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Synthesis and in vitro evaluation of novel 8-aminoquinoline–pyrazolopyrimidine hybrids as potent antimalarial agents

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ABSTRACT

In the search of novel chemotherapeutic agents for emerging drug resistant parasites, the hybridization approaches have successfully emerged as an efficient tool in malarial chemotherapy. Herein, a rational design and synthesis of novel 8-aminoquinoline and pyrazolopyrimidine hybrids and their antimalarial activity against wild type *Plasmodium falciparum* (*Pf*_NF54) and resistant strain (*Pf*_K1) is reported. The medicinal chemistry approach to expand the scope of this series resulted in an identification of potent compounds with nanomolar potency (best IC_{50} 5–10 nM). Systematic structure activity relationship (SAR) studies revealed that pyrazolopyrimidine and 8-aminoquinoline ring are essential for achieving good *P. falciparum* potency. The docking study revealed that the compound **6** can retain some of the critical interactions within pfDHODH drug target.

The last decade has witnessed the world's need of novel drugs to fight malaria. According to the World Health Organization, there were an estimated 207 million cases of malaria resulting in 627,000 deaths and 482,000 children under five years of age in 2012.[1] The vast majority of deaths are caused by *Plasmodium falciparum* and *Plasmodium vivax* infections. Artemisinin-based combination therapy is the most effective in treating patients with *P. falciparum* infection.[2] The rapid emergence of malarial parasite resistance to currently available antimalarial drugs, including artemisinin derivatives, pose a threat to derail the global efforts to cure malaria.[3] With the onset of drug-resistant *Plasmodium* parasites, new approaches are being developed to combat the widespread disease.[4] The hybridization strategy involves an incorporation of key pharmacophoric features from existing drugs to design a novel molecule with a different efficacy and resistance profile. This approach has seen some success in recent times in delivering novel chemical entities against protozoan parasites.[5]

Primaquine, an 8-aminoquinoline moiety is the only drug available to eliminate exoerythrocytic infection, and provide a radical cure for vivax malaria (Fig. 1).[6] A primaquine analog, tafenoquine with a long half-life (2–3 weeks) is currently in clinical trials for the prophylactic treatment of malaria.[7] A recent literature report revealed that modifications of 8-aminoquinoline moiety have been attempted to improve tissue and blood schizonticidal activity.[8] The triazolopyrimidine core (DSM1) has been reported as a potent inhibitor of pfDHODH and is active against *P. falciparum*.[9] Lead optimization identified a metabolically stable inhibitor of pfDHODH (DSM265) that successfully translated into efficacy against *P. falciparum* both in vitro and in vivo.[10] Recognizing the importance of these two bioactive cores, we were interested in hybridization of the pharmacophoric features of them into a single molecule to explore potential synergies. Herein, we report a rational design, synthesis and SAR relationship of aminoquinoline with ring bioisosteres of triazolopyrimidine.

The target compounds, **1**–**9** have been achieved as outlined in Schemes 1 and 2. The aromatic nucleophilic substitution, S_NAr of chloro-containing heterocycles with 8-aminoquinoline in the presence of NaH, DMF yielded the desired aminoquinolines, **1**, **4** and **6** as depicted in Scheme 1.[10b,11] To synthesize the aminoquinolines **2**, **5** and **7**, initially a S_NAr reaction was carried out to afford the intermediates **2a**, **5a** and **7a** which undergo further hydrogenation–dechlorination reaction in the presence of Pd/C in triethylamine under ambient hydrogen pressure resulting in desired products in good yields.[12]

Synthesis of aminoquinolines **3**, **8** and **9** is achieved as outlined in scheme 2. Commercially available amino pyrazoles treated with *N*,*N*-dimethylformamide dimethyl acetal, followed by cyclization with malononitrile in pyridine under microwave conditions afforded the corresponding intermediates, **19**, **20**. Subsequently, they were converted to the desired aminoquinolines, **8**, **9** under

