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Original article

Synthesis and antibacterial activity of 1H-pyrazolo[3,4-b]pyrazine and -pyridine derivatives

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Abstract

The investigations of new pyrazine and pyridine derivatives showing an antibacterial activity have been made. Upon treatment of 3-chloro-2-cyanopyrazine [1] and 2-chloro-3-cyanopyridine with 1,1-dimethyl-hydrazine, 1-aminopiperidine and 1-amino-4-methylpiperazine, either the pyrazolo-pyrazine (**1**), and -pyridine (**2**) derivatives, or ammonium salts (**3**–**8**) were obtained, according to the reaction conditions. Compound **1** was obtained in the reaction of the initial nitrile with methylhydrazine as well. The reactions of **1** gave the following derivatives: acylation—(**9**), that with *p*-chlorobenzoic aldehyde—(**10**), and with phenyl-isothiocyanate—(**11**). 3-Chloro-2-cyanopyrazine treated with hydrazine hydrate gave amidrazone (**12**), which upon condensation with *p*-chlorobenzoic aldehyde produced (**13**). The compounds obtained were tested in vitro for their tuberculostatic activity. The minimal inhibitory concentration (MIC) values were within 22–100 μg/cm^3. Compounds **1**, **5** and **6** were also tested in vitro for their activity towards 25 strains of anaerobic, and 25 strains of aerobic bacteria. They appeared to be of elevated activity towards the anaerobes and of low one towards the aerobes (Table 2).

Keywords: Cyclization; 1H-pyrazolo[3,4-b]pyrazines and -pyridines; Nitriles; Antibacterial activity

1. Introduction

The investigation of pyrazole derivatives condensed either with the benzene ring (indazoles), or with various heterocyclic systems confirmed their comprehensive biological activity. The indazo-lylquinazoline and indazolylbenzotriazine derivatives showed the antibacterial activity [2], just as the pyrazolquinoline derivatives [3]. Hoffman and Rayner [4] obtained the *N*-benzothienopyrazolo-amide derivatives, which have been used for effective treatment of the rhinovirus diseases. Japanese scientists obtained a series of aminoindazole derivatives, which were tested for their anti-phlogistic activity in alimentary canal treatment [5]. The reports of Roch et al. and Kuczyński et al. [6,7] showed the anticoagulative, hypotensive and antiarrythmic efficacy of pyrazolpyridines. 4-Hydroxypyrazolo[3,4-b]pyrimidine (allopurinol) appeared to be an effective 1-xanthine oxidase inhibitor, and the research on 4-hydroxypyra-zolo[3,4-b]pyridines proved their high enzymatic activity [8,9].

2. Chemistry

While going on with the search for new pyrazine and pyridine derivatives of antibacterial activity, 2-chloro-3-cyanopyridine [1] was treated with methylhydrazine, and the expected 1-methyl-1H-pyrazolo[3,4-b]pyrazinyl-3-amine (**1**) was obtained. Surprisingly, the same product was formed in the reaction with 1,1-dimethylhydrazine. The syntheses were carried on in varied conditions. In ether, an hour's heating gave the ammonium salt (**3**), which was transformed, in boiling ethanol, into compound **1**. Prolonged to 24 h heating in benzene allowed to obtain 1-methyl-1H-pyrazolo[3,4-b]pyrazinyl-3-amine (**1**) at one go.

2-Chloronicotinonitrile was also treated with 1-methylhydrazine, according to the method proposed by Kuczyński

