

ORIGINAL ARTICLE

Aminopyrazinoic acid esters as potential antimycobacterial drugs

Estery aminopyrazinkarboxylové kyseliny jako potenciální antimykobakteriální léčiva

Martin Doležal • Jana Vobicková • Barbora Servusová • Pavla Paterová

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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, ^1H NMR, ^{13}C NMR spectra, elementary analysis and melting points. Log P and Clog P 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 $\mu\text{g/mL}$.

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 autosamplrem Explorer 24, který sloužil k urychlení reakce. Připravené produkty byly charakterizovány pomocí IČ, ^1H NMR, ^{13}C NMR spekter, elementární analýzy a teplotou tání. Byly vypočteny

hodnoty log P a Clog P . U nasyntetizovaných 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 $\mu\text{g/mL}$.

Klíčová slova: proléčiva • estery pyrazinkarboxylové kyseliny • výpočet lipofily • *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 agents¹. Pyrazinamide (PZA) and pyrazinoic acid (POA) as potential analeptic drugs have been synthesised in Germany² and later in the USA as an intermediate compound on the pathway of aminopyrazine synthesis³, but the antimycobacterial activity of PZA was reported later, in 1952⁴⁻⁸. 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)⁹. PZA plays a unique role in modern TB chemotherapy¹⁰. 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 drugs¹¹. 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^{12, 13}.

Prof. PharmDr. Martin Doležal, Ph.D. (✉) • J. Vobicková • B. Servusová
Charles University in Prague, Faculty of Pharmacy, Department of Pharmaceutical Chemistry and Drug Control
Heyrovského 1203, 500 05 Hradec Králové, Czech Republic
e-mail: martin.dolezal@faf.cuni.cz

P. Paterová
Department of Clinical Microbiology, University Hospital, Hradec Králové, Czech Republic

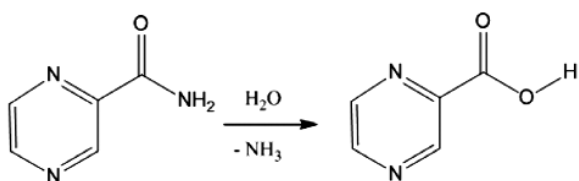


Fig. 1. Pyrazinamide is a prodrug of pyrazinoic acid, hydrolysed by pyrazinamidase¹⁴

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*¹⁴. 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^{15, 16}. Cynamon and colleagues^{15, 17} 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*^{16–20}. A classical quantitative structure-activity relationships model for the selected compounds was proposed^{18, 19}. 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 agents¹⁸; therefore substituted analogues of the POA prodrug with isosteric replacements of the hydroxylic 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)²⁷, i.e. prodrugs which can be easily activated by mycobacterial esterases.

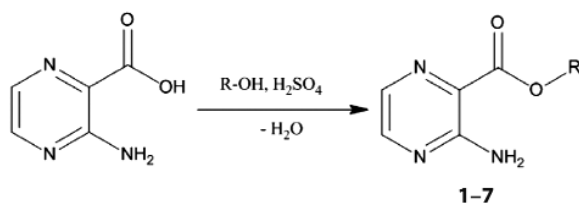


Fig. 2. General synthesis and structure of 3-aminopyrazine-2-carboxylates 1–7

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 (Schneidorf, 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 F₂₅₄ 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 ¹H and 125 MHz for ¹³C. 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. ²⁸) gives m.p. 189–191 °C; ¹H NMR (DMSO-*d*₆) δ 8.24 (d, 1H, *J*=2.2 Hz, H5), 7.88 (d, 1H, *J*=2.2 Hz, H6), 7.37 (bs, 2H, NH₂), 4.21 (q, 2H, *J*=7.1 Hz, OCH₂), 1.09 (t, 3H, *J*=7.1 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 166.3, 156.1, 147.9, 132.6, 123.5, 61.1, 14.3; IR [cm⁻¹] 3452 (–NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.24 (d, 1H, *J*=2.0 Hz, H5), 7.89 (d, 1H, *J*=2.0 Hz, H6), 7.32 (bs, 2H, NH₂), 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 (%)
1	-C ₂ H ₅	130	15	15	100	YES	4.0
2	-CH(CH ₃) ₂	120	20	15	100	YES	4.0
3	-C ₄ H ₉	120	15	15	100	YES	7.1
4	-CH ₂ CH(CH ₃) ₂	120	22	15	100	YES	7.9
5	-C ₅ H ₁₁	120	22	15	100	YES	9.5
6	-(CH ₂) ₂ CH(CH ₃) ₂	120	30	15	100	YES	3.3
7	-C ₆ H ₁₃	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)	
1	167.17	-0.15	0.5874	50	299	36526-32-6 ²⁸⁾
2	181.19	0.17	0.8964	100	552	1342973-72-1
3	195.22	0.76	1.6454	100	512	1259480-15-3
4	195.22	0.74	1.5154	100	512	1342715-59-6
5	209.25	1.18	2.1744	50	239	1340531-99-8
6	209.25	1.09	2.0444	100	478	1339690-33-3
7	223.27	1.59	2.7034	6.25	28	1341067-41-1

