s, 1H), 7.30-7.33 (m, 1H), 7.43 (dd, 1H, J = 2.20 Hz, 8.79 Hz), 7.60 (br s, 1H), 7.77 (d, 1H, J = 2.20 Hz), 8.26 (d, 1H, J = 8.79 Hz), 8.37 (d, 1H, J = 5.86 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.37, 26.65, 35.78, 42.19, 43.50, 60.56, 79.20, 98.65, 117.46, 124.01, 124.13, 127.40, 133.44, 149.01, 150.15, 154.29, 162.06, 162.66; Anal. calcd for $C_{19}H_{23}ClN_6O$: C, 58.99; H, 5.99; N, 21.72; found: C, 59.06; H, 6.08; N, 21.81. ESI-MS (m/z): 387.18 $(M + H)^+$, 389.19 $(MH + 2)^+$.

3-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)pyrimidin-2-yl)amino)propan-1-ol (**8p**): Off-white solid; yield: 86%; mp 102-104 °C; IR (ν_{max}/cm^{-1}, KBr): 3313, 2929, 2851, 1602, 1331, 1252, 1045, 796 ; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.59-1.62 (m, 4H), 1.66-1.70 (m, 2H), 3.21-3.29 (m, 6H), 3.39-3.43 (m, 2H), 3.72 (br s, 1H), 5.70 (d, 1H, J = 5.49 Hz), 6.38 (br s, 1H), 6.46 (d, 1H, J = 5.49 Hz), 6.95 (br s, 1H), 7.34 (br s, 1H), 7.43 (dd, 1H, J = 1.83 Hz, 8.54 Hz), 7.59 (br s, 1H), 7.77 (d, 1H, J = 1.83 Hz), 8.27 (d, 1H, J = 8.54), 8.37 (d, 1H, J = 5.49 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.32, 26.57, 30.63, 32.54, 37.73, 42.13, 58.66, 98.60, 117.39, 123.95, 124.11, 127.27, 133.38, 148.88, 150.14, 151.70, 161.61, 161.65, 162.58; Anal. calcd for $C_{20}H_{25}ClN_6O$: C, 59.92; H, 6.29; N, 20.96; found: C, 59.97; H, 6.36; N, 21.03; ESI-MS (m/z): 401.19 $(M + H)^+$, 403.18 $(MH + 2)^+$.

4-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)pyrimidin-2-yl)amino)butan-1-ol (**8q**): Off-white solid; yield: 90%; mp 124-126 °C; IR (ν_{max}/cm^{-1}, Film): 3295, 2925, 2856, 1582, 1455, 1369, 1136, 1082, 759; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.41-1.48 (m, 4H), 1.62-1.70 (m, 4H), 3.15-3.18 (m, 2H), 3.28-3.30 (m, 4H), 3.36-3.39 (m, 2H), 3.72 (br s, 1H), 5.70 (d, 1H, J = 5.49 Hz), 6.46 (d, 2H, J = 4.88 Hz), 6.99 (br s, 1H), 7.34 (br s, 1H), 7.43 (d, 1H, J = 9.16 Hz), 7.59 (br s, 1H), 7.77 (br s, 1H), 8.27 (d,

1H, J = 9.16 Hz), 8.37 (d, 1H, J = 4.88 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.35, 25.98, 26.61, 30.07, 40.44, 42.16, 60.64, 98.64, 117.40, 124.04, 124.14, 127.27, 133.46, 148.87, 150.19, 151.71, 162.59; Anal. calcd for $C_{21}H_{27}ClN_6O$: C, 60.79; H, 6.56; N, 20.25; found: C, 60.87; H, 6.61; N, 20.35. ESI-MS (m/z): 415.21 $(M + H)^+$, 417.22 $(MH + 2)^+$.

