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Substituted 5-aroylpyrazine-2-carboxylic acid derivatives: synthesis and biological activity

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Abstract

Homolytic aroylation of pyrazine nucleus with various substituted aromatic carbaldehydes afforded a series of 5-aroylpyrazine-2-carboxylic acid derivatives. The synthetic approach, analytical and spectroscopic data of all compounds synthesized, their preliminary in vitro evaluation of antituberculotic and antifungal activities, cytotoxicity data and subsequent SAR studies are presented. Among all derivatives prepared, only 5-(4-chlorobenzoyl)-pyrazine-2-carbothioamide (**3d**) showed promising activity (90% inhibition) against *Mycobacterium tuberculosis*. The highest antifungal effect (MIC < 1.95 μmol ml^{-1}) against *Trichophyton mentagrophytes*, the most susceptible fungal strain tested, was found for 5-benzoylpyrazine-2-carbothioamide (**3a**). Thioamides exhibited higher in vitro antimicrobial activity than the corresponding amides.
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1. Introduction

One-third of the world's population is infected with tuberculosis (TB), therefore, TB today still represents one of the major problems for public health worldwide. After a long period in which it seemed to be declining, an unexpected return has been recorded for this disease. Each year there are 8.7 million new cases of TB and estimated 1.7 million deaths. If current control efforts are not massively expanded, TB will kill more than 40 million people over the next 25 years. The existing recommended TB treatment is based on 2 months of four drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) followed by 4 months of two drugs (isoniazid and rifampicin). The current recommended strategy is facing two problems: multidrug resistance and HIV/AIDS pandemic [1,2]. Additionally, in patients with impaired cellular immunity (HIV-syndrom), mycobacterial and fungal (*Aspergillus*, *Histoplasma*, etc.) infections predominate and may coexist [3].

There are two basic approaches to get a new drug for TB: (i) synthesis of analogues, modifications or derivatives of existing compounds for shortening and improving TB treatment; and (ii) searching novel structures, that the TB organism 'has never seen before', for the treatment of multidrug-resistant TB [4].

Pyrazinamide (PZA), an analogue of nicotinamide, is a unique first-line TB drug, which is involved in shortening the TB therapy. PZA is a prodrug that requires activation or conversion at acid pH condition to its active form, pyrazinoic acid (POA), by the pyrazinamidase/nicotinamidase enzyme encoded by *pncA* gene of

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susceptible *Mycobacterium tuberculosis*. The target of PZA/POA appears to be the membrane, i.e. fatty acid synthase-I [5]. One of the effective methods that can lead to new drug discoveries is the bioisosteric replacement of a functional group.

This paper is the continuation of our interest in pyrazine-2-carboxylic acid derivatives, previous studies [6–11] showed that alkylation, amidation or substitution of the pyrazine ring with chlorine increases antituberculotic activity in series of functional derivatives pyrazine-2-carboxylic acid. Pyrazine nucleus readily undergoes radical aroylation reactions using the appropriate benzaldehyde with *tert*-butyl hydroperoxide/Fe^{2+} as the initiator [12]. Aim of this work is: (i) the radical aroylation of pyrazine nucleus study; (ii) in vitro screening of antituberculotic, antifungal activity, and cytotoxic properties of newly prepared aroylpyrazines; (iii) the comparison of their lipophilicity parameters; and finally (iv) the SAR study of functional modifications in 5-aroylpyrazine-2-carboxylic acid series, i.e. carboxamides, carbonitriles, carbothioamides, and methyl esters of pyrazine-2-carboxylic acid.

2. Experimental

2.1. Instrumentation

TLC was performed on Silufol UV 254 plates (Kavalier, Votice) using a mixture of acetone–toluene (1:1) or acetone–EtOAc (1:1). Silica gel plates were visualized in addition to the solution of 2,4-dinitrophenylhydrazine in NaOH (detection of carbonyl group). Melting points were determined on a Kofler block, and are uncorrected. Elemental analyses were obtained using an EA 1110 CHNS-O CE apparatus (Fisons Instruments S.p.A., Milan). The IR spectra were recorded on a Nicolet Impact 400 spectrometer in KBr pellets. The ^{1}H and ^{13}C NMR spectra were measured for DMSO (or $CDCl_3$) solutions with a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz (^{1}H NMR spectra) and 75 MHz (^{13}C NMR spectra). Chemical shifts were recorded as d values in parts per million (ppm), and were indirectly referenced to tetramethylsilane via the solvent signal (7.26 for ^{1}H and 77.0 for ^{13}C). Multiplicities are given together with the coupling constants (in Hz). Log *P* values were computed using a program ACD/log *P* ver. 1.0 (Advanced Chemistry Development Inc., Toronto).

