Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1015–1018
Bioorganic & Medicinal Chemistry Letters

Synthesis of ring-substituted 4-aminoquinolines and evaluation of their antimalarial activities

Peter B. Madrid,[a] John Sherrill,[a] Ally P. Liou,[b] Jennifer L. Weisman,[a,c] Joseph L. DeRisi[b] and R. Kiplin Guy[a,c,*]

[a]*Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143-2280, USA*
[b]*Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-2280, USA*
[c]*Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94143-2280, USA*

Received 21 October 2004; revised 10 December 2004; accepted 14 December 2004
Available online 20 January 2005

Abstract—A simple two-step synthesis method was used to make 51 B-ring-substituted 4-hydroxyquinolines allowing analysis of the effect of ring substitutions on inhibition of growth of chloroquine sensitive and resistant strains of *Plasmodium falciparum*, the dominant cause of malaria morbidity. Substituted quinoline rings other than the 7-chloroquinoline ring found in chloroquine were found to have significant activity against the drug-resistant strain of *P. falciparum* W2.

Malaria is one of the most devastating infectious diseases in the world, afflicting 200–500 million people and killing 1–2 million annually.[1] Of the four species of the protozoa that cause malaria in humans, *P. falciparum* is by far the most deadly. The worldwide increase in drug-resistance to many of the affordable chemotherapies, such as chloroquine, has created a demand for the development of new malaria treatments.

Quinoline containing compounds have long been used for treatment of malaria, beginning with quinine (Fig. 1).[2] Systematic modification of quinine led to the potent and inexpensive 4-aminoquinoline drug chloroquine. After the worldwide development of drug-resistance to chloroquine, focused chemistry and screening efforts produced mefloquine, another quinoline containing compound that was highly active against the chloroquine-resistant strains of *P. falciparum*.[3] Since the development of mefloquine, there have been several reports of new potent quinoline compounds.[4–7] Most of these contain the 7-chloroquinoline nucleus of chloroquine and vary in the length and nature of their basic amine side chain. Currently, compounds such as amodiaquine and AQ-13 are promising leads for the development of new drugs.[8]

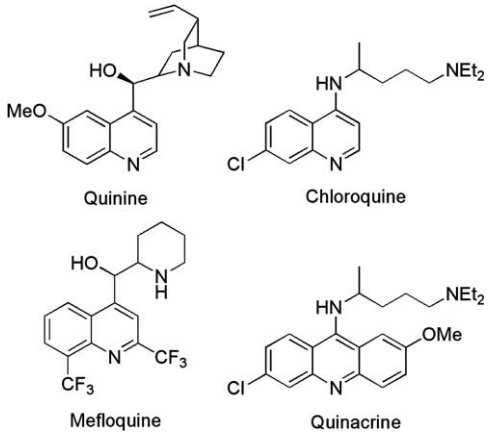


Figure 1. Quinoline antimalarial drugs with diverse substitutions around the quinoline ring.

While it is known that modification of the basic amine side chain can produce compounds active against drug-resistant *P. falciparum* strains, it has generally been assumed that changes to the quinoline nucleus itself will not. Changes to the ring system affect the pK_as of both the quinoline ring nitrogen and the side-chain nitrogen as well as other physical parameters such as lipophilicity,

Keyword: 4-Aminoquinoline malaria.
* Corresponding author. Tel.: +1 415 502 7051; fax: +1 415 514 0689; e-mail: rguy@cgl.ucsf.edu

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.12.037

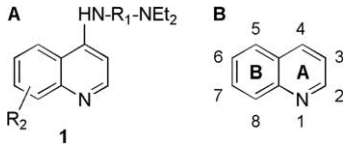


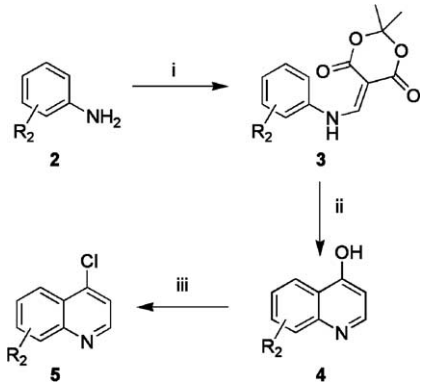
Figure 2. (A) Generalized structure of compound library **1**. (B) Lettering of rings and numbering of positions around the quinoline ring.

sterics, and electronegativity but have not significantly correlated with activity in drug resistant strains.[9] Recent work on ring substitutions has focused almost exclusively on the 7-position of the ring, so conclusions drawn from these data are limited. In this study, we explore the effects of B ring substitutions (Fig. 2, positions 5, 6, 7, and 8) on antimalarial activity against drug-resistant parasite strains.

