Assessment of the utility of alkylaminoquinolinyln-methanols as new antimalarial drugs

Dow GS\textsuperscript{1*}, Heady TN\textsuperscript{1}, Bhattacharjee AK\textsuperscript{1}, Caridha D\textsuperscript{1}, Gerena L\textsuperscript{1}, Gettayacamin M\textsuperscript{3}, Lanteri CA\textsuperscript{1}, Obaldia N \textsuperscript{III}\textsuperscript{1}, Roncal N\textsuperscript{1}, Shearer T\textsuperscript{1}, Smith PL\textsuperscript{1}, Tungtaeng A\textsuperscript{3}, Wolf L\textsuperscript{2}, Cabezas M\textsuperscript{2}, Yourick D\textsuperscript{2} and Smith, KS\textsuperscript{1}

1. Division of Experimental Therapeutics, Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910.

2. Division of Neuroscience, Walter Reed Army Institute of Research, 503 Robert Grant Ave, Silver Spring, MD 20910.

3. Department of Veterinary Medicine, United States Army Medical Component, Armed Forces Research Institute of Medical Sciences, 315/6, Rajthevi, Bangkok, Thailand 10400

4. Tropical Medicine Research/Gorgas Memorial Research Institute, Ave. Justo Arosemena # 3530, Panama City, Panama.

* Corresponding Author: Phone 1-310-319-9009, Fax 1-301-319-9954, Email geoffrey.dow@na.amedd.army.mil
ABSTRACT

Mefloquine has been one of the more valuable antimalarial drugs, but has never reached its full clinical potential due to concerns about its neurologic side effects, its greater expense relative to other antimalarials, and the emergence of resistance. The commercial development of mefloquine superseded that of another quinolinyl methanol, WR030090, which was used as an experimental antimalarial by the U.S. Army in the 1970s. We evaluated a series of related 2-phenyl substituted alkylaminoquinolinyl-methanols (AAQMs) for their potential as mefloquine replacement drugs, based on a series of appropriate in vitro and in vivo efficacy and toxicology screens and theoretical cost of goods. Generally, the AAQMs were less neurotoxic, exhibited greater antimalarial potency and are potentially cheaper than mefloquine, but showed poorer metabolic stability and pharmacokinetics and the potential for phototoxicity. These differences in physiochemical and biological properties are attributable to the ‘opening’ of the piperidine ring of the 4-position side chain. Modification of the most promising compound, WR069878, by substitution of an appropriate N functionality at the 4-position, optimization of quinoline ring substituents at the 6 and 7-positions, and deconjugation of quinoline and phenyl ring systems, is anticipated to yield a valuable new antimalarial drug.
INTRODUCTION

In the late 1960s to early 1970s, *Plasmodium falciparum* malaria in South East Asia had begun to develop resistance to all of the available antimalarial drugs (6). Cure rates were 11-20% and 26-50% for chloroquine and quinine, respectively, and had declined to only 90% for the triple combination of quinine/pyrimethamine/dapsone (6). All these regimens were associated with adverse side effects (6). As a consequence, the U.S. Army began routinely employing two experimental antimalarial drugs, WR030090 and WR033063, for the treatment of recrudescent malaria infections at the Walter Reed Army Medical Center (6). Subsequent field trials demonstrated that WR030090, a quinolinyl methanol, exhibited cure rates of at least 88% and was better tolerated than quinine (6, 21).

Shortly thereafter, mefloquine was discovered and was developed commercially by Hoffman La Roche and the U.S. Army. Mefloquine exhibited a long half-life in humans, and this desirable property facilitated its administration as a single dose for malaria treatment and as a once weekly dosing for prophylaxis (50). In contrast, WR030090 was only partially effective as a prophylactic agent, required a similar dosing regimen as quinine to effect cures, and was subsequently abandoned (6, 9, 21, 30). However, it is important to recognize that this occurred because of unfavorable pharmacokinetic characteristics, not as a consequence of unacceptable toxicity.

Mefloquine combined with artemisinate remains one of the most effective combination agents for treatment of malaria (41). Mefloquine is also the only once-weekly drug
approved for malaria chemoprophylaxis in the United States, that, barring the Thai border regions, is effective in almost all areas of the world. (7). However, mefloquine is relatively expensive compared to other antimalarials (8), which limits its accessibility to developing countries. Also, mefloquine use is associated with debilitating neurological effects in a small proportion of patients, and milder, but nevertheless concerning effects are also frequently observed (31, 40, 43, 47). These negative characteristics have limited the scope of the possible clinical utility of the drug.

In the present study, we report the antimalarial activity and pharmacological properties of a number of 2’ substituted alkyl amino quinoline methanols related to WR030090. Our goals were to (i) develop an understanding of structure activity relationships that might allow the synthesis of improved quinolinyl methanols and (ii) determine whether compounds related to WR030090 might prove to be more efficacious antimalarials.
MATERIALS AND METHODS

In vitro antimalarial activity of AAQMs. The in vitro activities of AAQMs against *P. falciparum* strains W2, D6, TM91C235 and TM90C2A were evaluated using the method of Desjardins et al. 1979 (12), as modified by Milhous et al. 1985 (32) and described in one of our earlier papers (16). W2 is chloroquine resistant and mefloquine sensitive, D6 is chloroquine sensitive but naturally less susceptible to mefloquine, TM91C235 is resistant to mefloquine, chloroquine and pyrimethamine as is TM90C2A, however this latter parasite is a two *pfmdr1* copy strain. We routinely run mefloquine in this screen as a control to ensure assay validity. Mefloquine has a mean IC50 +/- SD against *P. falciparum* TM91C235 of 15.7 +/- 2.7 ng/ml (last 15 assays).

