

## ORIGINAL ARTICLE

# Aminopyrazinoic acid esters as potential antimycobacterial drugs

## Estery aminopyrazinkarboxylové kyseliny jako potenciální antimykobakteriální léčiva

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### Summary

A series of esters of the 3-aminopyrazine-2-carboxylic acid as potential antimycobacterial drugs was synthesized. A CEM Discover microwave reactor with an autosampler Explorer 24 which served to accelerate the reaction was used for synthesis. The prepared products were characterized by IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectra, elementary analysis and melting points. Log P and ClogP values were calculated. Final products were evaluated *in vitro* for their antimycobacterial activity. The most active compound was hexyl 3-aminopyrazine-2-carboxylate (7), whose antimycobacterial activity (MIC) against *M. tuberculosis* H37Rv was 6.25 µg/mL.

**Keywords:** prodrugs • pyrazinoic acid esters • lipophilicity calculation • *in vitro* antimycobacterial activity

### Souhrn

Byla připravena série sedmi esterů 3-aminopyrazin-2-karboxylové kyseliny jako potenciální antimykobakteriální léčiva. Syntéza probíhala s využitím mikrovlnného reaktoru CEM Discover s autosamplerem Explorer 24, který sloužil k urychlení reakce. Připravené produkty byly charakterizovány pomocí IC, <sup>1</sup>H NMR, <sup>13</sup>C NMR spekter, elementární analýzy a teplotou tání. Byly vypočteny

hodnoty log P a ClogP. U nasynthetizovaných látek byla hodnocena *in vitro* antimykobakteriální aktivita. Nejúčinnější sloučeninou byl hexyl-3-aminopyrazin-2-karboxylát (7), jehož antimykobakteriální aktivita (MIC) proti *M. tuberculosis* H37Rv byla 6.25 µg/mL.

**Klíčová slova:** prodrugs • estery pyrazinkarboxylové kyseliny • výpočet lipofility • *in vitro* antimykobakteriální aktivita.

### Introduction

Tuberculosis (TB), the world's leading infectious disease, is caused by *Mycobacterium tuberculosis*, which is one of the most successful human pathogens. Treatment of TB requires an extra-long duration time using multiple drugs, divided into the first-line (isoniazid, rifampicin, pyrazinamide, ethambutol) and second line drugs. There is also an alarming emergence of multidrug resistant *M. tuberculosis*. As a result a need has arisen to develop novel anti-tubercular agents<sup>1</sup>. Pyrazinamide (PZA) and pyrazinoic acid (POA) as potential analeptic drugs have been synthesised in Germany<sup>2</sup> and later in the USA as an intermediate compound on the pathway of aminopyrazine synthesis<sup>3</sup>, but the antimycobacterial activity of PZA was reported later, in 1952<sup>4–8</sup>. It was soon shown that a mycobacterial enzyme called nicotinamidase hydrolyzed nicotinamide and pyrazinamide to the corresponding carboxylic acid, which is the actual active compound (see Fig. 1)<sup>9</sup>. PZA plays a unique role in modern TB chemotherapy<sup>10</sup>. Inclusion of PZA enables considerable shortening of the treatment period from the previously 9–12 months to 6 months, thus the drug plays a pivotal role in the current short-course chemotherapy for drug susceptible strains of TB. The powerful sterilizing activity of PZA is due to its ability to kill a population of persistent tubercle bacilli that are not killed by other TB drugs<sup>11</sup>. Furthermore, the synergistic activity of PZA with newly developed agents such as the diarylquinoline bedaquiline suggests that the use of PZA in regimens including novel agents could substantially improve efficacy, if the organism retains susceptibility to PZA<sup>12, 13</sup>.

