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Design, Synthesis, and SAR Studies of Mefloquine-Based Ligands as Potential Antituberculosis Agents

Sarva Jayaprakash,[a] Yasuyoshi Iso,[a] Baojie Wan,[b] Scott G. Franzblau,[b] and Alan P. Kozikowski*[a]

Tuberculosis (TB), a chronic infectious disease caused by *Mycobacterium tuberculosis*, is one of the leading causes of death in the world. According to the World Health Organization (WHO), one-third of the world's population is currently infected with the TB bacillus. It is estimated that 1.75 million deaths resulted from TB in 2003.[1] One-third of the increase in the incidence of TB in the past five years can be attributed to its co-infection with HIV, as this weakens the immune system and facilitates the development of multi-drug-resistant TB (MDR-TB).[2–4] It has been estimated that 50 million people have been infected with MDR-TB. As no new drugs have been introduced for TB over the last 40 years,[5] there is an urgent need to identify improved medications that are able to combat MDR-TB and to decrease the current six-month treatment protocol that is required for drugs in current use. To achieve this goal, we need to identify potent and nontoxic antituberculosis agents that kill both the replicating (R-TB) and nonreplicating persistent (NRP-TB) phenotypes; eradicating the latter is implicated as a necessity for shortening treatment. As part of our drug-discovery efforts aimed at developing novel antituberculosis agents, we report herein the design, synthesis, and structure–activity studies of mefloquine analogues.

Mefloquine is a well-known antimalarial drug still used today in spite of its neuropsychiatric side-effects that include dizziness, headache, insomnia, and vivid dreams.[6] Mefloquine and several of its analogues have been reported to have antibacterial activity.[7–10] From a screening program carried out at the Walter Reed Army Institute of Research, a series of mefloquine-related compounds with various substitutions on the quinoline ring were found to be more active than mefloquine itself against gram-positive bacteria.[7] Although mefloquine derivatives are also known to act as purine receptor antagonists,[11] the only prokaryotic target of mefloquine identified to date is an $F_0F_1H^+$ ATPase in *Streptococcus pneumoniae*.[12] The crystal structures of the rotor of the V-type and F-type Na^+ ATPases were disclosed recently.[13,14] In this context, it is important to note that the recently described diarylquinoline R207910, a potent antituberculosis agent, is also believed to owe its activity to interaction with a proton pump of the ATP synthase of *M. tuberculosis*.[15] From our own in-house screening of the Gen-Plus 960 compound library (MicroSource), mefloquine was found to have relatively potent activity against NRP-TB, which prompted us to choose it as a lead candidate for TB drug discovery.

The results of biological assays performed on the (+) and (−) forms of the *erythro* and *threo* isomers of mefloquine are shown in Table 1. The mefloquine isomers were tested against NRP-TB in the luciferase-based low oxygen recovery assay (LORA),[16] and these data were confirmed by the quantification of colony-forming units (cfu) immediately following the hypoxic incubation period. As expected through findings made in the malaria field, the *erythro* isomers were more active against *M. tuberculosis*, and the (+)-*erythro* isomer appeared to be less cytotoxic than the (−)-*erythro* isomer. The LORA-based MIC and MBC values are in the same range as those of rifampin, PA-824 (and for MBC, moxifloxacin), compounds that have previously demonstrated activity against NRP-TB. (The fluoroquinolines are the only class for which we have found the luciferase signal to significantly underestimate the activity relative to cfu.)

Table 1. Anti-TB activity of mefloquine isomers.

Compound	MIC [μM][a]			MBC [μM][c]	IC_{50} [μM]	SI[b]		
	MABA[d]	GFP[e]	LORA[f]	CFU	Vero[g]	MABA	GFP	LORA
(+)-*threo*-mefloquine·HCl	21.3	14.4	19.2	32	25.1	1.2	1.7	1.3
(−)-*threo*-mefloquine·HCl	12.8	11.7	17.3	32	30.1	2.3	2.6	1.7
(−)-*erythro*-mefloquine·HCl	7.8	11.2	7.2	8	17.2	2.2	1.5	2.4
(+)-*erythro*-mefloquine·HCl	6.7	3.8	7.3	4	38.1	5.6	9.9	5.2
moxifloxacin			42.3	2				
PA-824			7.6	4				
rifampin (RMP)	0.08	0.04	6.5	2	99.7	1246.4	2492.8	15.4

[a] MIC = minimum inhibitory concentration. [b] SI = selectivity index = IC_{50}/MIC. [c] MBC = minimal bactericidal concentration determined by quantification of colony forming units. [d] MABA = microplate Alamar Blue assay. [e] GFP = green fluorescent protein microplate assay. [f] LORA = low oxygen recovery assay (luciferase readout). [g] cytotoxicity towards Vero cells.

