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Short communication

1,5-Diaryl-2-ethyl pyrrole derivatives as antimycobacterial agents: Design, synthesis, and microbiological evaluation

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ABSTRACT

During the search of novel antitubercular drugs related to BM 212, new diarylpyrroles were designed and synthesized on the basis of a structure–activity relationship analysis of many pyrroles previously described by us. Among them, 1-(4-fluorophenyl)-2-ethyl-3-(thiomorpholin-4-yl)methyl-5-(4-methylphenyl)-1*H*-pyrrole (**2b**) proved to be particularly active, with a minimum inhibitory concentration (MIC, expressed as μg/mL) and a protection index (PI) better than or comparable to those of reference compounds. Also the remaining compounds were very active, although their MIC and PI were in general lower than those of their parent 2-methyl analogues.

1. Introduction

Mycobacterium tuberculosis (MTB), responsible for tuberculosis (TB) in humans, is positioned as the leading bacterial infectious agent [1,2]. Its synergy with the HIV-1 epidemic led TB to increase, in particular in some parts of the world, such as the World Health Organization (WHO) African Region, where a large part of new TB cases were attributable to HIV-1 co-infection [3]. Furthermore, the emergence of MTB strains resistant to all of the first-line drugs (leading to the multi drug-resistant TB, MDR-TB) [4] and to isoniazid and rifampin plus resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (leading to the extensive drug-resistant TB, XDR-TB) [5], is causing serious concern in some countries, because patients could become virtually untreatable using currently available anti-TB drugs. In addition, there have been no new drugs to treat TB in the last 40 years, with the exception of fluoroquinolones recently introduced [6], reflecting the inherent difficulties in discovery and clinical testing of new antitubercular agents and the lack of pharmaceutical industry research in the area [7]. As a consequence, the development of new drugs with potent activity toward MDR- and XDR-TB (as well as toward latent TB and infections caused by *Mycobacterium avium* complex) is urgently needed.

In continued attempts to identify new potent antimycobacterial compounds, we have previously described many pyrrole derivatives endowed with a high inhibitory activity toward MTB, including intramacrophagic mycobacteria and strains resistant to the antitubercular drugs currently used in therapy [8]. Among these compounds, 1,5-(4-chlorophenyl)-2-methyl-3-(4-methylpiperazin-1-yl)methyl-1*H*-pyrrole (BM 212, **1a**, Chart 1) proved to be the most active and it was considered the lead compound of this new series of derivatives. Structure–activity relationship (SAR) studies, combined with a pharmacophoric model for antimycobacterial compounds [8e], allowed us to find derivatives of **1a** (compounds **1b**–**h**, Chart 1) in which the following substituents and substitution pattern on the pyrrole ring were identified as responsible for the

Abbreviations: MTB, *Mycobacterium tuberculosis*; TB, tuberculosis; WHO, World Health Organization; MDR, multi drug-resistant; XDR, extensively drug-resistant; BM 212, 1,5-(4-chlorophenyl)-2-methyl-3-(4-methylpiperazin-1-yl)methyl-1*H*-pyrrole; SAR, structure–activity relationship; MIC, minimum inhibitory concentration; MNTD, maximum non-toxic dose; PI, protection index; DMEM, Dulbecco's minimum essential medium.