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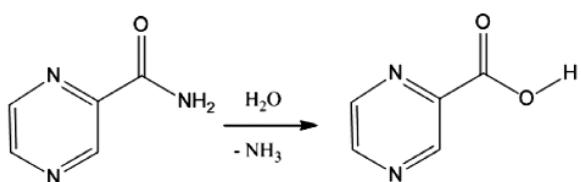


Fig. 1. Pyrazinamide is a prodrug of pyrazinoic acid, hydrolysed by pyrazinamidase<sup>14)</sup>

Substituted pyrazinecarboxylic acid esters have previously shown a very good *in vitro* activity against *M. avium* and *M. kansasii* as well as against *M. tuberculosis*<sup>14)</sup>. Pyrazinoic acid esters exhibit antimycobacterial activity probably due to better penetration through the mycobacterial cell wall, where they could be metabolized by mycobacterial esterases to the corresponding acid. While POA cannot pass through the mycobacterial cell walls due to its low lipophilicity, the concept of the POA prodrug is a suitable approach to increase the likelihood of its penetration into the resistant mycobacteria<sup>15, 16)</sup>. Cynamon and colleagues<sup>15, 17)</sup> synthesized a series of un- or substituted POA (pyrazinecarboxylic, 5-fluoropyrazine-2-carboxylic, 5-methylpyrazine-2-carboxylic, and 5-chloropyrazine-2-carboxylic acid) esters, exhibiting a good *in vitro* antimycobacterial activity against several mycobacterial strains, including the PZA-resistant strain of *M. tuberculosis*<sup>16–26)</sup>. A classical quantitative structure-activity relationships model for the selected compounds was proposed<sup>18, 19)</sup>. Some 5-hydroxypyrazine-2-carboxylic acid derivatives are up to 1000-fold more active *in vitro* against *M. tuberculosis* and other *Mycobacterium* strains than existing antituberculous agents<sup>18)</sup>; therefore substituted analogues of the POA prodrug with isosteric replacements of the hydroxyl moiety with the amino group were projected and prepared. The aim of our project was to develop some potential antimycobacterial prodrugs which can deliver the active agent to the site of the therapeutic action, to increase the drug bio-availability and selectivity of the therapeutic action and to present our results in the finding of new antimycobacterial active compounds based on esters **1–7** of 3-aminopyrazine-2-carboxylic acid (Fig. 2)<sup>27)</sup>, *i.e.* prodrugs which can be easily activated by mycobacterial esterases.

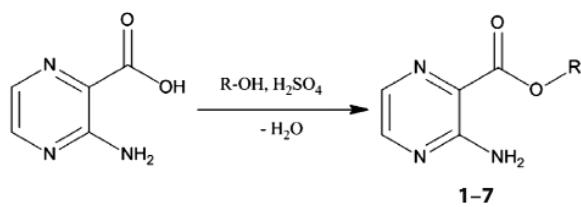


Fig. 2. General synthesis and structure of 3-aminopyrazine-2-carboxylates **1–7**

Two series of aliphatic alcohols were chosen for the synthesis of final structures **1–7**, namely four unbranched alcohols in the range from ethanol to *n*-hexanol and also three branched aliphatic alcohols – isopropanol, isobutanol and isopentanol.

## Experimental part

### Materials and methods

All organic solvents used for the synthesis were of analytical grade. All chemicals were purchased from Sigma-Aldrich (Schnelldorf, Germany). Compounds were synthesized using a microwave reactor CEM Discover with an autosampler Explorer 24 (CEM Corporation, Matthews, NC, USA). The reactions were monitored using Merck Silica 60 F<sub>254</sub> TLC plates (Merck, Darmstadt, Germany). Compounds were purified using an automated chromatograph CombiFlash Rf (Teledyne Isco, Lincoln, NE, USA) using columns filled with Kieselgel 60, 0.040–0.063 mm (Merck, Darmstadt, Germany); gradient elution (hexane/ethyl-acetate), detection wavelength 260 nm, monitor wavelength 280 nm. NMR analysis was performed on a spectrometer Varian Mercury-Vx BB 500 (Varian, Palo Alto, CA, USA) at 500 MHz for <sup>1</sup>H and 125 MHz for <sup>13</sup>C. Chemical shifts were recorded as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS). IR spectra were recorded in KBr blocks on a Nicolet Impact 400 (Nicolet, Madison, WI, USA). Elementary analysis was performed on a CE Instruments EA-1110 CHN analyser (CE Instruments, Wigan, UK). Melting points were determined on a Stuart SMP30 melting point apparatus (Bibby Scientific Limited, Staffordshire, UK) and are uncorrected.

