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Mefloquine–oxazolidine derivatives, derived from mefloquine and arenecarbaldehydes: In vitro activity including against the multidrug-resistant tuberculosis strain T113

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ABSTRACT

Ten new mefloquine–oxazolidine derivatives, 4-[(1*S*,8a*R*)-3-(aryl)hexahydro[1,3]oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (**1**: aryl = substituted phenyl) and 4-[(1*S*,8a*R*)-3-(heteroaryl) hexahydro[1,3]oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline [**2**: heteroaryl = 5-nitrothien-2-yl (**2a**); 5-nitrofuran-2-yl (**2b**) and 4*H*-imidazol-2-yl) (**2c**)], have been synthesized and evaluated against *Mycobacterium tuberculosis*. Compounds **1f** (aryl = 3-ethoxyphenyl), **1g** (Ar = 3,4,5-$(MeO)_3$-C_6H_2) and **2c** were slightly more active than mefloquine (MIC = 33 μM) with MICs = 24.5, 22.5 and 27.4, respectively, whereas compounds **1e** (aryl = 3,4-$(MeO)_2$-C_6H_3) and **2a** (MICs = 11.9 and 12.1 μM, respectively) were ca. 2.7 times more active than mefloquine, with a better tuberculostatic activity than the first line tuberculostatic agent ethambutol (MIC = 15.9). The compounds were also assayed against the MDR strain T113 and the same MICs were observed. Thus the new derivatives have advantages over such anti-TB drugs as isoniazid, rifampicin, ethambutol and ofloxacin, for which this strain is resistant. The most active compounds were not cytotoxic to Murine Macrophages Cells in a concentration near their MIC values.

1. Introduction

Widespread dissemination of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant TB (XDR-TB) is one of the major health issues faced by a number of countries. These forms of TB do not respond to standard treatments with first-line anti-TB drugs and can take up to two years or more to treat with other drugs that are less potent, more toxic and much more expensive.[1] Thus the development of new substances, which possess different mechanisms of action and improved effects when compared with the currently available drugs, is regarded as an urgent global health priority.

We have been particularly interested in the development of new mefloquine (MFL) derivatives for use in TB treatment. Beyond its irrefutable ability in malaria treatment and prophylaxis, MFL has a broad range activity against Gram-positive and Gram-negative bacteria.[2] Furthermore as shown by Bermudez and coworkers,[3] MFL can inhibit the growth of *Mycobacterium* species in a MIC range of 21.1–42.1 μM.

Studies utilizing the bacteria *Streptococcus pneumoniae* displayed that the prokaryotic target of MFL is the *c* subunit of the cytoplasmatic membrane sector F_0 of the enzyme F_0F_1 ATPase.[4] Furthermore, it was observed that the exposure of *Mycobacterium tuberculosis* to MFL at a 4× MIC concentration lead to upregulation of the expression of gene coding membrane proteins, suggesting that the target for mefloquine in *M. tuberculosis* is in the cell wall.[5] However, there are no data in the literature showing molecular interactions of mefloquine with its target.

We recently reported the synthesis and the antitubercular evaluation against *M. tuberculosis* H37Rv (ATCC 27294) of several

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mefloquine–oxazolidine derivatives, 4-[(1*S*,8a*R*)-3-(aryl)hexahydro[1,3] oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl) quinoline, **1**, obtained from mefloquine and monosubstituted benzaldehydes, see Scheme 1.[6] Compounds bearing electron-releasing substituents, for example, (**1k**: aryl = 2-MeOC_6H_4), displayed appreciable activities, being more active than mefloquine and displaying an activity comparable with that of the first line tuberculostatic agent ethambutol.

We have continued our studies with an investigation of derivatives, which includes compounds containing additional electron releasing groups, for example, (**1**: aryl = 3,4-dimethoxyphenyl and 3,4,5-trimethoxyphenyl) and heteroaryl units, 1-(2,8-bis(trifluoromethyl)quinolin-4-yl)-3-(aryl)-hexahydro-1*H*-oxazolo[3,4-α]pyridine, (**2**: heteroaryl = 5-nitrothien-2-yl, 5-nitrofuran-2-yl and 4H-imidazol-2-yl). Furthermore, we have tested the compounds, found to be most active against *M. tuberculosis* H37Rv (ATCC 27294), on the multidrug-resistant tuberculosis strain T113.