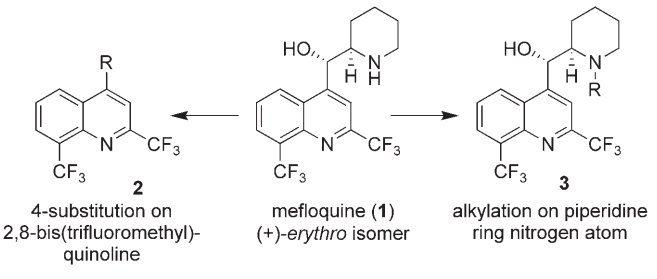
Mefloquine appears unique in that the MIC values against NRP-TB are similar to those against R-TB. In contrast, compounds such as isoniazid and ethambutol are inactive in the LORA, while only weak activity is observed with streptomycin.[16]

[a] *Dr. S. Jayaprakash, Dr. Y. Iso, Prof. Dr. A. P. Kozikowski*
Drug Discovery Program,
Department of Medicinal Chemistry and Pharmacognosy
University of Illinois at Chicago
833 South Wood Street, Chicago, IL 60612 (USA)
Fax: (+1) 312-996-7107
E-mail: kozikowa@uic.edu

[b] *Dr. B. Wan, Prof. Dr. S. G. Franzblau*
Institute for Tuberculosis Research
College of Pharmacy
University of Illinois at Chicago
833 South Wood Street, Chicago IL 60612 (USA)

*Supporting information for this article is available on the WWW under http://www.chemmedchem.org or from the author. (Experimental procedures and characterization data for compounds **6a–h, 8a–i, 10a–c, and 11a–j**)*

Based on the foregoing biological assays of the mefloquine isomers, we selected the (+)-*erythro* isomer as the lead candidate. Our synthesis of mefloquine analogues for biological evaluation is based on modifications to specific regions of the molecule, as shown in Scheme 1. As can be observed from the

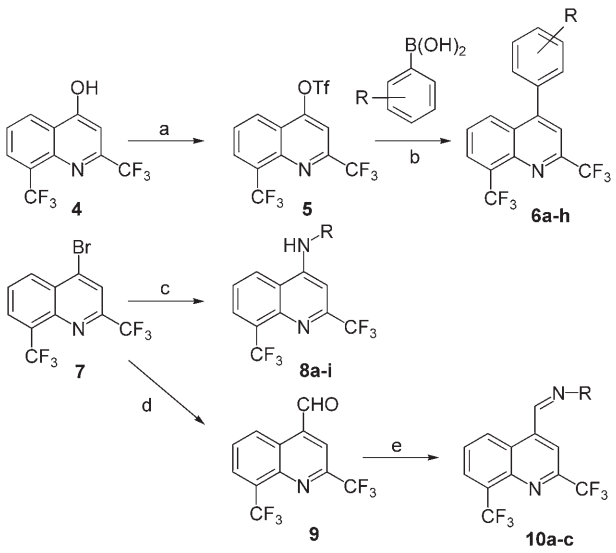


Scheme 1. Design of mefloquine-based antituberculosis agents.

mefloquine structure, its quinoline ring, the *sec*-hydroxy group, and the piperidine ring are the three regions available for modification. Thus, the functionalization and modification of each of these three sites can be explored in order to identify new structural analogues with potentially improved activity and reduced cytotoxicity compared with the lead compound **1**. In the initial stages of these studies, we carried out the synthesis of various analogues that have the same quinoline nucleus as mefloquine, but that possess varied substitutions at the 4-position, as shown in structure **2** (Scheme 1). We also prepared derivatives, like **3**, by carrying out a simple alkylation reaction of the basic piperidine nitrogen atom to study the effect of the N-substitution on the anti-TB activity of mefloquine.

