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STUDIES ON PYRAZINE DERIVATIVES. XLIX. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF 6-METHOXYPYRAZINE-2-CARBOXYLIC ACID HYDRAZIDE DERIVATIVES

Katarzyna Gobis,[1*] Henryk Foks,[1] Aleksandra Żuralska,[1] and Anna Kędzia[2]

[1]Department of Organic Chemistry, Medical University of Gdansk, 107 Gen. Hallera Str., 80-416 Gdansk, Poland
[2]Department of Oral Microbiology, Medical University of Gdansk, 14 Smoluchowskiego Str., 80-214 Gdansk, Poland

Abstract – The new 6-methoxy-pyrazine derivatives have been synthesized. 6-Methoxy-pyrazine-2-carboxylic acid hydrazide was used as an initial material to obtain mono- and dithioester of hydrazinecarbodithioic acid (**2** and **3**). Compound (**2**) in reaction with ethanolamine gave triazole derivative (**8**) with β–hydoxyethyl substituent in 4-position and hydroxyl group in 6-position of pyrazine ring. Dithioester (**3**) in a reaction with morpholine cyclized to 1,3,4-oxadiazole (**11**). The same substrate with alkyldiamines gave the few following derivatives: 1,3-diazacycloalkane derivatives (**9** and **10**), *S*-methyl-1,3,4-oxadiazole derivative (**12**) and 1,2,4-triazoletetrahydropyrimidine (**13**). The compounds obtained were tested in vitro for their activity towards pathogenic strains of anaerobic and aerobic bacteria. Derivative (**9)** was the most active against both types of tested strains.

INTRODUCTION

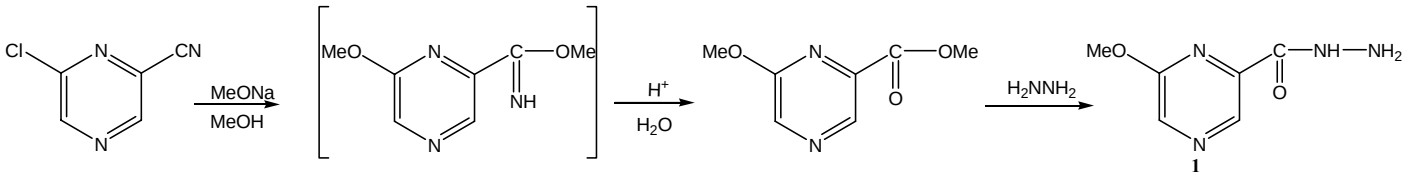
Our previous research works[1-3] as well as chemical literature[4-11] have indicated that pyrazine derivatives exhibit wide range of biological activity, inclusive of antibacterial action. Pyrazinamide and its less toxic derivative morinamide found an application in tuberculosis chemotherapy.[4] In group of sulfonamide drugs "Kelfizian" distinguishes for effectiveness against infections caused by strepto- and staphylococci.[5] Its molecule possesses methoxy group in 2-position of pyrazine ring and sulfanilamide substituent in

*Address correspondence to Katarzyna Gobis, Department of Organic Chemistry, Medical University of Gdansk
Al. Gen. Hallera 107, 80-416 Gdańsk, Poland
E-mail: katarzyna.gobis@wp.pl

3-position. Natural pyrazine products such as aspergillic and hydroxyaspergillic acids isolated from *Aspergillus flavus* culture[6] and emimycin from *Streptomyces griseochromogenes* also demonstrate strong antibacterial activity.[7] Among biologically active pyrazine derivatives many contain methoxy group closed to pyrazine ring. For example *N'*-substituted amidrazones[8] show high tuberculostatic activity and sulfonamides obtained by Camerino and Palemidessi[9-11] are widely applied as antibacterial drugs in infections of urinary tract, gall tract, respiratory system inflammation and meningitis caused by Gram-positive and Gram-negative bacteria.

RESULTS AND DISCUSSION

The intention of this work was to obtain 6-methoxypyrazine-2-carboxylic acid hydrazide and research its chemical reactivity in reactions leading to gain new derivatives of expected antibacterial activity. 6-Chloropyrazine-2-carbonitrile was used as starting material for that synthesis[12] It was transformed into 6-methoxypyrazine-2-carboximidic acid methyl ester in reaction with sodium methoxide in methanol. Acidification of reaction mixture with hydrochloric acid led to methyl ester formation which was next changed into hydrazide (**1**) (Scheme 1).