The heteroaryl derivatives were chosen since compounds bearing such moieties display very important roles in the treatment of bacterial infections. Classical examples are the antimicrobial agents: nitrofurantoin, metronidazole and nitazoxanide.[7] Another illustration of the importance of heterocyclic groups in antibacterial compounds is shown by the antibiotic ranbezolid, an oxazolidinone, which contains a nitrofuryl substituent, and possesses an expanded spectrum of activity compared to linezolid.[8] Specifically for TB, the nitroaromatic class of antibiotics is one of the few that have shown activity against latent *M. tuberculosis*. The most important examples are PA-824 and OPC-67683, currently in clinical trial for TB treatment.[9]

2. Results and discussion

2.1. Chemistry

The synthesis of mefloquine–oxazolidine derivatives, **1** and **2**, is summarized in Scheme 1. Initially, the reactions of mefloquine with the arenealdehydes were carried out in toluene, utilizing a Dean–Stark apparatus. However, in several cases, mainly when electron donating groups were present in the aromatic ring, a low reactivity was observed and, even after 48–72 h, much starting material had not reacted. Better results were reached when the reactions were carried out in the presence of molecular sieves 4 Å and amberlyst 15, in toluene. For the syntheses of compounds **1a–d** and **2a–c** an excess of the aldehyde was used with complete consumption of mefloquine observed by TLC after 24–48 h. The reaction mixtures were filtered through Celite and concentrated to yield a semi-solid, which was triturated with cold ethanol, in order to produce the pure product. Compounds **1e–g** were very soluble in ethanol and purification by column chromatography was required. In these cases, an excess of mefloquine was used, since the chromatographic separation was found to be easier in the absence of any aldehyde.

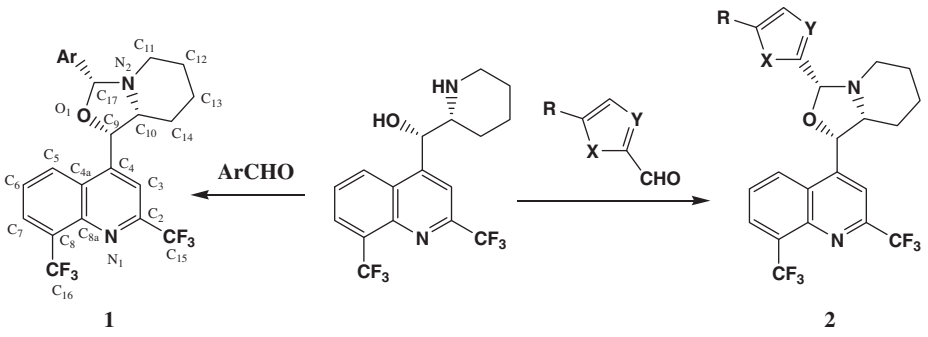
As observed in our previous work,[6] ring formation to give the oxazolidine system does not involve reaction at either of the chiral centres in the racemic *erythro* mefloquine reagent and hence the stereochemistries at these sites are preserved in the products. Two new chiral centres are generated in the oxazolidine product from the benzaldehyde carbonyl carbon and the piperidinyl nitrogen atom in mefloquine. As a racemic mefloquine reagent was used, so the product is formed as a racemic mixture. In most cases, except compound **2a**, the products were enantiomers with stereochemistries at C9, C10, C17 and N2 of (*R**), (*S**), (*S**) and (*R**), respectively. However, compound **2a** was formed as a mixture of diastereoisomers, as observed from the ^{1}H NMR spectrum, which displayed duplicated signals.

All compounds were identified by elementary analysis, ^{1}H NMR, ^{13}C NMR, IR spectra and MS data, and additionally in the case of **1g** by X-ray crystallography. The chemical shifts, multiplicities and coupling constants in the ^{1}H NMR spectra plus COSY experiments confirmed the proposed structures. For the oxazolidine ring, the protons, $H_{11'}$ appear as a characteristic singlet in the range 5.75–4.89 ppm. In the IR spectra, characteristic signals at 1311–1305 cm^{-1} were observed for the C–O axial deformation and at 1266–1080 cm^{-1} for the C–F axial deformation. Usually, more than one signal was observed for the CF_3 group as a result of the presence of rotational isomers.

The single crystal structure determination of **1g**, recrystallized from EtOH, was undertaken to confirm its stereochemistry and conformation.[10–12] The triclinic space group, P-1, was assigned, which being a non-chiral space group indicated that both enantiomers of **1g** are present in the single crystal examined. The chiral centres at C9, C10, C17 and N2 are assigned the stereochemistries (*R**), (*S**), (*S**) and (*R**), respectively. Figure 1 shows the atom arrangements in the (*R*), (*S*), (*S*), (*R*)-enantiomer. The bond angles and bond lengths are all in the expected regions. The piperidinyl ring has a near perfect chair conformation, while the five-membered 1,3-oxazolidine ring has an envelope shape with flap at the nitrogen atom. The angle between the plane of the quinoline moiety and (a) the plane of the phenyl ring is 35.9 Å and (b) with the best plane through the piperidinyl and oxazolidenyl rings is 87.9 Å. The only intermolecular interactions are weak C–H···O and C–H···F intermolecular hydrogen bonds and π···π stacking interactions.

2.2. Antimycobacterial activity against *M. tuberculosis* H37Rv (ATCC 27294)

The anti-mycobacterial activities of derivatives **1a–g** and **2a–c** are shown in Table 1. All compounds were assessed against



Scheme 1. Synthesis of mefloquine–oxazolidine derivatives.