Most of the current methods for synthesis of quinoline rings are variations of the Skraup method in which an aniline is heated with glycerol and an acid catalyst to form a stable intermediate that undergoes cyclization after a high-temperature Friedel–Crafts acylation.[10] Much of the recent work on quinoline ring formation reactions has been done using the method of Price and Roberts, which involves condensation of an aniline with ethoxymethylenemalonic ester.[9,11] We employed a modification of this method that uses methoxymethylene Meldrum's acid rather than ethoxymethylenemalonic ester.[12] This has the distinct advantage of giving the same products in two steps rather than four steps. A disadvantage to all of these reactions is they are usually carried out at very high-temperatures (>300 °C) and are notoriously messy. Another difficulty arises when using *meta* substituted anilines—the two different reactive *ortho* positions give regioisomeric products on the nucleophilic ring. The products favor the substituent being in the 7-position over the 5-position, but in almost all cases both products are formed. The separation of these isomers can be quite difficult depending on the size and nature of the substituent.

This paper outlines a simple two-step method for the synthesis of substituted quinoline rings and an evaluation of how these substitutions effect the resistance profile against a drug-sensitive and drug-resistant strain of *P. falciparum*.

The first step in the sequence is the condensation of an aniline with Meldrum's acid and trimethyl-*ortho*-formate (Scheme 1). The Meldrum's acid is refluxed in trimethyl-*ortho*-formate to form methoxymethylene Meldrum's acid in situ. The aniline **2** is then added to this solution where it enters into an addition–elimination reaction with the methoxymethylene moiety to afford the ene–amine precursor for cyclization. Strongly electron deficient anilines, such as nitro anilines, did not quantitatively form the intermediate under the standard conditions. Adding an equal volume of DMF to the reaction and increasing the reaction temperature overcame this sluggish reactivity. The ene–amine intermediates were then sealed into a glass reaction tube with



Scheme 1. Reagents and conditions: (i) Meldrum's acid, $CH(OCH_3)_3$, reflux, 1 h, then **2**, DMF, reflux 2 h; (ii) diphenylether, 300 °C, 300 W, 5 min; (iii) $POCl_3$ (neat), reflux, 3 h.

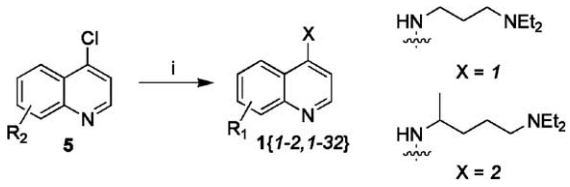
a small volume of phenyl ether as solvent and subjected to microwave irradiation for 5 min at 300 °C.

The crude reaction mixtures were then directly purified by silica chromatography to afford the desired pure 4-hydroxyquinolines in 20–70% overall yield. In some cases a mixture of isomers was formed. They could be separated by HPLC and the ratios of isomers formed is indicated in the Supplemental Materials (Table S1).

The hydroxyquinolines were dissolved in phosphorus oxychloride and heated to reflux for 3 h to give the desired 4-chloroquinolines, poised for nucleophilic addition of the side chain.

In order to better understand the structure–activity relationship (SAR) for relative effects of side chain structure and substitutions on the quinoline ring, two different amine side chains were attached to the four position of the ring. One of the side chains was (*N*,*N*-diethyl)-1,4-diaminopentane (X = 2) the side chain found in chloroquine, a series where there is substantial drug-resistance among clinical isolates. The other side chain was (*N*,*N*-diethyl)-1,3-diaminopropane (X = 1), a shortened analog that is known to restore activity against drug-resistant strains for the 7-chloroquinoline series.

Both of these side chains were attached to the 4-chloroquinoline rings by nucleophilic substitution (Scheme 2). The final products were obtained cleanly in 70–90% yields after workup. Compounds with purity below 80% were purified by reversed-phase preparative HPLC to give the final library **1**{**1**–**2**,**1**–**32**} with an average



Scheme 2. Reagents and conditions: (i) X = 1: 3-diethylaminopropylamine (neat), 135–155 °C, 2 h. or X = 2: 2-amino-5-diethylaminopentane, 135–155 °C, 2 h.

