Journal of Enzyme Inhibition, Vol. 16, pp. 425–432
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Antimycobacterial Activity of 3,4-dichlorophenyl-ureas, *N,N*-diphenyl-ureas and Related Derivatives

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(Received 11 July 2001)

Substituted urea derivatives were prepared by reacting 3,4-dichlorophenyl isocyanate with amino acids, dipeptides, histamine or dicyandiamide among others, or from *N,N*-diphenyl-carbamoyl chloride and amino acids, dipeptides, or histamine. Other derivatives were obtained by reaction of PABA or PAS with arylsulfonyl halides. Some of the new compounds showed appreciable activity as antimycobacterial agents against *Mycobacterium tuberculosis* H37Rv, producing an inhibition of growth in the range of 80–89%, at a concentration of 6.25 μM. Some derivatives of this series might constitute interesting lead molecules for designing novel types of drugs effective against *M. tuberculosis*, a re-emerging pathogen both in the developed and under-developed countries.

Keywords: 3,4-dichlorophenylureas; *N,N*-diphenylureas; *Mycobacterium tuberculosis*; Sulfonamides

INTRODUCTION

Although *Mycobacterium tuberculosis* (more precisely, two of its strains) was the first bacterial species to have the entire genome sequenced,[1] this has been of little help at present in identifying targets for the development of novel antimycobacterial drugs, mainly due to the complexity of this genoma (with at least 100 genes involved for example in the synthesis of mycobacterial cell wall). On the other hand, *M. tuberculosis* infection is a major global health problem, principally due to the constant emergence of drug resistant and multidrug resistant mycobacteria, correlated with such widespread infections in HIV positive patients.[2–4] Furthermore, no new antimycobacterial drugs have appeared in the last 30 years since rifampin was introduced,[5] whereas mycobacteria other than tuberculosis (such as *M. avium* complex—MAC; *M. xenopi*, *M. fortuitum*, *M. kansasii*) frequently produce severe disseminated infections in a growing number of patients.[6–9] Progress has ultimately been made in understanding the role of several proteins involved in susceptibility of mycobacteria to some of the clinically used drugs, or in the emergence of drug resistance. Examples of such enzymes are KatG, a multifunctional

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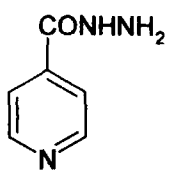

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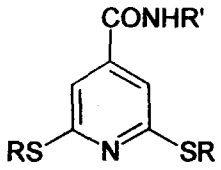
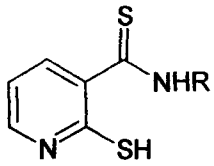


2: R, R' = alkyl



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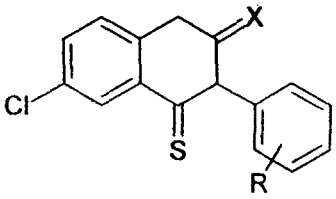
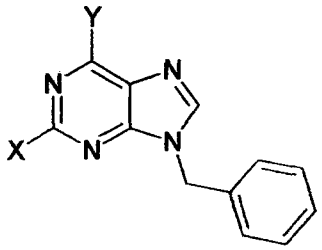
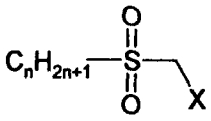


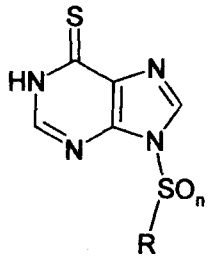
4: X = O,S; R = H, Me, Cl, etc.



5: X = Cl, Br; Y = phenylethynyl, *trans*-styryl, aryl, etc.



6: n = 6-18
X = $CONH_2$; SO_2NH_2, etc.



7: n = 0,2

heme enzyme possessing catalase–peroxidase and cytochrome P450-like oxidase activities (which is responsible for activation of the widely used drug isoniazid **1**);[10] InhA, an enoyl reductase which also seems to be a target for **1** and other antitubercular drugs;[11] DegP, a widely conserved heat shock protein possessing both general molecular chaperone and protease activities (this widely spread bacterial protease plays a major role in the degradation of proteins exported beyond the cytoplasm; its main function being most likely the removal of misfolded membrane and periplasmic proteins or not properly processed proteins)[12] or β-ketoacyl synthetase (the enzyme involved in fatty acid synthesis and elongation) among others.[5]