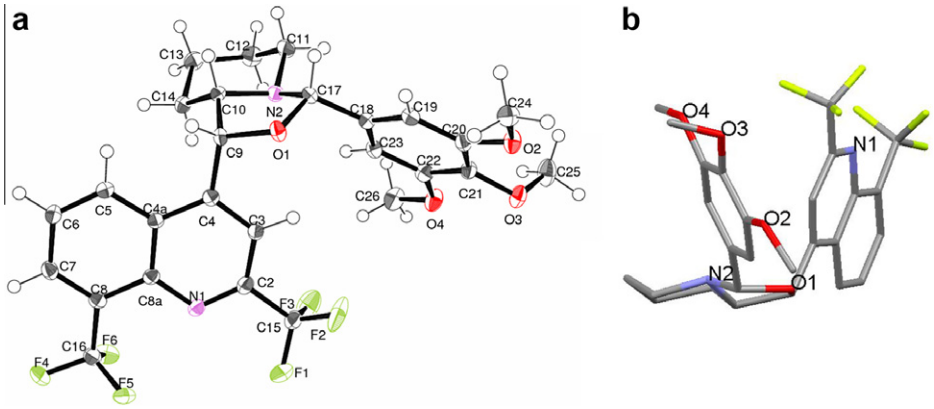


Figure 1. (a) Atom numbering scheme and atom arrangement for **1g**. Probability ellipsoids are drawn at the 50% level; (b) Conformation of **1g**. Hydrogen atoms have been omitted for clarity.

Table 1
Antimycobacterial activities against *M. tuberculosis* H37Rv (ATCC 27294) and MDR-TB strain T113

Compound	MIC[a] (μM) *M. tuberculosis* H37Rv(ATCC 27294)	MIC[a,b] (μM) MDR-TB strain T113
1a: Ar = 2,3-ClC_6H_3	>250	n.d.
1b: Ar = 3,4-ClC_6H_3	>250	n.d.
1c: Ar = 2,4-ClC_6H_3	>250	n.d.
1d: Ar = 2,6-ClC_6H_3	>250	n.d.
1e: Ar = 2,3-$(MeO)_2C_6H_3$	11.9	11.9
1f: Ar = 3-$EtOC_6H_4$	24.5	24.5
1g: Ar = 3,4,5-$(MeO)_3C_6H_2$	22.5	22.5
1h: Ar = 4-FC_6H_4	25.8[c]	25.8
1i: Ar = 2-HOC_6H_4	25.9[c]	25.9
1j: Ar = 4-HOC_6H_4	25.9[c]	25.9
1k: Ar = 2-$MeOC_6H_4$	12.6[c]	12.6
1l: Ar = 3-$MeOC_6H_4$	25.2[c]	25.2
1m: Ar = 4-$MeOC_6H_4$	25.2[c]	25.2
1n: Ar = C_6H_5	26.8[c]	26.8
2a: X = S, Y = CH, R = NO_2	12.1	12.1
2b: X = O, Y = CH, R = NO_2	99.8	99.8
2c: X = Y = N, R = H	27.4	27.4
Mefloquine	33	33
Ethambutol	15.9	61.2

[a] Minimum inhibitory concentration.
[b] Drug resistance profile: MDR strain resistant to isoniazid, rifampicin, ethambutol and ofloxacin.
[c] Data from Ref. 6.

M. tuberculosis H37Rv (ATCC 27294)[13] using the micro plate Alamar Blue assay (MABA),[14] a non-toxic methodology which employs a thermally-stable reagent and shows good correlation with proportional and BACTEC radiometric methods.[15,16] The assays were realized in triplicate and, in each case, a racemic mixture of the synthesized compound was used, with exception of compound **2a**, where the diastereoisomeric mixture was assayed.

In confirmation of the earlier findings, compounds, **1**, having electron donating substituent in the phenyl ring, exhibited excellent biological active.[6] The most active of the new compounds was the dimethoxy derivative (**1e**), with a MIC value of 11.9 μM, 2.8 times greater than that of the parent mefloquine (MIC = 33 μM). The new compound 3-ethoxy derivative (**1f**) had a similar activity to that previously reported for the 3-methoxy derivative (**1l**)[6] (24.5 and 25.2 μM, respectively), indicating that the longer alkyl chain length scarcely effected the biological activity. It is important to mention that the synthesis of the 2-ethoxy derivative was also attempted since, in our previous work,[6] the 2-methoxy derivative was identified as the most active compound. However, after several hours of reaction time, formation of the reaction product was not observed. This lack of reactivity can be attributed to the combination of the strong electron-donation character of the ethoxy group with steric effects caused by the presence of a longer alkyl chain in ortho position. The tri-substituted derivative (**1g**), while active, was less so than the dimethoxy derivative (**1e**). None of the dichlorophenyl compounds **1a-d** showed anti-tubercular activity, in keeping with the previous finding that compounds having electron withdrawing groups, such as chloro, had poor activities.

Among the three heteroaromatic compounds synthesized, **2a** displayed the best activity, with a MIC of 12.1 μM. However, the isosteric displacement of the 2-nitrothienyl moiety by a 2-nitrofuryl yielded the less active compound **2b**, with a MIC of 99.8 μM. The imidazole derivative **2c** displayed a modest activity with a MIC of 27.4 μM.

