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Antimycobacterial activity of new *ortho*-, *meta*- and *para*-toluidine derivatives

Mariangela Biava [a,*], Rossella Fioravanti [a], Giulio Cesare Porretta [a], Giancarlo Sleiter [b], Delia Deidda [c], Giorgio Lampis [c], Raffaello Pompei [c]

[a] *Dipartimento di Studi di Chimica e Tecnologia delle Sostanze Biologicamente Attive, Università 'La Sapienza', P. le A Moro 5, I-00185 Rome, Italy*

[b] *Dipartimento di Chimica and Centro CNR di Studio sui Meccanismi di Reazione, Università 'La Sapienza', P. le A Moro 5, I-00185 Rome, Italy*

[c] *Cattedra di Microbiologia Applicata, Facoltà di Scienze Matematiche Fisiche Naturali, Università degli Studi di Cagliari, Via Porcell 4, I-09124 Cagliari, Italy*

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Abstract

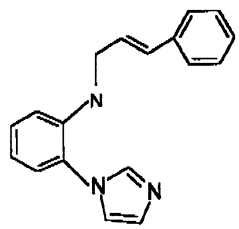
Novel toluidine derivatives are described. Some of them showed an interesting in vitro activity against *Mycobacterium tuberculosis*, *M. smegmatis*, *M. marinum*, *M. gordonae*, and *M. avium*. Some of them were more active than Streptomycin and Isoniazid, which were used as controls, against *M. avium* and *M. gordonae*. In particular, we confirm the good activity of biphenyl derivatives. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Antimycobacterial activity; *ortho*- *meta*- and *para*-Toluidines

1. Introduction

The resurgence of tuberculosis is due to the emergence of several factors. Among these, three are the most difficult to overcome: the multiple drug-resistant (MDR) strains of *Mycobacterium tuberculosis* versus the conventional therapeutical regimens [1–3], the particular virulence of *M. avium*, very often responsible for the death of HIV-infected patients, and the particularly resistant mycobacteria cell-wall structure, very waxy, hydrophobic, and having a high lipid content. It presents a lipidic bilayer and a peptidoglican layer (as that of the bacteria) and a thick mycolate rich outer covering, which functions as an exceptionally efficient barrier. So the search for new active compounds possessing a possible different mode of action could be very useful.

During the course of our investigation in the field of azole antimicrobial agents, we have described the synthesis and antimicrobial activity of many toluidine derivatives [4,5]. From this study we identified **1**, an imidazole derivative with good in vitro activity against mycobacteria and candidae [4].



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These findings prompted us to prepare a series of new azole derivatives in the hope of increasing activity. We thus modified, in a first study, the structure of **1** by substituting the amine function with the amidic one [5].

As an extension of our previous work and on the basis of the obtained results, we pursued a program to systematically modify the structure of **1**. In this paper we describe the synthesis and the microbiological activity of new derivatives **2**–**22** obtained by introducing structural fragments necessary for the activity (thiomorpholine, biphenyl and/or *p*-Cl-phenyl and the

* Corresponding author.
E-mail address: biava@axrma.uniroma1.it (M. Biava)

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trans-cinnamyl groups) necessary for the activity on the basis of what has been reported by Barbachyn et al. [6], and on what was observed by us during our earlier studies [7,8].

New synthesized compounds **11**–**22** were tested for the in vitro antimicrobial activity against *Candida albicans*, *Candida* sp, *Cryptococcus neoformans*, Gram-positive or Gram-negative bacteria, and isolates of pathogenic plant fungi, *M. tuberculosis*, *M. smegmatis*, *M. gordonae*, *M. marinum* and *M. avium*. The cytotoxicity results are also included. Compounds **2**–**10** were not tested because the Schiff bases were not stable at the experimental conditions.

The obtained results showed that many of the tested compounds are active against *M. tuberculosis*, some of them are active against *M. gordonae*, *M. marinum* and *M. avium*, and many of them are more active than Isoniazid and Streptomycin which were used as controls.

2. Chemistry

The titled compounds were prepared as illustrated in Scheme 1. Derivatives **2**–**10** were synthesized by a procedure previously described by us [4], by reaction of 4-chlorobenzaldehyde, cinnamaldehyde or biphenylaldehyde and the appropriate amine **24**. Reduction of the azomethine linkage with $NaBH_4$ led to secondary amines **11**–**19**, while amides **20**–**22** were synthesized by a procedure previously described by us [9], by reaction of 4-chlorobenzoyl, biphenoyl or cinnamoyl chloride and the appropriate amine **24**. Physicochemical data are reported in Tables 1 and 2.