2.2. 5-Aroylpyrazine-2-carboxamides (**1a**–**1d**)

2.2.1. General procedure

A stirred mixture of the carbaldehyde (120 mmol), and pyrazine-2-carboxamide (40 mmol) in 50% H_2SO_4 (80 ml) and 99% AcOH (80 ml) was cooled to −5 °C. To this mixture 80% *tert*-BuO_2H (13.5 g, 120 mmol) and a solution of $FeSO_4 \cdot 7H_2O$ (33.4 g, 120 mmol) in water were added simultaneously. The temperature must not exceed 10 °C. The resulting mixture was stirred for additional 2 h, during which the temperature was allowed to rise to 20 °C. The reaction product (yellow crystals) was filtered and crystallized from mixture EtOH/H_2O.

2.2.2. 5-Benzoylpyrazine-2-carboxamide (**1a**)

Yield 40%, m.p. 205–206 °C (205 °C [13]). *Anal.* Calc. for $C_{12}H_9N_3O_2$ (226.2): 68.43% C, 3.99%; H, 18.49% N; found: 68.29% C, 4.15% H, 18.41% N, log $P = 1.85 \pm 0.43$. IR spectrum: 3432 (NH amide), 1672 (CO amide), 1598 (CO aroyl), 1580 (Ph), 1413, 1274, 1178 (pyrazine). ^{1}H NMR spectrum: δ 9.25d 1H $J = 1.32$ (H3), 9.16–9.13m 1H (H6), 8.01–7.94m 2H (H2′, H6′), 7.76–7.67m 1H (H-4′), 7.61–7.52m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 192.4, 164.8, 151.5, 146.6, 144.1, 142.4, 135.4, 134.2, 131.0, 128.9.

2.2.3. 5-(2-Hydroxybenzoyl)-pyrazine-2-carboxamide (**1b**)

Yield 24%, m.p. 245–246 °C. *Anal.* Calc. for $C_{12}H_9N_3O_3$ (243.2): 59.26% C, 3.73% H, 17.28% N; found: 59.28% C, 3.70% H, 17.11% N, log $P = 2.14 \pm 0.47$. IR spectrum: 3427 (NH amide), 3233 (OH), 1673 (CO amide), 1625 (CO aroyl), 1601 (Ph), 1437, 1247, 1180 (pyrazine). ^{1}H NMR spectrum: δ 10.44s 1H (OH), 9.19d 1H $J = 1.50$ (H3), 9.03d 1H $J = 1.49$ (H6), 8.42bs 1H (NH_2), 7.99bs 1H (NH_2), 7.65–7.58m 1H (H6′), 7.53–7.44m 1H (H4′), 6.99–6.89m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 195.0, 164.8, 158.8, 152.7, 146.4, 142.6, 135.5, 131.7, 123.5, 119.6, 117.2.

2.2.4. 5-(4-Hydroxybenzoyl)-pyrazine-2-carboxamide (**1c**)

Yield 11%, m.p. 235–236 °C. *Anal.* Calc. for $C_{12}H_9N_3O_3$ (243.2): 55.17% C, 4.24% H, 16.09% N; found: 55.48% C, 4.34% H, 16.10% N, log $P = 1.54 \pm 0.45$. IR spectrum: 3420 (NH amide), 3231 (OH), 1701 (CO amide), 1634 (CO aroyl), 1600 (Ph), 1448, 1281, 1171 (pyrazine). ^{1}H NMR spectrum: δ 10.55s 1H (OH), 9.23d 1H $J = 1.35$ (H-3), 9.06d 1H $J = 1.35$ (H6), 8.43bs 1H (NH_2), 7.99bs 1H (NH_2), 7.96–7.88m 2H (H2′, H6′), 6.94–6.87m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 190.2, 164.9, 163.2, 152.6, 146.2, 143.8, 142.2, 134.0, 126.7, 115.6.