5-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)pyrimidin-2-yl)amino)pentan-1-ol (**8r**): Orange semi-solid; yield: 78%; IR (ν_{max}/cm^{-1}, Film): 3250, 2925, 2854, 1579, 1456, 1363, 1214, 756; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.20-1.30 (m, 2H), 1.35-1.49 (m, 4H), 1.64-1.71 (m, 4H), 3.19-3.21 (m, 2H), 3.33-3.36 (m, 6H), 4.52 (br s, 2H), 5.90 (br s, 1H), 6.51 (d, 1H, J = 5.50 Hz), 7.26 (br s, 1H), 7.45 (d, 1H, J = 9.16 Hz), 7.60-7.62 (m, 1H), 7.83 (d, 1H, J = 2.29 Hz), 7.90 (br s, 1H), 8.38 (d, 1H, J = 5.50 Hz), 8.42-8.44 (m 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 21.21, 22.91, 25.20, 26.20, 28.78, 32.18, 40.44, 42.18, 60.59, 98.54, 117.06, 124.43, 124.68, 125.64, 134.23, 146.92, 149.92, 151.15, 162.31, 162.39; Anal. calcd for $C_{22}H_{29}ClN_6O$: C, 61.60; H, 6.81; N, 19.59; found: C, 61.67; H, 6.83; N, 19.64; ESI-MS (m/z): 429.22 $(M + H)^+$, 431.21 $(MH + 2)^+$.

6-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)pyrimidin-2-yl)amino)hexan-1-ol (**8s**): Yellow semi-solid; yield: 82%; IR (ν_{max}/cm^{-1}, Film): 3252, 2928, 2857, 1578, 1451, 1212, 1053, 803, 747; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.21-1.26 (m, 4H), 1.35-1.46 (m, 4H), 1.65-1.70 (m, 4H), 3.16-3.21 (m, 2H), 3.32-3.36 (m, 7H), 5.93-5.94 (m, 1H), 6.56 (d, 1H, J = 5.95 Hz), 7.50 (dd, 2H, J = 2.29 Hz, 9.16 Hz), 7.61-7.62 (m, 1H), 7.85 (d, 2H, J = 2.29 Hz), 8.05 (br s, 1H), 8.39-8.44 (m, 2H); Anal. calcd for $C_{23}H_{31}ClN_6O$: C, 62.36; H, 7.05; N, 18.97; found: C, 62.41; H, 7.12; N, 19.04; ESI-MS (m/z): 443.24 $(M + H)^+$, 445.24 $(MH + 2)^+$.

2-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)-6-methyl pyrimidin-2-yl)amino)ethanol (**8t**): White solid; yield: 86%; mp 182-184 °C; IR (ν_{max}/cm^{-1}, Film): 3295, 2919, 854, 1576, 1362, 1136, 1075, 813; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.58-1.63 (m, 2H), 1.65-1.71 (m, 2H), 1.99 (s, 3H), 3.23-3.30 (m, 4H), 3.44-3.47 (m, 5H), 5.56 (s, 1H), 6.17 (br s, 1H), 6.46 (d, 1H, J = 5.13 Hz), 6.75 (br s, 1H), 7.30-7.32 (m, 1H), 7.42 (dd, 1H, J = 2.20 Hz, 8.79 Hz), 7.76 (d, 1H, J = 2.20 Hz), 8.26 (d, 1H, J = 8.79 Hz), 8.36 (d, 1H, J = 5.13 Hz); Anal. calcd for $C_{20}H_{25}ClN_6O$: C, 59.92; H, 6.29; N, 20.96; found: C, 59.97; H, 6.36; N, 21.03; ESI-MS (m/z) calcd: 401.21 $(M + H)^+$, 403.22 $(MH + 2)^+$.

3-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)-6-methyl pyrimidin-2-yl)amino)propan-1-ol (**8u**): Off-white solid; yield: 84%; mp 110°C; IR (ν_{max}/cm^{-1}, Film): 3297, 2929, 2859, 1580, 1332, 1247, 1136, 1078, 799; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.58-1.67 (m, 6H), 1.99 (s, 3H), 3.23-3.29 (m, 6H), 3.39-3.43 (m, 2H), 3.71 (br s, 1H), 5.55 (s, 1H), 6.45 (d, 2H, J = 5.49 Hz), 6.75 (br s, 1H), 7.30-7.32 (m, 1H), 7.43 (d, 1H, J = 8.54 Hz), 7.75-7.77 (m, 1H), 8.27 (d, 1H, J = 8.54 Hz), 8.37 (d, 1H, J = 5.49 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.33, 26.71, 32.69, 37.62, 42.14, 58.58, 98.63, 117.44, 123.94, 124.11, 127.43, 133.33, 149.07, 150.07, 151.88, 161.98, 163.26; Anal. calcd for $C_{21}H_{27}ClN_6O$: C, 60.79; H, 6.56; N, 20.25; found: C, 60.83; H, 6.61; N, 20.27; ESI-MS (m/z): 415.22 $(M + H)^+$, 417.21 $(MH + 2)^+$.

