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Synthesis and in vitro anti *Mycobacterium tuberculosis* activity of a series of phthalimide derivatives

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ABSTRACT

New phthalimide derivatives were easily prepared through condensation of phthalic anhydride and selected amines with variable yields (70–90%). All compounds (**3a–l**) were evaluated against *Mycobacterium tuberculosis* $H_{37}Rv$ using Alamar Blue susceptibility. The compounds **3c**, **3i**, and **3l** have the minimum inhibitory concentrations (MICs) of 3.9, 7.8, and 5.0 μg/mL, respectively, and could be considered new lead compounds in the treatment of tuberculosis and multi-drug resistant tuberculosis.

1. Introduction

Tuberculosis (TB) has become an important problem worldwide : about 2 million people die each year, particularly in developing countries; it is estimated that about one-third of the world population is currently infected with the bacillus in its latent form and that nearly 9 million new cases develop each year.[1] According to WHO, multiresistant tuberculosis is responsible for approximately 460 thousand new cases per year and for about 740 thousand new patients infected by both *Mycobacterium tuberculosis* and HIV/AIDS. Recent estimates show that 10% of all new TB infections are resistant to at least one anti-TB drug.[2]

To treat an infection, a cocktail of drugs including, for example, isoniazid, rifampin, ethambutol, and pyrazinamide is prescribed for 2 months followed by a continuation phase in which isoniazid and rifampin are taken. Long-term therapies lasting between 6 and 9 months have frequently led to patient non-compliance and, in turn, contributed to the emergence of multi-drug resistant TB (MDR-TB).[3]

The ever-increasing drug resistance, toxicity, and side effects of currently used antituberculosis drugs, and the absence of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds.

In the last 10 years the research on *M. tuberculosis* and possible drug candidates has undergone much progress with genome unrevealed and the discovery of different biological targets.[4,5] The potential activities of several natural and synthetic compounds have been described against *M. tuberculosis*.

Among this, compounds containing phthalimide subunit have been described as an scaffold (biophoro) to design new prototypes drug-candidates with different biological activities and are used in different diseases as, for example, leprosy, AIDS, tumor, diabetes, multiple myeloma, convulsion, inflammation, pain, bacterial infection among others.[6–8]

Several reports in the literature demonstrate the antimicrobial potential of phthalimide derivative.[9–11] Some of these derivatives with N-substituted phthalimide moieties possess minimal inhibitory concentrations (MICs) comparable with those of clinically used antibiotics.[12]

Previous results from our group demonstrated that molecular hybridization between phthalimide subunit present in thalidomide and the sulfonamide drugs, as for example dapsone, leads to antimycobacterial compounds with activity against *Mycobacterium leprae*. These compounds also showed immunomodulatory properties (unpublished results). All these results encourage us to evaluate these same compounds against the *M. tuberculosis.*

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The new phthalimide derivatives (**3a–l**) were evaluated for in vitro anti-TB activity against *M. tuberculosis* $H_{37}Rv$ and the cytotoxicity of those compounds was analyzed utilizing macrophage cell line (J774).

2. Results and discussion

2.1. Chemistry

The compounds were prepared through condensation reaction between phthalic anhydride and amines previously selected in acetic acid under reflux during 2–3 h (Scheme 1). After cooling the compounds were filtered and recrystallized in ethanol in 70–80% yields, except compound **3i** that was purified by preparative column chromatography.

The purity of synthesized compounds was checked by thin layer chromatography (TLC) and elemental analyses, and the structures were identified by spectral data. The IR spectra of compounds (**3a–j**), the bands representing carbonyl group of imide phthalimide ring appeared at 1782 cm^{-1} and 1713 cm^{-1}. The sulfonyl group appeared at 1350 cm^{-1} and 1140 cm^{-1}. The nuclear magnetic resonance spectra (^1H NMR) of **3**(**a–j**) showed multiple signals corresponding to resonances of phthalimide protons at 7.9–8.0 ppm and phenyl ring linked to phthalimide subunit at 7.6 ppm and 8.0 ppm. The nuclear magnetic resonance spectra (^{13}C NMR) of **3**(**a–j**) showed the signals corresponding to resonances of phthalimide protons at 123.7, 134.8, 131.4, and 166.2 ppm. The ^{13}C NMR showed the signals of carbon resonance of phenyl ring linked to phthalimide subunit at 132, 126, 128, and 137 ppm.

