Journal of Medicinal Chemistry

Article

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Lead Optimization of Antimalarial Propafenone Analogues

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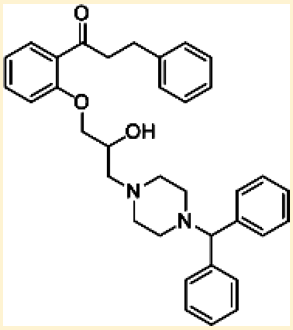
Supporting Information

ABSTRACT: Previously reported studies identified analogues of propafenone that had potent antimalarial activity, reduced cardiac ion channel activity, and properties that suggested the potential for clinical development for malaria. Careful examination of the bioavailability, pharmacokinetics, toxicology, and efficacy of this series of compounds using rodent models revealed orally bioavailable compounds that are nontoxic and suppress parasitemia in vivo. Although these compounds possess potential for further preclinical development, they also carry some significant challenges.



INTRODUCTION

In 2010, there were approximately 216 million cases of malaria worldwide with an estimated death toll of 655000.[1] Continued resistance to the currently available drugs[2,3] and recently observed slowing in patient responses to artemisinins[4−7] enforces a need for further research to discover new antimalarial therapies. Screening of a small collection of known drugs and bioactives revealed that propafenone, a class 1c antiarrhythmia drug,[8] potently inhibited growth of *Plasmodium falciparum* and was selectively more potent against the multidrug-resistant W2 and K1 strains.[9] Use of propafenone, or any other antiarrhythmic drug, as an antimalarial was previously unreported, and the increased activity against the multidrug resistant strains is very unusual. Therefore, the structure−activity relationships (SAR) of this series were explored to establish if the mammalian ion channel activity could be separated from the antimalarial activity and if the unusual strain specificity was maintained. Preliminary hit-to-lead studies focused attention upon a particular subseries of related compounds in which the alkyl amine side chain of propafenone was replaced by a bulkier, less basic amine (Figure 1, **1**−**6**).[10]

This hit-to-lead study carefully established structure−activity relationships (SAR) and led to a set of analogues with improved in vitro efficacy, low cytotoxicity, and a reasonable therapeutic index with respect to the ion channels targeted by propafenone itself, as seen in Table 1. In vitro testing of the ion channels targeted by propafenone (hERG, Nav1.5, and Kir6.2/SUR2A) revealed that activity against Nav1.5 and Kir6.2 could be completely suppressed and that against hERG lessened while also increasing the efficacy of the analogues compared to propafenone (Table 1).[10] This strongly suggests the resulting compounds would have significantly lessened cardiac effects relative to propafenone. With these two goals achieved, it was hoped the analogues would maintain the favorable pharmacokinetic properties of the parent compound despite the addition of the bulky side chain required to squelch ion channel activity. In the current study, six compounds from the lead optimization series were evaluated to determine their absorption, distribution, metabolism, and excretion (ADME), using both in vitro and in vivo methods in order to assess potential for development (Figure 1). The compounds were selected to include the three most potent analogues and the three with the best in vitro physiochemical properties which should translate to favorable in vivo pharmacokinetic behavior. Additionally, the compounds were assessed in vitro and in vivo for toxicology. Upon the basis of the examination of ADME and toxicology, two of the compounds were tested to determine in vivo efficacy in a rodent malaria model.