4-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)-6-methyl pyrimidin-2-yl)amino)butan-1-ol (**8v**): Pale-yellow solid; yield: 81%; mp 116-118°C; IR (ν_{max}/cm^{-1}, Film): 3303, 2925, 2856, 1580, 1366, 1136, 1079, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.39-1.49 (m, 4H), 1.59-1.71 (m, 4H), 1.99 (s, 3H), 3.15-3.20 (m, 2H), 3.25-3.28 (m, 4H), 3.36-3.39 (m, 2H), 3.81 (br s, 1H), 5.55 (s, 1H), 6.44 (d, 2H, J = 5.50 Hz), 6.77 (br s, 1H), 7.29-7.32 (m, 1H), 7.41 (dd, 1H, J = 2.29 Hz, 9.16 Hz), 7.77 (d, 1H, J = 2.29 Hz), 8.26 (d, 1H, J = 9.16 Hz), 8.36 (d, 1H, J = 5.50 Hz); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 25.34, 26.01, 30.07, 40.39, 42.15, 60.66, 98.62, 117.44, 123.94, 124.10, 127.41, 133.34, 149.06, 150.09, 151.85, 161.63, 163.26; Anal. calcd for $C_{22}H_{29}ClN_6O$: C, 61.60; H, 6.81; N, 19.59; found: C, 61.68; H, 6.85; N, 19.67; ESI-MS (m/z): 429.22 $(M + H)^+$, 431.23 $(MH + 2)^+$.

5-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)-6-methyl pyrimidin-2-yl)amino)pentan-1-ol (**8w**): Off-white solid; yield: 75%; mp 128-130 °C; IR (ν_{max}/cm^{-1}, Film): 3284, 2924, 2854, 1576, 1366, 1136, 1079, 802; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.21-1.46 (m, 6H), 1.62-1.69 (m, 4H), 2.01 (s, 3H), 3.15-3.19 (m, 2H), 3.26-3.31 (m, 4H), 3.33-3.36 (m, 2H), 4.12 (br s, 1H), 5.59 (s, 1H), 6.45 (d, 1H, J = 5.49 Hz), 7.35-7.38 (m, 2H), 7.42 (dd, 2H, J = 1.83 Hz, 9.16 Hz), 7.76-7.78 (m, 1H), 8.28 (d, 1H, J = 9.16 Hz), 8.36 (d, 1H, J = 5.49 Hz); ESI-HRMS (m/z) calcd for $C_{23}H_{31}ClN_6O$: 442.2248 (M^+), found: 443.2322 $(M + H)^+$, 445.2305 $(MH + 2)^+$.

6-((4-((4-((7-chloroquinolin-4-yl)amino)butyl)amino)-6-methyl pyrimidin-2-yl)amino)hexan-1-ol (**8x**): Off-white solid; yield: 72%; mp 136-138 °C; IR (ν_{max}/cm^{-1}, Film): 3284, 2925, 2857, 1577, 1365, 1280, 1136, 804; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.21-1.25 (m, 4H), 1.35-1.44 (m, 4H), 1.60-1.68 (m, 4H), 1.99 (s, 3H), 3.12-3.17 (m, 2H), 3.25-3.28 (m, 4H), 3.32-3.36 (m, 2H), 3.79 (br s, 1H), 5.54 (s, 1H), 6.44 (d, 2H, J = 5.34 Hz), 6.82 (br s, 1H), 7.31-7.33 (m, 1H), 7.42 (dd, 1H, J = 2.29 Hz, 9.16 Hz), 7.76-7.77 (m, 1H), 8.26 (d, 1H, J = 9.16 Hz), 8.35-8.36 (m, 1H); ESI-HRMS (m/z) calcd for $C_{24}H_{33}ClN_6O$: 456.2404 (M^+), found: 457.2468 $(M + H)^+$, 459.2459 $(MH + 2)^+$.