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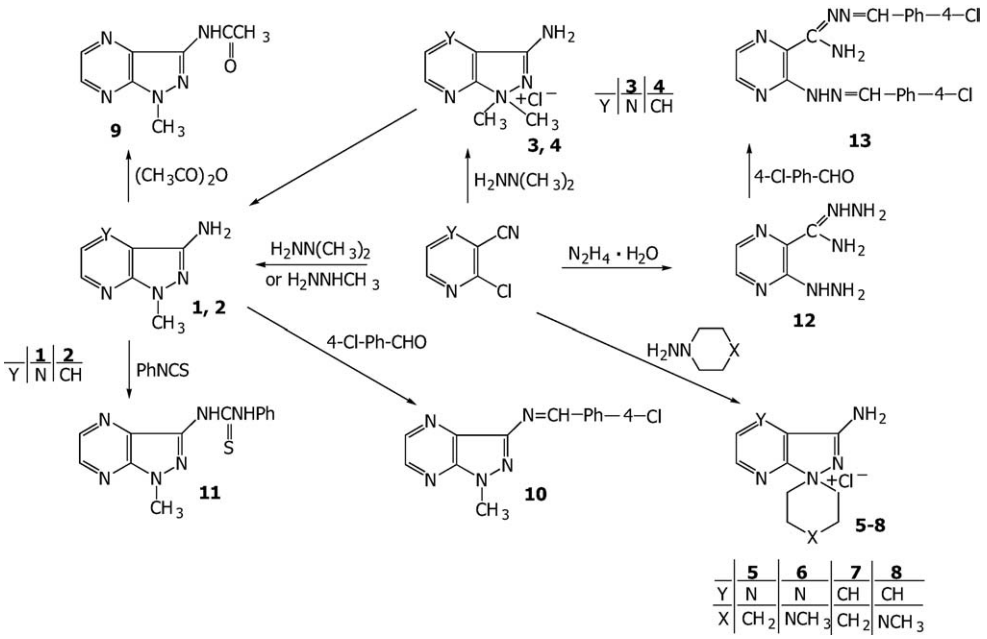

Scheme 1.

et al [7], which produced the aminopyrazolpyridine derivative (**2**). The reaction with 1,1-dimethylhydrazine, however, gave only the corresponding ammonium salt (**4**).

The reactions of 3-chloro-2-cyanopyrazine and 2-chloronicotinonitrile with cyclic hydrazine derivatives, 1-aminopiperidine and 1-amino-4-methylpiperidine, were carried on as well, and their only products were cyclic ammonium salts (**5**–**8**).

1-Methyl-1H-pyrazolo[3,4-b]pyrazino-3-yl-amine (**1**) was exposed to acylation with acetic anhydride, which gave the corresponding amide derivative (**9**), and to the action of *p*-chlorobenzoic aldehyde, or phenyl isothiocyanate, which gave, respectively, compound (**10**) or the thiourea derivative (**11**).

The reaction of 3-chloro-2-cyanopyrazine with hydrazine hydrate produced 3-hydrazinopyra-zino-2-amidrazone (**12**), which, condensed then with *p*-chlorobenzoic aldehyde, gave compound (**13**).

The reactions were shown on Scheme 1, and the characteristics of the compounds newly obtained, was given in Table 1.

3. Experimental

3.1. Chemistry

Melting points were determined with a Reichert apparatus and are uncorrected. The IR spectra were taken with a Satellite spectrophotometer. The ^{1}H NMR spectra were taken with a Varian Gemini 200 spectrometer. Reaction yields and the physical constants of the compounds obtained are given in Table 1. The results of elemental analyses for C and H for all the compounds obtained are in good agreement with the data calculated.

*3.1.1. 1-Methyl-1H-pyrazolo[3,4-b]pyrazin-3-ylamine (**1**)*

A. To 3-chloro-2-cyanopyrazine (5 mmol) dissolved in benzene (10 ml) methylhydrazine or 1,1-dimethylhydrazine (10 mmol) was added and stirred for 24 h at ambient temperature, then yellow precipitate of product **1** was filtered off.

B. Compound **3** (2.5 mmol) dissolved in ethanol (5 ml) was refluxed for 2 h; the solvent was evaporated then, and the residual **1** purified by crystallization.

*3.1.2. 1-Methyl-1H-pyrazolo[3,4-b]pyridin-3-ylamine (**2**)*

To 2-chloro-3-cyanopyridine (5 mmol) dissolved in dry xylene (20 ml) calcined soda (0.8 g) and methylhydrazine (10 mmol) were added and refluxed for 1 h. On cooling down, precipitated (**2**) was filtered off and crystallized.

