Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 14 (2006) 3113–3118
Bioorganic & Medicinal Chemistry

Synthesis, in vitro and in vivo antimycobacterial activities of diclofenac acid hydrazones and amides

Dharmarajan Sriram,* Perumal Yogeeswari and Ruth Vandana Devakaram

Medicinal Chemistry Research Laboratory, Pharmacy group, Birla Institute of Technology and Science, Pilani 333031, India

Received 29 November 2005; revised 15 December 2005; accepted 15 December 2005
Available online 18 January 2006

Abstract—Various diclofenac acid hydrazones and amides were synthesized and evaluated for in vitro and in vivo antimycobacterial activities against *Mycobacterium tuberculosis*. Preliminary results indicated that most of the compounds demonstrated better in vitro antimycobacterial activity (MIC: 0.0383–7.53 μM) than diclofenac (MIC: 21.10 μM) and ciprofloxacin (MIC: 9.41 μM). Among the synthesized compounds, 1-cyclopropyl-6-fluoro-8-methoxy-7-[[N^4-(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)-3-methyl]-N^1-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (**5d**) was found to be the most active compound in vitro with MIC of 0.0383 μM and was more potent than first line antitubercular drug isoniazid (MIC: 0.1822 μM). In the in vivo animal model **5d** decreased the bacterial load in lung and spleen tissues with 2.42 − and 3.66 − log10 protections, respectively, at 25 mg/kg body weight.

1. Introduction

Tuberculosis (TB) is the leading infections cause of death in the world, with approximately three million patients dying every year. Nearly one-third of the world's population is infected with *Mycobacterium tuberculosis* and the World Health Organization (WHO) estimates that about 30 million people will be infected within the next 20 years. Moreover, the resurgence of TB in industrialized countries and the worldwide increase in the prevalence of *Mycobacterium avium* complex (MAC) infections in immuno-compromised hosts (often accompanied by other bacterial infections) as well as the appearance of multi-drug resistant (MDR) strains of *M. tuberculosis* have prompted the quest for new antimycobacterial agents, without cross-resistance with known antituberculous agents. Development of resistance to existing drugs is a constantly growing phenomenon that has concerned researchers throughout the world, and now has reached alarming levels for certain infectious diseases. This combined with the recent decline in the development of new drugs to combat them can be anticipated to lead to infectious diseases lacking ready treatment regimens.[1–3] In view of the drug-resistant TB problem, it is important that new drugs inhibit targets different from those of currently used drugs.[4] In addition, development of new agents that can shorten the lengthy TB therapy requires the design of drugs that can inhibit targets involved in persistence or dormancy.

There are two basic approaches to develop a new drug for TB: (a) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment and (b) searching for novel structures, that the TB organism has never been presented with before, for the treatment of MDR TB.[5] To pursue this goal, our research efforts are directed to synthesize derivatives of reported antimycobacterial compound. Aryl acetic acid derivative such as diclofenac is an anti-inflammatory drug that also has in vitro and in vivo antimycobacterial activities.[6] Diclofenac sodium minimum inhibitory concentrations (MICs) ranged between 10 and 25 μg/mL against *M. tuberculosis*, *Mycobacterium smegmatis*, *Mycobacterium marinum*, *Mycobacterium scrofulaceum*, *Mycobacterium xenopi* and *M. avium*. Diclofenac are also active against MDR *M. tuberculosis*, suggesting that, diclofenac inhibits a new target in *M .tuberculosis*. In this paper, we reported the synthesis of various diclofenac acid hydrazones and amides, and their in vitro and in vivo antimycobacterial activities.