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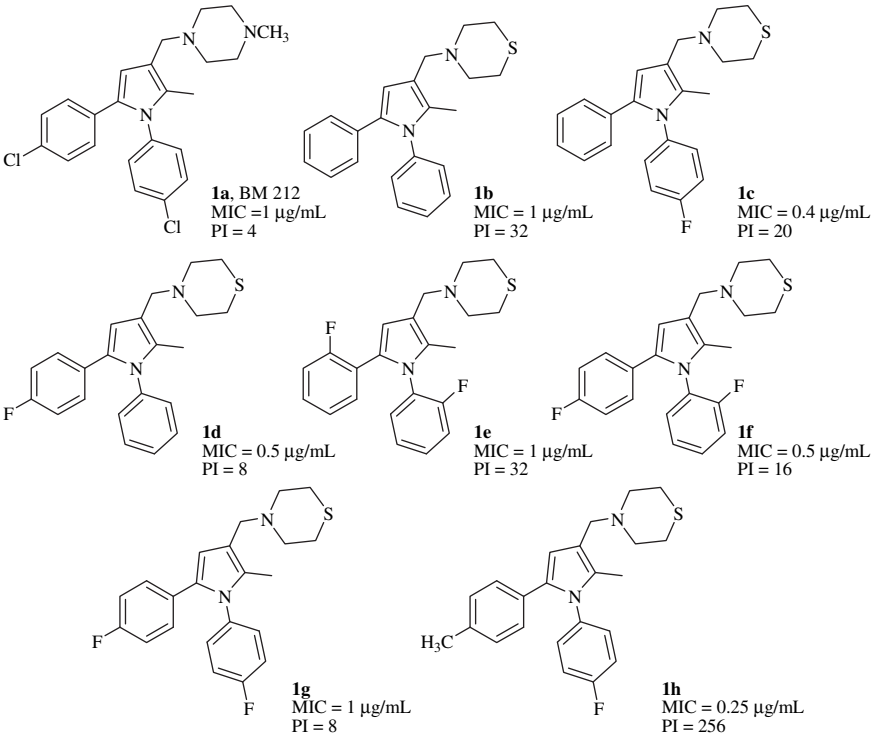
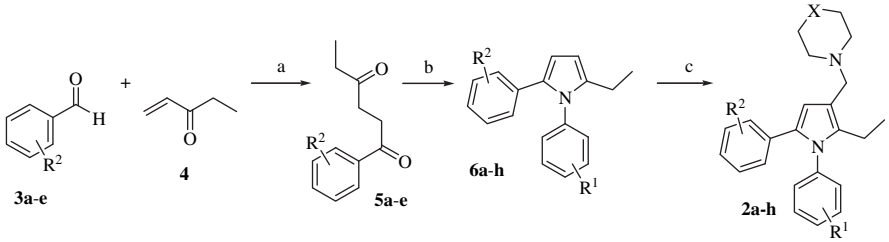


Chart 1. Schematic representation of 1,5-diarylpyrroles **1b–h** analogues of BM 212 (**1a**).

activity: (i) a substituted phenyl ring at both the positions 1 and 5 (F, Cl, and CH_3 were the best substituents) and (ii) an amino methyl group at position 3 (a thiomorpholinomethyl side chain was the optimal moiety) (Chart 1).

Moreover, SAR studies also suggested that a relationship does exist among MIC values and the lipophilic character of pyrrole derivatives [8a, 8b], leading us to the hypothesis that an increase of lipophilicity could improve antimycobacterial activity of such a class of compounds (as also stated by literature reports) [9–11]. As examples, the most active compounds (bearing a propyl or isopropyl substituent at one of the two phenyl rings of the diarylpyrrole scaffold) showed the highest calculated log *P* values among our pyrrole derivatives (7.23 and 7.03, respectively) and an activity higher with respect to that of their ethyl or methyl congeners [8a].

On this basis, to enlarge SAR considerations on such a class of compounds, and in view to verify the importance of the combination of both structural and lipophilic factors for the activity, we planned the synthesis of the new derivatives **2a–h** bearing an ethyl group at position 2 of the pyrrole nucleus, while keeping, on both N1 and C5 phenyl rings, the same substituents that gave the best results in terms of activity in previous 2-methyl derivatives [8b–8e]. Moreover, a thiomorpholinomethyl side chain at position 3 of the pyrrole was maintained, with the exception of compound **2a** (the corresponding ethyl derivative of the lead compound **1a**, in which the thiomorpholine was replaced by a *N*-methylpiperazine moiety). The new derivatives **2a–h** were evaluated for their activity toward various strains of MTB, in comparison to **1a** and current antitubercular drugs used as reference compounds.



Compounds. **3a**: R^2 = 4-Cl; **3b**: R^2 = 4-CH_3; **3c**: R^2 = H; **3d**: R^2 = 4-F; **3e**: R^2 = 2-F; **5a**: R^2 = 4-Cl; **5b**: R^2 = 4-CH_3; **5c**: R^2 = H; **5d**: R^2 = 4-F; **5e**: R^2 = 2-F; **6a**: R^2 = 4-Cl; R^1 = 4-Cl; **6b**: R^2 = 4-CH_3; R^1 = 4-F; **6c**: R^2 = H; R^1 = H; **6d**: R^2 = 4-F; R^1 = H; **6e**: R^2 = H; R^1 = 4-F; **6f**: R^2 = 4-F; R^1 = 4-F; 13g: R^2 = 4-F; R^1 = 2-F; **6h**: R^2 = 2-F; R^1 = 2-F; **2a**: R^1 = 4-Cl; R^2 = 4-Cl; X = N-CH_3; **2b**: R^2 = 4-CH_3; R^1 = 4-F; X = S; **2c**: R^2 = H; R^1 = H; X = S; **2d**: R^2 = 4-F; R^1 = H; X = S; **2e**: R^2 = H; R^1 = 4-F; X = S; **2f**: R^2 = 4-F; R^1 = 4-F; X = S; **2g**: R^2 = 4-F; R^1 = 2-F; X = S; **2h**: R^2 = 2-F; R^1 = 2-F; X = S. *Reagents*. a: 1) 3-Ethyl-5-(2-hydroxyethyl) -4-methylthiazolium bromide, NEt_3, mw, 15 min; 2) 2N HCl. b: amine, *p*-toluensulfonic acid, mw, 30 min. c: 1) *N*-methylpiperazine (**2a**) or thiomorpholine (**2b-h**), CH_3CN, HCHO, CH_3COOH, rt, 1 h; 2) NaOH 20% w/v.