### Synthesis of 3-aminopyrazine-2-carboxylates

Final compounds were prepared *via* esterification of 3-aminopyrazine-2-carboxylic acid (see Fig. 2). Special thick-walled tubes intended for use in a microwave reactor were filled with a mixture of 3-aminopyrazine-2-carboxylic acid (3 mmol), appropriate alcohol (4 mL, used in excess and as a solvent) and 95–97.5% sulphuric acid (1.9 mmol). Tubes fitted with a stirrer and closed with a special cap were inserted into the reactor. Reaction conditions for synthesis of individual compounds are listed in Table 1.

The reaction was monitored using a TLC with hexane/ethyl acetate 1 : 2 mixture as the eluent. Then the solution was evaporated till dryness with sea sand and purified using a flash column chromatography (40 g column, gradient elution hexane/ethyl acetate). The summary of prepared derivatives and their physico-chemical data are listed in Tables 1 and 2.

### Data of prepared compounds **1–7**

**Ethyl 3-aminopyrazine-2-carboxylate (1).** White crystalline compound. M.p. 199.9–202.4 °C (ref. <sup>28)</sup> gives m.p. 189–191 °C); <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.24 (d, 1H, *J*=2.2 Hz, H5), 7.88 (d, 1H, *J*=2.2 Hz, H6), 7.37 (bs, 2H, NH<sub>2</sub>), 4.21 (q, 2H, *J*=7.1 Hz, OCH<sub>2</sub>), 1.09 (t, 3H, *J*=7.1 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.6, 123.5, 61.1, 14.3; IR [cm<sup>−1</sup>] 3452 (−NH<sub>2</sub>), 2987, 1693 (C=O), 1604, 1304, 1190, 1106 (C–O), 815; Anal. Calcd. 50.29 % C, 5.43 % H, 25.14 % N; Found: 50.41 % C, 5.57 % H, 25.23 % N.

**Isopropyl 3-aminopyrazine-2-carboxylate (2).** White crystalline compound. M.p. 77.5–79.1 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.24 (d, 1H, *J*=2.0 Hz, H5), 7.89 (d, 1H, *J*=2.0 Hz, H6), 7.32 (bs, 2H, NH<sub>2</sub>), 5.19–5.09 (m, 1H,

Table 1. Reaction conditions for synthesis of prepared compounds **1–7**

| No.      | R  | Temp. (max) (°C) | Time (min) | Pressure(max) (bar) | Energy (max) (W) | Stirring | Yield (%) |
|----------|--|------------------|------------|---------------------|------------------|----------|-----------|
| <b>1</b> | -C <sub>2</sub> H <sub>5</sub>                                     | 130              | 15         | 15                  | 100              | YES      | 4.0       |
| <b>2</b> | -CH(CH <sub>3</sub> ) <sub>2</sub>                                 | 120              | 20         | 15                  | 100              | YES      | 4.0       |
| <b>3</b> | -C <sub>4</sub> H <sub>9</sub>                                     | 120              | 15         | 15                  | 100              | YES      | 7.1       |
| <b>4</b> | -CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>                 | 120              | 22         | 15                  | 100              | YES      | 7.9       |
| <b>5</b> | -C <sub>5</sub> H <sub>11</sub>                                    | 120              | 22         | 15                  | 100              | YES      | 9.5       |
| <b>6</b> | -(CH <sub>2</sub> ) <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub> | 120              | 30         | 15                  | 100              | YES      | 3.3       |
| <b>7</b> | -C <sub>6</sub> H <sub>13</sub>                                    | 120              | 27         | 15                  | 100              | YES      | 8.9       |

Table 2. Physico-chemical data of 3-aminopyrazine-2-carboxylic acid derivatives **1–7**, antimycobacterial activity against *M. tuberculosis* is expressed as the minimal inhibition concentration (MIC) in µg/mL and µmol/L

| No.      | M. w. (g/mol) | Log P | ClogP  | MIC     |          | CAS RNs, ref.             |
|----------|---------------|-------|--------|---------|----------|---------------------------|
|          |               |       |        | (µg/mL) | (µmol/L) |                           |
| <b>1</b> | 167.17        | -0.15 | 0.5874 | 50      | 299      | 36526-32-6 <sup>28)</sup> |
| <b>2</b> | 181.19        | 0.17  | 0.8964 | 100     | 552      | 1342973-72-1              |
| <b>3</b> | 195.22        | 0.76  | 1.6454 | 100     | 512      | 1259480-15-3              |
| <b>4</b> | 195.22        | 0.74  | 1.5154 | 100     | 512      | 1342715-59-6              |
| <b>5</b> | 209.25        | 1.18  | 2.1744 | 50      | 239      | 1340531-99-8              |
| <b>6</b> | 209.25        | 1.09  | 2.0444 | 100     | 478      | 1339690-33-3              |
| <b>7</b> | 223.27        | 1.59  | 2.7034 | 6.25    | 28       | 1341067-41-1              |