Keywords: Antimycobacterial; Anti-TB; Diclofenac derivatives; Acid hydrazones; Amides.
* Corresponding author. Tel.: +91 1596 244684; fax: +91 1596 244183; e-mail: dsriram@bits-pilani.ac.in

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.12.042

2. Results and discussion

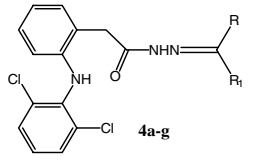
2.1. Synthesis

The general procedures for the preparation of target compounds **4a**–**g**[7] and **5a**–**d** (Tables 1 and 2) are described in Scheme 1. Methyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate (**2**) was prepared by the reaction of diclofenac (**1**) and methanol in the presence of a few drops of concentrated sulfuric acid. 2-(2-(2,6-Dichlorophenylamino)phenyl)acetohydrazide (**3**) was synthesized by heating hydrazine-hydrate and **2** in methanol. After condensing acid hydrazide with various substituted benzaldehyde and 4-bromo benzophenone, diclofenac acid hydrazones (**4a**–**g**) were obtained in 48–79% yield. 2-(2-(2,6-Dichlorophenylamino)phenyl)-acetic acid amides (**5a**–**d**) were prepared by reacting diclofenac with piperazine moiety of the fluoroquinolones in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (EDCI) as the carboxylate activator and 4-dimethylaminopyridine (DMAP) as catalyst in 71–88% yield. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data. UV spectra of **4a**–**g** exhibited characteristic K bands arising from chromophoric C═N group at 319–395 nm.[8] In the IR spectra of compounds **4a**–**g**, the bands representing azomethine and carbonyl groups appeared at 1625–1600 and 1662–1639 cm^{-1}, respectively. The nuclear magnetic resonance spectra (^{1}H NMR) of **4a**–**g** showed single signals corresponding to resonances of azomethine protons at 8.35 ppm. All the synthesized compounds showed D_2O exchangeable singlet of –NH– proton at 10.4 ppm, and singlet at 3.4 ppm for $–CH_2–$ protons. The elemental analysis results were within ±0.4% of the theoretical values.

2.2. Antimycobacterial activity

2.2.1. In vitro activity. All compounds were screened for their in vitro antimycobacterial activity against *M. tuberculosis* by agar dilution method similar to that recommended by the National Committee for Clinical Laboratory Standards[9] for the determination of MIC. The MIC is defined as the minimum concentration of compound required to complete inhibition of bacterial growth and MICs of the compounds were reported in (Tables 1 and 2) along with the standard drugs for comparison. The synthesized acid hydrazones (**4a**–**g**) and amides (**5a**–**d**) inhibit *M. tuberculosis* with MICs ranging from 0.3614 to 7.53 μM and 0.0382 to 0.3347 μM, respectively. All the synthesized compounds were found to be more active than the parent drug diclofenac. Among the acid hydrazones (**4a**–**g**), compound 2-{2-[(2,6-dichlorophenyl)amino]phenyl}-*N*′-[1-(4-bromophenyl)benzylidene]acetohydrazide (**4g**) was found to be the most active compound with MIC of 0.3614 μM and was more potent than fluoroquinolones ciprofloxacin

Table 1. Physical constants and in vitro antimycobacterial activities of diclofenac acid hydrazones



