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Original article

Synthesis and anti-mycobacterial evaluation of some pyrazine-2-carboxylic acid hydrazide derivatives

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ABSTRACT

A series of pyrazine-2-carboxylic acid hydrazide derivatives were synthesized and screened for their activity against *Mycobacterium tuberculosis*. The results show that pyrazine-2-carboxylic acid hydrazide–hydrazone derivatives **3a**–**l** were less active than pyrazinamide. In contrast, the N^4-ethyl-N^1-pyrazinoyl-thiosemicarbazide **4** showed the highest activity against *M. tuberculosis* $H_{37}Rv$ (IC_{90} = 16.87 μg/mL). Details of the structure–activity and structure–cytotoxicity relationships are discussed.

1. Introduction

The treatment of tuberculosis (TB) infections has become an important and challenging problem due to the emergence of multiple-drug-resistant organisms [1]. Different factors are responsible for the resurgence of TB, such as people infected with HIV virus, immigration, war, famine, homelessness, the lack of new drugs and multi-drug-resistant tuberculosis resulted from inconsistent or partial treatment. According to WHO global report, in 2006 there were an estimated 9.2 million worldwide new cases of TB with 0.5 million cases of multidrug-resistant TB (MDR-TB) and estimated 1.5 million deaths from TB in HIV-negative people and 0.2 million among people infected with HIV [2]. The recommended therapy for TB consists of an initial phase of treatment with four drugs – isoniazid, rifampicin, pyrazinamide and ethambutol, taken daily for two months, followed by a continuation phase of treatment with isoniazid and rifampicin for another four months [3,4]. Pyrazinamide is one of the frontline agents that played a significant role in shortening the duration of treatment of MDR-TB. It is considered to be a prodrug that requires activation by pyrazinamidase to pyrazinoic acid, which is believed to be the active form [5]. The SAR of pyrazinamide derivatives is still very limited because the drug is active only under acidic conditions and more efficacious *in vivo* than would be predicted by its *in vitro* potency with vaguely defined mechanism of action [3,6,7]. On the other hand, the hydrazone–hydrazide derivatives [8,9] as well as the N^4-alkyl thiosemicarbazides [10] of the anti-mycobacterial drug isoniazid were reported to improve its activity. Therefore, guided by the above mentioned data as well as the high activity of pyrazinamide against MDR-TB, we would like to report the synthesis, anti-mycobacterial screening as well as the cytotoxicity studies of a new series of pyrazine-2-carboxylic acid hydrazide derivatives namely hydrazones and N^4-alkyl thiosemicarbazides.

2. Results and discussion

2.1. Chemistry

The synthesis of the target compounds is outlined in Schemes 1 and 2. The core intermediate pyrazine-2-carboxylic acid hydrazide **2** was prepared from pyrazine-2-carboxylic acid **1** by esterification followed by hydrazinolysis to give **2** in a good yield.

The hydrazones **3a**–**l** were prepared in one step by the condensation of the hydrazide **2** with the appropriate aryl aldehydes or ketones in ethanol according to the procedure described in literature [11]. On the other hand the thiosemicarbazide **4** was prepared by reaction of **2** with ethyl isothiocyanate.

The commercially non-available 2-chloroquinoline-3-carboxaldehyde derivatives **6a**–**b** were prepared by heating a mixture of the acetanilide derivatives **5a**–**b** with phosphorus oxychloride in

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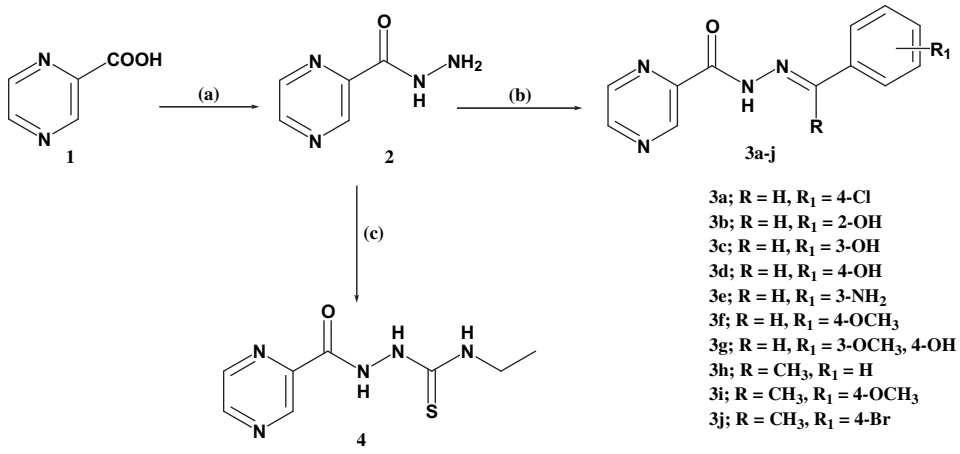


Scheme 1. Reagents and conditions; a) (i) Et_3N, ClCOOEt, MeOH, (ii) $NH_2NH_2 \cdot H_2O$ b) Aldehyde or Ketone, EtOH, reflux c) EtNCS, EtOH, reflux.

