

STUDIES ON PYRAZINE DERIVATIVES. XLVII. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NOVEL PYRAZINE DERIVATIVES WITH AMIDOXIME MOIETY

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Abstract: The new pyrazine derivatives exhibiting an antibacterial activity have been synthesized. Initial amidoxime **1** was obtained in the reaction of pyrazinecarbonitrile with hydroxylamine. Upon treatment of amidoxime with methyl iodide *O*-methyl derivative **2** was formed. Both amidoximes were transformed into imidoyl chlorides **3**, **4**. Then the chloride atom in those derivatives was substituted with various secondary amines giving appropriate oximes **5-18** and *O*-methyl-oximes **19** and **20**. The obtained compounds were tested *in vitro* for their tuberculostatic activity. The inhibiting concentration (MIC) values were within 25-100 µg/mL. Their activity towards 25 strains of anaerobic and 25 strains of aerobic bacteria was also studied. Three compounds exhibited activity against both types of bacteria.

Keywords: Pyrazine derivatives; Amidoximes; Antimicrobial activity; Antituberculosis agents

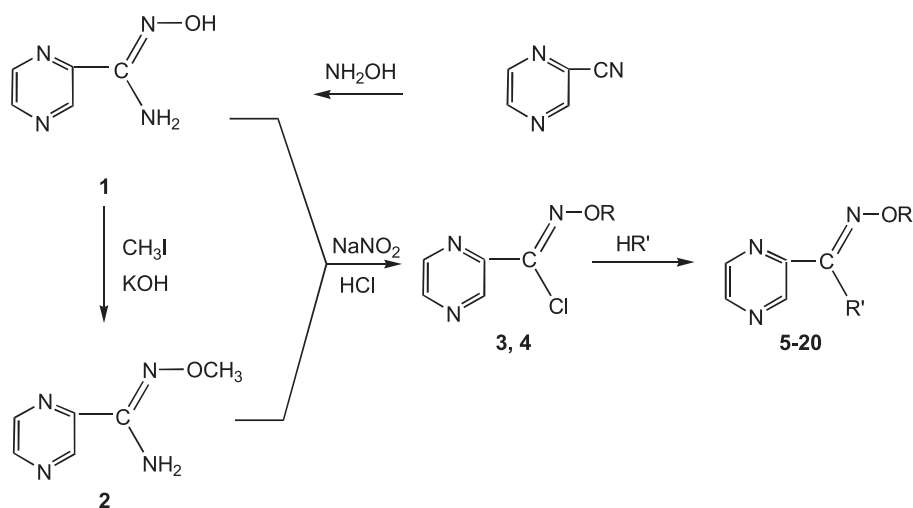
The emergence of drug-resistant pathogenic strains in recent years, e.g. *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Salmonella typhi*, has been of major concern (1-4). Among other infectious diseases, tuberculosis, caused by *Mycobacterium tuberculosis*, seems to be the most invasive, and the multidrug-resistance (MDR) phenomenon makes it the world's number one killer especially for immunosuppressed AIDS patients (5). Because of this, there is a great need for antibacterial and antituberculous drugs with improved properties such as enhanced activity against MDR strains and reduced toxicity.

Pyrazinamide is one of the most effective antituberculous drugs. Various pyrazine derivatives and pyrazinamide analogs also exhibit high antibacterial activity, e.g. pyrazinoic acid esters (6), pyrazine thiocarboxamide and *N*-hydroxymethylpyrazine thiocarboxamide (7) and ring substituted pyrazinyl-chalcones (8). Previously, we have described 4-mono- and 4-disubstituted 1-pyrazinoyl thiosemicarbazides with high tuberculostatic activity (9, 10). Continuing our extensive studies on antibacterial and antituberculous agents active against multidrug-resistant strains, a series of new heterocyclic ami-

doximes bearing pyrazine ring have been synthesized and their antibacterial and antituberculous activities *in vitro* have been assayed.

