

Antimycobacterial compounds. New pyrrole derivatives of BM212

Mariangela Biava,^{a,*} Giulio Cesare Porretta,^a Delia Deidda,^b Raffaello Pompei,^b
Andrea Tafi^c and Fabrizio Manetti^c

^aDipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università 'La Sapienza', P.le A Moro 5,
00185 Rome, Italy

^bCattedra di Microbiologia Applicata, Facoltà di Scienze Matematiche Fisiche Naturali, Università degli Studi di Cagliari, Via Porcell 4,
09124 Cagliari, Italy

^cDipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via Aldo Moro snc, 53100 Siena, Italy

Received 29 July 2003; accepted 22 December 2003

Abstract—We have identified **BM212** as a lead compound among a series of pyrrole derivatives with good in vitro activity against *mycobacteria* and *candidae*. First studies led us to synthesize some pyrrole compounds in which the thiomorpholine fragment was present. Some compounds revealed very active and these findings prompted us to prepare new pyrrole derivatives **2–15** in the hope of increasing the activity. The microbiological data showed interesting in vitro activity against *Mycobacterium tuberculosis* and atypical mycobacteria.

© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

The increase of tuberculosis (TB) cases due in particular to the increased incidence of *M. avium* complex infection in HIV-infected individuals, has prompted a vigorous search for new drugs for the treatment of the disease.

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.

In our previous work we have reported on the synthesis and both antimycobacterial and antifungal activities of some pyrrole derivatives,^{4–6} and most of the synthesized compounds showed interesting antimycobacterial activities. Among them, **BM 212** revealed the most active, and it appeared to be endowed with particularly potent and selective both antimycobacterial and antifungal properties.⁴

A program followed by us to systematically modify **BM 212** led us to individuate the importance of the substituents in C5, N1⁵ and C3.⁶ The microbiological results showed the importance of the presence of the thiomorpholine at C3 of the pyrrole and the *p*-chlorophenyl substituents in N1 and C5. The choice to employ the thiomorpholine has been done on the basis of what previously observed by Barbachyn,⁷ while the introduction of the *p*-Cl-phenyl rings on the basis of what was previously observed by us.

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

Moreover, we previously built and optimized a four-feature pharmacophore model for antimycobacterial compounds with different structure, and consisting in a hydrophobic, a hydrogen bond acceptor group and two aromatic ring pharmacophore features.⁸

More recently we synthesized new pyrrole derivatives⁹ and among them, compound **1** was found more potent, less toxic and more selective than the lead compound **BM 212**.

Keywords: Pyrrole derivatives; Thiomorpholine substituent; Antimycobacterial activity; Pharmacophore model.

* Corresponding author. Tel.: +39-6-49913812; fax: +39-6-49913133;
e-mail: mariangela.biava@uniroma1.it

All new compounds were identified by elemental analyses and NMR data, that were reported in the experi-

mental part only for compounds **2** and **3** as representative for thiomorpholine or *N*-methylpiperazine derivatives respectively.

Physicochemical data for compounds **2–15** are shown in Table 5.

3. Results

The in vitro activities of compounds **2–15** against *Mycobacterium tuberculosis* 103471, *M. gordonae* 6427, *M. smegmatis* 103599, *M. marinum* 6423 and *M. avium*

Table 1. Cytotoxicity, in vitro activity against *M. tuberculosis* and Protection Index (PI) of compounds **BM 212**, **1**, **2–15**, Isoniazid, Streptomycin and Rifampin

Compd	MIC (µg/mL)		
	MTD ₅₀ VERO cells	<i>M. tuberculosis</i> 103471	Protection Index (PI)
BM 212	4	0.70	5.6
1	8	0.4	20
2	8	8	1
3	2	> 16	—
4	4	4	1
5	2	16	—
6	2	16	—
7	4	> 16	—
8	2	16	—
9	4	> 16	—
10	4	> 16	—
11	2	> 16	—
12	4	> 16	—
13	4	> 16	—
14	32	1	32
15	4	16	—
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**, **2–15**, Isoniazid, Streptomycin and Rifampin