DMF (Scheme 2). The structure of the prepared compounds was fully characterized on the basis of melting points, IR, ^1H NMR, mass spectra and elemental analysis.

2.2. Biological assay

All of the prepared compounds (hydrazones **3a**–**l** and thiosemicarbazide **4**) are initially subjected to the TAACF's primary screen against *Mycobacterium tuberculosis* strain $H_{37}R_v$ (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the microplate Alamar Blue Assay (MABA) [12] in the dose–response assay. The assay returns IC_{90}, and IC_{50} values for all compounds where IC stands for 'inhibitory concentration' – i.e the concentration where a compound inhibits the TB strain by 90% or 50%, respectively. As shown in Table 1, all the prepared hydrazones **3a**–**l** as well as the parent hydrazide **2** were inactive with IC_{90}, and IC_{50} values more than 100 μg/mL.

A rationale for the design of the anti-mycobacterial drug isoniazid hydrazones is the increased lipophilicity of prepared compounds resulting in a beneficial influence on their activity. These hydrazones undergo hydrolysis to their parent hydrazide (isoniazid) which is responsible for their activity [8]. However this strategy unexpectedly was non effective for the prepared pyrazine-2-carboxylic acid hydrazide-hydrazones as none of them exhibited improved or similar anti-mycobacterial activity to pyrazinamide. These unforeseen results were not only in disagreement with a previous report showing good anti-mycobacterial activity (without exact MICs or detailed SARs) of some pyrazine-2-carboxylic acid hydrazide-hydrazones [13] but also against a reality that all the prepared hydrazones **3a**–**l** exhibited increased lipophilicity compared to pyrazinamide (see Clog*P* values, Table 1). A reason of the results obtained may be attributed to that the parent hydrazide **2** is less active than pyrazinamide itself.

Confronted by these results of the hydrazones, we pursued another venue of structural modification of the pyrazine-2-carboxylic acid hydrazide to N^4-ethyl-N^1-pyrazinoyl-thiosemicarbazide **4** as a representative example of the N^4-alkyl-N^1-pyrazinoyl thiosemicarbazides. We selected to synthesize **4** with the hope that it would possess high anti-mycobacterial activity based on a literature survey showing that the N^4-alkyl-N^1-isonicotinoyl thiosemicarbazides showed higher activity than their N^4-aryl analogs in the SAR of isoniazid derivatives [10]. Furthermore, some N^4-aryl-N^1-pyrazinoyl thiosemicarbazides were already reported to have low anti-mycobacterial activity [14,15].

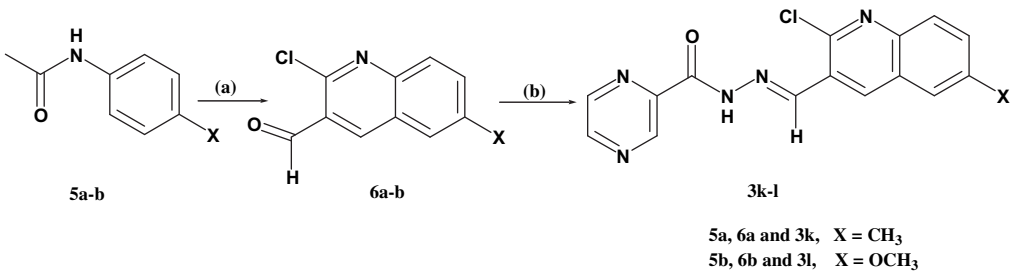
As anticipated, compound **4** was the most active in this series with IC_{90}, and IC_{50} values of 16.87 μg/mL and 11.37 μg/mL, respectively. This is a quite good activity compared with pyrazinamide having MIC_{90} value ranging from 6 to 50 μg/mL, specifically; MIC_{90} at pH 5.5 is 50 μg/mL [5].

The high anti-mycobacterial activity of **4** can be attributed to one or more of the following hypotheses: (1) the thiosemicarbazide molecule masks the acid group of pyrazinoic acid, and allowing it to pass through the bacterial cell membrane. (2) A synergistic anti-mycobacterial effect of both the thiosemicarbazide molecule (thiosemicarbazides were reported to exhibit anti-mycobacterial activity) with pyrazinoic acid produced from its hydrolysis (3) or that **4** can act as bacterial pyrazinamidase activator.

