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# Design, Synthesis, and Pharmacological Evaluation of Mefloquine-Based Ligands as Novel Antituberculosis Agents

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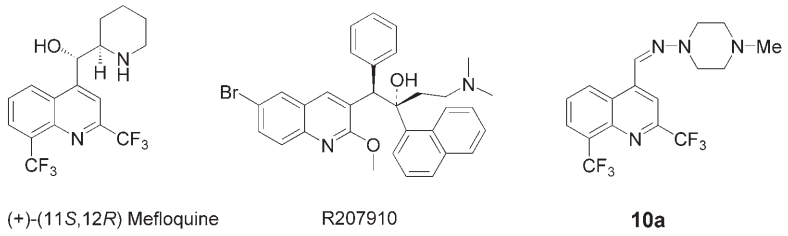
*Tuberculosis (TB) is presently regarded as one of the most dangerous infective diseases worldwide and one of the major AIDS-associated infections. To shorten the current treatment regimen, there is an urgent need to identify new anti-TB agents which are active against both replicating TB (R-TB) and nonreplicating TB (NRP-TB). Mefloquine, a well-known antimalarial drug was found to possess reasonable activity against NRP-TB, and accordingly, 30 new analogues were synthesized and evaluated for their anti-TB activity against* Mycobacterium tuberculosis *$H_{37}Rv$. As the target of mefloquine in* Mycobacterium tuberculosis *remains unknown, we resorted to modifying mefloquine in a variety of chemically convenient ways, which led us in turn to the active hydrazone **10a**. Further modifications of **10a** led to compound **7f**, with an improved anti-TB activity/selectivity profile with both less cytotoxicity and less predicted CNS side effects compared with mefloquine. The clear structure–activity relationships (SARs) derived from this study should facilitate our ultimate goal of identifying improved anti-TB agents.*

## Introduction

Tuberculosis (TB), often considered a historical disease which may no longer be a threat, is in fact among the top five deadly diseases in developing countries. Overall, one-third of the world's population is currently infected with the *Mycobacterium tuberculosis* and it has been estimated that 1.7 million died of TB in 2004.[1] The current TB chemotherapy regimen requires patients to take three to four drugs (isoniazid, rifampin, pyrazinamide, and ethambutol) for a minimum of six months. Although all of them target replicating *M. tuberculosis* (R-TB), only rifampin and pyrazinamide have shown activity against nonreplicating persistent TB (NRP-TB). The key to shortening the current long regimen (the primary goal of new TB chemotherapy) lies in effectively targeting this NRP-TB.[2,3] To this end, we report herein the design and synthesis of mefloquine-based analogues and the evaluation of their activity against R-TB and NRP-TB using the microplate Alamar Blue assay (MABA[4]) and the low-oxygen recovery assay (LORA[5]), respectively.




(+)-(11*S*,12*R*) Mefloquine R207910 **10a**

**Figure 1.** Structures of mefloquine, R207910, and **10a**.

As reported previously,[6] mefloquine (Figure 1), a well-known antimalarial drug,[7] was identified as having relatively potent activity against NRP-TB. There are data suggesting that the target of mefloquine in *Streptococcus pneumoniae* is an $F_0F_1$ bifunctional ATP synthase/ATPase.[8] There is also a remarkable similarity of mefloquine when superimposed with R207910, which is currently in phase II clinical trial for tuberculosis (Figure 1).[9] The target of R207910 has recently been shown to be an ATP synthase. Although these data are suggestive of an ATPase target for mefloquine-based compounds, this has yet to be proven. Therefore we pursued a ligand-based drug design approach to optimize this class of molecules. In the previous preliminary examination of mefloquine analogues we demonstrated modestly improved anti-TB activity of **10a**[6] (Figure 1). In the current study we further explored modifica-

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tions in various regions of **10a** to better understand the anti-TB structure-activity relationships (SARs) of this class.

## Results and Discussion

In addition to the previously reported anti-TB and mammalian cell toxicity data for mefloquine and the hydrazone derivative **10a**, the current biological profiling demonstrated the advantages of **10a** over mefloquine racemate (Tables 1 and 2).

The anti-TB activity of **10a** is better than or equal to that of mefloquine while demonstrating significantly less toxicity at a receptor, cellular, and animal level. In the latter case, mefloquine at 40 and 80 mg kg$^{-1}$ po per day for four days resulted in a 5% and 10% reduction in body weight with the higher dosage group showing matted fur. In contrast **10a**, at dosages up to 200 mg kg$^{-1}$ was well tolerated with an increase in body weight of 5% (the same as untreated mice of the same age) over the same time course (higher dosages have not been used to date). In vitro studies suggest that mefloquine interacts with a number of potential neurological targets, however, the human adenosine receptor A2a is a strong candidate for mefloquine's neuropsychiatric side effects, given its suspected role in sleep modulation and its potent inhibition by mefloquine.[10–12] Compound **10a** showed less inhibition for both the A2a and the related A1 receptor. In addition, the antimicrobial spectrum of activity of **10a** is narrower than that of mefloquine (Table 2), a positive trait for a disease such as TB that requires long treatment duration. The overall profile of this molecule thus qualifies it as a lead candidate, and further analogues were therefore prepared.