All new compounds, **11**–**22** were identified by elemental analyses and NMR data.

3. Experimental

3.1. Chemistry

Melting points were determined using a Stuart Scientific Melting Point SMP1 apparatus and are uncorrected. IR spectra were taken with a Perkin–Elmer 1310. ^{1}H NMR spectra were recorded on a Bruker AM 200 MHz spectrometer with TMS as internal standard; the values of the chemical shifts (δ) are given in ppm. Progress of the reaction was monitored by TLC on alumina oxide plates with fluorescent indicator (Fluka). Carlo Erba Alumina Oxide 1 (II–III according to Brockmann) was used for chromatography. Extracts were dried over Na_2SO_4, and solvents were removed under reduced pressure. Oily compounds were analyzed after chromatographic purification; solids were recrystallized from ethylacetate. New derivatives were analyzed for C, H, N and Cl; microanalyses data were within $\pm 0.4\%$ of the theoretical values. Microanalyses were performed by Laboratories of Dr M. Zancato, Department of Pharmaceutical Sciences, University of Padova (Italy). Yields refer to purified products and are not optimized.

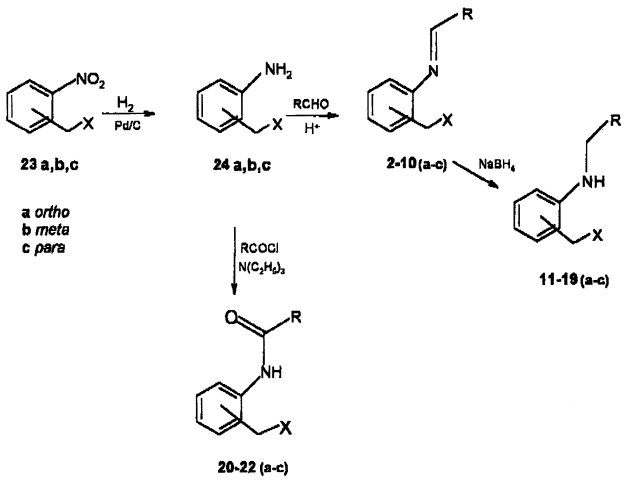
3.1.1. Nitrobenzylamines *23*

Compounds **23** were prepared according to the general procedure previously described [4]. Physicochemical data for compounds **23a**–**c** are reported in Table 1.

23a: ^{1}H NMR ($CDCl_3$): δ 2.7 (m, 8H, thiomorpholine), 3.6 (s, 2H, CH_2–N), 7.5–8.2 (m, 4H, aromatic protons); IR (cm^{-1}) $\nu(NO_2)$ 1520, 1340.

3.1.2. Benzeneamines *24*

Compounds **24** were prepared according to the general procedure previously described [4]. Physicochemical data for compounds **24a**–**c** are reported in Table 1.



	X	R	Isomers
2a/b/c	imidazolyl	*p*-Cl-phenyl	*ortho/meta/para*
3a/b/c	imidazolyl	Biphenyl	*ortho/meta/para*
4a/b/c	*N*-methyl piperazinyl	*p*-Cl-phenyl	*ortho/meta/para*
5a/b/c	*N*-methyl piperazinyl	Biphenyl	*ortho/meta/para*
6a/b/c	morpholinyl	*p*-Cl-phenyl	*ortho/meta/para*
7a/b/c	morpholinyl	Biphenyl	*ortho/meta/para*
8a/b/c	thiomorpholinyl	*p*-Cl-phenyl	*ortho/meta/para*
9a/b/c	thiomorpholinyl	Biphenyl	*ortho/meta/para*
10a/b/c	thiomorpholinyl	Cinnamyl	*ortho/meta/para*
11a/b/c	imidazolyl	*p*-Cl-phenyl	*ortho/meta/para*
12a/b/c	imidazolyl	biphenyl	*ortho/meta/para*
13 ab/c	*N*-methyl piperazinyl	*p*-Cl-phenyl	*ortho/meta/para*
14a/b/c	*N*-methyl piperazinyl	biphenyl	*ortho/meta/para*
15a/b/c	morpholinyl	*p*-Cl-phenyl	*ortho/meta/para*
16a/b/c	morpholinyl	biphenyl	*ortho/meta/para*
17a/b/c	thiomorpholinyl	*p*-Cl-phenyl	*ortho/meta/para*
18a/b/c	thiomorpholinyl	biphenyl	*ortho/meta/para*
19a/b/c	thiomorpholinyl	cinnamyl	*ortho/meta/para*
20a/b/c	thiomorpholinyl	*p*-Cl-phenyl	*ortho/meta/para*
21a/b/c	thiomorpholinyl	biphenyl	*ortho/meta/para*
22a/b/c	thiomorpholinyl	cinnamyl	*ortho/meta/para*

