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N-[4-(1,1'-BIPHENYL)METHYL]-4-(4-THIOMORPHOLINYLMETHYL) BENZENAMINES AS NON-OXAZOLIDINONE ANALOGUES OF ANTIMYCOBACTERIAL U-100480.

Marino Artico,#* Antonello Mai,# Gianluca Sbardella,# Silvio Massa,≠ Giorgio Lampis,† Delia Deidda† and Raffaello Pompei†

#*Dipartimento di Studi Farmaceutici, Università di Roma "La Sapienza", P.le Aldo Moro 5, 00185 Roma, Italy*
≠Dipartimento Farmaco Chimico Tecnologico, Università di Siena, Banchi di Sotto 55, 53100 Siena, Italy
† Cattedra di Microbiologia Applicata, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università di Cagliari, Via Porcell 4, 09124 Cagliari

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Abstract. Thiomorpholine analogues of U-100480 with the biphenylmethyl group replacing the acetamidomethyloxazolidinone moiety have been synthesized and tested as antimycobacterial agents together with various related derivatives. Some biphenyl derivatives were endowed with high activity against *Mycobacterium tuberculosis* and other non-tuberculous mycobacteria.

The worldwide resurgence of tuberculosis due to the emergence of multiple drug-resistant strains of *Mycobacterium tuberculosis* towards the conventional therapeutical regimens[1-3] necessitates new and more effective antitubercular agents. Also clinical management of acquired immune deficiency syndrome (AIDS) has become very difficult because of development of opportunistic infections, particularly those involving *Mycobacterium avium* and *M. tuberculosis*, which are often responsible of death of HIV-infected patients.

To hinder the rapid spread of tuberculosis some companies searched for novel classes of antimycobacterial agents in order to acquire drugs for new therapeutic protocols to replace current treatment regimens, which more and more frequently fail against this disease.

During investigations in the oxazolidinone antibacterial agent area,[4,5] the Upjohn Co. Laboratories developed intensive SAR studies on DuP 721 (**1**), a totally synthetic antibacterial agent, which has been previously reported to exhibit fairly good activity against *M. tuberculosis*.[6] As a result of their intensive studies the Upjohn Co. Laboratories discovered novel and potent oxazolidinones, represented by the general structure **2**, which were endowed with potent antibacterial activities.[7] In particular, replacement of the piperazine moiety with the thiomorpholine pharmacophore in structure **2** led to derivative **3** (U-100480)[8] exhibiting potent *in vitro* activity against *M. tuberculosis*. The *in vitro* activity of **3** extended to other mycobacterial species, including *M. avium* complex, an opportunistic pathogen which has been associated with tuberculosis in patients infected by HIV. A preliminary study on the mechanism of oxazolidinone antibacterial agents have revealed that they are bacterial protein synthesis inhibitors.[8]

Recently, we reported on the synthesis and antimycobacterial activities of various 1-aryl-2-(1-imidazolyl)ethanamines as miconazole-like nitrogen analogues bearing arylmethyl substituents such as the 4-(1,1'-biphenyl)methyl, 1-naphthylmethyl and 3-phenyl-2-propenyl at the nitrogen of amino group.[9] Among test derivatives only those having the 4-(1,1'-biphenyl)methyl chain, such as compound **4**, were

* E-mail: artico@uniroma1.it - Fax: +39 6 4462731.

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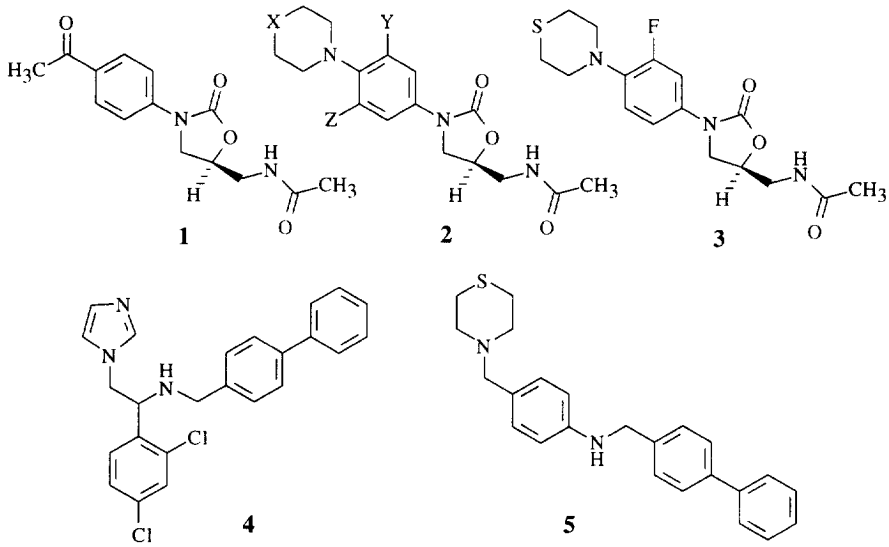


