Brief Articles

1,5-Diphenylpyrrole Derivatives as Antimycobacterial Agents. Probing the Influence on Antimycobacterial Activity of Lipophilic Substituents at the Phenyl Rings

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Synthesis and biological evaluation of new derivatives of 1,5-bis(4-chlorophenyl)-2-methyl-3-(4-methylpiperazin-1-yl)methyl-1*H*-pyrrole (BM 212, **16**) are reported. Variously substituted phenyl rings with different substitution pattern and lipophilicity were added to the pyrrole nucleus to evaluate their influence on the activity toward *Mycobacterium tuberculosis* (MTB) and atypical mycobacteria. The most active derivatives showed activity between 0.125−0.5 μg/mL (better than **16** and streptomycin) and protection index (64−256) higher than **16** (4) and similar to isoniazid and streptomycin (128).

Introduction

Mycobacterium tuberculosis (MTB[a]), responsible for tuberculosis (TB) in humans, causes the death of almost 3 million people each year, and it is positioned as the leading bacterial infectious agent.[1,2] Moreover, today more people die from tuberculosis than ever before;[2] therefore, the development of new drugs with activity against multidrug-resistant (MDR) TB, extensively drug-resistant (XDR) TB, and latent TB is a priority task, although new agents that will shorten the duration of current chemotherapy are also needed. Furthermore, the new compounds should have a novel mode of action, since such agents are likely to be more effective against drug resistant strains, and they must be selective for mycobacteria and compatible with HIV-1 drugs (further details in Supporting Information).

In the past, we have identified 1,5-diarylpyrrole derivatives with good activity toward MTB and non-tuberculosis mycobacteria, also showing low cytotoxicity. Compound **16** (BM 212) was the best hit compound of this new class of derivatives[3] and one of the most promising future antimycobacterial drug.[4] Moreover, structure−activity relationship (SAR) studies and a pharmacophore-based ligand design approach allowed us to identify chemical groups and their substitution pattern on the pyrrole, responsible for the activity. Analysis of the superposition pathway of compounds on the pharmacophoric model revealed that a hydrophobic feature was a crucial key for determining antitubercular activity, in agreement with literature reports showing that a more hydrophobic character of compounds was required to increase the antitubercular potency.[5] On this basis, attempts to find the optimal substituents for fulfilling the pharmacophoric features and thus improving antitubercular activity led us to the identification of five new hits.[6−10] Compound **1** (Table 1), bearing a *p*-F-phenyl ring at N1 of the pyrrole, a thiomorpholinomethyl chain at C3, and a *p*-methylphenyl moiety at C5, showed the best antimycobacterial activity.[11]

Moreover, log *P* values calculated for pyrrole derivatives previously synthesized showed that more active compounds had a marked hydrophobic character, corresponding to log *P* values from 5.58 to 6.70. To check the hypothesis that a further increase of lipophilicity could improve activity of compounds, facilitating their entrance through the lipid-rich mycobacterial membrane, we planned to design and synthesize new pyrrole derivatives **2**−**15** by modification of the molecular structure of **1** (Table 1). In particular, we chose to increase the size of the *p*-methyl group of **1** to an ethyl, propyl, and isopropyl chain. On the other hand, the *p*-F-phenyl group was maintained and the corresponding chloro analogue was also taken into account because it was reminiscent of N1 and C5 substituents of **16**. Each of the *p*-alkyl- and *p*-halophenyl substituents was alternately placed at position 1 or 5, respectively, following a suggestion coming from pharmacophore modeling (further details in Supporting Information). The new compounds were all tested against MTB and atypical mycobacteria. The most active of them toward MTB were also assayed for their activity toward the H37Rv strain of MTB, toward resistant and MDR clinical isolate strains, and toward intramacrophagic MTB.

Chemistry

Synthesis of the target compounds is shown in Scheme 1. Briefly, reaction of a suitable benzaldehyde **17a**−**f** with methyl vinyl ketone **18** was performed according to the Stetter reaction,

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[a] Abbreviations: MTB, *Mycobacterium tuberculosis*; TB, tuberculosis; MDR, multidrug-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 nontoxic dose; PI, protection index.