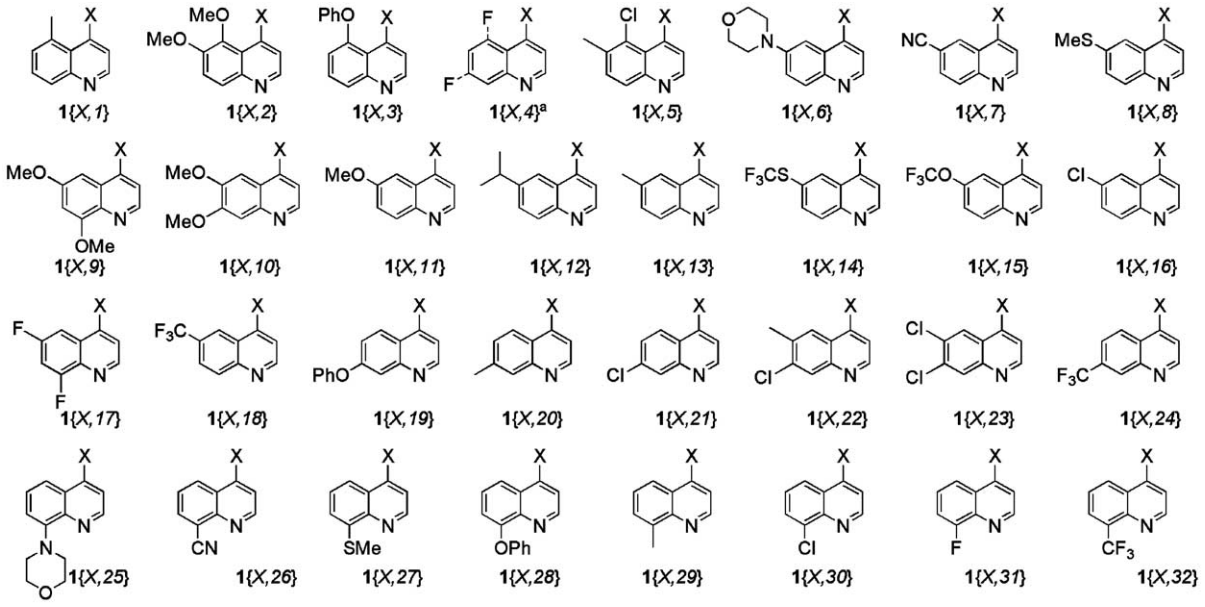


Figure 3. Structures of quinoline rings synthesized for this study and chemset nomenclature for the resulting library. [[a]Regioisomers were inseparable by reversed-phase HPLC. The 5-fluoro and 7-fluoro isomers were present in a ratio of 1:1.]

purity of 94%. Purity and identity was verified for all samples by LC/MS; additionally, ^{1}H NMR were obtained for 10% of the library. Many of the reactions utilizing side chain X = 2 failed due to substantial impurities found in the commercially available amine starting material.

Compound library **1**{**1**–**2**,**1**–**32**} (Fig. 3) was screened for growth inhibition activity against *P. falciparum* using a fluorescent-active cell sorting (FACS) assay (Fig. 4).[13] We chose to screen at two concentrations (30 nM and 200 nM) against both a drug-sensitive strain, 3D7, and a highly drug-resistant strain, W2. As expected, all compounds were more active against 3D7 than W2 and compounds with the shorter side chain were consistently more potent, especially against the drug-resistant W2 strain. It should also be noted that the most active compounds had substituents located at either the 6- or 7-position on the quinoline ring. In general, active substitutions tended to be small electron withdrawing groups with the notable exception of the 7-OPh compound **1**{**1**,**19**}, which proved to be one of the most active compounds.

After screening the activities of these compounds, the six most active compounds were chosen for full dose–response analysis. Along with this set, we also included the 6-chloro-2-methoxyacridine ring system found in quinacrine to compare a non-quinoline ring against the set of quinolines (Table 1). The shorter side chain variants consistently give higher potency than the

Table 1. Growth inhibitory activity, EC_{50} (nM)[a], of selected compounds against *P. falciparum* strains

Chemset number, substitution	3D7		W2	
	1{**1**,X}	**1**{**2**,X}	**1**{**1**,X}	**1**{**2**,X}
21, 7-Cl (CQ)	2	17	23	382
24, 7-CF_3	33	62	62	309
19, 7-OPh	34	115	71	267
22, 7-Cl, 6-Me	9	32	50	270
18, 6-CF_3	90	51	125	290
15, 6-OCF_3	82	62	102	287
Quinacrine[b]	5	8	8	32

[a]Values are means of three experiments.
[b]Acridine.

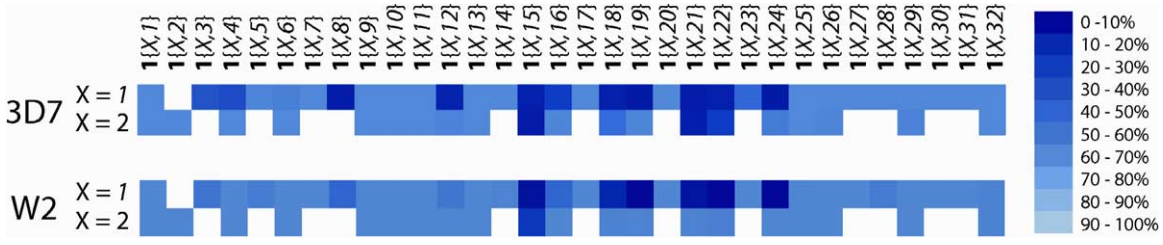


Figure 4. *P. falciparum* growth inhibition results for quinoline ring substitution library at 30 nM concentration. Colorimetric scale represents the percentage of growth of parasite relative to an untreated control population. White squares indicate a library member that could not be made or purified. Data represents the average of three separate measurements with standard deviation <10% of value.