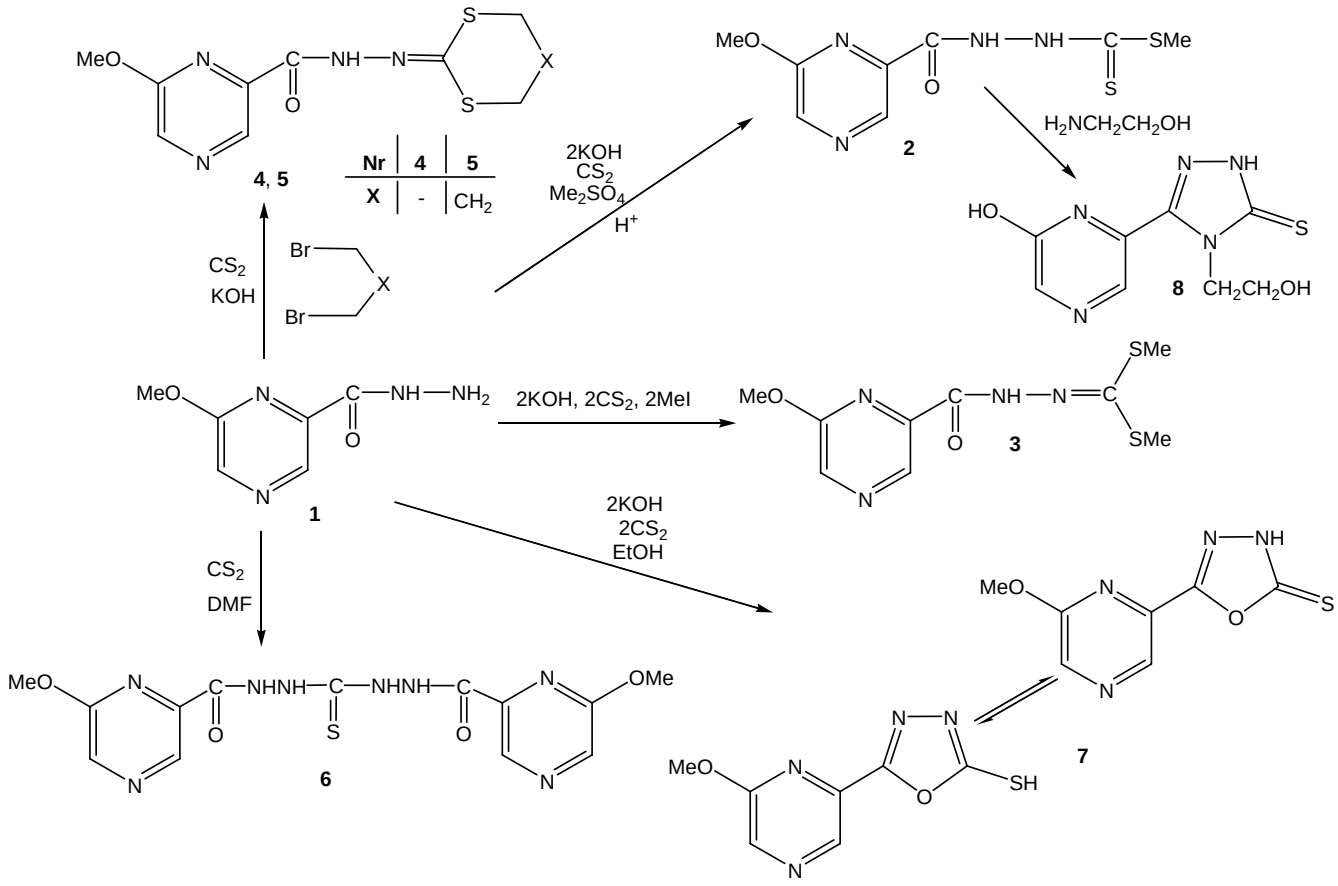


Scheme 1

In our previous paper[13] we have proved that pyrazine-2-carboxylic acid hydrazide is susceptible to carbon disulfide addition and the product of that reaction can be useful in synthesis of nitrogen heterocyclic systems. Within the framework of this work analogical reactions for hydrazide (**1**) were performed. The first step concerned an obtainment of hydrazinecarbodithioic acid mono- and dimethylthioesters (**2** and **3**). Monothioester (**2**) was obtained by acting with carbon disulfide on compound (**1**) in water-methanol milieu and dimethyl sulfate in the presence of an excess of potassium hydroxide (Scheme 2). A trial of methylation with methyl iodide led to formation of a mixture consisted of compounds (**2** and **3**), very difficult to separate. Using of 2 moles of methyl iodide towards hydrazide (**1**) and carbon disulfide in alkaline solution led to pure dithioester (**3**) formation with high yield.

Significant easiness of dithioester (**3**) formation prompted us to synthesize cyclic dithioesters (**4** and **5**) by exchanging methyl iodide with appropriate dibromoalkanes. 1,2-Dibromoethane and 1,3-dibromopropane were used in reaction resulting in obtainment of 6-methoxy-pyrazine-2-carboxylic acid

[1,3]dithiolan-2-ylidene-hydrazide (**4**) and 6-methoxy-pyrazine-2-carboxylic acid [1,3]dithian-2-ylidene-hydrazide (**5**) appropriately.