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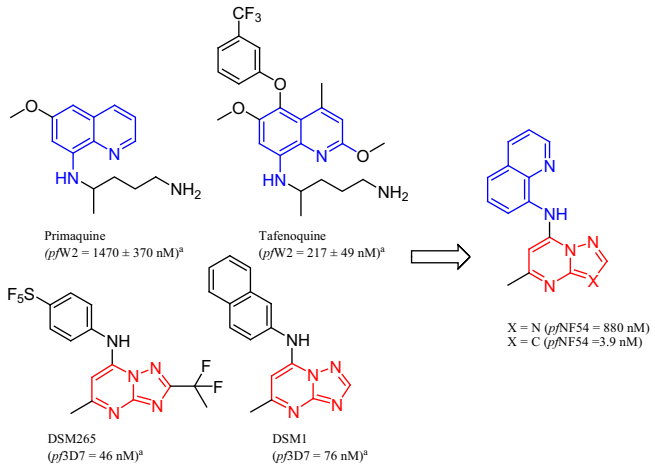
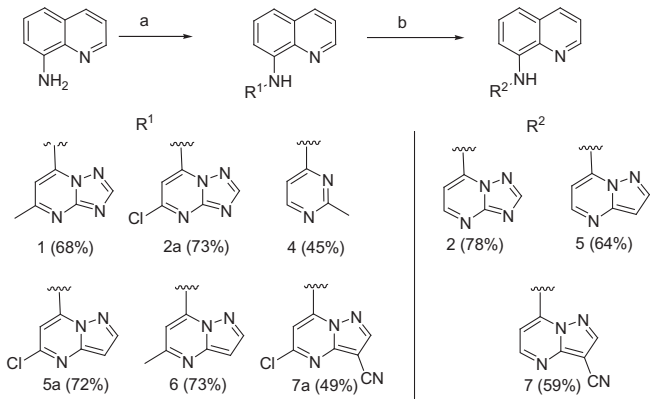
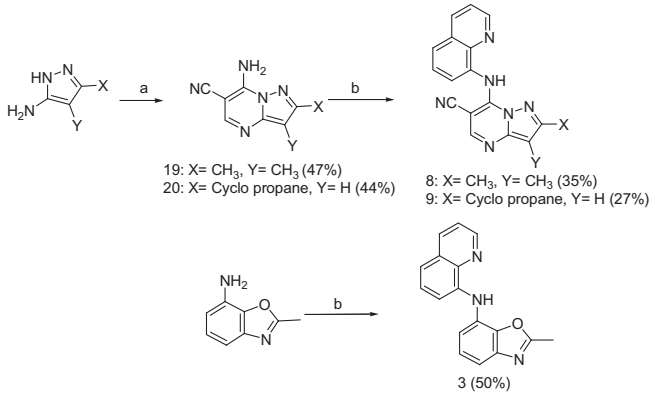


Figure 1. Potential antimalarial agents. [a]Literature reported IC50 value.



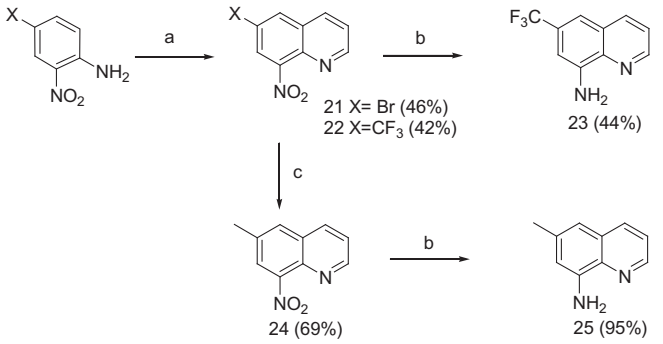
Scheme 1. Reagents and conditions: (a) Het-Cl, NaH, DMF, 0 °C to 60 °C, 5–12 h; (b) Pd/C, TEA, H_2 gas, DCM/MeOH (1:2), rt, 6–12 h.