Acknowledgements

DSR thanks Council of Scientific and Industrial Research [No. 02(0049)/12/EMR-II] New Delhi, India for financial support. MT is thankful to DST for the award of INSPIRE senior research fellowship. AT and PP are thankful to CSIR for the award of senior research fellowship and research associateship, respectively. SIK is thankful to the United States Department of Agriculture (USDA), Agricultural Research Service Specific Cooperative Agreement No. 58-6408-2-0009 for partial support of this work. The authors thank CIF-USIC, University of Delhi, Delhi for analytical data and DU-DST mass spectrometer facility for mass data. Mr John Trott is acknowledged for excellent technical support in testing biological activity at NCNPR.

Notes and references

[+]Dedicated to Dr DS Bhakuni on the occasion of his 85th Birthday.

[a] *Department of Chemistry, University of Delhi, Delhi-110007, India. Fax: +91-11-27667501; Tel: +91-11-27667465;E-mail: dsrawat@chemistry.du.ac.in;*

[b] *National Centre for Natural Products Research, University of Mississippi, MS-38677, USA.*

† Electronic Supplementary Information (ESI) available: Experimental protocol for computational studies and ^{1}H NMR/^{13}C NMR spectra of representative compounds. See DOI: 10.1039/b000000x/

1. W. Fox, *Bull. World Health Organization,* 1971, **45**, 559-572.
2. Beena and D. S. Rawat, *Med. Res. Rev.,* 2013, **33**, 693–764.
3. V. Rodriguez, J. S. Hart, E. J. Freireich, G. P. Bodey, K. B. McCredie, J. P. Whitecar and C. A. Coltman, *Cancer,* 1973, **32**, 69-75.
4. A. N. Chawira and D. C. Warhurst, *J. Trop. Med. Hyg.,* 1987, **90**, 1-8.
5. B. F. Eriksson and R. F. Schinazi, *Antimicrob. Agents Chemother.,* 1989, **33**, 663-669.
6. World Malaria Report: 2013, WHO Press, Geneva, 2013.
7. H. Noedl, Y. Se, K. Schaecher, B. L. Smith, D. Socheat and M. M. Fukuda, *N. Engl. J. Med*., 2008, **359**, 2619-2620.
8. A. M. Dondorp, F. Nosten, P. Yi, D. Das, A. P. Phyo, J. Tarning, K. M. Lwin, F. Ariey, W. Hanpithakpong, S. J. Lee, P. Ringwald, K. Silamut, M. Imwong, K. Chotivanich, P. Lim, T. Herdman, S. S. An, S. Yeung, P. Singhasivanon, N. P. Day, N. Lindegardh, D. Socheat and N. J. White, *N. Engl. J. Med*., 2009, **361**, 455-467.
9. A. P. Phyo, S. Nkhoma, K. Stepniewska, E. A. Ashley, S. Nair, R. McGready, C. ler Moo, S. Al-Saai, A. M. Dondorp, K. M. Lwin, P. Singhasivanon, N. P. Day, N. J. White, T. J. Anderson and F. Nosten, *Lancet*, 2012, **379**, 1960-1966.
10. B. Meunier, *Acc. Chem. Res.,* 2008, **41**, 69-77.
11. F. W. Muregi and A. Ishih, *Drug Develop. Res.,* 2010, **71**, 20-32.
12. S. Manohar, M. Tripathi and D. S. Rawat, *Curr. Top. Med. Chem*., 2014, **14**, 1706-1733.
13. F. Coslédan, L. Fraisse, A. Pellet, F. Guillou, B. Mordmüller, P. G. Kremsner, A. Moreno, D. Mazier, J. Maffrand, and B. Meunier, *Proc. Natl. Acad. Sci. U.S.A.*, 2008, **105**, 17579-17584.