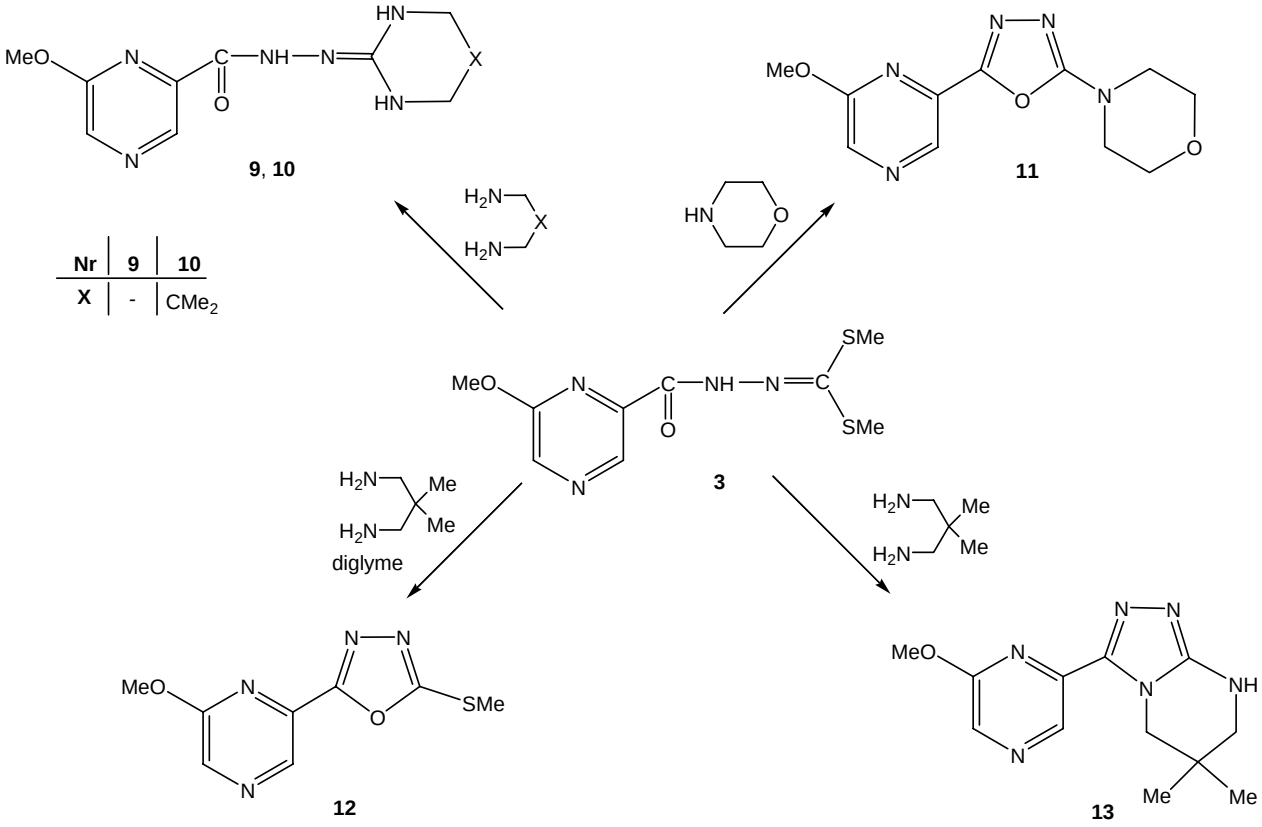
Scheme 2

A trial to obtain 5-(6-methoxypyrazine-2-yl)-[1,3,4]oxadiazole-2-thiol (**7**) from hydrazide (**1**) upon treatment with carbon disulfide and heating of the adduct formed in that way in DMF was without success. That reaction gave as a product 6-methoxypyrazine-2-carboxylic acid *N'*-(*N'*-6-methoxypyrazinoyl-2-hydrazinocarbothioyl)–hydrazide (**6**). IR spectrum of derivative obtained was identical with spectrum of the compound synthesized in another way.[14] That fact confirmed the structure of **6**. Desired 1,3,4-oxadiazole (**7**) was obtained during refluxing of hydrazide (**1**) with carbon disulfide and potassium hydroxide in alcohol solution for 1.5 h. Double signals for 6-methoxypyrazine protons observed in ^{1}H NMR spectrum of that product evidenced that derivative (**7**) existed in two tautomeric forms of 1,3,4-oxadiazole-2-thiol and 1,3,4-oxadiazole-2-thione.

Previously *N*'-(pyrazine-carbonyl)-hydrazinecarbodithioic acid methyl ester has been used successfully to obtain 4-substituted-1,2,4-triazole-thiones in reactions with various aminoalcoholes.[15] Analogical trials were performed for compound (**3**) without a success. Only with aminoethanol derivative (**8**)

(6-[4-(2-hydroxy-ethyl)-5-mercapto-4*H*-[1,2,4]triazol-3-yl]pyrazin-2-ol) was synthesized with rather low yield. IR and MS spectra of that product indicate a lack of methoxy group in 6-position.