2.3. Evaluation against a MDR-TB strain

After evaluation against the standard strain of *M. tuberculosis* H37Rv (ATCC 27294), the active compounds, **1**, mainly the alkoxy derivatives, but also **1h** and **1n**, were tested against a MDR-TB strain, see Table 1. This strain, T113, was isolated from a clinical case of pulmonary tuberculosis in the city of Rio de Janeiro and belonged to the collection of the Bacteriology and Bioassay Laboratory—Instituto de Pesquisa Clínica Evandro Chagas—PEC—FIOCRUZ. The utilization of this strain was motivated by the fact that it is resistant to the main drugs utilized in the first line TB treatment (isoniazid, rifampicin and ethambutol) and one important fluoroquinolone utilized in the second line TB treatment (ofloxacin). The susceptibility profiles were evaluated though the non radiometric Bactec MGIT 960 (BT960) system and the micro plate Alamar Blue assay (MABA), at two laboratories in an inter-laboratory control program. Of some significance, the same MICs were observed as found for the standard strain H37Rv (ATCC 27294). Thus the new derivatives differ from isoniazid, rifampicin, ethambutol and ofloxacin, for which MDR strain T113 is resistant. Considering the alarming situation of MDR and XDR-TB, the identification of substances which can target these strains can be considered of great importance for the development of new anti-TB drugs.

2.4. Cell viability assay

The cellular viability in the presence and absence of test compounds was determined by Mosmans's MTT

Table 2
Data of cytotoxic effects of test compounds on Murine Macrophages Cells 18 h after the treatment

Compound	% Cell viability/doses (μM)		
	100	10	1
1a	100	100	100
1b	100	100	100
1c	98	100	100
1d	59	92	93
1e	100	100	100
1f	95	100	100
1g	97	100	100
2a	100	100	100
2b	100	100	100
2c	100	100	100
Mefloquine	70	81	95

(3-(4,5-demethylthylthiazol-2-yl)-2,5-dimethyl tetrazolium bromide; Merck) microcultured tetrazolium assay[17] at three different concentrations, 100, 10 and 1 μM. The results were expressed as percentage cell viability (Table 2). The active compounds were not cytotoxic to the host cells at concentrations near the MIC. However, mefloquine displayed a low cytotoxicity.

3. Conclusion

In this work, a series of mefloquine–oxazolidine derivatives, containing phenyl and heteroaryl substituents on the oxazolidine nucleus, has been synthesized and tested against *M. tuberculosis*. Among them, six compounds displayed modest to good antimicobacterial activities in the in vitro microplate Alamar Blue assay. A slight improvement in the biological activity was observed for compounds **1f**, **1g** and **2c** when compared with the prototype mefloquine. On the other hand, compounds **1e** and **2a** (MICs = 11.9 and 12.1 μM, respectively) were around 2.7 times more active than mefloquine. The compounds were evaluated against a MDR-TB strain and the same MICs were observed. The cell viability assay displayed that the compounds were not cytotoxic to Murine Macrophages Cells in a concentration near the MIC. When compared with the first line tuberculostatic agent ethambutol (MIC = 15.9), the mefloquine–oxazolidine derivatives **1e** and **2a** can be considered an important start point in the development of new tuberculostatic drugs.

4. Experimental

4.1. General procedures

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (ESI assay insolution of ammonium chloride) were recorded on Micromass ZQ Waters mass spectrometer. Elementary analyses were performed on a Perkin-Elmer 2400 CHN Elemental Analyser and a microbalance AD-4 autobalance Perkin-Elmer. NMR spectra were recorded on a Bruker Avance 400 operating at 400.00 MHz (^{1}H) and 100.0 MHz (^{13}C) and Bruker Avance 500 spectrometer operating at 500.00 MHz (^{1}H) and 125.0 MHz (^{13}C), in deuterated acetone. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and J-coupling in Hertz (Hz). Proton and carbon spectra were typically obtained at room temperature. For TLC plates coated with silica gel were run in ethyl acetate/hexane mixture and spots were developed in ultraviolet (λ = 254 nm).

4.2. Synthesis of the mefloquine derivatives 1a–d and 2a–c

A toluene solution (5 ml) containing 0.3 g (0.8 mmol) of mefloquine, 0.96 mmol of the respective aldehyde, 1 g of molecular sieves 4 Å and a catalytic amount of amberlyst 15 was heated under reflux during 24–48 h. The consumption of mefloquine was followed by TLC. The reaction mixture was filtered through Celite and concentrated to yield a semi-solid, which was triturated with cold ethanol, in order to produce the pure product.