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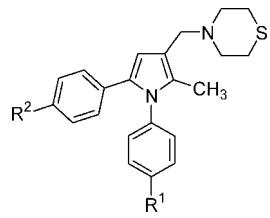
Table 1. Structure, in Vitro Activity, Cytotoxicity, Protection Index (PI), and Calculated log *P* Values of the New Pyrrole Derivatives **2**−**15** and Reference Compounds



compd[a]	R1	R2	MIC[b]	MNTD50[c]	PI[d]	log P[e]
1	F	methyl	0.25	64	256	5.86
2	F	ethyl	1	16	16	6.32
3	F	propyl	2	4	2	6.77
4	F	isopropyl	0.25	32	128	6.57
5	ethyl	F	2	2	1	6.32
6	propyl	F	1	4	4	6.77
7	isopropyl	F	16	8	0.5	6.57
8	Cl	methyl	0.5	64	128	6.32
9	Cl	ethyl	0.25	16	64	6.77
10	Cl	propyl	0.25	16	64	7.23
11	Cl	isopropyl	0.125	8	64	7.03
12	methyl	Cl	0.5	16	32	6.32
13	ethyl	Cl	0.5	16	32	6.77
14	propyl	Cl	0.25	32	128	7.23
15	isopropyl	Cl	0.25	32	128	7.03
16			1	4	4	6.02
isoniazid			0.125	32	128	
streptomycin			0.50	>64	>128	
rifampicin			0.25	64	256	

[a] Compounds **1**−**15** have been submitted to a PCT patent (ref 14). [b] MIC (μg/mL) toward *M. tuberculosis* 103471. [c] MNTD$_{50}$ (μg/mL) toward Vero cells. [d] PI = protection index, as the ratio of cytotoxicity (MNTD$_{50}$) to in vitro activity (MIC). [e] Calculated by the AlogP98 method (ref 15).

by employing the Discovery microwave system apparatus (optimizing time and yield of reaction),[11] leading to 1,4-diketones **19a**−**f**. In the presence of the appropriate amine, following the Paal−Knoor condensation, **19a**−**f** cyclized to yield the expected 1,5-diarylpyrroles **20a**−**n**. Construction of the side chain at C3 was achieved in good yield by reaction of compounds **20a-n** with formaldehyde and thiomorpholine, according to the Mannich reaction conditions, to give the final compounds **2**−**15**.

Biology

Compounds showing minimum inhibitory concentration (MIC) values of 16 μg/mL or lower in a preliminary assay were then studied for their inhibitory activity toward MTB 103471 and a panel of atypical mycobacteria, such as *M. marinum* 6423, *M. avium* 103317, and *M. smegmatis* 10359. MIC values were determined for each compound toward each of the test mycobacteria.

Cytotoxic activity assays were performed in Vero cells to determine the maximum nontoxic dose (MNTD, expressed as μg/mL), defined as the drug concentration that decreases cell multiplication less than 50% of the control.

In vitro activity of **2**−**15**, as well as cytotoxicity and protection index (PI), are reported in Tables 1 and 2. Compounds that were particularly active were also tested against MTB H37Rv and strains resistant to isoniazid and rifampicin (Table 2). Isoniazid, streptomycin, rifampicin, and **16** were used as reference compounds.

Selected compounds (namely, **4**, **14**, and **15**), which showed an interesting activity against reference strains and the highest PI values, were also evaluated for their activity against 32 clinical isolates of MTB (Table 3), while **14** and **15** were also tested toward intracellular MTB (Table 4).

Results and Discussion

Compounds **2**−**15** were tested in vitro against MTB and atypical mycobacteria, and for the most active of them, activity toward MTB H37Rv and resistant and multidrug-resistant clinical isolate strains was also evaluated. Biological data showed a general trend that confirmed previous suggestions on (i) the importance of the (thiomorpholin-4-yl)methyl moiety as a substituent of the pyrrole ring, (ii) the nature of the substituent present in the two aromatic rings at N1 and C5, and (iii) their substitution pattern.[9−11]

In principle, **2**−**15** showed a very good in vitro biological profile (Table 1). In fact, they had an impressive activity toward MTB (along with poor cytotoxicity) with a consequent high protection index. Moreover, in several cases, they were also active toward atypical mycobacteria (Table 2). As examples, *M. smegmatis* was sensitive to **5** and **6** (whose MIC values were 32- and 16-fold better than that of isoniazid and significantly improved with respect to that of **1**), while **4**, **10**, and **11** had interesting activity toward *M. avium* and *M. marinum*, comparable to **1** and better than isoniazid.