Scheme 1.

Table 1
Physicochemical data for compounds **2**–**10** and **23**–**24**

Comp.	M.p. (°C)	Yield (%)	Formula (MW)
2a [a]	100–103	85	$C_{17}H_{14}N_3Cl$ (295.76)
2b [b]	109–111	50	$C_{17}H_{14}N_3Cl$ (295.76)
2c [a]	97–99	90	$C_{17}H_{14}N_3Cl$ (295.76)
3a	137–138	96	$C_{23}H_{19}N_3$ (337.41)
3b	143–145	78	$C_{23}H_{19}N_3$ (337.41)
3c	184–185	95	$C_{23}H_{19}N_3$ (337.41)
4a [c]	39–40	50	$C_{19}H_{22}N_3Cl$ (327.84)
4b	oil	81	$C_{19}H_{22}N_3Cl$ (327.84)
4c	76–77	98	$C_{19}H_{22}N_3Cl$ (327.84)
5a	78–79	79	$C_{25}H_{27}N_3$ (369.50)
5b	85–86	94	$C_{25}H_{27}N_3$ (369.50)
5c	118–119	92	$C_{25}H_{27}N_3$ (369.50)
6a [c]	oil	60	$C_{18}H_{19}ClN_2O$ (314.80)
6b	oil	95	$C_{18}H_{19}ClN_2O$ (314.80)
6c	73–75	95	$C_{18}H_{19}ClN_2O$ (314.80)
7a	87–88	93	$C_{24}H_{24}N_2O$ (356.45)
7b	107–108	87	$C_{24}H_{24}N_2O$ (356.45)
7c	117–118	91	$C_{24}H_{24}N_2O$ (356.45)
8a	88–90	99	$C_{18}H_{19}ClN_2S$ (330.86)
8b	67–69	94	$C_{18}H_{19}ClN_2S$ (330.86)
8c	99–100	98	$C_{18}H_{19}ClN_2S$ (330.86)
9a	103–104	99	$C_{24}H_{24}N_2S$ (372.51)
9b	116–117	98	$C_{24}H_{24}N_2S$ (372.51)
9c [d]	147–148	93	$C_{24}H_{24}N_2S$ (372.51)
10a	oil	99	$C_{20}H_{22}N_2S$ (322.46)
10b	oil	98	$C_{20}H_{22}N_2S$ (322.46)
10c	92–93	90	$C_{20}H_{22}N_2S$ (322.46)
23a	95–96	90	$C_{11}H_{14}N_2O_2S$ (238.27)
23b	88–90	85	$C_{11}H_{14}N_2O_2S$ (238.27)
23c [d]	106–107	90	$C_{11}H_{14}N_2O_2S$ (238.27)
24a	75–76	98	$C_{11}H_{16}N_2S$ (208.29)
24b	80–81	99	$C_{11}H_{16}N_2S$ (208.29)
24c [d]	84–85	97	$C_{11}H_{16}N_2S$ (208.29)

[a] Ref [16].
[b] Ref. [9].
[c] Ref. [4].
[d] Ref. [17].