The pyrazineamidoxime (**1**) was obtained in a reaction of commercially available pyrazinecarbonitrile with hydroxylamine. *O*-Methylation of pyrazinecarboxamidoxime to *O*-methylpyrazinecarboxamidoxime (**2**) was performed with methyl iodide in alkaline solution. *N*-hydroxy (**3**) and *N*-methoxy-pyrazinimidoyl (**4**) chlorides were prepared from corresponding amidoximes on treatment with sodium nitrite in hydrochloric acid solution at 0°C. The chlorides were isolated in moderate yields. That method was published before as a simple procedure for the synthesis of *Z* isomers of pyridinecarboximidoyl chlorides (11). The obtained chlorides were used for the synthesis of appropriate oximes (**5-20**). The reactions of imidoyl chlorides with secondary amines were performed in mole ratio 1:2 in order to neutralize hydrochloride generated during the reactions. Anhydrous methanol or dioxane was used as the solvent. In the case of *O*-methyl-oxime only 1-phenylpiperazine reacted with *O*-methoxy-pyrazineimidoyl chloride (**4**) in good yield found for the reaction with *N*-hydroxypyrazinimidoyl chloride (**3**). 1-Benzylpiperazine did not react with

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Scheme 1.

O-methylpyrazineimidoyl chloride (**4**) under the reaction conditions in any of used solvents (dioxane, methanol, benzene, dimethylformamide). Probable reason was the fact that refluxing of **4** in various solvents from 8 to 48 h caused complete thermal isomerization to *E* isomer which did not react with primary and secondary amines. Because of that the reaction of *O*-methoxypyrazineimidoyl chloride (**4**) with 1-benzylpiperazine was performed in mole ratio 1:20 without solvent at room temperature, with good yield 84%. All the syntheses described above have been shown in Scheme 1, and the characteristics of the compounds obtained are in Table 1. The spectral data for newly synthesized compounds have been presented in Table 2.

EXPERIMENTAL

Chemistry

All materials and solvents were of analytical reagent grade. Thin-layer chromatography was performed on Merck Kieselgel 60F₂₅₄ plates and visualized with UV. Silica gel 60 (70-230 mesh, Fluka) was used for column chromatography. The results of elemental analyses (%C, H, N) for all the compounds obtained were in good agreement with the calculated data. The ¹H NMR spectra in CDCl₃ or DMSO-d₆ were recorded on Varian Unity Plus (500 MHz) and Varian Gemini (200 MHz) instruments. The IR spectra were determined on thin film of the neat liquid (compound **20**) or as KBr pellets of the solids on a Satellite FT-IR spectrophotometer. The mass spectra were taken on Finingan MAT 95 (70 eV). Melting points were determined on BOETIUS apparatus and were uncorrected.

Pyrazinecarboxamidoxime (**1**)

To a stirred solution of hydroxylamine hydrochloride (28 g, 0.4 mol) in methanol (50 mL) with water (70 mL) a solution of potassium hydroxide (24 g, 0.4 mol) in methanol (50 mL) with water (25 mL) was added. The precipitated potassium chloride was filtered and pyrazinecarbonitrile (30 mL, 0.28 mol) in portions with cooling was added to clear filtrate. The reaction mixture was refluxed for 0.5 h and after cooling the final solid was filtered, washed with water and dried at room temperature. The crude product was recrystallized to afford beige leaflets (37.8 g). MS: (*m/z*) = 138 (85.8 M⁺), 108 (100), 106 (20.5), 81 (19.1), 80 (11.2), 79 (18.6), 53 (13.4), 52 (26.2).

O-Methylpyrazinecarboxamidoxime (**2**)

A 2.8 g (20 mmol) quantity of **1** was dissolved in a solution of potassium hydroxide (1.2 g, 21 mmol) in methanol (20 mL) with water (5 mL). Next, methyl iodide (1.56 mL, 25 mmol) was added. The reaction mixture was stirred for 12 h and then methanol was evaporated. The residue was diluted with water (20 mL), cooled, filtered and recrystallized giving 1.33 g of beige needles. MS: (*m/z*) = 152 (83.7 M⁺), 107 (16.2), 106 (67.6), 80 (49.4), 79 (28.9), 53 (19.4), 47 (100.0), 43 (13.8).

