Synthesis and Antibacterial Activity of Novel Pyridine and Pyrazine Derivatives Obtained from Amidoximes

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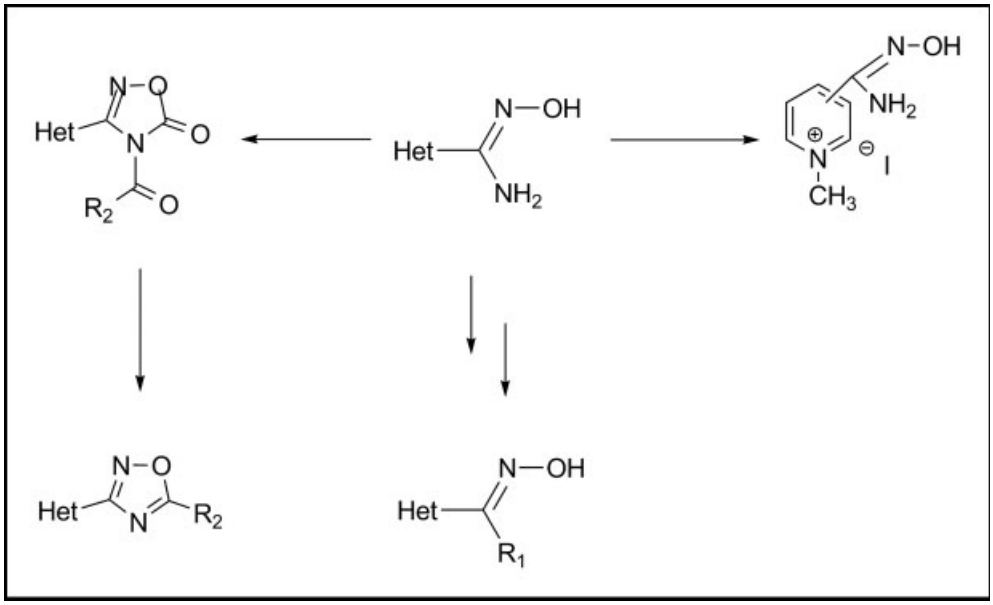

The new pyridine, 4-pyridine *N*-oxide and pyrazine derivatives exhibiting an antibacterial activity have been synthesized. Amidoximes were transformed into *N*-hydroxyimidoyl chlorides and then into appropriate oximes. Upon treatment of pyridinecaboxamidoximes with methyl iodide 1-methylpyridynium iodides were formed. Reaction of amidoximes with various carbamoyl chlorides led to corresponding 5-aminocarbonyl-1,2,4-oxadiazoles. Some of carboxamides have undergone thermal decarboxylation to tertiary amines. The newly synthesized compounds were tested *in vitro* for their tuberculostatic activity. MIC of the most active compound **9** was 12.5 μg/mL for $H_{37}Rv$ strain. Their activity towards 25 strains of anaerobic and 25 strains of aerobic bacteria was also studied. Derivative **18** was active against both aerobic and anaerobic types of the bacteria.

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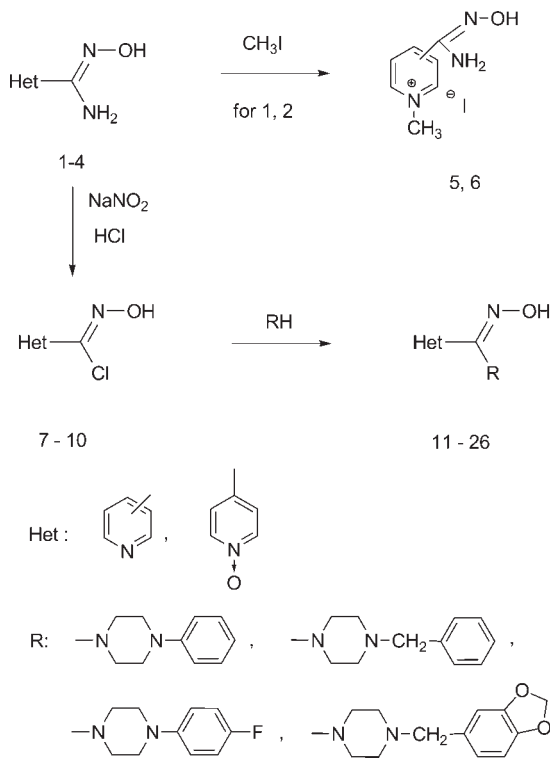
INTRODUCTION

Infections caused by *Mycobacteria* (*M. tuberculosis*, *M. avium*, *M. kansasaii*, *M. bovis*) are known to express multidrug-resistance toward most chemicals, disinfectants and number of antibiotics and chemotherapeutics as a consequence of single point mutations [1]. This phenomenon is very dangerous especially for HIV-infected individuals because of significantly increased risk of the infection progress to active disease. Other pathogenic strains, e.g. *Streptococcus pneumonia*, *Staphylococcus aureus*, *Enterococcus faecium*, also exhibit multidrug-resistance but mediated by other gene changes [2]. Thus antimicrobial therapy with a combination of different drugs is required and new active compounds for first-line therapy are needed.

In the last few years, many isoniazide and pyrazinamide derivatives have been synthesized. Few groups demonstrate high activity against *M. tuberculosis*: 2′-monosubstituted isonicotinohydrazides [3], isonicotinoylhydrazones [4], pyridine-2-carboxamidrazones [5,6] and 2-pyrazine or 3-pyridine-1,2,4-oxadiazole-5-ones [7]. Various pyridinium halides also exhibit antibacterial activity [8–10]. In our previous papers, we reported tuberculostatic activity of 4-mono- and 4-disubstituted pyridoyl thiosemicarbazides [11] and some derivatives

Scheme 1



of 5-substituted 3-pyrazine-1,2,4-oxadiazoles [12]. Further studies on antituberculosis agents active against multidrug- resistant strains prompted us to synthesize a series of new pyridine, pyridine-4-*N*-oxide and pyrazine derivatives possessing substituted amidoxime group or 1,2,4-oxadiazole ring substituted with amide in 4-position or amine group in 5-position. Both types of compounds could be synthesized from the same substrates, derivatives possessing carboxamidoxime functional group [13]. We now report on the synthesis and *in vitro* evaluation of their antibacterial and antituberculostatic activities *in vitro*.