OCH₂), 1.30 (d, 6H, *J*=6.4 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 165.8, 156.1, 147.8, 132.6, 123.8, 68.7, 21.8; IR [cm⁻¹] 3456 (-NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.25 (d, 1H, *J*=2.2 Hz, H5), 7.90 (d, 1H, *J*=2.2 Hz, H6), 7.32 (bs, 2H, NH₂), 4.26 (t, 2H, *J*=6.7 Hz, OCH₂), 1.72–1.62 (m, 2H, CH₂), 1.43–1.33 (m, 2H, CH₂), 0.91 (t, 3H, *J*=7.3 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 166.3, 156.1, 147.9, 132.7, 123.5, 64.7, 30.3, 18.9, 13.8; IR [cm⁻¹] 3439 (-NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.18 (d, 1H, *J*=2.0 Hz, H5), 8.03 (d, 1H, *J*=2.0 Hz, H6), 7.26 (bs, 2H, NH₂), 4.19 (t, 2H, *J*=6.9 Hz, OCH₂), 2.06–1.95 (m, 1H, CH), 0.94 (d, 6H, *J*=6.6 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 166.3, 156.1, 147.9, 132.7, 123.5, 70.8, 27.5, 19.1; IR [cm⁻¹] 3450 (-NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.24 (d, 1H, *J*=2.2 Hz, H5), 7.89 (d, 1H, *J*=2.2 Hz, H6), 7.32 (bs, 2H, NH₂), 4.25 (t, 2H, *J*=6.7 Hz, OCH₂), 1.73–1.64 (m, 2H, CH₂), 1.38–1.26 (m, 4H, CH₂), 0.87 (t, 3H, *J*=7.1 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 166.3, 156.1, 147.9, 132.7, 123.5, 65.0, 28.0, 27.8, 21.9, 14.0; IR [cm⁻¹] 3453 (-NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.24 (d, 1H, *J*=1.2 Hz, H5), 7.90 (d, 1H, *J*=1.2 Hz, H6), 7.32 (bs, 2H, NH₂), 4.29 (t, 2H, *J*=6.7 Hz, OCH₂), 1.73–1.60 (m, 1H, CH), 1.58 (q, 2H, *J*=6.8 Hz, CH₂), 0.91 (d, 3H, *J*=6.6 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) δ 166.3, 156.1, 147.9, 132.7, 123.5, 63.5, 36.9, 24.7, 22.5; IR [cm⁻¹] 3453 (-NH₂), 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; ¹H NMR (DMSO-*d*₆) δ 8.24 (d, 1H, *J*=2.2 Hz, H5), 7.90 (d, 1H, *J*=2.2 Hz, H6), 7.32 (s, 2H, NH₂), 4.25 (t, 2H, *J*=6.6 Hz, OCH₂), 1.72–1.63 (m, 2H, CH₂), 1.40–1.19 (m, 6H, CH₂), 0.85 (t, 3H, *J*=7.1 Hz, CH₃); ¹³C NMR (DMSO-*d*₆) 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⁻¹] 3445 (-NH₂), 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 µg/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	6.25
<i>M. avium</i> 80/70	6.25	>100	>100	>100	>100	>100	50	>100	12.5
<i>M. avium</i> 152	6.25	>100	>100	>100	>100	>100	50	>100	12.5
<i>M. tuberculosis</i> H ₃₇ Rv	1.56	12.5	50	100	100	100	50	100	6.25

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 µg/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, µg/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 2009²⁹. 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 plasma¹⁰. 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 plasma²⁹. 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 Vontor²⁸) via alkoxycarbonylation 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 ¹H a ¹³C 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*/Clog*P* values of compounds **1–7** were calculated using the program ChemBioDraw Ultra (ver. 12.0) and the results are shown in Table 3. The Clog*P* 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 easily¹⁹.