N-Hydroxypyrazineimidoyl chloride (**3**)

1 (2.8 g, 20 mmol) was dissolved in a mixture of concentrated hydrochloric acid (20 mL) and water (100 mL) at 0°C. Sodium nitrite (1.4 g, 20 mmol) in 10 mL of water were added dropwise and the reaction mixture was stirred for 1 h. Next, the precipitate was filtered, washed with ice-cool water and recrystallized from methanol giving 1.7 g of the product.

Table 1. Characteristics of the newly synthesized pyrazine derivatives

















Compound No.	R	R'	M.p. [°C] Solvent for crystallization	Yield [%]	Molecular formula MW
1	H	NH ₂	186-187 methanol	96	C ₃ H ₆ N ₄ O 138.13
2	CH ₃	NH ₂	84-85 water	42	C ₆ H ₈ N ₄ O 152.15
3	H	Cl	154-156 methanol	53	C ₃ H ₄ ClN ₃ O 157.56
4	CH ₃	Cl	82-83 cyclohexane/ petroleum ether	53	C ₆ H ₆ ClN ₃ O 171.58
5	H		91-95 cyclohexane	18	C ₁₁ H ₁₆ N ₄ O 220.27
6	H		98-102 cyclohexane	19	C ₉ H ₁₂ N ₄ O ₂ 208.28
7	H		106-108 toluene	54	C ₁₂ H ₁₆ N ₄ O ₃ 264.28
8	H		146-148 toluene	18	C ₁₅ H ₁₆ N ₆ O 296.33
9	H		130-132 dioxane	13	C ₁₄ H ₁₅ N ₃ O 269.30
10	H		139-141 toluene	86	C ₁₅ H ₁₇ N ₃ O 283.33
11	H		142-145 dioxane	54	C ₁₆ H ₁₉ N ₃ O 297.35
12	H		146-149 dioxane	77	C ₁₇ H ₁₇ N ₃ O ₃ 339.35
13	H		183-186 dioxane/ water	93	C ₁₅ H ₁₆ FN ₃ O 301.32
14	H		102-105 toluene	69	C ₁₆ H ₁₉ N ₃ O ₂ 313.35
15	H		197-201 dioxane/ water ₃	98	C ₁₅ H ₁₆ N ₆ O 328.33
16	H		164-166 toluene	44	C ₁₅ H ₁₃ Cl ₂ N ₃ O 352.22
17	H		195-198 methanol	97	C ₂₂ H ₂₃ N ₃ O 373.44
18	H		120-124 dioxane/ water	91	C ₁₈ H ₂₁ N ₃ O 323.39
19	CH ₃		83-86 methanol	64	C ₁₆ H ₁₉ N ₃ O 297.35
20	CH ₃		oil	84	C ₁₇ H ₂₁ N ₃ O 311.38