4a-g

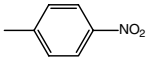
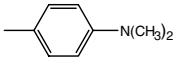
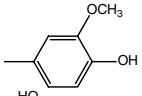
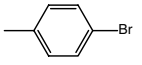
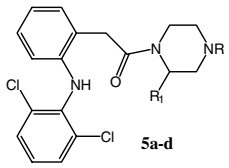
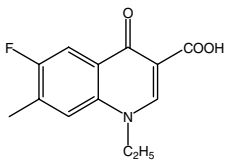
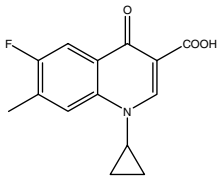
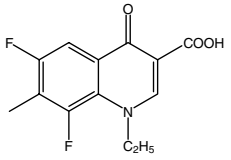
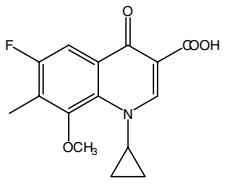
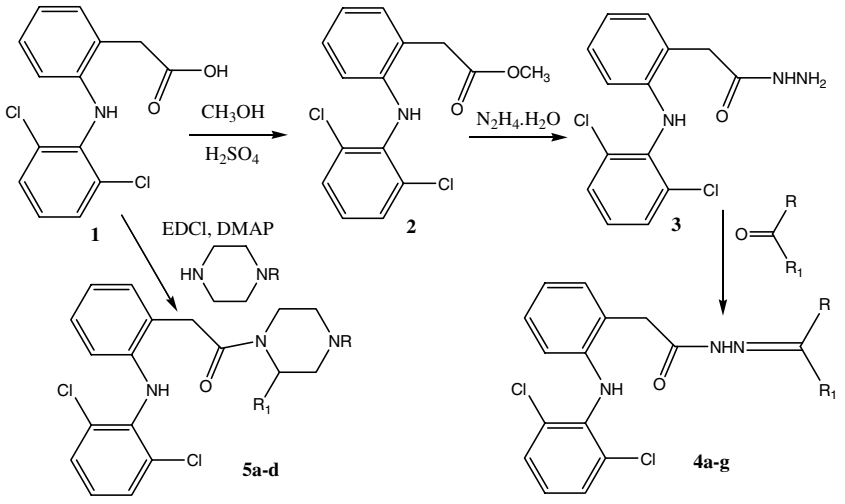
Compound	R	R_1	Yield (%)	Mp (°C)	Clog *P*	MIC (μM)	IC_{50} (μM)
4a		H	48	236	5.53	1.76	>22.62
4b		H	72	220	5.53	1.76	>22.62
4c		H	67	260	6.45	3.60	>23.10
4d		H	66	250	6.17	1.76	>22.65
4e		H	79	232	5.37	7.02	>22.56
4f		H	70	229	5.5	7.53	>24.13
4g			57	189	8.18	0.3614	>18.07
Diclofenac	—	—	—	—	4.12	21.10	>33.76

Table 2. Physical constants and in vitro antimycobacterial activities of diclofenac amides



5a-d

Compound	R	R_1	Yield (%)	Mp (°C)	Clog *P*	MIC (μM)	IC_{50} (μM)
5a		H	71	212	5.44	0.3347	>16.73
5b		H	88	218	5.39	0.1640	>16.40
5c		CH_3	86	213	5.91	0.3177	>15.88
5d		CH_3	81	208	5.59	0.0382	>15.30
Diclofenac	—	—	—	—	4.12	21.10	>33.76
Ciprofloxacin	—	—	—	—	1.32	9.41	>30.18
Gatifloxacin	—	—	—	—	1.51	2.07	>26.63
Isoniazid	—	—	—	—	−0.6	0.1822	7291.8



Scheme 1. Synthetic protocol of diclofenac acid hydrazones and amides.

and gatifloxacin. Acid hydrazones derived from 4-bromo benzophenone were found to be more active than substituted benzaldehyde derived acid hydrazones. Within substituted benzaldehyde derived acid hydrazones the order of activity with respect to substituents is $-NO_2$, $-N(CH_3)_2 > -Cl > -OH$ with $-OCH_3 > -OH$.

Hence, it is the electron-withdrawing group that is favourable. Among the diclofenac amides (**5a**–**d**), compound 1-cyclopropyl-6-fluoro-8-methoxy-7-[N^4-(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)-N^1-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (**5d**) was found to be the most active compound in vitro with MIC of 0.0383 μM and was four times more active than the first line antitubercular drug isoniazid (MIC: 0.1822 μM).

The lipophilicity of the compounds is well known to play an important role in the penetration of these compounds into bacterial cells.[10] Assuming that the issue of penetration is even more crucial for compound's activity against mycobacteria,[11] our results demonstrated that simply increasing the lipophilic character of compounds increased the activity, as shown with log *P* values of the synthesized compounds (5.39–8.18) (Tables 1 and 2) were much more than the parent compound (4.12).

