Bioorganic & Medicinal Chemistry Letters 20 (2010) 1347–1351

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Structure–activity relationships amongst 4-position quinoline methanol antimalarials that inhibit the growth of drug sensitive and resistant strains of *Plasmodium falciparum*

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ARTICLE INFO

Article history:
Received 17 December 2009
Revised 29 December 2009
Accepted 4 January 2010
Available online 7 January 2010

Keywords:
Antimalarials
Plasmodium falciparum
Plasmodium berghei
Mefloquine
Quinoline methanol
Structure–activity relationship

ABSTRACT

Utilizing mefloquine as a scaffold, a next generation quinoline methanol (NGQM) library was constructed to identify early lead compounds that possess biological properties consistent with the target product profile for malaria chemoprophylaxis while reducing permeability across the blood–brain barrier. The library of 200 analogs resulted in compounds that inhibit the growth of drug sensitive and resistant strains of *Plasmodium falciparum*. Herein we report selected chemotypes and the emerging structure–activity relationship for this library of quinoline methanols.

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Malaria continues to be a major health challenge in most tropical and many subtropical regions.[1] Of the 1–3 million annual fatalities due to malaria, the majority are pregnant women and children below age five. Our broad objective is to satisfy a projected future unmet medical need for cost-effective, intermittent preventative treatment (IPT) of malaria and prophylaxis in which a blood schizonticidal drug is given periodically as a single therapeutic dose to otherwise healthy, non-immune individuals.[2] We opted for the quinoline methanol scaffold due to the relative safety of mefloquine (MQ, WR142490) in pregnancy and its well characterized clinical profile.[3]

Mefloquine would be the US Army's drug of choice for prophylaxis but for its association with adverse central nervous system (CNS) effects.[4] Current justification for use is the long half-life (weekly dosing) and activity against chloroquine-resistant malaria.[5] The ability of mefloquine to accumulate in the CNS at micromolar concentrations and target multiple receptors has been well documented, but the relevant targets have not been identified.[6] We seek to prevent CNS penetrability from the outset by reengineering the quinoline methanol scaffold **5** (Fig. 1) to yield derivatives that exhibit fewer adverse CNS effects while retaining antimalarial efficacy. Research within the Walter Reed Army Institute of Research has shown that acyclic *N*-alkylaminoquinoline methanols display a selectivity index superior to mefloquine[7] and further evidence suggests the neurotoxicity of mefloquine appears to be associated with the piperidine ring.

Generally, the structure–activity relationships (SAR) were not adequately studied during the 1960s development of mefloquine, which relied upon in vivo models.[8] We chose to increase potency and reduce absorption through the blood–brain barrier by optimizing the 4-position aminoalcohol moiety and probing the quinoline scaffold by adding novel pentafluorosulfanyl (–SF5) substituents.[9]

In order to optimize the 4-position moiety, we studied the effect of alterations of physiochemical properties on activity while constructing over two hundred analogs. Due to the breadth and diversity of this endeavor, it was decided to report these data in two parts: (1) the structure–activity relationship that emerged and (2) the analysis of the relationship between physiochemical properties and biological data.[10] Herein we examine the 4-position SAR and emerging chemotypes of interest, which we are currently optimizing as part of a structure-based rational drug design program.

In order to efficiently prepare analogs with the 2,8-trifluoromethyl quinoline core, the vast majority of derivatives were

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0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.01.001

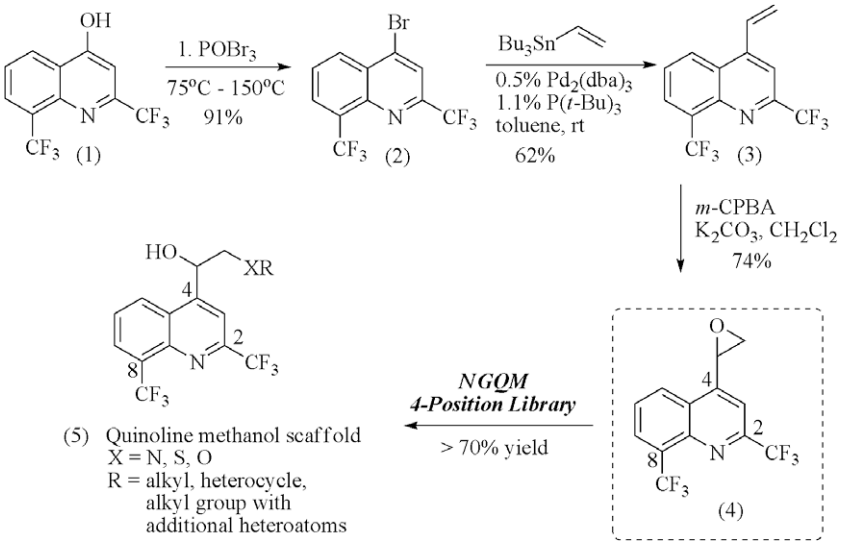


Figure 1. Synthesis of the next generation quinoline methanol scaffold.