*3.1.3. 1,1-Dimethyl-1H-pyrazolo[3,4-b]pyrazin-3-ylamine chloride (**3**)*

To 3-chloro-2-cyanopyrazine (5 mmol) in diethyl ether (10 ml) 1,1-dimethylhydrazine (5 mmol) was added and refluxed for 1 h. Reaction mixture was cooled down then, and the precipitated compound (**3**) filtered off.

*3.1.4. 1,1-Dimethyl-1H-pyrazolo[3,4-b]pyridin-3-ylamine chloride (**4**)*

To 2-chloro-3-cyanopyridine (5 mmol) dissolved in benzene (10 ml) 1,1-dimethylhydrazine (10 mmol) was added, stirred for 48 h at ambient temperature; the precipitated compound (**4**) was filtered off then and recrystallized.

*3.1.5. Spiro-piperidin-1,1′-1H-pyrazolo[3,4-b]pyrazin-3-ylamine chloride (**5**) and spiro-4-methyl-piperazin-1,1-1H-pyrazolo[3,4-b]pyrazin-3-ylamine chloride (**6**)*

3.1.5.1. General method. To 3-chloro-2-cyanopyrazine (5 mmol) dissolved in benzene (10 ml) either 1-amino-

Table 1
Characteristics of the newly synthesized compounds **1**–**13**

Compound	Formula molecular mass	Yield (%)	M.p. (°C) solvent for crystallization	^{1}H NMR solvent δ (ppm)
1	$C_6H_7N_5$ 149.16	67	147–150 cyclohexane	DMSO-d_6: 3.81 (s, 3H, CH_3); 5.9 (s, 2H, NH_2); 8.37, 8.46 (2d, 2H, CH pyrazin)
2	$C_7H_8N_4$ 148.17	54	100–104 cyclohexane	$CDCl_3$: 3.5 (s, 3H, CH_3); 4.5 (s, 2H, NH); 6.7 (d, 1H, CH pyrydin); 7.7 (d, 1H, CH pyrydin); 8.3 (m, 1H, CH pyrydin)
3	$C_7H_{10}N_5Cl$ 199.52	35	170 decompose	DMSO-d_6: 3.65 (s, 6H, CH_3); 8.41 (s, 2H, NH_2); 8.97, 9.22 (2d, 2H, CH pyrazin)
4	$C_8H_{11}N_4Cl$ 198.52		155 decompose	DMSO-d_6): 3.59 (s, 6H, CH_3); 7.92, 8.91 (2 m, 3H, CH pyrydin); 8.5 (b.s, 2H, NH_2)
5	$C_{10}H_{14}N_5Cl$ 239.55	72	165–167 absolutes EtOH/Et_2O	DMSO-d_6: 1.7–2.2 (m, 6H, CH_2); 3.55, 4.05 (2 m, 4H, CH_2); 8.5 (s, 2H, NH_2); 9.0, 9.25 (2d, 2H, CH pyrazin)
6	$C_{10}H_{15}N_6Cl$ 254.56	43	164 decompose	(DMSO-d_6: 2.41 (s, 3H, CH_3); 2.89 (s, 2H, CH_2); 3.12 (s, 2H, CH_2); 3.61 (s, 2H, CH_2); 4.14 (s, 2H, CH_2); 8.6 (s, 2H, NH_2); 9.02, 9.26 (2d, 2H, CH pyrazin)
7	$C_{11}H_{15}N_4Cl$ 238.56	55	164–170 absolutes EtOH/Et_2O	DMSO-d_6: 1.5–2.25 (m, 6H, CH_2); 2.95 (m, 2H, CH_2); 4.05 (m, 2H, CH_2); 7.96 (m, 1H, CH pyrydin); 8.25–8.75 (m, 2H, NH_2); 8.9 (m, 2H, CH) pyrydin)
8	$C_{11}H_{16}N_5Cl$ 253.57	62	183–187 absolutes EtOH/Et_2O	DMSO-d_6: 2.4 (s, 3H, CH_3); 2.89 (m, 2H, CH_2); 3.14 (m, 2H, CH_2); 3.46 (m, 2H, CH_2); 4.16 (m, 2H, CH_2); 7.99 (m, 1H, CH pyrydin); 8.35–8.7 (m, 2H, NH_2); 8.77 (m, 1H, CH pyrydin); 8.91 (m, 1H, CH pyrydin)
9	$C_8H_9N_5O$ 191.19	43	167–169 benzene	DMSO-d_6: 2.1 (s, 3H, CH_3); 4.02 (s, 3H, CH_3); 8.61 (s, 2H, CH pyrazin)
10	$C_{13}H_{10}N_5Cl$ 271.76	66	182–183 MeOH	$CDCl_3$: 3.3 (s, 1H, CH); 3.9 (s, 3H, CH_3); 7.1–7.4 (m, 2H, Ph); 7.7–7.9 (m, 2H, Ph); 8.3–8.5 (m, 2H pyrazin)
11	$C_{13}H_{12}N_6S$ 284.27	48	168–170 EtOH	$CDCl_3$: 4.0 (m, 3H, CH_3); 7.6 (m, 5H, Ph); 8.4 (s, 2H pyrazin); 11.2 (m, 2H, NH)
12	$C_5H_9N_7$ 167.18	56	169–175 EtOH	DMSO-d_6: 4.3–4.7 (b.s, 2H, NH); 5.4–5.68 (b.s, 2H, NH); 5.83 (s, 2H, NH); 7.71, 8.01 (2d, 2H, CH pyrazin); 9.83 (s, 1H, NH)
13	$C_{19}H_{15}N_7Cl_2$ 412.25	70	205–210 acetone	$CDCl_3$: 1.26 (s, 2H, CH); 7.44, 7.8 (2 m, 8H, Ph); 8.62 (s, 2H, CH pyrazin)