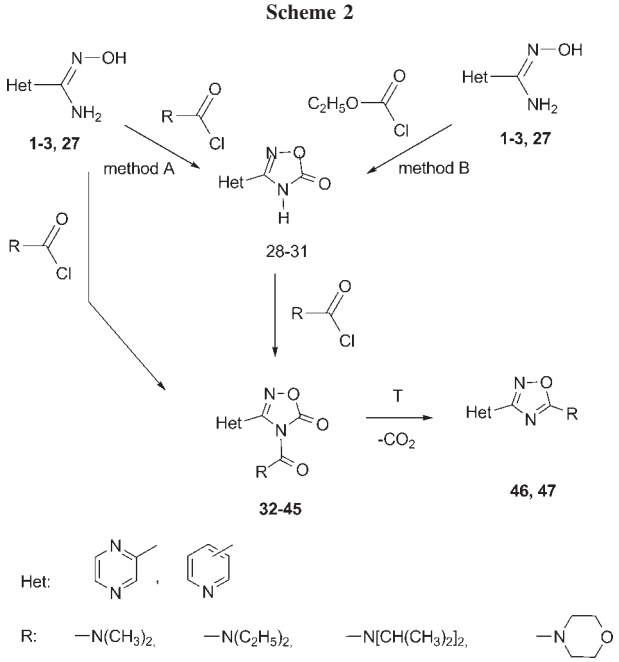
RESULTS AND DISCUSSION

Carboxamidoximes **1–4** and **27** were obtained in reaction of appropriate carbonitriles with hydroxylamine. Methylation of 3- and 4-pyridinecarboxamidoximes **2**, **3** on pyridine nitrogen atom was performed with methyl iodide in anhydrous dioxane and resulted in 1-methylpyridynium iodides **5**, **6** formation (Scheme 1).

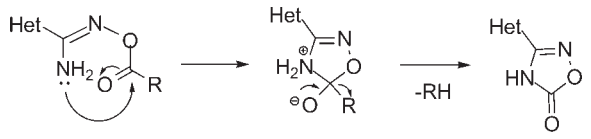
Compounds **2** and **3** also reacted with methyl iodide in alkaline solution but both pyridine nitrogen and amidoxime oxygen underwent methylation. *N*-hydroxycarboximidoylchlorides **7–10** were prepared from corresponding carboxamidoximes on treatment with sodium nitrite in hydrochloric acid solution at 0°C. Syntheses performed for pyridine derivatives required an use of sodium hydrogen carbonate to isolate products from reaction mixtures at pH 3. The obtained chlorides were used for the synthesis of appropriate oximes **11–26**. The reactions with secondary amines were performed in mole ratio 1:2 to neutralize hydrochloride generated during the reactions. Anhydrous dioxane was used as the solvent and reactions took about 15 min. In the case of oximes synthesized as pyridine *N*-oxide derivatives **23–26** refluxing time was prolonged to 1.5 h.

Amidoximes **1–4** and **27** were also treated with triple excess of appropriate carbamoyl chlorides in pyridine environment giving expected 3,4-disubstituted 5-oxo-[1,2,4]oxadiazoles **32–45** (Scheme 2) as a result of intramolecular nucleophilic attack of NH nitrogen electron pair for carbonyl carbon of carbamoyl moiety bound to OH oxygen instead proton. That attack followed substitution of OH proton and one of NH_2 protons by two carbamoyl moieties agreeably to mechanism proposed earlier by Marquez and DiPersia [14]. In two cases, the rate of that reaction was different resulting in formation of 3-monosubstituted 1,2,4-oxadiazole-5(4*H*)-ones **30** and **31** (method A). Those products forms probably as result of fast attack of NH_2 nitrogen electron pair for carbonyl carbon before NH_2 proton was substituted by carbamoyl (Scheme 3). Similar result was reached in control reactions between corresponding amidoximes and ethyl chloroformate (method B). Obtained compounds **28** and **29** were next transformed to 3,4-disubstituted derivatives by substitution in N4 position. Two amides **34** and **37** were undergone thermal decarboxylation at 215°C to tertiary amines **46** and **47**.

Scheme 2



Scheme 3



R: $N(CH_3)_2$, OC_2H_5

Characteristics of newly synthesized compounds have been presented in Table 1.

The investigations of aerobic and anaerobic bacteria susceptibility to the synthesized pyridine derivatives are summarized in Table 2. 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 μg/mL inhibited the growth of Gram-negative bacteria except single strains of *Bacteroides fragilis*, *B. forsythus* and *Fusobacerium necrophorum*. These results were 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 μg/mL). Among 26 tested derivatives 24 (92%) exhibited differential activity against anaerobic bacteria (8–52% of the tested strains). The anaerobes were the most susceptible at concentrations in ranges from $\leq$6.2 to 100 μg/mL to derivatives **24** and **26** (52% were susceptible) and to compound **9** (40% of susceptible strains). The aerobic bacteria were generally not susceptible to compounds **13** and **15** in mentioned range of concentrations. Among 24 derivatives active towards anaerobic bacteria, 21 were more effective to Gram-positive strains. Compounds **30** (MIC 25–100 μg/mL, 100% of susceptible strains), **9** and **24** (MIC $\leq$6.2–100 μg/mL, 89%) exhibited the highest activity. Derivatives **26**, **27**, and **29** were more active against Gram-negative anaerobic rods. Compound **27** was the most active one (MIC $\leq$6.2–100 μg/mL, 38%).

Only one from 30 (3%) tested compounds was active towards aerobic bacteria. Derivative **18** was active in concentration 50–100 μg/mL and inhibited the growth of 16% of the tested aerobic bacteria. Other compounds did not inhibit the growth of aerobic bacteria in the range of tested concentration ($\leq$6.2–200 μg/mL). Derivative **18** was 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 μg/mL). In the case of anaerobic *Fusobacterium nucleatum*, ATCC 25586 compounds **21** (MIC 100 μg/mL), **23** (MIC 100 μg/mL) and **15** (MIC 100 μg/mL) were active. Derivative **18** induced the growth inhibition of *Bacteroides vulgatus* ATCC 8482 in concentration of 100 μg/mL. That compound also inhibited the growth of two aerobic standard strains: *Klebsiella pneumoniae* ATCC 13883 and *Staphylococcus aureus* ATCC 25923 and MIC value for that derivative was 100 μg/mL in both casas.