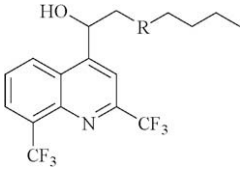
constructed according to the procedures outlined in Figure 1.[11] Bis(trifluoromethyl)quinolin-4-ol **1** was melted along with phosphorous oxybromide to provide 4-bromo-2,8-bis(trifluoromethyl) quinoline **2**,[12] which was subjected to a Stille[13] reaction in order to yield vinyl intermediate **3**. Simple oxidation[14] led to epoxide **4**, which was diversified at the 4-position through a regioselective S_N2 nucleophilic ring opening mechanism. Since this was an exploratory library meant to provide early-lead compounds, we chose to construct and screen racemic compounds **5**.

Initially, the significance of the homobenzylic amine moiety was explored. As shown in Table 1, while *n*-butyl amine (WR177000) was quite efficacious, chains with heteroatoms such as oxygen (WR308633), sulfur (WR308632), and carbon (WR308653) were devoid of activity. Based upon these results, only nitrogen-based moieties were further explored at the 4-position in the remainder of the exploratory library.

In regards to nitrogen functionalization, the data suggests that the nature of the 4-position amine and the degree of electron density around the nitrogen are most strongly associated with activity. The remainder of the discussion explores the relationship between 4-position amine substituents and antimalarial activity.

Antimalarial and selectivity data for a subset of compounds with aromatic side chains are presented in Table 2. The data suggests a reduction of electron density with respect to the amine, due to the adjacent benzene ring, diminishes potency. For example,

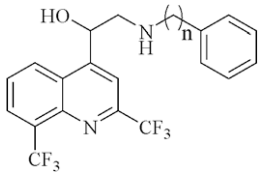
Table 1
P. falciparum IC_{90} (ng/mL) values resulting from probing the homobenzylic site of QMs



Compound	R	IC_{90} (ng/mL)[a]
WR177000	NH	2, 1, 9, 12
WR308633	0	>500[b]
WR308632	S	>500[b]
WR308653	C	>500[b]

[a] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.
[b] >500 ng/mL for each of four strains.

Table 2
P. falciparum IC_{90} (ng/mL) values and selectivity of phenyl, benzyl, and phenethylamino QMs



Compound	*n*	LC_{50}[a] (ng/mL)	IC_{90}[b] (ng/mL)
WR308251	0	22,298	484, 377, 437, 500
WR308252	1	20,427	19, 67, 86, 151
WR308253	2	9638	19, 23, 43, 23

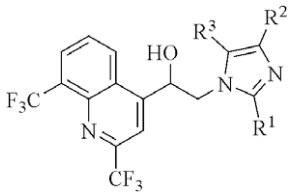
[a] Cytotoxicity: macrophage IC_{50} (ng/mL).
[b] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.

phenylamino QM (WR308251) has a much lower activity then benzylamino QM (WR308252) and phenethylamino QM (WR308253). All phenyl amines tested were nearly devoid of activity, and the addition of electron-withdrawing groups further decreased potency.

The imidazole class of compounds is interesting because the IC_{90} values for many of them are similar across the four drug-resistant strains with WR308437 and WR308623 in particular illustrating this reduction in cross-susceptibility to related drugs (Table 3). This is encouraging, since in vitro susceptibility of clinical isolates of *Plasmodium falciparum* to related aminoalcohol antimalarials such as halofantrine correlates strongly to that of mefloquine.[15] On the other hand, metabolic stability was poor, suggesting the compounds may have short half-lives in vivo. Consequently, efforts are underway to synthesize more metabolically stable compounds, since their cross-susceptibility profiles are of great interest.