Compd	MIC (µg/mL)			
	<i>M. smegmatis</i> 103599	<i>M. marinum</i> 6423	<i>M. gordonae</i> 6427	<i>M. avium</i> 103317
BM 212	25	100	> 100	0.4
1	> 16	> 16	> 16	2
2	> 16	> 16	> 16	> 16
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	> 16	> 16	> 16
10	> 16	> 16	> 16	> 16
11	> 16	> 16	> 16	> 16
12	> 16	> 16	> 16	> 16
13	> 16	> 16	> 16	> 16
14	> 16	> 16	> 16	16
15	> 16	8	16	> 16
Isoniazid	64	16	32	32
Streptomycin	8	32	16	8
Rifampin	32	0.6	0.6	0.3

103317 are listed in Tables 1 and 2. Cytotoxicity and Protection Index (PI) were also evaluated for all synthesized compounds and data are reported in Table 1.

4. Discussion

4.1. Antimycobacterial activity

Interesting results were obtained from data regarding antimycobacterial activity. In fact in general, by a comparison between *N*-methylpiperazine and thiomorpholine derivatives, we can confirm the importance of the thiomorpholine introduction in the pyrrole structure regarding the in vitro antimycobacterial activity.⁹ In any case some considerations have to be done regarding the substituent in N1 and C5. It is important to point out that, contrary to our expectations, compounds **2**, **4**, **6** and **8** were less active and more toxic than the corresponding *p*-F or *p*-Cl derivatives.⁹ These results underline that the fluorine atom in *para* position at both the phenyl rings in N1 and/or C5 of the pyrrole ring is fundamental for the activity. In fact, compounds **2** and **4** are 20- and 8-fold less active than the corresponding *para* fluoro derivative **1**, respectively, suggesting that the *ortho* substitution does not increase the superimposition with the aromatic areas. The results are even worse for compound **10** and **12**, that are completely inactive. On the contrary, for compound **14**, in which both the phenyl rings in N1 and C5 are unsubstituted, a very interesting profile was found. It besides to be less toxic than **1** and **BM 212** shows also a very good Protection Index (PI) (Table 1). In particular, even if **14** is not the most active compound, its activity is comparable to that of reference compounds and PI is the best among all the compounds synthesized till now. This findings is in contrast with what previously reported about the introduction of the fluorine atom in a structure, about a better activity and a lower toxicity.

As **14** is analogue of **1** and **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 **14**, and it exerts bactericidal activity also on intracellular mycobacteria. The MIC is the same of **BM 212** and lower than Rifampin and **1**. This result is very important because mycobacteria can reside for years inside lymphoid cells and macrophage, and traditional drugs are not able to get throw it. One other important aspect is the high selectivity of derivative **14** against mycobacteria. In fact, this compound is very active only against *M. tubercu-*

Table 3. Inhibition of intramacrophagic *Mycobacterium tuberculosis* of compound **1**, **14**, **BM 212** and Rifampin

Compd	MIC (µg/mL)	
	Inhibition of intramacrophagic mycobacteria	
14	1	
1	3	
BM 212	1	
Rifampin	3	

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

Strains resistant N.	Strept.	Isoniazid	Rifamp.	Ethamb.	BM 212	1	14
<i>M. tuberculosis</i> 15	s ^a	s	r	s	s	s	s
<i>M. tuberculosis</i> 150	s	s	r	s	s	s	s
<i>M. tuberculosis</i> 585	s	r	r	s	s	s	s
<i>M. tuberculosis</i> 535	s	s	s	r	s	s	s
<i>M. tuberculosis</i> 541	r ^b	r	s	s	s	s	s

^a Sensitive.^b Resistant.

losis, while it is completely inactive against atypical mycobacteria, with the exception of *M. avium* (see Table 2).