4.2.1. 4-[(1*S*,8a*R*)-3-(2,3-Dichlorophenyl)hexahydro[1,3]-oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1a)

Yield 58% as a white solid. m.p. 155–156 °C. ^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 8.5 Hz; H_5 or H_7); 8.35 (1H; d; J = 7.5 Hz; H_5 or H_7); 7.98–7.94 (2H; m; $H_{4''}$ and $H_{3''}$ or $H_{5''}$); 7.85 (1H; s; H_3); 7.75 (1H; dd; J = 8.0 and 1 Hz; $H_{3''}$ or $H_{5''}$); 7.61 (1H; t; J = 7.5 Hz; H_6); 6.38 (1H; d; J = 8.0 Hz; $H_{1'}$); 5.43 (1H; s; $H_{11'}$); 3.32 (1H; dddd; J = 11.0, 8.0 and 2.5 Hz; $H_{2'}$); 2.92 (1H; d; J = 10.5 Hz; $H_{7'}$ or $H_{8'}$); 2.19 (1H; ddd; J = 13.5, 11.0 and 2.5 Hz; $H_{7'}$ or $H_{8'}$); 1.71 (1H; d; J = 12.0 Hz; $H_{3'}$ or $H_{4'}$); 1.65–1.57 (2H; m; $H_{9'}$ and $H_{10'}$); 1.43–1.27 (2H; m; $H_{5'}$ and $H_{6'}$); 0.38 (1H; dddd; J = 16.0, 12.0 and 4.0 Hz; $H_{3'}$ or $H_{4'}$) ppm. ^{13}C NMR (125.0 MHz DMSO-d_6) δ 151.7; 144.4; 140.5; 137.8; 134.6; 134.2; 132.4; 130.3; 130.2; 129.8; 129.3; 128.4; 128.3; 125.4; 122.3; 119.2; 117.3; 93.8; 77.9; 66.9; 48.8; 27.8; 24.8; 24.3 ppm. LC/MS: m/z [M+H] = 536. IR ν_{max} (cm^{-1}; KBr pellets): 1308 (C–O); 1181, 1141, 1110, 1080 (C–F). Anal. ($C_{24}H_{18}Cl_2F_6N_2O$) Theoretical: C, 53.85; H, 3.39; N, 5.23. Found: C, 54.26; H, 3.24; N, 5.16.

4.2.2. 4-[(1*S*,8a*R*)-3-(3,4-Dichlorophenyl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1b)

Yield 70% as a white solid. m.p. 184–185 °C. ^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 10.5 Hz; H_5 or H_7); 8.35 (1H; d; J = 9.0 Hz; H_5 or H_7); 8.04 (1H; s; H_3); 7.98–7.94 (2H; m; $H_{1''}$ or $H_{4''}$ or $H_{5''}$); 7.78–7.70 (2H; m; H_6 and $H_{1''}$ or $H_{4''}$ or $H_{5''}$); 6.33 (1H; d; J = 10.0 Hz; $H_{1'}$); 4.99 (1H; s; $H_{11'}$); 3.25 (1H; dddd; J = 13.5, 10.0 and 2.5 Hz; $H_{2'}$); 2.87–2.81 (1H; m; $H_{7'}$ or $H_{8'}$); 2.18–2.12 (1H; m; $H_{7'}$ or $H_{8'}$); 1.67–1.51 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$); 1.38–1.23 (2H; m; $H_{5'}$ and $H_{6'}$); 0.39 (1H; dddd; J = 20.0, 15.5 and 4.5 Hz; $H_{3'}$ or $H_{4'}$) ppm. ^{13}C NMR (125.0 MHz DMSO-d_6) δ 151.8; 144.1; 139.2; 133.9; 133.4; 132.0; 131.3; 130.4; 130.3; 129.8; 129.6; 128.5; 128.4; 117.1; 96.2; 77.7; 67.1; 48.5; 27.8; 24.8; 24.3 ppm. LC/MS: m/z [M+H] = 536. IR ν_{max} (cm^{-1}; KBr pellets): 1307 (C–O); 1211, 1182, 1151, 1134, 1112 (C–F). Anal. ($C_{24}H_{18}Cl_2F_6N_2O$) Theoretical: C, 53.85; H, 3.39; N, 5.23. Found: C, 53.99; H, 3.19; N, 5.23.

4.2.3. 4-[(1*S*,8a*R*)-3-(2,4-Dichlorophenyl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1c)

Yield 52% as a white solid. m.p. 162–163 °C. ^{1}H NMR [400.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.36 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.03–7.95 (2H; m; H_6 and $H_{2''}$ or $H_{4''}$ or $H_{5''}$); 7.87 (1H; s; H_3); 7.67–7.63 (2H; m; $H_{2''}$ or $H_{4''}$ or $H_{5''}$); 6.37 (1H; d; J = 8.0 Hz; $H_{1'}$); 5.39 (1H; s; $H_{11'}$); 3.31 (1H; dddd; J = 12.0, 8.0 and 4.0 Hz; $H_{2'}$); 2.90–2.89 (1H; m; $H_{7'}$ or $H_{8'}$); 2.21–2.15 (1H; m; $H_{7'}$ or $H_{8'}$); 1.70 (1H; d; J = 12.0 Hz; $H_{3'}$ or $H_{4'}$); 1.65–1.56 (2H; m; $H_{9'}$ and $H_{10'}$); 1.42–1.26 (2H; m; $H_{5'}$ and $H_{6'}$); 0.38 (1H; dddd; J = 16.0, 12.0 and 4.0 Hz; $H_{3'}$ or $H_{4'}$) ppm. ^{13}C NMR (100.0 MHz DMSO-d_6) δ 151.8; 148.2; 144.0; 137.4; 136.4; 134.3; 131.3; 130.8; 130.4; 130.3; 129.8; 128.7; 128.5; 126.2; 123.5; 121.1; 117.2; 92.9; 77.8; 66.9; 48.7; 27.8; 24.8; 24.3 ppm. LC/MS: m/z [M+H] = 536. IR ν_{max} (cm^{-1}; KBr pellets): 1308 (C–O); 1212, 1186, 1159, 1110, 1089 (C–F). Anal.