Considering all these data, many groups have reported ultimately the synthesis and antimycobacterial activity of novel classes of compounds that hopefully will lead to new therapeutic agents useful in the fight against mycobacterial diseases. Among the new structures recently reported, of particular interest seem to be some 2,6-bis(alkylthio)-4-pyridine carboxamides, **2**;[13] dihydro-2-thioxo-3-pyridinecarbothioamides, **3**;[14] 6-chloro-3-phenyl-4-thioxo-2*H*-1,3-benzoxazine-2(3*H*)-ones and -dithiones, **4**;[15] 9-benzylpurines, **5**[16] or some sulfonyl-containing fatty acid derivatives (carboxamides and sulfonamides) of type **6**.[5] Our group also reported some sulfonylated/sulfenylated-6-mercaptopurine derivatives of type **7**, with very good antimycobacterial activity.[17]

Considering the structural elements present in some of the antimycobacterial compounds **1–7** mentioned above, and our interest in the design of biologically active compounds incorporating ureido and/or sulfonyl moieties (such as sulfonamide carbonic anhydrase inhibitors/activators,[18–22] sulfonylated amino acid hydroxamates as metalloprotease inhibitors,[23,24] or sulfonylated derivatives with antifungal[25,26] and anticancer activity,[27–29] we report here the serendipitous discovery of antimycobacterial activity of 3,4-dicholorophenyl-ureas, *N,N*-diphenyl-ureas and some related derivatives, which might constitute interesting lead compounds for the development of more efficient antimycobacterial agents.

MATERIALS AND METHODS

Chemistry

Melting points were determined with a heating plate microscope and are uncorrected IR spectra were obtained in KBr pellets with a Perkin–Elmer 16PC FTIR spectrometer and ^{1}H-NMR spectra with a Varian 300CXP apparatus in solvents specified in each case. ^{13}C-NMR spectra were recorded at 75.57 MHz, with the same apparatus. Chemical shifts are expressed as δ values relative to Me_4Si as standard. Elemental analyses were done by combustion for C, H, N with an automated Carlo Erba analyzer, and were ±0.4% of the theoretical values.

Compounds used in synthesis (3,4-dichlorophenyl-isocyanate; *N,N*-diphenylcarbamoyl chloride, arylsulfonyl halides, histamine, PABA, PAS, natural and non-natural amino acids, dipeptides, dicyandiamide, etc.) were commercially available (from Sigma–Aldrich or Acros). The new ureido/sulfonylureido-derivatives were prepared as described previously for some related derivatives,[27–29] by the reaction of 3,4-dichlorophenyl isocyanate with amines/amino acids/dipeptides (from Sigma or Aldrich). *N,N*-diphenyl-ureas were prepared from Ph_2NCOCl (E. Merek) and nucleophiles, as previously reported for related compounds.[27–29] Sulfonamides **20** and **21** were prepared by the reaction of arylsulfonyl chlorides and PABA/PAS, as described previously for related compounds.[22] Acetonitrile, acetone (E. Merck) or other solvents used in the synthesis were doubly distilled and kept on molecular sieves in order to maintain them in anhydrous conditions.

General Procedure for the Synthesis of Ureas 10, 12 And Carbamate 15

An amount of 188 mg (1 mmol) of 3,4-dichlorophenyl isocyanate, **8**, and the corresponding stoichiometric amount of amino acid/dipedtide/amine, **9a–i**, or dicyandiamide, **11**, or oxime, **14** (or derivative **12**) were suspended/dissolved in 100 ml of anhydrous acetone or acetonitrile, and heated at reflux for 2–6 h (TLC control). Catalytic amounts of Et_3N were sometimes used for the reaction of the less reactive compounds (e.g. **14** or **12**). The solvent was then evaporated *in vacuo* and the obtained crude product taken up in a small amount of water acidified with 1N HCl, filtered off and crystallized from ethanol or mixtures of ethanol–water. Yields were in the range of 90–95%, and generally no tar or other side products were formed.

General Procedure for the Synthesis of Urea 17a–m

An amount of 232 mg (1 mmol) of *N,N*-diphenyl carbamoyl chloride was suspended in 50 ml of dry acetonitrile and triethylamine (108 μl) dissolved in the same solvent together with 1 mmol of amino acid/dipeptide/amine, **9a–m**, were added to the reaction mixture, which was stirred at 4°C for 4–5 h (TLC control). The solvent was evaporated *in vacuo*, the residue taken up in

50 ml of cold water, brought to pH 5 with 5% citric acid, and the precipitated urea filtered and recrystallized from ethanol (yield in the range of 95–98%).