The later hypothesis is based on Zhang et al. [16] discovery that *M. tuberculosis* pyrazinamidase is a monomeric Fe^{2+}/Mn^{2+} protein and can be inhibited by an excess of other metals competing for the same binding-site such as Zn^{2+}.

2.3. Cytotoxicity assay

Cytotoxicity of all the prepared compounds is done in parallel with the TB dose response assay. Compounds are screened by serial



Scheme 2. Reagents and conditions; a) $POCl_3$, DMF b) EtOH, reflux.

Table 1
The *in-vitro* anti-mycobacterial activity of test compounds.

Compd. #	TAACF[a] ID	IC_{50} (μg/mL)	IC_{90} (μg/mL)	CC_{50} Vero cells (μg/mL)[b]	CLog*P*[c]
2	417781	>100	>100	>30	−1.16
3a	417771	>100	>100	>30	1.72
3b	417774	>100	>100	11.43	1.54
3c	417772	>100	>100	>30	0.91
3d	417773	>100	>100	>30	0.91
3e	417775	>100	>100	>30	0.31
3f	417779	>100	>100	>30	1.16
3g	417769	>100	>100	>30	0.71
3i	417770	>100	>100	>30	1.90
3j	417778	>100	>100	>30	2.84
3k	417777	>100	>100	>30	1.55
3l	417776	>100	>100	5.37	1.31
4	417780	11.37	16.87	29.75	−1.71
PZA	415101	–	6–50[d]	–	−0.68

[a] TAACF: Tuberculosis Antimicrobial Acquisition and Coordinating Facility.
[b] Measured by Cell Titer-Glo.
[c] CLog*P* calculated using ChemDraw Ultra 9.0.
[d] Data from Ref. [5].

dilution to assess toxicity to a VERO cell line. The VERO cell cytotoxicity assay returns a CC_{50} value. As shown in Table 1, all the tested compounds are showed CC_{50} values more than 30 μg/mL except **3b**, **3l**, **4** with values 11.43, 5.37 and 29.75 μg/mL, respectively. Although compound **4** exhibited the best anti-mycobacterial activity (Table 1), its cytotoxicity is relatively high thus having narrow selectivity index (1.76). The selectivity index is defined as the ratio of the measured CC_{50} (mammalian cell toxicity) to the IC_{90} ($H_{37}R_v$ *M. tuberculosis*) described above. According to TAACF rules, if the SI value is ≥10, then the compound may be considered for further screening. Therefore, compound **4** was not evaluated further although pyrazinamide itself is considered a narrow selectivity index drug.

Nevertheless, more information on the structure–activity relationships of these compounds and more potent anti-mycobacterial agents can be achieved by the preparation of N^1-pyrazinolyl-N^4-alkyl thiosemicarbazides and their cyclization products.

3. Experimental

3.1. Chemistry

Reactions were monitored by TLC analysis using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) with F_{254} indicator thin layer plates. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected.

IR spectra were recorded as KBr disks on a Brukar Vector 22 IR spectrophotometer. ^{1}H NMR spectra were obtained on a 300 MHz AL spectrophotometer and on 60 MHz Varian EM-360L NMR spectrophotometer using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) relative to $CDCl_3$ (7.29) or DMSO-d_6 (2.5) and coupling constants (J) in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet.

Accurate masses were obtained on Micromass LCT mass spectrometer, which was recorded in the positive ion mode with leucine enkephalin as an internal lock mass standard. Elemental analysis was performed on Vario EL III, Elemental analyzer, GMBH. D-63452 HANAU.

3.1.1. Synthesis of pyrazine-2-carboxylic acid hydrazide (**2**)

To a stirred solution of **1** (0.124 g, 1.0 mmol) in 30 mL dry chloroform at −10 °C, triethylamine (0.10 g, 1.0 mmol) was added, followed by ethyl chloroformate (0.108 g, 1.0 mmol) in a drop wise manner over a period of 10 min under stream of nitrogen gas, the mixture was continuously stirred for an additional 30 min. Methanol (0.32 g, 10.0 mmol) was added over a period of 15 min, the mixture was stirred for additional 12 h at room temperature. The solvent was evaporated under reduced pressure and the obtained crude oily product was dissolved in 30 mL methanol, hydrazine monohydrate (0.25 g, 5.0 mmol) was added and the mixture was refluxed for 6 h, the mixture was cooled to room temperature and the obtained precipitate was recrystallized from ethanol to give **2** as a yellowish white crystals (0.11 g; 80% yield), m.p. 169–170 °C. IR (KBr) ν_{max} (cm^{-1}): 3435 (NH_2), 1636 (C=O), 1582 (C=N). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 4.66 (s, 2H, NH_2), 8.72 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.85 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.16 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 10.18 (s, 1H, NH). MS: m/z (%) 138 [M^+] (100), 124 (5), 105 (5).