Received: February 28, 2012
Published: June 18, 2012

   dx.doi.org/10.1021/jm300286a | *J. Med. Chem.* 2012, 55, 6087−6093

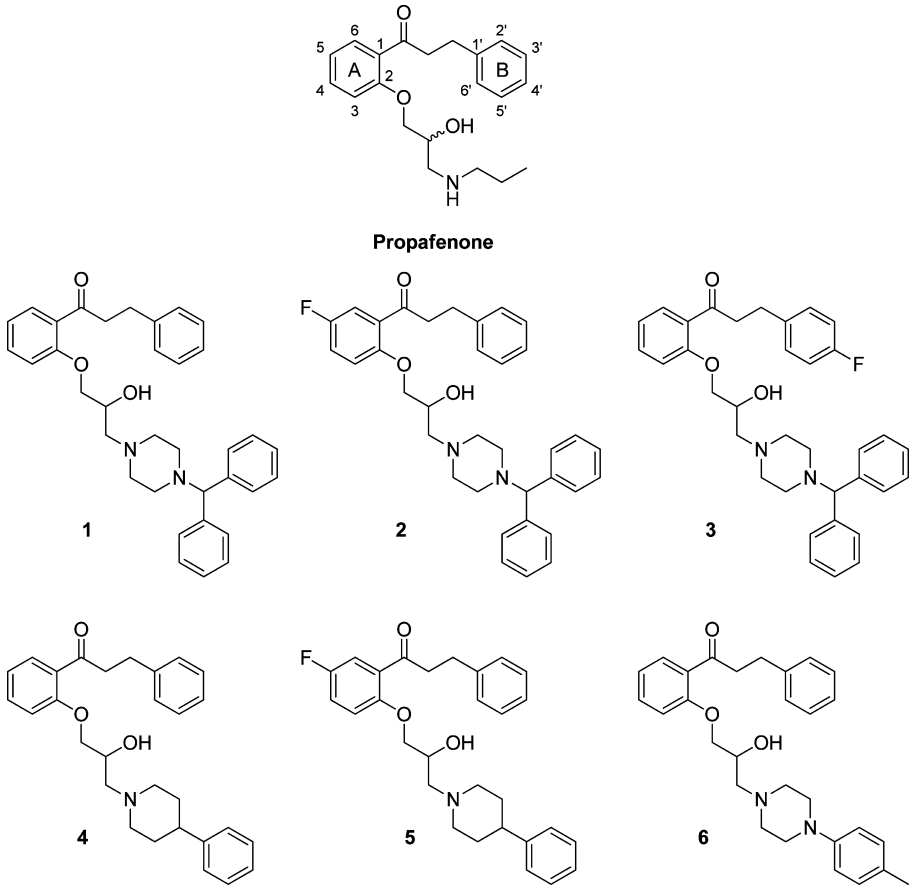


Figure 1. Propafenone and analogues chosen for further studies.

Table 1. In Vitro Potency and In Vitro Ion Channel Effects of the Tested Propafenone Analogues

					IC_{50} (μM)				
	3D7 EC_{50} (nM)	3D7 EC_{90} (nM)	K1 EC_{50} (nM)	K1 EC_{90} (nM)	hERG	Nav1.5 tonic	Nav1.5 phasic	Kir6.2	TI (hERG IC_{50}/K1 EC_{50})
1	60 ± 10	90 ± 20	100 ± 50	100 ± 10	2.6	N/A	N/A	N/A	26
2	240 ± 80	660 ± 70	50 ± 30	310 ± 50	4.84	>5	>5	>5	895
3	160 ± 20	450 ± 60	100 ± 40	210 ± 20	N/A	N/A	N/A	N/A	N/A
4	340 ± 90	750 ± 50	120 ± 40	240 ± 40	3.46	>5	>5	>5	65
5	1330 ± 880	2200 ± 600	260 ± 150	420 ± 50	N/A	N/A	N/A	N/A	N/A
6	390 ± 60	1300 ± 80	190 ± 90	640 ± 120	4.31	>5	>5	>5	23

RESULTS AND DISCUSSION

Chemistry. The synthesis of the targeted compounds (Figure 1, **1**–**6**) proceeded smoothly and in accordance with previous work.[10] The hydrogen transfer reductions, along with the microwave aminolysis of the epoxide rings, could be performed on up to a 1.5 g scale, in the Biotage Initiator 60. Removal of the palladium catalyst, post-reduction, was carried out using Celite filtration rather than through syringe filters, as previously reported. The final compounds were easily purified by recrystallization. The purity of all compounds was determined to be greater than 95% by UPLC/UV/ELSD/MS (Waters Affinity)[11] and the identity confirmed by NMR and MS.

As previously reported,[10] the diphenylmethyl-piperazine compounds **1**, **2**, and **3** were the most potent of the compounds, had the weakest activity in the ion channels targeted by the parent compound, and consistently proved to be more active against the K1 strain of *P. falciparum*. To allow for better pharmacological performance, the lower molecular weight, but less potent, compounds **4**, **5**, and **6** were tested as well.

ADME Studies: In Vitro. In vitro studies were aimed at identifying potential liabilities of the compounds including potential for drug–drug interactions, predicted bioavailability, and metabolism.