Table 2
Physicochemical data for compounds **11**–**22**

Comp.	M.p. (°C)	Yield (%)	Formula (MW)
11a [a]	99–101	63	$C_{17}H_{16}N_3Cl$ (297. 79)
11b [b]	107–109	60	$C_{17}H_{16}N_3Cl$ (297.79)
11c [a]	126–129	65	$C_{17}H_{16}N_3Cl$ (297. 79)
12a	110–111	25	$C_{23}H_{21}N_3$ (339.44)
12b	150–152	33	$C_{23}H_{21}N_3$ (339.44)
12c	160–162	57	$C_{23}H_{21}N_3$ (339.44)
13a [c]	oil	40	$C_{19}H_{24}N_3Cl$ (329.87)
13b	99–100	62	$C_{19}H_{24}N_3Cl$ (329.87)
13c	100–101	86	$C_{19}H_{24}N_3Cl$ (329.87)
14a	78–80	71	$C_{25}H_{29}N_3$ (371.53)
14b	92–93	50	$C_{25}H_{29}N_3$ (371.53)
14c	91–93	53	$C_{25}H_{29}N_3$ (371.53)
15a [c]	oil	60	$C_{18}H_{21}ClN_2O$ (316.83)
15b	71–72	87	$C_{18}H_{21}ClN_2O$ (316.83)
15c	72–73	78	$C_{18}H_{21}ClN_2O$ (316.83)
16a	87–88	30	$C_{24}H_{26}N_2O$ (358.48)
16b	100–101	87	$C_{24}H_{26}N_2O$ (358.48)
16c	125–126	87.5	$C_{24}H_{26}N_2O$ (358.48)
17a	55–56	78	$C_{18}H_{21}ClN_2S$ (332.89)
17b	94–95	75	$C_{18}H_{21}ClN_2S$ (332.89)
17c	99–100	70	$C_{18}H_{21}ClN_2S$ (332.89)
18a	93–94	45	$C_{24}H_{26}N_2S$ (374.54)
18b	118–120	50	$C_{24}H_{26}N_2S$ (374.54)
18c [d]	120–123	90	$C_{24}H_{26}N_2S$ (374.54)
19a	oil	60	$C_{20}H_{24}N_2S$ (324.48)
19b	oil	65	$C_{20}H_{24}N_2S$ (324.48)
19c	71–73	70	$C_{20}H_{24}N_2S$ (324.48)
20a	144–145	84	$C_{18}H_{19}ClN_2OS$ (346.87)
20b	130–131	70	$C_{18}H_{19}ClN_2OS$ (346.87)
20c	196–197	50	$C_{18}H_{19}ClN_2OS$ (346.87)
21a	175–176	55	$C_{24}H_{24}N_2OS$ (388.53)
21b	165–166	60	$C_{24}H_{24}N_2OS$ (388.53)
21c [c]	223–224	60	$C_{24}H_{24}N_2OS$ (388.53)
22a	155–156	70	$C_{20}H_{22}N_2OS$ (338.47)
22b	93–94	85	$C_{20}H_{22}N_2OS$ (338.47)
22c	133–135	80	$C_{20}H_{22}N_2OS$ (338.47)

[a] Ref. [16].
[b] Ref. [9].
[c] Ref. [4].
[d] Ref. [17].

24a: ^{1}H NMR ($CDCl_3$): δ 2.75 (s, 8H, thiomorpholine), 3.4 (s, 2H, CH_2N), 3.6 (broad, 2H, NH_2), 6.6–7.09 (unr m, 4H, aromatic protons); IR (cm^{-1}) $\nu(NH_2)$ 3460, 3420.

3.1.3. Azomethines *2*–*10*

Compounds **2**–**10** were synthesized and prepared according to the general procedure previously described [4]. Physicochemical data are reported in Table 1.

3a: ^{1}H NMR ($CDCl_3$): δ 2.1 (s, 1H, NH), 4.35 (s, 2H, –CH_2–imidazole), 5.1 (s, 2H, CH_2–N); 6.7–7.6 (m unr, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **5a**: ^{1}H NMR ($CDCl_3$): δ 2.3 (s, 3H, N–CH_3), 2.5 (m, 8H, *N*-methylpiperazine 2- and 3-CH_2), 3.7 (s, 2H, –CH_2–*N*-methylpiperazine), 6.9–8.4 (m unr, 13H, aromatic protons), 10.0 (s, 1H, N=CH–); IR (cm^{-1}) ν(NH) 3300. **7a**: ^{1}H NMR ($CDCl_3$): δ 2.4 (m, 4H, morpholine 3-CH_2), 3.6 (s, 6H, CH_2–*N*-morpholine and morpholine 2-CH_2), 6.9–8.3 (unr m, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **8a**: ^{1}H NMR ($CDCl_3$): δ 2.7 (m, 4H, thiomorpholine 3-CH_2), 2.8 (m, 4H, thiomorpholine 2-CH_2), 3.7 (s, 2H, CH_2–N), 6.7–8.3 (m, 8H, aromatic protons), 10.1 (s, 1H, N=CH–); IR (cm^{-1}) ν(NH) 3300. **9a**: ^{1}H NMR ($CDCl_3$): δ 2.6 (m, 4H, thiomorpholine 3-CH_2), 2.7 (m, 4H, thiomorpholine 2-CH_2), 3.6 (s, 2H, CH_2–N), 6.9–8.3 (m, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **10a**: ^{1}H NMR ($CDCl_3$): δ 2.6 (m, 4H, thiomorpholine 3-CH_2), 2.7 (m, 4H, thiomorpholine 2-CH_2), 3.6 (s, 2H, CH_2–N), 6.6-8.1 (m unr, 11H, aromatic protons and CH=CH–), 9.6 (s, 1H, N=CH–); IR (cm^{-1}) ν(NH) 3300.