Activity of dithioester (**3**) in reaction with morpholine, 1,2-diaminoethane, 2,2-dimethylpropane-1,3-diamine was also investigated. Reaction with morpholine run with cyclization to 1,3,4-oxadiazole (**11**) with morpholine substituent in 2-position. The course of that reaction was analogical as described for pyrazine-2-carboxylic acid (bis-methylsulfanyl-methylene)-hydrazide.[13] Similar course was also characteristic for the reactions with diamines carried out in ethanol or dioxane with 6-methoxy-pyrazine-2-carboxylic acid diazacycloalkylhydrazides (**9** and **10**) as the products.



Scheme 3

Dithioester (**3**) refluxed in excess of 2,2-dimethyl-propane-1,3-diamine gave compound (**13**) of two condensed rings: 1,2,4-triazole and tetrahydropyrimidine. An unexpected effect was gained by refluxing of compound (**3**) with the same amine in diglyme. A product remained to be a derivative of 1,3,4-oxadiazole-3-thione (**12**), and diamine acted as a base catalyzing a cyclization reaction. Close effect was observed during refluxing of compound (**3**) in pyridine.

The characteristics of all newly synthesized 6-methoxypyrazine derivatives are presented in Table 1.

Table 1 The characteristics of synthesized 6-methoxy-pyrazine derivatives

Compound	Yield [%]	mp Solvent for crystallization	Molecular formula MW	Analysis [%] Calcd./Found			IR [cm^{-1}]	^1H NMR δ [ppm]
				C	H	N		
2	36	184-186 benzene	$C_8H_{10}N_4O_2S_2$ 258.31	37.19 37.16	3.90 4.09	21.69 21.52	3247, 1584, 1544, 1140, 1005, 954, 852, 762, 516, 456	$CDCl_3$: 2.72 (s, 3H, SCH_3), 4.10 (s, 3H, OCH_3), 8.48 and 8.97 (2s, 2H, pyrazine), 11.30 and 11.75 (2brs, 2H, 2NH)
3	47	167-168 MeOH	$C_9H_{12}N_4O_2S_2$ 272.34	39.69 39.87	4.44 4.35	20.57 20.45	2997, 2923, 1698, 1543, 1505, 1334, 1134, 1008, 843, 457	$CDCl_3$: 2.59 (s, 6H, $2SCH_3$), 4.03 (s, 3H, OCH_3), 8.42 and 8.98 (2s, 2H, pyrazine), 10.80 (s, 1H, NH)
4	27	205-207 EtOH	$C_9H_{10}N_4O_2S_2$ 270.32	39.99 40.04	3.73 3.68	20.73 20.62	3295, 1692, 1548, 1517, 1394, 1332, 1129, 1008, 841, 454	$CDCl_3$: 3.50-3.70 (2m, 4H, $2CH_2$), 4.05 (s, 3H, OCH_3), 7.27 and 8.43 (2s, 2H, pyrazine), 9.97 (s, 1H, NH)
5	51	183-186 EtOH	$C_{10}H_{12}N_4O_2S_2$ 284.35	42.24 42.22	4.26 4.57	19.70 19.51	3264, 2945, 1696, 1549, 1393, 1330, 1127, 1008, 844, 630, 460	$CDCl_3$: 2.25 (m, 2H, CH_2), 3.20 (m, 4H, $2CH_2$), 4.05 (s, 3H, OCH_3), 8.43 and 9.)1 (2s, 2H, pyrazine), 10.73 (s, 1H, NH)
6	32	194-196 DMSO/H_2O	$C_{13}H_{14}N_8O_4S$ 378.36	41.26 40.89	3.73 3.75	29.62 29.58	3309, 2947, 1694, 1544, 1459, 1395, 1336, 1005, 625, 452	[12]
7	56	189-191 EtOH	$C_7H_6N_4O_2S$ 210.21	39.99 39.63	2.88 2.94	26.65 26.54	2949, 1679, 1543, 1459, 1384, 1309, 1225, 1145, 1003, 942	DMSO-d_6: 3.99 (2s, 3H, OCH_3), 8.46, 8.51, 8.66, and 8. 74 (4s, 2H, pyrazine), 9.90-10.30 (brs, 1H, NH) – *tautomeric forms*
8	20	242-246 EtOH/H_2O	$C_8H_9N_5O_2S$ 239.26	40.16 39.13	3.79 3.86	29.27 29.35	1601, 1538, 1430, 1284, 1190, 1144, 1055, 1012, 870, 518	DMSO-d_6: 3.76 and 4.50 (2m, 4H, $2CH_2$), 8.24 and 8.50 (2s, 2H, pyrazine), 11.80-12.40 (brs, 1H, NH), 14.10 (s, 1H, OH)