Scheme 1.

2. Chemistry

Synthesis of the target compounds **2a–h** is shown in Scheme 1. Reaction of a suitable benzaldehyde **3a–e** with ethyl vinyl ketone **4**, according to the Stetter reaction, by employing the Discovery Microwave System apparatus (150 W, 70 °C, 170 psi) [8a], afforded 1,4-diketones **5a–e**. In the presence of the appropriate amine, following the Paal–Knoor condensation conditions for 30 min, by employing the Discovery Microwave System apparatus (150 W, 170 °C, 170 psi) [8a], intermediates **5a–e** cyclized to yield the expected 1,5-diarylpyrroles **6a–h**. Construction of the side chain at C3 was achieved in good yield by reaction of **6a–h** with formaldehyde and *N*-methylpiperazine or thiomorpholine, according to the Mannich reaction conditions, to give the expected derivatives **2a** or **2b–h**, respectively.

3. Results and discussion

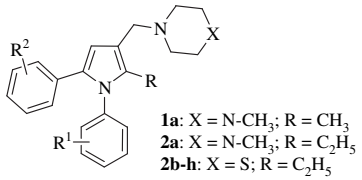
Compounds **2a–h** were preliminary evaluated for their in vitro activity toward MTB CIP 103471 and *M. avium* CIP 103317. Only compounds showing MIC values lower than 16 μg/mL were also assayed toward MTB H37Rv and MTB strains resistant to rifampicin. Cytotoxicity in VERO cells was determined (and expressed as maximum non-toxic dose, MNTD) and PI was calculated as the ratio between MNTD and MIC values. Rifampicin, streptomycin, and **1a** were used as reference compounds. Biological data are reported in Table 1.

Analysis of the activity data toward MTB CIP 103471 clearly showed that compounds **2a**, **2c–e**, and **2h** were all characterized by MIC values two- to fourfold higher than those found for the corresponding 2-methyl derivatives [8c, 8e] (even though some of them possessed log *P* values comparable to those of previous very active compounds of this class), with a significant cytotoxicity thus leading to low values of PI. Compound **2f** was inactive. On the other hand, the best compound **2b** showed the same MIC value previously found for the corresponding 2-methyl derivative (0.25 μg/mL) [8b], but a significantly higher PI value (>512 versus 256) due to the very low cytotoxicity of this new compound (MNTD > 128 μg/mL). The same compound was also very active toward both MTB H37Rv and MTB rifampicin-resistant strain (both activity values are 0.25 μg/mL). Such results showed that higher log *P* values were not always able to determine better activity and led us to revise our previous hypothesis that an increase of log *P* was in general associated with an improvement of activity. In particular, the fact that the increase of log *P* caused by the insertion at the position 2 of an ethyl group (instead of a methyl one) was not profitable to improve the activity, led to the suggestion that the substituent at position 2 was generally less important for activity in comparison to substituents at positions 1, 3, and 5. As a consequence, the correlation between higher lipophilicity and antimycobacterial activity may depend on structural modifications mainly involving the phenyl rings at N1 and C5, already identified as crucial keys in determining antimycobacterial activity, being also able to match the hydrophobic features of the pharmacophoric model (Fig. 1) [8a, 8b].