General Procedure for the Synthesis of Sulfonamides 20, 21

Amino acids, **18** or **19**, and arylsulfonyl chlorides were suspended in equimolar amounts in acetonitrile at room temperature and the stoichiometric amount of Et_3N was added. Stirring was continued at room temperature for 4–8 h (TLC control), the solvent was evaporated *in vacuo*, and the crude products obtained taken up in a small amount of 0.1N HCl solution. The crude products were filtered, air dried and crystallized from ethanol–water 1:2 (v/v). Yields of sulfonamides **20** and **21** were in the range of 85–90%.

All compounds, **10a–i, 12, 13, 15, 17a–m, 20** and **21** reported here were fully characterized by means of elemental analysis (±0.4% of the calculated, theoretical data of the proposed formulae), IR, ^{1}H- and ^{13}C-NMR data, which confirmed the proposed structures. Examples of a representative derivative of each series is provided below.

3,4-Dichlorophenylureido-L-phenylalanine, ***10f****;* as white crystals, m.p. 205–206°C. IR (KBr), cm^{-1}: 760, 995, 1023, 1040, 1520 (amide II), 1730 (amide I), 1770 (COOH), 3165 (NHCONH). ^{1}H-NMR (DMSO-d_6): 3.10–3.55 (m, 2H, CH_2CH of Phe), 4.08 (dd, $^3J_{HH} = 5.0$, $^3J_{HH} = 7.8$, 1H, CH_2CH of Phe), 7.33 (d, 1H, H-5 of 3,4-dichlorophenyl), 7.39–7.58 (m, 5H, H_{arom} of Phe), 7.60 (d, 1H, H-6 of 3,4-dichlorophenyl), 7.94 (s, 1H, H-2 of 3,4-dichlorophenyl), 9.67 (br s, 2H, NHCONH), 10.43 (br s, 1H, COOH). ^{13}C-NMR (DMSO-d_6): 41.3 (s, CH_2CH of Phe), 59.4 (s, CH_2CH of Phe), 130.8 (s, C_{para} of Phe), 132.7 (s, C of 3,4-dichlorophenyl), 133.4 (s, C_{meta} of Phe), 135.0 (s, C_{ortho} of Phe), 135.9 (s, C of 3,4-dichlorophenyl), 141.3 (s, C_{ipso} of Phe), 142.8 (s, NHCONH), 145.4 (s, C of 3,4-dichlorophenyl), 148.6 (s, C of 3,4-dichlorophenyl), 178.9 (s, CO_2H of Phe). Found: C, 54.33; H, 4.21; N, 7.85. $C_{16}H_{14}Cl_2N_2O_3$ ($M = 353.21$) requires: C, 54.41; H, 4.00; N, 7.93%.

1-Cyano-3-(3,4-dichlorophenylureido)-guanidine ***12****;* as white crystals, m.p. > 300°C. IR (KBr), cm^{-1}: 835, 950, 1020, 1033, 1045, 1524 (amide II), 1726 (amide I), 2200 (CN), 3165 (NHCONH). ^{1}H-NMR (DMSO-d_6): 7.36 (d, 1H, H-5 of 3,4-dichlorophenyl), 7.58 (d, 1H, H-6 of 3,4-dichlorophenyl), 7.93 (s, 1H, H-2 of 3,4-dichlorophenyl), 8.20 (br s, 1H, NHCN), 8.84 (br s, 1H, C=NH), 9.67 (br s, 2H, NHCONH). Found: C, 40.02; H, 2.48; N, 25.61. $C_9H_7Cl_2N_5O$ ($M = 272.10$) requires: C, 39.73; H, 2.59; N, 25.74%.