piperidine or 1-amino-4-methylpiperazine (5 mmol) was added and stirred for 24 h at ambient temperature. The filtered off precipitates of compounds **5** or **6**, respectively, were recrystallized.

*3.1.6. Spiro-piperidin-1,1'-1H-pyrazolo[3,4-b]pyridin-3-ylamine chloride (**7**) and spiro-4-methyl-piperazin-1,1'-1H-pyrazolo[3,4-b]pyridin-3ylamine chloride (**8**)*

3.1.6.1. General method. To 2-chloro-3-cyanopyridine (5 mmol) dissolved in benzene (10 ml) either 1-amino-piperidine or 1-amino-4-methylpiperazine (5 mmol) was added and stirred for 20 h at ambient temperature. The precipitated compounds **7** or **8**, respectively, were filtered off then.

*3.1.7. N-(1-Methyl-1H-pyrazolo[3,4-b]pyrazin-3-yl)-acetamide (**9**)*

Compound **1** (5 mmol) dissolved in acetic anhydride (5 ml) was refluxed for 1 h. On cooling down the mixture was poured on ice, neutralized with saturated Na_2CO_3 solution and extracted thrice with chloroform. The combined extracts were dried with anhydrous $MgSO_4$ and the solvent evaporated. Precipitated compound **9** was crystallized.

*3.1.8. (4-Chloro-benzylidene)-(1-methyl-pyrazolo[3,4-b]pyrazin-3-yl)-amine (**10**)*

To compound **1** (5 mmol) dissolved in 10 ml ethanol, 5 mmol *p*-chlorobenzaldehyde was added and refluxed for 1 h. On cooling down, the precipitated compound **10** was filtered off.

*3.1.9. 1-(1-Methyl-1H-pyrazolo[3,4-b]pyrazin-3-yl)-3-phenyl-thiourea (**11**)*

To compound **1** (5 mmol) dissolved in anhydrous pyridine (5 ml) phenyl isothiocyanate (5 mmol) was added and refluxed for 2 h. The product **11** was precipitated with petroleum ether and crystallized.

*3.1.10. 3-Hydrazinepyrazine-2-amidrazone (**12**)*

To 3-chloro-2-cyanopyrazine (5 mmol) dissolved in ethanol (10 ml) and cooled with ice, hydrazine hydrate (15 mmol) was added drop wise. After 1 h the precipitated compound **12** was filtered off.