9	30	255-258 H_2O	$C_9H_{12}N_6O_2$ 236.24	45.75 45.91	5.12 5.03	35.58 35.42	1693, 1605, 1391, 1303, 1202, 1141, 1003, 864, 628, 470	DMSO-d_6: 3.40 (m, 4H, $2CH_2$), 4.02 (s, 3H, OCH_3), 6.40 (brs, 2H, 2NH), 8.37 and 8.69 (2s, 2H, pyrazine)
10	56	240-246 EtOH	$C_{12}H_{18}N_6O_2$ 278.31	51.78 51.72	6.52 6.61	30.20 30.04	1668, 1634, 1557, 1439, 1393, 1293, 1170, 1007, 769, 686	DMSO-d_6: 1.03 (s, 6H, $2CH_3$), 3.02 (s, 4H, $2CH_2N$), 3.96 (s, 3H, OCH_3), 7.76 (brs, 1H, NH), 8.15 (m, 1H, pyrazine), 8.52 (brs, 1H, NH), 8.97 (m, 1H, pyrazine), 10.92 (brs, 1H, NH)
11	6	141-142 cyclohexane	$C_{11}H_{13}N_5O_3$ 263.25	50.18 50.05	4.98 5.02	26.61 26.58	2925, 1537, 1400, 1271, 1213, 1112, 1003, 910, 886, 471	$CDCl_3$: 3.60-3.80 (2m, 8H, morpholine), 4.06 (s, 3H, OCH_3), 8.28 and 8.80 (2s, 2H, pyrazine)
12	40	116-117 EtOH	$C_8H_8N_4O_2S$ 224.24	42.85 42.78	3.60 3.73	24.99 24.83	2950, 1582, 1544, 1460, 1424, 1300, 1213, 1109, 1003, 885, 467	$CDCl_3$: 2.80 (s, 3H, SCH_3), 4.07 (s, 3H, OCH_3), 8.34 and 8.88 (2s, 2H, pyrazine)
13	28	251-255 EtOH	$C_{12}H_{16}N_6O$ 260.30	55.37 55.19	6.20 6.28	32.29 32.14	2964, 1630, 1531, 1299, 1197, 1064, 895, 865, 473	DMSO-d_6: 1.05 (s, 6H, $2CH_3$), 3.03 (m, 2H, CH_2), 3.45 (m, 2H, CH_2), 3.96 (s, 3H, OCH_3), 4.36 (s, 1H, NH), 8.15 and 8.85 (2s, 2H, pyrazine)

Microbiology

The investigations of aerobic and anaerobic bacteria susceptibility to the synthesized 6-methoxypyrazine derivatives are summarized in Table 2A and 2B. The results have been compared with that obtained while testing the susceptibility of the same bacteria to metronidazole (for anaerobes) and amikacin (for aerobes).

Table 2 Antibacterial activity of tested compounds

A

Anaerobic bacteria	MIC [μg/mL]								
	Metronidazole*	**1**	**4**	**5**	**8**	**9**	**11**	**12**	**13**
Gram-positive:									
Peptococcus niger	≤6.2	25	50	100	12.5	25	25	100	100
Peptostreptococcus magnus	≤6.2	≥200	50	≥200	100	50	100	50	≥200
Peptostreptococcus micros	≤6.2	50	50	100	25	25	50	50	50
Actinomyces israeli	≤6.2	≥200	25	≥200	12.5	25	50	50	100
Actinomyces naeslundii	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Propionibacterium granulosum	≥200	≥200	≥200	100	50	≥200	100	≥200	≤6.2
Gram-negative:									
Prevotella bivia	≤6.2	100	100	≥200	100	25	25	100	≥200
Prevotella buccalis	≤6.2	≥200	≥200	100	≥200	≥200	≥200	≥200	100
Prevotella intermedia	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200
Prevotella loescheii	≤6.2	100	≥200	50	50	25	12.5	≥200	100
Porhyromonas asaccharolytica	≤6.2	100	≥200	≥200	≥200	≥200	25	≥200	≥200
Fusobacterium nucleatum	≤6.2	12.5	100	≥200	≥200	≥200	≥200	100	50
Fusobacterium necrophorum	≤6.2	50	≥200	100	50	≥200	≥200	≥200	25
Bacteroides forsythus	≤6.2	≥200	≥200	≥200	≥200	≥200	100	≥200	≥200
Bacteroides uedyticus	≤6.2	100	≥200	50	≥200	≥200	≥200	≥200	≥200

B

Aerobic bacteria	MIC [μg/ml]					
	Amikacin**	**1**	**4**	**9**	**10**	**12**
Gram-positive:						
Staphylococcus aureus	≤6.2	≥200	≥200	≥200	≥200	100
Corynebacterium spp.	50	25	100	≥200	25	≥200
Gram-negative:						
Klebsiella pneumoniae	≤6.2	50	50	25	≥200	25
Acinetobacter baumannii	≤6.2	≥200	50	≥200	≥200	100
Escherichia coli	≤6.2	25	≥200	≥200	≥200	≥200
Pseudomonas aeruginosa	≤6.2	≥200	≥200	≥200	25	≥200
Pseudomonas stutzeri	12.5	≥200	≥200	≥200	≥200	≥200