2.2.2. In vitro cytotoxicity. All the compounds were further examined for toxicity (IC_{50}) in a mammalian VERO cell line at concentrations of 10 μg/mL. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega Cell Titer 96 non-radioactive cell proliferation assay.[12] The compounds were found to be non-toxic up to 10 μg/mL. Compound **5d** showed selectivity index (IC_{50}/MIC) of more than 400.

2.2.3. In vivo activity. Subsequently, compound **5d** was tested for efficacy against *M. tuberculosis* at a dose of 25 mg/kg (Table 3) in six-week-old female CD-1 mice. In this model,[13] the mice were infected intravenously through caudal vein approximately 10^7 viable *M. tuberculosis* ATCC 35801. Drug treatment began after inoculation of the animal with microorganism for 10 days by oral route. After 35 days postinfection, the spleens and right lungs were aseptically removed and ground in a tissue homogenizer, the number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in ambient air for four weeks prior to counting. Bacterial counts were measured and compared with the counts from negative (untreated) controls (mean colony forming units (CFU) in lung: 8.78 ± 0.12 and in spleen: 6.84 ± 0.21). Compound **5d** decreased the bacterial load in lung and spleen tissues with 6.36 ± 0.19 and $3.18 \pm 0.11 - \log 10$ protections, respectively, and was considered to be promising in reducing bacterial count in lung and spleen tissues. When compared to isoniazid at the same dose level, **5d** decreases the bacterial load equally in spleen tissues.

Table 3. In vivo activity data of **5d** and isoniazid against *Mycobacterium tuberculosis* in mice

Compound	Lungs (log CFU ± SEM)	Spleen (log CFU ± SEM)
Control	8.78 ± 0.12	6.84 ± 0.21
5d (25 mg/kg)	6.36 ± 0.19	3.18 ± 0.11
Isoniazid (25 mg/kg)	5.80 ± 0.18	3.14 ± 0.12

3. Conclusion

Among the synthesized compounds, **5d** was found to be four times more potent than first line antitubercular drug isoniazid in vitro. The investigation on further structure–activity relationships and emergence of drug resistance is now in progress. The present results highlight the importance of increasing lipophilicity of the compounds to overcome transport barrier into the cells. The enhanced activity of amide derivatives might be due to the fluoroquinolone moiety which might inhibit the DNA gyrase enzymes of *M. tuberculosis*.

4. Experimental

Melting points were taken with an electrothermal melting point apparatus (Buchi BM530) in open capillary tubes and are uncorrected. Infrared spectra (KBr disc) were run on a Jasco IR Report 100 spectrometer. ^{1}H NMR spectra were scanned on a JEOL Fx 300 MHz NMR spectrometer using DMSO-d_6 as solvent. Chemical shifts are expressed in δ (parts per million) relative to tetramethylsilane. Elemental analyses (C, H and N) were performed on Perkin Elmer model 240C analyzer and the data were within ±0.4% of the theoretical values. C log *P* values were calculated using chem. Draw ultra 8.0 software.

4.1. Synthesis of methyl 2-(2-(2,6-dichlorophenylamino)-phenyl)acetate (2)

Diclofenac sodium (0.01 mol) and methanol (20 mL) refluxed for 3 h in a few drops of concentrated sulfuric acid as a catalyst. The reaction progress was monitored by TLC using a mixture of chloroform and methanol in the ratio of 9:1 as the mobile phase. The obtained solid was washed with $NaHCO_3$ solution (5%), dried and recrystallized twice from methanol to give **2** with 72% yield and mp of 87 °C.

4.2. Synthesis of 2-(2-(2,6-dichlorophenylamino)phenyl)-acetohydrazide (3)

To methanolic solution of methyl 2-(2-(2,6-dichlorophenylamino)phenyl)acetate (**2**) (25 mL, 0.01 mol) was added hydrazine-hydrate (4 mL) and refluxed for 2 h. The reaction progress was monitored by TLC using a mixture of chloroform and methanol in the ratio 9:1 as the mobile phase. After the completion of reaction, the reaction mixture was then cooled, diluted with water and allowed to stand overnight. The solid precipitated was washed with water, dried and recrystallized twice from methanol to give **3** with 72% yield and mp of 154 °C.