3.1.2. General procedure for preparation of hydrazones **3a–l**

To a stirred solution of **2** (0.138 g, 1.0 mmol) in 25 mL absolute ethanol, various substituted aldehydes or ketones (1.0 mmol) were added, the mixture was refluxed for 12 h, then cooled to room temperature and the obtained precipitate was recrystallized from ethanol to give **3a–l** in a good yield.

*3.1.2.1. 4-Chlorobenzylidene pyrazine-2-carboxylic acid hydrazide (**3a**) [13].* Yellowish white crystals in (0.213 g; 81.9% yield), m.p. 240–242 °C. IR (KBr) ν_{max} (cm^{-1}): 3450 (NH), 1651 (C=O), 1564 (C=N), 1534 (N–H), 1326, 1168, and 1137 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.42 (dd, 2H, J = 8.45 Hz, Ar–H), 7.81 (dd, 2H, J = 8.45 Hz, Ar–H), 8.55 (s, 1H, CH), 8.75 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.85 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.36 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 10.60 (s, 1H, NH). MS: m/z (%) 260 [M^+] (100), 262 (24), 264 (32), 141 (4). 140 (4).

*3.1.2.2. 2-Hydroxybenzylidene pyrazine-2-carboxylic acid hydrazide (**3b**).* Yellow crystals in (0.201 g; 83% yield), m.p. 210–211 °C. IR (KBr) ν_{max} (cm^{-1}): 3415 (OH), 3380 (NH), 1685 (C=O), 1570 (C=N), 1512 (N–H), 1355, 1309, and 1118 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 6.93 (t, 2H, J = 8.50 Hz, Ar–H), 7.33 (t, 1H, J = 5.76 Hz, Ar–H), 7.51 (d, 1H, J = 9.06 Hz, Ar–H), 8.80 (s, 1H, CH), 8.85 (dd, 1H, J = 2.50 Hz, J = 1.50 Hz, H5 of pyrazine), 8.95 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.31 (d, 1H, J = 1.50 Hz, H3 of pyrazine). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 117.71 (CH), 119.75 (CH), 120.58 (CH), 130.81 (CH), 132.72 (CH), 144.26 (CH), 145.07 (CH), 145.16 (C), 148.78 (CH), 151.18 (C), 158.35 (C), 160.17 (CO). MS: m/z (%) 242 [M^+] (100), 141 (4), 122 (5). Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.45; H, 4.19; N, 22.79.

*3.1.2.3. 3-Hydroxybenzylidene pyrazine-2-carboxylic acid hydrazide (**3c**).* White powder in (0.206 g; 85.1% yield), m.p. 264–265 °C. IR (KBr) ν_{max} (cm^{-1}): 3450 (OH), 3380 (NH), 1661 (C=O), 1592 (C=N), 1509 (N–H), 1356, 1310, and 1160 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 5.32 (s, 1H, OH), 6.63 (d, 1H, J = 2.00 Hz, Ar–H), 6.80 (d, 1H, J = 2.00 Hz, Ar–H), 7.05 (s, 1H, Ar–H), 7.13 (d, 1H, J = 3.60 Hz, Ar–H), 8.50 (s, 1H, CH), 8.82 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.92 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.30 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 12.18 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 114.04 (CH), 118.94 (CH), 120.17 (CH), 130.99 (CH), 136.39 (C), 144.19 (CH), 144.99 (CH), 145.53 (CH), 148.65 (CH), 150.74 (C), 158.42 (C), 160.16 (CO). MS: m/z (%) 242 [M^+] (100), 141 (6), 122 (5). Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.34; H, 4.34; N, 23.48.

*3.1.2.4. 4-Hydroxybenzylidene pyrazine-2-carboxylic acid hydrazide (**3d**).* Yellowish white crystals in (0.206 g; 85.1% yield), m.

p. > 300 °C. IR (KBr) ν_{max} (cm^{-1}): 3455 (OH), 3365 (NH), 1663 (C=O) 1593 (C=N), 1518 (N–H), 1352, 1295, and 1143 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 6.85 (dd, 2H, J = 6.00 Hz, Ar–H), 7.58 (dd, 2H, J = 6.00 Hz, Ar–H), 8.55 (s, 1H, Ar–H), 8.81 (dd, 1H, J = 2.40 Hz, J = 1.40 Hz, H5 of pyrazine), 8.92 (d, 1H, J = 2.40 Hz, H6 of pyrazine), 9.27 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 9.95 (s, 1H, OH), 12.10 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 116.99 (CH), 126.21 (C), 130.17 (CH), 144.13 (CH), 144.89 (CH), 145.67 (CH), 148.49 (CH), 150.94 (C), 159.85 (C), 160.37 (CO). MS: m/z (%) 242 [M^+] (100), 141 (6), 122 (7). Anal. Calcd. for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.16; N, 23.13. Found: C, 59.49; H, 4.17; N, 22.85.