The determined minimum concentrations inhibiting the growth of tuberculous strains (MIC) for most of the tested compounds were within the limits 12–100 μg/mL. MIC of the most active compound **9** was 12.5 μg/mL for H_{37}Rv strain and 25 μg/mL for other strains.

In conclusion, the present research showed that reaction between carboxamidoximes and carbamoyl chlorides can occur according to two different rates. One derivative (**18**) exhibited wide spectrum of antibacterial activity but it did not performe better that metronidazole against anaerobes and amikacin against aerobes. Other compound (**9**) exhibited interesting tuberculostatic activity and it can be good lead structure for further modifications.

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 of obtained compounds were in agreement with calculated values within ± 0.3 % range. ^{1}H NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on Varian Unity Plus (500 MHz) and Varian Gemini (200 MHz) instruments. IR Spectra (KBr) were determined as KBr pellets of the solids on a Satellite FT-IR spectrophotometer. Mass spectra for compounds **28**, **29**, and **46** were taken on Finingan MAT 95 by a chemical ionization method with isobutane. Melting points were determined on BOETIUS apparatus and were uncorrected.

Pyridinecarboxamidoximes (1–3). To a stirred solution of hydroxylamine hydrochloride (7 g, 0.1 mol) in methanol (50 mL) a solution of potassium hydroxide (6 g, 0.1 mol) in methanol was added. The precipitated potassium chloride was filtered off and appropriate pyridinecabonitrile (7 g, 60 mmol) was added to the clear filtrate. Reaction mixture was refluxed for 1 h and after cooling the final solid of **3** was filtered off, washed with water and dried at room temperature. The crude product was recrystallized to afford of bright leaflets (7.5 g). To isolate two other isomers final reaction mixtures were evaporated and 20 mL of water was added to the residue. The crude products were filtered off after cooling and purified by crystallization yielding 8 g of **1** and 6.7 g of **2**.

2-Pyridinecarboxamidoxime (1). This compound was obtained as colorless short needles. Yield 87%; m.p. 117–118°C. (ref. [17], m.p. 117°C).

3-Pyridinecarboxamidoxime (2). This compound was obtained as colorless small needles. Yield 73%; m.p. 131–133°C. (ref. [17], m.p. 131°C).

4-Pyridinecarboxamidoxime (3). This compound was obtained as colorless small needles. Yield 82%; m.p. 197–199°C. (ref. [17], m.p. 207°C).

Table 1

Characteristics of the newly synthesized derivatives.