2.2.5. 5-(4-Chlorobenzoyl)-pyrazine-2-carboxamide (**1d**)

Yield 16%, m.p. 225–226 °C. *Anal.* Calc. for $C_{12}H_8ClN_3O_2$ (261.7): 55.08% C, 3.08% H, 16.06% N; found: 55.46% C, 3.12% H, 15.99% N, log $P = 2.57 \pm 0.45$. IR spectrum: 3425 (NH amide), 1702 (CO amide), 1674 (CO aroyl), 1589 (Ph), 1477, 1400, 1274, 1177

(pyrazine). ^{1}H NMR spectrum: δ 10.73bs 2H (NH_2), 9.33d 1H $J = 1.51$ (H3), 9.20d 1H $J = 1.51$ (H6), 7.94–7.86m 2H (H2′, H6′), 6.94–6.86m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 189.3, 163.6, 152.6, 147.3, 145.7, 133.9, 130.9, 126.1, 116.1, 115.7.

2.3. 5-Aroylpyrazine-2-carbonitriles (**2a**–**2d**)

2.3.1. General procedure

A mixture of compound **1** (10 mmol) and $POCl_3$ (110 mmol) is stirred at 110 °C for 1 h. Then the mixture cooled at 10 °C and poured on ice. Temperature must not exceed 50 °C. The aqueous solution was extracted with $CHCl_3$. The solvent was dried over $MgSO_4$ and evaporated in vacuo. The reaction product (red crystals) was filtered and crystallized from mixture EtOH/H_2O.

2.3.2. 5-Benzoylpyrazine-2-carbonitrile (**2a**)

Yield 91%, m.p. 93–94 °C. *Anal.* Calc. for $C_{12}H_7N_3O$ (208.2): 68.89% C, 3.37% H, 20.09% N; found: 68.63% C, 3.28% H, 19.65% N, log $P = 1.86 \pm 0.43$. IR spectrum: 2220 (CN), 1670 (CO aroyl), 1597 (Ph), 1447, 1275, 1156 (pyrazine). ^{1}H NMR spectrum ($CDCl_3$): δ 9.36–9.34m 1H (H3), 9.30–9.28m 1H (H6), 8.01–7.95m 2H (H2′, H6′), 7.78–7.69m 1H (H4′), 7.62–7.54m 2H (H3′, H5′). ^{13}C NMR spectrum ($CDCl_3$): δ 191.5, 151.4, 147.4, 145.9, 134.8, 134.4, 131.3, 130.9, 128.8, 116.1.

2.3.3. 5-(2-Hydroxybenzoyl)-pyrazine-2-carbonitrile (**2b**)

Yield 92%, m.p. 132–133 °C. *Anal.* Calc. for $C_{12}H_7N_3O_2$ (225.2): 64.00% C, 3.13% H, 18.66% N; found: 64.33% C, 3.09% H, 18.27% N, log $P = 2.15 \pm 0.47$. IR spectrum: 3210 (OH), 2210 (CN), 1626 (CO aroyl), 1605 (Ph), 1447, 1248, 1149 (pyrazine). ^{1}H NMR spectrum: δ 10.49s 1H (OH), 9.30d 1H $J = 1.37$ (H3), 9.18d 1H $J = 1.37$ (H6), 7.62dd 1H $J = 7.69$ $J = 1.65$ (H6′), 7.56–7.47m 1H (H4′), 7.01–6.91m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 193.7, 158.7, 152.8, 147.7, 144.5, 135.7, 131.4, 130.9, 123.0, 119.5, 117.2, 116.1.

2.3.4. 5-(4-Hydroxybenzoyl)-pyrazine-2-carbonitrile (**2c**)

Yield 90%, m.p. 163–164 °C. *Anal.* Calc. for $C_{12}H_7N_3O_2$ (225.2): 64.00% C, 3.13% H, 18.66% N; found: 64.36% C, 2.95% H, 18.37% N, log $P = 1.54 \pm 0.44$. IR spectrum: 3397 (OH), 2244 (CN), 1640 (CO aroyl), 1586 (Ph), 1437, 1280, 1149 (pyrazine). ^{1}H NMR spectrum: δ 10.53s 1H (OH), 9.33d 1H $J = 1.51$ (H3), 9.20d 1H $J = 1.51$ (H6), 7.94–7.86m 2H (H2′, H6′), 6.94–6.86m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 189.3, 163.6, 152.6, 147.3, 145.7, 133.9, 130.9, 126.1, 116.1, 115.7.