3.1.4. Amines *11*–*19*

A solution of appropriate azomethine **2**–**10** (0.01 mol) in anhydrous ethanol (100 ml) was treated with

$NaBH_4$ (1.9 g, 0.05 mol) and heated at reflux for 4 h. Evaporation of the solvent gave a residue, which was dissolved in chloroform (100 ml). The organic phase was washed with water, dried, and evaporated. The residue was purified by passing it through an alumina column to give pure **11**–**19**. Physicochemical data are reported in Table 2.

12a: ^{1}H NMR ($CDCl_3$): δ 2.1 (s, 1H, NH), 4.35 (s, 2H, CH_2–imidazole), 5.1 (s, 2H, CH_2–N); 6.7–7.6 m unr, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **14a**: ^{1}H NMR ($CDCl_3$): δ 2.1 (s, 1H, NH), 2.2 (s, 3H, N–CH_3), 2.4 (m, 8H, *N*-methylpiperazine 2- and 3-CH_2), 3.5 (s, 2H, CH_2–*N*-methylpiperazine), 4.3 (m, 2H, CH_2–N), 6.5–7.5 (m unr, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **16a**: ^{1}H NMR ($CDCl_3$): δ 2.4 (m, 4H, morpholine 3-CH_2), 3.5 (s, 2H, NH–CH_2), 3.6–3.7 (m, 4H, morpholine 2-CH_2), 4.3 (s, 2H, CH_2–*N*-morpholine), 6.5–7.5 (unr m, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **17a**: ^{1}H NMR ($CDCl_3$): δ 1.2 (s, 1H, NH–), 2.6 (m, 4H, thiomorpholine 3-CH_2), 2.7 (m, 4H, thiomorpholine 2-CH_2), 3.5 (s, 2H, NH–CH_2), 4.4 (s, 2H, CH_2–N), 6.5–7.4 (m, 8H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **18a**: ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 1H, NH–), 2.4 (m, 4H, thiomorpholine 3-CH_2), 2.5 (m, 4H, thiomorpholine 2-CH_2), 3.4 (s, 2H, NH–CH_2), 4.3 (s, 2H, CH_2–N), 6.5–7.4 (m, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300. **19a**: ^{1}H NMR ($CDCl_3$): δ 1.4 (s, 1H, NH–), 2.6–2.7 (m, 8H, thiomorpholine), 3.6 (d, 2H, NH–CH_2), 3.9 (d, 2H, CH_2–N), 6.3–7.4 (m unr, 11H, aromatic protons and CH=CH–); IR (cm^{-1}) ν(NH) 3300.

3.1.5. Amides ***20***–***22***

A solution of the appropriate acid chloride (0.012 mol) in 20 ml of the appropriate solvent was dropped slowly into a well-stirred solution of the suitable benzeneamine **24** and triethylamine in 60 ml of the same solvent. The solutions were left to react, refluxing for 3 h. At the end of the reaction, the solvent was removed under reduced pressure and the oily residue was extracted with chloroform and washed with water. The organic phase was dried over anhydrous sodium sulfate. Finally the solid was recrystallized from a suitable solvent. The physicochemical data are reported in Table 2.