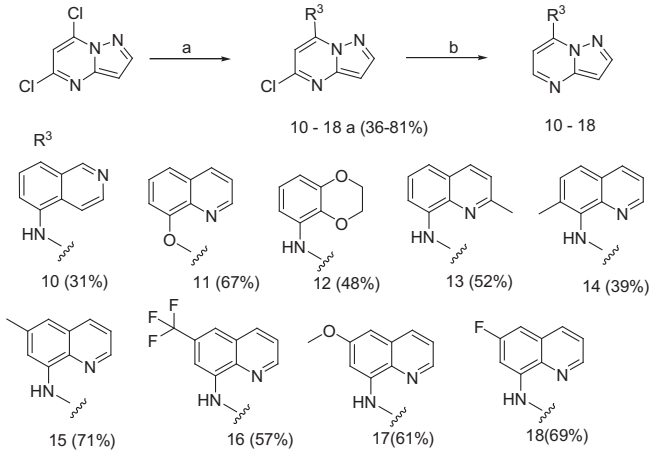
Scheme 2. Reagents and conditions: (a) (i) *N*,*N*-dimethylformamide dimethyl acetal, xylene, 3 h, 150 °C; (ii) malononitrile, pyridine, 85 °C, 20 min, MW; (b) 8-bromoquinoline, $Pd_2(dba)_3$, xantphos, *t*-BuONa, toluene, 110 °C, 12 h.

Buchwald conditions. Similar conditions were used to synthesize compound **3**.

The synthesis of aminoquinolines, **23**, **25** is outlined in Scheme 3. Condensation of glycerol with substituted 2-nitroaniline under acidic conditions afforded intermediates, **21**, **22**. Hydrogenation of the nitro group of quinoline, **22** in the presence of Pd/C led to compound **23**. Bromo substituted nitroquinoline, **21** when subjected to Suzuki reaction, followed by reduction afforded compound **25**.



Scheme 3. Reagents and conditions: (a) glycerol, NaI, H_2SO_4, 150 °C; (b) Pd/C, H_2 gas, MeOH, 16 h; (c) trimethylboroxine, $Pd(PPh_3)_4$, K_2CO_3, DMF, 110 °C, 16 h.



Scheme 4. Reagents and conditions: (a) R-NH_2 or R-OH, NaH, DMF, 0 °C to 60 °C, 5–12 h; (b) Pd/C, TEA, DCM/MeOH (1:2), rt, 6–12 h.

Syntheses of compounds (**10**–**18**) are outlined in Scheme 4. The analogs were synthesized by following the same procedure as illustrated in Scheme 1.

All of the synthesised compounds (**1**–**18**) were tested for their antimalarial activity against a sensitive (NF54) and a multidrug-resistant (K1) strain of *Pf* using a SYBR Green-based readout.[13,14] Chloroquine, pyrimethamine, and artesunate were used as reference drugs in all of the experiments.

Our initial focus to explore antiplasmodial activity for the triazolopyrimidine core (TP) hybridized with 8-aminoquinoline resulted in a hit, **1** with an IC50 of 0.88 μM (Table 1). Removal of methyl group at the C-5 in TP ring, **2** was tolerated for *P. falciparum* potency. Our medicinal chemistry efforts were focused on finding structurally similar rings for replacing the TP core and to expand the chemical scope of the series. We used bicyclic 5,6 and monocyclic 6 member ring systems. The replacement of TP ring with benzoxazole ring, **3** was found to be moderately active. However, the pyrimidine ring substitution, **4** was inactive against *P. falciparum*. On the other hand, converting TP ring into pyrazolopyrimidine ring by removing one nitrogen atom in compound, **5** showed >100 fold improvement in potency with single digit nanomolar compared to compound **2**. Encouraged by these results, we explored further SAR modifications on pyrazolopyrimidine ring as a core. Incorporation of a methyl at the C-5 position of pyrazolopyrimidine ring, **6** also retained the potency. Introduction of an electron withdrawing groups like nitrile at C-3 position, **7** showed moderate potency. The pyrazolopyrimidine, **8** with dimethyl substitution was 5 fold less active as compared to pyrazolopyrimidine, **5** and **6**. The replacement of dimethyl substitution with cyclopropyl group, **9**

