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NEW PYRROLE DERIVATIVES AS ANTIMYCOBACTERIAL AGENTS ANALOGS OF BM212

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Abstract. During the course of our investigations in the field of azole antimicrobial agents, we have identified **BM212** a pyrrole derivative with good *in vitro* activity against *mycobacteria* and *candidae*. These findings prompted us to prepare new pyrrole derivatives **2-6** in the hope of increasing the activity. The microbiological data showed interesting *in vitro* activity against *Mycobacterium tuberculosis* and atypical mycobacteria.

The recent increase of tuberculosis cases in the world, is in particular due to the emergence of Multidrug-Resistant tuberculosis (MTB) strains towards the conventional therapeutical regiments,[1-3] which had led to the spread of resistant disease in select groups, including hospital workers and patients, persons infected with human immunodeficiency virus (HIV), individuals with recent exposure to a person infected with MDR-TB.[4] The need for new antimycobacterial drugs is based also on the side-effect profiles of currently available drugs, so that new and effective compounds, endowed with different mode of action look like a possible solution of this problem. Moreover, since in immunocompromised patients, tubercular pathology is very often accompained by mycotic infections caused by *Candida albicans*, *Candida* sp. and *Cryptococcus neoformans*, this concomitance has suggested to search for new substances able to act both as antifungals and antimycobacterials.

Recently, we have reported on the synthesis and antimycobacterial and antifungal activities of some pyrrole derivatives,[5,6] and it is the first report regarding pyrrole compounds on this topic.

Most of the synthesized compounds showed interesting antifungal and antimycobacterial activities, but, among them, **BM 212-1** revealed the most active, and it appeared to be endowed with particularly potent and selective both antimycobacterial and antifungal properties.

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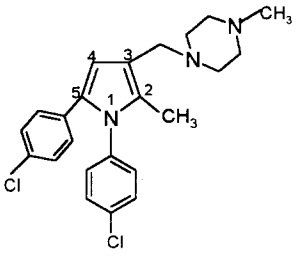


BM 212
1

It was also active against drug resistant mycobacteria of clinical origin, including strains resistant to ethambutol, isoniazid, amikacin, streptomycin, rifampin and rifabutin, and against intracellular mycobacteria, residing in the U937 human histiocytic lymphoma cell line, after 7 days of contact.[5]

Then, since **1** was also active against *Candida albicans* and *Candida* sp.,[6] we considered **1** was a promising lead for the discovery of more potent agents with both antifungal and antimycobacterial activities.

Consequently, we pursued a program to systematically modify the **1** structure but the first modifications have not increased its activity.[6]

On the basis of this results we have formulated some SAR:

1 – position C4 has to be unsubstituted;

2 – position N1 has to be substituted with a phenyl ring directly bound to the *N* atom of the pyrrole.

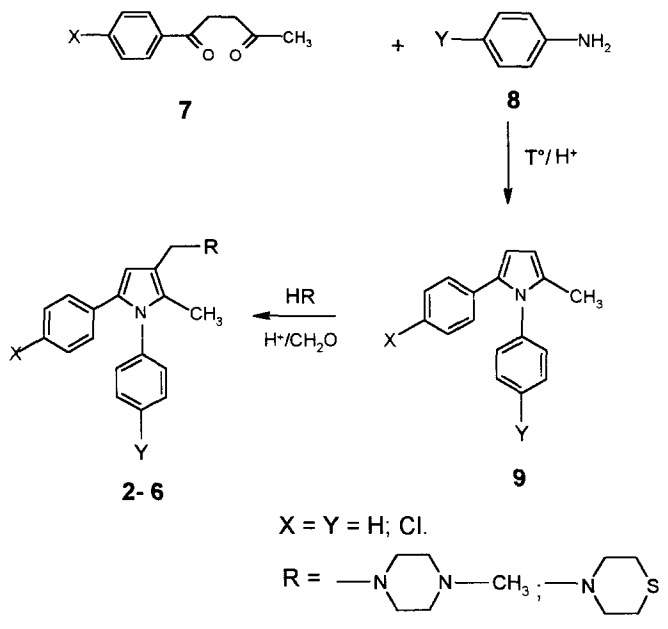
To better understand the importance of the presence of a phenyl ring bound to C5 of the pyrrole, we have synthesized new derivatives **2-6** introduced structural fragments necessary for the activity, as thiomorpholine or *N*-methylpiperazine, on the basis of what has been observed by Barbachyn[7] and us, and by substituted the halogen atoms at the phenyl ring in N1 and/or C5 of the pyrrole ring, or leaved both the phenyl rings in N1 and C5 unsubstituted.