Table 2. IR and ¹H NMR spectral data of newly synthesized compounds

Compound No.	IR ν_{max} [cm ⁻¹]	¹ H NMR δ [ppm]
1	3436, 3332, 3161, 2855, 1670, 1590, 1520, 1484, 1373, 953	200 MHz, DMSO-d ₆ : 5.97 (s; 2H, NH ₂), 8.62-8.66 (m; 2H, pyrazine), 9.06-9.07 (m; 1H, pyrazine), 10.26 (s; 1H, OH)
2	3472, 3352, 1645, 1167, 1061, 1042, 1017, 911, 849	500 MHz, CDCl ₃ : 3.96 (s; 3H, OCH ₃), 5.46 (s; 2H, NH ₂), 8.47 (s; 1H, pyrazine), 8.57 (s; 1H, pyrazine), 9.22 (s; 1H, pyrazine)
3	3144, 3004, 2858, 1604, 1494, 1477, 1457, 1410, 1278, 1168, 1069, 1019, 968	200 MHz, CDCl ₃ : 8.67-8.71 (m; 2H, pyrazine), 9.05 (s; 1H, pyrazine), 13.00 (s; 1H, OH)
4	2944, 1578, 1405, 1290, 1168, 2994, 1150, 1070, 1042, 1014, 985, 908, 862, 730	500 MHz, CDCl ₃ : 4.20 (s; 3H, OCH ₃), 8.66, 8.78 and 9.20 (3s; 3H, pyrazine)
5	3243, 2928, 2860, 1695, 1603, 1490, 1438, 1413, 1393, 1369, 1139, 1017, 942, 856	200 MHz, CDCl ₃ : 1.62 (s; 8H, CH ₂), 3.23 (t; 4H, NCH ₂ , <i>J</i> 5.6 Hz), 8.61 (d; 1H, pyrazine, <i>J</i> 2 Hz), 8.72-8.73 (m; 2H, pyrazine), 8.90 (s; 1H, OH)
6	3220, 2969, 2852, 1610, 1476, 1440, 1413, 1379, 1264, 1167, 1149, 1116, 1060, 1029, 969	200 MHz, CDCl ₃ : 3.07 (t; 4H, NCH ₂ , <i>J</i> 4.9 Hz), 3.63 (t; 4H, OCH ₂ , <i>J</i> 4.8 Hz), 8.58-8.85 (m; 3H, pyrazine), 9.44 (s; 1H, OH)
7	3215, 2965, 2878, 1621, 1410, 1387, 1364, 1229, 1170, 1141, 1090, 1056, 1030, 956, 896	200 MHz, CDCl ₃ d (ppm): 1.77 (t; 2H, CH ₂ CO ₂ , <i>J</i> 5.8 Hz), 1.85 (t; 2H, CH ₂ CO ₂ , <i>J</i> 5.8 Hz), 3.21 (t; 2H, NCH ₂ , <i>J</i> 5.6 Hz), 3.47 (t; 2H, NCH ₂ , <i>J</i> 5.6 Hz), 4.00 (d; 4H, OCH ₂ , <i>J</i> 5.4 Hz), 8.58 (s; 1H, OH), 8.60-8.63 (m; 1H, pyrazine), 8.73-8.81 (m; 1H, pyrazine), 8.88 (d; 1H, pyrazine, <i>J</i> 1.2 Hz)
8	3225, 2983, 2850, 1595, 1482, 1434, 1385, 1279, 1246, 1163, 980, 957, 773	200 MHz, CDCl ₃ : 3.22 (t; 4H, NCH ₂ , <i>J</i> 5 Hz), 3.63 (t; 4H, NCH ₂ , <i>J</i> 5.3 Hz), 6.65-6.71 (m; 2H, pyridyl), 7.48-7.57 (m; 1H, pyridyl), 7.64 (s; 1H, OH), 8.20-8.23 (m; 1H, pyridyl), 8.66 (d; 1H, pyrazine, <i>J</i> 2.6 Hz), 8.76 (t; 1H, pyrazine, <i>J</i> 2.6 Hz), 8.87 (d; 1H, pyrazine, <i>J</i> 1.4 Hz)
9	3148, 3029, 2935, 2809, 1631, 1455, 1410, 1378, 1309, 1288, 1025, 1006, 944, 905, 850, 792	200 MHz, CDCl ₃ : 2.37 (s; 3H, NCH ₃), 2.58 (t; 4H, NCH ₂ , <i>J</i> 4.5 Hz), 3.48 (t; 4H, NCH ₂ , <i>J</i> 4.7 Hz), 8.54-8.58 (m; 3H, pyrazine), 8.89 (s; 1H, OH)
10	3411, 2981, 2843, 1596, 1498, 1339, 1274, 1235, 1173, 1152, 1022, 984, 954, 758	500 MHz, CDCl ₃ : 3.24 (d; 8H, NCH ₂ , <i>J</i> 4.5 Hz), 6.90-7.04 (m; 3H, ArH), 7.26-7.36 (m; 2H, ArH), 8.58-8.73 (m; 3H, pyrazine), 8.85 (s; 1H, OH)