4. Conclusions

Among new 1,5-diarylpyrrole derivatives, the best compound **2b** showed a very good biological profile, having the same MIC value toward MTB previously found for the corresponding 2-methyl analogue (0.25 μg/mL) and a PI value significantly improved (>512 versus 256) because of the very low cytotoxicity (MNTD > 128 μg/mL). Compound **2b** proved also to be very active against both MTB H37Rv and MTB rifampicin-resistant strains, being 0.25 μg/mL both the MIC values. Since the biological data of **2b** were very promising in terms of high activity and low cytotoxicity, they prompted us toward in vivo investigations. In parallel,

Table 1
Structure, in vitro antimycobacterial activity (toward *Mycobacterium tuberculosis* CIP 103471, *M. tuberculosis* H37Rv, rifampicin-resistant *M. tuberculosis* and *M. avium* CIP 103317), cytotoxicity, and protection index of the new pyrrole derivatives **2a–h** and reference compounds.



1a: X = N-CH_3; R = CH_3
2a: X = N-CH_3; R = C_2H_5
2b-h: X = S; R = C_2H_5

Compounds[a]	R^1	R^2	MIC (μg/mL)				MNTD (μg/mL)	PI[c]	clog *P*[d]
			M. tuberculosis strain			*M. avium* 103317			
			103471	H37Rv	Rifa-R[b]				
1a, BM 212	4-Cl	4-Cl	1	1	ND	0.5	4	4	6.02
2a	4-Cl	4-Cl	2	4	4	4	8	4	6.69
2b	4-F	4-CH_3	0.25	0.25	0.25	16	>128	>512	6.53
2c	H	H	2	4	4	8	16	8	5.83
2d	H	4-F	1	1	1	8	8	8	6.04
2e	4-F	H	2	4	4	16	16	8	6.04
2f	4-F	4-F	16	ND	ND	16	8	0.5	6.25
2g	2-F	4-F	2	2	2	8	8	4	6.25
2h	2-F	2-F	4	8	8	16	8	2	6.25
S[e]			0.50	0.50	0.50	8	128	256	
R[f]			0.25	0.25	>100	0.25	64	256	

[a] Compounds **2a–h** have been submitted to a PCT patent (see Ref. [8d]).
[b] Rifa-R: rifampicin-resistant.
[c] PI: protection index, expressed as MNTD/MIC ratio.
[d] Calculated according to the Alog *P*98 method (Ref. [4]).
[e] S: streptomycin.
[f] R: rifampicin.

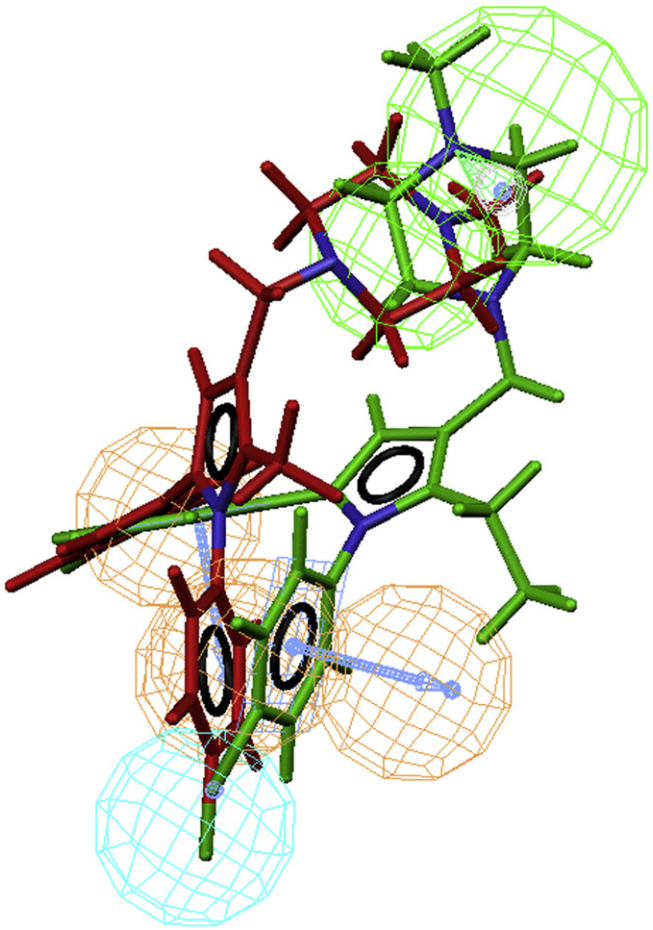


Fig. 1. Graphical representation of the superposition mode of **2a** (green) and **1a** (red) into the pharmacophoric model. Side chains at positions 2 and 3 of **2a** are oriented in opposite directions with respect to the pyrrole plane and are only able to partially satisfy the hydrogen bond acceptor group (green spheres) and the directionality constraint imposed by one of the aromatic ring features (orange spheres, bottom right corner). The hydrophobic region (cyan sphere) is filled by the p-Cl group of both compounds, as well as the other aromatic ring feature (orange spheres, middle left).

further efforts are planned for a structural optimization of this class of compounds.

