Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 515–520

BIOORGANIC & MEDICINAL CHEMISTRY

Importance of the Thiomorpholine Introduction in New Pyrrole Derivatives as Antimycobacterial Agents Analogues of BM 212

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Received 31 May 2002; accepted 19 September 2002

Abstract—During the course of our investigations in the field of azole antimicrobial agents, we have identified **BM 212**, a pyrrole derivative with good in vitro activity against mycobacteria and candidae. These findings prompted us to prepare new pyrrole derivatives **1**–**10** in the hope of increasing the activity. The microbiological data showed interesting in vitro activity against *Mycobacterium tuberculosis* and atypical mycobacteria.

Introduction

The resurgence of tuberculosis (TB) is by now sadly remarkable. Moreover, the drug-resistant TB has become a serious concern as increasing numbers of TB cases are reported to be caused by strains of *Mycobacterium tuberculosis* resistant to one or more antituberculosis drugs.[1–3] An urgent need exists for the development of new antimycobacterial agents with a unique mechanism of action that, 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 a search for new substances able to act both as antifungals and antimycobacterials.

Previously, we have reported on the synthesis of both antimycobacterial and antifungal activities of some pyrrole derivatives,[4–6] and it was 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** proved the most active, and it appeared to be endowed with particularly potent and selective antimycobacterial and antifungal properties.

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.[4]

Then, since **BM 212** was also active against *C. albicans* and *Candida* sp., we considered **BM 212** a promising lead for the discovery of more potent agents with both antifungal and antimycobacterial activities.

Consequently, we pursued a program to systematically modify structure **BM 212** but the first modifications have not increased its activity,[5] nevertheless they allowed us to point out the importance of substituents in C5 and N1. As a consequence, we synthesized other derivatives,[6] in which we alternatively introduced *N*-methylpiperazine or thiomorpholine at C3 of the pyrrole and introduced a phenyl ring in N1 and C5 of the pyrrole ring unsubstituted or substituted with a

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PII: S0968-0896(02)00455-8

chlorine atom. The choice to employ the thiomorpholine has been done on the basis of what was observed by Barbachyn,[7] while the introduction of the phenyl rings was on the basis of what was previously observed by us.

New compounds exhibited good in vitro activity against both *M. tuberculosis* and non tuberculosis species of mycobacteria and a similar behavior for both thiomorpholine and *N*-methylpiperazine derivatives; moreover the presence of the chlorine atom at C5 in the phenyl moiety seemed to be an important parameter for the activity against atypical mycobacteria.

In any case, none of them was found to be more active than the lead compound **BM 212**.

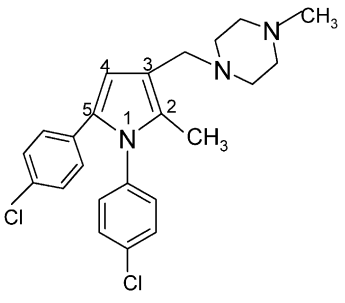
The above findings suggest further studies directed towards improving the inhibitory activities and reducing the cytotoxicity of **BM 212**.

In this paper, we report the synthesis of new compounds **1–10** alternatively introducing *N*-methylpiperazine or thiomorpholine at C3 of the pyrrole and substituting *para* position of the phenyl ring in N1 and/or C5 of the pyrrole ring with Cl or F atoms.

As reported in the Experimental we carried out the same biological screening previously performed to better compare the activity of new compounds with those previously tested. **BM 212** was obviously employed as the reference compound.

Moreover, we previously built and optimized a four-feature pharmacophore model for antitubercular compounds with different structures, and consisting of a hydrophobic, a hydrogen bond acceptor group and two aromatic ring pharmacophore features.[8]

A preliminay superimposition between the most active compound of this series and the model evidenced that **2** possessed appropriate structural elements to interact with all the features of the pharmacophore model itself. This finding led to the suggestion that the new compounds **1–10** were characterized by chemical functions similar (in physicochemical properties and three-dimensional arrangement) to those of the antitubercular compounds used to generate the pharmacophore model and, thus, they all can share the same receptor binding site.



BM 212

As a consequence, the pharmacophore model has been applied to rationalize the structure–activity relationship data of the new compounds described in this paper.

