Journal of Medicinal Chemistry

ARTICLE

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Synthesis and Evaluation of 7-Substituted 4-Aminoquinoline Analogues for Antimalarial Activity

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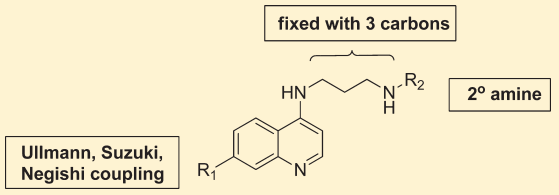
Supporting Information

ABSTRACT: We previously reported that substituted 4-aminoquinolines with a phenyl ether substituent at the 7-position of the quinoline ring and the capability of intramolecular hydrogen bonding between the protonated amine on the side chain and a hydrogen bond acceptor on the amine's alkyl substituents exhibited potent antimalarial activity against the multidrug resistant strain *P. falciparum* W2. We employed a parallel synthetic method to generate diaryl ether, biaryl, and alkylaryl 4-aminoquinoline analogues in the background of a limited number of side chain variations that had previously afforded potent 4-aminoquinolines. All subsets were evaluated for their antimalarial activity against the chloroquine-sensitive strain 3D7 and the chloroquine-resistant K1 strain as well as for cytotoxicity against mammalian cell lines. While all three arrays showed good antimalarial activity, only the biaryl-containing subset showed consistently good potency against the drug-resistant K1 strain and good selectivity with regard to mammalian cytotoxicity. Overall, our data indicate that the biaryl-containing series contains promising candidates for further study.



INTRODUCTION

Malaria, a devastating infectious disease caused by five species of *Plasmodium,* affects 200−500 million people and causes over 800 000 deaths annually.[1] The most lethal form of malaria is caused by *Plasmodium falciparum*.[2] Since the development of quinine, drugs containing the quinoline nucleus have been a mainstay of antimalarial therapy.[3,4] However, the spread of chloroquine (CQ) and mefloquine resistant strains of *P. falciparum* has curbed the usefulness of this class of drugs.[5,6] In the past 2 decades, a number of new potent quinolines that overcome chloroquine resistance have been reported.[7−9] Most contain the 7-chloroquinoline nucleus of chloroquine and vary in the length and nature of their basic amine side chain.[10−12]

We have reported extensive studies on the role of side chains for the antimalarial activity and efficacy.[13,14] Additional work in our laboratory systematically examined modification of the quinoline ring and the basic side chain of 4-aminoquinolines (**1**)[13−16] demonstrating that intramolecular hydrogen bonding (**2**) between the protonated amine and the hydroxyl group on the tertiary basic amine is crucial for the potency against CQ-resistant strains. In a similar systematic survey we found that most modifications to the quinoline nucleus resulted in lowered potency and had little effect on relative potency in multidrug resistant (MDR) strains, such as W2.[15,16] This finding was in line with the consensus model from the literature that requires a small, electron withdrawing group at this position (chlorine or trifluoromethyl). From these studies, one finding that did stand out was the high potency of the 7-PhO substitution in the context of a shorter amino containing side chain, which was generically more potent against MDR strains. In addition other studies showed that exchanging the quinoline ring system with an acridine ring system in the context of the same side chain gave improved antimalarial potency against both CQ-sensitive and CQ-resistant strains, suggesting that increased bulk and/or hydrophobicity might be advantageous.[17] Given these previous SAR results, we designed a new array of 4-aminoquinoline analogues (**3**) to explore this hypothesis by using parallel medicinal chemistry. Here we report the design, synthesis, and biological evaluation of this array.

Our strategy for the design of this compound array is shown in Figure 1. We fixed the side chain portion using a propyl spacer (three carbons) between two amines because such shorter side chain variants have consistently given better antimalarial potency than longer chains (more than four carbons).[16] A fixed set of substitutions on the terminal alkylamine was chosen to allow for combinatorial interactions with the quinoline nucleus within the highly potent "chemical space". This diversity set was based on previously obtained SAR for chloroquine analogues. The 7-position of the quinoline ring system was varied through a range of substituents that were accessible by the Ullmann coupling,[18,19] Suzuki coupling,[20,21] and Negishi coupling reactions,[22] which respectively generate diaryl ether, biaryl, and alkylaryl substitutions. These three subseries were chosen to systematically vary

Received: May 18, 2011
Published: September 12, 2011

the size, hydrogen bonding capabilities, electrostatics, and hydrophobicity at the 7-position. Overall this study was expected to firmly establish which types of substitutions are tolerated at the 7-position and how this position interacts with the side chain in defining potency in CQ-resistant *Plasmodium* species.

CHEMISTRY

The general approach used to produce this compound array followed our previously established route (Scheme 1). The main challenge in this work was the development of a robust, general route to 7-bromo-4-chloroquinoline **7a**, which served as the key intermediate for the diversification. Since chloro and bromo substituted carbons in general have strongly differing reactivity, this intermediate allowed for the selective synthesis of all targeted compounds. The synthetic route to the key intermediate **7a** followed a previously reported method.[16] Condensation of 3-bromoaniline with Meldrum's acid **4** and trimethyl orthoformate gave enamine **5**. Cyclization with microwave acceleration gave regioisomers **6a** and **6b** as a 2:1 mixture that was inseparable by both flash column chromatography and crystallization. Chlorination of the mixture with $POCl_3$ allowed the successful separation of 7-bromo-4-chloroquinoline **7a**, the key intermediate, from its isomer 5-bromo-4-chloroquinoline **7b** by column chromatography. Condensing **7b** with 20 equiv of 1,3-propyldiamine afforded compound **8**, which was used in the next coupling reaction without purification.

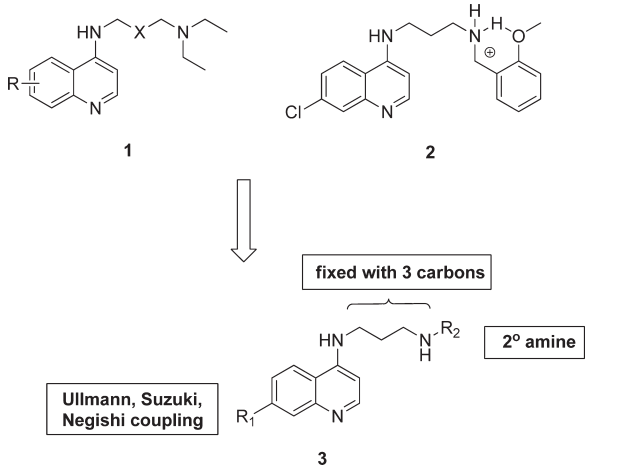


Figure 1. Rational design for 7-substituted 4-aminoquinolines. The three-carbon spacer between two amines and the terminal secondary amine was selected, since this pair consistently gave high potency in previous work. To explore substitution on the quinoline ring at the 7-position, metal assisted-coupling reactions including the Ullmann, Suzuki, and Negishi reactions were applied to generate diaryl ether, biaryl, and alkylaryl subsets, respectively.

Intermediate **8** was coupled with three sets of building blocks (diversity sets shown in Figure 2): phenols (x = 1, y = 1−8), boronic acids (x = 2, y = 1−8), and alkyl/benzyl zinc halides (x = 3, y = 1−12) in the presence of the corresponding metal catalyst. Each diversity set was designed to sample widely in sterics, electrostatics, and hydrophobicity. This parallel diversification step resulted in the production of the array of 7-substituted-4-aminoquinolines **9**$\{x,y\}$. The final array was produced by an orthogonal diversification at the terminal amine. Each member of array **9**$\{x,y\}$ was treated in parallel with the four aldehydes defined from our previous studies (z = 1−4) to give 4,7-disubstituted quinoline analogues **3**$\{x,y,z\}$.