4.3. General procedure for the synthesis of acid hydrazones (4a–g)

A solution of 0.01 mol of **3** and equimolar amount of appropriate aldehyde or ketone in 60 mL of ethanol were stirred at room temperature for 1 h. After

completion of the reaction, the precipitate obtained was filtered off, washed with water and cleaned twice with boiling ethanol to give **4a**–**g** with 48–79% yield.

4.3.1. 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}-*N*′-(2-nitrobenzylidene)acetohydrazide (4a). Yield: 48%; mp: 236 °C; IR (KBr): 3269, 1645, 162 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.37 (s, 2H, –CH_2), 6.35–7.6 (m, 11H, Ar–H), 8.32 (s, 1H, CH=N), 10.4 (s, 1H, –NH–, D_2O exchangeable), 12.1 (s, 1H, –CONH–, D_2O exchangeable). Calculated for $C_{21}H_{16}Cl_2N_4O_3$: C, 56.90; H, 3.64; N, 12.64. Found: C, 56.79; H, 3.71; N, 12.63.

4.3.2. 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}-*N*′-(4-dimethylaminobenzylidene)acetohydrazide (4d). Yield: 66%; mp: 250 °C; IR (KBr): 3272, 1642, 1630 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 2.85 (s, 6H, –$N(CH_3)_2$), 3.4 (s, 2H, –CH_2), 6.3–7.4 (m, 11H, Ar–H), 8.32 (s, 1H, CH=N), 10.42 (s, 1H, –NH–, D_2O exchangeable), 12.15 (s, 1H, –CONH–, D_2O exchangeable). Calculated for $C_{23}H_{22}Cl_2N_4O$: C, 62.59; H, 5.02; N, 12.69. Found: C, 62.79; H, 4.97; N, 12.7.

4.3.3. 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}-*N*′-(4-hydroxy-3-methoxybenzylidene)acetohydrazide (4e). Yield: 79%; mp: 232 °C; IR (KBr): 3269, 1642, 1630 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.37 (s, 2H, –CH_2), 3.73 (s, 3H, –OCH_3), 6.34–7.1 (m, 10H, Ar–H), 8.32 (s, 1H, CH=N), 10.4 (s, 1H, –NH–, D_2O exchangeable), 10.95 (s, 1H, –OH), 12.1 (s, 1H, –CONH–, D_2O exchangeable). Calculated for $C_{22}H_{19}Cl_2N_3O_3$: C, 59.47; H, 4.31; N, 9.46. Found: C, 56.4; H, 4.30; N, 9.43.

4.3.4. 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}-*N*′-(3-hydroxybenzylidene)acetohydrazide (4f). Yield: 70%; mp: 229 °C; IR (KBr): 3269, 1642, 1630 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.37 (s, 2H, –CH_2), 6.38–7.1 (m, 11H, Ar–H), 8.32 (s, 1H, CH=N), 10.4 (s, 1H, –NH–, D_2O exchangeable), 11.95 (2s, 2H, –CONH– and –OH). Calculated for $C_{21}H_{17}Cl_2N_2O_2$: C, 60.88; H, 4.14; N, 10.14. Found: C, 60.91; H, 4.04; N, 10.13.

4.3.5. 2-{2-[(2,6-Dichlorophenyl)amino]phenyl}-*N*′-[(4-bromophenyl)benzylidene]acetohydrazide (4g). Yield: 57%; mp: 189 °C; IR (KBr): 3270, 1645, 1628 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 3.37 (s, 2H, –CH_2), 6.3–7.79 (m, 16H, Ar–H), 10.42 (s, 1H, –NH–, D_2O exchangeable), 12.15 (s, 1H, –CONH–, D_2O exchangeable). Calculated for $C_{27}H_{20}BrCl_2N_3O$: C, 58.61; H, 3.64; N, 7.59. Found: C, 58.54; H, 3.59; N, 7.46.