Chemistry

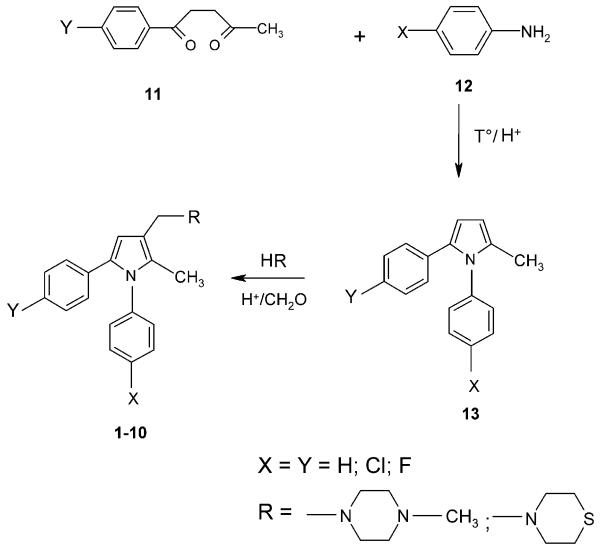
Compounds **1–10** were prepared as illustrated in Scheme 1, from the appropriate 1,4-diketone, obtained by reacting levulinic acid and chorobenzene in the presence of $AlCl_3$. The Mannich bases were obtained by a procedure previously described by us.[5]

All new compounds were identified by elemental analyses and NMR data are reported in the Experimental only for compounds **1** and **2** as representative of *N*-methylpiperazine and thiomorpholine derivatives, respectively.

Physicochemical data for compounds **1–10** are shown in the References section.

Results

The in vitro activities of compounds **1–10** against *M. tuberculosis* 103471, *M. gordonae* 6427, *M. smegmatis* 103599, *M. marinum* 6423 and *M. avium* 103317 are listed in Tables 1 and 2. The Cytotoxicity and Protection



Compd	X	Y	R
1	F	H	*N*-methylpiperazinyl
2	F	H	thiomorpholinyl
3	H	F	*N*-methylpiperazinyl
4	H	F	thiomorpholinyl
5	Cl	F	*N*-methylpiperazinyl
6	Cl	F	thiomorpholinyl
7	F	F	*N*-methylpiperazinyl
8	F	F	thiomorpholinyl
9	F	Cl	*N*-methylpiperazinyl
10	F	Cl	thiomorpholinyl

Scheme 1.

Index (PI) were also evaluated for all synthesized compounds and data are reported in Table 1. None of the investigated compounds was found to be active against *C. albicans*, *Candida* sp., *C. neoformans*, Gram-positive or Gram-negative bacteria, viruses and isolates of pathogenic plant fungi (data not shown).

Discussion

Antimycobacterial activity

Interesting results were obtained from data regarding antimycobacterial activity. In fact, compounds **2**, **4**, **6**, **8** and **10** not only show a remarkable in vitro activity against *M. tuberculosis*, but they were also only slightly toxic. In particular, compounds **2** and **4** were the most active.

It is important to point out that compound **2**, besides being more active than the lead compound **BM 212**, shows a very good Protection Index (PI). In particular, **2** proved to be the most active compound, and both its activity and PI are comparable to those of reference compounds. These findings confirm what was reported about the introduction of the fluorine atom in a structure, regarding better activity and lower toxicity.

As **2** is an analogue of **BM 212**, we also tested it against intracellular and resistant mycobacteria, and data are reported in Tables 3 and 4. All of the tested strains were inhibited by compound **2**, and it exerts bactericidal activity also on intracellular mycobacteria. The MIC is comparable to that of Rifampin, even though it is higher than **BM 212**. This result is very important because mycobacteria can reside for years inside lymphoid cells and macrophages, and traditional drugs are not able to get through it. One other important aspect is the high selectivity of derivatives **2**, **4**, **6**, **8** and **10** against mycobacteria. In fact, these compounds are very active only against *M. tuberculosis*, while they are completely inactive against atypical mycobacteria, with the exception of **2** that is also active against *M. avium* (see Table 2).

In conclusion, compound **2** is the most potent pyrrole derivative studied so far, more potent, less toxic and more selective than **BM 212**, the lead compound for this class of antimycobacterial agent, that shows, differently from **2**, also anticandida activity. A chloro atom simultaneously in the *para* position at both N1 and C5 of the pyrrole seems to be essential for the antimycotic activity.