Drug–drug interactions (DDIs) are a serious concern with antimalarial drugs due to their use in areas where cotreatment of HIV is common.[12] Propafenone is extensively metabolized by CYP2D6[8] and therefore has potential for adverse drug–drug interactions with currently used malaria drugs that inhibit CYP2D6, such as chloroquine,[13] quinine, and quinidine,[14] along with HIV protease inhibitors such as indinavir, ritonavir, and saquinavir.[12] Additionally, the new propafenones may bind to other common cytochrome P450s (CYP). To provide a preliminary assessment of the potential for DDIs, an in vitro CYP inhibition assay was carried out. Compounds **1**–**6** were incubated with control CYP substrates (indicated in parentheses in column headings, see Supporting Information),

dx.doi.org/10.1021/jm300286a | *J. Med. Chem.* 2012, 55, 6087–6093

human liver microsomes, and cofactors for 20 min at 37 °C. Known CYP inhibitors were used as controls. Formation of metabolites was measured by LC-MS/MS and compared to control incubations (incubation of substrates with microsomes and cofactors, no test articles or inhibitors). None of the propafenones inhibited CYP1A2, CYP2B6, CYP2C9, CYP2C19, or CYP3A4 (see Supporting Information, Table 1). However compounds **4** and **5** were weak CYP2D6 inhibitors (Figure 2). Compounds **4** and **5** also showed some oxidation of the phenyl ring of phenyl-piperidine moiety that is consistent with interaction with CYP2D6 in the microsomal models.

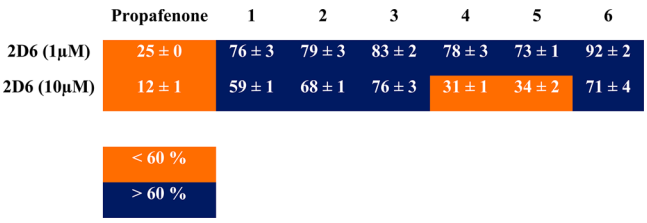


Figure 2. Heat map of CYP2D6 in presence of propafenones. Propafenone significantly inhibits CYP2D6 at both concentrations tested whereas the new propafenone analogues do not.

In earlier SAR studies, it was decided to incorporate fluorine into the propafenones at the points of metabolism of propafenone itself, which vary between mice and humans.[15,16] In humans, the primary metabolite of propafenone is 5-hydroxypropafenone, whereas in rodents it is 4′-hydroxypropafenone. Incorporation of the fluorine at the 4′- and 5-positions did not affect potency but generally led to decreased solubility relative to unsubstituted analogues (Table 2). Likewise, permeability was generally lowered for all of the analogues relative to propafenone. Thus the substitutions that allowed modulation of the cardiac ion channel activities of propafenone while retaining antimalarial potency introduced liabilities with respect to physiochemical properties that were exacerbated by fluorination.

Fluorination of the 5-position of the compounds slowed human microsomal metabolism for diphenylmethyl-piperazines **1** and **2**. However, fluorination of the 4′-position failed to slow metabolism of any analogue in the mouse microsome models. Overall, the mouse microsomes caused more oxidative cleavage at the β nitrogen of the piperazine ring whereas the human microsomes afforded more hydroxylation of the A ring. At this site, the enzymes could accommodate fluorination by moving the site of oxidation around the ring to "avoid" the fluorinated position if present.

Overall, the in vitro studies predicted that the new compounds would not have significantly worse potential for drug interactions or metabolism than propafenone itself. However, they also predicted that the bioavailability may be worse. Because the compounds were active in cellular models that required permeation through two membranes, it was decided to proceed with in vivo pharmacokinetic assessment even in light of modest to low permeability of the compounds.

ADMET: In Vivo. The in vivo pharmacokinetic behavior of all six compounds was established at oral doses of 20 and 200 mg/kg in female CD-1 mice (Table 3). Compounds **1**, **3**, and **4** were also tested at 500 mg/kg (see Supporting Information, Table 2). The C_{max} of methyl-diphenylpiperidine compounds rose approximately 10-fold when increasing dosing from 20 to 200 mg/kg. Interestingly, phenyl-piperidine compound **4** had a suprastoichiometric increase in C_{max}, almost 25-fold, with increasing the dose from 20 mg/kg and 200 mg/kg. One possible reason for this could be saturation of the metabolism mechanism. In contrast, phenyl-piperazine **5** showed only a minimal increase in C_{max} when increasing dosing from 20 mg to 200 mg/kg. None of the compounds tested at 500 mg/kg showed a significant increase in C_{max} compared to the 200 mg/kg dose, indicating that an absorption limit had been reached. Compound **4** also showed the highest value for AUC_{inf} at the higher concentrations, with approximately 5-fold improvement over compound **1** at 500 mg/kg. Overall, compounds **4** and **6** exhibited the best overall profiles.