11	3142, 3032, 2945, 2823, 1626, 1454, 1373, 1264, 1167, 1136, 1016, 995, 971, 851, 750, 703	200 MHz, CDCl ₃ : 2.58 (t; 4H, NCH ₂ , <i>J</i> 4.7 Hz), 3.46 (t; 4H, NCH ₂ , <i>J</i> 4.7 Hz), 3.59 (s; 2H, NCH ₂ Ar), 7.28-7.39 (m; 5H, ArH), 8.56-8.60 (m; 2H, pyrazine), 8.89 (s; 1H, pyrazine), 9.11 (s; 1H, OH)
12	3158, 3025, 2952, 2823, 1631, 1500, 1445, 1408, 1298, 1250, 1042, 1026, 1014, 997, 940, 909, 857	200 MHz, DMSO-d ₆ : 2.38 (t; 4H, NCH ₂ , <i>J</i> 4 Hz), 3.24 (t; 4H, NCH ₂ , <i>J</i> 4.4 Hz), 3.57 (s; 2H, NCH ₂ Ar), 5.98 (s; 2H, OCH ₂ O), 6.80 (m; 3H, ArH), 8.68 (m; 3H, pyrazine), 10.49 (s; 1H, OH)
13	3191, 3055, 2859, 2838, 1633, 1508, 1449, 1407, 1381, 1330, 1213, 1148, 1021, 929, 836	200 MHz, CDCl ₃ : 3.11 (t; 4H, NCH ₂ , <i>J</i> 5 Hz), 3.42 (t; 4H, NCH ₂ , <i>J</i> 5 Hz), 6.92-7.10 (m; 4H, ArH), 8.64-8.68 (m; 2H, pyrazine), 8.78 (d; 1H, pyrazine, <i>J</i> 1.2 Hz), 10.60 (s; 1H, OH)
14	3222, 3061, 2887, 2834, 1630, 1609, 1501, 1449, 1241, 1150, 1019, 919, 748	200 MHz, CDCl ₃ : 3.18 (t; 4H, NCH ₂ , <i>J</i> 4.7 Hz), 3.62 (t; 4H, NCH ₂ , <i>J</i> 4.8 Hz), 3.89 (s; 3H, OCH ₃), 6.96 (m; 4H, ArH), 7.38 (s; 1H, OH), 8.61 (m; 2H, pyrazine), 8.94 (d; 1H, pyrazine, <i>J</i> 1.2 Hz)
15	3287, 2852, 1598, 1506, 1476, 1390, 1323, 1251, 1115, 1023, 959, 855, 828	200 MHz, DMSO-d ₆ : 3.10 (s; 4H, NCH ₂), 3.52 (s; 4H, NCH ₂), 7.04 (d; 2H, ArH, <i>J</i> 9 Hz), 8.07 (d; 2H, ArH, <i>J</i> 9 Hz), 8.67-8.79 (m; 3H, pyrazine), 9.78 (s; 1H, OH)
16	3245, 2833, 1599, 1554, 1488, 1454, 1413, 1386, 1239, 1175, 1161, 1027, 956	200 MHz, CDCl ₃ : 3.21 (s, 8H, NCH ₂), 6.70-6.77 (m; 1H, ArH), 6.94-6.98 (m; 1H, ArH), 7.26-7.36 (m; 1H, ArH), 8.60-8.75 (m; 3H, pyrazine), 8.85 (s; 1H, OH)
17	3061, 3024, 2954, 2849, 2818, 1624, 1450, 1305, 1282, 1267, 1170, 1151, 1024, 1002, 708	200 MHz, CDCl ₃ : 2.47 (s; 4H, NCH ₂), 3.08 (s; 2H, NCH ₂), 3.42 (s; 2H, NCH ₂), 4.27 (s; 1H, NCHAr ₂), 7.14-7.44 (m; 10H, ArH), 8.54-8.76 (m; 3H, pyrazine), 8.86 (s; 1H, OH)
18	3051, 3027, 2915, 2837, 1626, 1518, 1410, 1391, 1304, 1114, 1021, 997, 971, 742	200 MHz, DMSO-d ₆ : 2.67 (s; 4H, NCH ₂), 3.26 (d; 2H NCH ₂ CH=, <i>J</i> 6.6 Hz), 3.50 (s; 4H, NCH ₂), 6.25-6.6.39 (m; 1H, CH=CH), 6.52-6.60 (m; 1H, CH=CH), 7.20-7.41 (m; 5H, ArH, 1H pyrazine), 8.57 (d; 2H, pyrazine, <i>J</i> 4.8 Hz), 8.90 (s; 1H, OH)
19	2901, 2821, 1604, 1497, 1150, 1062, 1036, 1015, 853, 766, 696	500 MHz, CDCl ₃ d (ppm): 3.26 (s; 4H, NCH ₂), 3.55 (s; 4H, NCH ₂), 3.92 (s; 3H, OCH ₃), 6.89 (s; 3H, ArH), 7.82 (m; 2H, ArH), 8.56 (s; 2H, pyrazine), 8.90 (s; 1H, pyrazine)
20	2935, 2811, 1675, 1600, 1453, 1395, 1348, 1269, 1172, 1146, 1065, 1017, 742, 699	500 MHz, CDCl ₃ : 2.52 (t; 4H, NCH ₂ , <i>J</i> 4.9 Hz), 3.39 (t; 4H, NCH ₂ , <i>J</i> 4.9 Hz), 3.55 (s; 2H, NCH ₂ Ar), 3.87 (s; 3H, OCH ₃), 7.29-7.34 (m; 5H, ArH), 8.54-8.56 (m; 2H, pyrazine), 8.84 (d; 1H, pyrazine <i>J</i> 1 Hz)