Computational investigations

Considering biological data, it is possible to observe that activity of compounds **1–10** is markedly affected by replacement of the *N*-methylpiperazine moiety with a thiomorpholine group, with compounds **2** and **4** showing the best values of in vitro activity toward *M. tuberculosis* (0.4 and 0.5 μg/mL, respectively). Moreover, in the thiomorpholino series, monofluoro derivatives **2** and **4** are more active than the corresponding dihalogenated counterparts **6**, **8**, and **10**.

To further analyze the relationships between the structural properties of compounds **1–10** and their relative activity data, we have applied a pharmacophore model recently built[8] and optimized[9] by our research group starting from compounds belonging to different structural classes of known antitubercular agents.

The aim of the current computational study was the investigation of the structural features of compounds

Table 1. Cytotoxicity, in vitro activity against *M. tuberculosis* and Protection Index (PI) of compounds **BM 212**, **1–10**, Isoniazid, Streptomycin and Rifampin

Compound	MIC (μg/mL)		Protection Index (PI)
	MTD_{50} VERO cells	*M. tuberculosis* 103471	
BM 212	4	0.70	5.6
1	16	16	1
2	8	0.4	20
3	4	16	0.25
4	4	0.5	8
5	8	16	0.5
6	8	2	4
7	16	16	1
8	8	1	8
9	16	2	8
10	8	1	8
Isoniazid	32	0.25	128
Streptomycin	>64	0.50	128
Rifampin	64	0.3	213

Table 2. In vitro activity against *M. smegmatis*, *M. marinum*, *M. gordonae* and *M. avium* of compounds **BM 212**, **1–10**, Isoniazid, Streptomycin and Rifampin

Compound	MIC (μg/mL)			
	M. smegmatis 103599	*M. marinum* 6423	*M. gordonae* 6427	*M. avium* 103317
BM 212	25	100	>100	0.4
1	>16	>16	>16	>16
2	>16	>16	>16	2
3	>16	16	>16	16
4	>16	>16	>16	16
5	>16	>16	16	>16
6	>16	>16	>16	>16
7	>16	>16	>16	>16
8	>16	16	>16	16
9	>16	8	>16	>16
10	>16	>16	16	16
Isoniazid	64	16	32	32
Streptomycin	8	32	16	8
Rifampin	32	0.6	0.6	0.3

Table 3. Inhibition of intramacrophagic *M. tuberculosis* of compound **2** and Rifampin

Compound	MIC (μg/mL) Inhibition of intramacrophagic mycobacteria
2	3
BM 212	1
Rifampin	3

Table 4. Sensitivity of different strains of *M. tuberculosis* resistant to different inhibitors

Strains resistant N.	Streptomycin	Isoniazid	Rifampicin	Ethambutol	BM 212	2
M. tuberculosis 15	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
M. tuberculosis 150	Sensitive	Sensitive	Resistant	Sensitive	Sensitive	Sensitive
M. tuberculosis 585	Sensitive	Resistant	Resistant	Sensitive	Sensitive	Sensitive
M. tuberculosis 535	Sensitive	Sensitive	Sensitive	Resistant	Sensitive	Sensitive
M. tuberculosis 541	Resistant	Resistant	Sensitive	Sensitive	Sensitive	Sensitive

1–10 responsible for antitubercular activity toward *M. tuberculosis*, with a view to contributing to the understanding of the interaction of all these compounds with the corresponding putative receptor. In this context, we have assumed that all these compounds bind in the same way to the receptor itself.

Moreover, because no experimental data on the biologically relevant conformations of the selected compounds are available, we resorted to a molecular mechanics approach (the 2D–3D sketcher of Catalyst)[10] to build the conformational models to be used in the fitting procedure to the pharmacophore.

Evaluation of how well derivatives **1–10** are able to fit the pharmacophore model highlighted that the chemical functionalities of the model are all matched by the chemical groups of **2** (Fig. 1), the most active compound of the whole set, taken as a representative example of all pyrrole derivatives. In particular, while the fluorine atom occupies the hydrophobic feature (HY) of the model, RA1 is matched by the phenyl ring bound to the nitrogen of the pyrrole nucleus. Moreover, the second aromatic ring feature (RA2) is mapped by the phenyl ring at the 2-position of the pyrrole, and the sulfur atom of the thiomorpholine ring is located inside the hydrogen bond acceptor feature, HBA. The last finding is worthy of further consideration. Particularly, the location of the sulfur atom allows the rationalization of the best activity generally associated with the thiomorpholino derivatives with respect to the corresponding *N*-methylpiperazine analogues. In fact, for compounds **1**, **3**, **5**, **7**, and **9** we were unable to find a similar conformation characterized by the piperazine N4 nitrogen atom as the hydrogen bond acceptor. Accordingly, for such compounds, the N1 nitrogen atom fulfills the HBA feature.