No	Het	R	Mp [°C] Solvent	Yield [%]	Molecular formula	MW	Calcd/found					
							C		H		N	
4	1-oxide-pyridin-4-yl	NH_2	258-260 water	75	$C_6H_7N_3O_2$	153.14	47.05	47.11	4.61	4.65	27.44	27.49
5	3-(1-methyl)-pyridinium iodide	NH_2	165-167 methanol	90	$C_7H_{10}IN_3O$	279.07	30.13	30.10	3.61	3.56	15.06	15.12
6	4-(1-methy)-pyridinium iodide	NH_2	199-201 methanol	42	$C_7H_{10}IN_3O$	279.07	30.13	30.18	3.61	3.66	15.06	15.01
10	1-oxide-pyridin-4-yl	Cl	147-149 methanol	97	$C_6H_5ClN_2O_2$	172.57	41.76	41.73	2.92	2.81	16.24	16.28
11	pyridin-2-yl	4-phenyl-piperazin-1-yl	190-192 toluene	78	$C_{16}H_{18}N_4O$	282.33	68.06	68.12	6.43	6.29	19.85	19.88
12	pyridin-2-yl	4-benzyl-piperazin-1-yl	152-154 toluene	67	$C_{17}H_{20}N_4O$	296.36	68.89	68.84	6.80	6.83	18.91	18.96
13	pyridin-2-yl	4-(4-fluorophenyl-piperazin-1-yl	154-156 toluene	88	$C_{16}H_{17}FN_4O$	300.34	63.98	64.17	5.71	5.67	18.66	18.70
14	pyridin-2-yl	4-piperonyl-piperazin-1-yl	167-169 toluene	56	$C_{18}H_{20}N_4O_3$	340.37	63.51	63.48	5.92	6.09	16.46	16.54
15	pyridin-3-yl	4-phenyl-piperazin-1-yl	130-132 toluene	89	$C_{16}H_{18}N_4O$	282.33	68.06	67.81	6.43	6.51	19.85	19.99
16	pyridin-3-yl	4-benzyl-piperazin-1-yl	159-161 toluene	74	$C_{17}H_{20}N_4O$	296.36	68.89	68.93	6.80	6.73	18.91	18.86
17	pyridin-3-yl	4-(4-fluorophenyl-piperazin-1-yl	143-145 toluene	83	$C_{16}H_{17}FN_4O$	300.34	63.98	63.79	5.71	5.86	18.66	18.52
18	pyridin-3-yl	4-piperonyl-piperazin-1-yl	156-158 toluene	65	$C_{18}H_{20}N_4O_3$	340.37	63.51	63.45	5.92	6.08	16.46	16.42
19	pyridin-4-yl	4-phenyl-piperazin-1-yl	78-80 toluene	76	$C_{16}H_{18}N_4O$	282.33	68.06	68.31	6.43	6.41	19.85	19.88
20	pyridin-4-yl	4-benzyl-piperazin-1-yl	83-85 acetonitrile	86	$C_{17}H_{20}N_4O$	296.33	68.89	68.84	6.80	6.92	18.91	19.07
21	pyridin-4-yl	4-(4-fluorophenyl-piperazin-1-yl	167-169 acetonitrile	71	$C_{16}H_{17}FN_4O$	300.34	63.98	64.31	5.71	5.83	18.66	18.62
22	pyridin-4-yl	4-piperonyl-piperazin-1-yl	150-152 toluene	60	$C_{18}H_{20}N_4O_3$	340.37	63.51	63.33	5.92	5.87	16.46	16.59
23	1-oxide-pyridin-4-yl	4-phenyl-piperazin-1-yl	195-197 DMF/water	49	$C_{16}H_{18}N_4O_2$	298.33	64.41	64.57	6.08	6.05	18.78	18.51
24	1-oxide-pyridin-4-yl	4-benzyl-piperazin-1-yl	194-196 DMF/water	33	$C_{17}H_{20}N_4O_2$	312.36	65.36	65.42	6.45	6.31	17.94	18.06
25	1-oxide-pyridin-4-yl	4-(4-fluorophenyl-piperazin-1-yl	232-234 methanol	36	$C_{16}H_{17}FN_4O_2$	316.34	60.74	60.78	5.42	5.56	17.71	17.60
26	1-oxide-pyridin-4-yl	4-piperonyl-piperazin-1-yl	165-167 toluene	33	$C_{18}H_{20}N_4O_4$	356.37	60.66	60.29	5.66	5.78	15.72	15.48
28	pyridin-3-yl	–	229-231 water	A: 28 B: 52	$C_7H_5N_3O_2$	163.13	51.54	51.46	3.09	3.11	25.76	25.83
29	pyridin-4-yl	–	240-245 water	A: 26 B: 55	$C_7H_5N_3O_2$	163.13	51.54	51.47	3.09	3.17	25.76	25.88
31	pyridin-2-yl	–	185-190 water	97	$C_7H_5N_3O_2$	163.13	51.54	51.43	3.09	3.08	25.76	25.79
32	pyrazin-2-yl	$(C_2H_5)_2N$	133-134 toluene	50	$C_{11}H_{13}N_5O_3$	263.25	50.19	50.11	4.98	4.79	26.60	26.52
33	pyridin-2-yl	$(C_2H_5)_2N$	111-112 ethanol/water	73	$C_{12}H_{14}N_4O_3$	262.26	54.96	54.98	5.38	5.27	21.36	21.38
34	pyridin-3-yl	$(C_2H_5)_2N$	117-118 ethanol/water	52	$C_{12}H_{14}N_4O_3$	262.26	54.96	54.85	5.38	5.39	21.36	21.25
35	pyridin-4-yl	$(C_2H_5)_2N$	88-89 ethanol/water	52	$C_{12}H_{14}N_4O_3$	262.26	54.96	54.87	5.38	5.26	21.36	21.17
36	pyrazin-2-yl	$[(CH_3)_2CH]_2N$	105-107 ethanol/water	32	$C_{13}H_{17}N_5O_3$	291.31	53.60	53.69	5.88	5.97	24.04	24.06
37	pyridin-2-yl	$[(CH_3)_2CH]_2N$	145-148 ethanol/water	77	$C_{14}H_{18}N_4O_3$	290.32	57.92	57.90	6.25	6.32	19.30	19.35
38	pyridin-3-yl	$[(CH_3)_2CH]_2N$	66-69 *CCh*	62	$C_{14}H_{18}N_4O_3$	290.32	57.92	57.97	6.25	6.36	19.30	19.42
39	pyridin-4-yl	$[(CH_3)_2CH]_2N$	110-112 *CCh*	9	$C_{14}H_{18}N_4O_3$	290.32	57.92	57.83	6.25	6.14	19.30	19.32
40	pyrazin-2-yl	$(CH_3)_2N$	182-184 water	21	$C_9H_9N_5O_3$	235.20	45.96	45.89	3.86	3.77	29.78	29.69
42	pyridin-3-yl	$(CH_3)_2N$	101-103 water	5	$C_{10}H_{10}N_4O_3$	234.21	51.28	51.47	4.30	4.35	23.92	23.71
43	pyridin-4-yl	$(CH_3)_2N$	129-131 water	13	$C_{10}H_{10}N_4O_3$	234.21	51.28	51.39	4.30	4.21	23.92	23.80
44	pyrazin-2-yl	morpholin-4-yl	170-171 A: water B: *CCh*	A: 6 B: 57	$C_{11}H_{11}N_5O_4$	277.24	47.66	47.54	4.00	3.98	25.26	25.37
45	pyridin-2-yl	morpholin-4-yl	143-145 *CCh*	54	$C_{12}H_{12}N_4O_4$	276.25	52.17	52.29	4.38	4.53	20.28	20.20
46	pyridin-3-yl	$(C_2H_5)_2N$	30-31 *CCh*	39	$C_{11}H_{14}N_4O$	218.26	60.53	60.55	6.47	6.54	25.67	25.78
47	pyridin-2-yl	$[(CH_3)_2CH]_2N$	80-83 *CCh*	51	$C_{13}H_{18}N_4O$	246.31	63.39	63.24	7.37	7.24	22.75	22.56

Table 2

In vitro antibacterial activity of newly synthesized compounds

	MIC [μg/mL]														
	G+					G−									
No	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
A															
5	12.5	≤6.2	≥200	100	50	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
6	≤6.2	≤6.2	≥200	50	≥200	25	50	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
7	25	≤6.2	≥200	≥200	≥200	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
8	25	≤6.2	≥200	≥200	25	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	100	
9	50	≤6.2	≥200	12.5	12.5	≤6.2	≤6.2	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
10	50	≤6.2	25	25	≥200	12.5	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
11	≤6.2	12.5	≥200	12.5	≥200	12.5	100	≥200	≥200	≥200	≥200	100	≥200	≥200	
12	≥200	≤6.2	≥200	25	100	100	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
14	25	25	≤6.2	12.5	12.5	12.5	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
16	≥200	≤6.2	100	100	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
17	≥200	12.5	≥200	≤6.2	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
18	≥200	25	≥200	≥200	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
19	≥200	50	≥200	25	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
20	≥200	50	≥200	25	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
21	≥200	≤6.2	12.5	≤6.2	≥200	12.5	≥200	≥200	≥200	≥200	≥200	≥200	≥200	≥200	
22	12.5	≤6.2	≥200	100	≥200	100	≥200	50	≥200	≥200	≥200	≥200	≥200	≥200	
23	≥200	50	25	25	≥200	≤6.2	≤6.2	≥200	12.5	≤6.2	≥200	≥200	≥200	100	
24	100	≤6.2	≤6.2	25	25	≤6.2	≤6.2	≥200	≤6.2	≤6.2	≥200	≥200	≥200	100	
25	≤6.2	≤6.2	≥200	≤6.2	≤6.2	≤6.2	≤6.2	≥200	≤6.2	≤6.2	≥200	≥200	≥200	≥200	
26	≥200	≤6.2	–	25	≥200	≤6.2	≤6.2	≥200	≤6.2	≤6.2	≥200	≥200	50	50	
27	≥200	≤6.2	≤6.2	≥200	≥200	≤6.2	12.5	≥200	50	12.5	≥200	≥200	100	100	
28	≥200	25	50	100	100	12.5	25	≥200	≥200	25	≥200	≥200	100	50	
29	≥200	12.5	12.5	≥200	≥200	≤6.2	≤6.2	≥200	≥200	50	≥200	≥200	≥200	100	
30	50	50	25	100	100	≤6.2	50	≥200	≥200	100	≥200	≥200	≥200	100	
Metronidazole[a]	0.8	0.4	1.6	6.2	12.5	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	≤0.4	
	1	2				3	4	5	6	7					
B															
18	100	100				≥200	50	≥200	≥200	≥200					
Amikacin[b]	≤6.2	50				≤6.2	≤6.2	≤6.2	≤6.2	12.5					