**Table 2.** Spectrum of Activity of **10a**.

| Organism | ATCC[a] # | MIC (μM) | |
|---|---|---|---|
| | | Mefloquine | **10a** |
| *Enterococcus faecalis* | 29212 | 33.1 | >252.8 |
| *Staphylococcus aureus* | 29213 | 66.2 | >252.8 |
| *Klebsiella pneumoniae* | 35657 | 66.2 | >252.8 |
| *Acinetobacter baumannii* | Baa-747 | 264.8 | >252.8 |
| *Escherichia coli* | 25922 | 264.8 | >252.8 |
| *Candida krusei* | 6258 | 264.8 | >252.8 |
| *Enterobacter cloacae* | 49141 | >264.8 | >252.8 |
| *Pseudomonas aeruginosa* | 27853 | >264.8 | >252.8 |
| *Candida albicans* | 90028 | >264.8 | >252.8 |
| *Candida glabrata* | 90030 | >264.8 | >252.8 |

[a] American Type Culture Collection (Rockville, MD).

**Table 1.** Comparison of mefloquine and **10a**.

| Assay | Mefloquine | **10a** |
|---|---|---|
| MABA MIC, μM (μg/mL) | 13 (4.91) [a] | 9 (3.56) [a] |
| LORA MIC, μM (μg/mL) | 7 (2.65) [a] | 16 (7.46) [a] |
| Cytotoxicity for VERO cells, $IC_{50}$, μM | 11[b] | >128 [a] |
| Mouse maximum tolerated dose, po, mg kg$^{-1}$ | 40 | >200 |
| Human adenosine receptor A1, % inhibition at 0.1 μM | 28 | 2 |
| Human adenosine receptor A1, % inhibition at 1.0 μM | 89 | -1 |
| Human adenosine receptor A1, % inhibition at 10 μM | 105 | 12 |
| Human adenosine receptor A2a, % inhibition at 0.1 μM | 32 | -14 |
| Human adenosine receptor A2a, % inhibition at 1.0 μM | 81 | 2 |
| Human adenosine receptor A2a, % inhibition at 10 μM | 97 | 10 |

[a] Reference [6]. [b] In Reference [6] this was mistakenly reported as >128 μM.

First, we replaced the methyl substitution at the 4-position of the piperazine ring with longer aliphatic or unsaturated chains to explore the presumed binding cavity, and introduced additional basic groups to possibly improve binding affinity (Figure 2). The starting material, 2,8-bis-trifluoromethylquinolin-4-ol (**1**) was transformed to the bromide **2** by reaction with phosphorous oxybromide. The aldehyde **3** was prepared by treatment of the bromide with *n*-butyllithium and then DMF.[13] One of the required 4-substituted- piperazin-1-ylamines **6a** was commercially available, but the other building blocks **6b–f** were not. These were prepared by N-nitrosation of the requisite piperazine followed by Zn-HCl reduction.[14][15] The hydrazones **7a–f** were then generated by condensation of the aldehyde **3** with the piperazine derivatives **6a–f** in THF (Scheme 1).

The distal nitrogen atom present in the piperazine ring was replaced by oxygen to ascertain whether a basic amine group was necessary for the activity. The fact that compound **7a** showed no anti-TB activity (Table 3) led us to next consider appending different functional groups to the more remote nitrogen atom. The allyl substituted compound **7b**, the hydroxyethyl compound **7c**, and the unsubstituted compound **7e** all showed better activity than **10a** in the MABA (and less activity in the LORA). The best compound in this series, **7f**, bears a dimethylaminoethyl group which suggested that the longer chain containing a basic terminus may better complement the binding pocket (in the as yet unknown target protein), or that this group may result in better transport through the cell wall. These notions were also supported in part by the fact that protected nitrogen **7d** was not active in either the MABA or LORA. In addition, compound **7f** may not pass through the