In regards to alkyl amino quinoline methanols, an intricate relationship between steric bulk and lipophilicity of the alkyl groups has emerged and merits further study in order to determine the discrete contributions to efficacy. Although the mechanism of action for mefloquine is not fully understood, the lipophilic nature of MQ and quinine is known to correlate with the delivery of drug to the parasite.[16] MQ is also known for high-affinity binding to erythrocytes and other cells, which may provide a reservoir of drug and contribute to the long half-life.[17]

Table 3
P. falciparum IC_{90} (ng/mL) values, selectivity, and metabolic stability of heterocyclic amino quinoline methanols (HAQMs)



Compound	R^1	R^2	R^3	LC_{50}[a] (ng/mL)	IC_{90}[b] (ng/mL)	Mouse[d] (mm)
WR308437	Et	Me	H	25,707	69, 43, 56, 57	22
WR308623	n-Pr	H	H	50,082	360, 320, 250, 240	8
WR308626	i-Pr	H	H	50,082	>500[c]	10
WR308763	Me	–Benzene–		ND[e]	110, 220, 310, 320	9
WR308764	H	–Benzene–		ND[e]	120, 410, 480, 450	4

[a] Cytotoxicity: macrophage IC_{50} (ng/mL).
[b] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.
[c] Selectivity index = LC_{50} against macrophages/IC_{90} against PF W2.
[d] Half life in human liver micosomes. The half-life of mefloquine in this test system is >60 min.
[e] ND = no data.

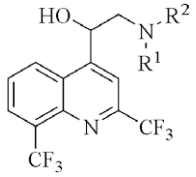
In general, these compounds were more potent than MQ and displayed a one-log increase in selectivity. The primary amine WR308314 is nearly devoid of activity, while the addition of methyl, ethyl, propyl, and butyl groups substantially increases efficacy (Table 4). Interestingly, branched alkyl substituents such as *i*-Pr and *i*-Bu prove quite active, while *t*-Bu displays moderate activity. Chain length also appears to affect activity since *n*-Bu (WR177000) and *n*-hexyl (WR308442) derivatives have different levels of potency, presumably resulting from the addition of the two methylene units. *N*-Methyl (WR308245), *i*-Pr (WR308257), and *t*-Bu (WR183545) derivatives all displayed favorable metabolic stability profiles, presumably due to inhibition of N-dealkylation.[18]

A variety of alkyl amino quinoline methanols containing side chains with additional heteroatoms were also constructed (Table 5). In this series, it became apparent that efficacy is reduced by the addition of an alcohol or fluorine within the side chain. In particular, when the hydroxyl group of WR308258 is transformed into the methyl ether (WR308412), potency increases by nearly an order of magnitude. Thioether WR308278, ether WR308622, and benzyl amine WR308396 were more potent and demonstrated a superior selectivity index compared to mefloquine. In this series, the ethers, thioethers, and secondary amines proved most promising, and second generation libraries based on these lead compounds are currently being constructed.

After constructing a small series of cyclic diamines, we observed promising activity emerging within the series as illustrated by WR308621 and WR319535, which correlate with WR308396 (Table 6). Likewise, a second generation library based upon cyclic motifs is underway.

Representative compounds from this library were evaluated for their in vivo efficacy against *P. berghei* in a mouse model. The bidirectional permeability of most of the compounds was measured across MDR1-transformed MDCK cell monolayers in the presence of the PgP inhibitor cyclosporin A. This assay is normally used as an indicator for permeability properties across the blood–brain barrier.

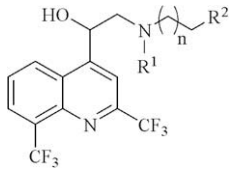
Table 4
P. falciparum IC_{90} (ng/mL) values and selectivity of alkyl amino quinoline methanols (AAQMs)



Compound	R^1	R^2	LC_{50}[a] (ng/mL)	IC_{90}[b] (ng/mL)	Mouse[c] 1/2 life (mm)
WR308314	H	H	8074	470, 400, >500, >500 ND	
WR308245	H	Me	13,667	17, 45, 58, 80	>60
WR308246	Me	Me	24,626	20, 69,79, 173	ND
WR308254	Et	Et	2092	9, 26, 37, 67	ND
WR183544	n-Pr	n-Pr	ND[d]	1,7, 14, 16	ND
WR308257	H	i-Pr	5201	5,23,23,39	>60
WR308277	n-Pr	n-Pr	33,160	5, 20, 20, 30	ND
WR177000	H	n-Bu	2965	2, 7, 9, 12	ND
WR176990	n-Bu	n-Bu	20,075	2, 11,27, 19	ND
WR308607	H	i-Bu	27,117	2, 13, 15,21	27
WR183545	H	i-Bu	10,459	19, 101, 137, 154	>60
WR308442	H	n-hex	ND[d]	124, 372, 409, 481	ND

[a] Cytotoxicity: macrophage IC_{50} (ng/mL).
[b] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.
[c] Half life in mouse liver micosomes (min).
[d] ND = no data.