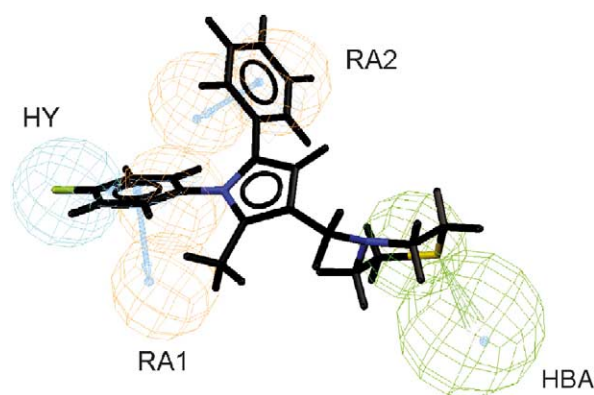
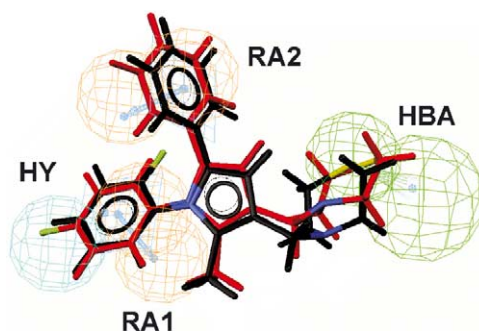
In conclusion compound **14** is the most interesting pyrrole derivative studied so far, as active and selective as **1** but less toxic than both **1** and **BM 212**.

5. Computational investigations

The new pyrrole derivatives have been computationally analysed by means of the Catalyst software¹¹ for their fit properties to a pharmacophoric model previously built from us for antitubercular compounds. As a result, it has been highlighted that a fluorine atom at the para position of one of the two aromatic rings directly linked to the pyrrole nucleus, plays a crucial role for antitubercular activity, according to our previously reported data. In fact, compounds **2** (MIC=8 µg/mL) and **4** (MIC=4 µg/mL) were found 20- and 8-fold less active than *p*-fluoro derivative (Figs 1 and 2) **1**. This trend in activity could be justified considering that the fluorine atom at the *para* position is accommodated into the hydrophobic region HY of the pharmacophoric model. As a consequence, lacking such a substituent corresponds to the impossibility of interacting with the hydrophobic zone, with a consequent decrease in activity. Moreover, introduction of a halogen atom at the *ortho* position instead of the *para* position was detrimental to activity. In fact, comparison of biological data associated with **2**, **4** and **8** suggested that increasing the substituent size led to a decreased activity of the corresponding compound. In addition, analysis of the superposition model of the above three compounds into

the pharmacophoric model led to the finding that the *ortho*-halogen is located in a region of space where any pharmacophoric feature lies. It also suggested that a decreased activity associated with an increased halogen size could derive from an unfavorable steric interaction between the same halogen atom and the corresponding receptor counterpart.

Regarding compounds **10** and **12** bearing a 1-naphthyl moiety at the nitrogen atom or at the position 5 of the pyrrole nucleus, we found that such a large ring was unable to satisfy at the same time the hydrophobic portion HY and the aromatic region RA1 of the pharmacophore, with a consequent decrease in activity in respect to the corresponding *p*-fluorophenyl derivatives.

**Figure 1.** **1** mapped to the pharmacophore model.**Figure 2.** Compound **2** (MIC=8 µg/mL) and the corresponding *p*-fluoro derivative **1** (MIC=0.4 µg/mL) mapped to the pharmacophore model. It is important to note that the orientation of both compounds into the pharmacophore is very similar. The most important difference in orientation is represented by the fact that **2** is unable to occupy the HY hydrophobic region, with a consequent decrease of 20-fold in antitubercular activity.**Table 5.** Chemical-physical data of compounds **2–15**

Compd	mp (°C)	Yield (%)	Formula (MW)
2	95–96	45	C ₂₂ H ₂₃ N ₂ SF (366.50)
3	Oil	35	C ₂₃ H ₂₆ N ₃ F (363.48)
4	90–91	75	C ₂₂ H ₂₃ N ₂ SF (366.50)
5	73–74	40	C ₂₃ H ₂₆ N ₃ F (363.48)
6	70–71	45	C ₂₂ H ₂₃ N ₂ SCl (382.96)
7	oil	60	C ₂₃ H ₂₆ N ₃ Cl (379.94)
8	98–99	50	C ₂₂ H ₂₃ N ₂ SCl (382.96)
9	73–74	30	C ₂₃ H ₂₆ N ₃ Cl (379.94)
10	130–131	30	C ₂₆ H ₂₆ N ₂ S (398.57)
11	Oil	30	C ₂₇ H ₂₉ N ₃ (395.55)
12	120–121	98	C ₂₆ H ₂₆ N ₂ S (398.57)
13	112–113	70	C ₂₇ H ₂₉ N ₃ (395.55)
14	Oil	96	C ₂₂ H ₂₄ N ₂ S (348.51)
15	104–107	55	C ₂₃ H ₂₇ N ₃ (345.49)