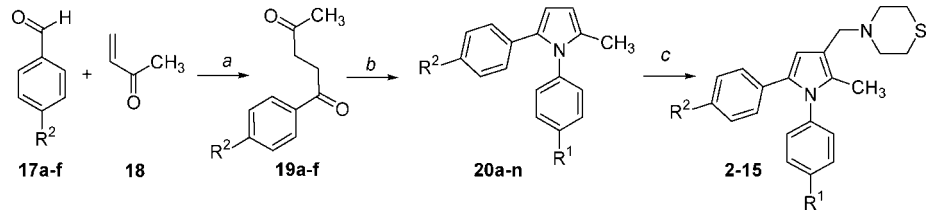
Derivatives bearing a halogen at the N1 phenyl ring were generally more active than the corresponding C5 analogues, confirming our previous findings based on the pharmacophoric model. It is also important to note that compounds with a chlorine atom on the phenyl ring at N1 or, alternatively, at C5 were in principle more active and less cytotoxic than the corresponding fluoro analogues, thus showing a high PI. As an example, **9** and **10** showed a biological profile in terms of MIC and PI better than that found for the corresponding fluoro analogues **2** and **3**, respectively (Table 1). The only exception to this trend was **11**, showing a PI lower than that of **4** (64 versus 128, respectively), although it was characterized by a better MIC value (0.125 versus 0.25 μg/mL, respectively).

The most interesting microbiological results were represented by activity values found toward MTB H37Rv and its clinical strain resistant to rifampicin. In fact, for all the new compounds tested, MIC values ranging from 0.125 to 0.5 μg/mL were found (Table 2), comparable to **1** and, in several cases, better than (**10** and **11**) or comparable to (**4**, **9**, **14**, and **15**) that of isoniazid (0.25 μg/mL). On the other hand, compounds were inactive against isoniazid-resistant strains, with the sole exception of **11**, which retained some activity (8 μg/mL). Also in this case, **8**−**11**, bearing a N1 *p*-halo (namely, chloro) substituent, were generally more active than the corresponding C5 *p*-halo (namely, chloro) substituted analogues **12**−**15**. The experimental evidence makes these compounds extremely interesting when compared to the compounds now used in therapy, which tend to be less active against drug-resistant mycobacteria.

An analysis of the lipophilic character of the new compounds showed that their calculated log *P* values ranged from 6.32 to 7.23 (Table 1). In general, the increase of log *P* values (in comparison to the previous series of pyrrole derivatives)[11] was found to be associated with an improvement of activity. In fact, 9 out of the 14 new compounds (**4**, **8**−**15**, about 64%) had MIC values toward MTB 103471 in the range between 0.5 and 0.125 μg/mL, with respect to only 4 out of 28 congeneric derivatives (about 14%) described in our previous work.[11] Four of the remaining new pyrroles (**2**, **3**, **5**, and **6**) had activity between 1 and 2 μg/mL, while **7** was the sole exception (MIC of 16 μg/mL).

Moreover, it is also very important to point out that two of the most active compounds (**10** and **11**), showing the best MIC

Scheme 1[a]