Table 3. Pharmacokinetic Profile in Mice

test article	dose (mg/kg)	C_{max} (nM)	T_{max} (h)	AUC_{inf} (nM/ml)	V/F (L/kg)	Cl/F (mL/h/kg)
1	20	73.7	1	248.7	318.2	141107
	200	667	0.5	3103.1	602.9	113136
	500	597.7	1	3368.5	2020	260562
2	20	98.7	1	ND[a]	ND[a]	ND[a]
	200	907.5	1	6166.5	275.8	55189
3	20	92.4	0.5	298.1	369.6	114161
	200	1140.1	1	5665.8	287.6	60066
	500	860.6	0.5	4078.8	1758	208599
4	20	69.6	0.3	224.0	1093.3	186546
	200	1622.9	0.5	6799.7	277.5	61460
	500	1745.4	0.5	15332.9	623	68135
5	20	434.4	1	1596.1	223.8	25233
	200	480.7	1	3748.5	981.7	107444
6	20	301.8	8	ND[a]	ND[a]	ND[a]
	200	866.5	0.5	6486.8	271.8	60268

[a]ND: not determined due to limited number of time-points in the elimination phase.

Table 2. Calculated Physical Properties, Measured Permeability (PAMPA), and Measured Stability in Microsomal Models

compd	calcd Log $D7.4$[a]	Pe ($\times 10^{-6}$ cm/s)	soln (μM)	mouse microsomes $Cl_{int'}$ (mL/min/kg)	human microsomes $Cl_{int'}$ (mL/min/kg)
propafenone	2.5	1500 ± 360	70 ± 20	46 ± 5	4 ± 1
1	6.5	2.3 ± 1	0.1 ± 0	70 ± 10	14 ± 1
2	6.7	0.8 ± 0.1	0.2 ± 0.1	34 ± 3	5 ± 2
3	6.7	und[b]	0.6 ± 0.2	43 ± 3	13 ± 1
4	5.5	490 ± 70	7.3 ± 1	27 ± 2	11 ± 1
5	5.7	und[b]	0.2 ± 0.1	29 ± 2	19 ± 1
6	5.7	und[b]	2.2 ± 0.3	30 ± 6	38 ± 4

[a]calculated using pipeline pilot. [b]under the detectable limit.