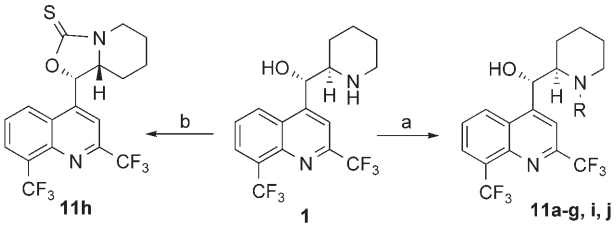
The synthesis of the 4-aryl quinolines **6a–h** was carried out using the Suzuki-coupling protocol as previously described.[17] The reaction between triflate **5** and the appropriate arylboronic acid in the presence of $[Pd(PPh_3)_4]$ catalyst gave the corresponding aryl-substituted products in a very good yield (Scheme 2). The triflate **5** was prepared from 2,8-bis(trifluoromethyl)-4-quinolinol (**4**),[18] using triflic anhydride in pyridine.[19,20] The 4-amino substituted quinolines **8a–i** were prepared in a single-step reaction between 4-bromo-2,8-bis(trifluoromethyl)quinoline (**7**)[18] and an excess of the appropriate amine to give a good yield of the corresponding 4-aminoquinolines.[21,22] The Schiff bases **10a–c** were synthesized by treatment of 4-formyl-2,8-bis(trifluoromethyl)quinoline (**9**)[23] with the respective amine in THF, resulting in a yield of over 70%, as shown in Scheme 2.

For the preparation of the N-alkylated compounds **11a–d, f,** and **g**, mefloquine hydrochloride **1**[24] was allowed to react with the corresponding bromide in *N*,*N*-dimethylformamide using K_2CO_3 to give the desired products in good to excellent yields. Compound **11e** was prepared from mefloquine by reaction with 4-(bromomethyl)benzenesulfonamide[25] in THF in the presence of triethylamine. The urea **11i** was obtained in 52% yield from the reaction of mefloquine in THF with 4-chlorophenyl isocyanate at 0 °C and warmed to room temperature for 4 h. Protection of mefloquine with *tert*-butoxycarbonyl



Scheme 2. Synthesis of 4-substituted 2,8-bis(trifluoromethyl)quinoline derivatives: a) Tf_2O, pyridine, −78–0 °C, 2 h, 96%; b) KBr, K_3PO_4, $[Pd(PPh_3)_4]$, 1,4-dioxane, reflux, 14 h; c) Neat RNH_2, 1,4-dioxane, reflux, 2 h; d) *n*BuLi, DMF, ether, −78 °C to RT, 3 h; 73%; e) RNH_2, THF, RT, 3 h.

(Boc) was carried out using Boc_2O in THF to give **11j** in excellent yield (Scheme 3).[26]

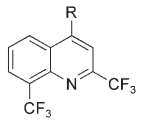


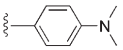
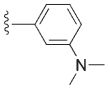
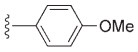
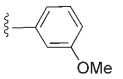
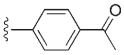
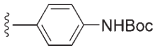
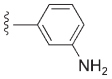
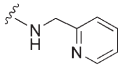
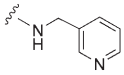
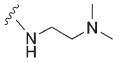
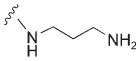
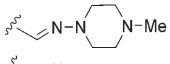
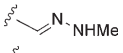
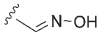
Scheme 3. Modification at the piperidine ring nitrogen atom in mefloquine: a) for compounds **11a–d**, **f**, **g**: RBr, K_2CO_3, DMF, RT, 14 h; for **11e**: 4-(bromomethyl)benzenesulfonamide, Et_3N, THF, 12 h, 15%; for **11i**: 4-chlorophenyl isocyanate, THF, 0 °C to RT, 52%, 4 h; for **11j**: Boc_2O, Et_3N, 1,4-dioxane, 0 °C to RT, 2 h, 98%; b) 1,1′-thiocarbonyldiimidazole, Et_3N, THF, 2 h, 20%.