N,N-*Diphenylureido-valine* ***17c****;* as white crystals, m.p. 240–241°C. 1R (KBr), cm^{-1}: 1040, 1525 (amide II), 1710 (amide I), 1770 (COOH), 3165 (NHCONH). ^{1}H-NMR (DMSO-d_6): 1.13 (d, $^3J_{HH} = 6.7$, 6H, $CH(CH_3)_2$ of Val), 2.29–2.54 (m, 1H, $CH(CH_3)_2$ of Val), 3.79 (d, $^3J_{HH} = 4.3$, 1H, NHCHCH of Val), 7.08–7.75 (m, 10H, ArH), 9.50 (br s, 2H, NHCONH), 10.21 (br s, 1H, COOH. ^{13}C-NMR (DMSO-d_6): 22.1 (s, $CH(CH_3)_2$ of Val), 23.7 (s, $CH(CH_3)_2$ of Val), 34.3 (s, $CH(CH_3)_2$ of Val), 64.5 (s, NHCHCH of Val), 130.5 (s, C_{para} of Ph), 133.7 (s, C_{meta} of Ph), 135.3 (s, C_{ortho} of Ph), 139.3 (s, NHCONH), 141.0 (s, C_{ipso} of Ph), 178.3 (s, CO_2H of Val). Found: C, 69.53; H, 6.32; N, 8.80. $C_{18}H_{20}N_2O_3$ ($M = 312.37$) requires: C, 69.21; H, 6.45; N, 8.97%.

4-(4-Toluenesulfonylamido)-benzoic Acid ***20****;* as white crystals, m.p. 285–287°C, dec.). IR (KBr), cm^{-1}: 560, 720, 815, 1035, 1130 (SO_2^{sym}), 1350 (SO_2^{as}), 1450, 1780 (COOH), 3190 (SO_2NH), 3420 (OH). ^{1}H-NMR (DMSO-d_6), δ, ppm: 2.50 (s, 3H, Me), 7.06–7.46 (m, AA′BB′ system, 4H, $J_{AB} =$ 7.4 Hz, ArH from tosyl), 7.18–7.75 (m, AA′BB′ system, 4H, $J_{AB} = 7.9$ Hz, ArH from 4-carboxyphenyl), 8.11 (br s, 1H, SO_2NH), 10.66 (br s, 1H, COOH). Found: C, 57.86; H, 4.23; N, 4.63. $C_{14}H_{13}NO_4S$ (291.33) requires: C, 57.72; H, 4.50; N, 4.81%.

Antimycobacterial Activity Assay

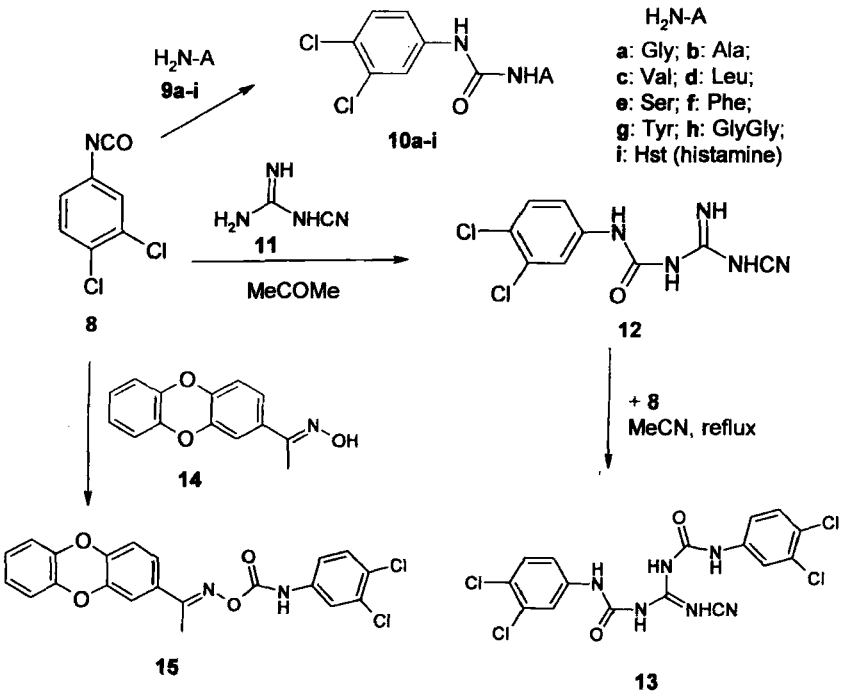
The new compounds reported here were tested for antimycobacterial activity, against *M. tuberculosis* strain H37Rv, by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Frederick, MD, USA, by using the method of Collins and Franzblau.[30] The primary screening was conducted at 6.25 μg/ml of test compound, in the BACTEC 12B medium, using the Microplate Alamar Blue Assay (MABA). Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system.[30]