dx.doi.org/10.1021/jm300286a | J. Med. Chem. 2012, 55, 6087−6093

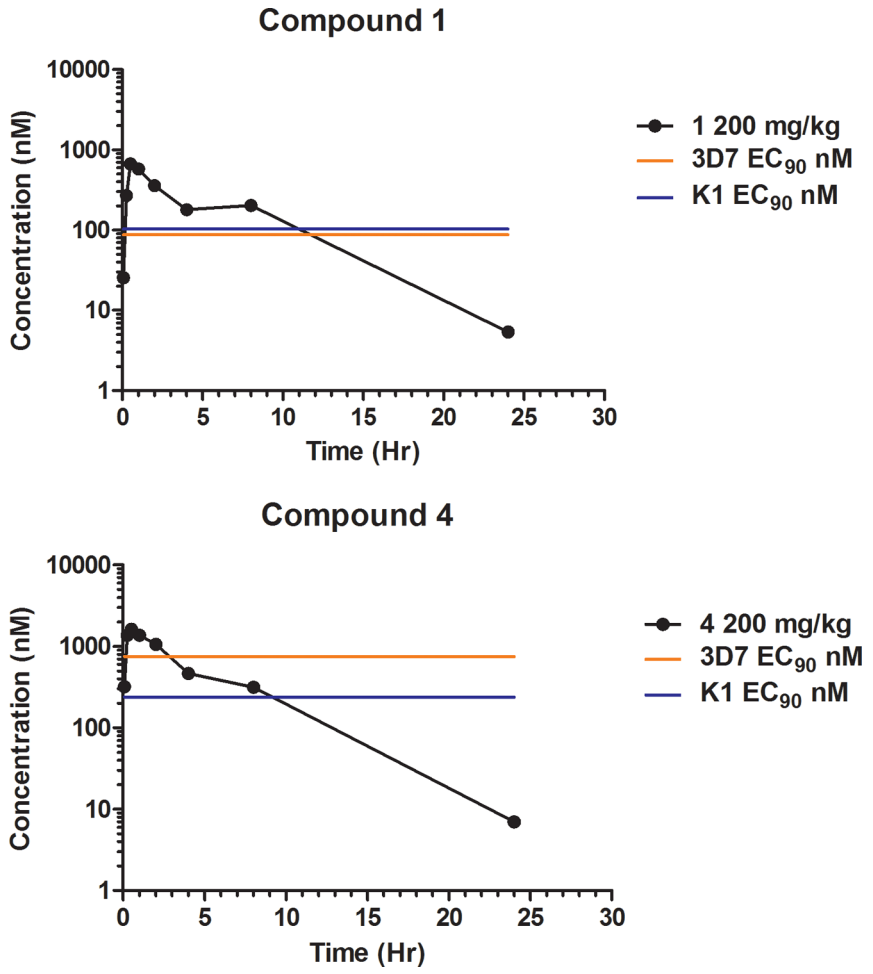


Figure 3. Plasma drug concentrations of compounds **1** and **4** vs time after oral dosing of 200 mg/kg. The in vitro EC_{90} efficacies against 3D7 and K1 *P. falciparum* are shown for reference.

In choosing which compounds to examine in efficacy models, we compared the plasma exposure of compounds (as a marker for the target tissue, erythrocytes) and the in vitro potencies against *P. falciparum*. Unfortunately, the species of malaria that infects rodents, *P. berghei*, cannot be cultured in vitro, so potency against this organism could not be used. In assessing the six compounds, the nonfluorinated methyldiphenylpiperazine **1** showed the greatest in vitro efficacy and reasonable PK parameters. The second compound chosen was piperidine compound **4**, which gave much improved PK parameters at the cost of lower potency (Figure 3). Compound **1** was expected to maintain plasma levels above the measured EC_{90} for *P. falciparum* for over 10 h after a single oral dose of 200 mg/kg, whereas compound **4** was expected to maintain appropriate levels for roughly 5 h. Therefore, efficacy experiments were justified with compounds **1** and **4** in order to determine if correlations could be made between in vitro activity and in vivo efficacy.

Toxicity Studies in Mice. Toxicity studies were carried out in female CD-1 mice on all six propafenone compounds to determine the maximum tolerated dose (MTD). This was done in parallel with the pharmacokinetics studies described above in order to establish dose−toxicity relationships. The mice were treated with a single oral dose of 10, 100, 200, and 500 mg/kg and observed for 48 h. No adverse clinical signs were observed in this time period at any of the dosage concentrations for any of the compounds. This finding can be explained by the relatively low C_{max} values and the poor dose linearity that were observed, together with the lack of cytotoxicity observed during in vitro testing.[8] Propafenone has an LD_{50} of 605 mg/kg in female mice when administered orally, so no toxic effects should be expected.[8] Therefore, the modifications made to propafenone did not manifest new patent toxicology and efficacy experiments could be justified with any of the lead compounds.

■ IN VIVO EFFICACY OF 1 AND 4

The studies summarized above provided justification for carrying efficacy modeling with two compounds (**1** and **4**) that should have sufficient exposure to lead to significant suppression of parasitemia and were nontoxic at doses expected to be efficacious. Propafenone analogues **1** and **4** were tested against *P. berghei* (NK-65) infected ICR mice using the modified Thompson's test[17] to establish oral efficacy (see Supporting Information, Table 3). Chloroquine was used as a control. Both compounds were tested at 100 and 200 mg/kg, administered orally on days 3, 4, and 5 after initial inoculation with parasites (Figure 4).