4.4. General procedure for the synthesis of diclofenac amides (5a–d)

To a solution of diclofenac (0.44 mmol) in dry methylene chloride (10 mL) were added 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide·HCl (0.48 mmol) and 4-dimethylaminopyridine (44 μmol) followed by appropriate fluoroquinolone derivative (0.5 mmol). The reaction mixture was stirred at room temperature overnight. The mixture was diluted with water (~30 mL) and extracted with ethyl acetate (2× 30 mL). The combined organic solution was washed with water (2× 50 mL), dried (magnesium sulfate) and filtered and the solvent was removed in vacuo. The crude product was purified by chromatography on silica gel (EtOAc–hexanes, 20:80 then 50:50) and obtained as a bright yellow solid **5a**–**d** in 71–88% yield.

4.4.1. 1-Ethyl-6-fluoro-7-[[*N*^{4}-(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)]-3-methyl]*N*^{1}-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (5a). Yield: 71%; mp: 212 °C; IR (KBr): 3270, 3010, 2850, 2840, 1736, 1645, 1630, 1620, 1596, 1506, 1236, 1125 cm; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.28 (t, 3H, CH_3 of C_2H_5), 3.37 (s, 2H, –CH_2), 3.7–4.1 (m, 8H, –piperazine-H), 4.25 (q, 2H, CH_2 of C_2H_5), 6.58–8.0 (m, 9H, Ar–H), 8.6 (s, 1H, C_2-H), 12.15 (s, 1H, –CONH–, D_2O exchangeable), 14.86 (br s, 1H, COOH). Calculated for $C_{30}H_{27}Cl_2FN_4O_5$: C, 60.31; H, 4.55; N, 9.38. Found: C, 60.32; H, 4.61; N, 9.35.

4.4.2. 1-Cyclopropyl-6-fluoro-8-methoxy-7-[[*N*^{4}-(2-(2-(2,6-dichlorophenylamino)phenyl)acetyl)-3-methyl]-*N*^{1}-piperazinyl]-4-oxo-1,4-dihydro-3-quinoline carboxylic acid (5d). Yield: 81%; mp: 208 °C; IR (KBr): 3270, 3010, 2862, 2850, 1742, 1640, 1620, 1506, 1240, 1125 cm; ^{1}H NMR (DMSO-d_6) δ (ppm): 032–0.54 (m, 4H, cyclopropyl-H), 1.28 (s, 3H, CH_3 of piperazine), 1.4 (m, 1H, cyclopropyl-H), 2.5–3.3 (m, 7H, –piperazine-H), 3.37 (s, 2H, –CH_2), 3.73 (s, 3H, methoxy), 6.3–6.88 (m, 8H, Ar–H), 7.96 (s, 1H, C_2-H), 12.15 (s, 1H, –CONH–, D_2O exchangeable), 14.86 (br s, 1H, COOH). Calculated for $C_{33}H_{31}Cl_2FN_4O_5$: C, 60.65; H, 4.78; N, 8.57. Found: C, 60.59; H, 4.69; N, 8.67.

4.5. Biological activity

4.5.1. In vitro antimycobacterial activity (Agar dilution method). Compounds were evaluated for their in vitro antimycobacterial activity against *M. tuberculosis* H37Rv procured from the Tuberculosis Research Center, Indian Council of Medical Research, Chennai, India. The agar dilution method was performed using Middlebrook 7H10 medium supplemented with Middlebrook OADC medium (Hi-Media). After solidification of the agar, the plates were inoculated with 0.1 ml of 10^{-2} and 10^{-4} dilutions of a McFarland 1.0 concentration of a suspension of organism. The inoculated plates were then incubated at 37 °C for four weeks. The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculums.