A: (1) *Peptostreptococcus magnus*, (2) *Peptostreptococcus micros*, (3) *Actinomyces israelii*, (4) *Actinomyces naeslundii*, (5) *Propionibacterium acnes*, (6) *Prevotella bivia*, (7) *Prevotella buccalis*, (8) *Prevotella intermedia*, (9) *Prevotella loescii*, (10) *Porhyromonas asacharolytica*, (11) *Fusobacterium nucleatum*, (12) *Fusobacterium necrophorum*, (13) *Bacteroides forsythus*, (14) *Bacteroides fragilis*.

B: (1) *Staphylococcus aureus*, (2) *Corynebacterium spp*., (3) *Klebsiella pneumonia*, (4) *Acinetobacter baumanii*, (5) *Acinetobacter baumanii*, (6) *Pseudomonas aeruginosa*, (7) *Pseudomonas stutzeri*.

[a] Metronidazole (Sigma).

[b] Amikacin sulfate salt (Sigma).

1-Oxy-isonicotincarboxamidoxime (4). A 7.2 g (60 mmol) quantity of 4-cyanopyridine N-oxide was dissolved in 50 mL of hot water and then water solution of hydroxylamine was added in small portions with stirring. Hot reaction mixture was left at room temperature for 1 h then cooled and precipitate was filtered and recrystallized giving 6.85 g of small colorless needles. Hydroxylamine solution was prepared by mixing 7 g (0.1 mol) of hydroxylamine hydrochloride in 10 mL of water with 6 g (0.1 mol) of potassium hydroxide in 10 mL of water. IR: 3416, 3296, 3180, 2834, 1644, 1608, 1500, 1439, 1379, 1226, 1189, 1099, 1036, 946, 863 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 6.03 (s; 2H, NH_2), 7.65 (m; 2H, 4-pyridyl), 8.20 (m; 2H, 4-pyridyl), 10.07 (s; 1H, OH) ppm.

***N*-hydroxycarbamimidoyl-1-methyl-pyridynium iodides (5, 6).** 1.3 g (10 mmol) of **2** or **3** was dissolved in hot anhydrous dioxane. After cooling to room temperature 2.5 mL (40 mmol) of methyl iodide was added. Reaction mixture was refluxed for 1 h then left at room temperature for next 1 h. After cooling precipitate was filtered and recrystallized to afford 2.6 g (**5**) and 2.7 g (**6**) of the product.

N-hydroxycarbamimidoyl-1-methyl-3-pyridynium iodide (5). This compound was obtained as light yellow prisms. IR: 3389, 3286, 1639, 1592, 1506, 1462, 1416, 1361, 1299, 1206, 953, 898, 876, 669 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.37 (s; 3H, N^+CH_3), 6.33 (s; 2H, NH_2), 8.14 (q; 1H, 3-pyridyl, J_1 8.3 Hz, J_2 6 Hz), 8.70 (d; 1H, 3-pyridyl, *J* 8.3 Hz), 8.96 (d; 1H, 3-pyridyl, *J* 6 Hz), 9.19 (s; 1H, 3-pyridyl), 10.39 (s; 1H,OH) ppm.

N-hydroxycarbamimidoyl-1-methyl-3-pyridynium iodide (6). This compound was obtained as light yellow prisms. IR: 3462, 3342, 1649, 1624, 1557, 1525, 1410, 1360, 1289, 1225, 1196, 1081, 952, 839, 824 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 4.31 (s; 3H, N^+CH_3), 6.42 (s; 2H, NH_2), 8.26 (d; 2H, 4-pyridyl, *J* 6.4 Hz), 8.94 (d; 2H, 4-pyridyl, *J* 6.4 Hz), 10.92 (s; 1H, OH) ppm.

***N*-Hydroxy-pyridinecarboximidoyl chlorides (7–9).** Appropriate pyridinecarboxamidoxime **1–3** (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 (1.6 g, 23 mmol) in 10 mL of water was added dropwise and reaction mixture was stirred for 1 h at 0°C. Next saturated solution of sodium hydrogen carbonate was slowly added to the reaction mixture until pH 3 was reached. The precipitate was filtered, washed with ice-cold water and purified by crystallization giving 2.8 g (**7**), 1.6 g (**8**), and 2.5 g (**9**) of the product.

N-Hydroxy-2-pyridinecarboximidoyl chloride (7). This compound was obtained as white small crystals. Yield: 80%; m.p. 120–122°C (ref. [18], m.p. 126–128°C.

N-Hydroxy-2-pyridinecarboximidoyl chloride (8). This compound was obtained as white small crystals. Yield: 80%; m.p. 129–131°C (ref. [18], m.p. 142–145°C).

N-Hydroxy-2-pyridinecarboximidoyl chloride (9). This compound was obtained as white small crystals 138–139°C (ref. [18], m.p. 148–150°C).

1-Oxide-*N*-hydoxy-4-pyridinecarboximidoyl chloride (10). 5.6 g of **4** (10 mmol) was dissolved in 40 mL of concentrated hydrochloric acid. The reaction mixture was cooled to temperature 0°C. Next sodium nitrite (5.5 g, 80 mmol) in water (10 mL) was added dropwise and mixture was stirred for 0.5 h. The precipitate was filtered and washed with ice-cold water. The crude product was recrystallized yielding bright needles (4.2 g). IR: 3098, 2520, 1603, 1550, 1476, 1444, 1232, 1213, 1183,1029, 959, 838, 623 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.73 (d; 2H, 4-pyridyl, *J* 7.5 Hz) 8.26 (d;2H, 4-pyridyl, *J* 20 Hz), 12.85 (s;1H, OH) ppm.