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 µg/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^{30, 31} and antifungal³² 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.

References

1. WHO. *Global Tuberculosis Report 2012*. Available online: http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf (accessed on 14th February 2013).
2. Dalmer O., Eugen W. (E. Merck) Pyrazine Derivatives. 1936, DE 632257.
3. Hall S. A., Spoerri P. E. Syntheses in the Pyrazine Series. II. Preparation and Properties of Aminopyrazine. *J Am Chem Soc* 1940; 62, 664–665.
4. Dessau F. I., Burger F. J., Yeager R. L., Kulish M. A Method for the Determination of *in vitro* Sensitivity of Tubercle Bacilli to Pyrazinamide (Aldinamide). *Am Rev Tuberc* 1952; 65, 635–636.
5. Yeager R. L., Munroe W. G. C., Dessau F. I. Pyrazinamide (Aldinamide) in the Treatment of Pulmonary Tuberculosis. *Am Rev Tuberc* 1952; 65, 523–545.
6. Malone L., Schurr A., Lindh H., McKenzie D., Kiser J. S., Williams J. H. The Effect of Pyrazinamide (Aldinamide) on Experimental Tuberculosis in Mice. *Am Rev Tuberc* 1952; 65, 511–518.
7. Solotorovsky M., Gregory F. J., Ironson E. J., Bugie E. J., O'Neill R. C., Pfister K. Pyrazinoic Acid Amide; an Agent Active against Experimental Murine Tuberculosis. *Proc Soc Exp Biol Med* 1952; 79, 563–565.
8. Kushner S., Dalalian H., Sanjurjo J. L., Bach F. L. Jr., Safir S. R., Smith V. K. Jr., Williams J. H. Experimental Chemotherapy of Tuberculosis. 2. The Synthesis of Pyrazinamides and Related Compounds. *J Am Chem Soc* 1952; 74, 3617–3621.
9. Konno K., Feldmann F. M., McDermott, W. Pyrazinamide Susceptibility and Amidase Activity of Tubercle Bacilli. *Am Rev Respir Dis* 1967; 95, 461–469.
10. Zhang Y., Mitchison D. The Curious Characteristics of Pyrazinamide: a Review. *Int J Tuberc Lung Dis* 2003; 7, 6–21.
11. Mitchison D. A. The Action of Antituberculosis Drugs in Short Course Chemotherapy. *Tubercle* 1985; 66, 219–225.
12. Ibrahim M., Andries K., Lounis N., Chauffour A., Truffot-Pernot C., Jarlier V., Veziris N. Synergistic Activity of R207910 Combined with Pyrazinamide against Murine Tuberculosis. *Antimicrob Agents Chemother* 2007; 51, 1011–1015.
13. Diacon A. H., Pym A., Grobusch M., Patientia R., Rustumjee R., Page-Shipp L., Pistorius Ch., Krause R., Bogoshi M., Churchyard G., Venter A., Allen J., Palomino J. C., De Marez T., van Heeswijk R. P. G., Lounis N., Meyvisch P., Verbeeck J., Parys W., de Beule K., Andries K., Mc Neeley D. F. The Diarylquinoline TMC207 for Multidrug-Resistant Tuberculosis. *N Engl J Med* 2009; 360: 2397–2405.
14. Doležal M., Kešetović D., Zitko J. Antimycobacterial Evaluation of Pyrazinecarboxylic Acid Derivatives. *Curr Pharm Design* 2011; 17, 3506–3514.
15. Cynamon M. H., Klemens S. P., Chou T. S., Gimi R. H., Welch J. T. Antimycobacterial Activity of a Series of Pyrazinoic Acid-Esters. *J Med Chem* 1992; 35, 1212–1215.
16. Cynamon M. H., Gimi R., Gyenes F., Sharpe C. A., Bergmann K. E., Han H. J., Gregor L. B., Rapolu R., Luciano G., Welch J. T. Pyrazinecarboxylate Esters with Broad Spectrum *in vitro* Antimycobacterial Activity. *J Med Chem* 1995; 38, 3902–3907.
17. Cynamon M. H., Welch J. T. Preparation of Pyrazinoic Acid Esters as Anti-*Mycobacterium avium* Agents. U.S. Pat. 1997, US 5643912 A 19970701.