4.5.2. In vitro cytotoxicity assay. Cytotoxicity was assessed against VERO cells (CCL-81, American Type Culture Collection) by exposing monolayers in 96-well plates to 3-fold dilutions of test compounds for 72 h. Cell viability was measured using the CellTiter96 aqueous non-radioactive cell proliferation assay (Promega Corp, Madison, WI), which determines the extent of reduction of a tetrazolium dye by measuring the absorbance of the product at 490 nm. Untreated cells and cells lysed with sodium dodecyl sulfate were used to determine 0% and 100% inhibition, respectively.

4.5.3. In vivo antimycobacterial activity. Six-week-old female CD-1 mice were infected intravenously through a caudal vein. Each mouse received approximately 10^7 viable *M. tuberculosis* ATCC 35801 suspended in 0.2 ml of modified 7H10 broth. They were divided into three groups of 10 mice each after two days. One group received daily for 10 days the aqueous suspension of the test compounds (25 mg/kg body weight) by oral (po) route. The second group received isoniazid at 25 mg/kg body weight for 10 days by po route, whereas the third group served as the control receiving no drug. Animals were kept under observation for 35 days post infection. All mice were euthanized by CO_2 inhalation. The spleens and right lungs were aseptically removed and ground in a tissue homogenizer. The number of viable organisms was determined by serial 10-fold dilutions and subsequent inoculation onto 7H10 agar plates. Cultures were incubated at 37 °C in ambient air for four weeks prior to counting. Viable cell counts were converted to logarithms, which were then evaluated by one- or two-variable analyses of variance. Statistically significant effects from the analyses of variance were further evaluated by Tukey's honestly significant difference tests[14] to make pair-wise comparisons among means.

Acknowledgment

The authors are thankful to Council of Scientific and Industrial Research, New Delhi, India for their financial assistance (No. 01 (1979)/05/EMR/-II).

References and notes

1. WHO Web site, <http://www.who.org/> and NIAID Web site, <http://www.niaid.nih.gov/factsheets/tb.htm/>.
2. Snider, D. E.; Castro, K. G.. *N. Eng. J. Med.* **1998**, *338*, 1689.
3. Sbardella, G.; Mai, A.; Artico, M.; Loddo, R.; Setzu, M. G.; La Colla, P. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1537.
4. Zhang, Y.; Amzel, L. M. *Curr. Drug. Targets* **2002**, *3*, 131.
5. Crabb, C. *Bull. World Health Organ.* **2002**, *80*, 517.
6. Dutta, N. K.; Kumar, K. A.; Mazumdar, K.; Dastidar, S. G. *Indian J. Exp. Biol.* **2004**, *42*, 922.
7. Abbas, S. E.; Abou-Youssef, H. E.; Matta, K. L. *Egypt. J. Pharm. Sci.* **1991**, *32*, 515.
8. Komurcu, S. G.; Rollas, S.; Ulgen, M.; Gorrod, J. W. *Boll. Chim. Farmac.* **1995**, *134*, 375.
9. National Committee for Clinical Laboratory Standards. Antimycobacterial susceptibility testing for *Mycobacterium tuberculosis*. Proposed standard M24-T. National Committee for Clinical Laboratory Standards, Villanova, PA, 1995.
10. Novak, K.; Noszal, B.; Hermecz, I.; Kereszturi, G.; Podanyi, B.; Szasz, G. *J. Pharm. Sci.* **1990**, *79*, 1023.
11. Young, D. B. Strategies for new drug development. In *Tuberculosis*; Bloom, B. R., Ed.; ASM Press: Washington, DC, 1994; pp 559–567.
12. Gundersen, L. L.; Nissen-Meyer, J.; Spilsberg, B. *J. Med. Chem.* **2002**, *45*, 1383.
13. Sriram, D.; Yogeeswari, P.; Basha, S. J.; Radha, D. R.; Nagaraja, V. *Bioorg. Med. Chem.* **2005**, *13*, 5774.
14. Kirk, R. E. *Experimental design procedures for the behavioral sciences*; Brooks-Cole: Belmont, Calif, 1986.