inhibitors of *M. tuberculosis* and *M. avium*, whereas the related N-(1-naphthyl)methyl and N-(3-phenyl-2-propenyl) derivatives were totally inactive. Therefore, the biphenylmethyl moiety seems to play a fundamental role as a pharmacophore in antimycobacterial 1-aryl-2-(1-imidazolyl)ethanamines such as **4**. As a further approach to novel compounds active against *M. tuberculosis* we then decided to synthesize compound **5**, an U-100480 analogue having the oxazolidinone moiety replaced by the 4-(1,1'-biphenyl)methyl pharmacophore and the thiomorpholine ring linked to aniline residue by a methylene group. The derivative **5** combined the active portions of both derivatives **3** and **4** and was expected to be active against mycobacteria species. Unlike **3**, which is obtained by a multistep synthetic pathway, the novel derivative **5** can be prepared by a simple procedure.

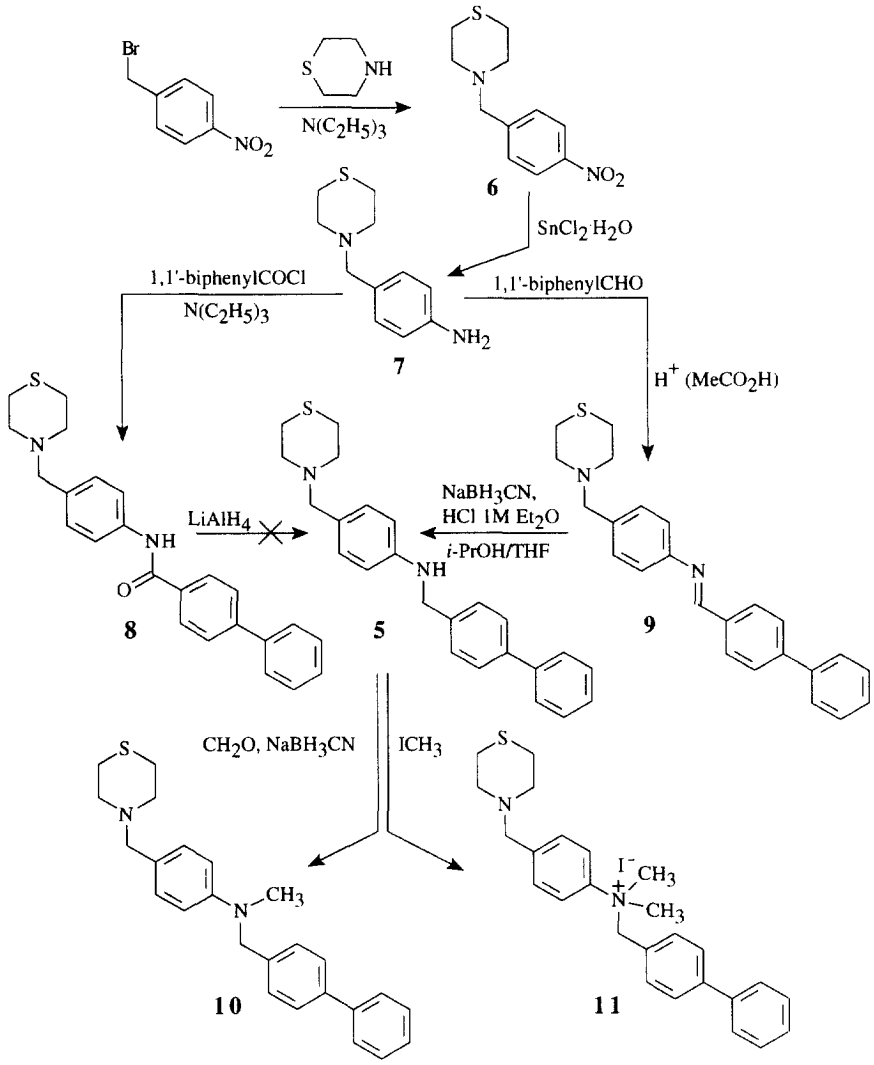
Chemistry

Reaction of 4-nitrophenylmethyl bromide with thiomorpholine in the presence of triethylamine furnished 4-(4-nitrophenylmethyl)thiomorpholine (**6**), which was then reduced to 4-(4-thiomorpholinylmethyl) benzenamine (**7**) with stannous dichloride. 1,1'-Biphenyl-4-carboxylic acid chloride was then reacted with **7** in the presence of triethylamine to yield the corresponding 1,1'-biphenyl-4-carboxyamide **8**. Lithium aluminum hydride reduction of amide **8** to the title derivative **5** was unsuccessful. However, compound **5** was easily obtained by reacting 1,1'-biphenyl-4-carboxaldehyde with amine **7** in glacial acetic acid and then reducing without isolation the crude imine **9** with sodium cyanoborohydride in acidic medium. Attempts to prepare the *N*-methyl derivative (**10**) of **5** with methyl iodide failed and the related iodomethylate **11** was isolated from reaction as the sole product. Compound **10** was then obtained easily from **5** by treatment with formaldehyde in the presence of sodium cyanoborohydride (Scheme 1). The physicochemical data for compounds **5-11** are shown in the reference section.[10]