Antimalarial activity of AAQMs in mice and monkeys. The *P. berghei*-mouse efficacy data were obtained from the WRAIR chemical information system (subcutaneous testing) or from recent tests conducted at AFRIMS, Thailand or at the University of Miami using a modified version of the Thompson test. Basically, groups of five mice were inoculated through i.p. injection on day 0 (usually with 1 x 10^6 *P. berghei*-parasitized erythrocytes). Drugs were administered either subcutaneously or orally for three days (usually on days 3-5) at doses of 1.25 – 160 mg/kg in two to four fold increments. Cure was defined as survival until day 60 (subcutaneous dosing) or day 31 post-treatment (oral dosing). Non-treated control mice usually die on day 6-10 post-infection. The minimum effective dose was the lowest dose level that cured at least one of five mice. The *Aotus* studies were performed as previously described (35, 36). Briefly, groups of two *Aotus lemurinus*

*lemurinus* of the karyotype VIII or IX (27) male and female monkeys with weights ranging from 742-970 g were inoculated with $5 \times 10^6$ parasites of either the FVO strain of *P. falciparum* or the AMRU1 strain of *P. vivax*. Both of these strains are chloroquine resistant. The course of infection with *P. falciparum* is usually lethal, whilst the AMRU1 *P. vivax* strain induces a potentially lethal thrombocytopenia if left untreated. Monkeys were examined and thick Giemsa-stained blood smears were prepared and enumerated daily (17) to monitor the course of infection. When parasitemias increased to greater than 5000 parasites per µl, monkeys were treated orally with the test drug. If drug treatment failed, *i.e.* parasitemia did not decline, or increased again to > 5000 parasites per µl, the monkeys were rescued with a single dose of orally administered mefloquine (20 mg/kg). In some instances, a high dose of the test drug was used to retreat monkeys instead of the mefloquine rescue. Monkeys are considered cured if they are parasite free 90 days post treatment. For the *P. falciparum* studies, the dose rate selected was 10 mg/kg/day x 7, since earlier studies suggested that WR030090, a compound that later proceeded to clinical studies, cleared but did not cure infections at this dose (WRAIR archival data). Cure at this dose for related analogs would therefore be a good indicator of their superiority over a compound that had already proceeded into clinical development. Plasma samples were taken from the monkeys in some studies for quantification of metabolites. Additional blood samples were taken as appropriate for complete blood counts and serum chemistry. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication, 1996 edition (34).
**Metabolic stability analysis and metabolite determination.** Mefloquine analogs (10 µM) were added to a mixture containing an NADPH-regenerating buffer (1.25 mM β-NADP⁺, 3.3 mM glucose-6-phosphate, and 3.3 mM MgCl₂) and 0.5 mg/ml pooled human liver microsomes to a final volume of 125 µl. The mixtures were incubated for 5 minutes at 37°C and the reactions were initiated by adding 25 µl glucose-6-phosphate dehydrogenase to a final concentration of 1 unit/ml. The reactions were maintained at 37°C until they were terminated by the addition of an equal volume of 100% ice cold acetonitrile at 0, 10, 30, 60, and 120 minutes. Samples were centrifuged to pellet the proteins and the supernatant was analyzed by LC-MS/MS in duplicate using fast LC gradient or isocratic methods. Parent drug was quantified with external calibration, using plots of parent drug response v. amount. Chromatograms were analyzed using the mass spectrometry software Xcalibur™ QuanBrowser (for ThermoFinnigan® instruments) or MassLynx™ (for Waters® instruments). Concentrations of parent drug remaining at each time point were calculated using the unknown peak areas and corresponding calibration curves. In order to calculate the half-life, a first-order rate of decay was assumed. A plot of the natural log (LN) of the drug concentration versus time was generated, where the slope of that line was -k. Half-life was calculated as 0.693/k. The positive control tested with each of these assays was nifedipine, which exhibited a mean half-life +/- SD of 31.7 +/- 5.3 min with human liver microsomes and 27.6 +/- 2.6 min with mouse liver microsomes (based on 5 assays). Mefloquine, which has been run >5 times in this assay, consistently exhibits a half-life of > 120 min in the presence of both human and mouse
microsomes. All reagents were purchased from Sigma except for the microsomes, which were obtained from BD Gentest™.

For metabolite identification, samples were prepared as described above with human liver microsomes. Additional samples were prepared with each drug using mouse, rat, and rhesus monkey liver microsomes. Samples were separated using an LC gradient method and analyzed by full scan LC-MS and LC-MS/MS. Parent compound and putative metabolites were all fragmented, and these MS/MS experiments were used in combination with the no-NADPH control experiments to confirm the assignment of peaks as metabolites. These MS/MS data were also used to do preliminary structural elucidation. Although dealkylated metabolites could be straightforwardly identified, the regioselectivity of other modifications, specifically the position(s) of hydroxylation, could not be definitively ascertained. The relative percentage of formation of each metabolite was determined in a semi-quantitative manner since standards of each metabolite were not available. Peak areas of each metabolite and the internal standard were determined, and their ratios were calculated as metabolite area/internal standard area. The percent formation of each metabolite was determined as the area ratio divided by the sum of all the metabolite area ratios.

**Cytochrome P450 inhibition assays.** Two assays were used to evaluate the drug-drug interaction potential of the compounds. An LC-MS-based assay was first used as a prescreen of all the compounds against each isozyme, then confirmation of significant inhibition was performed using the standard fluorometric assay. The LC-MS assay was
conducted in a 96-well format using a cocktail of four CYP substrates specifically metabolized by four of the CYP isozymes. A mixture of tolbutamine (106 µM), omeprazole (10.6 µM), bufuralol (10.6 µM) and midazolam (10.6 µM) was used to evaluate the activity of CYP2C9, CYP2C19, CYP2D6, and CYP3A4, respectively. The test compounds were incubated for five minutes at four different concentrations (single replicates) in the presence of the CYP substrate cocktail and 0.5 mg/ml human liver microsomes in NADPH-regenerating buffer. The reaction was initiated by the addition of 1 U/ml glucose-6-phosphate dehydrogenase (G6PD). The reactions were stopped after 25 minutes by the addition of an equal volume of 100% ice cold methanol containing a dextromethorphan internal standard (5 µM). The samples were then centrifuged and transferred to a new plate for analysis.

Quantification of the production of specific metabolites from each CYP substrate was performed using LC-MS. Known inhibitors for each isoenzyme (quinidine, sulfaphenazole, tranylcypromine and ketoconazole) were used as positive controls for the inhibition of specific enzymatic activity. The inhibition obtained from the test drug was expressed as a percentage of the controls without inhibitors. In general, inhibition of enzymatic activity seen at test drug concentrations of >50 µM were not considered to be significant enough for further evaluation. Values between 10 and 50 µM were considered to be moderate inhibition and lower than 10 µM were significant.

Confirmation of CYP2D6 inhibition by phenylquinolinyl methanols was done using a modified version of the standard fluorometric assay recommended by the supplier (BD
Gentest™). Briefly, the assay used cDNA-expressed CYP2D6 isoenzyme at 15 pmol/ml protein and 3-[2-(N,N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AAMC) as the 2D6-specific fluorescent substrate. The reaction mixtures were placed in a 96-well plate with both a quinidine control and a range of concentrations of the test compound. The plate was incubated for 30 minutes at 37ºC and the production of 3-[2-(N,N-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin hydrochloride (AHMC), the fluorescent metabolite, was quantified using a fluorescence plate reader. IC$_{50}$ values were then calculated as described using the two concentrations that bracket 50% inhibition. IC$_{50}$ values for the control, quinidine, were in each case found to be within the accepted range of 0.001 - 0.05 µM as specified by the manufacturer.