O-Methylpyrazinehydroximoyl chloride (4)

1.52 g of **2** (10 mmol) was dissolved in 10 mL of concentrated hydrochloric acid at 0°C. Sodium nitrite (0.83 g, 12 mmol) in water (5 mL) was added dropwise. Next, 20 mL of water were added and mixture was stirred for 0.5 h. The precipitate was filtered off and washed with ice-cool water. The crude product was recrystallized yielding bright prisms (0.95 g).

General procedure for the preparation of pyrazin-2-yl-methanone oximes (5-18)

A 0.78 g (5 mmol) quantity of **3** was dissolved in 10 mL of anhydrous dioxane or methanol. Next, 10 mmol of appropriate secondary amine was added dropwise. Solid amines were dissolved in a small volume of the solvent (5 mL). The reaction mixture was refluxed for 15 min and the solvent was evaporated. Next, 40 mL of ice-cooled water were added to the residue. The precipitate was filtered, washed with water and recrystallized. In the case of oily emulsion (oximes **5-9**) the extraction with chloroform was performed. The combined chloroform fractions were dried and evaporated and the oily residue was either extracted several times with hot cyclohexane (**5, 6**) or recrystallized from suitable solvent.

4-Phenylpiperazin-1-yl-pyrazin-2-yl-methanone O-methyl-oxime (19)

A 0.86 g (5 mmol) quantity of **4** was dissolved in 5 mL of anhydrous dioxane and 1.53 mL (1.62 g, 10 mmol) of 1-phenylpiperazine was added dropwise and then the reaction mixture was refluxed for 3 h. The solvent was evaporated and 30 mL of ice-cool water were added to the residue. The precipitate was filtered, washed with cold water and recrystallized from methanol yielding 0.96 g of beige prisms.

4-Benzylpiperazin-1-yl-pyrazin-2-yl-methanone O-methyl-oxime (20)

A 0.7 g (4 mmol) of **4** and 13.9 mL (14.1 g, 80 mmol) of 1-benzylpiperazine were mixed at 5°C. The solution was stirred at room temperature for 24 h, then ice (100 g) was added with stirring to the solution. The precipitate was filtered off and the filtrate was extracted with chloroform. The combined chloroform fractions were dried and evaporated. The crude product was separated and purified by column chromatography of methanol solution on silica gel using toluene – acetone (1:1, v/v) as a liquid phase. Yield: 1.04 g of yellow oil.