($C_{24}H_{18}Cl_{12}F_6N_2O$) Theoretical: C, 53.85; H, 3.39; N, 5.23. Found: C, 53.83; H, 3.10; N, 5.26.

4.2.4. 4-[(1*S*,8a*R*)-3-(2,6-Dichlorophenyl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1d)

Yield 65% as a white solid. m.p. 224–225 °C. ^{1}H NMR [200.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.63–8.59 (2H; m); 8.35 (1H; d; J = 8.0 Hz; H_5 or H_7); 7.97 (1H; t; J = 8.0 Hz; H_6); 7.57–7.45 (3H; m); 6.30 (1H; d; J = 10.0 Hz; $H_{1'}$); 5.75 (1H; s; $H_{11'}$); 3.24–3.14 (1H; m; $H_{2'}$); 2.90–2.69 (1H; m; $H_{7'}$ or $H_{8'}$); 2.21–2.04 (1H; m; $H_{7'}$ or $H_{8'}$); 1.66–1.20 (5H; m; $H_{5'}$, $H_{6'}$, $H_{9'}$, $H_{10'}$ and $H_{3'}$ or $H_{4'}$); 0.53 (1H; dddd; J = 16.0, 12.0 and 4.0 Hz; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 151.1; 144.1; 139.4; 136.2; 132.5; 132.4; 130.6; 130.3; 130.1; 129.7; 129.1; 128.5; 128.3; 122.2; 117.2; 94.0; 76.9; 66.9; 48.2; 27.9; 24.8; 24.7 ppm. LC/MS: m/z [M+H] = 536. IR ν_{max} (cm^{-1}; KBr pellets): 1308 (C–O); 1215, 1188, 1140, 1108 (C–F). Anal. ($C_{24}H_{18}Cl_{12}F_6N_2O$) Theoretical: C, 53.85; H, 3.39; N, 5.23. Found: C, 53.83; H, 3.49; N, 5.13.

4.2.5. 4-[(1*S*,8a*R*)-3-(5-Nitro-2-thienyl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (2a)

Yield 50% as a brown solid. m.p. 160 °C (dec.). ^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.67 (1H; d; J = 8.3 Hz; H_5 or H_7); 8.36 (1H; d; J = 7.3 Hz; H_5 or H_7); 8.25 (1H; s; H_3); 8.09 (1H; d, J = 4.4 Hz; $H_{3''}$); 7.97 (1H; t; J = 7.8 Hz; H_6); 7.55 (1H; d, J = 4.4 Hz; $H_{4''}$); 6.40 (1H; d; J = 8.3 Hz; $H_{1'}$); 5.34 (1H; s; $H_{11'}$); 3.33–3.31 (1H; m; $H_{2'}$); 3.03 (1H; d; J = 10.7 Hz; $H_{7'}$ or $H_{8'}$); 2.31–2.23 (1H; m; $H_{7'}$ or $H_{8'}$); 1.63–1.59 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$); 1.35–1.28 (2H; m; $H_{5'}$ and $H_{6'}$); 0.44–0.35 (1H; m; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 153.6; 151.1; 150.6; 148.6; 144.1; 130.4; 130.4; 130.3; 129.9; 129.8; 129.7; 129.4; 128.9; 128.5; 128.2; 127.4; 125.8; 123.7; 117.0; 92.4; 88.2; 78.9; 77.9; 67.1; 61.2; 48.6; 45.6; 27.7; 24.7; 24.2; 24.1; 23.9; 21.9 ppm. LC/MS: m/z [M+H] = 518. IR ν_{max} (cm^{-1}; KBr pellets): 1309 (C–O); 1266, 1212, 1188, 1147, 1110, 1087 (C–F). Anal. ($C_{22}H_{17}F_6N_3O_3S$) Theoretical: C, 51.07; H, 3.31; N, 8.12. Found: C, 50.92; H, 3.18; N 8.05.