All these considerations led to the suggestions that 1) an aromatic system with a reduced overall size (with respect to the naphthyl group) but more extended in length (i.e., a biphenyl moiety), or (2) a phenyl ring bearing a lipophilic group (such as a methyl, ethyl, or isopropyl substituent) at the para position could have profitable interactions with the pharmacophoric model (with a consequent enhancement in activity), especially with its HY lipophilic portion.

The thiomorpholino sulphur atom of each pyrrole derivative corresponded to the hydrogen bond acceptor feature of the pharmacophoric model.

Finally, the methyl group at the position 2 of the pyrrole nucleus seemed to be not important for interacting directly with the pharmacophoric elements. On the contrary, it played a crucial role in determining the conformational properties of each compound, in particular the orientation of the aromatic ring directly linked to the pyrrole nitrogen with respect to the side chain bearing the thiomorpholine system. On the basis of all the above results, compounds with unsubstituted phenyl rings, such as **14**, were found unexpectedly to have an interesting profile that the pharmacophoric model is at the moment unable to justify.

6. 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 Bruker spectrometer using deuteriochloroform as solvent and TMS as internal standard. Microanalyses of compounds **2–15** 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.

6.1. Syntheses

6.1.1. Diketones 18. To a solution of the suitable arylaldehyde (0.09 mol), triethylamine (0.14 mol), methyl vinyl ketone (0.07 mol) and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (0.014 mol) were added. The mixture was heated at 70 °C for 24 h under nitrogen atmosphere. At the end the mixture was treated with 2 N HCl (10 mL) and after extraction with methylene chloride (200 mL), the organic layer was washed with aqueous sodium bicarbonate (100 mL) and water (200 mL). The organic fractions were dried over Na₂SO₄, filtered and concentrate to give a crude product that was chromatographed (Al₂O₃; hexane/ethylacetate, 1/1).

6.1.2. Pyrroles 19. The title compounds were prepared according to the general procedure previously described.⁵

6.1.2. Mannich bases 2–1. To a stirred solution of the appropriate pyrrole **19** (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) (100 mL) and extracted with ethyl acetate (200 mL). The organic extracts were combined, washed with water (200 mL) 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.

2: Yield: ¹H NMR (CDCl₃) δ: 1.97 (s, 3H, pyrrole 2-CH₃), 2.56–2.69 (m, thiomorpholine 8H), 3.39 (s, 2H, 3-CH₂-thiomorph), 6.24 (s, 1H, pyrrole 4H), 6.83–7.29 (m, 9H, aromatic protons).

3: Yield: ¹H NMR (CDCl₃) δ: 1.98(s, 3H, N-CH₃), 2.29 (s, 3H, pyrrole 2-CH₃), 2.45 (m, 8H, *N*-methylpiperazine 2 and 3-CH₂), 3.42 (s, 2H, CH₂-N), 6.30 (s, 1H, pyrrole 4H), 7.1–7.34 (m, 10H, aromatic protons).

6.2. Microbiology

6.2.1. Compounds. All compounds **2–15** and drug references were dissolved in DMSO at a concentration of 10 mg/mL and stored cold until used.

6.2.2. 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.¹² 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 g/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. goodii* 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 turbidity. Stock solutions of substances were prepared by dissolving a known weight of agent in DMSO. The stock solutions were sterilized by passage through 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.

6.2.3. Inhibitory activity of BM 212, 1 and 14 on multi-drug-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, 1** and **14** as 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, 1** and **14** on intracellular mycobacteria was studied on U₉₃₇ cells (INC-FLow), 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.

7. 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-3-D sketcher of the program. A representative family of conformations were generated for each molecule using the poling algorithm and the 'best quality conformational analysis' method¹¹. The parameter set employed to perform all the conformational calculations derives from the CHARMM force field,¹³ opportunely modified and corrected.¹⁴

The best quality conformational analysis approach has been selected because it provides the best possible conformational coverage within Catalyst.¹⁵

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

The Compare/Fit command within 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.