5. Experimental section

5.1. Chemistry

The general procedure for the preparation of hexane-1,4-diones **5a**–**e** and 1,5-diarylpyrroles **6a**–**h** is described in Supporting Information.

5.2. General procedure for the preparation of compounds *2a*–*h*

Following the Mannich reaction, to a stirred solution of an appropriate pyrrole **6** (5.6 mmol) in acetonitrile (20 mL), a mixture of thiomorpholine (0.57 g, 5.6 mmol) or *N*-methylpiperazine (0.56 g, 5.6 mmol), formaldehyde (0.18 g, 5.6 mmol) (40% in water) and 5 mL of acetic acid, was added dropwise. After the addition was complete, the mixture was stirred at room temperature for 1 h. The mixture was then treated with a solution of sodium hydroxide (20%, w/v) and extracted with ethyl acetate. The organic extracts were combined, washed with water and dried. After removal of solvent, the residue was purified by column chromatography, using silica gel and petroleum ether/ethyl acetate (3:1 v/v). The eluates were combined after TLC control and the solvent was removed to give **2a**–**h** as solids in satisfactory yield. Re-crystallization from diethyl ether gave the required products. Structure of the final compounds was confirmed by observing the disappearance of the singlet of the proton at the position 3 of the pyrrole (6.09 ppm) and the appearance of the singlet corresponding to the methylene group at 3.50 ppm [8a]. The elemental analyses for compounds **2a**–**h** are reported in Table 2 (Supporting Information).

5.3. 2-Ethyl-1-(4-methylphenyl)–3-(thiomorpholin-4-yl)-methyl-5-(4-fluorophenyl)-1H-pyrrole (*2b*)

Mp 165 °C (yield 45%); ^{1}H NMR ($CDCl_3$) 7.26 (m, 4H), 7.16 (m, 2H), 7.00 (m, 2H), 6.27 (s, 1H), 3.46 (s, 2H), 2.78 (s broad, 4H), 2.71 (s broad, 4H), 2.25 (q, 2H), 2.18 (m, 3H), 0.85 (t, 3H). ^{13}C NMR ($CDCl_3$) 160.18 (d, 1C), 139.25 (s, 1C), 135.90 (s, 1C), 132.17 (s, 1C), 129.53 (s, 1C), 129.49 (s, 1C), 128.99 (s, 2C), 128.73 (s, 2C), 127.64 (s, 1C), 114.91 (s, 2C), 114.69 (s, 2C), 110.58 (s, 1C), 55.44 (s, 2C), 54.89 (s, 1C), 28.17 (s, 2C), 18.02 (s, 2C), 14.87 (s, 1C). Anal. ($C_{24}H_{27}FN_2S$) C, H, N, S, F.

5.4. Microbiology

5.4.1. Compounds

Compounds **2b**–**h** and reference drugs were dissolved in DMSO at a concentration of 5 mg/mL and stored cold until used. Compound **2a** was dissolved in ethanol at 6 mg/mL.

5.4.2. Antimycobacterial activity

Compounds were preliminarily assayed according to protocol already described [8b], following a standard twofold agar dilution method in Middlebrook 7H11 agar medium (Difco) containing 10% of OADC (oleic acid, albumin and dextrose complex) [12].

Compounds with better preliminary activity were tested toward *M. tuberculosis* CIP 103471, *M. tuberculosis* H37Rv ATCC 27294, rifampicin-resistant *M. tuberculosis* ATCC 35838, and atypical mycobacteria, such as *M. avium* CIP 103317. In all cases, MIC value for each compound was determined for each mycobacterial strain. Further details are in Supporting Information.

5.4.3. Cytotoxic activity assays

Cytotoxic activity assays were performed in Vero cells to determine the maximum non-toxic dose defined as the highest drug concentration showing no morphological changes as determined microscopically by the observation at 72 h of incubation.

5.5. Computational details

The QSAR+ module of Cerius2 [13] was used to calculate Alog *P*98 values of the studied compounds. Such a descriptor is an implementation of the atom type-based Alog *P* method using the latest published set of parameters [14].

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2009.06.005.

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