*3.1.2.5. 3-Aminobenzylidene pyrazine-2-carboxylic acid hydrazide (**3e**).* Yellow crystals in (0.198 g; 82.1% yield), m.p. 253–254 °C. IR (KBr) ν_{max} (cm^{-1}): 3435 (NH_2), 3260 (NH), 1679 (C=O), 1609 (C=N), 1511 (N–H), 1366, 1315, and 1140 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 5.30 (s, 2H, NH_2), 6.65 (d, 1H, J = 8.01 Hz, Ar–H), 6.81 (d, 1H, J = 7.50 Hz, Ar–H), 7.01 (s, 1H, Ar–H), 7.07–7.12 (m, 1H, Ar–H), 8.50 (s, 1H, CH), 8.82 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.93 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.30 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 12.16 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 112.72 (CH), 117.06 (CH), 117.49 (CH), 130.32 (CH), 135.61 (CH), 144.16 (CH), 144.96 (CH), 145.59 (C), 148.59 (CH), 149.89 (C), 151.51 (C), 160.04 (CO). MS: m/z (%) 241 [M^+] (100), 141 (12), 121 (13). Anal. Calcd. for $C_{12}H_{11}N_5O$: C, 59.74; H, 4.60; N, 29.03. Found: C, 59.50; H, 4.57; N, 28.75.

*3.1.2.6. 4-Methoxybenzylidene pyrazine-2-carboxylic acid hydrazide (**3f**).* Yellow crystals in (0.21 g; 82% yield), m.p. 225–226 °C. IR (KBr) ν_{max} (cm^{-1}): 3450 (NH), 1667 (C=O), 1596 (C=N), 1497 (N–H), 1392, 1290, and 1140 (pyrazine). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.85 (s, 3H, O$\underline{CH_3}$), 7.04 (dd, 2H, J = 8.76 Hz, Ar–H), 7.68 (dd, 2H, J = 8.76 Hz, Ar–H), 8.58 (s, 1H, CH), 8.80 (dd, 1H, J = 2.50 Hz, J = 1.50 Hz, H5 of pyrazine), 8.95 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.28 (d, 1H, J = 1.50 Hz, H3 of pyrazine), 12.20 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 53.69 (O$\underline{CH_3}$) 111.90 (CH), 124.28 (C), 126.25 (CH), 140.42 (CH), 141.57 (CH), 142.10 (CH), 144.89 (CH), 147.10 (C), 156.45 (C), 158.28 (CO). MS: m/z (%) 256 [M^+] (100), 141 (5), 136 (7). Anal. Calcd. for $C_{13}H_{12}N_4O_2$: C, 60.93; H, 4.72; N, 21.86. Found: C, 60.92; H, 4.71; N, 21.33.

*3.1.2.7. 4-Hydroxy-3-methoxybenzylidene pyrazine-2-carboxylic acid hydrazide (**3g**) [17].* Yellow crystals in (0.228 g, 83.8% yield), m. p. 249–250 °C. IR (KBr) ν_{max} (cm^{-1}): 3460 (OH), 3310 (NH), 1671 (C=O), 1595 (C=N), 1499 (N–H), 1385, 1309, and 1144 (pyrazine). ^{1}H NMR (60 MHz, DMSO-d_6) δ (ppm): 4.20 (s, 3H, O$\underline{CH_3}$), 7.30–7.75 (m, 2H, Ar–H), 8.00 (s, 1H, Ar–H), 9.30 (s, 1H, CH), 9.70 (d, 2H, J = 8.06 Hz, CH of pyrazine), 10.20 (s, 1H, CH of pyrazine), 11.20 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 57.54 (O$\underline{CH_3}$), 110.41 (CH), 116.71 (CH), 123.62 (CH), 126.62 (C), 144.16 (CH), 144.89 (CH), 145.71 (CH), 148.51 (CH), 148.90 (C), 150.11 (C), 151.14 (C), 159.86 (CO). MS: m/z (%) 272 [M^+] (100), 152 (5), 141 (4).