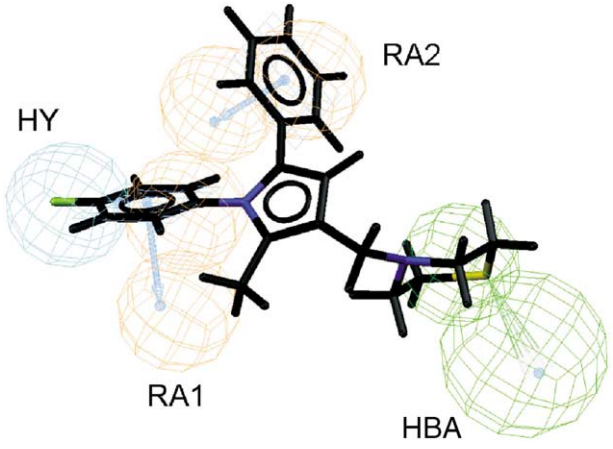


Figure 1. Compound **2** mapped to the pharmacophore model.

Analysis of the fitting properties of the studied compounds to the pharmacophore model allowed additional considerations. In fact, within the thiomorpholino subset, compound **4** showed a very similar orientation with respect to **2**, the fluorine atom occupying the hydrophobic feature and the pyrrole nucleus simply reversing its orientation of 180°. Similarly, compounds **8** and **10** possess a fluorine atom (bound to the phenyl ring attached to the pyrrole nitrogen) mapping HY. On the contrary, the best orientation of **6** showed the chlorine substituent matching HY and the fluorine atom lying in an empty region of space adjacent to RA2.

In summary, the pharmacophore model was able to rationalize two major structure–activity relationships for these compounds. In particular, structures with a thiomorpholine ring were found to better fit the model with respect to the corresponding *N*-methylpiperazine derivatives, in agreement with in vitro activity data toward *M. tuberculosis*. Finally, a fluorine atom lying in the region of HY was shown as a well-suited substituent to allow a good fit of such compounds into the pharmacophore model and enhance their antitubercular activity.

Experimental

Melting points were uncorrected and taken on a Fischer-Jones apparatus. Infrared spectra (Nujol mulls) were run on a 297 Perkin-Elmer spectrophotometer. NMR spectra were recorded for all the synthesized compounds on a 200 Brucker spectrometer using deuterochloroform as solvent and TMS as internal standard. Microanalyses of compounds **1–10** were performed by the Servizio di Microanalisi dell'Area di Ricerca di Roma del CNR (Dr. F. Tarli, Dr. Petrilli and Mr. Dianetti). Carlo Erba aluminum oxide (activity II–III, according to Brockmann) was used for chromatographic purifications. Fluka Stratocrom aluminum oxide plates with fluorescent indicator were used for thin-layer chromatography (TLC) to check the purity of the compounds.

Syntheses

Pyrroles 13. The title compounds were prepared according to the general procedure previously described.[5]

Mannich bases 1–10. To a stirred solution of the appropriate pyrrole **13** (5.6 mmol) in 20 mL of acetonitrile, a mixture of *N*-methylpiperazine or thiomorpholine (5.6

mmol), formaldehyde (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. The eluates were combined after TLC control and the solvent was removed to give the pure product.

Physicochemical data are reported in Table 5.

1: ^{1}H NMR ($CDCl_3$) δ: 1.98 (s, 3H, N–CH_3), 2.29 (s, 3H, pyrrole 2-CH_3), 2.45 (m, 8H, *N*-methylpiperazine 2 and 3-CH_2), 3.42 (s, 2H, CH_2–N), 6.30 (s, 1H, pyrrole 4H), 7.1–7.34 (m, 10H, aromatic protons).

2: ^{1}H NMR ($CDCl_3$) δ: 1.97 (s, 3H, pyrrole 2-CH_3), 2.56–2.69 (m, thiomorpholine 8H), 3.39 (s, 2H, 3-CH_2-thiomorph), 6.24 (s, 1H, pyrrole 4H), 6.83–7.29 (m, 9H, aromatic protons).