*Metronidazole (Sigma)
**Amikacin sulfate salt (Sigma)

Low metronidazole concentrations in range from ≤ 6.2 to 12.5 μg/mL inhibited the growth of Gram-negative bacteria. Gram-positive rods were the most resistant to metronidazole (MIC > 200 μg/mL). These results were coincided with those obtained by other authors.[16, 17] Activity against anaerobic bacteria

exhibited 8 of 11 tested 6-methoxypirazine derivatives: (**1**, **4**, **5**, **8**, **9**, **11**-**13**). The anaerobes were the most susceptible at concentrations in range from ≤ 6.2 to 25 μg/mL to derivative (**9**) (20 % were susceptible) and to compounds (**8** and **11)** (16% of susceptible strains). Moreover from 4 to 12 % of anaerobic strains were susceptible at concentrations in range from ≤ 6.2 to 25 μg/mL to derivatives (**4** and **13**). The aerobic bacteria were generally not susceptible to compounds (**2**, **3** and **10**) in mentioned range of concentrations. Tested 6-methoxypyrazine derivatives at concentrations in range from ≤ 6.2 to 100 μg/mL inhibited growth of 24 to 48 % of anaerobic strains. All derivatives active towards anaerobic bacteria were more effective to Gram-positive strains.

Aerobic bacteria were less susceptible to tested compounds than anaerobes. The growth of aerobic bacteria was inhibited at concentrations in range from 25 to 50 μg/mL. Only 1-2 of tested aerobic strains were susceptible to derivatives (**1**, **9**, **10** and **12**) at concentration 25 μg/mL. Also individual strains of aerobic bacteria exhibited susceptibility to compound (**4**) at 50 μg/mL. Other compounds did not inhibit the growth of aerobic bacteria in the range of tested concentration (≤ 6.2-200 μg/mL). The standard strains of both types of bacteria exhibited rather high resistance towards tested compounds (MIC ≥ 200 μg/mL). In the case of anaerobic *Bacteroides ovatus* ATCC 8483 compounds (**11**) (MIC 50 μg/mL), (**4**) (MIC 100 μg/mL), (**5**) (MIC 100 μg/mL), (**8)** (MIC 100 μg/mL) and (**9**) (MIC 100 μg/mL) were active. Derivative (**5**) induced the growth inhibition of *Bacteroides vulgatus* ATCC 8482 at concentration of 100 μg/mL. Compound (**1**) inhibited the growth of *Peptostreptococcus anaerobius* ATCC 27337 at 100 μg/mL. The same activity level was exhibited by derivative (**13**) against *Propionibacterium acnes* ATCC 11827. Among standard aerobic strains only *Pseudomonas aeruginosa* ATCC 27853 was susceptible to compound (**9**) at concentration 100 μg/mL.

In summery, derivative (**9**) was the most active against both types of tested strains. It inhibited 20 % of anaerobic and 4 % of aerobic strains at concentration 25 μg/mL. That fact makes it an interesting lead structure for further syntheses. Although only 2 compounds (**2** and **3**) seemed to be inactive, the MIC values obtained for the other derivatives were not satisfactory. None of the tested compounds performed better than metronidazole against anaerobes and amikacin against aerobes.

EXPERIMENTAL

All materials and solvents were of analytical reagent grade. Thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates and visualized with UV. The results of elemental analyses (%C, H, N) for all the compounds obtained were in good agreement with the data calculated. ^{1}H NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on Varian Gemini (200 MHz) instrument. IR Spectra were determined as KBr pellets of the solids on a Satellite FT-IR spectrophotometer. Mass spectra (MS) for compound (**4** and **8**)

were taken on Finingan MAT 95 (70 eV). Melting points were determined on BOETIUS apparatus and were uncorrected.

Synthesis of 6-methoxypyrazine-2-carboxylic acid hydrazide (1): Metallic sodium (2.8 g, 0.12 mol) and dry methanol (60 mL) were placed into round-bottomed flask (250 mL) furnished with condenser and dropping funnel. After vigorous reaction 12 mL (0.12 mol) of 6-chloropyrazine-2-carbonitrile was added.[11] After 15 min, mixture was treated with 40 mL of 20 % hydrochloric acid. Next 30 mL of water was added until clear solution was obtained. The solution was stirred at room temperature for about 0.5 h. Then the mixture was poured into 100 mL of ice-water mixture. The solid of 6-methoxypyrazine-2-carboxylic acid methyl ester was filtered and washed with ice-cold water. Dry ester (10.4 g, 0.06 mol) was dissolved in 30 mL of hot EtOH and 10 mL of 99 % hydrazine hydrate was added, and mixture was refluxed for 1h. The precipitate was filtered after cooling, washed with ice-cold water and recrystallized. Melting point in agreement with reference.[12]