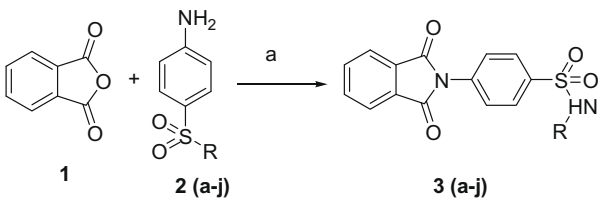
The compound **3h** was obtained through condensation reaction between phthalyc anhydride and isoniazid. The IR spectra showed that the carbonyl group of imide phthalimide ring appeared at 1782 cm^{-1} and 1713 cm^{-1} and the hydrazide carbonyl group appeared at 1662 cm^{-1}. The nuclear magnetic resonance spectra (^1H NMR) of **3h** showed multiple signals corresponding to resonances of phthalimide protons at 8.0 ppm and of pyridinic protons at 8.85 ppm and 7.88 ppm. The ^{13}C NMR showed the signals of phthalimide ring at 124, 135, 131, and 164 ppm and of the isoniazid subunit derivative at 165, 139, 121, and 150 ppm.

The elemental analysis results were within ±0.4% of the theoretical values. The lipophilicities of the synthesized compounds (**3a–l**) were calculated using CHEMDRAW ultra 8.0 (Table 1). All compounds presented lipophilicity higher than drug such as isoniazid.

2.2. Antimycobacterial activity in vitro determination

The compounds (**3a–l**) were evaluated against *M. tuberculosis* $H_{37}Rv$ (ATTC 27294) using Microplate Alamar Blue Assay (MABA). Isoniazid was used as control drug in the tests. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration required to complete inhibition of bacterial growth. The MICs of the compounds were reported in Table 1.

The compounds **3c** and **3l** inhibited *M. tuberculosis* growth with MICs of 3.9 and 5 μg/mL, respectively. Structure–activity relationship showed that if pyrimidine ring (**3c–f)** is substituted in any positions or changed by isosteric replacement the activity against *M. tuberculosis* decreases. The compound **3i** inhibited the growth at 7.8 μg/mL. When the amino group of compound **3i** was duplicated and substituted by another phthalimide ring (**3j**) there was decrease in activity.



Scheme 1. Reagents and conditions: acetic acid, reflux, 2 h, 37–90%.

Molecular modifications in pyridine ring of compound **3l** decrease anti-tubercular activity. Furthermore, the result of compound **3l** is consistent with the structure–activity relationship described for isoniazid in which the substitution of N-2 can lead to active compounds, but less than isoniazid. The introduction of phthalimide group by molecular hybridization strategy does not improve the activity of parental drug-isoniazid.

Antimicrobial activity assay of those three compounds (**3c**, **3i**, and **3l**) provide evidence that they are potential against tuberculosis agent. Although the MIC values obtained here are larger than those of isoniazid (0.02–0.03 μg/mL), these compounds have stronger in vitro activity than pyrazinamide (MIC of 50–100 μg/mL), another first line antitubercular drug.[13]

2.3. In vitro cytotoxicity evaluation

The compounds were tested for cytotoxicity (IC_{50}) in J774 cells at concentrations until 150× the MIC for *M. tuberculosis* $H_{37}Rv$. Table 2 shows the cytotoxicity to host cells of compounds **3c**, **3i**, and **3l**. The selective index was calculated as ratio of MIC in μg/mL and cytotoxicity (IC_{50}).[14]

The selective index showed that J774 cells containing compounds (**3c**, **3i**, and **3l**) have higher activity to *M. tuberculosis* than eukaryotic cells.

3. Conclusions

All compounds were obtained with good yields (37–90%) from commercially available materials.

These compounds **3c**, **3i**, and **3l** showed to be more potent than the other phthalimide derivatives in inhibition of *M. tuberculosis* growth with MICs of 3.9, 7.8, and 5.0 μg/mL, respectively. These compounds are not cytotoxic to host cell at the concentrations effective in inhibiting *M. tuberculosis* infection. The lipophilicity is an important physico-chemical property related to the capacity of the compounds across the membrane. All compounds presented lipophilicity higher than drugs such as isoniazide and pyrazinamide used in the treatment. These compounds could be considered new lead compounds in the treatment of tuberculosis, multi-drug resistant, and latent tuberculosis.