General procedure for the synthesis of pyridylmethanone oximes (11–26). A 0.78 g (5mmol) quantity of carboximidoyl chlorides **7–10** was dissolved in 10 mL of anhydrous dioxane. Next 10 mmol of appropriate secondary amine was added dropwise. Solid amines, 1-(4-flourophenyl)piperazine and 1-piperonylpiperazine, were dissolved in a small volume of the solvent (5 mL). The reaction mixture was heated under reflux for 15 min. In the case of 1-oxy-isonicitin derivatives, **23–26** refluxing time was prolonged for 1.5 h. The solvent was evaporated and 40 mL of ice-cold water was added to the residue. The precipitate was filtered, washed with water and recrystallized from suitable solvent yielding the solid.

4-Phenylpiperazin-1-yl-pyridin-2-yl-methanone oxime (11). This compound was obtained as white powder. IR: 3457, 2844, 1615, 1595, 1502, 1448, 1382, 1340, 1276, 1236, 1167, 1153, 951, 789, 754, 685 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.27 (s; 8H, NCH_2), 6.89–7.00 (m; 3H, ArH), 7.28 (m; 3H, 2H ArH and 1H 2-pyridyl), 7.38 (M; 1H, 2-pyridyl), 7.61 (m; 1H, 2-pyridyl), 7.85 (m; 1H, 2-pyridyl), 8.72 (s; 1H, OH) ppm.

4-Benzylpiperazin-1-yl-pyridin-2-yl-methanone oxime (12). This compound was obtained as white powder. IR: 3168, 3053, 2826, 1621, 1588, 1566, 1447, 1432, 1385, 1275, 1172, 1140, 966, 943, 791, 754, 704 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.53 (s; 4H, NCH_2), 3.09 (s; 4H, NCH_2), 3.57 (s; 2H, NCH_2Ar), 7.31 (s; 6H, 5H ArH and 1H 2-pyridyl), 7.53 (s; 1H, 2-pyridyl), 7.79 (s; 1H, 2-pyridyl), 8.69 (s; 1H, 2-pyridyl), 9.18 (s;1H,OH) ppm.

4-(4-Fluorophenyl)piperazin-1-yl-pyridin-2-yl-methanone oxime (13). This compound was obtained as white powder. IR: 3207, 2828, 1631, 1512, 1455, 1434, 1390, 1236, 1170, 988, 953, 818, 789 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): 3.15–3.23 (dd; 8H, NCH_2, J_1 38 Hz, J_2 5 Hz), 6.90 (m; 4H, ArH), 7.50 (d; 3H, 2-pyridyl, *J* 7.8 Hz), 7.84 (m; 1H, 2-pyridyl), 8.72 (s; 1H, OH) ppm.

4-Piperonylpiperazin-1-yl-pyridin-2-yl-methanone oxime (14). This compound was obtained as white powder. IR: 3179, 2831, 1608, 1590, 1487, 1439, 1370, 1244, 1169, 1141, 1039, 975, 799 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.49 (s; 4H, NCH_2), 3.08 (s; 4H, NCH_2), 3.47 (s;2H, NCH_2Ar), 5.93 (s; 2H, OCH_2O), 6.73 (s; 2H, ArH), 6.87 (s; 1H, ArH), 7.33–7.53 (m; 2H, 2-pyridyl), 7.80 (s;1H, 2-pyridyl), 8,69 (s;1H, 2-pyridyl), 9.07 (s; 1H, OH) ppm.

4-Phenylpiperazin-1-yl-pyridin-3-yl-methanone oxime (15). This compound was obtained as white powder. IR: 3151, 3037, 2843, 1599, 1497, 1446, 1337, 1242, 1153, 1014, 923, 899, 765, 695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.21, (s; 4H, NCH_2), 3.57 (s; 4H, NCH_2), 6.89–6.95 (m; 3H, ArH), 7.28–7.41 (m;4H, 2H ArH and 2H 3-pyridyl), 7.86 (s; 1H, OH), 8.75 (m; 2H, 3-pyridyl) ppm.

4-Benzylpiperazin-1-yl-pyridin-3-yl-methanone oxime (16). This compound was obtained as white powder. IR: 3173, 2820, 1615, 1593, 1454, 1416, 1380, 1266, 1157, 1142, 1031, 1010, 965, 742, 714, 699 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.47 (s; 4H, NCH_2), 3.05 (s; 4H, NCH_2), 3.54 (s; 2H, NCH_2ArH), 7.25–7.37 (m; 6H, 5H ArH and 1H 3-pyridyl), 7.80 (m; 1H, 3-pyridyl), 8.37 (s; 1H, OH), 8.65 (m; 2H, 3-pyridyl) ppm.

4-(4-Fluorophenyl)piperazin-1-yl-pyridin-3-yl-methanone oxime (17). This compound was obtained as white powder. IR: 3176, 3056, 2839, 1624, 1592, 1510, 1417, 1382, 1267, 1235, 1152, 1025, 982, 957, 815, 713 cm^{-1}; ^1H NMR 500 MHz, $CDCl_3$: 3.15 (d; 8H, NCH_2, *J* 48 Hz), 6.87–6.90 (m; 4H, ArH), 7.42 (m; 1H, 3-pyridyl), 7.88 (d; 1H, 3-pyridyl, *J* 7 Hz), 8.22 (brs;1H,OH) 8.70 (d; 2H, 3-pyridyl, *J* 45 Hz).

4-Piperonylpiperazin-1-yl-pyridin-3-yl-methanone oxime (18). This compound was obtained as white powder. IR: 3162, 2827, 1600, 1592, 1487, 1440, 1368, 1265, 1246, 1159, 1142, 1033, 969, 921, 869, 809, 714 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.45 (s; 4H, NCH_2), 3.04 (s; 4H, NCH_2), 3.42 (s; 2H, NCH_2Ar), 5.92 (s; 2H, OCH_2O), 6.72 (s; 2H, ArH), 6.84 (s; 1H, ArH), 7.37 (s; 1H, 3-pyridyl), 7.81 (s; 1H, 3-pyridyl), 8.18 (brs; 1H, OH), 8.62–8.69 (d; 2H, 3-pyridyl, *J* 33 Hz) ppm.