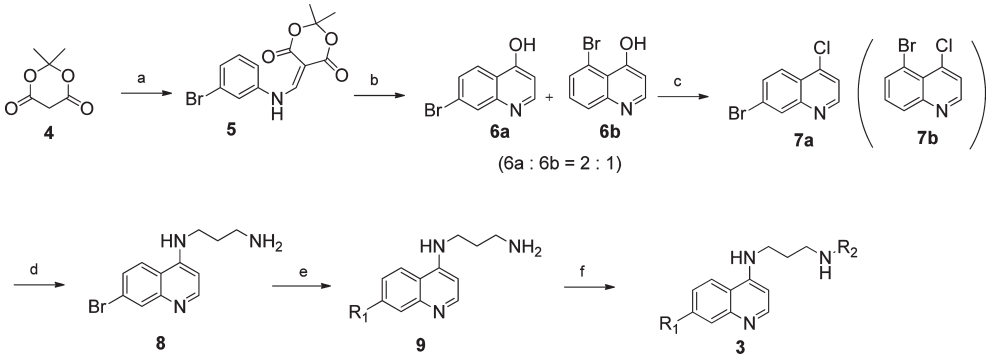
The crude products were purified by flash chromatography using a SP-1 (Biotage) purification system with dichloromethane and methanol mixture (1% → 50% gradient condition). Each purified product was then dissolved in a saturated methanolic solution of hydrochloric acid and then evaporated to dryness to afford the HCl salts. Purity and identity were verified for all compounds by UPLC/MS and ^{1}H NMR. The yield, purity, and NMR data of final compounds are available in the Supporting Information. All purified products were dissolved in DMSO to a standard concentration of 10 mM for biological testing.

BIOLOGICAL AND PHARMACOLOGICAL TESTING

The compounds in array **3**$\{x,y,z\}$ were tested against CQ-sensitive (3D7) and CQ-resistant (K1) strains of *P. falciparum* in concentration−response experiments in a 3-fold dilution schema spanning 15 μM to 0.7 nM, using a previously established method.[23] All experiments were carried out in triplicate, each triplicate experiment being replicated on two separate days for a total of 9 replicates. Data are reported as mean EC_{50} values with 95% confidence limits.

The compounds in array **3**$\{x,y,z\}$ were also tested for growth inhibition against four human cell lines: Raji, a Burkett's lymphoma derived line; BJ, an immortalized foreskin fibroblast derived line;

Scheme 1. Synthesis of the Compound Array 3$\{x,y,z\}$[a]



[a] Reagents and conditions: (a) 3-bromoaniline, triethyl orthoformate, reflux, 1 h; (b) 225 °C, microwave, Ph_2O, 5 min; (c) $POCl_3$, toluene, 100 °C, 1 h; (d) 1,3-aminopropane, K_2CO_3, TEA, NMP, 140 °C, 1 h. (e) For Ullmann reagent: phenols, CuI, Cs_2CO_3, ligand, 1,4-dioxane, 4 Å molecular sieves, reflux, 24 h. For Suzuki reagents: boronic acids, $PdCl_2$(dppf), Cs_2CO_3, toluene, 4 Å molecular sieves, 100 °C, 24 h. For Negishi reagents: zinc halides, $Pd(PPh_3)_4$, THF, reflux, 16 h.

dx.doi.org/10.1021/jm200636z |J. Med. Chem. 2011, 54, 7084–7093

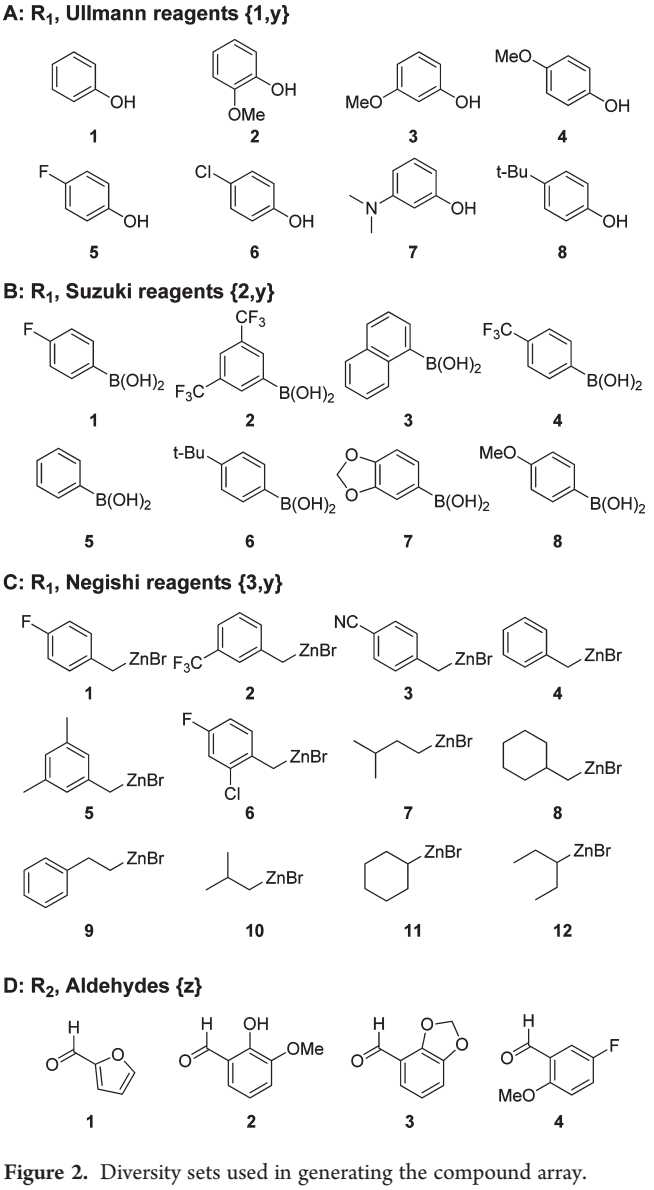


Figure 2. Diversity sets used in generating the compound array.

HEK 293, an embryonic kidney fibroblast derived line; and HepG2, a hepatocellular carcinoma derived line. The cytotoxicity was determined by detection of total cellular ATP levels (Cell Titer Glo). The same concentration−response plates were used for both antimalarial and growth proliferation experiments. All experiments were carried out in triplicate with one replicate for a total of three replicates. Data are reported as mean EC_{50} values with 95% confidence limits.

Finally, the kinetic solubility and passive permeability of the molecules in array **3**(*x*,*y*,*z*) were investigated to provide guidance in interpreting cell based assay results and to inform considerations about the development potential of the compounds. Solubility was determined using the pIon method by dilution from DMSO stocks into PBS buffer containing citric acid at neutral pH (7.4).[24] Permeability was assessed using the pIon implementation of the parallel artificial membrane permeation assay (PAMPA) in PBS buffer containing citric acid at neutral pH (7.4), thus reflecting the conditions required for activity in cell-based assays.[25] Both assays emulate the conditions used to determine cytotoxicity and antimalarial activity. The results of this testing schema are discussed below, broken out by subset of the analogues at the 7-position of the quinoline nucleus.