Next, all of the compounds were evaluated for their activity against the *M. tuberculosis* strain $H_{37}Rv$ by the MABA[27] and LORA methods, and the results are displayed as the calculated MIC values.[28] Prior to the determination of luminescence in the LORA, serial dilutions of cultures were plated on 7H11 agar, and cfu were counted after 18 days incubation at 37 °C. The lowest concentration yielding a 90% reduction in cfu relative to untreated controls was considered as the MBC. Cytotoxicity toward Vero cells was determined after 72 h exposure to the test compounds and observing their subsequent ability to reduce a tetrazolium dye.[29] The activity data including cytotoxicity (IC_{50}) and selectivity indices (MIC/IC_{50}) of compounds **6a–h**, **8a–i,** and **10a–c** of the 4-substituted 2,8-bis(trifluoromethyl)quinolines are presented in Table 2, and data for the N-alkylated mefloquine derivatives **11a–j** are shown in Table 3.

Among the compounds shown in Table 2, **6h**, **8a**, **8c,** and **8f** exhibited moderate in vitro activity against *M. tuberculsis*

Table 2. In vitro activity of products prepared from triflate **5** and bromide **7** against *M. tuberculosis* $H_{37}Rv$.



Compd	R	MIC [μM] MABA	MIC [μM] LORA	IC_{50} [μM] Vero cells	SI MABA	SI LORA	C log $P^{[a]}$
mefloquine		13	7	128	0.9	1.7	3.6
rifampin		0.1	2	122	1929	65	–
6a		>128	>128	35	–	–	6.1
6b		>128	>128	39	–	–	6.1
6c		118	>128	45	0.4	–	5.8
6d		>128	>128	57	–	–	5.8
6e		>128	>128	95	–	–	5.3
6f		>128	>128	>128	–	–	5.3
6g		7	55	15	2	0.3	6.7
6h		60	55	45	0.8	0.8	4.8
8a		66	30	35	0.5	1	3.9
8b		>128	>128	>128	–	–	–
8c		31	94	52	2	0.5	4.7
8d		>128	>128	>128	–	–	4.2
8e		>128	>128	>128	–	–	3.9
8f		59	62	>128	>2	>2	3.9
8g		>128	>128	114	–	–	4.7
8h		>128	>128	15	–	–	3.7
8i		118	>128	32	0.3	–	3.9
10a		9	16	>128	>14	>8	3.9
10b		>128	>128	>128	–	–	3.7
10c		>128	>128	27	–	–	4.2

[a] C log*P* calculated by using web site http://www.daylight.com/daycgi/clogp.

very good activity (MIC = 7 μM) against R-TB. The remaining compounds in Table 2 showed little or no activity.

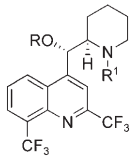
As is apparent, most of the 4-aryl-substituted quinoline derivatives prepared from the Suzuki-coupling protocol were inactive, except for **6g** and **6h**. Compounds with methoxyphenyl substitution on the quinoline ring (compounds **6c**–**e**) were inactive, as were compounds with a dimethylamino group in the 3- or 4- position of the phenyl ring (compounds **6b** and **6a**). In comparison with these dimethylamino-containing compounds **6a** and **6b**, the unsubstituted aniline analog **6h** shows some activity in the MABA assay, whereas the Boc derivative **6g** is even more active than mefloquine in the same assay.

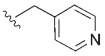
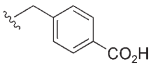
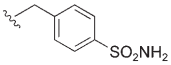
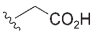
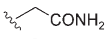
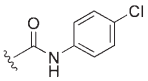
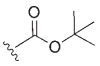
Compounds **8a**–**i**, prepared by the direct amination of the 4-bromoquinoline showed higher activities than those of the 4-arylquinolines. Among these 4-aminoquinolines, **8a, 8c,** and **8f** showed moderate activity (MIC = 30–90 μM). The N-demethylated one-carbon structural **8c** homologue, **8d**, was devoid of any activity, indicating that the length of the alkyl chain between the two amino groups may play an important role. It would appear that the 1,2-diamino group is preferred over a 1,3-diamino group for activity. This is also evident from the activity shown by compounds **8a** and **8f** in comparison with **8c**. Moreover, when one of the nitrogen atoms of the 1,2-diamine is replaced with an oxygen atom (for example, **8f** and **8e**), activity is lost. The remaining compounds prepared by amination chemistry were inactive. Unlike the potent analogue **10a**, the hydrazones **10b** and **10c** prepared from *N*-methylhydrazine and hydroxylamine respectively, were inactive.