[a] Compounds. **17a**, R^2 = F; **17b**, R^2 = Cl; **17c**, R^2 = CH_3; **17d**, R^2 = C_2H_5; **17e**, R^2 = C_3H_7; **17f**, R^2 = *i*-C_3H_7; **19a**, R^2 = F; **19b**, R^2 = Cl; **19c**, R^2 = CH_3; **19d**, R^2 = C_2H_5; **19e**, R^2 = C_3H_7; **19f**, R^2 = *i*-C_3H_7; **20a**, R^1 = F, R^2 = C_2H_5; **20b**, R^1 = F, R^2 = C_3H_7; **20c**, R^1 = F, R^2 = *i*-C_3H_7; **20d**, R^1 = Cl, R^2 = CH_3; **20e**, R^1 = Cl, R^2 = C_2H_5; **20f**, R^1 = Cl, R^2 = C_3H_7; **20g**, R^1 = Cl, R^2 = *i*-C_3H_7; **20h**, R^1 = C_2H_5, R^2 = F; **20i**, R^1 = C_3H_7, R^2 = F; **20j**, R^1 = *i*-C_3H_7, R^2 = F; **20k**, R^1 = CH_3, R^2 = Cl; **20l**, R^1 = C_2H_5, R^2 = Cl; **20m**, R^1 = C_3H_7, R^2 = Cl; **20n**, R^1 = *i*-C_3H_7, R^2 = Cl; **2**, R^1 = F, R^2 = C_2H_5; **3**, R^1 = F, R^2 = C_3H_7; **4**, R^1 = F, R^2 = *i*-C_3H_7; **5**, R^1 = C_2H_5, R^2 = F; **6**, R^1 = C_3H_7, R^2 = F; **7**, R^1 = *i*-C_3H_7, R^2 = F; **8**, R^1 = Cl, R^2 = CH_3; **9**, R^1 = Cl, R^2 = C_2H_5; **10**, R^1 = Cl, R^2 = C_3H_7; **11**, R^1 = Cl, R^2 = *i*-C_3H_7; **12**, R^1 = CH_3, R^2 = Cl; **13**, R^1 = C_2H_5, R^2 = Cl; **14**, R^1 = C_3H_7, R^2 = Cl; **15**, R^1 = *i*-C_3H_7, R^2 = Cl. Reagents: (a) (1) 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide, NEt_3, mw, 15 min, (2) 2 N HCl; (b) $H_2NC_6H_4R^1$, *p*-toluensulfonic acid, EtOH, mw, 30 min; (c) (1) thiomorpholine, CH_3CN, HCHO, CH_3COOH, room temp, 3 h; (2) NaOH 20% w/v.

Table 2. In Vitro Activity of the New Pyrrole Derivatives **2**−**15** and Reference Compounds toward Atypical Mycobacteria, *M. tuberculosis* H37Rv, and Its Rifampicin-Resistant and Isoniazid-Resistant Strains

	MIC (μg/mL)					
compd	*M. avium*	*M. marinum*	*M. smegmatis*	MTB H37Rv	R-resistant[a]	I-resistant[b]
1	8	8	16	0.125	0.5	32
2	>16	>16	>16	ND[c]	ND[c]	ND[c]
3	>16	>16	>16	ND[c]	ND[c]	ND[c]
4	4	8	>16	0.25	0.25	>16
5	>16	>16	2	ND[c]	ND[c]	ND[c]
6	>16	16	4	ND[c]	ND[c]	ND[c]
7	>16	>16	8	ND[c]	ND[c]	ND[c]
8	16	>16	>16	0.5	0.5	16
9	16	8	8	0.25	0.25	16
10	4	4	>16	0.125	0.125	>16
11	8	4	8	0.125	0.125	8
12	>16	16	16	0.5	0.5	>16
13	16	4	8	0.5	0.5	16
14	16	16	>16	0.25	0.25	>16
15	16	16	>16	0.25	0.25	>16
16	0.5	100	25	2	1	2
isoniazid	32	16	64	0.25	0.25	>16
streptomycin	8	32	8	4	4	16
rifampicin	0.25	0.5	32	0.25	>64	0.25

[a] R-resistant: rifampicin-resistant. [b] I-resistant: isoniazid-resistant. [c] ND: not determined.

Table 3. In Vitro Antimicrobial Activity (μg/mL) of Selected Compounds **4**, **14**, and **15** toward 32 Clinical Isolates of *M. tuberculosis*, As Determined by the Agar Dilution Method

compd	MIC range	MIC_{50}	MIC_{90}
4	0.25–0.5	0.50	0.5
14	0.125–2	0.50	1
15	0.25–1	0.25	0.5
isoniazid	0.125–1	0.125	0.25

Table 4. Activity of Decreasing Concentrations of Compounds **14** and **15** in Macrophages J774 Infected with *M. tuberculosis* H37Rv[a]

	concentrations (μg/mL)		
compd	1	0.5	0.25
14	98.79	85.79	21.05
15	98.74	84.21	47.37
rifampicin	99.79	98.42	95.79

[a] Results are expressed as % of reduction of the number of surviving bacteria with respect to the untreated control.

values, also had the highest log *P* values (7.23 and 7.03, respectively). In a similar way, their corresponding regioisomers **14** and **15** were the most active among compounds belonging to the subclass consisting of **12**−**15**. These results, showing that antimycobacterial activity was in general improved with the increase of lipophilicity, provided support for the hypothesis that an increase of lipophilicity could improve antimycobacterial activity of such a class of compounds.