Diaryl Ether Series. Thirty-two diaryl ethers derived from the Ullmann reaction were synthesized and evaluated. The data are summarized in the heat map in Table 1, which was produced by using the EC_{50} values determined by fitting curves to the concentration−response experiments described above. The tabulated experimental values and errors can be found in the Supporting Information. Of 32 diaryl ethers, 21 compounds showed reasonable potency according to the MMV lead criteria (EC_{50} < 50 nM) against the CQ-sensitive 3D7 strain. However, only two compounds (**3**{1,4,1} and **3**{1,7,4}) exhibited highly potent activity (20 and 31 nM EC_{50}, respectively) against the CQ-resistant strain K1. This is in stark contrast to the results with the analogous chloro compounds, which are all equivalent compounds that are roughly equipotent against the two strains.[13] One set of compounds stands out: **3**{1,1,4}, **3**{1,3,4}, and **3**{1,5,4}, all carrying the 3-F-6-MeO-Bn alkylamine substituent, showed enhanced potency in strain K1. Generally the furfuryl (z = 1) and 3-F-6-MeO-Bn (z = 4) substituted alkylamines in this series exhibited better potency against strain K1 than the 2-HO-3-MeO-Bn (z = 2) and piperonyl (z = 3) substituents. All of 2-HO-3-MeO-Bn (z = 2) substituted alkylamines had poor antimalarial activity against strain K1, whereas they were highly potent against strain 3D7. Electronic effects were not strongly evident for the diaryl ethers, but electron-donating substituents showed slightly better activity against strain 3D7.

The compounds were tested against a panel of four mammalian cell lines (HepG2, HEK293, Raji, and BJ). In general HepG2 cells were the most sensitive, while other lines were less sensitive against compounds (EC_{50} > 5 μM). Table 1 shows only HepG2 data, while all cytotoxicity data are summarized in the Supporting Information. Most of the compounds active against strain 3D7 (<50 nM EC_{50}) showed moderate cytotoxicity (EC_{50} of 3−15 μM) in HepG2, resulting in a reasonable average selectivity for the parasite (>100-fold). Actives against strain K1 (<50 nM EC_{50}) also showed at least 100-fold selectivity. All compounds in this series had reasonable solubility (14.5−46 μM) and good permeability ((317−1309) × 10^{-6} cm/s) in pH 7.4 buffer (Table 1 in Supporting Information).

Biaryl Series. Next we evaluated the biological activity of the biaryl series derived from the Suzuki reaction. All data for this series are summarized in the Supporting Information, and selected data are shown in Table 2. Most compounds in this series showed high potency against strain 3D7. Interestingly, many of them were also highly potent (<50 nM EC_{50}) against strain K1. Thus, there is a stark contrast between the diaryl ether and the biaryl series with respect to their activity against strain K1, which expresses the PfCRT transporter, the primary mediator of CQ resistance. However, compounds **3**{2,1,2} and **3**{2,5,2}, the most potent against 3D7 (both 9 nM EC_{50}), only showed weak potency against K1 (850 and 1950 nM EC_{50} values, respectively), resulting in high selectivity for 3D7 (93- and 213-fold). As was the case with the diaryl ether series, all 2-HO-3-MeO-Bn substituted alkylamines showed very good activity in strain 3D7.

A notable trend was observed: increasing hydrophobicity in this series was accompanied by decreasing antimalarial activity. For example, all *tert*-Bu-Ph (y = 6) substituted, naphthyl substituted (y = 3), and 3,5-CF_3-Ph substituted (y = 2) compounds showed poor activity in both 3D7 and K1. In contrast to the diaryl ether series, compounds with 2-HO-3-MeO-Bn (z = 2)

Table 1. Biological and Pharmacological Evaluation of Diaryl Ethers: Antimalarial Activity and Cytotoxicity[c]

3{x,y,z}	R_1	R_2	3D7[a] EC_{50} (μM)	K1[a] EC_{50} (μM)	K1/3D7	HepG2[b] EC_{50} (μM)	HepG2 /3D7	HepG2 /K1
CQ	--	--	0.03	1.0	33	25	833	33
3{1,4,1}	4-MeO-PhO	Furfuryl	0.004	0.020	5.6	5.3	1479	265
3{1,7,4}	3-Me_2N-PhO	3-F-6-MeO-Bn	0.007	0.031	4.7	3.3	514	110
3{1,1,3}	PhO	Piperonyl	0.018	0.078	4.3	2.7	149	34
3{1,2,1}	2-MeO-PhO	Furfuryl	0.001	0.087	66.9	6.5	5014	75
3{1,5,1}	4-F-PhO	Furfuryl	0.026	0.096	3.6	16.0	611	167
3{1,6,4}	4-Cl-PhO	3-F-6-MeO-Bn	0.031	0.109	3.5	4.5	143	41
3{1,5,4}	4-F-PhO	3-F-6-MeO-Bn	0.297	0.135	0.5	6.6	22	49
3{1,1,4}	PhO	3-F-6-MeO-Bn	0.162	0.138	0.9	1.5	9	11
3{1,3,1}	3-MeO-PhO	Furfuryl	0.009	0.153	17.4	15.5	1763	102
3{1,7,1}	3-Me_2N-PhO	Furfuryl	0.027	0.155	5.7	16.0	588	103
3{1,8,2}	4-*tert*Bu-PhO	2-HO-3-MeO-Bn	0.054	0.249	4.6	1.5	28	6
3{1,3,4}	3-MeO-PhO	3-F-6-MeO-Bn	2.360	0.296	0.1	7.1	3	24
3{1,6,3}	4-Cl-PhO	Piperonyl	0.038	0.320	8.4	7.2	189	22
3{1,4,2}	4-MeO-PhO	2-HO-3-MeO-Bn	0.004	0.375	91.5	5.5	1347	15
3{1,2,3}	2-MeO-PhO	Piperonyl	0.014	0.517	37.7	5.9	427	11
3{1,3,3}	3-MeO-PhO	Piperonyl	0.012	0.600	50.0	14.1	1178	24
3{1,4,4}	4-MeO-PhO	3-F-6-MeO-Bn	0.054	0.660	12.2	7.0	129	11
3{1,5,3}	4-F-PhO	Piperonyl	0.015	0.667	44.5	16.0	1067	24
3{1,6,2}	4-Cl-PhO	Furfuryl	0.011	0.785	70.7	5.7	514	7
3{1,6,1}	4-Cl-PhO	Furfuryl	0.040	0.793	19.8	7.8	194	10
3{1,2,4}	2-MeO-PhO	3-F-6-MeO-Bn	0.022	0.960	44.0	6.4	293	7
3{1,4,3}	4-MeO-PhO	Piperonyl	0.046	1.424	30.9	7.9	172	6
3{1,1,2}	PhO	2-HO-3-MeO-Bn	0.015	1.843	122.0	6.8	450	4
3{1,7,2}	3-Me_2N-PhO	2-HO-3-MeO-Bn	0.010	2.044	215.1	7.3	765	4
3{1,8,3}	4-*tert*Bu-PhO	Piperonyl	0.137	2.451	17.9	0.9	7	0.4
3{1,8,4}	4-*tert*Bu-PhO	3-F-6-MeO-Bn	0.106	2.780	26.2	7.2	68	3
3{1,3,2}	3-MeO-PhO	2-HO-3-MeO-Bn	0.072	3.026	42.0	5.3	74	2
3{1,5,2}	4-F-PhO	2-HO-3-MeO-Bn	0.011	3.132	282.2	11.0	990	4
3{1,7,3}	3-Me2N-PhO	Piperonyl	1.363	3.930	2.9	16.0	12	4
3{1,8,1}	4-*tert*Bu-PhO	Furfuryl	1.110	4.809	4.3	3.1	3	1
3{1,2,2}	2-MeO-PhO	2-HO-3-MeO-Bn	0.009	5.701	626.4	11.2	1227	2
3{1,1,1}	PhO	Furfuryl	0.314	5.971	19.0	15.2	48	3