Biological activity**Antibacterial activity**

The investigations included 25 strains of anaerobic bacteria and 25 strains of aerobic bacteria iso-

lated from the oral cavity, respiratory system and abdominal cavity as well as 12 standard strains. The anaerobes belonged to the following genera: *Peptostreptococcus* (5 strains), *Actinomyces* (2), *Propionibacterium* (2), *Prevotella* (6), *Porphyromonas* (2), *Fusobacterium* (3), *Bacteroides* (5), and standard strains: *Bacteroides fragilis* ATCC 25285, *Bacteroides vulgatus* ATCC 8482, *Bacteroides ovatus* ATCC 8483, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337 and *Propionibacterium acnes* ATCC 11827. There were also the following aerobes: *Staphylococcus aureus* (4 strains), *Corynebacterium spp.* (2), *Klebsiella pneumoniae* (3), *Acinetobacter baumannii* (2), *Escherichia coli* (6), *Pseudomonas aeruginosa* (6), *Pseudomonas stutzeri* (2) and 6 standard strains: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Acinetobacter baumannii* ATCC 19606, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

The susceptibility of the anaerobic bacteria was determined by means of the plate dilution technique in Brucella agar, supplemented with 5% sheep's blood (12, 13). For aerobic bacteria experiments agar dilution technique with Miller-Hinton agar was used. The derivatives were dissolved in 1 mL of DMSO immediately before the experiment. Sterile distilled water was used for further dilutions. The following concentrations of derivatives were used: 200, 100, 50, 25, 12.5 and 6.2 µg/mL. The inoculum containing 10⁶ CFU/spot applied to the agar plates with Steers replicator. For aerobes, the inoculated agar plates and agar plates without derivatives were incubated for 24 h at 37°C. For anaerobes, agar plates were incubated in anaerobic jars for 48 h at 37°C in 10% CO₂, 10% H₂ and 80% N₂ with palladium catalyst and indicator for anaerobiosis. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the derivative that inhibited growth of the anaerobes.

Mycobacterium tuberculosis

The compounds were examined for their tuberculostatic activity towards *Mycobacterium tuberculosis* H₃₇Rv strain and two "wild" strains isolated from tuberculous patients: one (Spec. 210) resistant to p-aminosalicylic acid (PAS), isonicotinic acid hydrazide (INH), ethambutol (ETB) and rifampicin (RFP), another (Spec. 192) fully sensitive to the administered drugs. *In vitro* investigations were performed by a classical test tube method of successive dilution with Youman's liquid medium containing 10% of bovine serum (14).

Table 3. *In vitro* antibacterial activity of some newly synthesized compounds

A

Anaerobic Bacteria	Metronidazole*	MIC [$\mu\text{g/mL}$]														
		1	2	3	6	8	11	12	13	14	15	16				
<u>Gram-positive:</u>																
<i>Peptostreptococcus magnus</i>	0.8	≤ 6.2	≥ 200	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	12.5	≥ 200	≥ 200	25	≤ 6.2	≤ 6.2	12.5
<i>Peptostreptococcus micros</i>	0.4	≤ 6.2	100	≤ 6.2	≥ 200	-	≥ 200	≥ 200	≥ 200	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2
<i>Actinomyces israelii</i>	1.6	≥ 200	≥ 200	≥ 200	≤ 6.2	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Actinomyces neaslundii</i>	6.2	100	≥ 200	100	≥ 200	≤ 6.2	≥ 200	≥ 200	25	25	25	25	12.5	≥ 200	≥ 200	≥ 200
<i>Propionibacterium acnes</i>	12.5	100	≥ 200	≥ 200	≤ 6.2	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	50	50	≥ 200	≥ 200	≥ 200	≥ 200
<u>Gram-negative:</u>																
<i>Prevotella bivia</i>	≤ 0.4	100	100	50	25	25	≤ 6.2	≤ 6.2	≤ 6.2	≤ 6.2	12.5	12.5	25	≤ 6.2	≤ 6.2	≥ 200
<i>Prevotella buccalis</i>	≤ 0.4	≥ 200	≥ 200	100	≤ 6.2	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	50	50	≥ 200	≤ 6.2	≥ 200	≥ 200
<i>Prevotella intermedia</i>	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Prevotella loeschii</i>	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Porphyromonas asaccharolytica</i>	≤ 0.4	25	25	25	50	≥ 200	≥ 200	≥ 200	≥ 200	12.5	≥ 200	≥ 200	≥ 200	50	25	≥ 200
<i>Fusobacterium nucleatum</i>	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Fusobacterium necrophorum</i>	≤ 0.4	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Bacteroides forsythus</i>	≤ 0.4	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200
<i>Bacteroides fragilis</i>	≤ 0.4	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200	100	≥ 200	≥ 200	≥ 200	≥ 200	≥ 200