Table 1
Core modification of triazolopyrimidine ring

Compound	NF54 IC_{50} (μM)	K1 IC_{50} (μM)
1	0.88	0.76
2	1.4	0.84
3	1.3	1.6
4	>0.5	>0.5
5	0.0053	0.0078
6	<0.0039	0.0067
7	0.071	0.051
8	0.026	0.027
9	1.6	2
Chloroquine	0.017	0.347
Artemisinin	0.007	0.0065
Pyrimethamine	0.028	8.6
Atovaquone	0.0014	0.0023

was detrimental for *P. falciparum* activity. This indicates that bulky group is not well tolerated at C-3 position for *P. falciparum* potency.

Having identified the pyrazolopyrimidine ring as the best core for antiplasmodial activity, we started exploration around the 8-aminoquinoline ring (Table 2). To understand the essential features of the 8-aminoquinoline, we synthesised aminoquinoline, **10** by moving the nitrogen one position away and quinoline, **11** that introduced an ether linkage. Both the compounds led to decreased antiplasmodial activity. Further, the replacement of the 8-aminoquinoline with 2,3-dihydrobenzo[*b*][1,4]dioxin-5-amine, **12** was also inactive against *P. falciparum*. This modification clearly suggested that 8-aminoquinoline motif may play a critical role in contributing to antiplasmodial activity.

Next, we embarked on introducing small substitutions in the 8-aminoquinoline ring for further SAR understanding. Introducing methyl group at the C-2, **13** and C-7, **14** was not favorable. These results indicated that ring planarity and chelation may be required antiplasmodial activity. Introduction of fluorine at the C-5, **18** retained single digit nanomolar potency. Also electron donating or withdrawing group like methyl, methoxy or CF3 substitution at the C-5, **15**, **16** and **17** exhibited good antiplasmodial activity. However, they were 3–5 folds less potent than unsubstituted analogs, **5**, **6**.

Triazolopyrimidine scaffold binding interaction with pfDHODH is well known in the PDB.[15] Hence we docked compounds **5**, **6**, **17** and **18** of the 8-aminoquinoline–pyrazolopyrimidine class in to the published crystal structure of pfDHODH (PDB ID: 3I65).

Figures 2 and 3 show the comparison of binding mode of compound **6** and compound **5**, **17** and **18** with crystal bound triazolopyrimidine inhibitor, respectively. In general the compounds can bind to a site adjacent to the FMN cofactor site and are capable of making similar hydrophobic and polar interactions in the binding site. The quinoline group overlaps with the naphthyl group of DSM1 whereas pyrazolopyrimidine ring overlaps with triazolopyrimidine core. The pyrazolopyrimidine core binds in the pocket lined by hydrophobic residues like Leu 172, Leu 176, Gly 181,

Table 2
SAR in 8-aminoquinoline core

Compound	NF54 IC_{50} (μM)	K1 IC_{50} (μM)
10	1.2	1.6
11	>2	>2
12	>2	>2
13	1.7	1.2
14	>2	>2
15	0.063	0.038
16	0.042	0.018
17	0.014	0.026
18	0.0039	0.0039