Heat map	3D7 EC_{50} (μM)	K1 EC_{50} (μM)	K1/3D7	HepG2 EC_{50}	HepG2 /3D7	HepG2 /K1
	<0.01	<0.01	>100	>15	>1000	>1000
	<0.05	<0.05	>50	>10	>500	>500
	<0.1	<0.1	>10	>5	>100	>100
	<0.5	<0.5	>1	>1	>50	>50
	>0.5	>0.5	<1	<1	<50	<50

[a] Values are the mean of three independent experiments in triplicate. [b] Values are the mean of one experiment in triplicate. [c] Activity summary of the compounds was sorted by EC_{50} value against K1 strain.

and piperonyl (z = 3) substituents on the terminal alkylamine exhibited good activity against strain K1 (EC_{50} < 50 nM). Although this series displayed moderate overall cytotoxicity (0.7−15 μM EC_{50} in HepG2), all compounds with low nanomolar EC_{50} values against 3D7 and K1 had a good selectivity for the parasite relative to mammalian cells. All compounds in this series also have good solubility (32−42 μM) and good permeability ((237−2101) × 10^{-6} cm/s) in pH 7.4 buffer (Table 2 in Supporting Information)

Alkylaryl Series. The alkylaryl series, derived from Negishi couplings, was evaluated in the same test cascade (Table 3). This set of compounds also showed good antimalarial activity against strain 3D7. However, their activity against strain K1 was very weak (EC_{50} > 200 nM). Most of the active compounds in this series showed moderate to strong cytotoxicity (0.4−16 μM) in HepG2, resulting in a reasonable selectivity relative to mammalian cells (>100). However, given the poor potency on K1, there was almost no selectivity relative to mammalian cells of that strain. Interestingly, the most potent compound **3** with 4 nM EC_{50} against 3D7 was quite toxic (403 nM EC_{50}) to HepG2 cell, but it still gave good selectivity (>100-fold) between parasite and mammalian cells. Overall this series is the least promising with respect to potential for development. In general the compounds in this series tended to be less soluble and less permeable than the other series studied. They had values ranging from modest to good solubility (4.2−41 μM) and modest to good permeability ((23−2110) × 10^{-6} cm/s) in pH 7.4 buffer (Table 3 in Supporting Information)

DISCUSSION

In earlier work we identified a previously unappreciated aspect to the structure−activity relationships of the 4-aminoquinoline antimalarials in which the 7-chloro substituent of the quinolone

dx.doi.org/10.1021/jm200636z |J. Med. Chem. 2011, 54, 7084–7093

Table 2. Biological and Pharmacological Evaluation for Biaryls: Antimalarial Activity and Cytotoxicity[c]

No	R_1	R_2	3D7[a] EC_{50} (μM)	K1[a] EC_{50} (μM)	K1/3D7	HepG2[b] EC_{50} (μM)	HepG2 /3D7	HepG2 /K1
CQ	--	--	0.03	1.0	33	25	833	33
3{2,8,1}	4-MeO-Ph	Furfuryl	0.011	0.023	2.2	6.00	565.7	261.9
3{2,7,4}	Piperonyl	3-F-6-MeO-Bn	0.031	0.024	0.8	6.04	194.7	255.8
3{2,4,2}	4-CF_3-Ph	2-HO-3-MeO-Bn	0.013	0.024	1.8	2.21	166.2	91.0
3{2,8,3}	4-MeO-Ph	Piperonyl	0.029	0.026	0.9	5.63	192.9	218.3
3{2,7,3}	Piperonyl	Piperonyl	0.026	0.027	1.0	4.79	185.0	178.8
3{2,8,4}	4-MeO-Ph	3-F-6-MeO-Bn	0.096	0.029	0.3	5.96	62.1	205.5
3{2,5,3}	Ph	Piperonyl	0.017	0.029	1.7	4.07	234.0	138.9
3{2,1,3}	4-F-Ph	Piperonyl	0.018	0.035	2.0	8.77	495.4	252.0
3{2,5,4}	Ph	3-F-6-MeO-Bn	0.011	0.045	4.2	2.96	276.4	66.3
3{2,7,1}	Piperonyl	Furfuryl	0.019	0.046	2.4	6.50	340.2	142.2
3{2,7,2}	Piperonyl	2-HO-3-MeO-Bn	0.008	0.050	6.0	1.14	135.4	22.7
3{2,1,1}	4-F-Ph	Furfuryl	0.032	0.069	2.1	7.76	240.4	113.2
3{2,8,2}	4-MeO-Ph	2-HO-3-MeO-Bn	0.013	0.078	6.0	1.60	122.9	20.6
3{2,4,4}	4-CF_3-Ph	3-F-6-MeO-Bn	0.054	0.089	1.7	5.19	96.8	58.4
3{2,1,4}	4-F-Ph	3-F-6-MeO-Bn	0.018	0.097	5.5	0.77	44.0	7.9
3{2,4,3}	4-CF_3-Ph	Piperonyl	0.025	0.098	3.9	1.89	74.5	19.2
3{2,5,1}	Ph	Furfuryl	0.020	0.128	6.3	5.92	291.6	46.4
3{2,2,2}	3,5-CF_3-Ph	2-HO-3-MeO-Bn	0.029	0.209	7.2	0.94	32.4	4.5
3{2,4,1}	4-CF_3-Ph	Furfuryl	0.020	0.223	11.0	3.47	171.0	15.5
3{2,6,1}	4-*tert*-Bu-Ph	Furfuryl	0.704	0.325	0.5	3.15	4.5	9.7
3{2,6,2}	4-*tert*-Bu-Ph	2-HO-3-MeO-Bn	0.251	0.418	1.7	0.69	2.8	1.7
3{2,6,3}	4-*tert*-Bu-Ph	Piperonyl	0.223	0.490	2.2	1.37	6.2	2.8
3{2,3,3}	1-Naphtyl	Piperonyl	0.598	0.538	0.9	6.57	11.0	12.2
3{2,2,3}	3,5-CF_3-Ph	Piperonyl	0.284	0.604	2.1	1.16	4.1	1.9
3{2,2,4}	3,5-CF_3-Ph	3-F-6-MeO-Bn	0.092	0.812	8.8	0.87	9.4	1.1
3{2,1,2}	4-F-Ph	2-HO-3-MeO-Bn	0.009	0.854	92.8	6.84	742.9	8.0
3{2,2,1}	3,5-CF_3-Ph	Furfuryl	0.285	0.972	3.4	2.41	8.5	2.5
3{2,3,4}	1-Naphtyl	3-F-6-MeO-Bn	1.154	1.034	0.9	16.00	13.9	15.5
3{2,3,2}	1-Naphtyl	2-HO-3-MeO-Bn	0.033	1.119	33.9	2.52	76.3	2.3
3{2,3,1}	1-Naphtyl	Furfuryl	0.031	1.295	41.2	5.60	178.2	4.3
3{2,6,4}	4-*tert*-Bu-Ph	3-F-6-MeO-Bn	0.144	1.594	11.0	0.62	4.3	0.4
3{2,5,2}	Ph	2-HO-3-MeO-Bn	0.009	1.936	212.7	3.21	353.0	1.7

Heat map	3D7 EC_{50} (μM)	K1 EC_{50} (μM)	K1/3D7	HepG2 EC_{50} (μM)	HepG2 /3D7	HepG2 /K1
	<0.01	<0.01	>100	>15	>1000	>1000
	<0.05	<0.05	>50	>10	>500	>500
	<0.1	<0.1	>10	>5	>100	>100
	<0.5	<0.5	>1	>1	>50	>50
	>0.5	>0.5	<1	<1	<50	<50

[a] Values are the mean of three independent experiments in triplicate. [b] Values are the mean of one experiment in triplicate. [c] Activity summary of the compounds was sorted by EC_{50} value against K1 strain.