4.2.6. 4-[(1*S*,8a*R*)-3-(5-Nitro-2-furyl)hexahydro[1,3]oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (2b)

Yield 45% as a yellow solid. m.p. 185–186 °C. ^{1}H NMR [500.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 8.5 Hz; H_5 or H_7); 8.35 (1H; d; J = 7.5 Hz; H_5 or H_7); 8.32 (1H; s; H_3); 7.96 (1H; t; J = 8.0 Hz; H_6); 7.65 (1H; d; J = 3.5 Hz; $H_{3''}$); 7.15 (1H; d; J = 4.0 Hz; $H_{4''}$); 6.39 (1H; d; J = 8.0 Hz; $H_{1'}$); 5.14 (1H; s; $H_{11'}$); 3.21 (1H; dddd; J = 11.0, 8.0 and 2.5 Hz; $H_{2'}$); 3.01 (1H; d; J = 10.5 Hz; $H_{7'}$ or $H_{8'}$); 2.27–2.22 (1H; m; $H_{7'}$ or $H_{8'}$); 1.65 (1H; d; J = 12.5 Hz; $H_{3'}$ or $H_{4'}$); 1.61–1.57 (2H; m; $H_{9'}$ and $H_{10'}$); 1.34–1.23 (2H; m; $H_{5'}$ and $H_{6'}$); 0.34 (1H; dddd; J = 15.5, 12.0 and 3.5 Hz; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 154.8; 151.2; 130.3; 130.2; 129.6; 128.5; 128.3; 117.4; 114.6; 113.0; 90.5; 77.9; 66.9; 48.6; 27.4; 24.7; 24.1 ppm. LC/MS: m/z [M+H] = 502. IR ν_{max} (cm^{-1}; KBr pellets): 1306 (C–O); 1186, 1140, 1111 (C–F). Anal. ($C_{22}H_{17}F_6N_3O_4$) Theoretical: C, 52.70; H, 3.42; N, 8.38. Found: C, 52.81; H, 3.38; N, 8.37.

4.2.7. 4-[(1*S*,8a*R*)-3-(1*H*-Imidazol-2-yl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (2c)

Yield 52% as a yellow solid. m.p. 210–212 °C. ^{1}H NMR [400.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.67 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.48 (1H; s; H_3); 8.34 (1H; d; J = 8.0 Hz; H_5 or H_7); 7.93 (1H; t; J = 8.0 Hz; H_6); 7.25 (1H; sl; $H_{2''}$); 7.12 (1H; sl; $H_{3''}$); 6.31 (1H; d; J = 8.0 Hz; $H_{1'}$); 5.10 (1H; s; $H_{11'}$); 3.19 (1H; dddd; J = 12.0, 8.0 and 4.0 Hz; $H_{2'}$); 2.95 (1H; d; J = 12.0 Hz; $H_{7'}$ or $H_{8'}$); 2.22–2.11 (1H; m; $H_{7'}$ or $H_{8'}$); 1.67–1.53 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$); 1.35–1.22 (2H; m; $H_{5'}$ and $H_{6'}$); 0.37 (1H; dddd; J = 16.0, 12.0 and 4.0 Hz; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (100.0 MHz DMSO-d_6) δ 150.7; 147.9; 133.5; 144.8; 143.0; 142.7; 129.2; 129.1; 128.8; 127.4; 127.3; 125.3; 124.4; 122.6; 117.0; 90.7; 76.6; 65.9; 47.7; 26.7; 23.7; 23.3 ppm. LC/MS: m/z [M+H] = 457. IR ν_{max} (cm^{-1}; KBr pellets): 1305 (C–O); 1212, 1184, 1144, 1112 (C–F). Anal. ($C_{21}H_{18}F_6N_4O$) Theoretical: C, 55.27; H, 3.98; N, 12.28. Found: C, 54.78; H, 3.98; N, 12.78.

4.3. Synthesis of the mefloquine derivatives 1e–g

A toluene solution (5 ml) containing 0.3 g (0.8 mmol) of mefloquine, 0.67 mmol of the respective aldehyde, 1 g of molecular sieves 4 Å and a catalytic amount of amberlyst 15 was heated under reflux during 24–48 h. The consumption of the aldehyde was observed by TLC. After, the reaction mixture was filtered through Celite and concentrated to yield a semi-solid, which was purified by chromatographic column using hexane/ethyl acetate as the eluent.

4.3.1. 4-[(1*S*,8a*R*)-3-(2,3-Dimethoxyphenyl)hexahydro[1,3]oxazolo-[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1e)