*3.1.2.8. 1-(Phenylethylidene) pyrazine-2-carboxylic acid hydrazide (**3h**).* White crystals in (0.195 g, 81.2% yield), m.p. 212–213 °C. IR (KBr) ν_{max} (cm^{-1}): 3226 (NH), 3045-3004 (Ar–CH), 2960–2830 (Aliph–CH), 1599 (C=N), 1567 (Ar C=C), 1375, 1312, and 1140 (pyrazine). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.40 (s, 3H, CH_3), 7.00–7.00 (m, 5H, Ar–H), 7.88–7.94 (m, 2H, CH of pyrazine), 9.50 (d, 1H, J = 1.4 Hz, CH of pyrazine), 10.60 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.10 (CH_3), 124.88 (CH), 126.52 (CH), 128.00 (CH), 135.57 (C), 140.68 (CH), 142.00 (C), 142.42 (CH), 145.80 (CH), 152.23 (C), 156.37 (CO). MS: m/z (%) 241 (100) [M^+], 242 (15), 141 (12). 121 (13) Anal. Calcd. for $C_{13}H_{12}N_4O$: C, 64.99; H, 5.03; N, 23.32. Found: C, 64.86; H, 5.10; N, 23.30.

*3.1.2.9. [1-(4-Methoxyphenyl)ethylidene]pyrazine-2-carboxylic acid hydrazide (**3i**).* White powder in (0.222 g, 82.2% yield), m.p. 179–180 °C. IR (KBr) ν_{max} (cm^{-1}): 3425 (NH), 1666 (C=O), 1633 (C=C), 1520 (N–H), 1388, 1305, and 1147 (pyrazine). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.40 (s, 3H, $\underline{CH_3}$), 3.85 (s, 3H, O$\underline{CH_3}$), 6.93 (dd, 2H, J = 8.45 Hz, Ar–H), 7.88 (dd, 2H, J = 8.45 Hz, Ar–H), 8.55 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.82 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.50 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 10.65 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.19 (CH_3), 57.02 (O$\underline{CH_3}$) 114.71 (CH), 129.09 (CH), 130.77 (C), 131.28 (C), 142.78 (CH), 143.29 (CH), 144.60 (CH), 148.35 (C), 158.89 (C), 161.48 (CO). MS: m/z (%) 270 [M^+] (100), 150 (8), 141 (4). Anal. Calcd. for $C_{14}H_{14}N_4O_2 \cdot H_2O$: C, 58.32; H, 5.59; N, 19.43. Found: C, 58.27; H, 5.54; N, 19.37.

*3.1.2.10. [1-(4-Bromophenyl)ethylidene]pyrazine-2-carboxylic acid hydrazide (**3j**).* White crystals in (0.255 g, 80% yield), m.p. 198–199 °C. IR (KBr) ν_{max} (cm^{-1}): 3310 (NH), 1689 (C=O), 1597 (C=N), 1506 (N–H), 1387, 1298, and 1142 (pyrazine). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.42 (s, 3H, CH_3), 7.58 (dd, 2H, J = 8.45 Hz, Ar–H), 7.80 (dd, 2H, J = 8.45 Hz, Ar–H), 8.55 (dd, 1H, J = 2.50 Hz, J = 1.50 Hz, H5 of pyrazine), 8.80 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.52 (d, 1H, J = 1.50 Hz, H3 of pyrazine), 10.70 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 13.18 (CH_3), 126.58 (CH), 129.41 (CH), 138.34 (C), 141.15 (CH), 141.92 (CH), 145.81 (CH), 145.90 (C), 153.03 (C), 156.57 (C), 159.46 (CO). MS: m/z (%) 319 [M^+] (100), 321 (8), 323 (6), 198 (5), 141 (4). Anal. Calcd. for $C_{13}H_{11}BrN_4O$: C, 48.92; H, 3.47; N, 17.55. Found: C, 48.55; H, 3.81; N, 17.56.

*3.1.2.11. (2-Chloro-6-methylquinolin-3-ylmethylene)pyrazine-2-carboxylic acid hydrazide (**3k**).* Yellowish white crystals in (0.27 g, 83% yield), m.p. 230–231 °C. IR (KBr) ν_{max} (cm^{-1}): 3445 (NH), 1666 (C=O), 1593 (C=N), 1514 (N–H), 1382, 1262, and 1143 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.50 (s, 3H, CH_3), 7.73 (d, 1H, J = 1.92 Hz, Ar–H), 7.89 (d, 1H, J = 8.49 Hz, Ar–H), 8.00 (s, 1H, Ar–CH), 8.83 (s, 1H, CH), 8.87 (dd, 1H, J = 2.50 Hz, J = 1.40 Hz, H5 of pyrazine), 8.94 (d, 1H, J = 2.50 Hz, H6 of pyrazine), 9.17 (s, 1H, Ar–CH), 9.33 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 12.82 (s. 1H, NH). MS: m/z (%) 325 [M^+] (100), 327 (20), 329 (40), 205 (3), 141 (4). Anal. Calcd. for $C_{16}H_{12}ClN_5O$: C, 58.99; H, 3.71; N, 21.50. Found: C, 58.86; H, 3.70; N, 21.39.