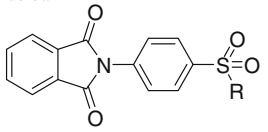
4. Experimental

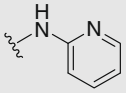
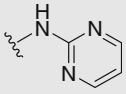
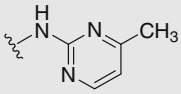
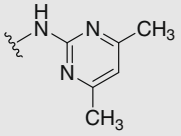
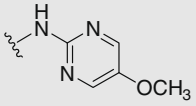
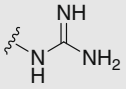
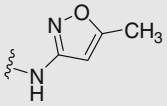
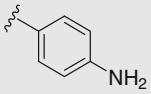
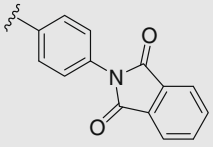
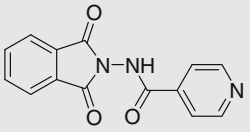
Melting points were taken with an electrothermal melting point apparatus (SMP3 Bibby Stuart Scientific) in open capillary tubes and are uncorrected. Infrared spectra (KBr disc) were run on a FTIR-8300 Shimadzu and the frequencies were expressed in cm^{-1}. ^1H NMR and ^{13}C NMR spectra were scanned on a Bruker DRX-400 (400 MHz) NMR spectrometer using DMSO-d_6 as solvent. Chemical shifts were expressed in ppm (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. General procedure for the synthesis of phthalimide derivatives (3a–l)

A mixture of selected amine (**2 a–l**) (4,0 mmol), phthalic anhydride (0.5 g, 3.4 mmol), and 20 mL of glacial acetic acid

Table 1
Physical constants and in vitro antimycobacterial activities of compounds **3a–l**



Compound	R	Yields (%)	Mp (°C)	Clog *P*[a]	MIC (μM)
3a	H	90	303–306	0.91	>250 μg/mL
3b		73	277–280	2.31	>250 μg/mL
3c		77	308–310	1.57	3.9 μg/mL
3d		76	275–278	2.07	>250 μg/mL
3e		74	297–301	2.57	>250 μg/mL
3f		71	262–265	2.12	>250 μg/mL
3g		70	269–271	0.24	125 μg/mL
3h		74	215–218	2.04	125 μg/mL
3i		37	217–220	2.36	7.8 μg/mL
3j		83	306–310	3.84	>250 μg/mL
		77	133–136	−0.44	5 μg/mL
3l	Isoniazid	—	—	−1.15	0.02 μg/mL

[a] Calculated on CHEM DRAW ULTRA 8.0 software.

Table 2
Cytotoxicity and selective index

Compound	MIC (μg/mL)	Cytotoxicity (μg/mL)	Selective index
3c	3.9	312.5	80
3i	7.8	312.5	40
3l	5.0	625	125
Isoniazid	0.015–0.03	—	—

was stirred under nitrogen at 130 °C for 2 h. After that the mixture was cooled and 30 mL of cold water was added. The product was filtered and washed with cold water. The product was recrystallized in ethanol without further purification to lead to compounds with variable yields (37–90%).

4.1.1. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)benzenesulfonamide (3a)

Yield: 90%, mp: 303–306 °C.

IR ν_{max} (cm^{-1}; KBr pellets):1779 and 1715 (C=O imide); 1312 and 1137 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.1 (2H; d; J = 8.5 Hz; H_7); 8.0 (2H; m; H_2); 7.9 (2H; m; H_1); 7.8 (2H; d; J = 8,5 Hz; H_6) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 167.4; 136.7; 135.6; 132.7; 130.7; 128.4; 128.0; 124.4 ppm. Calcd for $C_{14}H_{10}N_2O_4S$: C, 55.62; H, 3.33; N, 9.27. Found: C, 55.2; H, 3.11; N, 9.53.

4.1.2. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-pyridin-2-yl benzenesulfonamide (3b)

Yield: 73, mp: 277–280 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1782 and 1713 (C=O imide); 1311 and 1139 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.01 (5H; m; H_2, H_7 e $H_{1'}$); 7.77 (1H; ddd; J = 8,5 Hz; $H_{3'}$); 7.64 (2H; dd; J = 8,73 Hz; H_6); 7.24 (1H; dd; J = 8,5 Hz; $H_{2'}$); 6.87 (1H; dd; J = 8,5 Hz; $H_{4'}$) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.6; 141.1; 136; 134.9; 134.8; 132; 131.4; 129; 128; 124; 123.6; 114.3; 113.3 ppm. Calcd for $C_{19}H_{13}N_3O_4S$: C, 60.15; H, 3.45; N, 11.08. Found: C, 60.55; H, 3.33; N, 11.5.