B

Aerobic Bacteria	Amikacin**	MIC [$\mu\text{g/mL}$]		
		1	2	3
<u>Gram-positive:</u>				
<i>Staphylococcus aureus</i>	≤ 6.2	≤ 200	≥ 200	≥ 200
<i>Corynebacterium spp.</i>	50	25	≥ 200	≥ 200
<u>Gram-negative:</u>				
<i>Klebsiella pneumoniae</i>	≤ 6.2	12.5	50	25
<i>Acinetobacter baumannii</i>	≤ 6.2	12.5	50	100
<i>Escherichia coli</i>	≤ 6.2	≤ 6.2	50	100
<i>Pseudomonas aeruginosa</i>	≤ 6.2	≥ 200	≥ 200	100
<i>Pseudomonas stutzeri</i>	12.5	≥ 200	≥ 200	≥ 200

*Metronidazole (Sigma)

**Amikacin sulfate salt (Sigma)

RESULTS AND DISCUSSION

The investigations of aerobic and anaerobic bacteria susceptibility to the synthesized pyrazine derivatives are summarized in Table 3A and 3B. The results have been compared with that obtained while testing the susceptibility of the same bacteria to metronidazole (for anaerobes) and amikacin (for aerobes).

Low metronidazole concentrations in range $\leq 0.1 - 3.1 \mu\text{g/mL}$ inhibited the growth of Gram-negative bacteria except single strains of *Bacteroides fragilis*, *B. forsythus* and *Fusobacterium necrophorum*. These results coincided with those obtained by other authors (15, 16). The lowest susceptibility to metronidazole exhibited Gram-positive rods from *Propionibacterium acnes* species (MIC $> 12.5 \mu\text{g/mL}$). Among 19 derivatives 9 (47%) exhibited differential activity against anaerobic bacteria (16 – 44% of strains). The anaerobes were the most susceptible at concentrations in ranges from ≤ 6.2 to $100 \mu\text{g/mL}$ to derivative **3** (44% were susceptible). The aerobic bacteria were generally not susceptible to compounds **4**, **5**, **9**, **10**, **17-20** in the mentioned range of concentrations. Among 9 derivatives active towards anaerobic bacteria, 8 were more effective to Gram-positive strains. Compound **13** (MIC $\leq 6.2 - 25 \mu\text{g/mL}$, 66% of susceptible strains) exhibited the highest activity. However, derivative **2** (MIC $50 - 100 \mu\text{g/mL}$, 12.5% of susceptible strains) was the most active against Gram-negative anaerobic rods. Only 3 from 19 (16%) tested derivatives exhibited activity towards aerobic bacteria: compounds **1**, **2** and **3**. Derivative **1** was the most active one (MIC = $6.2 - 50 \mu\text{g/mL}$, 32% of susceptible strains). Other compounds did not inhibit the growth of aerobic bacteria in the range of tested concentration ($\leq 6.2 - 200 \mu\text{g/mL}$). Derivatives **1**, **2** and **3** were active against both aerobic and anaerobic types of bacteria.

The standard strains of both types of bacteria exhibited rather high resistance towards tested compounds (MIC $\geq 200 \mu\text{g/mL}$). In the case of anaerobic *Fusobacterium nucleatum* ATCC 25586 compounds **3** (MIC $25 \mu\text{g/mL}$), **14** (MIC $50 \mu\text{g/mL}$) and **15** (MIC $100 \mu\text{g/mL}$) were active. Derivative **1** induced the growth inhibition of *Peptostreptococcus anaerobias* ATCC 27337 in concentration of $50 \mu\text{g/mL}$. Compounds **1**, **2** and **3** inhibited the growth of only one aerobic standard strain *Klebsiella pneumoniae* ATCC 13883 and MIC values for those derivatives were $50 \mu\text{g/mL}$, $50 \mu\text{g/mL}$ and $100 \mu\text{g/mL}$, respectively.

The determined minimum concentrations inhibiting the growth of tuberculous strains (MIC) for the tested compounds were within the limits of $25 - 100 \mu\text{g/mL}$ what indicates low antituberculosis activity.

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