20a: ^{1}H NMR ($CDCl_3$): δ 1.5 (s, 1H, NH), 2.7 (s, 8H, thiomorpholine), 3.5 (s, 2H, CH_2–N), 7.1–8.1 (m unr, 8H, aromatic protons); IR (cm^{-1}) ν(NH) 3300, ν(CO) 1680. **21a**: ^{1}H NMR ($CDCl_3$): δ 1.5 (s, 1H, NH), 2.7 (s, 8H, thiomorpholine), 3.6 (s, 2H, CH_2–*N*), 7.0–8.0 (m unr, 13H, aromatic protons); IR (cm^{-1}) ν(NH) 3300, ν(CO) 1680. **22a**: 1.2 (s, 1H, NH), 2.7 (s, 8H, thiomorpholine), 3.5 (s, 2H, CH_2–N), 6.6–8.3 (m unr, 11H, aromatic protons and CH=CH); IR (cm^{-1}) ν(NH) 3300, ν(CO) 1680.

3.2. Microbiology

3.2.1. Compounds

All compounds **11**–**22** and drug references were dissolved in DMSO at a concentration of 10 mg/ml and stored cold until used.

3.2.2. 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 [10]. The derivatives found active in the preliminary test were assayed for inhibitory activity against a variety of *Mycobacterium* strains in Middlebrok 7H9 broth using the NCCLS procedure designed for rapidly growing mycobacteria. They are reported in Table 3. The mycobacteria used were *M. tuberculosis* 103471, *M. smegmatis* 103599, *M. gordonae* 6427, *M. marinum* 6423, and *M. avium 103317*. In all cases, minimum inhibitory concentrations (MICs in μg/ml) for each compound were determined. The MIC was defined at 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 throughout a 0.2 μm nylon membrane filter. Serial two-fold dilutions of the compounds with water were prepared. The tubes were incubated at 37°C for 72 h. A control tube without any drug was included in each experiment.

3.2.3. Cytotoxicity

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. Six-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 the 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.

3.2.4. Antimycotic activity

Antiyeast activity was tested with a broth microdilution method [11], the minimal inhibitory concentration (MIC) has been calculated elsewhere [8]. Ketoconazole and Miconazole were used for comparative studies.

3.2.5. Inhibitory activity on mycelial radial growth of plant pathogenic fungi isolates

This test was carried out as previously reported [12], *Drechslera graminea* DG2, *Phomopsis* sp. 717, *Rhizoctonia solani* 433 and *Botrytis cinerea* 644 isolates were used for this assay. The isolates used were supplied by the Istituto Sperimentale per la Patologia Vegetale,

Table 3
Antimycobacterial activity against *M. tuberculosis*, *M. smegmatis*, *M. marinum*, *M. gordonae* and *M. avium*, of compounds **11**–**22**, Isoniazid and Streptomycin

Comp.	Cytotoxicity	MIC μg/ml				
		Mycobacterium				
	MTD_{50} VERO cells	*M. tuberculosis* 103471	*M. smegmatis* 103599	*M. marinum* 6423	*M. gordonae* 6427	*M. avium* 103317
11a	2	8	>16	>16	>16	>16
11b	8	4	>16	8	1	4
11c	2	4	>16	2	0.5	1
12a	8	8	>16	>16	>16	>16
12b	64	4	>16	4	1	1
12c	8	8	>16	>16	>16	4
13a	8	>16	>16	>16	>16	>16
13b	4	>16	>16	>16	16	16
13c	4	>16	>16	>16	4	>16
14a	4	4	8	1	1	2
14b	1	8	>16	2	1	>16
14c	1	4	>16	1	4	8
15a	4	8	>16	8	4	4
15b	16	>8	>16	8	1	>16
15c	16	>16	>16	8	8	>16
16a	16	>16	>16	2	8	4
16b	4	8	>16	8	4	>16
16c	8	4	>16	2	1	8
17a	8	>16	>16	8	1	>16
17b	8	8	>16	>16	>16	>16
17c	4	>16	>16	>16	>16	8
18a	8	>16	>16	>16	>16	>16
18b	32	>16	>16	>16	>16	>16
18c	4	>16	>16	>16	>16	>16
19a	64	>16	>16	>16	>16	>16
19b	16	>16	>16	4	8	4
19c	4	8	>16	4	1	8
20a	64	>16	>16	>16	>16	>16
20b	32	8	>16	8	8	>16
20c	64	>16	>16	>16	>16	>16
21a	64	>16	>16	>16	>16	>16
21b	8	>16	>16	>16	>16	>16
21c	64	>16	>16	>16	>16	>16
22a	64	>16	>16	>16	>16	>16
22b	32	8	>16	8	>16	>16
22c	32	>16	>16	8	4	4
Isoniazid	32	0.25	64	16	32	32
Streptomycin	>64	0.5	8	32	16	8

Rome. Ketoconazole was used as a positive control. All the tested compounds and controls were dissolved in acetone (5 mg/ml); further dilution in the test medium furnished the required concentration in the range 6.25–100 μg/ml. The cultures were obtained on potato dextrose agar (Oxoid) at pH 5.6. Data were recorded after 72 h at 22°C. The activity of the compounds was estimated on the basis of percentage of growth inhibition by comparing the diameter of the zone of mycelial growth with that of the reference control in acetone.