2.3.5. 5-(4-Chlorobenzoyl)-pyrazine-2-carbonitrile (**2d**)

Yield 16%, m.p. 225–226 °C. *Anal.* Calc. for $C_{12}H_8ClN_3O_2$ (261.7): 55.08% C, 3.08% H, 16.06% N; found: 55.46% C, 3.12% H, 15.99% N, log $P = 2.58 \pm 0.44$. IR spectrum: 2225 (CN), 1674 (CO aroyl), 1589 (Ph), 1477, 1400, 1274, 1177 (pyrazine). ^{1}H NMR spectrum ($CDCl_3$): δ 9.53–9.50m 1H (H3), 9.22–9.21m 1H (H-6), 8.04–7.97m 2H (H2′, H6′), 7.68–7.59m 2H (H3′, H5′). ^{13}C NMR spectrum ($CDCl_3$):δ 191.9, 155.5, 147.2, 143.6, 139.2, 135.1, 129.1, 128.0, 117.2.

2.4. 5-Aroylpyrazine-2-carbothioamides (**3a**–**3d**)

2.4.1. General procedure

A compound **2** (4 mmol) was dissolved in MeOH (50 ml) and $(NH_4)_2S$ (4 ml, 20% wt.% solution in water) was added. A reaction mixture was kept at 5 °C for 24 h. Then the mixture was filtered, MeOH evaporated and the product (yellow crystals) crystallized from EtOH.

2.4.2. 5-Benzoylpyrazine-2-carbothioamide (**3a**)

Yield 81%, m.p. 119–120 °C. *Anal.* Calc. for $C_{12}H_9N_3OS$ (242.2): 59.24% C, 3.73% H, 17.27% N, 13.18% S; found: 59.56% C, 3.77% H, 17.22% N, 12.93% S, log $P = 2.04 \pm 0.49$. IR spectrum: 3443 (NH amide), 1660 (CO aroyl), 1598 (Ph), 1447, 1273, 1173 (pyrazine). ^{1}H NMR spectrum: δ 10.50bs 1H (NH_2), 10.19bs 1H (NH_2), 9.60d 1H $J = 1.51$ (H3), 9.09d 1H $J = 1.51$ (H-6), 8.01–7.95m 2H (H2′, H6′), 7.74–7.67m 1H (H-4′), 7.60–7.52m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 192.4, 192.3, 150.6, 147.9, 144.1, 143.1, 135.5, 134.2, 131.0, 128.8.

2.4.3. 5-(2-Hydroxybenzoyl)-pyrazine-2-carbothioamide (**3b**)

Yield 85%, m.p. 171–173 °C. *Anal.* Calc. for $C_{12}H_9N_3O_2S$ (259.2): 55.59% C, 3.50% H, 16.21% N, 12.36% S; found: 56.85% C, 3.62% H, 15.9% N, 12.23% S, log $P = 2.33 \pm 0.52$. IR spectrum: 3440 (NH amide), 1636 (CO aroyl), 1601 (Ph), 1437, 1247, 1180 (pyrazine). ^{1}H NMR spectrum: δ 10.50bs 1H (NH_2), 10.39bs 1H (OH), 10.19bs 1H (NH_2), 9.60d 1H $J = 1.51$ (H3), 9.09d 1H $J = 1.51$ (H6), 8.01–7.95m 1H (H-6′), 7.74–7.67m 1H (H4′), 7.60–7.52m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 195.0, 192.5, 158.7, 151.7, 147.9, 144.3, 141.6, 135.5, 131.7, 123.5, 119.6, 117.2.

2.4.4. 5-(4-Hydroxybenzoyl)-pyrazine-2-carbothioamide (**3c**)

Yield 88%, m.p. 199–200 °C. *Anal.* Calc. for $C_{12}H_9N_3O_2S$ (259.2): 51.98% C, 4.00% H, 15.15% N, 11.56% S; found: 52.04% C, 3.97% H, 15.19% N, 11.44% S, log $P = 1.73 \pm 0.50$. IR spectrum: 3432 (NH amide), 1647 (CO aroyl), 1601 (Ph), 1438, 1278, 1148 (pyrazine). ^{1}H NMR spectrum: δ 10.50bs 1H (OH), 9.93d 1H $J =$

1.47 (H3), 9.17d 1H $J = 1.47$ (H6), 8.53bs 1H (NH_2), 8.06–7.97m 2H, (H2′, H6′), 7.21–7.18m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 193.1, 191.9, 164.1, 162.6, 151.7, 144.8, 142.5, 129.2, 116.3.