4-Phenylpiperazin-1-yl-pyridin-4-yl-methanone oxime (19). This compound was obtained as light beige powder. IR: 3063, 2889, 2851, 1633, 1600, 1496, 1410, 1383, 1243, 1150, 1023, 962, 927, 830, 760, 692 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.18 (s; 8H, NCH_2), 6.89 (m; 3H, ArH), 7.25 (t; 2H, ArH, *J* 7.5 Hz), 7.42 (d; 2H, 4-pyridyl, *J* 4.5 Hz), 8.03 (s; 1H, OH), 8.74 (d; 2H, 4-pyridyl, *J* 4.5 Hz) ppm.

4-Benzylpiperazin-1-yl-pyridin-4-yl-methanone oxime (20). This compound was obtained as small white crystals. IR: 3054, 2920, 2820, 1623, 1597, 1457, 1402, 1300, 1154, 1002, 967, 833, 743 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.50 (s; 2H, NCH_2), 2.60 (s; 2H, NCH_2), 3.06 (s; 2H, NCH_2), 3.45 (s; 2H, NCH_2), 3.57 (s; 1H, NCH_2Ar), 3.64 (s; 1H, NCH_2Ar), 7.26–7.39 (m; 7H, 5H ArH and 2H 4-pyridyl), 8.10 (brs; 1H, OH), 8.59 (d; 1H, 4-pyridyl, *J* 6 Hz), 8,68 (d; 1H, 4-pyridyl, *J* 5.5 Hz) ppm.

4-(4-Fluorophenyl)piperazin-1-yl-pyridin-4-yl-methanone oxime (21). This compound was obtained as white powder. IR: 3193, 3070, 2837, 1640, 1598, 1508, 1449, 1386, 1285, 1267, 1231, 1147, 1023, 959, 927, 825 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 3.15 (d; 8H, NCH_2, *J* 43 Hz), 6.83–7.14 (m; 4H, ArH), 7.26–7.51 (m; 3H, 2H 4-pyridyl and 1H OH), 8.15 (brs; 1H, OH) 8.75 (m; 2H, 4-pyridyl) ppm.

4-Piperonylpiperazin-1-yl-pyridin-4-yl-methanone oxime (22). This compound was obtained as white powder. IR: 3180, 3024, 2815, 1599, 1488, 1441, 1369, 1248, 1159, 1142, 1037, 973, 826 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.43 (s; 4H, NCH_2), 3.01 (s; 4H, NCH_2), 3.43 (s; 2H, NCH_2Ar), 5.92 (s; 2H, OCH_2O), 6.72 (s; 2H, ArH), 6.83 (s; 1H, ArH), 7.35 (s; 2H,4-pyridyl), 8.25 (s; 1H, OH), 8,69 (s; 2H, 4-pyridyl) ppm.

4-Phenylpiperazin-1-yl-1-oxide-pyridin-4-yl-methanone oxime (23). This compound was obtained as white powder. IR: 3141, 3027, 2832, 1597, 1481, 1453, 1383, 1235, 1173, 1157, 1012, 971, 860, 735 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 2.49 (s; 4H, NCH_2), 3.16 (s; 4H, NCH_2), 6.78 (t; 1H, ArH, *J* 7 Hz), 6.94 (d; 2H, ArH, *J* 8.5 Hz), 7.21 (t; 2H, ArH, J_1 8.5 Hz, J_2 7 Hz), 7.50 (d; 2H, 4-pyridine-N-oxide, *J* 6.5 Hz), 8.17 (d; 2H, 4-pyridine-N-oxide, *J* 6.5 Hz), 10.49 (s; 1H, OH) ppm.

4-Benzylpiperazin-1-yl-1-oxide-pyridin-4-yl-methanone oxime (24). This compound was obtained as white powder. IR: 3139, 3078, 2845, 1623, 1596, 1481, 1453, 1408, 1336, 1226, 1177, 1156, 1012, 928, 763, 632 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.05 (s; 4H, NCH_2), 3.17 (s; 4H, NCH_2), 3.33 (s; 2H, NCH_2Ar), 6.94–7.23 (m; 5H, ArH), 7.47 (d; 2H, 4-pyridine-N-oxide, *J* 6.5 Hz), 8.26 (d; 2H, 4-pyridine-N-oxide, *J* 6 Hz), 9.99 (s; 1H, OH) ppm.

4-(4-Fluorophenyl)piperazin-1-yl-1-oxide-pyridin-4-yl-methanone oxime (25). This compound was obtained as white powder. IR: 3174, 3059, 2848, 1637, 1614, 1442, 1375, 1361, 1221, 1186, 1147, 1037, 989, 965, 854, 834, 819 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 3.07 (dd; 8H, NCH_2, J_1 28 Hz, J_2 8 Hz), 6.94–7.06 (m; 4H, ArH), 7.45 (m; 2H, 4-pyridine-N-oxide), 8.25 (m; 2H, 4-pyridine-N-oxide), 9.98 (s;1H,OH) ppm.

4-Piperonylpiperazin-1-yl-1-oxide-pyridin-4-yl-methanone oxime (26). This compound was obtained as white powder. IR: 3055, 2901, 2839, 1612, 1488, 1439, 1369, 1230, 1179, 1156, 1035, 970, 854 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 2.44 (m; 4H, NCH_2), 2.89 (s; 2H, NCH_2Ar), 3.42 (m; 4H, NCH_2), 5.97 (s; 2H, OCH_2O), 6.73–6.87 (m;3H, ArH), 7.42 (dd; 2H, 4-pyridine-N-oxide, J_1 25 Hz, J_2 7 Hz), 8.20 (dd; 2H, 4-pyridine-N-oxide, J_1 27 Hz, J_2 7 Hz), 9.89 (s; 1H,OH) ppm.

Pyrazinecarboxamidoxime (27). This compound was obtained as light beige needles according to method described earlier [19]. Yield: 96%; m.p. 186–187°C (lit. ref. [19], m.p. 186–187°C).

1,2,4-Oxadiazol-5-ones (28, 29). *Method A*. In 10 mL of dry pyridine 0.01 mol of appropriate carboxamidoxime was dissolved and 0.03 mol of carbamoyl chloride was added. The mixture was refluxed for 3 h. Then pyridine was evaporated and residue was cooled. Precipitate was filtered, washed with cold water and recrystallized. *Method B*. In 10 mL of dry pyridine 5 mmol of appropriate carboxamidoxime was dissolved and 0.956 mL (10 mmol) of ethyl chloroformate was added. The mixture was refluxed for 6 h. Then pyridine was evaporated and residue was cooled. Precipitate was filtered, washed with cold water and recrystallized with addition of active carbon.