As a general trend for the activity toward MTB 103471, chloro derivatives **8**−**15** were more active and less cytotoxic with respect to the corresponding fluoro analogues and they were also characterized by a higher lipophilicity. Moreover, compounds with the *p*-Cl-phenyl ring at N1 (**8**−**11**) showed a very slight improvement of activity with respect to the corresponding C5 derivatives (**12**−**15**). This was in good agreement with what arose from the analysis of the superposition mode of such a class of compounds to the pharmacophoric model. In fact, 1,5-diarylpyrrole derivatives with a substitution pattern similar to that of **1** (as in **8**−**11**) showed their preferred orientation characterized by the C5 *p*-alkylphenyl moiety able to match a hydrophobic (with the *p*-alkyl group) and an aromatic (with the phenyl ring at C5) portion of the pharmacophore, while the N1 *p*-halophenyl substituent filled the remaining aromatic ring feature of the model. Inverting the substitution pattern at positions 1 and 5 (as in **12**−**15**) caused a rotation of about 180° around the pyrrole plane, leading to fit all pharmacophoric features in a less profitable way (i.e., the fit score, which is the sum of how well each chemical feature of compounds fits each pharmacophoric feature, was lower in comparison to the alternative orientation).[11]

Among fluoro derivatives, the most interesting activity (0.25 μg/mL) was shown by **4**, also characterized by one of the best protection index (128).

Compounds **4**, **14**, and **15** were also assayed toward clinical isolates of MTB (showing activity comparable to that of isoniazid used as control, Table 3), while **14** and **15** were also evaluated for their activity toward intracellular MTB (Table 4). It is important to note that while the inhibitory activity toward extracellular MTB accounts for the ability of test compounds to treat active tuberculosis, assays on intracellular MTB assess the ability of compounds to inhibit mycobacteria during the latent phase of TB, before latent TB infection itself progresses to active disease. Biological results reported in Table 4 indicate that both compounds showed a concentration-dependent inhibition of the growth of intracellular mycobacteria, comparable to that of rifampicin, used as the reference compound. This result is very important because mycobacteria can reside for years inside lymphoid cells and macrophages (during the latent phase of TB) and many traditional drugs are unable to get at it. Moreover, combating latent TB infection is one of the major challenges mainly for reducing the high rate of progression to active disease in immunocompromised individuals (in fact, progression is higher in persons with concomitant HIV-1 infection).

In summary, biological results on the new pyrroles showed an antimycobacterial activity generally improved with respect to analogues previously described. On the basis of the low cytotoxicity, several compounds also showed a high PI value, comparable to that of current antitubercular drugs such as isoniazid. Compounds were active toward rifampicin-resitant strains, as well as toward a wide panel of clinical isolates, further confirming the great significance reached in recent years by the pyrrole class of compounds in the field of new antimycobacterial compounds.[2]

Conclusions

New pyrrole derivatives related to **16** were designed, synthesized, and tested to probe the influence of lipophilicity on their antimycobacterial activity, based on the hypothesis that an increase of lipophilicity could improve inhibitory activity toward mycobacteria. In general, the new compounds showed a very high antimycobacterial activity, and the best results were found for compounds with higher lipophilicity (with respect to the parent compounds),[11] in agreement with recent literature showing that the most active compounds among a set of oxadiazole derivatives were those characterized by the highest log *P* values (namely, 8.14 and 9.20).[12] Further efforts are required to design and synthesize new derivatives with appropriate lipophilicity. In fact, although a relationship between MIC values and log *P* seemed to exist for the current pyrrole derivatives, we are aware that in general molecules with too high a lipophilicity are destined to have a very low solubility (not enough for assays to be performed) and to be trapped in hydrophobic media (i.e., membranes) that avoid compounds themselves to reach their site of action. On this basis, suitable formulations should be studied to improve pharmacokinetic properties of compounds and allow them to reach their site of action.

Finally, considering that no new antitubercular drug has been introduced in the market during the past 50 years, results reported in this paper are of crucial importance in the field of antitubercular agents, and they further support the evidence that this class of compounds can be considered very promising in this field. However, additional derivatives have been designed to further improve the biological profile and to allow for in vivo experiments, while studies on the mechanism of action of pyrrole derivatives at the molecular level are ongoing in our laboratories to shed light on their molecular target.