Chemistry

Compounds **2-4** were prepared as illustred in the Scheme 1, from the appropriate 1,4-diketone, obtained by reacting levulinic acid and chorobenzene in the presence of $AlCl_3$. The Mannich bases were obtained by a procedure previously described by us.[6] All new compounds were identified by elemental analyses and NMR data. Physicochemical data for compounds **2-4** are shown in the reference section.

Compounds **5** and **6** are prepared according to literature.[8]

SCHEME 1



Compd	X	Y	R
2	Cl	H	thiomorpholinyl
3	H	Cl	thiomorpholinyl
4	Cl	Cl	thiomorpholinyl
5	Cl	H	*N*-methylpiperazinyl
6	H	Cl	*N*-methylpiperazinyl

Results and Discussion

The compounds **2-6** were submitted for an evaluation of their *in vitro* activity against *M. tuberculosis*, *C. albicans* and *C.* sp. The cytotoxicity of these derivatives was also determined using VERO cells after dissolution in DMSO at the initial concentration of 10 μg/ml.

All of the tested compounds **2-6** revealed inactive against *Candida albicans* and *C.* sp, and data was not shown. As shown in Table 1, to antimycobacterial activity, compounds **2-6** showed a good activity even though smaller than that of compound **1**; encouraged by these results, further assays against atypical mycobacteria were performed, since nontuberculosis mycobacteria are usually resistant to isoniazid and other antitubercular agents[5]. We therefore tested compounds **2-6** against a pannel of mycobacterial species, including *M. avium*, *M. gordonae*, *M. marinum* and *M. smegmatis*.

Table 1. Cytotoxicity and *in vitro* activity against *M. tuberculosis* of compounds **2-6**, **BM 212**, **isoniazid, streptomycin** and **rifampin.**

Compd	MIC[a] (μg/ml)	
	MTD_{50}[b] VERO cells	*M. tuberculosis* 103471
1	4	0.70
2	4	2
3	8	2
4	32	4
5	4	4
6	4	4
isoniazid	32	0.25
streptomycin	>64	0.50
rifampin	64	0.3

[a] For minimum inhibitory concentrations (MICs in μg/ml) the compounds were incorporated into Middlebrook 7H9 broth using the broth microdilution test (see references 9, 10 and 11). The MIC was defined as the lowest concentration of drug that yielded an absence of visual torbidity. The Mycobacterium tuberculosis test organisms were grown in Middlebrook 7H9 medium (Difco) with 10% of ADC (albumine dextrose complex).Stock solutions of substances were prepared by dissolving a known weight of agent in DMSO. The stock solutions were sterilized by passage throught a 0.2 μm Nylon membrane filter. Serial 2-fold dilutions of the compounds with water were prepared. The tubes were incubated at 37 °C for 3-21 days. A control tube without any drug was included in each experiment.

[b] Cytotoxicity of compounds was tested on VERO cell monolayers (ICN-Flow), grown in Dulbecco's modified MEM (GIBCO Lab. Inc.) with 2% fetal calf serum. 6 well culture plates were inoculated with 9×10^4 cells. After 24 h the compounds were added and after 5 further days the cells were detached from wells, trypsinized and counted in a Neubauer chamber. The minimal toxic dose (MTD_{50}) was the concentration of drugs that induced a reduction of 50% of cell growth with respect to the control.

As reported in Table 2, the tested compounds showed an interesting activity, and they were in general more active than controls with the exception of rifampin; in particular compound **6** was very active against *M. marinum*, *M. gordonae* and *M. smegmatis*.

Table 2. *In vitro* activity against *M. smegmatis*, *M. marinum*, *M. gordonae* and *M. avium* of compounds **1**, **2-6**, **isoniazid**, **streptomycin** and **rifampin.**

Compd	MIC (μg/ml)			
	M. smegmatis 103599	*M. marinum* 6423	*M. gordonae* 6427	*M. avium* 103317
1	25	100	>100	0.4
2	>16	>16	>16	>16
3	16	8	>16	16
4	>16	>16	>16	>16
5	8	16	16	16
6	8	1	8	16
isoniazid	64	16	32	32
streptomycin	8	32	16	8
rifampin	32	0.6	0.6	0.3

Conclusion

In summary, compounds **2-6** exhibit a good *in vitro* activity against both *M. tuberculosis* and non tuberculosis species of mycobacteria. From these first microbiological data it is possible to observe a similar behaviour for thiomorpholine and *N*-methylpiperazine derivatives, and the more interesting compound against the atypical mycobacteria tested in this study is compound **6**. Moreover the presence of the chlorine atom at C5 in the phenyl moiety seems to be an important parameter (compound **1** and **4** are much less efficient against these mycobacteria). The above findings suggest further studies directed towards improving the inhibiting activities and reducing the cytotoxicity of **1**, the lead compound of a new class of antimycobacterial agents.