8. Table of microanalyses

Compd	%C	%H	%N	%S	F	Cl
2	72.10	6.33	7.64	8.75	5.18	Calc
	72.14	6.30	7.65	8.72	5.21	found
3	76.00	7.21	11.56		5.23	Calc
	75.98	7.24	11.59		5.19	found
4	72.10	6.33	7.64	8.75	5.18	Calc
	72.10	6.31	7.65	8.74	5.22	found
5	76.00	7.21	11.56		5.23	Calc
	76.02	7.24	11.53		5.26	found
6	69.00	6.05	7.31	8.37		9.26 Calc
	69.04	6.05	7.31	8.39		9.23 found
7	72.71	6.90	11.06			9.33 Calc
	72.69	6.87	11.09			9.31 found
8	69.00	6.05	7.31	8.37		9.26 Calc
	69.03	6.06	7.30	8.34		9.28 found
9	72.71	6.90	11.06			9.33 Calc
	72.70	6.93	11.10			9.30 found

Compd	%C	%H	%N	%S	F	Cl
10	78.35	6.58	7.03	8.04		Calc
	78.31	6.59	7.02	8.07		found
11	81.99	7.39	10.62			Calc
	81.97	7.38	10.62			found
12	78.35	6.58	7.03	8.04		Calc
	78.55	6.60	7.03	8.05		found
13	81.99	7.39	10.62			Calc
	81.98	7.38	10.65			found
14	75.82	6.94	8.04	9.20		Calc
	75.79	6.98	8.03	9.23		found
15	79.96	7.88	12.16			Calc
	79.94	7.90	12.16			found

Acknowledgements

Supported by a grant of MIUR of Italy (40%). Università di Roma 'La Sapienza'.

References and notes

- Hamilton, C. D. *Curr. Infect. Dis. Rep.* **1999**, *1*, 80.
- Migliori, G. B.; Ambrosetti, M.; Fattorini, L.; Penati, V.; Vaccarino, P.; Besozzi, G.; Ortona, L.; Saltini, C.; Orefici, G.; Moro, M. L.; Lona, E.; Cassone, A. *Int. J. Tuberc. Lung Dis.* **2000**, *4*, 940.
- Fitzgerald, D. W.; Morse, M. M.; Pape, J. W.; Johnson, W. D., Jr. *Clin. Infect. Dis.* **2000**, *31*, 1495.
- Deidda, D.; Lampis, G.; Fioravanti, R.; Biava, M.; Porretta, G. C.; Zanetti, S.; Pompei, R. *Antimicrob. Agents Chemother.* **1998**, 3035.
- Biava, M.; Fioravanti, R.; Porretta, G. C.; Sleiter, G.; Ettore, A.; Deidda, D.; Lampis, G.; Pompei, R. *Med. Chem. Res.* **1999**, 19.
- Biava, M.; Fioravanti, R.; Porretta, G. C.; Deidda, D.; Maullu, C.; Pompei, R. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2983.
- 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.
- Manetti, F.; Corelli, F.; Biava, M.; Fioravanti, R.; Porretta, G. C.; Botta, M. *Il Farmaco* **2000**, *55*, 484.
- Biava, M.; Porretta, G. C.; Deidda, D.; Pompei, R.; Manetti, F.; Tafi, A. *Bioorg. Med. Chem.* **2003**, *11* (4), 515.
- Khanna, I. K.; Weier, R. M.; Yu, Y.; Collins, P. W.; Miyashiro, J. M.; Koboldt, C. M.; Veenhuizen, A. W.; Currie, K. S.; Seibert, K.; Isakson, P. C. *J. Med. Chem.* **1997**, *40*, 1619.
- Catalyst software, Accelrys, Inc.
- Hawkins, J. E.; Wallace, R. J., Jr.; Brown, A. Anti-bacterial susceptibility test: Mycobacteria. In *Manual of Clinical Microbiology*, 5th edn.; Balows, A., Hausler, W. J., Jr., Hermann, K. L., Isenberg, H. D., Shadomy, H. J., Eds.; American Society for Microbiology: Washington, D, 1991.
- (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.
- Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comp. Chem.* **1983**, *4*, 187.
- 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.