Microbiology

Compounds. All compounds **1–10** and drug references were dissolved in DMSO at a concentration of 10 mg/mL and stored cold until used.

Antimycobacterial activity. All compounds were preliminarily assayed against two freshly isolated clinical strains, *M. fortuitum* CA10 and *M. tuberculosis* B814, according to the dilution method in agar.[11] Growth media were Mueller–Hinton (Difco) containing 10% of OADC (oleic acid, albumine and dextrose complex) for *M. fortuitum* and Middlebrook 7H11 agar (Difco) with 10% of OADC (albumine dextrose complex) for *M. tuberculosis*. Substances were tested at the single dose of 100 μg/mL. The active compounds were then assayed for inhibitory activity against a variety of mycobacterium strains in Middlebrook 7H9 broth using the NCCLS procedure. They are reported in Tables 1 and 2. The mycobacterium species used were *M. tuberculosis* 103471 and among the atypical mycobacteria *M. smegmatis* 103599, *M. gordonae* 6427, *M. marinum* 6423 and *M. avium* 103317 (from the Institute Pasteur collection).

In all cases, minimum inhibitory concentrations (MICs in μg/mL) for each compound were determined. The MIC was defined as the lowest concentration of drug that yielded an absence of visual torbidity. 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. Isoniazid (INH), Streptomycin and pyrrolnitrin were used as controls.

Table 5. Chemical–physical data of compounds **1–10**

Compound	Mp (°C)	Yield (%)	Formula (MW)
1	137–140	60	$C_{23}H_{26}N_3F$ (363.47)
2	95–97	54	$C_{22}H_{23}N_2SF$ (366.43)
3	102–105	58	$C_{23}H_{26}N_3F$ (363.47)
4	107–110	50	$C_{22}H_{23}N_2SF$ (366.43)
5	115–118	35	$C_{23}H_{25}N_3FCl$ (397.91)
6	90–93	85	$C_{22}H_{22}N_2SFCl$ (400.92)
7	109–111	68	$C_{23}H_{25}N_3F_2$ (381.46)
8	110–111	88	$C_{22}H_{22}N_2SF_2$ (384.47)
9	59–60	80	$C_{23}H_{25}N_3FCl$ (397.91)
10	149–150	50	$C_{22}H_{22}N_2SFCl$ (400.92)

Inhibitory activity of BM 212 and 2 on multidrug-resistant and intramacrophagic mycobacteria. The mycobacteria used were *M. tuberculosis* 15, *M. tuberculosis* 150, *M. tuberculosis* 585, *M. tuberculosis* 535 and *M. tuberculosis* 541. The MIC of the compound **BM 212** was tested on multiresistant *M. tuberculosis* strain in Middlebrook 7119 broth enriched with 10% ADC (Difco) using the macrodilution broth method. The bactericidal activity of **BM 212** on intracellular mycobacteria was studied on U_{937} cells (INCFLOW), a human histiocytic cell line. The cells were differentiated into macrophages with 20 ng/mL of phorbol myristate acetate (PMA, Sigma) and grown in RPMI 1640 medium with 10% fetal calf serum.

Antimycotic activity. Antiyeast activity was tested with a broth microdilution method[12] and the minimal inhibitory concentration (MIC), the range of MICs and the mean susceptibility (nX) have been calculated as described elsewhere.[13] Ketoconazole and miconazole were used for comparative studies.

Antiviral activity. Antiviral activity was assayed on VERO cell monolayers infected with Herpes simplex virus type 2 (HSV2) and Poliovirus type 1 (1S) (all from NIH, USA). VERO cells in 24-cell culture plates were infected with the viruses at a ratio of about 1000 cells/viral infectious unit. After 48 h of incubation, the viral cytopathic effect was detected under a light microscope and the antiviral effect of drugs was reported as the dose which inhibited by 50% the cytopathic effect of viruses (ID_{50}). Foscarnet was used as control.

Computational methods

All calculations and graphic manipulations were performed on a Silicon Graphics O2 workstation by means of the Catalyst 4.6 software package.

All the compounds used in this study were built using the 2D–3D sketcher of the program. A representative family of conformations was generated for each molecule using the poling algorithm and the 'best quality conformational analysis' method.[10] The parameter set employed to perform all the conformational calculations derives from the CHARMm force field,[14] opportunely modified and corrected.[15]

The best quality conformational analysis approach has been selected because it provides the best possible conformational coverage within the catalyst.[16]

Conformational diversity was emphasized by selection of the conformers that fell within the 20 kcal/mol range above the lowest energy conformation found.