The majority of the N-alkylated mefloquine derivatives are reasonably active (Table 3). The N-benzylated compound **11a** showed better activity (MIC = 7 μM (MABA), MIC = 8 μM

$H_{37}Rv$, and in particular the hydrazone **10a** prepared from quinoline aldehyde **9** and 1-amino-4-methylpiperazine was the most active compound in this series. The antituberculosis activity of **10a** (MIC = 9 μM) towards R-TB was higher than the lead compound **1** (MIC = 13 μM), whereas it showed lower activity (MIC = 16 μM) against NRP-TB. Compound **6g** exhibited

Table 3. In vitro activity of products prepared by N-alkylation of the mefloquine piperidine ring against *M. tuberculosis* $H_{37}Rv$.



Compd[a]	R	R^1	MIC [μM] MABA	MIC [μM] LORA	IC_{50} [μM] Vero cells	SI MABA	SI LORA	C log*P*[b]
(±)-**11a**	H		7	8	50	7	7	5.9
(±)-**11b**	H		30	25	>128	>4	>5	4.8
(±)-**11c**	H		22	31	35	2	1	4.4
(±)-**11d**	H		63	94	98	2	1	3.4
(±)-**11e**	H		15	22	38	3	2	4.1
(±)-**11f**	H		>128	>128	>128	–	–	1.9
(±)-**11g**	H		>128	>128	>128	–	–	3.2
(±)-**11h**			>128	>128	–	–	–	3.3
(+)-**11i**	H		29	21	10	0.3	0.5	5.8
(+)-**11j**	H		31	61	10	0.3	0.2	5.6

[a] For the preparation of **11a**–**h**, racemic erythro isomer was used as starting material. [b] C log*P* calculated by using web site http://www.daylight.com/daycgi/clogp.

(LORA)) than the lead compound **1**. By changing from a benzyl to a 4-pyridylmethyl group (compound **11c**), the activity is reduced by ~70%. The N-allylated and N-Boc protected mefloquine derivatives, **11b** and **11j** respectively, showed moderate activity (MIC = 25–60 μM). Of the two 4-substituted benzyl derivatives containing more polar electron-withdrawing groups (**11d** and **11e**), the compound containing a 4-sulfonamido group (**11e**) showed good activity (MIC = 15 μM (MABA), MIC = 22 μM (LORA)). Interestingly, when the piperidine nitrogen atom and the hydroxyl group in mefloquine were joined together through the formation of an oxazolidinethione ring system (compound **11h**), the activity of the parent compound was completely lost. Lastly, to evaluate the effect of conversion of the piperidine to its urea derivative, we prepared urea **11i**. This compound was found to be moderately active against R-TB (MIC = 29 μM) and NRP-TB (MIC = 21 μM). From these structure–activity studies it is apparent that some of the N-benzylated analogues of mefloquine show interesting activity profiles, whereas the attachment of a simple acetic acid or acetamide appendage to the piperidine ring nitrogen atom totally obliterates activity. It is also important to note that the best compounds **10a** and **11a** were found to have low cytotoxicities in the Vero assay and thus, show good selectivity indices.

In conclusion, some mefloquine-based compounds have been prepared and evaluated for their activity against *M. tuberculosis*. By making use of both the MABA and LORA methods, the anti-TB activity of these compounds was tested against the R-TB and NRP-TB phenotypes. Although some of the analogues prepared that possess the same quinoline nucleus as mefloquine showed moderate biological activity, compound **10a** in particular, showed an improved activity and selectivity against R-TB. These findings provide support for the investigation of other structural analogues of **10a**, possibly by modifiying its quinoline and piperazine moieties to identify lead candidates with improved biological activity against both replicating and nonreplicating TB. Additionally, in light of our finding that the N-benzylated mefloquine derivative **11a** retains activity, other modifications to this site of the molecule can be made in an effort to identify compounds showing reduced CNS side effects.

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Keywords: alkylation · amination · hydrazones · mefloquine · tuberculosis

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