**Neurocytotoxicity, neuroprotection assays and confocal microscopy.** The neurocytotoxicity assay of AAQMs was conducted as previously described (16). This assay utilizes primary rat forebrain neurons and is a multi-endpoint screen. In this system a large component of the neurotoxicity of mefloquine can be attributed to the disruption of calcium homeostasis via discharge of the endoplasmic reticulum calcium store and activation of ill-defined plasma membrane calcium channels (14, 15). Mefloquine may also induce other, uncharacterized effects in these cells. Compounds were screened at 10, 100 and 1000 µM in triplicate, and the reduction in viability observed was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described (16). Approximate IC$_{50}$ values were calculated on the basis of the level of inhibition observed at each concentration. Full dose response assays are run for compounds of interest (e.g. WR069878) with two fold dilutions (n=6 wells per dilution).
of each drug. IC50s were calculated using Prism. Each time the assay is conducted, we routinely include 20 µM mefloquine as a control. This concentration of mefloquine reduces cellular viability by a mean +/- SD of 52 +/- 4.4%. Assays are re-run if the loss of viability induced by mefloquine is < 40% or > 60%.

Neuroprotection experiments with 6,7-dinitroquinoxaline-2,3-dione (DNQX) and magnesium were performed as described previously (14). Basically, neurons were exposed to DNQX (100 µM) or magnesium (12 mM) for 5 min, followed by mefloquine (25 µM) or WR069878 (250 µM) for 20 min after which reduction in cell viability was determined. Neither DNQX nor magnesium altered cellular viability alone. Each combination of treatments was tested in quadruplicate on two occasions and similar trends were observed each time. DNQX is an inhibitor of non-N-methyl-D-aspartate (NMDA) receptors, whilst magnesium inhibits the functioning of the inositol 1,4,5-trisphosphate (IP3)-mediated calcium signaling pathway at several points.

The effects of mefloquine and WR069878 on neuronal calcium homeostasis were assessed utilizing confocal microscopy as described elsewhere (16). Briefly neurons were loaded with calcium sensitive dye Fluo 3-A for 1 h and were washed prior to image experiments. Neurons were ‘spiked’ with 100 µM mefloquine or WR069878. Subsequent changes in neuronal calcium homeostasis were recorded as fluctuations in the emitted fluorescence of fluo-3-complexed calcium at 530 nm (excitation was 488 nm). Sequential image scans of fields containing 5-25 neurons were used to construct temporal profiles. Scans were made at 10 s intervals. Fluorescence levels for each neuron were normalized.
to time zero values. Data were then pooled from three and five independent experiments for mefloquine and WR069878 respectively.

**Phototoxicity pharmacophore and 3T3 neutral red uptake phototoxicity test.** We attempted to develop a pharmacophore for phototoxicity to be used as an *in silico* screening tool. An earlier study reported the minimum phototoxic concentration for a range of different quinolinyl methanols in a yeast assay system (22). These data were used to generate a three-dimensional pharmacophore model using the HypoGen algorithm of the CATALYST® (Accelrys software Inc.) methodology as previously described (2, 16). The structures of clinically used quinoline methanols were mapped onto the pharmacophores and estimated values for minimum phototoxic concentration were generated.

The 3T3 neutral red uptake (NRU) phototoxicity test, conducted by MB Research Laboratories (Spinnerstown, PA), was used to identify quinoline methanols that have the potential to exert *in vivo* phototoxicity after systemic application. Briefly, the central 60 wells of two 96-well plates per test compound were seeded with Balb/c 3T3 mouse fibroblast cells and maintained in culture for 24 hrs. These plates were then pre-incubated with a range of eight different concentrations of test compound (six wells per concentration) for one hour. Next, one plate was treated with a UVA dose of 5 J/cm² by irradiating for 50 min at 1.7 mW/cm², whereas the other plate remained non-treated and in the dark. Next, the treatment medium was replaced with culture medium and cell viability was determined after 24 hrs by measuring neutral red uptake for 3 hrs. Finally,
the 3T3 NRU Phototox Prediction Software (version 2.0, ZEBET) was used to calculate EC$_{50}$ values and Photo-Irritant Factors (PIF) for each compound. Compounds showing potential for phototoxicity have a PIF > 5.0.

**In silico toxicity screening.** We utilized TOPKAT® (Accelrys software Inc., see TOPKAT User Guide 6.1; Accelrys, Inc., San Diego, CA, 2003) for assessment of the 2-phenyl substituted AAQMs for their potential toxicity to human health and surrounding ecosystem by screening through thirty different models that are currently available in the software. Data are presented as the total number of tests passed by each compound (Table 1). This software package allows a rapid assessment of potential toxicity of chemicals solely from 2D molecular structures. The qualitative models in TOPKAT® provide dichotomous output (yes/no) for rodent carcinogenicity, Ames mutagenicity, developmental toxicity, skin sensitization, skin irritancy, ocular irritation, and aerobic biodegradability. The quantitative models provide point estimates for the lowest observed adverse effect level (LOAEL), oral rat lethal dose (LD$_{50}$), lethal concentration (LC$_{50}$), maximum tolerated dose (MTD), and octanol-water partition coefficient (VlogP) along with 95% confidence limits for each. Within a class of chemicals, a relatively high number of failed tests may be indicative of potential toxicity.

**Physiochemical Properties.** Important physiochemical properties including LogP, LogD, predicted solubility, polar surface area, pKA, number of H donors and acceptors, etc. were calculated using Advanced Chemistry Development LogD Sol Suite.
Cost and ease of synthesis analysis. The bench-scale cost analysis for mefloquine presented here is based on a 1971 synthesis (37). The syntheses of WR069878 and WR030090 in Figure 5 are hypothetical. Each synthetic step in these syntheses is strongly supported in the literature, and we have included the immediately relevant literature citations for each step. The isatin starting material for WR069878 was not commercially available at the time of this publication. The cost analysis for WR069878 incorporates synthesis of the starting 3-chloro-4-methyl isatin. Yields for the hypothetical syntheses are averages from the literature values. The pricing of the bench-scale mefloquine synthesis and WR069878 and WR030090 was done using current reagent pricing of commonly used laboratory quantities, not bulk reagent quantities (Aldrich catalog 2000-2001). As in the mefloquine synthesis, condensation of the appropriate isatin and ketone should afford the quinoline core (37). Conversion of the quinoline acid to the acid chloride could be accomplished with either thionyl chloride or oxalyl chloride (25). Conversion of the acid halide to the \(\alpha\)-halo ketone could be accomplished through the diazoketone (1, 19, 25, 26). Treatment of the \(\alpha\)-halo ketone with sodium borohydride should afford the epoxide (3, 29). Lastly, the epoxide could be opened with dibutyl amine to give an enantiomeric secondary alcohol (4, 18, 33).
RESULTS