14. V. V. Kouznetsov and A. Gómez-Barrio, *Eur. J. Med. Chem.*, 2009, **44**, 3091-3113.
15. K. Singh, H. Kaur, K. Chibale, J. Balzarini, S. Little and P.V. Bharatam, *Eur. J. Med. Chem*., 2012, **52**, 82-97.
16. S. I. Pretorius, W. J. Breytenbach, C. De Kock, P. J. Smith and D. D. N'Da, *Bioorg. Med. Chem.*, 2013, **21**, 269-277.
17. K. Singh, H. Kaur, P. Smith, C. D. Kock, K. Chibale and J. Balzarini, *J. Med. Chem*., 2014, **57**, 435-448.
18. A. P. Gorka, A. de Dios and P. D. Roepe, *J. Med. Chem*., 2013, **56**, 5231-5246.
19. K. A. de Villiers and T. J. Egan, *Molecules*, 2009, **14**, 2868-2887.
20. S. Manohar, S. I. Khan and D. S. Rawat, *Bioorg. Med. Chem. Lett*., 2010, **20**, 322-325.
21. S. Manohar, S. I. Khan and D. S. Rawat, *Chem. Biol. Drug Des*., 2013, **81**, 625-630.
22. S. Manohar, S. I. Khan and D. S. Rawat, *Chem. Biol. Drug Des*., 2011, **78**, 124-136.
23. S. Manohar, U. C. Rajesh, S. I. Khan, B. L. Tekwani and D. S. Rawat, *ACS. Med. Chem. Lett*., 2012, **3**, 555-559.
24. A. Thakur, S. I. Khan and D. S. Rawat, *RSC Adv*., 2014, **4**, 20729-20736.
25. D. Kumar, S. I. Khan, B. L. Tekwani, P. Ponnan and D. S. Rawat, *RSC Adv.*, 2014, **4**, 63655-63669.
26. D. Kumar, S. I. Khan, B. L. Tekwani, P. Ponnan and D. S. Rawat, *Eur. J. Med. Chem.*, 2015, **89**, 490-502.
27. M. T. Makler and D. J. Hinrichs, *Am. J. Trop. Med. Hyg*., 1993, **48**, 205-210.
28 M. Jain, S. I. Khan, B. L. Tekwani, M. R. Jacob, S. Singh, P. P. Singh and R. Jain, *Bioorg. Med. Chem*. 2005, **13**, 4458-4466.
29. P. G. Bray, S. R. Hawley, M. Mungthin and S. A. Ward, *Mol. Pharmacol.*, 1996, **50**, 1559-1566.
30. M. Foley and L. Tilley, *Pharmacol. Ther.*, 1998, **79**, 55-87.
31 Glide, version 5.8, Schrödinger, LLC, New York, NY, 2012.
32. J. Yuvaniyama, P. Chitnumsub, S. Kamchonwongpaisan, J. Vanichtanankul, W. Sirawaraporn, P. Taylor, M. D. Walkinshaw and Y. Yuthavong, *Nat. Struct. Biol*., 2003, **10**, 357-365.
33. A. Ali, R. M. Bandaranayake, Y. Cai, N. M. King, M. Kolli, S. Mittal, J. F. Murzycki, M. N. L. Nalam, E. A. Nalivaika, A. Özen, M. M. Prabu-Jeyabalan, K. Thayer and C. A. Schiffer, *Viruses*, 2010, **2**, 2509–2535
34. Y. Yuthavong, B. Tarnchompoo, T. Vilaivan, P. Chitnumsub, S. Kamchonwongpaisan, S. A. Charman, D. N. McLennan, K. L. White, L. Vivas, E. Bongard, C. Thongphanchang, S. Taweechai, J. Vanichtanankul, R. Rattanajak, U. Arwon, P. Fantauzzi, J. Yuvaniyama, W. N. Charman and D. Matthews, *Proc. Natl. Acad. Sci. USA*, 2012, **109**, 16823-16828.
35. QikProp, version 3.5, Schrödinger, LLC, New York, NY, 2012.
36. C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Deliv. Rev.,* 2001, **46**, 3-26..
37. D. Kumar, S. I. Khan, P. Ponnan and D. S. Rawat, *New J. Chem.*, 2014, **38**, 5087-5095.
38. V. R. Solomon, S. K. Puri, K. Srivastava and S.B. Katti, *Bioorg. Med. Chem.*, 2005, **13**, 2157-2165.