Experimental Section

Chemistry. General Procedure for the Preparation of Pentane-1,4-diones 19a−f. Following the Stetter reaction, a mixture of the appropriate benzaldehyde **17a−f** (0.09 mol), triethylamine (19.5 mL, 0.14 mol), methyl vinyl ketone **18** (0.09 mol), and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (3.53 g, 0.014 mol) (Scheme 1) was put into a round-bottom flask equipped with a stir bar. The flask was inserted into the cavity of a Discovery microwave system apparatus and heated (150 W for 15 min, internal temperature 70 °C, and internal pressure 60 psi). The residue was treated with 2 N HCl (10 mL). After extraction with ethyl acetate, the organic layer was washed with aqueous sodium bicarbonate and water. The organic fractions were dried over Na_2SO_4, filtered, and concentrated to give a crude orange liquid. After chromatography on aluminum oxide (activity II−III, according to Brockmann) (cyclohexane/ethyl acetate, 3:1 v/v), the desired **19a−c** were isolated as a light-yellow solids which, after recrystallization from cyclohexane, gave an analytical sample as white needles, and compounds **19d−f** as oils. Example, 1-(4-ethylphenyl)-pentane-1,4-dione (**19d**): oil (yield 72%); ^{1}H NMR ($CDCl_3$) δ 7.89 (m, 2H), 7.26 (m, 2H), 3.24 (t, 2H), 2.85 (t, 2H), 2.69 (q, 2H), 2.47 (s, 3H), 1.22 (t, 3H). Anal. ($C_{13}H_{16}O_2$) C, H.

General Procedure for the Preparation of 1,5-Diarylpyrroles 20a−n. Following the Paal−Knorr reaction, the proper diketone **19** (2.28 mmol) was dissolved in ethanol (2 mL) into a round-bottom flask equipped with a stir bar. The suitable amine (2.28 mmol) and *p*-toluensulfonic acid (30 mg, 0.17 mmol) were added. The flask was inserted into the cavity of the Discovery microwave system apparatus and heated (150 W for 30 min, internal temperature 160 °C, and internal pressure 150 psi).[13] The reaction mixture was cooled and concentrated. The crude material was purified by chromatography on aluminum oxide (activity II−III, according to Brockmann) with cyclohexane as the eluant to give the expected 1,5-diarylpyrroles **20a**,**c−n** as solids in satisfactory yield and compound **20b** as an oil. Example, 2-methyl-1-(4-fluorophenyl)-5-(4-ethylphenyl)-1*H*-pyrrole (**20a**): mp 118 °C (yield 80%); ^{1}H NMR ($CDCl_3$) δ 7.12 (m, 2H), 7.05 (m, 2H), 6.99 (m, 2H), 6.96 (m, 2H), 6.36 (d, 1H), 6.31 (d, 1H), 2.56 (q, 2H), 2.13 (s, 3H), 1.17 (t, 3H). Anal. ($C_{19}H_{18}FN$) C, H, N, F.

General Procedure for the Preparation of Compounds 2−15. Following the Mannich reaction, to a stirred solution of an appropriate pyrrole **20a−n** (5.6 mmol) in acetonitrile (20 mL), a mixture of thiomorpholine (0.57 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 3 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 **2−15** as solids in satisfactory yield. Recrystallization from diethyl ether gave the final products. Example, 2-methyl-1-(4-fluorophenyl)-3-(thiomorpholin-4-yl)-methyl-5-(4-ethylphenyl)-1*H*-pyrrole (**2**): mp 98 °C (yield 40%); ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 2H), 7.06 (m, 2H), 6.92 (m, 4H), 6.26 (s, 1H), 3.42 (s, 2H), 2.74 (s broad, 4H), 2.68 (s broad, 4H), 2.58 (q, 2H), 2.03 (s, 3H), 1.15 (t, 3H). Anal. ($C_{24}H_{27}FN_2S$) C, H, N, S, F.

Microbiology. Antimycobacterial activity and cytotoxic activity assays were performed following a protocol previously reported[11] (further details in Supporting Information).