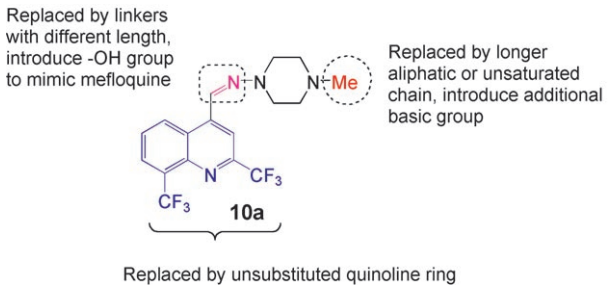


**Figure 2.** Modification of **10a**.

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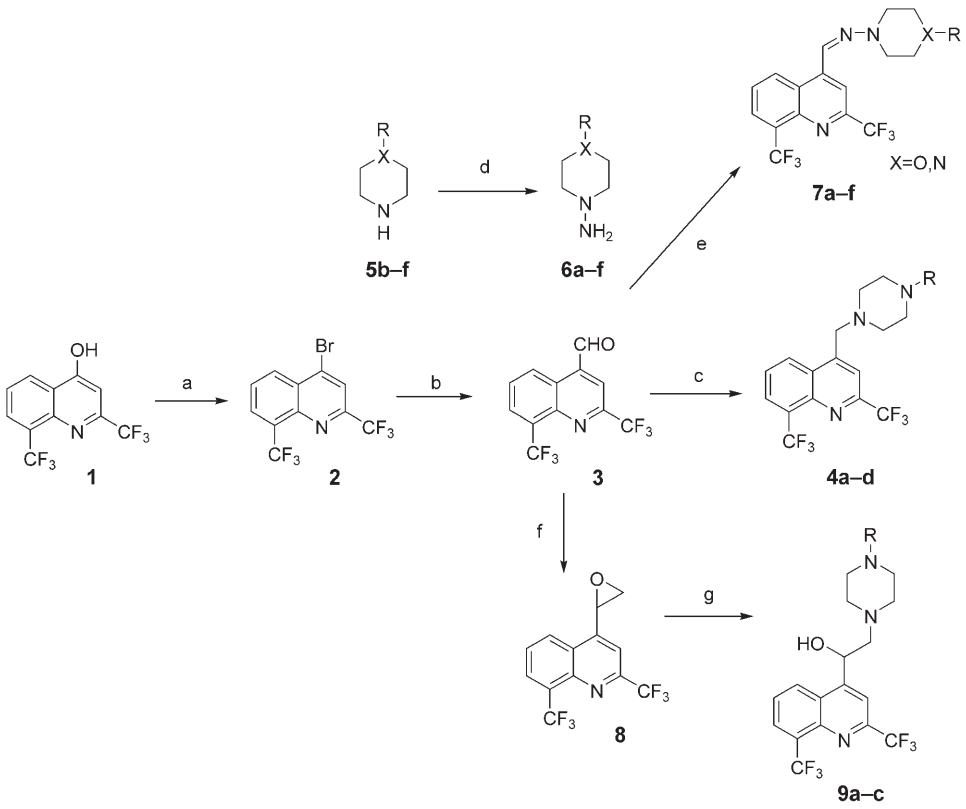


**Scheme 1.** Synthesis of mefloquine analogues displayed in Table 3. a. $POBr_3$, 75 °C for 10 min, 150 °C for 2 h, 95%, b. 1) *n*BuLi, ether, −78 °C, 15 min, 2) DMF, ether, −78 °C, 3 h, 53% c. piperazine derivatives, $NaBH(OAc)_3$, DCE, $N_2$, overnight, d. 1) $NaNO_2$, HCl, MeOH, 30 min, 2) Zn, HCl, MeOH, 30 min, e. PTSA, THF, overnight, f. trimethylsulfonium iodide, NaH/DMSO, THF, 2 h, 56%, g. piperazine derivatives, water, rt, overnight.

blood-brain barrier as its calculated LogBB[16] is around 0.07, which should preclude the CNS side effects observed with the parent compound mefloquine (LogBBs of mefloquine and **10a** are 0.32 and 0.33, respectively).

Second, to gauge the importance of the hydrazone bond, we replaced it with a one-carbon linker between the quinoline and piperazine moiety by carrying out a reductive amination reaction between aldehydes **3** and the piperazine derivatives in the presence of sodium triacetoxyborohydride[17] (Scheme 1).

Although compounds **4a**, **4c**, and **4d** had modest antituberculosis activities in MABA and were relatively nontoxic to the mammalian cells, none was better than the corresponding hydrazones. However, the MABA result indicates that a basic nitrogen terminus contributed to its anti-TB activity as compound **4d** showed the best MIC among this series, whereas the nitrogen protected compound **4b** did not show any anti-TB activity.

Furthermore, considering that most of the compounds **4a–d** were not toxic toward Vero cells, a hydroxyl group was introduced into a two-carbon linker, thus leading to analogues more closely resembling mefloquine. Whereas the Schiff base nitrogen can serve as an H-bond acceptor, this hydroxyl group could serve as a donor group. This simple change will again provide valuable information to build up a larger body of SAR results. The aldehyde **3** underwent the Corey–Chaykovsky reaction to give the corresponding epoxide **8**,[18] which was reacted in turn with nucleophilic amines in water and in the absence of any catalyst[19] to give the $\beta$-hydroxy-ethyl piperazines alcohol **9a–c** (Scheme 1).

All of the compounds **9a–c** were prepared and tested as racemates. Surprisingly, **9c** had little activity against *M. tuberculosis*. This may possibly be related to the different length of the linker between the dimethylamine terminus and the piperazine ring (three-carbon in **9c** versus two-carbon in **7f**). Despite a lack of cytotoxicity of this hydroxyl containing series, the anti-TB activities were much less than the corresponding hydrazones (**9a** versus **10a** and **9b** versus **7b**).

To evaluate the importance of the 2,8-bis-trifluoromethylquinoline moiety, the unsubstituted compound **12a** was prepared in the same way as compound **4d**. As was apparent from the data, the two trifluoromethyl group enhanced the anti-TB activity of **4d** as **12a** did not show any activity.