Yield 58% as a white solid. m.p. 130–131 °C. ^{1}H NMR [400.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.35 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.24 (1H; s; H_3); 7.96 (1H; t; J = 8.0 Hz; H_6); 7.35 (1H; d; J = 3.0 Hz; $H_{1''}$); 7.24 (1H; dd; J = 8.0 and 3.0 Hz; $H_{5''}$); 7.08 (1H; d; J = 8.0 Hz; $H_{4''}$); 6.26 (1H; d; J = 4.0 Hz; $H_{1'}$); 4.89 (1H; s; $H_{11'}$); 3.90 (3H; s; OCH_3); 3.88 (3H; s; OCH_3); 3.20–3.15 (1H; m; $H_{2'}$); 2.94–2.90 (1H; m; $H_{7'}$ or $H_{8'}$); 2.09–2.05 (1H; m; $H_{7'}$ or $H_{8'}$); 1.63–1.53 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$) 1.36–1.25 (2H; m; $H_{5'}$ and $H_{6'}$); 0.38 (1H; dddd; J = 16.0, 12.0 and 4.0 Hz; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 152.5; 151.5; 150.8; 148.4; 144.0; 130.3; 130.2; 129.8; 128.5; 128.4; 122.4; 117.4; 117.3; 112.6; 112.3; 97.9; 77.0; 67.1; 55.9; 56.2; 48.5; 27.8; 24.9; 24.4 ppm. LC/MS: m/z [M+H] = 527. IR ν_{max} (cm^{-1}; KBr pellets): 1311 (C–O); 1172, 1136, 1107 (C–F). Anal. ($C_{26}H_{24}F_6N_2O_3$) Theoretical: C, 59.32; H, 4.59; N, 5.32. Found: C, 59.45; H, 4.68; N, 5.27.

4.3.2. 4-[(1*S*,8a*R*)-3-(3-Ethylphenyl)hexahydro[1,3]oxazolo[3,4-*a*]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinoline (1f)

Yield 55% as a white solid. m.p. 110–112 °C. ^{1}H NMR [200.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.67 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.34 (1H; d; J = 6.0 Hz; H_5 or H_7); 8.22 (1H, s); 7.95 (1H; t; J = 8.0 Hz); 7.44 (1H; t; J = 8.0 Hz); 7.30–7.27 (2H, m); 7.05 (1H, d, J = 8.0 Hz); 6.28 (1H; d; J = 8.0 Hz; $H_{1'}$); 4.91 (1H; s; $H_{11'}$); 4.14 (2H; q; J = 6.0 Hz; OCH_2CH_3); 3.23–3.14 (1H; m; $H_{2'}$); 2.88–2.83 (1H; m; $H_{7'}$ or $H_{8'}$); 2.15–2.04 (1H; m; $H_{7'}$ or $H_{8'}$); 1.66–1.46 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$); 1.42–1.16 (5H; m; $H_{5'}$, $H_{6'}$ and OCH_2CH_3); 0.42–0.36 (1H; m; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 160.6; 152.3; 144.0; 139.6; 130.7; 130.3; 130.2; 129.8; 128.4; 125.3; 121.6; 117.4; 116.6; 115.1; 97.8; 77.3; 67.1; 64.1; 48.5; 27.8; 24.9; 24.4; 15.2 ppm. LC/MS: m/z [M+H] = 511. IR ν_{max} (cm^{-1}; KBr pellets): 1307 (C–O); 1203, 1184, 1143, 1109, 1087 (C–F). Anal. ($C_{26}H_{24}F_6N_2O_2$) Theoretical: C, 61.17; H, 4.74; N, 5.49. Found: C, 61.10; H, 4.92; N, 5.51.

4.3.3. 2,8-bis(Trifluoromethyl)-4-[(1*S*,8a*R*)-3-(3,4,5-trimethoxyphenyl)hexahydro[1,3]oxazolo[3,4-*a*]pyridin-1-yl]-quinoline (1g)

Yield 72% as a white solid. m.p. 147–149 °C. ^{1}H NMR [200.00 MHz (FIDRES ± 0.12 Hz), $(CH_3)_2CO$-d_6] δ: 8.68 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.35 (1H; d; J = 8.0 Hz; H_5 or H_7); 8.22 (1H; s; H_3); 7.96 (1H; t; J = 8.0 Hz; H_6); 7.05 (2H; s; $H_{1''}$ and $H_{5''}$); 6.28

(1H; d; J = 8.0 Hz; $H_{1'}$); 4.89 (1H; s; $H_{11'}$); 3.92 (9H; s; OCH_3); 3.24–3.15 (1H; m; $H_{2'}$); 2.92–2.82 (1H; m; $H_{7'}$ or $H_{8'}$); 2.13–2.04 (1H; m; $H_{7'}$ or $H_{8'}$); 1.64–1.43 (3H; m; $H_{3'}$ or $H_{4'}$, $H_{9'}$ and $H_{10'}$); 1.37–1.23 (2H; m; $H_{5'}$ and $H_{6'}$); 0.47–0.31 (1H; m; $H_{3'}$ or $H_{4'}$) ppm.

^{13}C NMR (50.0 MHz DMSO-d_6) δ 154.9; 152.4; 149.0; 148.2; 144.1; 140.3; 133.4; 130.3; 130.2; 129.8; 128.4; 117.2; 107.6; 106.6; 98.0; 77.1; 67.13; 60.6; 56.7; 56.4; 48.6; 27.8; 24.9; 24.4 ppm. LC/MS: m/z [M+H] = 557. IR ν_{max} (cm^{-1}; KBr pellets): 1305 (C–O); 1232, 1211, 1180, 1163, 1128, 1111 (C–F). Anal. ($C_{27}H_{26}F_6N_2O_4$) Theoretical: C, 58.27; H, 4.71; N, 5.03. Found: C, 58.13; H, 4.24; N, 5.01.

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