2.4.5. 5-(4-Chlorobenzoyl)-pyrazine-2-carbothioamide (**3d**)

Yield 83%, m.p. 219–220 °C. *Anal.* Calc. for $C_{12}H_8ClN_3OS$ (277.7): 51.90% C, 2.90% H, 15.13% N, 11.55% S; found: 51.82% C, 2.86% H, 15.03% N, 11.39% S, log $P = 2.76 \pm 0.50$. IR spectrum: 3439 (NH amide), 1647 (CO aroyl), 1601 (Ph), 1438, 1278, 1148 (pyrazine). ^1H NMR spectrum ($CDCl_3$): δ 9.91d 1H $J = 1.51$ (H3), 9.22bs 1H (NH_2), 9.15d 1H $J = 1.51$ (H6), 8.17–8.09m 2H (H2′, H6′), 7.75bs 1H (NH_2), 7.55–7.47m 2H (H3′, H5′). ^{13}C NMR spectrum ($CDCl_3$): δ 192.3, 190.1, 150.5, 145.0, 144.5, 142.9, 140.6, 133.5, 132.5, 128.8.

2.5. 5-Aroylpyrazine-2-carboxylic acids (**4a**–**4b**)

2.5.1. General procedure

A compound **1** (40 mmol) was refluxed in 10% solution of NaOH for 10 h, then the solution was filtered with charcoal and the solution was neutralized by 10% HCl. White crystalline product was obtained.

2.5.2. 5-Benzoylpyrazine-2-carboxylic acid (**4a**)

Yield 94%, m.p. 148–150 °C. *Anal.* Calc. for $C_{12}H_8N_2O_3$ (228.2): 63.16% C, 3.53% H, 12.28% N; found: 63.26% C, 3.75% H, 12.07% N, log $P = 2.45 \pm 0.43$. IR spectrum: 1692 (CO acid), 1645 (CO aroyl), 1589 (Ph), 1437, 1280, 1149 (pyrazine), (in literature 1692 and 1658 [14]). ^1H NMR spectrum: δ 9.27s 1H (H3), 9.24s 1H (H6), 8.03–7.95m 2H H-2′ (H6′), 7.77–7.68m 1H (H4′), 7.63–7.52m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 192.2, 164.8, 151.4, 145.4, 145.0, 144.1, 135.3, 134.1, 130.9, 128.7.

2.5.3. 5-(2-Hydroxybenzoyl)-pyrazine-2-carboxylic acid (**4b**)

Yield 91%, m.p. 141–142 °C. *Anal.* Calc. for $C_{12}H_8N_2O_4$ (244.2): 59.02% C, 3.30% H, 11.47% N; found: 59.12% C, 3.39% H, 11.32% N, log $P = 2.47 \pm 0.46$. IR spectrum: 3395 (OH), 1700 (CO acid), 1620 (CO aroyl), 1589 (Ph), 1447, 1280, 1149 (pyrazine). ^1H NMR spectrum: δ 10.46bs 1H (OH), 9.22d 1H $J = 1.38$ (H3), 9.12d 1H $J = 1.37$ (H6), 7.62dd 1H $J = 7.69$ $J = 1.38$ (H6′), 7.54–7.46m 1H (H4′), 7.00–6.91m 2H (H3′, H5′). ^{13}C NMR spectrum: δ 194.8, 164.9, 158.7, 152.6, 145.2, 144.4, 143.5, 135.4, 131.4, 123.4, 119.4, 117.2.

2.6. Methyl-5-aroylpyrazine-2-carboxylates (**5a**–**5b**)

2.6.1. General procedure

An anhydrous compound **4** (20 mmol) was dissolved in anhydrous MeOH (100 ml). To the solution 98% H_2SO_4 (0.5 ml) was added and the mixture was refluxed for 2 h under the Soxhlet-extractor, where dry Potasite (70 g) was located. Then the mixture was cooled, neutralized with $KHCO_3$ and filtered. MeOH was evaporated and the product (white crystals) was crystallized from EtOH.