A-ring could be replaced by a hydrophobic substituent with retention of potency. In order to more fully explore this observation, we applied three different coupling chemistries to a common synthetic intermediate to give access to fairly widely ranging substituents at this position (R_1 group). This array of compounds provided a wide range of physicochemical properties for the quinoline including variant pK_a of the quinoline nitrogen; overall electrostatics and sterics, based on the Topliss and Hammett approaches; and overall lipophilicity.

It is known that the pK_a of the ring and the basic side chain affect the activity against both CQ-sensitive and CQ-resistant strains of *Plasmodium*.[12,26,27] We calculated overall lipophilicity and pK_a of quinoline nitrogens (data summarized in Supporting Information).[28] Surprisingly, the biaryl series (x = 2) and alkylaryl series (x = 3) showed identical pK_a values (9.47) on the nitrogen of the quinoline ring, whereas the diaryl ether series (x = 1) showed a slightly lower pK_a value (9.28). Overall this did not appear to correlate with activity trends. Interestingly, no strong stereoelectronic effects were observed for substituents on the R_1 group. Unfortunately, we did not find a good correlation between antimalarial activity and pK_a.

Kaschula et al. reported that a more lipophilic group at the 7-position of 4-aminoquinoline enhanced antimalarial activity by increasing hematin affinity.[27] Therefore, correlations with ALogP were explored to elucidate the relationship between activity and lipophilicity (Supporting Information, Figure 2). Lipophilic compounds in our tests exhibited slightly weaker antimalarial activity in both the 3D7 and K1 strains when all series were combined (Figure 3A). The same trend was observed in the diaryl ether series alone (Figure 3B) and in the biaryl series (Figure 3C). However, the trend was reversed in the alkylaryl series (Figure 3D). We noted furthermore that in the diaryl ether series the antimalarial activity in strain 3D7 was more sensitive to lipophilicity than in strain K1. However, none of these trends were strong enough to have any real interest or to warrant further mechanistic studies with hematin. Overall, there were no strong trends in activity that could be easily explained using any conventional models for chloroquine mechanism of action.

dx.doi.org/10.1021/jm200636z |*J. Med. Chem.* 2011, 54, 7084–7093

Table 3. Biological and Pharmacological Evaluation for Alkylaryls: Antimalarial Activity and Cytotoxicity[c]

No	R_1	R_2	3D7[a] EC_{50} (μM)	K1[a] EC_{50} (μM)	K1/3D7	HepG2[b] EC_{50} (μM)	HepG2 /3D7	HepG2 /K1
CQ	--	--	0.03	1.0	33	25	833	33
3	1-Et-Pr	2-HO-3-MeO-Bn	0.004	0.273	68.3	0.41	103.3	1.5
3{3,5,2}	3,5-Me-Bn	2-HO-3-MeO-Bn	0.009	1.360	160.0	2.68	315.8	2.0
3{3,5,1}	3,5-Me-Bn	Furfuryl	0.009	0.678	77.0	6.27	712.1	9.2
3{3,3,2}	4-CN-Bn	2-HO-3-MeO-Bn	0.011	5.443	513.5	7.15	674.8	1.3
3{3,4,4}	Bn	3-F-6-MeO-Bn	0.011	1.424	129.5	7.99	726.7	5.6
3{3,6,2}	2-Cl-4-F-Bn	2-HO-3-MeO-Bn	0.013	5.136	404.4	2.35	185.4	0.5
3{3,9,3}	PhEt	Piperonyl	0.013	1.533	117.1	4.98	380.3	3.2
3{3,1,2}	4-F-Bn	2-HO-3-MeO-Bn	0.015	7.150	473.5	4.78	316.6	0.7
3{3,9,2}	PhEt	2-HO-3-MeO-Bn	0.017	5.517	324.5	6.80	400.0	1.2
3{3,4,2}	Bn	2-HO-3-MeO-Bn	0.017	6.971	400.6	5.72	328.6	0.8
3{3,11,2}	cHex	2-HO-3-MeO-Bn	0.019	16.000	864.9	1.08	58.1	0.1
3{3,6,1}	2-Cl-4-F-Bn	Furfuryl	0.020	1.223	62.1	5.73	290.8	4.7
3{3,10,2}	iso-butyl	2-HO-3-MeO-Bn	0.020	8.130	402.5	2.92	144.5	0.4
3{3,7,2}	iso-pentyl	2-HO-3-MeO-Bn	0.022	8.000	365.3	5.39	245.9	0.7
3{3,3,3}	4-CN-Bn	Piperonyl	0.023	3.069	136.4	6.08	270.2	2.0
3{3,2,2}	3-CF_3-Bn	2-HO-3-MeO-Bn	0.025	2.189	87.9	1.72	69.0	0.8
3{3,6,3}	2-Cl-4-F-Bn	Piperonyl	0.027	0.953	35.9	16.00	603.8	16.8
3{3,12,3}	1-Et-Pr	Piperonyl	0.027	1.065	39.5	6.35	235.1	6.0
3{3,7,3}	iso-pentyl	Piperonyl	0.027	0.206	7.6	2.80	103.1	13.5
3{3,3,4}	4-CN-Bn	3-F-6-MeO-Bn	0.028	0.770	27.5	6.05	216.2	7.9
3{3,5,4}	3,5-Me-Bn	3-F-6-MeO-Bn	0.034	0.794	23.1	0.94	27.4	1.2
3{3,3,1}	4-CN-Bn	Furfuryl	0.039	0.382	9.7	7.91	201.9	20.7
3{3,2,3}	3-CF_3-Bn	Piperonyl	0.039	1.314	33.5	2.20	56.1	1.7
3{3,4,1}	Bn	Furfuryl	0.040	3.550	89.9	16.00	405.1	4.5
3{3,2,4}	3-CF_3-Bn	3-F-6-MeO-Bn	0.040	0.323	8.1	0.44	11.0	1.3
3	1-Et-Pr	3-F-6-MeO-Bn	0.040	1.933	48.0	7.86	194.9	4.1
3{3,8,3}	cHexmethyl	Piperonyl	0.041	0.701	17.3	6.29	155.2	9.0
3{3,5,3}	3,5-Me-Bn	Piperonyl	0.041	1.040	25.6	2.25	55.5	2.2
3{3,9,1}	PhEt	Furfuryl	0.046	2.930	63.7	16.00	347.8	5.5
3{3,8,2}	cHexmethyl	2-HO-3-MeO-Bn	0.050	2.610	52.7	0.97	19.6	0.4
3{3,8,1}	cHexmethyl	Furfuryl	0.055	0.621	11.2	1.70	30.7	2.7
3{3,2,1}	3-CF_3-Bn	Furfuryl	0.056	0.743	13.2	3.19	56.7	4.3
3{3,1,1}	4-F-Bn	Furfuryl	0.057	1.659	28.9	5.60	97.5	3.4
3{3,10,3}	iso-butyl	Piperonyl	0.066	1.814	27.7	2.38	36.3	1.3
3{3,1,4}	4-F-Bn	3-F-6-MeO-Bn	0.075	0.580	7.8	3.53	47.1	6.1
3{3,12,1}	1-Et-Pr	Furfuryl	0.086	4.300	50.1	6.76	78.7	1.6
3{3,10,4}	iso-butyl	3-F-6-MeO-Bn	0.089	2.119	23.9	2.90	32.7	1.4
3{3,11,4}	cHex	3-F-6-MeO-Bn	0.090	1.596	17.7	2.29	25.4	1.4
3{3,7,4}	iso-pentyl	3-F-6-MeO-Bn	0.116	0.360	3.1	0.89	7.7	2.5
3{3,11,1}	cHex	Furfuryl	0.118	3.900	33.1	4.76	40.4	1.2
3{3,1,3}	4-F-Bn	Piperonyl	0.127	0.256	2.0	5.55	43.7	21.7
3{3,11,3}	cHex	Piperonyl	0.128	4.709	36.7	3.02	23.6	0.6
3{3,9,4}	PhEt	3-F-6-MeO-Bn	0.132	2.143	16.2	4.24	32.1	2.0
3{3,8,4}	cHexmethyl	3-F-6-MeO-Bn	0.138	0.687	5.0	1.83	13.3	2.7
3{3,7,1}	iso-pentyl	Furfuryl	0.176	4.605	26.1	11.24	63.7	2.4
3{3,6,4}	2-Cl-4-F-Bn	3-F-6-MeO-Bn	0.208	1.112	5.4	2.46	11.8	2.2
3{3,10,1}	iso-butyl	Furfuryl	0.581	4.500	7.7	16.00	27.5	3.6
3{3,4,3}	Bn	Piperonyl	0.720	1.237	1.7	6.28	8.7	5.1