OCH<sub>2</sub>), 1.30 (d, 6H, *J*=6.4 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 165.8, 156.1, 147.8, 132.6, 123.8, 68.7, 21.8; IR [cm<sup>-1</sup>] 3456 (-NH<sub>2</sub>), 2984, 2349, 1688 (C=O), 1604, 1302, 1187, 1091 (C-O), 816; Anal. Calcd. 53.03 % C, 6.12 % H, 23.19 % N; Found: 53.20 % C, 6.08 % H, 23.22 % N.

*Butyl 3-aminopyrazine-2-carboxylate (3).* White crystalline compound. M.p. 67.8–70.2 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.25 (d, 1H, *J*=2.2 Hz, H<sub>5</sub>), 7.90 (d, 1H, *J*=2.2 Hz, H<sub>6</sub>), 7.32 (bs, 2H, NH<sub>2</sub>), 4.26 (t, 2H, *J*=6.7 Hz, OCH<sub>2</sub>), 1.72–1.62 (m, 2H, CH<sub>2</sub>), 1.43–1.33 (m, 2H, CH<sub>2</sub>), 0.91 (t, 3H, *J*=7.3 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.7, 123.5, 64.7, 30.3, 18.9, 13.8; IR [cm<sup>-1</sup>] 3439 (-NH<sub>2</sub>), 2963, 2349, 1694 (C=O), 1603, 1309, 1192, 1104 (C-O), 816; Anal. Calcd. 55.37 % C, 6.71 % H, 21.52 % N; Found: 55.47 % C, 6.80 % H, 21.48 % N.

*Isobutyl 3-aminopyrazine-2-carboxylate (4).* White crystalline compound. M.p. 84.9–87.5 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.18 (d, 1H, *J*=2.0 Hz, H<sub>5</sub>), 8.03 (d, 1H, *J*=2.0 Hz, H<sub>6</sub>), 7.26 (bs, 2H, NH<sub>2</sub>), 4.19 (d, 2H, *J*=6.9 Hz, OCH<sub>2</sub>), 2.06–1.95 (m, 1H, CH), 0.94 (d, 6H, *J*=6.6 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.7, 123.5, 70.8, 27.5, 19.1; IR [cm<sup>-1</sup>] 3450 (-NH<sub>2</sub>), 2966, 1693 (C=O), 1609, 1310, 1194, 1095 (C-O), 814; Anal. Calcd. 55.37 % C, 6.71 % H, 21.52 % N; Found: 55.49 % C, 6.83 % H, 21.39 % N.

*Pentyl 3-aminopyrazine-2-carboxylate (5).* White crystalline compound. M.p. 62.7–65.0 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.24 (d, 1H, *J*=2.2 Hz, H<sub>5</sub>), 7.89 (d, 1H, *J*=2.2 Hz, H<sub>6</sub>), 7.32 (bs, 2H, NH<sub>2</sub>), 4.25 (t, 2H, *J*=6.7 Hz, OCH<sub>2</sub>), 1.73–1.64 (m, 2H, CH<sub>2</sub>), 1.38–1.26 (m, 4H, CH<sub>2</sub>), 0.87 (t, 3H, *J*=7.1 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.7, 123.5, 65.0, 28.0, 27.8, 21.9, 14.0; IR [cm<sup>-1</sup>] 3453 (-NH<sub>2</sub>), 2960, 2348, 1692 (C=O),

1606, 1300, 1192, 1102 (C-O), 816; Anal. Calcd. 57.40 % C, 7.23 % H, 20.08 % N; Found: 57.52 % C, 7.26 % H, 20.01 % N.