RESULTS AND DISCUSSION

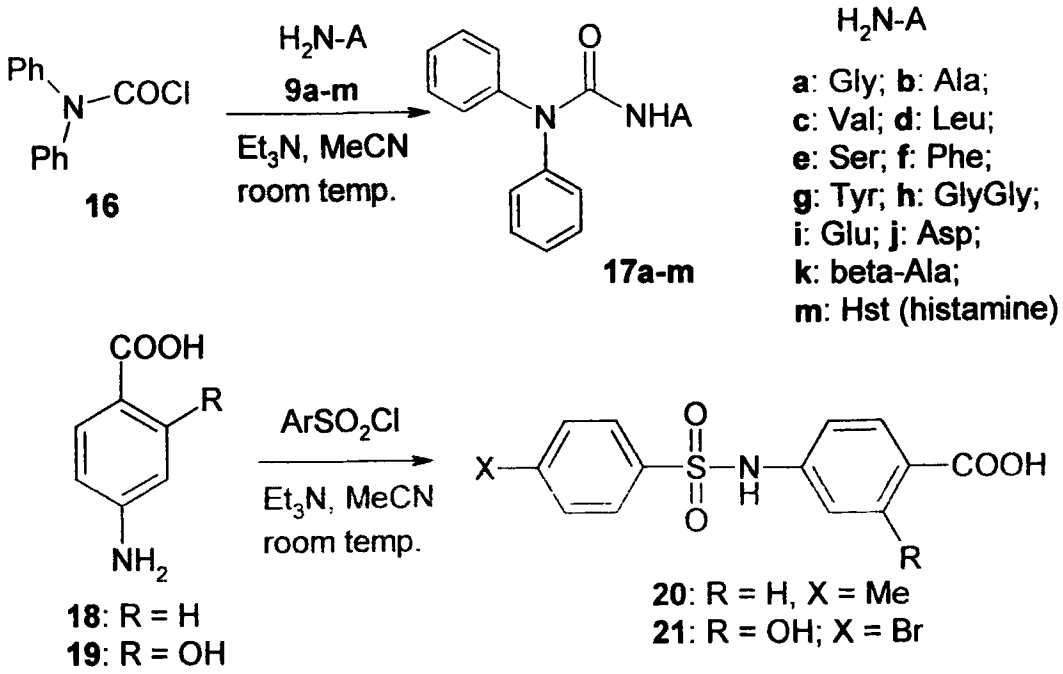
Dichlorophenylureas, **10a–i**, **12**, **13** and the related carbamate **15** were prepared by the reaction of 3,4-dichlorophenyl isocyanate, **8**, with nucleophiles, **9a–i**, **11**, **12** or **14**, as reported earlier for structurally related derivatives (Scheme 1).[27,29]

The *N,N*-diphenylureas, **17a–m**, were prepared by reaction of *N,N*-diphenyl-carbamoyl chloride, **16**, with amino acids, dipeptides or amines, **9a–m**, as reported previously for sulfonamides incorporating the same moiety,[26] whereas derivatives **20** and **21** from the aromatic amino acids PABA and PAS and arylsulfonyl halides, respectively, were determined by a procedure already used for the preparation of carbonic anhydrase inhibitors, as shown in Scheme 2.[22]

Reaction of 3,4-dichlorophenyl isocyanate, **8**, with different nucleophiles, such as the amino acid/dipeptides/amine, **9a–i**, dicyandiamide, **11**, or the oxime, **14**, afforded with very good yields the expected ureas/carbamates, **10a–i**, **12** and **15**, respectively. The cyanoguanidine derivative, **12**, was further reacted with another equivalent of **8**, leading to the bis-urea **13**. The trisubstituted ureas, **17a–m**, were on the other



SCHEME 1



SCHEME 2

hand prepared by acylation of the nucleophiles **9a–m** with *N,N*-diphenyl-carbamoyl chloride, again in very good yields, whereas arylsulfonylation of the aromatic amino acid derivatives, **18** and **19**, afforded the secondary sulfonamides, **20** and **21**.

Biological activity data for the synthesized compounds reported here are shown in Table I, with rifampin and isoniazid as standard drugs.