Synthesis of *N*'-(6-methoxypyrazine-2-carbonyl)hydrazinecarbodithioic acid methyl ester (2): Compound (**1**) (5 g, 30 mmol) was treated with a solution of KOH (3.4 g, 60 mmol) in water (20 mL) and EtOH (30 mL). Next 1.8 mL (30 mmol) of CS_2 was added. After 0.5 h, 1.4 mL (15 mmol) of Me_2SO_4 was added dropwise. Reaction mixture was stirred at room temperature for 1 h. After that time it was poured in 100 mL of cold water and acidified with 4 mL of AcOH. Precipitate was filtered and recrystalized.

Synthesis of 6-methoxypyrazine-2-carboxylic acid (bis-methylsulfanylmethylene)hydrazide (3): 1.68 g (10 mmol) of compound (**1**) was added to a stirred solution of 1.4 g (25 mmol) of KOH in 25 mL of water and 15 mL of EtOH. Next 1.5 mL (25 mmol) of CS_2 was added and after 15 min 1.6 mL (25 mmol) of MeI. The reaction mixture was stirred for 0.5 h, poured on 100 g of ice. Yellow precipitate was filtered and recrystalized.

Synthesis of 6-methoxypyrazine-2-carboxylic acid [1,3]dithiolan-2-ylidenehydrazide (4): Compound (**1**) (1.68 g, 10 mmol) was dissolved in a solution of 1.2 g (30 mmol) of KOH in 3 mL of water and 10 mL of EtOH. Next 0.6 ml (10 mmol) of CS_2 was added and after 15 min 0.87 mL (10 mmol) of 1,2-dibromoethane. After 1 h stirring the precipitate (0.16 g) was filtered and filtrate was concentrated and diluted with 15 mL of water. Next solution was extracted with $CHCl_3$, dried with $MgSO_4$ and evaporated resulting in another 0.6 g of the product. MS: m/z = 270.1 (100 M^+), 210.1 (41.4), 137.1 (22.3), 109.1 (79.1), 66.1 (26.1), 60.0 (44.1), 59.0 (37.2), 45.0 (54.1), 44.0 (39.8), 43.1 (27.3).

Synthesis of 6-methoxypyrazine-2-carboxylic acid [1,3]dithian-2-ylidenehydrazide (5): Compound (**1**) (0.84 g, 5 mmol) was dissolved in mixture of EtOH (15 mL) and Et_3N (2mL). Next 0.3 mL (5 mmol) of

CS_2 and 0.5 mL (5 mmol) of 1,3-dibromopropane were added. Reaction mixture was stirred for 12 h and then the precipitate was filtered and recrystalized.

Synthesis of 6-methoxypyrazine-2-carboxylic acid *N*'-(*N*'-6-methoxypyrazinoyl-2-hydrazinocarbothioyl)hydrazide (6): 1.68 g (10 mmol) of compound (**1**) was refluxed in 5 mL of N,N-dimethylformamide and 2 mL of CS_2 for 1 h. After that time the mixture was cooled and diluted with 30 mL of water. The precipitate was filtered and recrystalized.

Synthesis of 5-(6-methoxypyrazin-2-yl)[1,3,4]oxadiazole-2-thiol (7): 0.84 g (5 mmol) of compound (**1**) was dissolved in the solution of KOH (0.8 g, 10 mmol), water (2 mL) and EtOH (20 ml). Next 1 mL (10 mmol) of CS_2. The mixture was refluxed for 1.5 h, evaporated, diluted with 10 mL of water and acidified with HCl. The precipitate was filtered and recrystalized.

Synthesis of 6-[4-(2-hydroxyethyl)-5-mercapto-4*H*-[1,2,4]triazol-3-yl]pyrazin-2-ol (8): Compound (**2**) (2.4 g, 10 mmol) was refluxed in 3 mL of ethanolamine. After cooling 10 mL of water was added and mixture was acidified with 3.5 mL of AcOH. The whole was cooling in freezer for 2 weeks and after that time the crystals of product were filtered. MS: m/z = 239.1 (9.7 M^+), 197.1 (9.2), 196.1 (100), 195.1 (10.9).

Synthesis of 6-methoxypyrazine-2-carboxylic acid imidazolidin-2-ylidenehydrazide (9): 0.84 g (3 mmol) of **3** was refluxed in the mixture of 10 mL of dioxane and 1 mL of 1,2-diaminoethane. After evaporation of solvent the product was purified by crystallization.

Synthesis of 6-methoxy-pyrazine-2-carboxylic acid (tetrahydro-pyrymidin-2-ylidene)hydrazide (10): 0.84 g (3 mmol) of **3** was refluxed in the mixture of 10 mL of EtOH and 1 mL of 2,2-dimethylpropane-1,3-diamine for 8 h. After cooling product was filtered and recrystalized.