The compare/fit command within the catalyst has been used to predict activity values of the studied compounds. Particularly, the best fit option has been selected which manipulates the conformers of each compound to find, when possible, different mapping modes of the ligand within the model.

Acknowledgements

Supported by a contribution of the Istituto Pasteur Fondazione Cenci-Bolognetti Università di Roma 'La Sapienza'.

Appendix. Microanalysis data for compounds 1–10

Compd	%C	%H	%N	%S	%F	%Cl	
1	76.03	7.16	11.57		5.23		Calcd
	76.05	7.12	11.59		5.19		Found
2	72.13	6.28	2.19	8.74	5.19		Calcd
	72.00	6.30	2.19	8.70	5.20		Found
3	76.03	7.16	11.57		5.23		Calc
	76.08	7.18	11.55		5.27		Found
4	72.13	6.28	2.19	8.74	5.19		Calcd
	72.22	6.32	2.15	8.72	5.22		Found
5	69.43	6.29	10.57		4.78	8.93	Calcd
	69.41	6.20	11.00		4.80	8.89	Found
6	65.92	5.49	10.49	7.99	4.74	8.86	Calcd
	65.96	5.52	10.44	8.00	4.71	8.89	Found
7	72.44	6.56	11.02		9.97		Calcd
	72.49	6.50	10.98		10.02		Found
8	68.75	5.73	7.29	8.33	9.89		Calcd
	68.93	5.69	7.30	8.34	9.93		Found
9	69.52	6.30	10.58		4.79	8.93	Calcd
	69.55	6.27	10.60		4.83	8.89	Found
10	65.92	5.49	10.49	7.99	4.74	8.86	Calcd
	65.89	5.52	10.44	8.00	4.69	8.91	Found

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.
2. Davidson, P. T.; Le, H. Q. *Drugs* **1992**, *43*, 651.
3. Grosset, J. H. *Bull. Int. Union Tuberc. Lung Dis.* **1990**, *65*, 86.
4. Deidda, D.; Lampis, G.; Fioravanti, R.; Biava, M.; Porretta, G. C.; Zanetti, S.; Pompei, R. *Antimicrob. Agents Chemother.* **1998**, 3035.
5. Biava, M.; Fioravanti, R.; Porretta, G. C.; Sleiter, G.; Ettorre, A.; Deidda, D.; Lampis, G.; Pompei, R. *Med. Chem. Res.* **1999**, 19.
6. Biava, M.; Fioravanti, R.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2983.
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.
8. Manetti, F.; Corelli, F.; Biava, M.; Fioravanti, R.; Porretta, G. C.; Botta, M. *Il Farmaco* **2000**, *55*, 484.
9. Biava, M.; Fioravanti, R.; Porretta, G.C.; Deidda, D.; Maullu, C.; Pompei, R. *Med. Chem. Res.* **2002**, *11* (1), 50–66.
10. Catalyst Software; Accelrys, Inc.
11. Hawkins, J. E.; Wallac, R. J. Jr.; Brown, A. Antibacterial Susceptibility Test: Mycobacteria. In Balows, A., Hausler, W. J. Jr., Hermann, K. L., Isenberg, H. D., Shadomy, H. J., Eds. *Manual of Clinical Microbiology*, 5th ed.; American Society for Microbiology: Washington, DC, 1996.
12. McGinnis, M. R.; Rinaldi, M. G. *Antibiotics in Laboratory Medicine*; Williams & Wilkins: Baltimore, 1986; p 223.
13. Fioravanti, R.; Biava, M.; Porretta, G. C.; Lampis, G.; Deidda, D.; Pompei, R. *Med. Chem. Res.* **1999**, 162.
14. (a) Smellie, A.; Teig, S. L.; Towbin, P. *J. Comp. Chem.* **1995**, *16*, 171. (b) Smellie, A.; Kahn, S. D.; Teig, S. L. *J. Chem. Inf. Comp. Sci.* **1995**, *35*, 285. (c) Smellie, A.; Kahn, S. D.; Teig, S. L. *J. Chem. Inf. Comp. Sci.* **1995**, *35*, 295.
15. Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comp. Chem.* **1983**, *4*, 187.
16. Catalyst force field and potential energy functions are described in the file REFenergy.doc.html provided by Accelrys along with the program Catalyst 4.6.