18. Bergmann K. E., Cynamon M. H., Welch J. T. Quantitative Structure-Activity Relationships for the *in vitro* Antimycobacterial Activity of Pyrazinoic Acid Esters. *J Med Chem* 1996; 39, 3394–3400.
19. Fernandes J. P. S., Pasqualoto K. F. M., Felli V. M. A., Ferreira E. I., Brandt C. A. QSAR Modeling of a Set of Pyrazinoate Esters as Antituberculosis Prodrugs. *Arch Pharm (Weinheim, Germany)* 2010; 343, 91–97.
20. Yamamoto S., Toida I., Watanabe N., Ura T. *In vitro* Antimycobacterial Activities of Pyrazinamide Analogs. Results of Screening Tests. *Kekkaku* 1996; 71, 253–258.
21. Seitz L. E., Suling W. J., Reynolds R. C. Synthesis and Antimycobacterial Activity of Pyrazine and Quinoxaline Derivatives. *J Med Chem* 2002; 45, 5604–5606.
22. Terasawa T., Shigenaga S., Itoh S., Maeda J., Watanabe H., Kubo S., Ishii N. Preparation of Heterocyclic Carboxamide Compounds, in Particular Nicotinamides as ROCK Inhibitors. PCT Int. Appl. 2010, WO 2010032875 A2 20100325.
23. Chen S., Corbett W. L., Guertin K. R., Haynes N. E., Kester R. F., Mennona F. A., Mischke S. G., Qian Y., Sarabu R., Scott N. R., Thakkar K. C. Preparation of Pyrazines and Related Compounds as Glucokinase Activators for the Treatment of Type II Diabetes. PCT Int. Appl. (2004), WO 2004052869 A1 20040624.
24. Caudill J., Cooney M., Nigam S. C. Preparation of 5-Methylpyrazine-2-carboxylic Acid 4-Oxide Esters and Salts via Oxidation Using Oxone in a Halogenated Solvent. U.S. Pat. Appl. Publ. 2005, US 20050239803 A1 20051027.
25. Sayahi H., Pugliese K. M., Zimhony O., Jacobs W. R. Jr., Shekhtman A., Welch J. T. Analogs of the Antituberculous Agent Pyrazinamide Are Competitive Inhibitors of NADPH Binding to *M. tuberculosis* Fatty Acid Synthase I. *Chem Biodivers* 2012; 9, 2582–2596.
26. Ngo S. C., Zimhony O., Chung W. J., Sayahi H., Jacobs W. R. Jr., Welch J. T. Inhibition of Isolated *Mycobacterium tuberculosis* Fatty Acid Synthase I by Pyrazinamide Analogs. *Antimicrob Agents Chemother* 2007; 51, 2430–2435.
27. Weijlard J., Tishler M., Erickson A. E. New Aminopyrazines and their Sulfanilamide Derivatives. *J Am Chem Soc* 1945; 67, 802–806.
28. Vontor T., Palát K., Lyčka A. Homolytic Carbamoylation and Alkoxy-carbonylation of 2-Aminopyrazine. *Coll Czech Chem Commun* 1989; 54, 1306–1310.
29. Simões M. F., Valente E., Gómez J. R. M., Anes E., Constantino L. Lipophilic Pyrazinoic Acid Amide and Ester Prodrugs Stability, Activation and Activity against *M. tuberculosis*. *Eur J Pharm Sci* 2009; 37, 257–263.
30. Jones R. N., Barry A. L. Optimal Dilution Susceptibility Testing Conditions, Recommendations for MIC Interpretation, and Quality-Control Guidelines for the Ampicillin-Sulbactam Combination. *J Clin Microbiol* 1987; 25, 1920–1925.
31. Zitko J., Doležal M., Svobodová M., Vejsová M., Kuneš J., Kučera R., Jílek P. Synthesis and Antimycobacterial Properties of *N*-Substituted 6-Amino-5-cyanopyrazine-2-carboxamides. *Bioorg Med Chem* 2011; 19, 1471–1476.
32. Servusová B., Eibinová D., Doležal M., Kubíček V., Paterová P., Peško M., Králová K. Substituted *N*-Benzylpyrazine-2-carboxamides: Synthesis and Biological Evaluation. *Molecules* 2012; 17, 13183–13198.