Synthesis of 4-[5-(6-methoxypyrazin-2-yl)-[1,3,4]oxadiazol-2-yl]morpholine (11): Compound (**3**) (0.97 g, 5 mmol) was refluxed in 3 mL of morpholine for 2 h. The mixture was cooled, diluted with 20 mL of water and extracted with $CHCl_3$. The collected $CHCl_3$ fractions were dried with $MgSO_4$. After evaporation of the solvent residual oil crystallized.

Synthesis of 2-methoxy-6-(5-methylsulfanyl-[1,3,4]oxadiazol-2-yl)pyrazine (12): 0.84 g (3 mmol) of **3** was refluxed in the mixture of 3 mL of diglyme (diethylene glycol dimethyl ether) and 1 mL of 2,2-dimethylpropane-1,3-diamine for 8 h. After cooling 5 mL of water was added, product was filtered and recrystalized.

Synthesis of 3-(6-methoxypyrazin-2-yl)-6,6-dimethyl-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]-pyrimidine (13): 0.84 g (3 mmol) of **3** was refluxed with 4 mL of 2,2-dimethylpropane-1,3-diamine for

1h. After 10 min semi-product precipitated and next dissolved gradually. After cooling product was filtered and recrystalized.

Antibacterial activity

The investigations included 25 strains of anaerobic bacteria and 25 strains of aerobic bacteria isolated from the oral cavity, respiratory system and abdominal cavity as well as 12 standard strains. The anaerobes belonged to the following genera: *Peptococcus* (1 strain), *Peptostreptococcus* (4 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.[18, 19] For aerobic bacteria experiments agar dilution technique with Miller-Hinton agar was used. The derivatives were dissolved in 1ml 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 10^6 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 % CO_2, 10 % H_2 and 80 % N_2 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.

REFERENCES

1. H. Foks, K. Wisterowicz, B. Damasiewicz, A. Nasal, J. Petrusewicz, and A. Radwańska, *Acta Polon. Pharm.*, 1997, **54**, 55.
2. H. Foks, M. Janowiec, Z. Zwolska, and E. Augustynowicz-Kopeć, *Phosphorus Sulfur Silicon Relat. Elem.*, 2004, **179**, 2519.
3. J. Petrusewicz, R. Gami-Yilinkou, R. Kaliszan, B. Pilarski, and H. Foks, *Gen. Pharmacol.*, 1993, **24**, 17.

4. E. Felder, D. Pitre, and U. Tiepolo, *Arzneim.-Forsch.*, 1964, **14**, 1225.
5. M. Ghione, C. Bertazzoli, A. Buogo, T. Chieli, and V. Zaváglio, *Chemotherapia*, 1963, **6**, 344.
6. E. White and H. Hill, *J. Bacteriol*., 1943, **45**, 433.
7. M. Tarao, K. Kurasawa, N. Tanaka, H. Yonehera, and H. Umezawa, *J. Antibiot.*, Ser. A, 1960, **13**, 401.
8. C. Orlewska, H. Foks, M. Janowiec, and Z. Zwolska-Kwiek, *Pharmazie*, 1995, **50**, 565.
9. B. Camerino and G. Palamidessi, *Gzz.Chim. Ital.*, 1960, **90**, 1815.
10. B. Camerino and G. Palamidessi, *Gzz.Chim. Ital.*, 1960, **90**, 1807.
11. B. Camerino and G. Palamidessi, *Gzz.Chim. Ital.*, 1961, **91**, 1438.
12. H. Foks, M. Buraczewska, W. Manowska, and J. Sawlewicz, *Dissert. Pharm. Pharmacol.*, 1971, **23**, 49.
13. H. Foks, J. Mieczkowska, and M. Sitarz, *Phosphorus, Sulfur and Silicon*, 2000, **158**, 107.
14. D. Pancechowska-Ksepko, H. Foks, M. Janowiec, and Z. Zwolska-Kwiek, *Acta Polon. Pharm.*, 1988, **45**, 373.
15. H. Foks and W. Manowaska, *Pol. J. Pharmacol. Pharm.*, 1976, **28**, 49.
16. L. Boyanova, V. Djambazov, G. Gregorova, D. Iotov, D. Petrov, D. Osmanliev, Z. Minchev, and I. Mitov, *Anaerobe*, 2004, **10**, 261.
17. D. B. Hoellman, L. M. Kelly, M. R. Jacobs, and P. C. Appelbaum, *Antimicrob. Agents Chemother*., 2001, **45**, 589.
18. A. Balows, H. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy, 'Manual of Clinical Microbiology'; 5th ed., Am. Soc. Microbiol., Washington, 1991.
19. E. J. Baron and S. M. Finegold, 'Bailey and Scotts Microbiology', 8th ed., C.V. Mosby Co., St. Louis, 1990.