In vitro antimalarial activity of AAQMs. A sub-structure search of the WRAIR chemical library identified 21 compounds structurally related to WR030090. These compounds were subjected to batteries of in vitro and in vivo tests to assess their potential utility as antimalarials. Many of the AAQMs exhibited much greater antimalarial activity and decreased neurotoxicity in vitro relative to mefloquine (Table 1). These characteristics appear to be correlated with ‘opening’ of the piperidine ring of mefloquine since ‘open-chain’ N,N-dialkylaminoquinolines display a superior therapeutic index compared to 4-quinoline carbinolamines that contain phenyl substituents at the 2-position (Table 2). In general, those alkylaminoquinoline compounds showing relatively potent in vitro antimalarial activity and neurotoxicity contained either short alkyl amino chains (e.g. WR041294 vs WR029252, Table 1) or had one chain removed (e.g. WR007524 vs WR029252, Table 1). The in vitro efficacy of AAQMs tracked with mefloquine, since in most cases IC\textsubscript{50}s were in the rank order for \textit{P. falciparum} strains TM90C2A > TM91C235 > D6 > W2 (data not shown). The most promising compound, WR069878, exhibited IC\textsubscript{90}s against TM90C2A, TM91C235, D6 and W2 of 16, 11.7, 5.3 and 0.49 ng/ml, respectively. Comparable values for mefloquine were 101, 89, 20 and 3.9 ng/ml.

Relative metabolic stability of AAQMs. Many of the AAQMs tested were less metabolically stable than mefloquine in vitro (Table 1). Singly alkylated analogs (secondary amines) showed metabolic stability similar to that associated with mefloquine (WR176399 and WR007524, Table 1). In contrast, many of the dialkylated analogs were
less metabolically stable (Table 1). The N,N-dialkyl analogs were metabolized in all species primarily (> 90%) by N-dealkylation (26), yielding the corresponding secondary amine metabolite, as in WR069878 (Figure 2). Hydroxylation was a minor but secondary route of metabolic transformation. Singly alkylated analogs were metabolized to a much lesser extent via hydroxylation (WR176399), and/or N-dealkylation (WR041294), or not at all (WR007524). Metabolism was similar across all the species tested (rat, human, mouse, and rhesus). In cytochrome P450 inhibition assays, many of the analogs were more potent inhibitors of cytochrome CYP2D6 relative to inhibition caused by mefloquine (IC$_{50}$ of 18.6 µM), and one compound (WR069878) showed inhibitory activity equivalent to mefloquine (Table 1).

**In vivo antimalarial activity of AAQMs.** In terms of *in vivo* activity, almost all of the AAQMs exhibited some curative effects in the *P. berghei*-mouse model when administered subcutaneously (Table 1). With the exception of WR030090 and WR211679 (which had higher IC$_{50}$ values), all AAQMs with an IC$_{50}$ < 10 ng/mL were screened orally in the mouse. With the exception of WR177973, all were substantially more effective by the oral route, with minimum curative doses ranging from < 1.25-10 mg/kg/day for 3 days for most analogs (Table 1), although one must consider the fact that for the oral dosing experiment, survival was assessed at day 31 instead of day 60. The least neurotoxic of these were then tested for efficacy against *P. falciparum* in *Aotus*. Cures were observed with WR069878 and WR035058 (Table 1). WR074086 and WR176399 cleared parasitemias, but recrudescence was subsequently observed (Table 1). Clinical failure was associated with high *in vitro* IC$_{90}$ (≥20 ng/ml) against *P.*
falciparum TM91C235 and/or relatively low plasma concentrations (Table 3). No monkeys became ill, died, or showed signs of toxicity after treatment with the analogs. None of the observed changes in complete blood counts, serum chemistry, or body weight were attributable to toxicity. Adverse changes in hematocrit, hemoglobin, platelet, white blood cell and reticulocyte counts were observed with malaria infection, but resolved upon treatment with either mefloquine (rescue) or the AAQMs.

**AAQM plasma concentrations in monkeys.** Overall, plasma concentrations of the six AAQMs in Aotus monkeys were relatively low (mean peak concentrations of 28-413 ng/ml with a 10 mg/kg/day oral dose x 7, Table 3) compared to what one would usually observe after a single mefloquine treatment dose (peak concentrations of 800 – 8000 ng/ml, (49)). Lower than expected plasma concentrations for AAQMs may be due to a combination of several contributing factors. Firstly, some of the AAQMs tested were less metabolically stable than mefloquine, which could potentially result in lower overall plasma concentrations of the parent compound (Table 1). Secondly, most of the AAQMs were several orders of magnitude less soluble than mefloquine (Table 1). It is likely that the higher solubility of mefloquine is attributable to the more polar secondary amine of the piperidine ring, whereas most of the other AAQMs contained more aliphatic tertiary amines. Finally, the predicted Log P values for most of the AAQMs were substantially higher than mefloquine, and furthermore, substantially greater than that normally observed for commercially available drugs (Table 1, (24). Indeed, poor solubility and permeability are the two leading causes for poor drug absorption (24).
**WR069878 selected for further evaluation in monkeys.** WR069878 was chosen for further *Aotus* studies since, of the two curative analogs, it exhibited the least potent inhibitory effects against CYP2D6 (Table 1). Single doses at 20 or 40 mg/kg of WR069878 were not uniformly curative against *P. vivax* (Table 4). In contrast, 40 mg/kg for three days cured both monkeys. At lower dose rates, a seven day treatment regimen is apparently required, since 10 mg/kg/day for three days did not cure *P. vivax* infections, but 10 mg/kg/day for seven days did cure *P. falciparum* infections. No monkeys became ill, died, or showed signs of toxicity after treatment with WR069878. Plasma concentrations of all the analogs peaked within two hours of dosing, and declined to < 80% of their peak value by 24 hr post treatment. Collectively, these observations suggest that AAQMs require daily administration to induce cures. The approximate dose of WR069878 that would be required to treat a human *Plasmodium* infection would most likely be 10 mg/kg/day for three days (assuming a scaling factor of approximately 4 based on surface area, (48).