Heat map	3D7 EC_{50} (μM)	K1 EC_{50} (μM)	K1/3D7	HepG2 EC_{50} (μM)	HepG2 /3D7	HepG2 /K1
	<0.01	<0.01	>100	>15	>1000	>1000
	<0.05	<0.05	>50	>10	>500	>500
	<0.1	<0.1	>10	>5	>100	>100
	<0.5	<0.5	>1	>1	>50	>50
	>0.5	>0.5	<1	<1	<50	<50

[a] Values are the mean of three independent experiments in triplicate. [b] Values are the mean of one experiment in triplicate. [c] Activity summary of the compounds was sorted by EC_{50} value against 3D7 strain.

Another possible explanation for variations in activity would be variant cellular bioavailability of the compounds. The quinolines are well-known to accumulate in the acidic food vacuole of the parasite, so permeability is usually a poor predictor of potency, beyond a requirement that the compounds be able to cross a membrane. As expected, we observed no strong

dx.doi.org/10.1021/jm200636z |J. Med. Chem. 2011, 54, 7084–7093

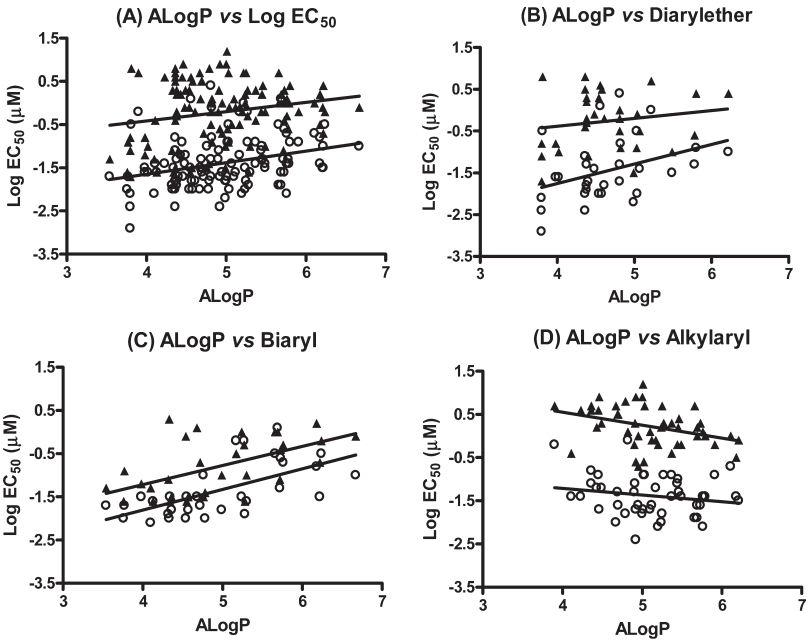


Figure 3. Relationship between lipophilicity and antimalarial activity (3D7 strain (○) and K1 strain (▲)): (A) ALogP vs log EC_{50}, complete compound array; (B) ALogP vs log EC_{50}, diaryl ethers; (C) ALogP vs log EC_{50},biaryls; (D) ALogP vs log EC_{50}-, alkylaryls.

correlations between permeability or solubility and potency. The most striking finding was that the alkylaryl series had fairly extreme cross-resistance with CQ-resistant strains. Presumably, this substitution at the 7-position increases transport by PfCRT.

CONCLUSIONS

A parallel synthetic strategy was employed to generate a rationally designed array of 7-substituted 4-aminoquinolines. Utilizing 7-bromo-4-chloroquinoline as a key intermediate enabled the exploration of two-dimensional diversity, using a fixed propyl spacer between the quinoline and the distal basic center and a small set of amine substituents previously shown to be active in CQ-resistant *Plasmodium* strains when used with the chloroquine nucleus. The diversity explored at the 7-position included diaryl ethers, biaryls, and alkylaryls and was produced using the Ullmann, Suzuki, and Negishi reactions, respectively. The compounds were screened against CQ-sensitive and CQ-resistant strains of *P. falciparum* and a panel of mammalian cell line to establish antimalarial potency and selectivity for mammalian cells.

Physicochemical properties of 4-aminoquinolines such as pK_a and lipophilicity can be influenced by the substitution at the 7-position, resulting in changes of the potency against malaria. However, in the series explored, the calculated pK_a of the compounds was not significantly changed by substitution at the 7-position, and no correlation was observed between pK_a and antimalarial activity. However, lipophilicity did weakly correlate with antimalarial activity. Strikingly, less lipophilic compounds were generally more potent against both the CQ-sensitive (3D7) and CQ-resistant strain (K1), an effect that was especially strong for the biaryl compounds.

Taken as a whole, this study highlights the potential for developing novel 4-aminoquinolines including a biaryl at the 7-position of the quinoline. Further exploration in preclinical studies is warranted.