*Isopentyl 3-aminopyrazine-2-carboxylate (6).* White crystalline compound. M.p. 79.2–84.6 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.24 (d, 1H, *J*=1.2 Hz, H<sub>5</sub>), 7.90 (d, 1H, *J*=1.2 Hz, H<sub>6</sub>), 7.32 (bs, 2H, NH<sub>2</sub>), 4.29 (t, 2H, *J*=6.7 Hz, OCH<sub>2</sub>), 1.73–1.60 (m, 1H, CH), 1.58 (q, 2H, *J*=6.8 Hz, CH<sub>2</sub>), 0.91 (d, 3H, *J*=6.6 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.7, 123.5, 63.5, 36.9, 24.7, 22.5; IR [cm<sup>-1</sup>] 3453 (-NH<sub>2</sub>), 2961, 2347, 1690 (C=O), 1605, 1300, 1187, 1098 (C-O), 816; Anal. Calcd. 57.40 % C, 7.23 % H, 20.08 % N; Found: 57.43 % C, 7.14 % H, 20.07 % N.

*Hexyl 3-aminopyrazine-2-carboxylate (7).* White crystalline compound. M.p. 77.6–79.8 °C; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.24 (d, 1H, *J*=2.2 Hz, H<sub>5</sub>), 7.90 (d, 1H, *J*=2.2 Hz, H<sub>6</sub>), 7.32 (s, 2H, NH<sub>2</sub>), 4.25 (t, 2H, *J*=6.6 Hz, OCH<sub>2</sub>), 1.72–1.63 (m, 2H, CH<sub>2</sub>), 1.40–1.19 (m, 6H, CH<sub>2</sub>), 0.85 (t, 3H, *J*=7.1 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 166.3, 156.1, 147.9, 132.7, 123.5, 65.1, 31.1, 28.3, 25.3, 22.2, 14.1; IR [cm<sup>-1</sup>] 3445 (-NH<sub>2</sub>), 2954, 2349, 1692 (C=O), 1600, 1310, 1189, 1105 (C-O), 817; Anal. Calcd. 59.17 % C, 7.67 % H, 18.82 % N; Found: 59.24 % C, 7.79 % H, 18.86 % N.

#### Lipophilicity calculation

Log *P* (the logarithm of the partition coefficient for *n*-octanol/water) and ClogP (the logarithm of *n*-octanol/water partition coefficient *P* based on established chemical interactions) were calculated using the program CS ChemBioDraw Ultra ver. 12.0 (CambridgeSoft, Cambridge, MA, USA). The results are shown in Table 2.

Table 3. *In vitro* antimycobacterial activity of studied compounds expressed as MIC in  $\mu\text{g}/\text{mL}$ 

| Mycobacterial strains                     | INH         | PZA         | 1    | 2    | 3    | 4    | 5  | 6    | 7           |
|---|-------------|-------------|------|------|------|------|----|------|-------------|
| <i>M. kansasii</i> 235/80                 | 50          | >100        | 50   | >100 | >100 | >100 | 50 | >100 | <b>6.25</b> |
| <i>M. avium</i> 80/70                     | 6.25        | >100        | >100 | >100 | >100 | >100 | 50 | >100 | <b>12.5</b> |
| <i>M. avium</i> 152                       | 6.25        | >100        | >100 | >100 | >100 | >100 | 50 | >100 | <b>12.5</b> |
| <i>M. tuberculosis</i> H <sub>37</sub> Rv | <b>1.56</b> | <b>12.5</b> | 50   | 100  | 100  | 100  | 50 | 100  | <b>6.25</b> |

### *In vitro* antimycobacterial activity

Antimycobacterial evaluation using the microdilution panel method was shielded by the Department of Clinical Microbiology, University Hospital and Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic. Four mycobacterial strains were used: *M. tuberculosis* H37Rv CNCTC My 331/88, *M. avium* CNCTC My 80/72, *M. avium* CNCTC My 152/73 and *M. kansasii* CNCTC My 235/80 (Czech National Collection of Type Cultures, National Institute of Public Health, Prague, Czech Republic). Tested compounds were dissolved in DMSO (to final concentrations 100, 50, 25, 12.5, 6.25, 3.125 and 1.563  $\mu\text{g}/\text{mL}$ ), diluted with Šula's semisynthetic medium (Trios, Prague, Czech Republic) and placed into the microdilution panel. Tested species were added in the form of a suspension in an isotonic saline solution. The final concentration of DMSO did not exceed 1% (v/v), this concentration of DMSO did not affect the growth of mycobacteria. The cultures were grown in Šula's semisynthetic medium at pH 5.7 and 37 °C. The antimycobacterial activity was determined visually after 14 days (6 days for *M. kansasii*) of incubation as the minimal inhibition concentration (MIC,  $\mu\text{g}/\text{mL}$ ), *i.e.*, the lowest concentration of the tested substance which inhibited the growth of mycobacteria.