**WR069878 is less neurotoxic than mefloquine.** We further investigated the neurological effects of WR069878 in our test system. WR069878 was clearly less neurotoxic than mefloquine (Table 1 & Figure 3). This effect does not result from a difference in solubility, since both drugs dissolved without evidence of precipitation across the concentration range tested. The mechanism of neurotoxicity of mefloquine also appears to be different, since WR069878 did not disrupt calcium homeostasis to the same extent as mefloquine (Figure 3). In addition, the neurotoxicity of WR069878 was not
inhibited by DNQX and supra-physiological magnesium, agents shown to partially protect neurons from mefloquine-induced neurotoxicity (Figure 3).

**Assessment of phototoxicity potential of AAQMs.** Phototoxicity may be associated with quinolinyl methanols that are phenyl substituted at the 2-position (44). We evaluated two approaches for assessing the possible phototoxicity of our most promising compound, WR069878. First, we generated a pharmacophore for phototoxicity based on published data for a yeast model (22). Features required for phototoxicity include a hydrogen bond acceptor and an aliphatic hydrophobic and two aromatic hydrophobic functionalities (Figure 4). The structures of three quinoline methanols were mapped to the pharmacophore. WR030090 and WR007930 both mapped all the features of the pharmacophore with estimated minimum phototoxic concentrations of 110 and 170 mg/ml, respectively (Figure 4, Table 6). WR007930 is historically associated with phototoxicity in Phase I clinical trials, whereas, WR030090 progressed to Phase II clinical trials without phototoxicity being deemed a significant concern (30, 42). Mefloquine mapped all the features of the pharmacophore, with the exception of an aromatic hydrophobic functionality associated with the phenyl ring at the 2-position, with an estimated minimum phototoxic concentration of 5600 mg/ml (Table 6). In addition to the pharmacophore assessment, these quinoline methanols were also tested in the 3T3 Neutral Red Uptake (NRU) Phototoxicity Test. In this test, compounds with a resultant Photo-Irritant Factor (PIF) value greater than 5.0 are likely to be phototoxic when administered systemically. The results of the 3T3 NRU assay indicate that WR007930 and WR030090 have the potential to exert phototoxicity (PIF= 29.0 and 105.7,
respectively), whereas mefloquine scored a PIF of 1.76, and therefore, as expected, is not considered phototoxic (Table 6). Thus, the pharmacophore, and the 3T3 NRU test, and other models outlined in Table 6 are all able to predict the absence of phototoxicity, but cannot grade the degree of potential phototoxicity. WR069878 exhibited a PIF of 107.7 in the 3T3 assay and a minimum phototoxic concentration of 1200 mg/ml in the pharmacophore model, suggesting the potential for phototoxicity.

**Synthesis scheme for WR069878 and WR030090.** We developed a synthetic scheme for WR069878 and WR030090 modeled after one published for mefloquine (Figure 5). Based on a bench-scale synthesis, the two dialkylaminoquinolinyl methanols were much less costly to produce than mefloquine (Table 5). The cost of a treatment course of each drug can be extrapolated based on the known or estimated effective dose. Using this scheme, the cost of treatment for WR069878 or a similar drug would likely be substantially lower than mefloquine. This analysis has two caveats. First, difference in cost of production between bench and industrial scale was assumed to be equivalent to mefloquine. Second, the synthesis of WR069878 is complicated by the lack of a commercially available source of starting material (Figure 5). Furthermore, “from-scratch” synthesis of this material would result in the synthesis of two isomers, the resolution of which could potentially be complicated for an industrial scale synthesis.
DISCUSSION

We have identified WR069878 as the most promising molecule from a group of structurally similar compounds. WR069878 was shown to be orally active without toxicity against *P. berghei* in mice and chloroquine-resistant *P. falciparum* and *P. vivax* in monkeys. The compound is also an order of magnitude more potent than mefloquine against mefloquine-resistant strains of *P. falciparum in vitro*. We have also shown that WR069878 is intrinsically less neurotoxic than mefloquine, and appears to exhibit a different mechanism of neurotoxicity *in vitro*. Furthermore, the cost of treatment with WR069878 (and a related quinoline, WR030090) has been estimated to be much lower than mefloquine. These favorable characteristics argue for further development of the compound. However, the potential phototoxicity of the molecule, its relative lack of metabolic stability, and the lack of commercially available starting materials suggest a more cautious approach. In the discussion that follows, we outline the structural basis for each of these characteristics and suggest modification to the molecular scaffold that is expected to yield a new antimalarial agent. Finally we discuss the likely clinical use of such a drug given the concern about potential cross-resistance to mefloquine.

Several lines of evidence suggest that the neurotoxicity of mefloquine, as assessed in the present study, appears to be associated with the piperidine ring. We showed in an earlier study (16) that 4-quinolinecarbinolamines, which possess the piperidine functionality at the 4-position, exhibit calcium-dependent neurotoxicity at a similar level of potency to mefloquine. In the present study, we show that ‘opening’ of the piperidine ring correlates
with lower neurotoxic potency. Furthermore, the reduced neurotoxicity of both WR069878 (this study) and chloroquine (14, 16), is associated with a failure to disrupt calcium homeostasis in the same manner as mefloquine. It is likely that this screen has \textit{in vivo} relevance, since mefloquine induces neuronal degeneration consistent with a necrotic effect in rats (13). Of course, it remains to be determined whether this physiological effect caused by mefloquine is solely responsible for its neuropathological effects \textit{in vivo}. Indeed, mefloquine accumulates in the central nervous system and has been shown to interact with numerous cellular targets within a concentration range that one could reasonably consider pharmacological (10, 15, 20, 23, 28, 39, 51). Furthermore, it is interesting to note that others have reported that the substitution of the piperidine ring with a pyridine ring also reduces the potency of mefloquine against at least one of these other potential neurological targets (28).

Replacement of the piperidine ring with 2 non-cyclic alkyl side chains to form a tertiary amine renders these compounds more metabolically labile and susceptible to \textit{N}-dealkylation. Moreover, the nitrogen side chain appears to be less vulnerable to \textit{N}-dealkylation if the chains are shortened or if only a single chain is present (a secondary amine). This modification, interestingly, also appears to make the compounds more biologically active. Therefore, it seems possible to modify the SAR of the \textit{N}-alkyl side chain, such that the ideal balance between metabolic stability, biological activity, and cost of goods is obtained. One approach to achieve this goal might be to include an \textit{N}-functionality in a non-piperidine ring system, either in the form of a pyridine ring (shown by Maertens et al. 2000 to be less toxic \textit{in vitro} to anion channels), a quinuclidine ring


quinine is not neurotoxic in our test system, (16) or a heterocyclic ring of smaller, different size. Indeed, a recent study reported the synthesis of a non-neuroactive molecule in which the piperidine ring of mefloquine was replaced with a quinuclidine ring (46), although the veracity of this claim is difficult to judge given that mefloquine was not included as a positive control in these neurological studies.