4.1.3. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-pyrimidin-2-yl benzenesulfonamide (3c)

Yield: 77%, mp: 308–310 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1786 and 1720 (C=O imide); 1317 and 1141 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.54 (2H; dd; J = 8,73 Hz; H_7); 8.13 (2H; dd; J = 8.73 Hz; $H_{2'}$); 7.99 (2H; m; H_2); 7,92 (2H; m; H_1); 7.69 (2H; dd; J = 8.73 Hz; H_6); 7.07 (1H; dd; J = 8.73 Hz; $H_{3'}$) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 165.8; 158; 150.2; 136; 135.2; 131.9; 129.3; 124; 122.1; 110 ppm. Calcd for $C_{18}H_{12}N_4O_4S$: C, 56.84; H, 3.18; N, 14.73. Found: C, 56.54; H, 3.31; N, 14.5.

4.1.4. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(4-methylpyrimidin-2-yl)benzenesulfonamide (3d)

Yield: 76%, mp: 275–279 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1789 and 1712 (C=O imide); 1361 and 1130 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.35 (1H; d; J = 8,1 Hz; $H_{1'}$); 8.11 (2H; d; J = 8.3 Hz; H_7); 7.98 (2H; m; H_2); 7.91 (2H; m; H_1); 7.67 (2H; dd; J = 8.3 Hz; H_6); 6.91 (1H; d; J = 8.1 Hz; $H_{2'}$) 2.32 (3H; s) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.5; 156.3; 150.2; 139.8; 135.5; 134.8; 131.4; 129; 128.3; 126.9; 123.5; 109.5; 23.1 ppm. Calcd for $C_{19}H_{14}N_4O_4S$: C, 57.86; H, 3.58; N, 14.21. Found: C, 57.4; H, 3.29; N, 14.2.

4.1.5. *N*-(4,6-Dimethylpyrimidin-2-yl)-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)benzenesulfonamide (3e)

Yield: 74%, mp: 297–301 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1786 and 1718 (C=O imide); 1361 and 1168 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.11 (2H; d; J = 8,7 Hz; H_7); 7.98 (2H; m; H_2); 7.90 (2H; m; H_1); 7.64 (2H; d; J = 8.7 Hz; H_6); 6.75 (1H; s; $H_{3'}$) 2.26 (6H; s) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.5; 155.9; 135.2; 134.8; 131.6; 131.4; 129.1; 128.5; 126.7; 123.5; 109.5; 23.3 ppm. Calcd for $C_{20}H_{16}N_4O_4S$: C, 58.81; H, 3.95; N, 13.72. Found: C, 58.7; H, 3.77; N, 13.41.

4.1.6. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(5-methoxypyrimidin-2-yl)benzenesulfonamide (3f)

Yield: 71%, mp: 262–265 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1785 and 1715 (C=O imide); 1367 and 1162 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.32 (2H; s; $H_{1'}$); 8.1 (2H; d; J = 8.7 Hz; H_7); 7.99 (2H; m; H_2); 7.91 (2H; m; H_1); 7.68 (2H; d; J = 8.7 Hz; H_6); 3.79 (3H; s) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 167; 151.4; 150.2; 145.2; 136; 132; 134.8; 131.9; 129.1; 127.7; 124; 56.7 ppm. Calcd for $C_{19}H_{14}N_4O_5S$: C, 55.6; H, 3.44; N, 13.65. Found: C, 55.5; H, 3.1; N, 13.2.

4.1.7. *N*-[Amino(imino)methyl]-4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)benzenesulfonamide (3g)

Yield: 70%, mp: 269–271 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 3495 (N–H); 1797 and 1713 (C=O imide); 1367 and 1134 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 7.99 (2H; m; H_2); 7.93 (2H; m; H_1); 7.90 (2H; d; J = 8.2 Hz; H_7); 7.60 (2H; d; H_6); 6.77 (4H; N–H; s) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.6; 158.2; 143.7; 134.8; 136.2; 131.4; 127.2; 126.2; 123.5 ppm. Calcd for $C_{15}H_{12}N_4O_4S$: C, 52.32; H, 3.51; N, 16.27. Found: C, 52.77; H, 3.69; N, 16.6.