Dosing compound **1** orally at 100 mg/kg gave a mean survival rate of 7 days. One mouse died on day 6, four on day 7, and one on day 8, post inoculation. The mean parasitemia for the group of the 5 surviving mice was 53.6%. At 200 mg/kg, the

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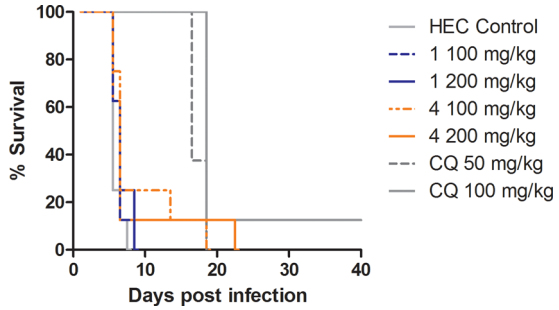

Figure 4. In Vivo Efficacy of compounds **1** and **4**.

mean survival rate was 7 days. One mouse died on day 6, four died on day 7, and two on day 9. The mean parasitemia for the four surviving mice was 55.3%. Comparatively at 50 mg/kg chloroquine showed a mean survival time of 17 days and, at 100 mg/kg, 22 days. Thus, the performance of compound **1** was not significantly better than that of the negative controls despite the fact that it had sufficient exposure to ensure that parasites should have been killed.

For compound **4** dosed at 100 mg/kg, the mean survival time was 9 days. Two mice died on day 6, four on day 7, and one each on days 13 and 18. The mean parasitemia on day 6 for the surviving mice was 33.3%. At 200 mg/kg, the mean survival time was 10 days. Three mice died on day 6, three on day 7, and one on day 22. The average parasitemia of the surviving mice on day 6 was 44.5%, and the lone surviving mouse had 28.4% parasitemia on day 13 and 50.4% on day 20. Thus, compound **4** had some efficacy in suppressing parasitemia and life extension but did not provide durable cures.

Although methyldipheny-piperazine compound **1** was more potent in vitro, piperidine compound **4** showed slightly improved in vivo efficacy, especially at higher doses. The slightly improved activity of **4** could be due to differing sensitivity of the *P. berghei* to the compounds relative to *P. falciparum* or the increased plasma concentration on initial administration as seen above. Overall, the efficacy and potency of the compounds agree qualitatively with the pharmacokinetic behaviors and potencies observed in earlier studies.

■ CONCLUSIONS

Evaluation of the compounds from the propafenone hit-to-lead campaign and comparison of the in vitro and in vivo properties has led to some insights in to how to proceed with this compound series. Although in vitro potency is acceptable, at a level comparable to early leads from other programs, there is poor in vivo efficacy. This is attributable mostly to the poor drug-like properties of the series that limit exposure. Use of the piperidine moiety appears to improve physical properties although it leads to modest CYP2D6 inhibition. Further exploration of both the lower part of the molecule and the linker between the A and B rings could lead to greater increases in potency and compounds with improved permeability and solubility while retaining the reduced ion channel activity already achieved. Clearly the most important challenge will be to raise exposure and/or increase potency while maintaining acceptable toxicology.

■ EXPERIMENTAL SECTION

Potency. *Growth of Parasites and IC_{50} Determinations.* The two *P. falciparum* strains were used in this study, 3D7 and K1, were provided by the MR4 Unit of the American Type Culture Collection (ATCC, Manassas, VA). Asynchronous parasites were maintained in culture based on the method of Trager.[18] Parasites were grown in presence of fresh group O-positive erythrocytes (Lifeblood Memphis, TN) in Petri dishes at a hematocrit of 4−6% in media consisting of 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, and 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 8807BC 384-well microtiter plate). Then 40 nL of each compound, previously serial diluted in a separate assay plate (Corning 3657 384-well white polypropylene plate), were dispensed in the assay plate followed by 20 μL of a synchronized culture suspension (2% rings, 10% hematocrit), thus giving a final hematocrit and parasitemia of 5% and 1%, respectively. Assay plates were incubated for 72 h, and the parasitemias were determined by a method previously described.[19] Briefly, 10 μL of the development solution (10X Sybr Green I, 0.5% v/v Triton, 0.5 mg/mL saponin, in RPMI) was added per well, assay plates were shaken for 30 s, incubated in the dark for 4 h, and then read with the Envision spectrophotomer at Ex/Em 485 nm/535 nm. EC_{50}s were calculated with the Robust Investigation of Screening Experiments (RISE) software.