Phototoxicity is potentially a problem with this class of drugs (42). However, for various reasons there are no useful data available to determine whether WR069878 or analogs would be likely to induce clinically important phototoxicity. First, the one study that reported in vivo phototoxicity (in mice) with WR069878 did not specify the route of administration beyond the fact that the compound was ‘injected’ (22). Second, quinolinyl methanols are not uniformly phototoxic in the clinical setting (Table 6). Third, amongst the various test systems for phototoxicity, there are no comparable data for all the quinolinyl methanols (Table 6). We therefore attempted to resolve this issue by developing a phototoxicity pharmacophore and utilizing the 3T3 NRU assay to rank the three compounds with which there is clinical experience. If successful, this approach may have had utility as an in silico screen for phototoxicity.

Pharmacophore mapping correctly identified mefloquine as a non phototoxic drug, however WR030090 and WR007930 were indistinguishable (Table 6). This mapping also indicated that the feature which did not map to mefloquine was the aromatic hydrophobic functionality associated with the phenyl group at the 2-position. The 3T3 assay was also able to clearly distinguish the lack of phototoxicity of mefloquine but did not rank
WR007930 and WR030090 correctly. Thus, the pharmacophore, 3T3 assay and other screens listed in Table 6 appear to be capable of ruling out phototoxicity, but not ranking related molecules in terms of the degree to which they could induce phototoxicity. The results of both of these screens suggest the potential for phototoxicity with WR069878. However, it may be possible to engineer out the phototoxicity of WR069878, since it is thought that the phototoxicity of quinoline methanols is due to extensive π-conjugation of the quinoline and phenyl ring systems (44). With mefloquine, this was avoided by addition of a trifluromethyl group at the 2-position. However, addition of a phenyl ring system improves intrinsic antimalarial activity. Future studies should be directed towards investigating the SAR balance between phototoxicity and antimalarial activity at the 2-position. The phototoxicity pharmacophore could then be used as an in silico screening tool to rule out phototoxicity prior to synthesis of new analogs.

Cost of goods is an important factor to consider when evaluating new antimalarial drugs, given that the disease is prevalent in developing countries. We have estimated that WR069878 and related compounds are likely to be less expensive to produce than mefloquine based on comparing the respective bench-scale syntheses for these compounds. Using this method, we determined that the starting materials needed for WR069878 are generally less expensive and also feature more reliable and higher yielding chemical steps. Furthermore, we anticipate that commercial process chemistry using more cost-effective bulk reagents will further improve the cost-basis of the AAQMs versus mefloquine.
The hypothetical synthesis of WR069878 and related compounds, whilst intrinsically cheaper than mefloquine at a bench-scale, is likely to be complicated by the lack of a commercially available starting material needed for large scale synthesis. This is because the synthesis of the appropriate starting material as shown would involve separation of isomers on a large scale (Figure 5). One approach to resolving this issue would be the strategic placement of blocking groups that could be easily removed on a large scale. For example, installing a methoxy functional group that would block cyclization at the undesired position and could be removed, for example with boron tribromide (BBr₃), should yield only the desired isomer. Alternatively, this issue might be avoided by introduction of different ring substituents at the 6, 7 and 8-positions. The present study did not, however, attempt to address the effect that such substitutions may have on intrinsic antimalarial activity, neurotoxicity, or metabolic stability.

Clinical resistance to mefloquine is mediated for the most part by amplified copy number of the gene \textit{pfmdr1} (41). \textit{Pfmdr1} encodes PgH-1, a malaria drug efflux protein homologous to mammalian P-glycoprotin (PgP). It has been suggested that this protein is linked mechanistically to mefloquine resistance, since it acts to detoxify the malaria parasite by extrusion of the drug into the food vacuole (45). However, clinical resistance to mefloquine may also occur in the absence of \textit{pfmdr1} amplification in some cases (41), and some parasites of West African origin exhibit a naturally lower susceptibility to mefloquine. The \textit{P. falciparum} strains TM90C2A, TM91C235 and D6 used here are representative of these scenarios. In an earlier study we observed that the susceptibility of different \textit{Plasmodium} strains to a series of 4-quinolinecarbinolamines tracks with that of
mefloquine (16). A similar observation was made in the present study with AAQMs. It is worth briefly considering the clinical implications of this observation.

*In vitro* susceptibility assays do not in themselves predict clinical efficacy, since resistance occurs when the MIC is not exceeded by plasma drug concentrations for a sufficient period of time. Therefore, AAQMs which are more potent than mefloquine should exhibit greater *in vivo* efficacy assuming similar pharmacokinetics, regardless of *in vitro* cross-susceptibility of parasites to mefloquine and AAQMs. However the *in vitro* data do imply that deployment of AAQMS as monotherapy for treatment of malaria may eventually result in reduced clinical utility through similar mechanisms as mefloquine. In order to avoid this, it would be necessary to appropriately combine AAQMS appropriately with other drugs. In South-East Asia, where mefloquine resistance is most prevalent, mefloquine remains effective in combination with artesunate (11, 38, 52) despite ten years of continuous use and a high background of *pfmdr1*-mediated resistance to mefloquine monotherapy (41). Furthermore, there is some evidence that the background of mefloquine resistance has declined (5). There is therefore every reason to suspect that a future WR069878 analog would be effective as a malaria treatment agent if used in combination with other antimalarials.