2.6.2. Methyl-5-benzoylpyrazine-2-carboxylate (**5a**)

Yield 60%, m.p. 101–103 °C. *Anal.* Calc. for $C_{13}H_{10}N_2O_3$ (242.2): 64.46% C, 4.16% H, 11.56% N; found: 64.77% C, 4.04% H, 11.46% N, log $P = 1.90 \pm 0.41$. IR spectrum: 1700 (CO acid), 1670 (CO aroyl), 1600 (Ph), 1437, 1280, 1149 (pyrazine). ^1H NMR spectrum ($CDCl_3$): δ 9.22s 1H (H3), 9.10s 1H (H6), 7.58–7.47m 2H (H2′, H6′), 7.39–7.22m 3H (H3′, H4′, H5′), 4.01s 3H (CH_3). ^{13}C NMR spectrum ($CDCl_3$): δ 192.0, 164.2, 159.0, 145.4, 145.3, 144.2, 134.0, 130.9, 128.5, 126.9, 53.1.

2.6.3. Methyl-5-(2-hydroxybenzoyl)-pyrazine-2-carboxylate (**5b**)

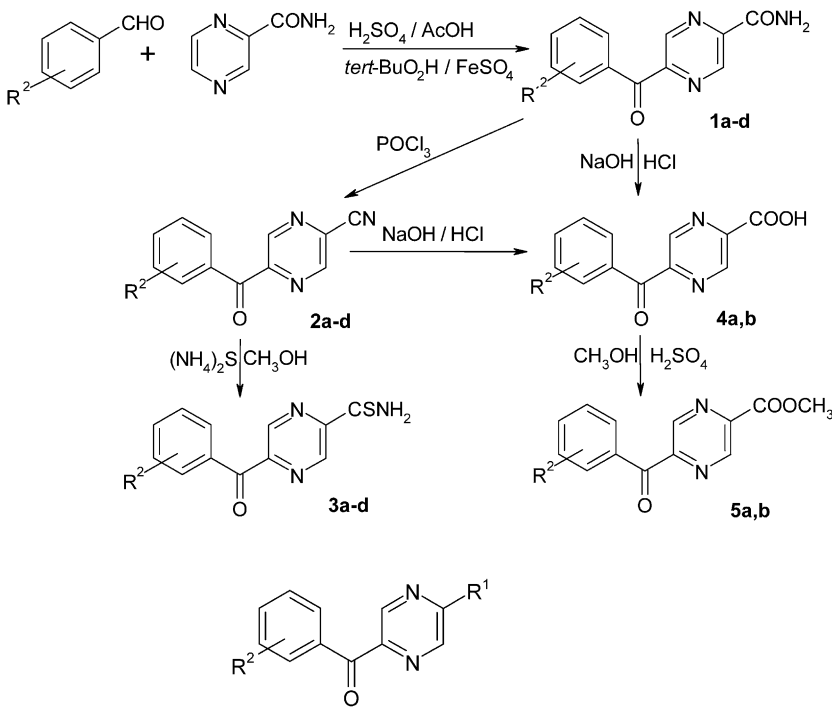
Yield 83%, m.p. 126–127 °C. *Anal.* Calc. for $C_{13}H_{10}N_2O_4$ (258.2): 60.47% C, 3.90% H, 10.85% N; found: 60.25% C, 3.60% H, 10.75% N, log $P = 2.19 \pm 0.44$. IR spectrum: 3398 (OH), 1730 (CO acid), 1640 (CO aroyl), 1595 (Ph), 1437, 1280, 1149 (pyrazine). ^1H NMR spectrum ($CDCl_3$): δ 11.85s 1H (OH), 9.41–7.38m 1H (H3), 9.24–9.22m 1H (H6), 8.04dd 1H $J =$ 8.24, $J = 1.38$ (H6′), 7.62–7.53m 1H (H4′), 7.11–7.07m 1H (H3′), 6.97–6.89m 1H (H5′), 4.10s 3H (CH_3). ^{13}C NMR spectrum ($CDCl_3$): δ 194.9 164.2, 163.7, 152.4, 145.1, 144.1, 144.0, 137.8, 133.7, 119.2, 118.7, 118.2, 53.5.

2.7. Antimycobacterial assay

Antimycobacterial evaluation was carried out in Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 μg ml^{-1} against *M. tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using the BACTEC 460 radiometric system [15]. Compounds showing at least 90% inhibition at 6.25 μg ml^{-1} in this primary screening were retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the minimum inhibitory concentration (MIC) in a broth microdilution Alamar Blue assay (MABA). The MIC was defined as the lowest concentration causing a decrease in fluorescence of 99% relative to controls.