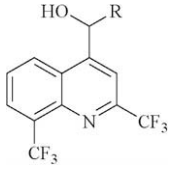
Table 5
P. falciparum IC_{90} (ng/mL) values and selectivity of alkyl amino quinoline methanols containing additional heteroatoms within the side-chain)

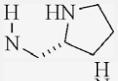
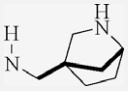


Compound	*n*	R^1	R^2	LC_{50}[a] (ng/mL)	IC_{90}[b] (ng/mL)
WR308258	1	H	OH	ND[d]	260, 490, 485, 388
WR308412	1	H	OMe	38,230	15, 59, 93, 100
WR308622	2	H	OMe	25,167	12, 51, 84, 86
WR308278	2	H	SMe	8618	10, 36, 39, 54
WR308247	1	H	F	ND[d]	>500[c]
WR308411	0	H	CF3	32,053	>500[c]
WR308396	1	H	NHBn	8279	6, 29, 40, 48

[a] Cytotoxicity: macrophage IC_{50} (ng/mL).
[b] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.
[c] >500 ng/mL for each of four strains.
[d] ND = no data.

Table 6
P. falciparum IC_{90} (ng/mL) values and selectivity of selected diamines



Compound	R	LC_{50}[a] (ng/mL)	IC_{90}[b] (ng/mL)
WR308621		21,790	46, 92, 100, 198
WR319535		4110	275, 75, 260, 251

[a] Cytotoxicity: macrophage IC_{50} (ng/mL).
[b] In vitro IC_{90} values against four different strains of *P. falciparum*, W2, D6, C235, and C2A.

As illustrated in Table 7, WR308278, WR177000, and WR308245 were active in vivo after oral dosing but exhibited greater permeability relative to mefloquine across MDCK cell monolayers. The latter property suggests greater propensity for accumulation into the central nervous system. Taking all data into consideration, only WR308396 and WR319535/WR309581 exhibited efficacy in vivo after oral dosing, lower or equivalent permeability across MDCK cell monolayers relative to mefloquine, and reduced permeability across MDCK cell monolayers relative to lipophilic alkyl compounds such as WR177000.

Table 7
Permeability across MDR1-transfected MDCK Cell monolayers[a] and in vivo efficacy of selected quinoline methanols in the blood schizonticidal *P. berghei*–ICR mouse model[b]

Compound	Permeability efficacy	
	A–B[a]	In Mice[b]
Racemic mefloquine	9.4	17
WR177000	25	8
WR308245	24	25
WR308278	38	17
WR308396	8.4	10
WR308412	32	15
WR308413	ND[c]	2
WR308622	ND[c]	9
WR308446	ND[c]	3
WR319535/WR319581	2.0	7

[a] Apparent permeability ($\times 10^{-6}$ cm/s).
[b] Determined after oral administration of 160 mg/kg/d × 3 (delay in mortality in days relative to vehicle control).
[c] ND = no data.

Structure–activity relationships were studied amongst a library of 200 4-position analogs of mefloquine. The 4-position alcohol and at least one diamine met the minimum requirement for equivalent potency to mefloquine. Decreased electron density around the homobenzylic side chain amine greatly diminished potency. Moderately enhanced potency, increased selectivity, and altered cross-susceptibility patterns were achieved with imidazole-containing analogs. Ten-fold greater potency and selectivity than mefloquine without altered cross-susceptibility patterns were achievable in analogs with alkyl side chains. Introduction of heteroatoms into these latter analogs generally reduced potency, although select analogs exhibited equivalent potency to mefloquine.

Taken together, these observations suggest a hypothesis for further rational lead optimization: Diamino-QMs should retain the potency of mefloquine but exhibit significantly reduced permeability across the blood–brain barrier. Second-generation libraries that test this hypothesis are currently underway. Due to the breadth of this library, the compounds were synthesized as racemates. We are currently optimizing a cost-effective, enantiopure synthetic route to epoxide **4**. The active lead compounds will be tested in vitro and in vivo as pure enantiomers or diastereomers, respectfully.

Acknowledgments

We thank the Military Infectious Diseases Research Program (MIDRP) for financial support.

This manuscript was reviewed by the Walter Reed Army Institute of Research and the US Army Medical Research and Materiel Command, and there is no objection to its publication or dissemination. The opinions expressed herein are those of the authors and do not necessarily reflect the views or opinions of the Department of the Army and the Department of Defense. Animal care and use was conducted under IACUC protocol # 06-05 approved by the Institutional Animal Care and Use Committee in accordance with national guidelines. The in vivo studies were conducted at USAMC-AFRIMS in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) international accredited animal facility. Research was conducted in compliance with the Animal Welfare Act, other federal statutes and regulations that relate to animals and experiments involving animals, and principles stated in the 'Guide for the Care and Use of Laboratory Animals (Guide for Care and Use of Laboratory Animals. National Academy Press, 1996).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.001.

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