*3.1.2.12. (2-Chloro-6-methoxyquinolin-3-ylmethylene) pyrazine-2-carboxylic acid hydrazide (**3l**).* Yellow crystals in (0.273 g, 80% yield), m.p. 260–261 °C. IR (KBr) ν_{max} (cm^{-1}): 3275 (NH), 1681 (C=O), 1591 (C=N), 1507 (N–H), 1382, 1299, and 1143 (pyrazine). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.92 (s, 3H, O$\underline{CH_3}$), 7.52 (d, 1H, J = 2.73 Hz, Ar–H), 7.70 (s, 1H, Ar–H), 7.89 (d, 1H, J = 7.30 Hz, Ar–H), 8.85 (s, 1H, CH), 8.93 (dd, 1H, J = 2.40 Hz, J = 1.40 Hz, H5 of pyrazine), 8.97 (d, 1H, J = 2.40 Hz, H6 of pyrazine), 9.18 (s, 1H, Ar–CH), 9.35 (d, 1H, J = 1.40 Hz, H3 of pyrazine), 12.80 (s, 1H, NH). MS: m/z (%) 341 [M^+] (100), 343 (19), 345 (41), 221 (3), 141 (4). Anal. Calcd. for $C_{16}H_{12}ClN_5O_2$: C, 56.23; H, 3.54; N, 20.49. Found: C, 56.22; H, 3.58; N, 20.10.

*3.1.3. Synthesis of N-ethyl-N′-(pyrazine-2-carbonyl) thiosemicarbazide (**4**)*

To a stirred solution of **2** (0.138 g, 1.0 mmol) in 25 mL absolute ethanol, ethyl isothiocyanate (1.0 mmol) was added, the mixture was refluxed for 12 h, then cooled to room temperature and the obtained precipitate was recrystallized from ethanol to give **4** as white crystals in (0.185 g; 82.2% yield), m.p. 218–219 °C. IR (KBr) ν_{max} (cm^{-1}): 3450 and 3215 (NH), 1680 (C=O), 1542 (C=S), 1385, 1316, and 1144 (pyrazine). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.05 (t, 3H, J = 7.10 Hz, CH_3), 3.43 (q, 2H, J = 6.87 Hz and 6.03 Hz, CH_2),

8.05 (t, 1H, $J = 5.49$ Hz, NH), 8.75 (dd, 1H, $J = 2.50$ Hz, $J = 1.40$ Hz, H5 of pyrazine), 8.90 (d, 1H, $J = 2.50$ Hz, H6 of pyrazine), 9.20 (d, 1H, $J = 1.40$ Hz, H3 of pyrazine), 9.38 (s, 1H, NH) 10.70 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 14.54 (CH_3), 38.77 (CH_2), 141.68 (CH), 142.50 (CH), 142.78 (C), 146.08 (CH), 160.10 (CO), 179.40 (C=S). MS: m/z (%) 225 [M^+] (100), 141 (4), 105 (10). Anal. Calcd. for $C_8H_{11}N_5OS$: C, 42.65; H, 4.92; N, 31.09. Found: C, 42.82; H, 4.92; N, 31.03.

*3.1.4. Synthesis of the 2-chloro-6-(substituted)quinoline-3-carboxaldehyde (**6a**–**b**) [18]*

A mixture of acetanilide derivative **5a**–**b** (1.0 mmol) and phosphorus oxychloride (7.0 mmol) in dimethyl formamide (2.5 mmol) was heated at 75 °C for 10 h, cooled to room temperature then poured on 250 mL ice-water and the formed precipitate was collected by filteration. The obtained crude product was recrystallized from ethyl acetate to give title compounds **6a**–**b**.

*3.1.4.1. 2-Chloro-6-methoxyquinoline-3-carboxaldehyde **6a**.* Yellowish white crystals in (0.125 g; 56.6% yield), m.p. 146–147 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.94 (s, 3H, OCH_3), 7.18 (d, 1H, $J = 2.8$ Hz, H-5), 7.46 (dd, 1H, $J = 9.0$ Hz and 2.8 Hz, H-7), 7.90 (d, 1H, $J = 9.0$ Hz, H-8), 8.56 (s, 1H, H-4), 10.50 (s, 1H, CHO).