### Results and discussion

We decided to test whether the isosteric replacements of the hydroxylic moiety with the amino group approach could be used to improve the activity of POA ester. Our results are in good correlation with similar series published in 2009<sup>29</sup>. However, there is some limitation to the use of such esters as drugs; efficacy studies in mice failed to show any antitubercular activity likely due to poor stability of the esters in plasma<sup>10</sup>. But another series of more lipophilic ester prodrugs (*i.e.* tetradecyl ester) were found to be active in concentrations 10-fold lower than those needed for PZA to kill sensitive *M. tuberculosis* and also have suitable stability in the presence of plasma<sup>29</sup>. 3-Aminopyrazine-2-carboxylates 1–7 have been synthesized using a microwave reactor and evaluated regarding to their antimycobacterial activity; six of them (2–7) are new compounds, ethyl 3-aminopyrazine-2-carboxylate (1) has been prepared by Vontor<sup>28</sup> via alkoxy carbonylation of aminopyrazine. The esterification was an easy and quick process, but title esters were obtained by microwave assisted reaction in low yields (3.3–9.5%). We suppose that the main reasons are low stability in solution and also decarboxylation of 3-aminopyrazine-2-carboxylic acid. 2-Aminopyrazine, the main side product, was detected by <sup>1</sup>H and <sup>13</sup>C NMR spectra. We

also planned to prepare and evaluate propyl ester of the starting compound, but the yields were too low and we were not successful to isolate it from the reaction mixture.

### Lipophilicity

Lipophilicity, one of the most important physicochemical properties of the compound, which seems to be a key factor related to the cell transmembrane transport and other biological processes, can either be determined experimentally or predicted by means of the commercially available programmes. Log *P*/ClogP values of compounds 1–7 were calculated using the program ChemBioDraw Ultra (ver. 12.0) and the results are shown in Table 3. The ClogP value is correlated directly to the molecular hydrophobicity, and, thereby, to the diffusion through the biological membranes, *i.e.* into the mycobacterial cell wall. The lowest lipophilicity was shown by ethyl 3-aminopyrazine-2-carboxylate (1), while hexyl 3-aminopyrazine-2-carboxylate (7) was the most lipophilic compound of this series. Based on log *P* values, lipophilicity increased with the extension of the alkyl chain. This can be easily understood because mycolic acids in the cell wall provide high hydrophobicity to the mycobacteria, and then, highly hydrophobic compounds can cross the cell wall more easily<sup>19</sup>.

### *In vitro* antimycobacterial evaluation

3-Aminopyrazine-2-carboxylate esters 1–7 have been evaluated regarding to their antimycobacterial activity against *M. tuberculosis* and several Mycobacteria Other Than Tuberculosis (MOTTs) (see Table 3). Only hexyl 3-aminopyrazine-2-carboxylate (7) possessed activity against *M. tuberculosis* H37Rv (MIC = 6.25  $\mu\text{g}/\text{mL}$ ) which was slightly better than MIC for PZA. More importantly, this compound also showed activity against the MOTTs tested, which are naturally unsusceptible to PZA. Other compounds did not exhibit any interesting activity against the tested strains. The obtained results provide some insight into the SAR in this series. The activity is probably lipophilicity dependent and culminates in the compound with hexyl substitution.

### *In vitro* antibacterial and antifungal evaluation

All prepared compounds were tested for their *in vitro* antibacterial<sup>30, 31</sup> and antifungal<sup>32</sup> activity. None of the synthesized compounds exhibited any activity against the strains tested.

### Conclusion

The present study has shown that long chain esters of 3-aminopyrazine-2-carboxylic acid possess better antimycobacterial properties and that higher lipophilicity

of prepared compounds could also facilitate passage through the mycobacterial cell wall.

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**Conflicts of interest:** none.

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