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Supporting Information Available: Additional information on tuberculosis and pyrrole compounds as antimycobacterial agents, experimental details (chemistry and microbiology, spectroscopic, and elemental analysis data), and details of computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cegielski, J. P.; Chin, D. P.; Espinal, M. A.; Frieden, T. R.; Rodriquez Cruz, R.; Talbot, E. A.; Weil, D. E.; Zaleskis, R.; Raviglione, M. C. The global tuberculosis situation. Progress and problems in the 20th century, prospects for the 21st century. *Infect. Dis. Clin. North Am.* **2002**, *161*, 1–58.
(2) Spigelman, M. K. New tuberculosis therapeutics: a growing pipeline. *J. Infect. Dis.* **2007**, *196*, S28–S34.
(3) Deidda, D.; Lampis, G.; Fioravanti, R.; Biava, M.; Porretta, G. C.; Zanetti, S.; Pompei, R. Bactericidal activities of the pyrrole derivative BM 212 against multidrug-resistant and intramacrophagic *Mycobacterium tuberculosis* strains. *Antimicrob. Agents Chemother.* **1998**, *42*, 3035–3037.
(4) Tomioka, H. Type II pneumocytes in the evaluation of drug antimycobacterial activity. *Expert Opin. Pharmacother.* **2003**, *4*, 127–139.
(5) Ragno, R.; Marshall, G. R.; Di Santo, R.; Costi, R.; Massa, S.; Pompei, R.; Artico, M. Antimycobacterial pyrroles: synthesis, anti-*Mycobac-*

terium tuberculosis activity and QSAR studies. *Bioorg. Med. Chem.* **2000**, *8*, 1423–1432.

(6) Biava, M.; Fioravanti, R.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R. New pyrrole derivatives as antimycobacterial agents analogs of BM 212. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2983–2988.

(7) Biava, M.; Porretta, G. C.; Deidda, D.; Pompei, R.; Tafi, A.; Manetti, F. Importance of the thiomorpholine introduction in new pyrrole derivatives as antimycobacterial agents, analogs of BM 212. *Bioorg. Med. Chem.* **2003**, *11*, 515–520.

(8) Biava, M.; Porretta, G. C.; Deidda, D.; Pompei, R.; Tafi, A.; Manetti, F. Antimycobacterial compounds. New pyrrole derivatives of BM 212. *Bioorg. Med. Chem.* **2004**, *12*, 1453–1458.

(9) Biava, M.; Porretta, G. C.; Poce, G.; Deidda, D.; Pompei, R.; Tafi, A.; Manetti, F. Antimycobacterial compounds. Optimization of the BM 212 structure, the lead compound for a new pyrrole derivative class. *Bioorg. Med. Chem.* **2005**, *13*, 1221–1230.

(10) Biava, M.; Porretta, G. C.; Manetti, F. New derivatives of BM 212, a class of antimycobacterial compounds based on the pyrrole ring as a scaffold. *Mini-Rev. Med. Chem* **2007**, *7*, 65–78.

(11) Biava, M.; Porretta, G. C.; Poce, G.; Supino, S.; Deidda, D.; Pompei, R.; Molicotti, P.; Manetti, F.; Botta, M. Antimycobacterial agents. Novel diarylpyrrole derivatives of BM 212 endowed with high activity toward *Mycobacterium tuberculosis* and low cytotoxicity. *J. Med. Chem.* **2006**, *49*, 4946–4952.

(12) Navarrete-Vazquez, G.; Molina-Salinas, G. M.; Duarte-Fajardo, Z. V.; Vargas-Villarreal, J.; Estrada-Soto, S.; Gonzalez-Salazar, F.; Hernandez-Nunez, E.; Said-Fernandez, S. Synthesis and antimycobacterial activity of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridines. *Bioorg. Med. Chem.* **2007**, *15*, 5502–5508.

(13) Minetto, G.; Raveglia, L. F.; Taddei, M. Microwave-assisted Paal−Knorr reaction. A rapid approach to substituted pyrroles and furans. *Org. Lett.* **2004**, *3*, 389–392.

(14) Biava, M.; Botta, M.; Deidda, D.; Manetti, F.; Pompei, R.; Porretta, G. C. Derivatives of 1-{[1,5-Bis(4-chlorophenyl)-2-methyl-1*H*-pyrrol-3-yl]methyl}-4-methylpiperazine, Synthesis Process and Uses Thereof WO2006092822, OC/ACT/PCT 92767, 2006.

(15) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. Prediction of hydrophobic (lipophilic) properties of small organic molecules using fragmental methods: analysis of ALOGP and CLOGP methods. *J. Phys. Chem.* **1998**, *102*, 3762–3772.

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