*3.1.11. 2-N (4-Chlorobenzylideneamidrazone)-3-(4-chlorobenzylidenehydrazin)-pyrazine (**13**)*

To compound **12** (2.5 mmol) dissolved in ethanol, *p*-chlorobenzoic aldehyde (5 mmol) was added and refluxed for 1 h. On cooling down, the precipitated compound **13** was filtered off.

3.2. Pharmacology

The compounds newly obtained (**1**–**13**) were tested for their tuberculostatic activity towards the standard Myc. tbc $H_{37}Rv$ strain, as well as two *wild* strains isolated from tuberculotic patients: one resistant to *p*-aminosalicylic acid (PAS), isonicotinic acid hydrazide (INH), ethambutol (EMB) and rifampycine (RFP), the other, fully susceptible to the drugs administered.

Tuberculostatic activity was tested in vitro with classical test tube method on Youman's liquid medium containing 10%

Table 2
The MIC for the compounds **1**, **5**, **6**

Anaerobic bacteria	Number of strains	MIC (μg/ml)																					
		Metronidazol						Compound **1**						Compound **5**						Compound **6**					
		≥ 200	100	50	25	12.5	≤ 6.2	≥ 200	100	50	25	12.5	≤ 6.2	≥ 200	100	50	25	12.5	≤ 6.2	≥ 200	100	50	25	12.5	≤ 6.2
Gram-positive: *Peptococcus niger*	1						1						1					1						1	
Peptostreptococcus magnus	2						2	2						2						1			1		
Peptostreptococcus micros	2						2	2						2							1		1		
Actinomyces israelii	1						1	1						1						1					
Actinomyces naeslundii	1						1	1						1						1					
Propionibacterium granulosum	2	2						1				1		1				1		1		1			
Gram-negative: *Prevotella bivia*	1						1			1						1				1					
Prevotella buccalis	2						2	2						2						1		1			
Prevotella intermedia	2					1	1	2						2						2					
Prevotella loescheii	1						1					1						1						1	
Porphyromonas asaccharolytica	2					1	1	2						2						2					
Fusobacterium nucleatum	1						1	1						1								1			
Fusobacterium necrophorum	2					1	1	2						2						1	1				
Bacteroides forsythus	3					1	2	3						3						1	1	1			
Bacteroides ureolyticus	2						1	2						2						2					

of bovine serum [10]. The obtained values of minimum growth inhibiting concentration (MIC): 25–100 μg/ml allowed to conclude, that the group of compounds tested is of rather weak tuberculostatic activity.

Compounds **1**, **5** and **6** were also tested in vitro towards 25 strains of anaerobic and 25 of aerobic bacteria. The results were given in Table 2.

3.2.1. Aerobic bacteria

The investigations included 25 strains of aerobes isolated from the oral cavity, respiratory tract and abdominal cavity, as well as six standard strains. There were following aerobes: *Staphylococcus aureus* (four strains), *Corynbacterium* spp. (2), *Klebsiella pneumoniae* (3), *Acinetobacter baumanii* (2), *Escherichia coli* (6), *Pseudomonas aeruginosa* (6), *Pseudomonas stutzeri* (2) and six standard strains: *Staphylococcus aureus* ATCC 25923, *Entorococcus faecalis* ATCC 29212, *K. pneumoniae* ATCC 13883, *A. baumanii* ATCC 19606, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

The susceptibility of the aerobic bacteria to the derivatives was determined using agar dilution technique with Mueller–Hinton agar [11,12]. It uses sterile distilled water for ultimate dilutions. The inoculum containing 10^6 CFU per spot was applied to the agar plates with Steers replicator. The inoculated agar plates and "blank" agar plates were incubated for 24 h at 37 °C. The minimal incubatory concentration MIC was defined as the lowest concentration of the derivative that inhibited growth of aerobic bacteria.