Cytotoxicity. 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 assay plates and incubated overnight at 37 °C in a humidified incubator with atmosphere controlled at 5% CO_2 and 100% humidity. DMSO inhibitor stock solutions were added the following day to a final concentration of 25 μM, 0.25% DMSO, 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. Solubility assays were carried out on a Biomek FX lab automation workstation (Beckman Coulter, Inc., Fullerton, CA) using μSOL Evolution software (pION Inc., Woburn, MA). The detailed method is as follows: 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 and 4) to make the reference plate, and the UV spectrum (250 nm −500 nm) of the reference plate was read. Then 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., Woburn, MA). Finally, 75 μL of filtrate was mixed with 75 μL of 1-propanol to make the sample plate, and the UV spectrum of the sample plate was read. Calculation was carried out by μSOL Evolution software based on the AUC (area under curve) of UV spectrum of the sample plate and the reference plate. All compounds were tested in triplicate.

Permeability Assay. The parallel artificial membrane permeability assay (PAMPA) was conducted on a Biomek FX lab automation workstation (Beckman Coulter, Inc., Fullerton, CA) using the PAMPA Evolution software (pION Inc., Woburn, MA). The detailed method is described as follows: 3 μL of 10 μM test compound stock was mixed with 600 μL of SSB (system solution buffer, pH 7.4 or 4, pION Inc., Woburn, MA) to make diluted test compound. Then 150 μL of diluted test compound was transferred to a UV plate (pION Inc., Woburn, MA), and the UV spectrum was read as the reference plate. The membrane on preloaded PAMPA sandwich (pION Inc., Woburn, MA) was painted with 4 μL of GIT lipid (pION Inc., Woburn, MA). The acceptor chamber was then filled with 200 μL of ASB (acceptor solution buffer, pION Inc., Woburn, MA), and the donor chamber was filled with 180 μL of diluted test compound. The PAMPA sandwich was assembled, placed on the Gut-box, and stirred for 30 min. The aqueous boundary layer was set to 40 μm for stirring. The UV

dx.doi.org/10.1021/jm300286a | J. Med. Chem. 2012, 55, 6087−6093

spectrum (250−500 nm) of the donor and the acceptor were read. The permeability coefficient was calculated using PAMPA Evolution software (pION Inc., Woburn, MA) based on the AUC of the reference plate, the donor plate, and the acceptor plate. All compounds were tested in triplicate.

Liver Microsomal Stability. First, 0.633 mL of mouse liver microsomes (20 mg/mL, female CD9 mice, Fisher Scientific, NC9567486) or human liver microsomes (20 mg/mL, 200 pooled mixed gender, Fisher Scientific 50-722-552) was mixed with 0.051 mL of 0.5 M EDTA solution and 19.316 mL of potassium phosphate buffer (0.1M, pH 7.4, 37 °C) to make 20 mL of liver microsome solution. One part of 10 mM DMSO compound stock was mixed with 4 parts of acetonitrile to make 2 mM diluted compound stock in DMSO and acetonitrile. Then 29.1 μL of the diluted compound stock was added to 2.3 mL of liver microsomal solution and vortexed to make a microsomal solution with compound. Then 180 μL of the microsomal solutions with different compounds were dispensed into respective rows of a 96-well storage plate (pION Inc., MA, 110323). For 0 h time point, 450 μL of precooled (4 °C) internal standard (10 μM warfarin in methanol) was added to the first three columns before the reaction starts. Microsome assay solution A (1.25 mL, Fisher Scientific, NC9255727) was combined with 0.25 mL of solution B (Fisher Scientific, NC9016235) in 3.5 mL of potassium phosphate buffer (0.1 M, pH 7.4). Then 45 μL of this A + B solution was added to each well of the 96-well storage plate (reaction plate). Liquid in the first three columns was moved to another storage plate (quenched plate). The reaction plate was then sealed and incubated at 37 °C and shaken at a speed of 60 rpm. The solutions were sampled at 0.5, 1, and 2 h time points. At each time point, 450 μL of precooled internal standard was added to three rows in the reaction plate, and the liquid was then transferred to the quenched plate. The quenched plate was then centrifuged (model 5810R, Eppendorf, Westbury, NY) at 4000 rpm for 20 min. Then 200 μL of supernatant was transferred to a 96-well plate and analyzed by UPLC-MS (Waters Inc., Milford, MA). The compounds and internal standard were detected by SIR. The log peak area ratio (compound peak area/internal standard peak area) was plotted against time, and the slope was determined to calculate the elimination rate constant [k = (−2.303)slope]. The half-life (hour) was calculated as $t(1/2) = 0.693/k$.