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References and Notes

1. Bass, J.B.,Jr.; Farer,L.S.; Hopewell,P.C.; O Brien, R.; Jacobs, R.F.;Ruben, F.; Snider, D.E., Jr.; Thornton, G. *Am. J. Respir. Crit.Care Med.* **1994**, *149*,1359-1374.
2. Davidson, P.T.; Le, H.Q. Drugs, **1992**, *43*, 651-673.
3. Grosset, J.H. *Bull. Int. Union Tuberc. Lung Dis.* **1990**, *65*, 86-91.
4. Irwin, L.L.; Pharm,D.; Rickman, L.S. *Infect. Med.* **1995**, *12*, 32-39.
5. Deidda, D.; Lampis, G.; Fioravanti, R.; Biava, M.; Porretta, G.C.; Zanetti, S.; Pompei, R. *Antimicrob. Agents Chemother.*, **1998,** 3035-3037.
6. Biava, M.; Fioravanti, R.; Porretta, G.C.; Sleiter, G.; Ettorre, A.; Deidda, D.; Lampis, G.; Pompei, R. *Med. Chem. Res.*, **1999**, 19-34.

7. Barbachyn, M.R.; Hutchinson, D.K.; Brickner, S.J.; Cynamon, H.; Kilburn, J.O.; Klemens, S.P.; Glickman, S.E.; Grega, K.C.; Hendges, S.K.; Toops, D.S.; Ford, C.W.; Zurenko, G.E.; *J. Med. Chem.*, **1996**, *39*, 680-685.
8. Cerreto, F.; Villa, A.; Retico, A.; Scalzo, M. Eur. J. Med. Chem. **1992**, *27*, 701-708.
9. Hawkins, J.E.; Wallace Jr., R.J.; Brown, A.; *Antibacterial susceptibility test: Mycobacteria*: in A. Balows, W.J. Hausler Jr., K.L. Hermann, H.D. Isenberg, H.J. Shadomy (eds.) Manual of Clinical Microbiology, 5th edn., American Society for Microbiology, Washington, D.C. **1991**, 1138-1152.
10. *National Committee for Clinical Laboratory standards. Antimycobacterial susceptibility testing.* **1990**, Proposed Standard M24, Villanova, PA.
11. Brown, B.A.; Sweson, J.M.; Wallace, R.J., Jr. Brith microdilution test for rapidly growing mycobacteria. In: Clinical microbiology procedures Handbook, Vol. I, AMS Press Washington, DC, **1992**.

12. **Characterization data:**

2: Yield: 15 %: mp: 147-150 °C; ^{1}H NMR ($CDCl_3$) δ: 1.97 (s, 3H, pyrrole 2-CH_3), 2.52-2.67 (m, thiomorpholine 8H), 3.36 (s, 2H, 3-CH_2-thiomorph), 6.24 (s, 1H, pyrrole 4H), 6.83-7.26 (m, 9H, aromatic protons).Anal. calcd. for. $C_{22}H_{23}N_2$ SCl: C, 70.00; H, 6.06; N, 7.32; S, 8.36. Found: C, 69.89; H, 6.16; N, 7.56; S, 8.51.

3: Yield: 63%: mp: 102-103 °C; ^{1}H NMR ($CDCl_3$) δ: 2.03 (s, 3H, pyrrole 2-CH_3), 2.62-2.72 (m, thiomorpholine 8H), 3.42 (s, 2H, 3-CH_2-thiomorph), 6.30 (s, 1H, pyrrole 4H), 6.97-7.31 (m, 9H, aromatic protons); Anal. calcd. for. $C_{22}H_{23}N_2$ SCl: C, 70.00; H, 6.06; N, 7.32; S, 8.36. Found: C, 70.18; H, 5.98; N, 7.10; S, 8.31.

4: Yield: 45%: oil: ^{1}H NMR ($CDCl_3$) δ: 2.00 (s, 3H, pyrrole 2-CH_3), 2.55-2.69 (m, thiomorpholine 8H), 3.38 (s, 2H, 3-CH_2-thiomorph), 6.26 (s, 1H, pyrrole 4H), 6.86-7.31 (m, 8H, aromatic protons); Anal. calcd. for. $C_{22}H_{22}N_2$ SCl_2: C, 63.27; H, 5.36; N, 6.71; S, 7.67. Found: C, 63.21; H, 5.42; N, 6.79; S, 7.55.