2.8. In vitro antifungal susceptibility testing

The broth microdilution test [16,17] was used for the assessment of in vitro antifungal activity of the synthe-



Compound	R^1	R^2	Compound	R^1	R^2
1a	$CONH_2$	H	**3a**	$CSNH_2$	H
1b	$CONH_2$	2-OH	**3b**	$CSNH_2$	2-OH
1c	$CONH_2$	4-OH	**3c**	$CSNH_2$	4-OH
1d	$CONH_2$	4-Cl	**3d**	$CSNH_2$	4-Cl
2a	CN	H	**4a**	COOH	H
2b	CN	2-OH	**4b**	COOH	2-OH
2c	CN	4-OH	**5a**	$COOCH_3$	H
2d	CN	4-Cl	**5b**	$COOCH_3$	2-OH

Scheme 1.

sized compounds and ketoconazole (standard) against *Candida albicans* ATCC 44859, *Candida tropicalis* 156, *Candida krusei* E28, *Candida glabrata* 20/I, *Trichosporon beigelii* 1188, *Trichophyton mentagrophytes* 445, *Aspergillus fumigatus* 231, and *Absidia corymbifera* 272. The procedure was performed with twofold dilutions of the compounds in RPMI 1640 buffered to pH 7.0 with 0.165 mol of 3-morpholino-propane-1-sulfonic acid. The final concentrations of the compounds ranged from 1000 to 0.975 μmol l^{-1}. Drug-free controls were included. The MICs were determined after 24 h and 48 h of static incubation at 35 °C. With *T. mentagrophytes*, the final MICs were read after 72 and 120 h of incubation.

2.9. Cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test was first described by Mosmann [18] and improved in subsequent years by several other investigators [19]. Cytotoxicity measurements were based on the growth inhibition of *Caco-2 cells* (human intestinal epithelial cells). An assay was performed after 24 h exposure to 50 μl ml^{-1} of 5% DMSO solutions of the tested agents. After incubation, 20 μl of MTT solution (5 mg ml^{-1}) were added to each well. The mitochondria of viable cells reduce the pale yellow MTT to a violet formazan: the more viable cells are present in well, the more MTT will be reduced to formazan. When

incubation for 90 min was stopped, 100 μl DMSO were added to each well. When formazan crystals had been dissolved, the optical densities at 550 nm wavelength of the samples were read on a Bio-rad Microplate reader (Bio-rad Laboratories, Austria). The results of in vitro cytotoxic activity were expressed as an EC_{50} – the dose of compound (in mmol l^{-1}) that inhibits proliferation rate of *Caco-2 cells* by 50% as to the control untreated cells.

3. Results and discussion

Pyrazine-2-carboxamide was used as the starting material for radical aroylation using substituted benzaldehydes. Radical aroylation is a homolytic chain oxidation in liquid phase catalysis by means of salts of transition metals (redox homogeneous catalysis). The principle of this method is described [20] and according to various different modification of radical aroylation, some substituted pyrazin-2-carboxylic acid derivatives were prepared [13,14,21].

Prepared 5-aroylpyrazine-2-carboxamides **1a**–**d** (method according to Heinisch) [14] were transformed to appropriate carbonitriles **2a**–**d** and carbothioamides **3a**–**d**. Corresponding carboxylic acids **4a**, **4b** and methyl esters **5a**, **5b** were prepared from **1a**, **1b** (Scheme 1). Our study describes the synthesis and properties of 16 compounds; two of them were characterized previously by Sato [13] (**1a**) and Heinisch [14] (**4a**), 14 derivatives of them were prepared newly. Primary in vitro antimycobacterial screening was provided for all 16 compounds, in vitro antifungal screening was performed for 11 compounds, cytotoxic assay was performed for eight derivatives.

In vitro antimycobacterial activity tended to be proportional to molecular weight and lipophilicity of these compounds. Therefore, in carbothioamides and methyl esters studied some considerable in vitro biological activity was determined in the functional derivatives carboxylic acid series. Both the highest activity (90% inhibition) against *M. tuberculosis* and the highest lipophilicity (log $P = 2.76$) of all compounds studied was found for 5-(4-chlorobenzoyl)-pyrazine-2-carbothioamide (**3d**). This compound was retested (MABA) to determine the actual minimal inhibitory concentration (MIC > 12.5 μg ml^{-1}). Only other two compounds (**3b**, **5b**) exhibited more than 50% inhibition, both of them with hydroxylic group in *ortho* position of benzene moiety of the structure (Table 1).