The new compounds reported here were tested for antimycobacterial activity, against *M. tuberculosis* strain H37Rv, by the TAACF,

TABLE I Antimycobacterial activity data against *M. tuberculosis* H37Rv of compounds **10–21** synthesized in the present study, and isoniazid and rifampin as standard antimycobacterial drugs

Compound	% Inhibition (at 6.25 μM) *M. tuberculosis* H37Rv	Compound	% Inhibition (at 6.25 μM) *M. tuberculosis* H37Rv
10a	80	**17b**	15
10b	83	**17c**	0
10c	82	**17d**	9
10d	89	**17e**	3
10e	89	**17f**	4
10f	78	**17g**	0
10g	84	**17h**	20
10h	84	**17I**	4
10I	20*	**17j**	20
12	13	**17k**	13
13	82	**17m**	15
15	80	**20**	3
17a	18	**21**	87
Isoniazid **1**	85	Rifampin	93

* IC_{50} = 23 mM, representing the cytoxicity of the compound in VERO cells.

Southern Research Institute, Frederick, MD, USA.

As seen from Table I, several of the new compounds reported here showed good inhibitory activity against *M. tuberculosis* H37Rv, leading to inhibition of bacterial growth in the range of 78–89% at a concentration of drug of 6.25 μM. Such compounds included the 3,4-dichlorophenylureas, **10a–10h**, **13** and **15**, as well as the *p*-bromophenylsulfonyl derivative of PAS, **21**. In this sub-series of compounds incorporating 3,4-dichlorophenylureas moieties, best activity was seen for the Leu and Ser derivatives, followed by the Tyr, Gly–Gly, Ala, Val, Gly and Phe derivatives. Very good activity was also observed for the bis-substituted dicyandiamide **13** (but not for the monosubstituted derivative **12**), as well as for the carbamate **15** (80–82% inhibition). These compounds practically possess comparable inhibitory activity with isoniazid and rifampin, two widely used antimycobacterial drugs, which also inhibit the growth of mycobacteria in the range of 85–93% at the same concentrations as the new compounds reported here (Table I).

Much less active against *M. tuberculosis* H37Rv were some of the remaining 3,4-dichlorophenylureas, such as **10i** and **12**, as well as several diphenylurea derivatives, such as **17a**, **17b**, **17h**, **17j**, **17k** and **17m**, which exhibited inhibition of growth in the range 13–20%, at a drug concentration of 6.25 μM (Table I). Finally, a very weak inhibitory activity (in the range of 3–9%) was shown by the derivatives **17d**, **17e**, **17i** and **20**. Two derivatives (**17c** and **17g**) did not inhibit at all the growth of *M. tuberculosis* H37Rv.

The following preliminary SAR data emerged: (i) the 3,4-dichlorophenylureas/carbamate were much more active in inhibiting the growth of mycobacteria, than the corresponding *N,N*-diphenylurea derivatives (compare for example the pairs **10a–17a**, **10b–17b**, **10d–17d**, etc.), (ii) in each sub-series of urea derivatives (i.e. the 3,4-dichlorophenylurea sub-series, and the *N,N*-diphenylurea sub-series, respectively) antimycobacterial activity was observed both for highly lipophilic derivatives (such as the Val, Leu and Phe derivatives) as well as for more hydrophilic compounds (such as the Gly, Gly–Gly and Ser derivatives), (iii) the presence of the bulky moieties in compounds **13** and **15** seems to be beneficial for the antimycobacterial activity, (iv) very different biological activity was shown by the structurally related sulfonamides **20** and **21**, with the *p*-aminosalicylic (PAS) derivative behaving as a potent antimycobacterial agent, whereas the *p*-aminobenzoic acid (PABA) derivative was inactive.

The cytotoxicity against VERO cells (IC_{50}) for compound **10i** was also determined, as this parameter is of great importance for the pharmacology of a potential antimycobacterial agent (Table I). The cytotoxicity of **10i** is in fact comparable to that of standard antimycobacterial drugs, such as isoniazid or rifampin (data not shown).

In conclusion, we report here the serendipitous discovery of good antimycobacterial activity for a series of substituted ureas and a preliminary SAR for this class of biologically active compounds. Some of these new compounds show the same level of activity in inhibiting the growth of mycobacteria, as the two clinically used drugs isoniazid and rifampin. Since tuberculosis is an "orphan disease", with no new drugs being developed for more than 30 years, the derivatives reported here may be considered as interesting leads for the potential development of new antimycobacterial agents.

Acknowledgements

Antimycobacterial data presented here were provided by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Frederick, MD, USA, through a research and development contract between our university and the US

National Institute of Allergy and Infectious Diseases. Thanks are addressed to Dr Joseph A. Maddry (TAACF) for his invaluable help. This research was financed in part by a grant from the Italian CNR-Target Project Biotechnology.

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