WR030090, one of the alkylaminoquinoline methanols investigated here, progressed into Phase II trials and was used to successfully treat emerging multi drug-resistant malaria in South East Asia during the Vietnam War (6). However, it was eventually abandoned in favor of mefloquine, not because of toxicity issues, but as a consequence of the
apparently superior pharmacokinetics of mefloquine. Many of the compounds investigated in the present study are superior to WR030090 in terms of oral activity and pharmacokinetics, and are superior to mefloquine in terms of neurotoxicity, intrinsic potency, and cost of goods. Structural improvement of the lead, WR069878, along the lines suggested, is likely to result in a valuable new antimalarial agent.
ACKNOWLEDGEMENTS

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We acknowledge the contribution made by the University of Miami, in the form of the screening of various AAQMs against *P. berghei* in mice. The authors thank William Otero and Jorge Aparicio for performing the microscopic analysis of blood films generated during the *Aotus* studies; Camilo Marin and the animal caretakers for supervision and animal husbandry at Gorgas Memorial Institute Aotus facility in Panama City, Panama; and Ms. Maritza Brewer and Gladyz Calviño for secretarial and administrative support. The authors also acknowledge the support of Laurie Brown, Sandra Simpson and Denise Anderson at WRAIR for secretarial and administrative support.
Table 1: See attached.
Table 2: Alkylaminoquinoline methanols exhibit a greater therapeutic index than 4-quinoline carbinolamines.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mefloquine</th>
<th>AAQMs</th>
<th>4QCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median IC50 against <em>P. falciparum</em> TM91C235 (nM)</td>
<td>35</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>Median IC50 against rat neurons (µM)</td>
<td>20</td>
<td>600</td>
<td>33</td>
</tr>
<tr>
<td>Therapeutic index (*1000)</td>
<td>0.57</td>
<td>35</td>
<td>1.3</td>
</tr>
<tr>
<td>Therapeutic index relative to mefloquine</td>
<td>1.0</td>
<td>61</td>
<td>2.3</td>
</tr>
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</table>
Table 3: Relationship between efficacy of selected AAQMs in *Aotus*, *in vitro* antimalarial activity, and plasma concentrations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Outcome in <em>Aotus</em></th>
<th>TM91C235 IC90 (ng/ml)</th>
<th>Plasma conc. on day 1 (ng/ml)</th>
<th>Plasma conc. on day 7 (ng/ml)</th>
<th>Mean peak concentration (ng/ml)</th>
<th>Mean peak conc/IC90</th>
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</thead>
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<tr>
<td>WR035058</td>
<td>Cure</td>
<td>8.5</td>
<td>454</td>
<td>84</td>
<td>371</td>
<td>105</td>
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<tr>
<td>WR069878</td>
<td>Cure</td>
<td>3.3</td>
<td>38</td>
<td>16</td>
<td>91</td>
<td>40</td>
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<tr>
<td>WR074086</td>
<td>Clear (R25)</td>
<td>7.0</td>
<td>179</td>
<td>4.0</td>
<td>78</td>
<td>7.8</td>
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<td>WR176399</td>
<td>Clear (R21)</td>
<td>&lt; 0.5</td>
<td>27</td>
<td>2.7</td>
<td>30</td>
<td>3.8</td>
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<td>WR029252</td>
<td>Failure</td>
<td>20</td>
<td>67</td>
<td>1.0</td>
<td>21</td>
<td>1.0</td>
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<tr>
<td>WR030090</td>
<td>Failure</td>
<td>52</td>
<td>597</td>
<td>11</td>
<td>16</td>
<td>5.6</td>
</tr>
</tbody>
</table>
Table 4: Summary of efficacy of WR069878 against *Plasmodium spp.* in *Aotus*.

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Number of Days</th>
<th>Type of treatment</th>
<th>Species</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1</td>
<td>Retreatment</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Cure</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>Retreatment</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Clear (8)</td>
</tr>
<tr>
<td>80</td>
<td>1</td>
<td>Retreatment</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Clear (11)</td>
</tr>
<tr>
<td>0.625</td>
<td>3</td>
<td>Primary</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Failure</td>
</tr>
<tr>
<td>0.625</td>
<td>3</td>
<td>Primary</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Failure</td>
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<tr>
<td>2.5</td>
<td>3</td>
<td>Primary</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Failure</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>Primary</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Cure</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>Primary</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Cure</td>
</tr>
<tr>
<td>40</td>
<td>3</td>
<td>Retreatment</td>
<td><em>P. vivax</em>-AMRU1</td>
<td>Cure</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Primary</td>
<td><em>P. falciparum</em> FVO</td>
<td>Cure</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>Primary</td>
<td><em>P. falciparum</em> FVO</td>
<td>Cure</td>
</tr>
</tbody>
</table>

Notes:

a. Primary treatment refers to the initial treatment given when parasitemia reached 5000 parasites/µL. Retreatment refers to administration of an additional course of treatment in the event of recrudescence after, or failure of, the primary treatment.

b. These strains/species are chloroquine-resistant.

c. Where the outcome of treatment was clearance, the number in brackets indicates the number of days before parasites recrudesced.
Table 5: Cost of goods analysis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Bench cost per 100g ($)</th>
<th>Bench cost per mg (c)</th>
<th>Market price of 228 mg base pill (c)</th>
<th>Market price per mg (c)</th>
<th>Treatment dose (mg)</th>
<th>Treatment cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloquine WR030090</td>
<td>13969</td>
<td>14</td>
<td>571c</td>
<td>2.50d</td>
<td>1250f</td>
<td>31</td>
</tr>
<tr>
<td>WR069878</td>
<td>1360</td>
<td>1.4</td>
<td>NA</td>
<td>0.24f</td>
<td>2100h</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes:

a Calculated based on hypothetical bench synthesis described in Figure 5, and published prices for starting materials from Sigma.
b Value in column 2 divided by 10000.
c Cost of a 250 mg Lariam tablet (228 mg mefloquine base, from Chambers 2003).
d Calculated by dividing cost of Lariam tablet in column 4 by 228.
e Estimated by assuming that scale up costs for WR069878 and WR030090 are equivalent to mefloquine.
f Standard mefloquine treatment dose is 1250 mg (Rendi-Wagner 2002).
g Total dose required for cure based on published treatment dose of 690 mg/kg/day for six days (Martin et al., 1973).
h Dose in mg assuming a scaling factor of 3.8 between Aotus and humans based on surface area, and that the average human weighs 70 kg.
i Calculated by multiplying the cost per mg in $ by treatment dose in mg.
Table 6: Clinical and preclinical phototoxicity data for three quinoline methanols

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Clinical endpoint</th>
<th>Mice</th>
<th>Yeast</th>
<th>Pharmacophore</th>
<th>(PIF)$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WR007930</td>
<td>Irritation/erythema after sun exposure (12 mg/kg po for 14 days)$^a$</td>
<td>Minimum phototoxic dose is 7 mg/kg by ‘injection’$^e$</td>
<td>Minimum phototoxic concentration (MPC) = 31 mg/ml</td>
<td>Estimated MPC = 170 mg/ml</td>
<td>29.0</td>
</tr>
<tr>
<td>WR030090</td>
<td>Clinically insignificant phototoxicity (10 mg/kg/day for 6 days in 4/124 people)$^b$</td>
<td>Doses of 25 or 50 mg/kg orally are phototoxic$^d$</td>
<td>Minimum phototoxic concentrations were 25 and 500 mg/ml</td>
<td>Estimated MPC = 110 mg/ml</td>
<td>105.7</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Not considered phototoxic</td>
<td>Not phototoxic at tolerated doses</td>
<td>Not tested</td>
<td>Estimated MPC = 5600 mg/ml</td>
<td>1.76</td>
</tr>
</tbody>
</table>