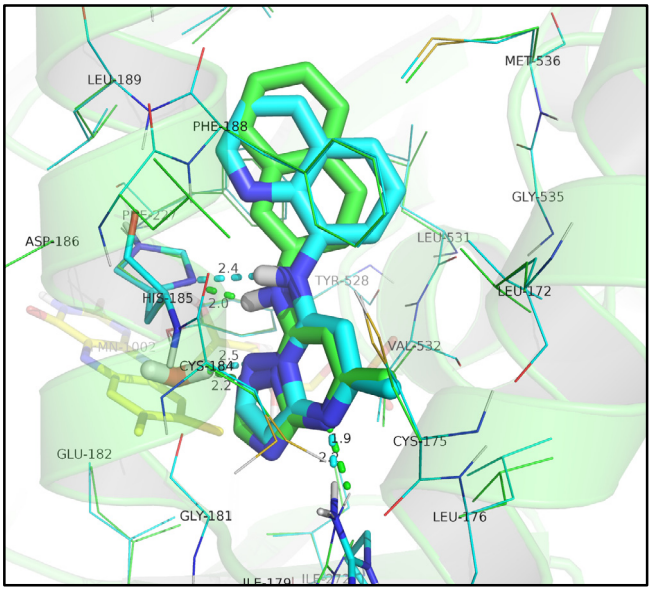


Figure 2. Docking binding mode of compound **6** (Cyan, Stick model) in the active site of *pf*DHODH (pdb ID: 3I65). The 5 Å active site residues are depicted as wireframe where as FMN (stick model) is shown in yellow color. The dotted (cyan and yellow) line indicates hydrogen bond interactions between compound **6** and crystal structure (DSM1) ligand.

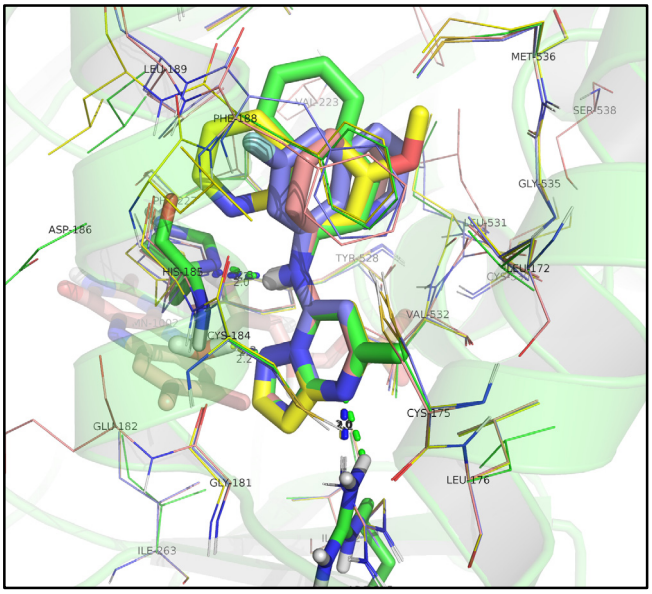


Figure 3. Comparison of docking binding mode of compound **5** (brick red), **17** (yellow), **18** (blue) and crystal structure bound (DSM1) ligand in the active site of *pf*DHODH.

Cys 184 and Val 532 and polar residues like His 185 and Arg 265. A few polar contacts were observed between ring N of pyrazolopyrimidine and Arg 265 and water molecule. The hydrogen of amino group makes a hydrogen bond interaction with His 185 and appears to be critical for activity as indicated by loss of activity for compound 11. This observation is consistent with the SAR of published triazolopyridine (DSM) series. The quinoline ring binds in the hydrophobic pocket lined by Leu 172, Phe 188, Leu 189, Leu 197, Phe 227, Ile 237 Leu 240 and Met 536. The orientation of the quinolone ring may be influenced by the position and nature of its substitution, as well as by the plasticity of the aforementioned hydrophobic pocket. Based on the substitution pattern the

quinoline core makes either face-to-face or face-to-edge aromatic stacking interactions with Phe 188 & 237. Docking studies revealed that the hybridized pyrazolopyrimidine scaffold with quinoline group can retain some of the crucial interactions needed for activity. These include HB interactions (His 185 and Arg 265) and aromatic stacking interactions (Phe 188 and 227) (precedence in literature).[15] Thus, we hypothesize that pfDHODH is a likely target for *P. falciparum* activity of this pyrazolopyrimidine scaffold. Over all docking studies show that compounds **5**, **6**, **17** and **18** has affinity for pfDHODH, thereby indicating a likely mechanism of action.

In conclusion, we have designed and synthesized novel 8-amino-quinoline–pyrazolopyrimidine hybrids and established SAR for this series. We have developed a new set of hybrids that show excellent antimalarial activities against both wild type *Plasmodium falciparum* (*Pf*_NF54) and resistant strain (*Pf*_K1). We plan to progress this series further by optimizing DMPK properties before further testing for in vivo efficacy.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.003.