## Conclusions

In the present study, we have detailed the synthesis and evaluation of mefloquine-based hydrazone analogues. These compounds were tested for their MICs against both replicating and non-replicating persistent *M. tuberculosis* $H_{37}Rv$ strain by MABA and LORA respectively, and clear SARs were observed. In addition, the cytotoxicity data toward Vero cells ($IC_{50}$) and the desired selectivity indices provided useful information in directing our synthetic efforts. We confirmed the importance of having a quinoline ring present as the main scaffold, as well as two trifluoromethyl groups to maintain anti-TB activity. For substitution at the remote nitrogen of the piperazine ring, various aliphatic and unsaturated chains are tolerated and a basic terminus is preferred (see Figure 3). As compound **7f** is more active than mefloquine, and shows a reduced mammalian cell toxicity and less potential for CNS side effects compared with mefloquine, it may serve as a better lead candidate for TB drug discovery. We believe that further elaboration of this compound class is warranted, and these studies will be reported in due course.

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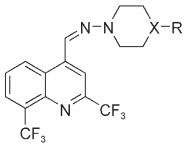
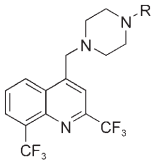
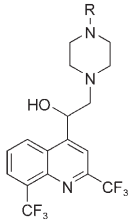
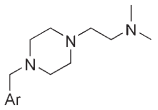
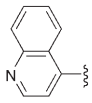
**Table 3.** In vitro activity of mefloquine-based ligands against *M. tuberculosis* $H_{37}Rv$.

| Compd. | Structure X | R | MIC (μM) MABA | LORA | $IC_{50}$ (μM) Vero cells | SI[a] MABA | LORA | Clog*P*[b] |
|---|---|---|---|---|---|---|---|---|
| | | |  | | | | | |
| **1** | - | Mefloquine | 13 | 7 | 11 | 0.9 | 1.6 | 3.9 |
| RMP | - | Rifampin | 0.1 | 2 | 122 | 1220 | 61 | - |
| **10a** | N | $CH_3$ | 9 | 16 | >128 | >14.2 | >8 | 3.3 |
| **7a** | O | - | >128 | >128 | >128 | - | - | 3.3 |
| **7b** | N | $CH_2CH{=}CH_2$ | 7.5 | 80.8 | 25.3 | 3.4 | 0.3 | 4.1 |
| **7c** | N | $CH_2CH_2OH$ | 7.3 | 31.3 | 24.2 | 3.3 | 0.8 | 2.3 |
| **7d** | N | $COOC(CH_3)_3$ | >128 | >128 | 11.4 | <0.1 | <0.1 | 4.9 |
| **7e** | N | H | 7 | 27.4 | 34.2 | 5 | 1.3 | 3.1 |
| **7f** | N | $CH_2CH_2N(CH_3)_2$ | 4 | 26.7 | 36.7 | 9 | 1.4 | 3.0 |
| | | |  | | | | | |
| **4a** | | $CH_3$ | 60 | 122.9 | >128 | >2.1 | >1.0 | 3.1 |
| **4b** | | $COOC(CH_3)_3$ | >128 | >128 | >128 | - | - | 4.3 |
| **4c** | | H | 52.8 | 100 | >128 | 2 | >1.3 | 2.8 |
| **4d** | | $CH_2CH_2N(CH_3)_2$ | 24.2 | 93.6 | 77.9 | 3 | 0.8 | 2.8 |
| | | |  | | | | | |
| **9a** | | $CH_3$ | 31.6 | >128 | >128 | 4 | - | 2.4 |
| **9b** | | $CH_2CH{=}CH_2$ | 31.1 | 119.8 | >128 | >4.1 | >1.0 | 3.2 |
| **9c** | | $(CH_2)_3N(CH_3)_2$ | 98.1 | >128 | >128 | >1.3 | - | 2.6 |
| | | |  | | | | | |
| **12a** | |  | >128 | >128 | >128 | - | - | 2.7 |

[a] Selectivity Index (SI) = $IC_{50}$/MIC. [b] Clog*P* (KOWWIN) calculated by using the website http://www.vcclab.org/lab/alogps/start.html.

## Experimental Section

### Biology:

**MICs**: MICs against *M. tuberculosis* were performed by microplate Alamar Blue assay (MABA[4]) and low oxygen recovery assay (LORA[5]).

MICs against all other microorganism were determined at equal weight/volume basis using a standard microbroth dilution assay,[20] and the data were expressed in μM.

**Human adenosine receptor A2a and A1 assay**: This radioisotope-based receptor-binding assay was outsourced to NovaScreen. Duplicates were performed at 0.1, 1, and 10 μM concentrations by modification of Jarvis's protocol.[21]

**Maximal tolerance dose**: Mefloquine and **10a** were administered by oral gavage to pairs of female BALB/c mice once daily for 5 day cycles, and mice were observed for overt signs of toxicity (weight loss, ruffled fur, huddling, etc.). The maximum administered dosages of mefloquine and **10a** were 40 and 200 mg $kg^{-1}$ of body weight, respectively.