4.1.8. 4-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-(5-methylisoxazol-3-yl)benzenesulfonamide (3h)

Yield: 74%, mp: 215–218 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1782 and 1712 (C=O imide); 1380 and 1172 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.0 (2H; d; J = 8.3 Hz; H_7); 7.99 (2H; m; H_2); 7.91 (2H; m; H_1); 7.64 (2H; d; J = 8.3 Hz; H_6); 6.18 (1H; s; $H_{1'}$) 2.35 (3H; s) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 170.4; 166.4; 143.7; 138.3; 134.8; 131.4; 129.6; 127.6; 124; 119.1; 95.4; 12 ppm. Calcd for $C_{18}H_{13}N_3O_5S$: C, 56.4; H, 3.42; N, 10.9. Found: C, 56.5; H, 3.3; N, 11.2.

4.1.9. 2-{4-[(4-Aminophenyl)sulfonyl]phenyl}-1*H*-isoindole-1,3(2*H*)-dione (3i)

The compound **3i** after precipitation was purified by preparative column chromatography (hexane/ethyl acetate 8:2). Yield: 37%, mp: 217–220 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 3446 and 3530 (N–H); 1786 and 1712 (C=O imide); 1371 and 1142 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.1 (2H; d; J = 8.5 Hz; H_7); 7.99 (2H; m; H_2); 7.94 (2H; d; $H_{1'}$); 7.91 (2H; m; H_1); 7.7 (2H; d; J = 8.5 Hz; H_6); 7.82 (2H; d; $H_{2'}$) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.1; 151.9; 136; 134.8; 132.3; 132; 131.7; 131.4; 129; 128; 123.6; 118.9 ppm. Calcd for $C_{20}H_{14}N_2O_4S$: C, 63.4; H, 3.73; N, 7.4. Found: C, 63.5; H, 3.5; N, 7.2.

4.1.10. Bis-(phenylsulfonyl-1*H*-isoindole-1,3(2*H*)-dione) (3j)

Yield: 83%, mp: 306–310 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1784 and 1713 (C=O imide); 1366 and 1137 (S=O).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.2 (4H; d; J = 8.6 Hz; H_7); 8.0 (4H; m; H_2); 7.93 (4H; d; $H_{1'}$); 7.8 (4H; d; J = 8.6 Hz; H_6) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 166.4; 135.4; 134.8; 131.4; 129.5; 127.6; 127.1; 113 ppm. Calcd for $C_{28}H_{16}N_2O_6S$: C, 66.14; H, 3.17; N, 5.51. Found: C, 66.4; H, 3.3; N, 5.3.

4.1.11. *N*-(1,3-Dioxo-1,3-dihydro-2*H*-isoindol-2-yl)isonicotinamide (3l)

Yield: 77%, mp: 133–136 °C.

IR ν_{max} (cm^{-1}; KBr pellets): 1783 and 1715 (C=O imide); 1662 (C=O amide).

NMR ^1H (400 MHz, DMSO-d_6) δ: 8.85 (2H; d; J = 8.8 Hz; $H_{2'}$); 8.03 (2H; m; H_2); 7.98 (2H; m; H_1); 7.88 (2H; d; J = 8.8 Hz; $H_{1'}$) ppm; ^{13}C NMR (400 MHz, DMSO-d_6) δ: 165; 164.2; 150.7; 137.7; 135.4; 129.4; 123.9; 121.4 ppm. Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.72. Found: C, 62.5; H, 3.3; N, 15.41.