References and notes

1. World Malaria Report 2013. World Health Organization. (http://www.who.int/malaria/publications/world_malaria_report_2013/en/).
2. Eastman, R. T.; Fidock, D. A. *Nat. Rev. Microbiol.* **2009**, *7*, 864.
3. Ariey, F.; Witkowski, B.; Amaratunga, C.; Beghain, J.; Langlois, A. C.; Khim, N.; Kim, S.; Duru, V.; Bouchier, C.; Ma, L.; Lim, P.; Leang, R.; Duong, S.; Sreng, S.; Suon, S.; Chuor, C. M.; Bout, D. M.; Ménard, S.; Rogers, W. O.; Genton, B.; Fandeur, T.; Miotto, O.; Ringwald, P.; Le Bras, J.; Berry, A.; Barale, J. C.; Fairhurst, R. M.; Benoit-Vical, F.; Mercereau-Puijalon, O.; Ménard, D. *Nature* **2014**, *505*, 7481.
4. (a) Held, J.; Kreidenweiss, A.; Mordmüller, B. *Expert Opin. Drug Discov.* **2013**, *11*, 1325; (b) Biot, C.; Chibale, K. *Infect. Disord. Drug Targets* **2006**, *6*, 173.
5. (a) Muregi, F. W.; Ishih, A. *Drug Dev. Res.* **2010**, *71*, 20; (b) Walsh, J. J.; Bell, A. *Curr. Pharm. Des.* **2009**, *15*, 2970.
6. Edgcomb, J. H.; Arnold, J.; Young, E. H., Jr.; Alving, A. S.; Eichelberger, J. J. *Nat. Malar. Soc.* **1950**, *9*, 285.
7. Nasveld, P. E.; Edstein, M. D.; Reid, M.; Brennan, L.; Harris, I. E.; Kitchener, S. J.; Leggat, P. A.; Pickford, P.; Kerr, C.; Ohrt, C.; Prescott, W. *Antimicrob. Agents Chemother.* **2010**, *54*, 792.
8. Vennerstrom, J. L.; Nuzum, E. O.; Miller, R. E.; Dorn, A.; Gerena, L.; Dande, P. A.; Ellis, W. Y.; Ridley, R. G.; Milhous, W. K. *Antimicrob. Agents Chemother.* **1999**, *43*, 598.
9. Phillips, M. A.; Gujjar, R.; Malmquist, N. A.; White, J.; Mazouni, F. E.; Baldwin, J.; Rathod, P. K. *J. Med. Chem.* **2008**, *51*, 3649.
10. (a) Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; Mazouni, F. E.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I.; Ferrer, S. B.; Jimenez-Díaz, M. B.; Gamo, F. J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A. *J. Med. Chem.* **2011**, *54*, 5540; (b) Marwaha, A.; White, J.; Mazouni, F. E.; Creason, S. A.; Kokkonda, S.; Buckner, F. S.; Charman, S. A.; Phillips, M. A.; Rathod, P. K. *J. Med. Chem.* **2012**, *55*, 7425.
11. Saito, T.; Obitsu, T.; Minamoto, C.; Sugiura, T.; Matsumura, N.; Ueno, S.; Kishi, A.; Katsumata, S.; Nakai, H.; Toda, M. *Bioorg. Med. Chem.* **2011**, *19*, 5955.
12. Monguchi, Y.; Kume, A.; Hattori, K.; Maegawa, T.; Sajiki, H. *Tetrahedron* **2006**, *62*, 7926.
13. Bennett, T. N.; Paguio, M.; Gligorijevic, B.; Seudieu, C.; Kosar, A. D.; Davidson, E.; Roepe, P. D. *Antimicrob. Agents Chemother.* **1807**, *2004*, 48.
14. Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **1803**, *2004*, 48.
15. Deng, X.; Gujjar, R.; Mazouni, F. E.; Kminsky, W.; Malmquist, N. A.; Goldsmith, E. J.; Rathod, P. K.; Phillips, M. A. *J. Biol. Chem.* **2009**, *284*, 26999.