4. Results and discussion

The investigations of anaerobic bacteria susceptibility to the compounds **1**, **5** and **6** were summarized in Table 2. The results were compared with that obtained while testing the susceptibility of the same bacteria to metronidazol. Despite of having been in therapeutic use for many years, metronidazol maintains high activity towards the anaerobes, esp. The Gram-negative ones, incl. Bacterioides genus [13–20]. It seems to be important, as the rod-bacteria strains from Bacterioides gen. are frequently involved in the infections, and appear to be resistant towards commonly used antibiotics.

The tests showed, that 22 from among 25 strains used, i.e. 88% were susceptible to metronidazol. This is consistent to the other reports [13–15,17–20]. The highest refractoriness exhibited the Gram-positive rod-bacteria strains from Propionibacterium gen. (MIC > 200 μg/ml). This is confirmed by other reports [15,16,19].

The highest, from three tested, activity towards the anaerobes showed derivative **6**. Low concentration ranging from 12.5 to 25 μg/ml inhibited growth of four (16%) strains, and the higher ones –50 and 100 μg/ml inhibited growth of four (16%) and three (12%) strains. Gram-positive rod-bacteria from Propionibacterium gen., whose was inhibited by metronidazol in concentrations higher than 200 μg/ml, appeared to be susceptible to the concentrations 50–200 μg/ml. Within the limits 12.5–200 μg/ml derivative **6** showed the activity towards 16 (16%) from among all the anaerobes tested. To inhibiting growth of the remaining nine (36%) strains the concentrations > 200 μg/ml were necessary. The growth inhibition of two from the standard strains, i.e. Propionibacterium acnes ATCC 11827, and Peptostreptococcus anaerobius ATCC 27337 was induced by concentration of 200 μg/ml.

The derivatives **1** and **5** were of similar activity towards the anaerobes. The activity of **1** appeared to be slightly higher. The lowest concentrations ≤ 6.2–12.5 μg/ml inhibited growth of three (12%) strains, and 50 μg/ml of one (4%) strain. From among the remaining 21 strains, 10 (40%) were inhibited by concentration 200 μg/ml and the last 11 (44%) strains—concentrations > 200 μg/ml.

Compound **5** inhibited growth of three (12%) strains in low concentrations 12.5–25 μg/ml. The remaining 22 (88%) strains required for growth inhibition the use of concentrations ≥ 200 μg/ml. MIC value for seven (25%) strains was 200 μg/ml.

All the derivatives evaluated were more active towards the tested Gram-positive microbes. The strains of Gram-positive rod-bacteria from Propionobacterium granulosum genus were more sensitive to the derivatives tested than to metronidazol. The growth of above-mentioned bacteria was inhibited by concentrations of the derivatives **5** and **6** within the limits 25–200 μg/ml, while by **1** within 12.5–200 μg/ml. The necessary metronidazol concentrations were ≥ 200 μg/ml. The tested strains of Gram-positive granulosum anaerobes were susceptible to metronidazol in concentrations ≤ 6.2 μg/ml, which is consistent to the results reported by other authors [13,17,18]. However, the lower in comparison with ours —susceptibility results were obtained by Goldstein et al. [15], Hoellman et al. [16] and Wexler et al. [20] (MICs for 90% strains were 0.25–2.0 μg/ml). From among the derivatives tested the highest activity towards the strains from Peptostreptococcus gen. was shown by compound **6**, which inhibited growth of three (60%) strains in concentrations from 12.5 to 25 μg/ml.

From Gram-negative rod-bacteria, the comparable susceptibility to metronidazol and two derivatives tested was exhibited by the strain from Porphyromonas loescheii gen. The growth inhibiting metronidazol concentration for this strain was 6.2 μg/ml, while that of derivatives **1** and **5** –12.5 μg/ml:

- the three derivatives evaluated **1**, **5**, **and 6** showed the differentiated activity towards anaerobic bacteria;
- the highest activity towards Gram-negative microbes was shown by derivative **6**;
- all the derivatives evaluated showed higher activity towards the Gram-positive anaerobes;
- the aerobic bacteria tested showed no susceptibility towards derivatives **1**, **5**, **6** within the limits of concentrations used.

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