### Chemistry:

**General information.** All starting materials were purchased from Sigma–Aldrich. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on Bruker spectrometer at 400 MHz and 100 MHz or 300 and 75 MHz respectively with TMS as an internal standard. The following abbreviations are used: DCM = dichloromethane; THF = tetrahydrofuran; Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, t = triplet, q = quadruplet and m = multiplet. Mass spectra were measured in the EI or ESI mode at an ionization potential of 70 *eV*; HRMS experiment was performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 250 mm $60F_{254}$ silica gel plates. Column chromatography was performed using Merck silica gel (40–60 mesh). Analytical HPLC was carried out on an Ace 5AQ column (25 cm × 4.6 mm), with detection at 254 and 366 nm on a Shimadzu SPD-10 A VP detector; flow rate = 2.0 mL $min^{-1}$; from 10% acetonitrile in water to 100% acetonitrile with 0.05% TFA.

**Synthesis of 4-bromo-2,8-bis-trifluoromethyl quinoline (2)**: Phosphorous oxybromide (1.02 g, 3.56 mmol) under nitrogen atmosphere was added to a 50 mL round bottom flask fitted with a condenser and stirred at 70 °C until all the solid was dissolved (usually within 5–10 min). 4-quinolinol **1** (1 g, 3.56 mmol) portion wise was added to this hot solution and the bath temperature was increased to 150 °C. Stirring was continued at this temperature for 2 h. After allowing to cool to room temperature the reaction mixture was quenched by the addition of ice-cold water (10 mL) and stirring was continued

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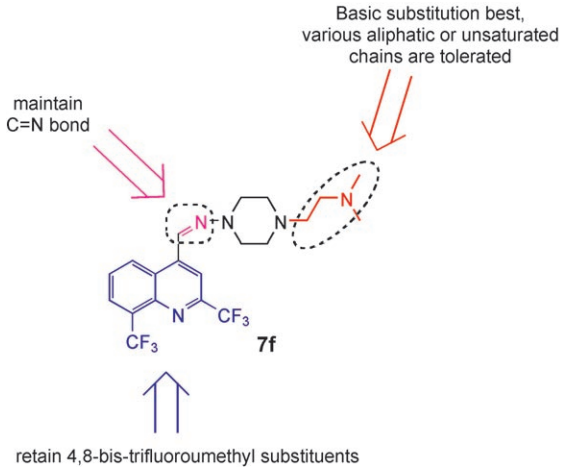


**Figure 3.** SAR of mefloquine-based ligands.

**Table 4.** Log*P*, PSA, FRB, and logBB values for **7f**, mefloquine, and **10a**.

| | **7f** | mefloquine | **10a** |
|---|---|---|---|
| log*P* | 3.0 | 3.9 | 3.3 |
| PSA | 35.0 | 45.1 | 31.7 |
| FRB | 7 | 4 | 4 |
| logBB | 0.07 | 0.32 | 0.33 |

for another 1 h. The precipitated bromide was then filtered through a sintered funnel by washing with an excess of purified water and dried under vacuum to obtain a colorless solid (1.16 g) in 95% yield. The bromide was then carried to the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.51 (d, J = 8.5 Hz, 1H), 8.26 (d, J = 7.2 Hz, 1H), 8.15 (s, 1H), 7.82 (t, J = 7.6 Hz, 1H); MS (APCI-LC MS) *m/z* 343.9 ($M^+$ + H, 100)

**Synthesis of 4-formyl- 2, 8-*bis*-trifluoromethyl quinoline (3)**: Refer to reference [13].

**General procedure to prepare compounds 4a–d**: 4-Formyl-2,8-bis-trifluoromethyl quinoline **3** (24 mg, 0.09 mmol) and 1-methyl piperazine (8.2 mg, 0.09 mmol) were mixed in 1, 2-dichloroethane (15 mL) and then treated with sodium triacetoxyborohydride (29 mg, 0.135 mmol). The mixture was stirred at room temperature under nitrogen atmosphere overnight. The reaction mixture was quenched by adding aqueous saturated $NaHCO_3$, and the product was extracted with EtOAc. The EtOAc extract was dried and the final product **4a** was purified by flash chromatography (EtOAc : Hexane = 1:4) with 94% yield. $^1$H NMR (400 MHz, $CDCl_3$): 8.43 (d, *J* = 8.4 Hz, 1H), 8.20 (d, *J* = 7.2 Hz, 1H), 7.87 (s, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 4.18 (s, 2H), 3.29 (m, 4H), 2.84 (m, 4H), 2.35 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$148.0, 144.8, 142.8, 129.3, 128.4, 127.6, 126.4, 126.0, 125.6, 123.5, 117.6, 58.8, 49.3, 43.1, 38.0; MS (APCI-LC MS) *m/z* 378.2 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{17}H_{17}F_6N_3 + H]^+$: 378.1399; found: 378.1397; HPLC purity: 98%.