Results and Discussion

Biphenylmethyl thiomorpholine **5** and the related compounds **8**,**10** and **11** were submitted for a preliminary evaluation of their *in vitro* activity against *M. tuberculosis*. The cytotoxicity of these derivatives was also determined using VERO cells after dissolution in DMSO at the initial concentration of 10 mg/mL.

SCHEME 1



As shown in Table 1 compounds **5** and **10** were highly active against a screening strain of *M. tuberculosis*, with activities comparable or two-fold inferior to those of streptomycin and isoniazid, respectively, used as reference compounds. Interestingly, the basicity of the amino group plays an important role in determining the antimycobacterial activity. In fact, the amido derivative **8** was found to be totally inactive and transformation of

the methylamino derivative **10** into the corresponding quaternary iodomethylate **11** also abated the antimycobacterial activity, probably because of the great increase in hydrophilicity. Interestingly, methyl substitution at the amine nitrogen did not alter either the activity or the cytotoxicity (compare **5** with **10**).

The good *in vitro* activity observed for **5** and **10** against *M. tuberculosis* and the acceptable degree of cytotoxicity encouraged further assays against atypical mycobacteria, including *Mycobacterium avium* often responsible for disseminated nontuberculous mycobacterial infections in immunologically compromised patients with AIDS. Since nontuberculous mycobacteria are usually resistant to isoniazid and other antitubercular agents used in current therapy regimens, the identification of novel antimycobacterial agents with broad spectrum activity is desiderable for possible clinical utilization. We therefore tested derivatives **5**, **8**,**10** and **11** against a panel of mycobacterial species, including *M. avium*, *M. smegmatis*, *M. gordonae* and *M. marinum*.

Table 1. Cytotoxicity and *In Vitro* Activity of Thiomorpholine Derivatives **5**, **8**, **10** and **11** against *M. tuberculosis*

organism	MIC[a] (MTD$_{50}$)[b]					
	5	**8**	**10**	**11**	streptomycin	isoniazid
M. tuberculosis CIP 103471	0.5 (8)	>16 (16)	0.5 (8)	8 (16)	0.5 (>64)	0.25 (32)

[a] For minimum inhibitory concentration (MIC, μg/mL) the compounds were incorporated into Middlebrook 7H9 broth using the broth microdilution test (see reference 11, 12 and 13). The MIC was defined as the lowest concentration of drug that yielded an absence of visual turbidity in liquid media. 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 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 10-15 days. A control tube without any drug was included un each experiment.

[b] Cytotoxicity of test compounds was tested in VERO cell monolayers (ICN-Fow), grown in Dulbecco's modified MEM (GIBCO Lab. Inc.) with 2% fetal calf serum. Six-well culture plates were inoculated with $9 \cdot 10^4$ cells. After 24 hours the compounds were added and after five further days the cell were detached from wells, trypsinized and counted in a Neubauer chamber. The minimal toxic dose (MTD50, μg/mL) was the concentration of drugs that induced a reduction of 50% of cell growth with respect to the control.

As reported in Table 2, test compounds showed very interesting activities, being comparable in potency to or more active than the clinical reference drugs. In fact, **5** and **10** exhibited potent *in vitro* activity against *M. avium*, with MICs of 4 μg/mL similar to that of streptomycin and eight-fold higher than that of isoniazid. To a lesser extent, they inhibited *M. gordonae* and *M. marinum*, whereas they were inactive against *M. smegmatis*.

Conclusion

In summary, N-[4-(1,1'-biphenyl)methyl]-4-(4-thiomorpholinylmethyl)benzenamines **5** and **10** exhibit potent *in vitro* activity against *M. tuberculosis*. The *in vitro* activities of these compounds extend to other mycobacterial species, including *M. avium*, an opportunistic pathogen associated with the acquired immune deficiency syndrome (AIDS). In general benzenamines **5** and **10** are as active or more active than the clinical drugs streptomycin and isoniazid against nontuberculosis species of mycobacteria.