CYP Inhibition. Test articles were incubated with model CYP substrates (indicated in parentheses in column headings), human liver microsomes, and cofactors for 20 min at 37 °C. Incubations containing known CYP inhibitors (listed in the first column) were also included. Formation of metabolites was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and compared to control incubations (incubation of substrates with microsomes and cofactors, no test articles or inhibitor).

In Vivo PK. Female CD-1 mice (Charles River Laboratories, Wilmington, MA) aged 8 weeks (25.0 ± 1.2 g) were housed in hanging polycarbonate cages. Animals were provided food and water ad libitum. The dose formulations were a suspension in 0.5% methylcellulose in sterile water and were administered by oral gavage (10 mL/kg). Blood was collected from three mice at each time point (5 min to 24 h), and two blood collections were made per mouse. Blood samples were mixed with K3EDTA anticoagulant, processed to plasma using standard methods, and stored frozen (−70 °C) until analysis. Protocols for all animal studies were approved by the Institutional Animal Care and Use Committee. The studies were in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health).

Sample Processing and Analysis. Plasma sample aliquots (50 μL) aliquots were transferred to microfuge tubes containing acetonitrile (200 μL) to precipitate the plasma proteins. These mixtures were vortexed for 10 min and centrifuged (18000g for 5 min). The resulting supernatants were transferred to new tubes, evaporated under vacuum, and reconstituted with 50 μL of 10 mM Na2EDTA in water containing 50 ng/mL of the internal standard for the assay (warfarin). The reconstituted samples were centrifuged (18000g for 5 min) and transferred to HPLC vials fitted with glass inserts for liquid chromatography−tandem mass spectrometry (LC-MS/MS) analysis.

Pharmacokinetics Analysis. The plasma pharmacokinetics of the test compounds **1**−**6** were determined using WinNonlin Professional (version 5.2) with noncompartmental analysis. The curve fitting of the terminal phase slope used uniform weighting and area under the concentration−time curve (AUC) was calculated using the linear up/log down trapezoidal method. Reported parameters were observed maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), AUC extrapolated to infinity (AUC_{inf}), clearance (Cl/F), and volume of distribution (V/F). Time points from the terminal elimination phase were selected manually for curve fitting analysis, and a minimum of three nonzero plasma concentrations after the T_{max} were required.

In Vivo Efficacy of 1 and 4. *Animals and Parasites.* All experiments were conducted in Swiss outbreed (ICR) female mice weighing 15−20 g, purchased form Harlan (North America). The animals were maintained in cages in an animal facility with alternative light and dark cycles in pellet food and tap water ad libitum. *Plasmodium berghei* (NK-65) were maintained in mice by serial passaging of infected blood from female donors until the experiment was initiated. Animal experiment protocols were approved by University of South Florida IACUC, and experiments were conducted in accordance to animal care policies.

In Vivo Efficacy Study and Treatment. The oral antimalarial efficacy of selected propafenone analogues **1** and **4** compounds were tested in *P. berghei* (NK-65) infected ICR mice by the Thompson's test.[17] Briefly, the experiment was initiated by inoculating 1×10^6 infected red blood cells from donor mice in plasma at day 0. Patent infection was confirmed by making tail vein blood smears on day 3 before dosing the test compounds. The animals were treated with single oral dose of either the test compound or reference drug chloroquine with a concentration ranging from 50 to 500 mg/kg on days 3, 4, and 5 post infection. The drugs were dissolved in 0.5% hydroxyethylcellulose (HEC), and the control mice received only the vehicle. The condition of the mice was monitored daily, and the survival was recorded. Parasitemia was monitored by preparing thin blood films on days 3 and 6 post inoculation and then at weekly intervals (days 13, 20, and 27) through day 30. Parasitemia was determined by microscopically observing at least 1000 Giema stained cells (magnification, ×1000).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Full CYP panel activity, pharmacokinetics table, and in vivo efficacy of compounds **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

WHO, World Health Organization; MMV, Medicines for Malaria Venture; HTS, high throughput screening; SAR, structure−activity relationship; hERG, human ether-à-go-go related gene; TI, therapeutic index; MTD, maximum tolerated dose; PK, pharmacokinetic; CYP, cytochrome P450; ADMET, absorption, distribution, metabolism, excretion, and toxicity; AUC, area under the curve; C_{max}, maximum plasma concentration; $t_{1/2}$, elimination half-life; t_{max}, time to maximum concentration

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