**Compound 4b**: 90% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.54 (d, *J* = 8.4 Hz, 1H), 8.18 (d, *J* = 6.4 Hz, 1H), 7.86 (s, 1H), 7.75 (t, *J* = 8.0 Hz, 1H), 4.02 (s, 2H), 3.47 (m, 4H), 2.50 (m, 4H), 1.48(s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 154.7, 148.3, 147.6, 146.8, 143.9, 129.4, 128.8, 128.6, 126.9, 124.9, 122.5, 119.8, 79.9, 59.8, 53.2, 28.4; MS (APCI-LC MS) *m/z* 464.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{21}H_{23}F_6N_3O_2 + H]^+$: 464.1773; found: 464.1771; HPLC purity: 98%.

**Compound 4c**: 92% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.46 (d, *J* = 8.5 Hz, 1H), 8.23 (d, *J* = 7.2 Hz, 1H), 7.85 (s, 1H), 7.79 (t, *J* = 7.6 Hz, 1H), 4.16 (s, 2H), 3.30 (m, 4H), 2.90 (m, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.4, 144.0, 142.4, 129.6, 128.4, 127.0, 126.8, 126.3, 125.6, 123.9, 117.6, 58.8, 49.0, 43.5, MS (APCI-LC MS) *m/z* 364.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{16}H_{15}F_6N_3 + H]^+$: 364.1243; found: 364.1240; HPLC purity: 98%.

**Compound 4d**: 88% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.50(d, *J*=8.5 Hz, 1H), 8.23 (d, *J* = 8.5 Hz, 1H), 7.92 (s, 1H), 7.53(t, *J*=7.6 Hz, 1H), 4.10 (s, 2H), 2.82 (m, 4H), 2.68 (m, 8H), 2.36 (m, 6H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.6, 144.0, 142.0, 129.5, 128.7, 127.3, 126.6, 126.0, 125.6, 123.6, 117.7, 58.9, 49.5, 43.4, 40.4; MS (APCI-LC MS) *m/z* 435.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{20}H_{24}F_6N_4 + H]^+$: 435.1978; found: 435.1971; HPLC purity: 99%.

**General procedure to prepare the compound 6b–f**: Piperazine derivatives (0.79 mmol) in 6N HCl (0.47 mL) were cooled to −10 °C and a solution of $NaNO_2$ (58 mg, 0.79 mmol) in $H_2O$ (5 mL) was added slowly (keeping the temperature below 0 °C). After the addition was completed, the precipitate formed. It was filtrated, and the filtrate was adjusted to pH 10 using NaOH, then extracted with chloroform, dried over $Na_2SO_4$, and evaporated. To the crude extraction in 5 mL of $H_2O$ and 5 mL of MeOH, 1 mL of concentrated HCl was added (while striving vigorously) followed by Zn powder (1.284 g, 19.75 mmol), 5 mL of HCl was added dropwise, and the reaction was kept at around 20–30 °C for 30 min. Then NaOH solution was added until formation of zincate. The mixture was extracted with $CHCl_3$, dried over $Na_2SO_4$, and the solvent was evaporated to give the product.

**Compound 6b**: 90% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 5.88-(m, 1H), 5.17 (m, 2H), 3.10 (d, *J* = 6.4 Hz, 2H), 3.02 (m, 4H), 2.75(m, 4H); MS (APCI-LC MS) *m/z* 142.1 ($M^+$ + H, 100)

**Compound 6c**. 89% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.68 (t, *J* = 4.4 Hz, 2H), 2.88(m, 4H), 2.63(m, 4H), 2.51 (t, *J* = 4.4 Hz, 2H); MS (APCI-LC MS) *m/z* 146.2 ($M^+$ + H, 100)

**Compound 6d**: 85% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.66 (m, 4H), 3.21 (m, 4H), 1.38 (s, 9H); MS (APCI-LC MS) *m/z* 202.2 ($M^+$ + H, 100)

**Compound 6e**: 89% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.23 (m, 4H), 2.58(m, 4H); MS (APCI-LC MS) *m/z* 102.1 ($M^+$ + H, 100)

**Compound 6f**: 89% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 3.27 (m, 6H), 2.97(m, 2H), 2.89(m, 10H); MS (APCI-LC MS) *m/z* 173.1 ($M^+$ + H, 100)

**General Procedure to Prepare the compound 7a–f**: 1-mino-piperazine derivative **6a** (12.2 mg, 0.12 mmol) in the presence of *p*-toluene sulfonic acid (catalytic amount) under an argon atmosphere was added to a solution of aldehyde **3** (17.4 mg, 0.06 mmol) in anhydrous THF at room temperature

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and stirred the reaction mixture overnight. The reaction mixture was then concentrated under reduced pressure and the residue was purified by preparative thin layer chromatography (10% MeOH in $CH_3Cl$) to afford the product **7a** with 90% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.75(d, $J$=8.4 Hz, 1H), 8.16 (m, 2H), 8.08 (s, 1H), 7.73(t, $J$=7.6 Hz, 1H), 3.98 (t, $J$=4.8 Hz, 4H), 3.45 (t, $J$=4.8 Hz, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.5, 141.1, 140.2, 128.7, 128.3, 128.2, 127.6, 126.8, 126.4, 125.8, 120.5, 113.4, 101.2, 65.8, 50.7; MS (APCI-LC MS) $m/z$ 378.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{16}H_{13}F_6N_3O + H]^+$: 378.1041; found: 378.1041; HPLC purity: 98%.