4.2. Biological activity

4.2.1. Antimycobacterial activity in vitro determination

The anti-*M. tuberculosis* activity of the compounds was determined using the MABA as analytical method.[15] Stock solutions of the tested compounds were prepared in dimethyl sulfoxide and diluted in broth medium Middlebrook 7H9 (Difco), supplemented with oleic acid, albumin, dextrose, and catalase (OADC enrichment—BBL/Becton-Dikinson, Sparks, MD, USA), to obtain final drugs with concentration ranges of 0.15–250 μg/mL. The isoniazid was solubilized with distilled water according to the manufacturers' recommendations (Difco laboratories, Detroit, MI, USA) and was used as standard drug. *M. tuberculosis* $H_{37}Rv$ ATCC 27294 was grown for 7–10 days in Middlebrook 7H9 supplemented with 0.05% Tween 80 OADC to avoid clumps. Suspensions were prepared and their turbidities matched to a McFarland No. 1 (turbidity standard). After further dilution of 1:25 in Middlebrook 7H9 supplemented with OADC, the inoculum was added to each well of the 96-well microtiter plate (Falcon 3072; Becton Dickinson, Lincoln Park, NJ) together with the compounds. Samples were set up in triplicate. Cultures were incubated for 7 days at 37 °C, and after this resazurin was added for the reading. The minimum inhibitory concentration (MIC) was defined as the lowest concentration resulting in 90% inhibition of growth of *M. tuberculosis*[15] measuring the fluorescence (excitation/emission of 530/590 nm filters, respectively) in a SPECTRAfluor Plus (Tecan).[13] For standard test, the MIC value of isoniazid was determined each time. The acceptable MIC of isoniazid ranged from 0.015 to 0.05 μg/mL.[13]

4.2.2. In vitro cytotoxicity evaluation

In vitro cytotoxicity evaluation was carried out on cultured murines tumor cell lines J774 as recommended by Ahmed et al.[16] The cells were routinely maintained with RPMI medium supplemented with 10% fetal bovine serum (FBS), at 37 °C in a humidified 5% CO_2 atmosphere. After reaching confluence, the cells were detached and counted. For the cytotoxicity assay, 2×10^4 cells well^{-1} were seeded in 200 μL of complete medium in 96-well plates (Corning Costar). The plates were incubated at 37 °C in 5% CO_2 for 24 h to allow cell adhesion prior to drug testing. The compounds were dissolved in sterile DMSO and diluted to concentrations of 625, 312.5, 156.3, 78, 39, 19.5, 9.75, 4.87, 2.43, 1.21, and 0.6 μg/mL. Cells were exposed to the compounds for a 24 h period. Resazurin solution was added to cell cultures and incubated for a 6-h period. Cell respiration, as an indicator of cell viability, was determined by resazurin (redox potential), the pink color means cell viability and the blue color means cell death. Thus, the IC_{50} value was defined as the bigger drug concentration at which 50% of the cells are viable relative to the control and measuring the fluorescence (excitation/emission of 530/590 nm filters, respectively) in a SPECTRAfluor Plus (Tecan).[13]

4.2.3. Selectivity index

A selectivity index (SI) can then be calculated by dividing the IC_{50} by the MIC; if the SI is >10, the compound is then evaluated further.[14]

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References and notes

1. Zhang, Y. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 529.
2. WHO Web site, <http://www.who.org/>.
3. Bentrup, K. H.; Russell, D. G. *Trends Microbiol.* **2001**, *9*, 597.
4. Camus, J. C.; Pryor, M. J.; Medigue, C.; Cole, S. T. *Microbiology* **2002**, *148*, 2967.
5. Nayyar, A.; Jain, R. *Curr. Med. Chem.* **2005**, *12*, 1873.
6. Bansal, C. R.; Karthikeyan, N. S.; Moorthy, H. N.; Trivedi, P. *Arkivoc* **2007**, *15*, 66.
7. Mendyk, A.; Salat, K.; Librowski, T.; Czarnecki, R.; Malawska, B. *Pol. J. Pharmacol.* **2001**, *53*, 689.
8. Kamal, A.; Satyanarayana, M.; Devaiah, V.; Rohini, V.; Yadav, J. S.; Mullick, B.; Nagaraja, V. *Lett. Drug Des. Discovery* **2006**, *3*, 494.
9. Patel, H. S.; Mistry, H. J.; Patel, N. K.; Desai, S. N. *Bulg. Chem. Commun.* **2004**, *36*, 167.
10. Rajora, S.; Banu, T.; Khatri, D.; Talesara, G. L. *Asian J. Chem.* **1999**, *11*, 1528.
11. Dilber, S.; Bogavac, M.; Radulovic, M. *Mikrobiologija* **1996**, *33*, 89.
12. Orzeszko, A.; Kaminska, B.; Starosciak, B. J. *Il Farmaco* **2002**, *57*, 619.
13. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
14. Orme, I. *Antimicrob. Agents Chemother.* **2001**, *45*, 1943.
15. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362.
16. Ahmed, S. A.; Gogal, R. M.; Walsh, J. E. *J. Immunol. Methods* **1994**, *170*, 211.