*3.1.4.2. 2-Chloro-6-methylquinoline-3-carboxaldehyde **6b**.* Yellowish white crystals in (0.145 g; 70.7% yield), m.p. 124–125 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.54 (s, 3H, CH_3), 7.53 (dd, 1H, $J = 8.9$ Hz and 2.9 Hz, H-7), 7.64 (d, 1H, $J = 2.9$ Hz, H-5), 7.99 (d, 1H, $J = 8.9$ Hz, H-8), 8.72 (s, 1H, H-4), 10.52 (s, 1H, CHO).

3.2. Biological evaluations

3.2.1. Determination of a 90% inhibitory concentration (IC_{90})

The initial screening is conducted against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) [17]. Compounds are tested in ten 2-fold dilutions, typically from 100 μg/mL to 0.19 μg/mL. The IC_{90} is defined as the concentration effecting a reduction in fluorescence of 90% relative to controls. This value is determined from the dose-response curve using a curve-fitting program.

3.2.2. Determination of mammalian cell cytotoxicity (CC_{50})

The VERO cell cytotoxicity assay is done in parallel with the TB Dose Response assay. After 72 h exposure, viability is assessed using Promega's Cell Titer Glo Luminescent Cell Viability Assay, a homogeneous method of determining the number of viable cells in culture based on quantitation of the ATP present. Cytotoxicity is determined from the dose–response curve as the CC_{50} using a curve-fitting program. Ultimately, the CC_{50} is divided by the IC_{90} to calculate an SI (Selectivity Index) value.

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References

[1] E.C. Rivers, R.L. Mancera, Drug Discov. Today 13 (2008) 1090–1098.
[2] WHO global tuberculosis report.http://www.who.int/tb/publications/global_report/2008/download_centre/en/index.html (2008) at:.
[3] Z. Ma, A.M. Ginsberg, M. Spigelman, in: J.B. Taylor, D.J. Triggle (Eds.), Comprehensive Medicinal Chemistry II, vol. 7, Elsevier, 2006, pp. 699–730.
[4] L. Kremer, G.S. Besra, Expert Opin. Invest. Drugs 11 (2002) 1033–1049.
[5] TB Alliance, Tuberculosis 88 (2008) 141–144.
[6] L. Palek, J. Dvorak, M. Svobodova, V. Buchta, J. Jampilek, M. Dolezal, Arch. Pharm. Chem. Life Sci. 341 (2008) 61–65.
[7] M. Dolezal, P. Cmedlova, L. Palek, J. Vinsova, J. Kunes, V. Buchta, J. Jampilek, K. Kralova, Eur. J. Med. Chem. 43 (2008) 1105–1113.
[8] J. Vinosova, A. Imramovsky, J. Jampılek, J.F. Monreal, M. Dolezal, Anti-Infective Agents Med. Chem. 7 (2008) 12–31.
[9] A. Imramovsky, S. Polanc, J. Vinsova, M. Kocevar, J. Jampılek, Z. Reckova, J. Kaustova, Bioorg. Med. Chem. 15 (2007) 2551–2559.
[10] M.C. Cardia, S. Distinto, E. Maccioni, A. Plumitallo, M. Saddi, M.L. Sanna, A. Delogu, J. Heterocycl. Chem. 43 (2006) 1337–1342.
[11] A. Manvar, A. Malde, J. Verma, V. Virsodia, A. Mishra, K. Upadhyay, H. Acharya, E. Coutinho, A. Shah, Eur. J. Med. Chem. 43 (2008) 2395–2403.
[12] L. Collins, S.G. Franzblau, Antimicrob. Agents Chemother. 41 (1997) 1004–1009.
[13] R. Govindarajan, H.J. Jameela, A.R. Bhat, Indian J. Heterocycl. Chem. 12 (2003) 229–232.
[14] W. Rudnicka, H. Foks, M. Janawiec, Z. Zwolska-Kwick, Acta Pol. Pharm. 43 (1986) 523–528.
[15] K. Gobis, H. Foks, J. Francuz, Z. Zwolska, E. Augustynowicz-Kopec, Phosphorus, Sulfur Silicon Relat. Elem. 181 (2006) 977–986.
[16] H. Zhang, J.-Y. Deng, L.-J. Bi, Y.-F. Zhou, Z.-P. Zhang, C.-G. Zhang, Y. Zhang, X.-E. Zhang, FEBS J. 275 (2008) 753–762.
[17] T.V. Gortinskaya, K.M. Murav'eva, M.N. Shchukina, Zh. Obshch. Khim. 25 (1955) 2313–2317 [Through Chemical Abst. 50:48790].
[18] O. Meth-Cohn, B. Narine, B. Tarnowski, Tetrahedron Lett. 20 (1979) 3111–3114.