EXPERIMENTAL SECTION

General. All materials were obtained from commercial suppliers and used without further purification. All solvents were dried using an aluminum oxide column. Thin-layer chromatography was performed on precoated silica gel 60 F254 plates. Purification of synthesized compounds was carried out by normal phase column chromatography (SP1 [Biotage], silica gel 230−400 mesh) followed by evaporation (HT-4X evaporator [Genevac]). Initiator [Biotage] was used for microwave reaction. ^{1}H NMR spectra were recorded using a Bruker 400 MHz spectrometer, in $CDCl_3$ or DMSO-d_6 solvent. Chemical shifts were reported as parts per million (ppm) downfield from solvent reference. Coupling constants (J) were measured in hertz (Hz). NMR peaks were assigned by MestReNova (5.2.2). Identity of all compounds was confirmed by proton and carbon NMR and by mass spectrometry. The purity of all compounds was assessed using LC/MS/UV/ELSD/CLND with the purity being assigned as the average determined by UV/ELSD/CLND. All compounds used for subsequent studies had a minimum purity of 95%.

Chemistry. 5-((3-Bromophenylamino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (5). A solution of 2,2-dimethyl-1,3-dioxane-4,6-dione (**4**) (21.6 g, 150 mmol) in triethyl orthoformate (100 mL) was refluxed for 1 h. After the mixture was cooled to room temperature, 3-bromoaniline (17.2 g, 100 mmol) was added to the solution. The mixture was refluxed for 2 h, then cooled to room temperature. The precipitates were collected and washed with ether to give compound **5** (32.8 g, quantitative yield) as pale yellow crystals. ^{1}H NMR ($CDCl_3$) δ ppm 11.51−10.89 (m, 1H), 8.60 (d, J = 14.1 Hz, 1H), 7.47−7.37 (m, 2H), 7.31 (t, J = 8.0 Hz, 1H), 7.21−7.15 (m, 1H), 1.76 (s, 6H).

7-Bromoquinolin-4-ol (6a) and 5-Bromoquinolin-4-ol (6b). Compound **5** (1.0 g, 3.1 mmol) and diphenyl ether (10 mL) were irradiated by microwave at 225 °C for 5 min. Ether (10 mL) was added to the mixture, and the resulting brown precipitates were collected and washed with ether to give a mixture of compounds **6a** and **6b**. Replicating this process 16 times in parallel gave 9.4 g (85% yield) of the product mixture. Caution: The reaction vessel develops high pressure. ^{1}H NMR (DMSO-d_6) δ ppm 11.79 (brs, 1H), 7.99 (d, J = 8.6 Hz, 2/

3H), 7.92 (d, J = 7.4 Hz, 2/3H), 7.82 (d, J = 7.4 Hz, 1/3H), 7.75 (d, J = 1.9 Hz, 2/3H), 7.52 (dd, J = 1.2, 8.0 Hz, 1/3H), 7.48 (dd, J = 1.4, 8.0 Hz, 1/3H), 7.45 (dd, J = 1.8, 8.6 Hz, 2/3H), 7.44 (dd, J = 8.0, 8.4 Hz, 1/3H), 6.05 (d, J = 7.5 Hz, 2/3H), 6.01 (d, J = 7.5 Hz, 1/3H).

7-Bromo-4-chloroquinoline (7a). To a suspension of a mixture of compounds **6a** and **6b** (9.4 g, 42 mmol) in toluene (70 mL) was added phosphorus oxychloride (13 g, 84 mmol). The mixture was stirred for 1 h at 100 °C, then cooled to room temperature. Excess phosphorus oxychloride was quenched by adding a small portion of ice−water and then stirring for 1 h. Additional water (100 mL) was added, and the resulting aqueous portion was extracted with dichloromethane. The organic phase was dried over $MgSO_4$, and the solvent was removed in vacuo. The crude product was purified by flash chromatography (EtOAc in hexane = 5% → 25%) to give compound **7a** (6.2 g, 61% yield) as white crystals and compound **7b** (3.0 g, 30% yield) as white crystals. **7a**: ^{1}H NMR ($CDCl_3$) δ ppm 8.79 (d, J = 4.8 Hz, 1H), 8.32 (d, J = 1.9 Hz, 1H), 8.12 (d, J = 9.0 Hz, 1H), 7.74 (dd, J = 9.0, 1.9 Hz, 1H), 7.51 (d, J = 4.7 Hz, 1H). **7b**: ^{1}H NMR ($CDCl_3$) δ ppm 8.73 (d, J = 4.6 Hz, 1H), 8.12 (dd, J = 8.4, 1.2 Hz, 1H), 7.96 (dd, J = 7.6, 1.2 Hz, 1H), 7.56 (d, J = 4.4 Hz, 1H), 7.52 (t, J = 8.4, 7.6 Hz, 1H).

***N*^{1}-(7-Bromoquinolin-4-yl)propane-1,3-diamine (8).** To a mixture of compound **7a** (6.18 g, 25.6 mmol) and 1,3-diaminopropane (37.9 g, 512 mmol) in *N*-methylpyrrolidinone (60 mL) was added triethylamine (777 mg, 7.68 mmol) and K_2CO_3 (1.17 g, 8.45 mmol). The resulting mixture was stirred at 140 °C for 1 h and then cooled to room temperature. Water (200 mL) was added to the solution, and the product was precipitated by cooling the mixture to 4 °C. The resulting white precipitates were collected, washed with water, and dried to give compound **8** (5.69 g, 80% yield) as white crystals. 1H NMR (DMSO-d_6) δ ppm 8.38 (d, J = 5.4 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.93 (d, J = 2.1 Hz, 1H), 7.54 (dd, J = 9.0, 2.1 Hz, 1H), 7.52 (brs, 1H), 6.48 (d, J = 5.5 Hz, 1H), 3.32 (brs, 2H), 2.68 (t, J = 6.5 Hz, 2H), 1.68−1.77 (m, 2H).

Procedure for Parallel Synthesis of Compounds 9{*x*=1}: Ullmann Coupling. To a mixture of compound **8** (279 mg, 1 mmol) in 1,4-dioxane (6 mL) were added the phenol reagent (4 mmol), Cs_2CO_3 (1.3 g, 4 mmol), 4 Å powder molecular sieves (∼280 mg, dried in oven), salicylaldoxime (55 mg, 0.4 mmol), and copper(I) iodide (38 mg, 0.2 mmol). The mixture was heated to reflux and maintained at that temperature for 24 h and then cooled to room temperature. A solution of dichloromethane (10 mL) and methanol (1 mL) was added to the mixture. After filtration the solvents were removed in vacuo. The crude product was purified by flash chromatography (MeOH/TEA in CH_2Cl_2 = 10%/1% to 40%/4% gradient) to give the corresponding compound **9**{x=1}.

Procedure for Parallel Synthesis of Compounds 9{*x*=2}: Suzuki Coupling. To a mixture of compound **8** (279 mg, 1 mmol) in toluene (6 mL) were added the boronic acid reagent (4 mmol), Cs_2CO_3 (1.3 g, 4 mmol), 4 Å powder molecular sieves (∼280 mg), and $PdCl_2$(dppf) (41 mg, 0.05 mmol). The mixture was heated to 100 °C for 24 h and then cooled to room temperature. Water (50 mL) was added and the resulting aqueous portion extracted with dichloromethane and 10% methanol. The organic phase was dried over $MgSO_4$, filtered, and dried in vacuo. The crude product was purified by flash chromatography (MeOH/TEA in CH_2Cl_2 = 10%/1% to 80%/8% gradient) to give the corresponding compounds **9**{x=2}.