**Compound 7b**: 88% yield. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.74(d, $J$=8.4 Hz, 1H), 8.15 (m, 2H), 8.00 (s, 1H), 7.71(t, $J$=7.6 Hz, 1H), 5.92 (m, 1H), 5.26 (m, 2H), 3.49 (t, $J$=4.8 Hz, 4H), 3.13 (d, $J$=6.6 Hz, 2H), 2.72(t, $J$=4.8 Hz, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.4, 144.6, 142.6, 134.4, 129.2, 128.9, 128.5, 126.6, 126.4, 126.1, 122.3, 120.0, 118.6, 113.4, 61.4, 52.0, 50.4; MS (APCI-LC MS) $m/z$ 417.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{19}H_{18}F_6N_4 + H]^+$: 417.1508; found: 417.1506; HPLC purity: 97%.

**Compound 7c**: 92% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.74(d, $J$=8.4 Hz, 1H), 8.15 (m, 2H), 8.02 (s, 1H), 7.71(t, $J$=7.6 Hz, 1H), 3.72 (t, $J$=4.4 Hz, 2H), 3.50 (t, $J$=4.8 Hz, 4H), 2.81(t, $J$=4.8 Hz, 4H), 2.69 (t, $J$=4.4 Hz, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.4, 144.6, 142.5, 129.3, 129.0, 128.7, 126.8, 126.6, 126.1, 122.7, 120.0, 113.4, 59.1, 58.0, 52.0, 50.4; MS (APCI-LC MS) $m/z$ 421.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{18}H_{18}F_6N_4O + H]^+$: 421.1458; found: 421.1455; HPLC purity: 98%.

**Compound 7d**: 94% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.72 (d, $J$=8.4 Hz, 1H), 8.15 (m, 2H), 8.04 (s, 1H), 7.77 (t, $J$=7.8 Hz, 1H), 3.71 (t, $J$=4.8 Hz, 4H), 3.46 (t, $J$=4.8 Hz, 4H), 1.50 (s, 9H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 154.1, 148.0, 144.3, 142.0, 129.0, 128.6, 128.3, 126.7, 126.4, 124.6, 121.9, 119.6, 113.2, 80.1, 49.9, 42.0, 29.0; MS (APCI-LC MS) $m/z$ 477.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{21}H_{22}F_6N_4O_2 + H]^+$: 447.1720; found: 448.1716; HPLC purity: 99%.

**Compound 7e**: 90% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.70 (d, $J$=8.4 Hz, 1H), 8.23 (m, 2H), 8.01 (s, 1H), 7.77 (t, $J$=7.8 Hz, 1H), 3.50 (t, $J$=4.8 Hz, 4H), 2.66 (t, $J$=4.8 Hz, 4H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.0, 144.5, 142.7, 129.0, 128.5, 128.0, 126.5, 126.1, 123.7, 121.4, 113.2, 102.1, 54.1, 50.3; MS (APCI-LC MS) $m/z$ 377.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{16}H_{14}F_6N_4$ + H]$^+$: 377.1195; found: 377.1190; HPLC purity: 99%.

**Compound 7f**: 90% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.70 (d, $J$=8.4 Hz, 1H), 8.17(d, $J$=7.2 Hz, 1H), 8.14(s, 1H), 8.04 (s, 1H), 7.73 (t, $J$=8.4 Hz, 1H), 3.50 (m, 4H), 3.29 (t, $J$=6.0 Hz, 2H), 2.99 (t, $J$=6.0 Hz, 2H), 2.91 (m, 10H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.2, 144.4, 142.6, 129.3, 128.4, 128.1, 126.6, 126.3, 123.4, 121.3, 113.0, 102.4, 59.1, 53.4, 52.8, 52.4, 43.4; MS (APCI-LC MS) $m/z$ 448.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{20}H_{23}F_6N_5 + H]^+$: 447.1930; found: 448.1923; HPLC purity: 99%.