**Notes**

$^a$ From Pullman et al., 1946  
$^b$ From Martin et al., 1973  
$^c$ From Ison and Davis, 1969  
$^d$ WRAIR archival data, method based on similar principles as Ison and Davis (1969).  
$^e$ Photo Irritant Factor (PIF), as calculated by the 3T3 Neutral Red Uptake Phototox Prediction Software (version 2.0, developed by ZEBET); compounds with the potential to be phototoxic have a PIF > 5.0.
Figure Legends

Figure 1: Structures of mefloquine and alkylaminoquinolinyl methanols (AAQMs). The AAQMs are substituted at the positions indicated in the bottom structure and as described in Table 1.

Figure 2: Metabolic scheme for AAQMs. As indicated for WR069878, the $N,N$-dialkyl analogs were metabolized in all species primarily by $N$-dealkylation (> 90%), yielding the corresponding secondary amine metabolite. Hydroxylation was a minor but secondary route of metabolic transformation. Singly alkylated analogs were metabolized to a much lesser extent via either hydroxylation and or $N$-dealkylation.

Figure 3: WR069878 is less neurotoxic and has a different mechanism of toxicity than mefloquine. A: Mefloquine, with an IC$_{50}$ of 27 µM is more neurotoxic than WR069878 with an IC$_{50}$ of 242 µM. B: Mefloquine, but not WR069878 at a concentration of 100 µM increases intracellular calcium concentrations, as indicated by the relative increase in Fuo3 fluorescence, as measured by confocal microscopy. The disruption of neuronal calcium caused by mefloquine occurs as a consequence of discharge of ER calcium store and influx of extracellular calcium through unknown mechanisms. Drugs were added at the time indicated by the arrow. C: The neurotoxicity of mefloquine, but not WR069878, is blocked by DNQX and supra-physiological magnesium, indicating that the compounds have different mechanisms of toxicity. Bars represent standard errors in all cases.
Figure 4: Phototoxicity pharmacophore maps. The phototoxicity pharmacophore was generated based on published studies (Ison and Davis, 1969), reporting the minimum phototoxic concentrations of various quinoline methanols in an *in vitro* yeast growth inhibition assay. All the features of the pharmacophore map to WR007930 and WR030090. However, the aromatic hydrophobic functionality associated with the 2-position phenyl group does not map to mefloquine.

Figure 5: Hypothetical synthesis of WR069878 and starting materials.
REFERENCES


### Table 1: Structures and Biological Data for Various Alkyl Amino Quinolines.

<table>
<thead>
<tr>
<th>Compound</th>
<th>2a*</th>
<th>2b*</th>
<th>2c*</th>
<th>3*</th>
<th>4a*</th>
<th>4b*</th>
<th>4c*</th>
<th>6*</th>
<th>7*</th>
<th>8*</th>
<th>Predicted solubility pH 5.5 (mg/ml)</th>
<th>Log P</th>
<th>Activity against P. falciparum TM911C235 (IC₅₀ in µM)</th>
<th>Neurotoxicity ranking (IC₅₀ in µM)</th>
<th>Metabolic stability (half-life in minutes)</th>
<th>CYP2D6 Inhibition (IC₅₀ in µM)</th>
<th>TOPKAT (Passed tests/30)</th>
<th>SC activity in mice (min effective dose in mg/kg/day)</th>
<th>Oral activity in mice (min effective dose in mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloquine</td>
<td>CF₃</td>
<td>H</td>
<td>Piperidine</td>
<td>CF₃</td>
<td>H</td>
<td>H</td>
<td>8.29</td>
<td>2.87</td>
<td>15</td>
<td>20</td>
<td>&gt;120</td>
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<td>10</td>
<td>20</td>
<td>5, 5* Cure</td>
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<td></td>
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<td>WR030990</td>
<td>H</td>
<td>Cl</td>
<td>Cl</td>
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<td>Bu</td>
<td>H</td>
<td>Cl</td>
<td>Cl</td>
<td>Cl</td>
<td>0.004</td>
<td>8.18</td>
<td>11</td>
<td>2000</td>
<td>&gt;120</td>
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<td>Bu</td>
<td>Bu</td>
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<td>&gt;30</td>
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<td>Me</td>
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<td>&gt;120</td>
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<td>Cl</td>
<td>H</td>
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**Notes**
- a Denotes position on quinoline ring at which substitution, as in Figure 1. Groups 2a-c denote either substitution at the 2-position or modifications to the phenyl ring attached to the quinoline ring at the 2 position. Groups 4a-c denote modifications to the N side chain at the 4 position.
- b Trifluoromethyl group is attached at 2-position of the quinoline ring.
- c t-Butyl group is attached to the 2-position of the quinoline ring.
- d Piperidine ring as in Figure 1.
- e N-oxide.
- f N-methyl.
- g Calculated using ACD/LogD Sol Suite.
- h Minimum daily dose that cured at least one of five *P. berghei*-challenged mice. Drugs were given s.c. for three days.
- i Minimum daily dose that cured at least one of five *P. berghei*-challenged mice. Drugs were given orally for three days. Compounds were tested at University of Miami unless otherwise indicated. Greater/less than symbols indicate that the MED was outside the dose range tested as indicated.
- j These compounds were tested at AFIRMIS, Thailand.
- k Clinical outcome in groups of two *P. falciparum*-challenged *Aotus*. Cure means that both monkeys were parasite free 90 days after treatment. Failure means that monkeys were rescued with mefloquine. R means that infections were cleared, and the number indicates the average day of recrudescence for both monkeys.
- l NT = Not tested due to insolubility or insufficient compound.
2-Phenyl substituted dialkylquinoliny1 methanols

Mefloquine
WR069878
C_{26}H_{32}Cl_{2}N_{2}O
Exact Mass: 458.19
m/z 459.2 [M+H]^+

Dealkylation (> 92%)

C_{22}H_{24}Cl_{2}N_{2}O
Exact Mass: 402.13
m/z 403.1

Hydroxylation

C_{24}H_{32}Cl_{2}N_{2}O_{2}
Exact Mass: 474.18
m/z 475.1

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Scheme 1: Proposed synthesis of WR 069878 with possible yields.

Scheme 2: Synthesis of isatin starting material.