The evaluation of in vitro antifungal activity of the synthesized compounds was performed against eight fungal strains. The results showed no activity against majority of fungal strains tested. Only compound **1d** and especially carbothioamides **3a**–**c** exhibited a promising in vitro antifungal activity against *T. mentagrophytes* (MIC = 1.95–62.5 μmol ml^{-1}). Other carbothioamide, 5-(4-chlorobenzoyl)-pyrazine-2-carbothioamide (**3d**) precipitated in the RPMI medium. 5-Benzoylpyrazine-2-carbothioamide (**3a**) had comparable activity against

Table 1
Antituberculotic activity, antifungal activity, cytotoxicity, selectivity index (SI), and lipophilicity (calculated log P) of compounds **1a**–**4d** in comparison with standards

Comp.	*Mycobacterium tuberculosis* $H_{37}Rv$ % inhibition at 6.25 (μg ml^{-1})	*Trichophyton mentagrophytes* 445 MIC (μmol ml^{-1}) [d]	*Caco-2 cells* EC_{50} (mmol l^{-1})	SI	log P
1a	8	250/ > 250	0.67	2.7	1.85 ± 0.43
1b	2	250/ > 250	12.50	50.0	2.14 ± 0.47
1c	0	> 500/ > 500	[b]		1.54 ± 0.45
1d	16	> 62.5/ > 62.5	[b]		2.57 ± 0.45
2a	10	500/500	[a]		1.86 ± 0.43
2b	5	125/250	14.95	119.6	2.15 ± 0.47
2c	3	250/500	7.22	28.9	1.54 ± 0.44
2d	12	250/500	[a]		2.58 ± 0.44
3a	39	< 1.95/ < 1.95	10.05	5153.9	2.04 ± 0.49
3b	60	3.91/7.81	7.48	1913.0	2.33 ± 0.52
3c	21	3.91/7.81	[a]		1.73 ± 0.50
3d	90	[b]	[a]		2.76 ± 0.50
4a	15	> 500/ > 500	18.96	37.9	2.45 ± 0.43
4b	4	> 500/ > 500	4.72	9.4	2.47 ± 0.46
5a	48	> 500/ > 500	[a]		1.90 ± 0.41
5b	53	> 250/ > 250	[a]		2.19 ± 0.44
Pyrazinamide	[c]				0.37 ± 0.35
Ketoconazole		0.98/1.95			4.01 ± 0.66

[a] Not tested.
[b] Precipitated in the testing medium.
[c] MIC = 12.5 μg ml^{-1}, data from Refs. [6–8].
[d] After 72/120 h.

T. mentagrophytes with ketoconazole (MIC = 1.95 μmol ml^{-1} after 120 h, Table 1). The negative results of antifungal screening do not allow us to draw conclusions on some structure–activity relationships.

The values of calculated lipophilicity (log *P*) of compounds ranged from 1.72 to 2.33 (Table 1).

MTT assay is a colorimetric assay system which measures the reduction of a tetrazolium component (MTT) into an insoluble formazan product by the mitochondria of viable cells. After incubation of the cells with the MTT reagent for approximately 2–4 h, a detergent solution is added to lyse the cells and solubilize the coloured crystals. The samples are read using a microplate reader at a wavelength of 550 nm. The intensity of colour produced is directly proportional to the number of viable cells. Therefore, the measurement of optical densities by MTT assay of some tested compounds was problematic due to interference of coloured samples (yellow or red) with formazan absorbance maximum at 550 nm. Lower solubility, resp. precipitation in the testing medium of compounds studied was other difficult trouble. The selectivity index (SI) is defined as the ratio of the measured IC_{50} in *Caco-2 cells* to the MIC described above (Table 1, note column labelled SI). 5-Benzoylpyrazine-2-carboxamide (**1a**) possessed the highest cytotoxicity ($IC_{50} = 0.67$ mmol l^{-1}, SI = 2.7). The in vitro antifungal most effective compound 5-benzoylpyrazine-2-carbothioamide (**3a**) exerted $EC_{50} = 10.05$ mmol l^{-1}, SI > 5000. There is no general result about the structure–cytotoxicity relationships in this limited series of compounds measured.

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