3.2.6. Antibacterial activity

The MICs against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were assayed in a liquid medium dilution test by using the Mueller–Hinton medium (DIFCO) [11–13]. Nalidixic acid was used as a control.

4. Results and discussion

The in vitro active compounds against *M. tuberculosis* 103471, *M. gordonae* 6427, *M. smegmatis* 103599,

M. marinum 6423, and *M. avium* 103317 are listed in Table 3. None of the investigated compounds was found to be active against *Candida albicans*, *Candida* sp, *Cryptococcus neoformans*, Gram-positive or Gram-negative bacteria, and isolates of pathogenic plant fungi, and data are not displayed, with the exception of **12a** against *Phomopsis* sp. (MIC = 0.25 μg/ml).

4.1. Antimycobacterial activity

Interesting results were obtained from these assays, and data are reported in Table 3. Among the tested compounds, **11b**, **11c**, **12b**, **14a**, **14c**, **16c**, and **18c** exhibited an interesting activity against *M. tuberculosis*, even though all of them proved to be less active than the controls. Against *M. marinum* not only were compounds **11c**, **14a**, **14b**, **14c**, **16a** and **16c** active, but they were also found to be more active than the reference compounds and in particular **14a** and **14c** were the most active. However, compounds **11b**, **15a**, **15b**, **15c**, **16b**, **16c**, **20b**, **22b** and **22c**, showed good activity, considering the fact that all of them are more active than Isoniazid and Streptomycin. Compounds **11b**, **11c**, **12b**, **14a**, **14b**, **15b**, **16c**, **17a** and **19c** proved to be active against *M. gordonae*, and all of them were much more active than the controls. Against *M. avium* the most active compounds were **11c**, **12b** and **14a**, but interesting activity was also shown by compounds **11b**, **12c**, **15a**, **16a**, **19b** and **22c** which were more active than Isoniazid and Streptomycin.

It is important to point out the generally low toxicity shown by all the new compounds, but in particular by compound **12b**, which proved to be very active against *M. marinum*, *M. gordonae*, and *M. avium*. This result is very important considering what has been reported about disseminated *M. avium* complex infections making a substantial contribution to both increased illness and death in patients with AIDS [14].

From the microbiological data the following SAR considerations can be brought forth:

- As previously observed [5], the amino derivatives are generally more active than the corresponding amides, and it is probably due to both the presence of a non-hindered lone pair of nitrogen atoms according to Mailman's hypothesis [15] and the increased rigidity conferred upon the structure.
- In fact, compounds **20a**, **20c**, **21a–c**, and **22a** are completely inactive and compounds **20b**, **22b**, and **22c** exhibited a moderate activity.
- We confirmed that *ortho* and *para* derivatives are the most active with the exception of the *meta* derivative **12b**.
- In general, contrary to what was reported by Barbachyn et al. [6], the introduction of the thiomorpholine disactivates both aminic and amidic derivatives. In fact, only derivatives **19b**, **19c**, and **22c**, where cinnamyl moiety is present, proved to be active.
- Regarding the replacement of the imidazole with *N*-methylpiperazine, morpholine and thiomorpholine moieties, no particular difference was observed when the cinnamyl substituent was maintained, whereas the activity against atypical mycobacteria increases when imidazole or *N*-methylpiperazine or morpholine moieties and *p*-Cl-phenyl or biphenyl ones are simultaneously introduced in the molecule, with the exception of *M. smegmatis*.

In conclusion, comparing the new derivatives with the other compounds mentioned above and described in depth in earlier works [4,5], we can affirm that in general the new derivatives are less toxic, more active against *M. gordonae* and *M. marinum*, and less active against *M. avium*, while their activity against *M. tuberculosis* is almost the same.

The obtained results suggest further modifications to the lead compound **1**, and studies are in progress on this topic.

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