chloroquine side chain variants against both 3D7 and W2. Interestingly, the pattern in EC_{50}s for the propyl side chain compounds on W2 mirrors that for 3D7 with the EC_{50}s being elevated by 1–2-fold, except in the case of the compounds containing a 7-chloroquinoline substructure. The 7-chloroquinoline and the 7-chloro-6-methylquinoline rings had EC_{50}s elevated by 11.5-fold and 5.5-fold, respectively.

When comparing the activities of the compounds with the chloroquine side chain against the two strains, it can be seen that the EC_{50}s increase, consistent with the notion that drug resistance is primarily modulated through the side chain identity. A feature worth noting is that the only compound that is notably less potent against W2 than the others is chloroquine (**1**{**2**,**21**}), which contains the 7-chloroquinoline ring. This indicates that perhaps the evolved resistance mechanism does have some specificity for the 7-chloroquinoline ring and therefore changes to the ring could be useful activity modulators against drug-resistant parasite strains. Another interesting feature of these data is that quinacrine retains its strong potency against the W2 strain despite the fact that it contains the side chain of chloroquine. The 7-OPh quinoline **1**{**1**,**19**} also contains an additional third ring, but perhaps because of its flexible nature as opposed to the rigid three ring system of the acridine, the drug-resistant parasite is able to survive in the presence of this compound.

In conclusion, we have used a simple two-step method to synthesize quinoline rings with diverse substitutions at the C-5, C-6, C-7, and C-8 positions. Modifications to the substituents around the ring led to several new active antimalarials, but their activity against the drug-resistant W2 strain of *P. falciparum* was weak when they contained the chloroquine side chain. This further supports the belief that the length and nature of the basic side chain is the primary modulator of activity against drug-resistant parasite strains. Despite this fact, modifications to the quinoline ring do somewhat improve the activity of this series of compounds against the W2 parasite strain. Also, exchanging the quinoline ring system with an acridine ring system has a profound affect on the activity of these compounds against drug-resistant strains. Further studies elucidating these trends are underway.

Acknowledgements

The Sandler Research Foundation and the NIH (AI053862).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.12.037.

References and notes

1. Gilles, H. M. *Management of Severe Malaria: A Practical Handbook*, 2nd ed.; World Health Organization: Geneva, 2000.
2. Coatney, G. R.; Cooper, W. C.; Eddy, N. B.; Greenberg, J. *Public Health Monogr.* **1953**, *15*, 1–322.
3. Rieckmann, K. H.; Trenholme, G. M.; Williams, R. L.; Carson, P. E.; Frischer, H.; Desjardins, R. E. *Bull. World Health Organ.* **1974**, *51*, 375–377.
4. De, D.; Krogstad, F. M.; Cogswell, F. B.; Krogstad, D. J. *Am. J. Trop. Med. Hyg.* **1996**, *55*, 579–583.
5. O'Neill, P. M.; Mukhtar, A.; Stocks, P. A.; Randle, L. E.; Hindley, S.; Ward, S. A.; Storr, R. C.; Bickley, J. F.; O'Neil, I. A.; Maggs, J. L.; Hughes, R. H.; Winstanley, P. A.; Bray, P. G.; Park, B. K. *J. Med. Chem.* **2003**, *46*, 4933–4945.
6. Ridley, R. G.; Hofheinz, W.; Matile, H.; Jaquet, C.; Dorn, A.; Masciadri, R.; Jolidon, S.; Richter, W. F.; Guenzi, A.; Girometta, M. A.; Urwyler, H.; Huber, W.; Thaithong, S.; Peters, W. *Antimicrob. Agents Chemother.* **1996**, *40*, 1846–1854.
7. Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. *J. Med. Chem.* **2002**, *45*, 4975–4983.
8. Olliaro, P.; Mussano, P. *Cochrane Database Syst. Rev.* **2003**, CD000016.
9. Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531–3539.
10. Davies, D. I. *Aromatic Heterocyclic Chemistry*; Oxford University: Oxford, New York, 1991.
11. De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. *J. Med. Chem.* **1998**, *41*, 4918–4926.
12. Briehl, H.; Lukosch, A.; Wentrup, C. *J. Org. Chem.* **1984**, *49*, 2772–2779.
13. Madrid, P. B.; Wilson, N. T.; DeRisi, J. L.; Guy, R. K. *J. Comb. Chem.* **2004**, *6*, 437–442.