Table 2. Cytotoxicity and *In Vitro* Activity of Derivatives **5**, **8**, **10** and **11** against Atypical Mycobacteria

compd	MIC (μg/mL)			
	Mycobacterium			
	avium CIP 103317	*smegmatis* CIP 103599	*gordonae* CIP 6427	*marinum* CIP 6423
5	4	>16	8	8
8	>16	>16	>16	>16
10	4	>16	>16	8
11	16	>16	8	16
streptomycin	4	8	16	32
isoniazid	32	64	32	16

The above findings indicate compound **5** (or **10**) as a lead compound for further studies directed towards improving the inhibiting activities and reducing the cytotoxicity of this novel antimycobacterial agent.

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10. **Characterization data:**
5: Yield: 100%: mp: 108-109°C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ : 2.67 (s, 8H, thiomorpholine), 3.41 (s, 2H, CH_2-thiomorph), 4.07 (brs, 1H, NH), 4.36 (s, 2H, CH_2-biphenyl), 6.59-6.63 (d, 2H, 2",6"-Ar-H), 7.08-7.12 (d, 2H, 3",5"-Ar-H), 7.30-7.48 (m, 5H, 3,5,3',4',5'-Ar-H), 7.55-7.61 (m, 4H, 2,6,2',6'-Ar-H); IR (KBr): 3360 cm^{-1} (NH); Anal. calcd. for $C_{24}H_{26}N_2S$: C, 76.96; H, 7.00; N, 7.48. Found: C, 77.13; H, 7.08; N, 7.22.
6: Yield: 94%: mp: 106-107°C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ : 2.71 (s, 8H, thiomorpholine), 3.61 (s, 2H, CH_2-Ar), 7.49-7.53 (d, 2H, 2,6-Ar-H), 8.16-8.20 (d, 2H, 3,5-Ar-H); IR (KBr): 1340, 1490 cm^{-1} (NO_2); Anal. calcd. for $C_{11}H_{14}N_2O_2S$: C, 55.44; H, 5.92; N, 11.76; S, 13.45. Found: C, 55.62; H, 6.01; N, 11.65; S, 13.37.
7: Yield: 96%: mp: 84-85°C (cyclohexane); ^{1}H NMR ($CDCl_3$) δ : 2.66 (s, 8H, thiomorpholine), 3.40 (s, 2H, CH_2-Ar), 3.63 (brs, 2H, NH_2), 6.61-6.65 (d, 2H, 2,6-Ar-H), 7.05-7.09 (d, 2H, 3,5-Ar-H); IR (KBr): 3320, 3410 cm^{-1} (NH_2); Anal. calcd. for $C_{11}H_{16}N_2S$: C, 63.42; H, 7.74; N, 13.45. Found: C, 63.57; H, 7.75; N, 13.26.
8: Yield: 54%: mp: 276-277°C dec. (DMF); ^{1}H NMR (DMSO-d_6) δ : 2.59 (s, 8H, thiomorpholine), 3.44 (s, 2H, CH_2-Ar), 7.22-7.26 (d, 2H, 3",5"-Ar-H), 7.39-7.52 (m, 3H, 2",6",4'-Ar-H), 7.71-7.83 (m, 6H, 2,6,2',3',5',6'-Ar-H), 8.01-8.06 (d, 2H, 3,5-Ar-H), 10.23 (s, 1H, NH); IR (KBr): 1640 (CO), 3350 cm^{-1} (NH); Anal. calcd. for $C_{24}H_{24}N_2OS$: C, 74.19; H, 6.23; N, 7.21; S, 8.25. Found: C, 74.27; H, 6.29; N, 7.33; S, 8.14.
9: Yield: 100%: mp: 151.0-151.5°C (acetonitrile); ^{1}H NMR ($CDCl_3$) δ : 2.71 (s, 8H, thiomorpholine), 3.54 (s, 2H, CH_2-Ar), 7.18-7.51 (m, 7H, 2",3",5",6",3',4',5'-Ar-H), 7.64-7.73 (m, 4H, 2,6,2",6"-Ar-H), 7.95-8.00 (d, 2H, 3,5-Ar-H), 8.51 (s, 1H, CH=N); IR (KBr): 1610 cm^{-1} (C=N).
10: Yield: 100%; mp: 120-121°C (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ : 2.67 (s, 8H, thiomorpholine), 3.04 (s, 3H, NCH_3), 3.42 (s, 2H, CH_2-thiomorph), 4.56 (s, 2H, CH_2-biphenyl), 6.70-6.74 (d, 2H, 2",6"-Ar-H), 7.12-7.16 (d, 2H, 3",5"-Ar-H), 7.24-7.52 (m, 5H, 3,5,3',4',5'-Ar-H), 7.56-7.60 (m, 4H, 2,6,2',6'-Ar-H); Anal. calcd. for $C_{25}H_{28}N_2S$: C, 77.28; H, 7.26; N, 7.21. Found: C, 77.09; H, 7.17; N, 7.43.
11: Yield: 82% ; mp: >280°C.
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