**Synthesis of 4-Oxiranyl-2, 8-bis-trifluoromethyl-quinoline (8)**: Sodium hydride (5.6 mg of 80% suspension in mineral oil) was placed in a dry flask under argon. Dry dimethyl sulfoxide (3 mL) was introduced through the syringe. With stirring, the flask was immersed into a 70 °C bath for 1 h. Then the solution was cooled, THF (10 mL) was added, and the flask was immersed in an ice bath. A solution of trimethylsulfonium iodide (32 mg, 0.16 mmol) in dimethyl sulfoxide (3 mL) was added dropwise during a 10 min period and the reaction was allowed to proceed for 15 min. Next, a solution of 4-formyl-2,8-*bis*-trifluoromethyl quinoline **3** (38 mg, 0.13 mmol) in dimethyl sulfoxide-THF (1:1, 6 mL) was added dropwise and the brown solution was stirred for 15 min. Then the contents of the flask were poured into water and extracted with DCM. The combined extracts were washed with aqueous NaCl, dried ($Na_2SO_4$), and evaporated. The residue was purified by preparative thin layer chromatography (20% hexane in EtOAc) to afford the product (56%). $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.39(d, $J$=7.2 Hz, 1H), 8.22 (d, $J$=8.5 Hz, 1H), 7.81 (s, 2H), 4.56 (s, 1H), 3.44(d, $J$=2.4 Hz, 1H), 2.84 (d, $J$=2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.6 (q, $J$=34.9 Hz), 146.3, 142.9, 129.4, 128.8 (q, $J$=5.4 Hz), 127.2, 126.6, 124.3, 121.6, 119.3, 112.9, 50.4, 48.8 MS (APCI-LC MS) $m/z$ 306.0 ($M^+$ + H, 100)

**General Procedure to Prepare the Compound 9a–c**: The epoxide **8** (45 mg, 0.15 mmol) and water (3 mL) were placed in a flask equipped with a magnetic stirrer. Then an amine (0.73 mmol) was added in one portion and kept the reaction at room temperature under vigorous magnetic stirring overnight. The reaction mixture was extracted with diethyl ether and dried over $Na_2SO_4$. The solvent was removed under reduced pressure to give the product in the almost pure form.

**Compound 9a**: 95% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.20 (m, 3H), 7.75 (t, $J$=7.6 Hz, 1H), 5.58 (m, 1H), 2.91 (m, 6H), 2.38-(m, 7H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 153.0, 147.7, 143.4, 128.9, 128.5, 127.8, 127.3, 126.6, 125.0, 124.9, 114.5, 67.2, 63.1, 53.2, 42.1, 39.0; MS (APCI-LC MS) $m/z$ 408.2 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{18}H_{19}F_6N_3O + H]^+$: 408.1505; found: 408.1501; HPLC purity: 99%.

**Compound 9b**: 94% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.21-(m, 3H), 7.81 (t, $J$=7.6 Hz, 1H), 6.0 (m, 1H), 5.90(m, 1H), 5.62 (m, 2H), 3.65 (d, $J$=7.2 Hz, 2H), 3.22(m, 8H), 3.19 (d, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.8, 144.6, 142.3, 134.2, 129.5, 128.9, 128.7, 126.8, 126.4, 126.0, 122.2, 120.0, 113.2, 67.8, 63.5, 53.0, 42.5; MS (APCI-LC MS) $m/z$ 434.1 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{20}H_{21}F_6N_3O + H]^+$: 434.1662; found: 434.1655; HPLC purity: 99%.

**Compound 9c**: 90% yield. $^1$H NMR (400 MHz, $CD_3OD$): $\delta$ 8.55(d, $J$=8.8 Hz, 1H), 8.27(d, $J$=6.8 Hz, 1H), 8.16 (s, 1H), 7.88 (t, $J$=7.6 Hz, 1H), 5.76 (m, 1H), 3.48 (d, 2H), 2.75 (m, 12H), 2.58 (s, 6H), 1.96(m, 2H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 148.4, 144.5, 142.5, 129.3, 128.9, 128.4, 126.7, 126.4, 126.1, 122.4, 113.5, 67.6, 63.3, 53.2, 44.9, 42.5, 29.3; MS (APCI-LC MS) $m/z$ 479.2 ($M^+$ + H, 100); FAB-HRMS calcd for $[C_{22}H_{28}F_6N_4O + H]^+$: 479.2240; found: 479.2239; HPLC purity: 99%.

**Compound 12a**: The same procedure as for compounds **4a–d** was used but with quinoline aldehyde. 94% yield. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 8.85(d, $J$=8.5 Hz, 1H), 8.62 (t, $J$=7.6 Hz, 2H), 7.68 (s, $J$=8.5 Hz, 1H), 3.98 (s, 2H), 3.68 (m, 4H), 3.05 (t, 2H), 2.82 (t, 2H), 2.64 (m, 12H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ 149.4, 147.5, 144.3, 129.6, 129.1, 126.6, 124.1, 121.4, 102.3, 70.5, 59.1, 53.8, 52.8, 43.6; MS (APCI-LC MS) $m/z$ 299.1

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($M^+ + H$, 100); FAB-HRMS calcd for $[C_{18}H_{26}N_4 + H]^+$: 299.2230; found: 299.2229; HPLC purity: 97%.

**Calculation of Log BB**: ACD/LogBB Equation: = (0.205 × LogP)-(0.0094 × PSA)-(0.055 × FRB) + 0.18, and the PSA and FRB data were calculated by using website http://www.molinspiration.com/cgi-bin/properties (see Table 4 for values).

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**Keywords:** hydrazones · ligand-based drug design · mefloquine · structure–activity relationships · tuberculosis

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