Procedure for Parallel Synthesis of Compounds 9{*x*=3}: Negishi Coupling. To a solution of compound **8** (279 mg, 1 mmol) and $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) was added the alkylzinc bromide reagent (8 mL, 0.5 M in THF, 4 mmol). The mixture was refluxed for 16 h, then cooled to room temperature (1 h is sufficient for benzylzinc halide). Then 1 N HCl (10 mL) was added to the mixture and stirred. Additional water (40 mL) was added to the mixture, and the solution was then neutralized by adding solid K_2CO_3. The mixture was extracted with 10% methanol in dichloromethane, the organic phase dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (MeOH/TEA in CH_2Cl_2 = 10%/1% to 80%/8% gradient) to give the corresponding compounds **9**{x=3}. Benzylzinc bromide solution was prepared as follows: To a suspension of Zn powder (261 mg, 4 mmol) in THF (6 mL) was added benzyl bromide (4 mmol). The solution was heated to 50 °C for 30 min. The solution was used immediately for Negishi coupling.

Procedure for Parallel Synthesis of Compounds 3{*x*,*y*,*z*}: Reductive Amination. To a solution of compound **9**{x} (0.1−0.2 mmol) in methanol (1−2 mL) was added 1 M aldehyde stock solution in methanol (1 equiv) After the mixture was stirred for 1 h, sodium borohydride (0.5 equiv) was added to the reaction mixture and the resulting mixture stirred for 30 min. The reaction was quenched by addition of water (2 mL). The aqueous portion was extracted with CH_2Cl_2, and the sample was concentrated in vacuo. The crude product was purified by flash chromotography (1% methanol in CH_2Cl_2 (12 mL) and 30% methanol in CH_2Cl_2 (16 mL)). The solvent was removed in vacuo, and the product was suspended in 1.25 M HCl in methanol solution (1 mL). The solvent and excess of HCl were evaporated *in vacuo* to give the corresponding HCl salt.

Antimalarial Activity. Two *P. falciparum* strains were used in this study and were provided by the MR4 Unit of the American Type Culture Collection (ATCC, Manassas, VA). Those two strains were the chloroquine sensitive strain 3D7 and the chloroquine resistant strain K1.

Asynchronous parasites were maintained in culture based on the method of Trager.[29] Parasites were grown in the presence of fresh group O-positive erythrocytes (Lifeblood Memphis, TN) in Petri dishes at a hematocrit of 4−6% in RPMI based medium (RPMI 1640 supplemented with 0.5% AlbuMAX II, 25 mM HEPES, 25 mM $NaHCO_3$ (pH 7.3), 100 μg/mL hypoxanthine, and 5 μg/mL gentamycin). Cultures were incubated at 37 °C in a gas mixture of 90% N_2, 5% O_2, 5% CO_2. For IC_{50} determinations, 20 μL of RPMI 1640 with 5 μg/mL gentamycin were dispensed per well in an assay plate (Corning 384-well microtiter plate, clear bottom, tissue culture treated, catalog no. 8807BC). An amount of 40 nL of compound, previously serial diluted in a separate 384-well white polypropylene plate (Corning, catalog no. 8748BC), was dispensed to the assay plate by hydrodynamic pin transfer (FP1S50H, V&P Scientific Pin Head) and then an amount of 20 μL of a synchronized culture suspension (1% rings, 4% hematocrit) was added per well, thus making a final hematocrit and parasitemia of 2% and 1%, respectively. Assay plates were incubated for 72 h, and the parasitemia was determined by a method previously described:[30] Briefly, an amount of 10 μL of the following solution in RPMI (10× Sybr Green I, 0.5% v/v Triton, 0.5 mg/mL saponin) was added per well. Assay plates were shaken for 30 s, incubated in the dark for 90 min, then read with the Envision spectrophotometer at Ex/Em of 485 nm/535 nm. IC_{50} values were calculated with the robust investigation of screening experiments (RISE) with four-parameter logistic equation.

Cytotoxicity in Mammalian Cells. BJ, HEK293, Hep G2, and Raji cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and were cultured according to recommendations. Cell culture media were purchased from ATCC. Cells were routinely tested for mycoplasma contamination using the MycoAlert mycoplasma detection kit (Lonza). Exponentially growing cells were plated in Corning 384-well white custom assay plates and incubated overnight at 37 °C in a humidified 5% CO_2 incubator. DMSO compound solutions were added the following day to a top final concentration of 25 μM and then diluted $^1/_3$ for a total of 10 testing concentrations. Cytotoxicity was determined following a 72 h incubation using Promega Cell Titer Glo reagent according to the manufacturer's recommendation. Luminescence was measured on an Envision plate reader (Perkin-Elmer).

Solubility. The solubility assay was carried out on Biomek FX lab automation workstation (Beckman Coulter, Inc.). An amount of 10 μL

of compound stock was added to 190 μL of 1-propanol to make a reference stock plate. Then 5 μL from this reference stock plate was mixed with 70 μL of 1-propanol and 75 μL of phosphate buffered saline (PBS, pH 7.4) to make the reference plate, and the UV spectrum (250−500 nm) of the reference plate was measured using a SPECTRAmax PLUS plate reader (Molecular Devices). An amount of 6 μL of 10 mM test compound stock was added to 600 μL of PBS in a 96-well storage plate and mixed. The storage plate was sealed and incubated at room temperature for 18 h. The suspension was then filtered through a 96-well filter plate (pION Inc.). Then 75 μL of filtrate was mixed with 75 μL of 1-propanol to make the sample plate for UV spectroscopic analysis. A single experiment was performed in triplicate for each compound. Solubility was calculated using μSOL Evolution software based on the AUC (area under the curve) of the UV spectrum of the sample plate and the reference plate.

Permeability Assay. The parallel artificial membrane permeability assay (PAMPA) was carried out on a Biomek FX lab automation workstation (Beckman Coulter, Inc.). An amount of 3 μL of test compound stock (10 mM in DMSO) was mixed with 600 μL of SSB (system solution buffer, pH 7.4 or 4, pION Inc.) to dilute the test compound. Then 150 μL of diluted test compound in SSB was transferred to a UV plate (pION Inc.) and the UV spectrum was measured on a SPECTRAmax PLUS plate reader (Molecular Devices) to establish a reference plate. The membrane on a preloaded PAMPA sandwich (pION Inc.) was painted with 4 μL of GIT lipid (pION Inc.). The acceptor chamber was then filled with 200 μL of ASB (acceptor solution buffer, pION Inc.), and the donor chamber was filled with 180 μL of test compound diluted in SSB. The PAMPA sandwich (donor and acceptor chambers) was assembled, placed on the Gut-box (pION Inc.), and stirred for 30 min. The aqueous boundary layer was set to 40 μM for stirring, and the UV spectra (250−500 nm) of the donor and the acceptor chambers were read. A single experiment was performed in triplicate for each compound. The permeability coefficient was calculated using PAMPA Evolution 96 Command software (pION Inc.) based on the AUC of the reference, donor, and acceptor plates.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Biological data (antimalarial, cytotoxicity in mammalian cells, solubility, and permeability), ^{1}H NMR spectra, and purity of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported by NIH/NIAID (Grant Al075517), the American Lebanese Syrian Associated Charities (ALSAC), and St. Jude Children's Research Hospital.

■ ABBREVIATIONS USED

CQ, chloroquine; MDR, multidrug resistant; PAMPA, parallel artificial membrane permeation assay; WHO, World Health Organization; MMV, Medicines for Malaria Venture; HTS, high throughput screening; SAR, structure−activity relationship; Clint′, intrinsic clearance

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