3-Pyridin-3-yl-4H-[1,2,4]oxadiazol-5-one (28). This compound was obtained as small beige needles. IR: 3060, 2936, 1772, 1604, 1558, 1511, 1477, 1416, 1359, 1223, 1141, 1124, 1051, 1029, 915, 892, 761 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 7.63 (t; 1H, 3-pyridyl, J_1 3.0 Hz, J_2 5.0 Hz), 8.17 (q; 1H, 3-pyridyl, J_1 4.7 Hz, J_2 1.8 Hz), 8.79 (d; 1H, 3-pyridyl, *J* 4.9 Hz), 8.97 (s; 1H, 3-pyridyl), 12.8–13.4 (brs; 1H, NH) ppm. MS: m/z 164 (100 MH^+), 120 (32.4).

3-Pyridin-4-yl-4H-[1,2,4]oxadiazol-5-one (29). This compound was obtained as small white needles. IR: 3104, 3057, 1636, 1547, 1471, 1409, 1308, 1259, 1223, 1151, 1099, 1000, 933, 884, 846, 778 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 7.75 (q; 2H, 4-pyridyl, J_1 1.8 Hz, J_2 2.7 Hz), 8.82 (q; 2H, 4-pyridyl, J_1 1.7 Hz, J_2 2.8 Hz), 12.40–13.40 (brs; 1H, NH) ppm. MS: m/z 164 (62.6 MH^+), 120 (100).

1,2,4-Oxadiazole-5-ones (30, 31). Synthesis was performed according to procedure described above for compounds **28**, **29** *method B*.

3-Pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (30). This compound was obtained as small beige crystals. Yield: 63%; m.p. 259–261°C (ref. [12], m.p. 260–262°C).

3-Pyridin-2-yl-4H-[1,2,4]oxadiazol-5-one (31). This compound was obtained as small beige needles. IR: 3061, 1788, 1585, 1567, 1496, 1460, 1419, 1301, 1114, 1096, 990, 953, 893, 798 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 7.62–7.86 (m; 1H, 2-pyridyl), 7.95–8.09 (m; 2H, 2-pyridyl), 8.75 (m; 1H, 2-pyridyl), 12.85–13.40 (brs; 1H, NH) ppm.

4-Aminocarbonyl-[1,2,4]oxadiazol-5-ones (32–41). Synthesis was performed according to procedure described for compounds **28**, **29** *method A*.

4-Diethylaminocarbonyl-3-pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (32). This compound was obtained as small colorless crystals. IR: 2981, 1790, 1733, 1584, 1568, 1474, 1453, 1425, 1378, 1268, 1181, 1166, 1147, 1018, 905, 857, 759 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.31–1.39 (m; 6H, $2CH_2CH_3$), 3.47–3.63 (m; 4H, $2NCH_2CH_3$), 8.63 (t; 1H, pyrazinyl, *J* 1.8 Hz) 8,79 (d, 1H, pyrazinyl, *J* 2.5 Hz), 9.28 (d; 1H, pirazyna, *J* 1.5 Hz) ppm.

4-Diethylaminocarbonyl-3-pyridin-2-yl-4H-[1,2,4]oxadiazol-5-one (33). This compound was obtained as small colorless crystals. IR 2977; 1780; 1724; 1565, 1476; 1454; 1426; 1400; 1272; 1212; 1177; 1059; 903; 859; 756 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): 1.31–1.39 (m; 6H, $2CH_2CH_3$), 3.44–3.55 (m; 4H, $2NCH_2CH_3$), 7.46–7.53 (m; 1H, 2-pirydyl), 7.86–7.94 (m; 1H, 2-pyridyl), 8.62 (m; 1H, 2-pyridyl) ppm.

4-Diethylaminocarbonyl-3-pyridin-3-yl-4H-[1,2,4]oxadiazol-5-one (34). This compound was obtained as small beige crystals. IR: 2976, 1786, 1722, 1589, 1550, 1426, 1383, 1273, 1255, 1211, 1140, 1056, 1023, 901, 857, 814, 796, 758 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.23–1.34 (m, 6H, $2CH_2CH_3$), 3.46–3.52 (m; 4H, $2NCH_2CH_3$), 7,46–7,53 (q; 1H, 3-pyridyl, J_1 5.1 Hz, J_2 3.0 Hz), 8.01–8.07 (m; 1H, 3-pyridyl), 8.81 (q; 2H, 3-pyridyl, J_1 1.8 Hz, J_2 3.3 Hz) ppm.

4-Diethylaminocarbonyl-3-pyridin-3-yl-4H-[1,2,4]oxadiazol-5-one (35). This compound was obtained as small beige crystals. IR: 2978, 1792, 1725, 1659, 1637, 1600, 1583, 1408, 1270, 1251, 1212, 1064, 991, 909, 859, 827, 780, 759 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.25–1.35 (q; 6H, $2CH_2CH_3$, J_1 7.1 Hz, J_2 6.3 Hz), 3.45 (m; 4H, $2NCH_2CH_3$), 7.57 (d; 2H, 4-pyridyl, *J* 5.0 Hz), 8.83 (d; 2H, 4-pyridyl, *J* 6.2 Hz) ppm.

4-Diisopropylaminocarbonyl-3-pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (36). This compound was obtained as beige powder. IR: 2995, 1801, 1721, 1581, 1434, 1375, 1312, 1245, 1206, 1176, 1029, 1016, 900, 866, 825, 763 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.27–1.49 (m; 12H, $2CH(CH_3)_2$), 3,57–3,71 (m; 1H, $NCH(CH_3)_2$), 4,05–4,19 (m; 1H, $NCH(CH)_3$), 8,57 (q; 1H, pyrazinyl, J_1 1.5 Hz, J_2 1.0 Hz), 8.76 (d; 1H, pyrazinyl, *J* 2.5 Hz); 9,23 (d; 1H, pyrazinyl, *J* 1.5 Hz) ppm.

4-Diisopropylaminocarbonyl-3-pyridin-2-yl-4H-[1,2,4]oxadiazol-5-one (37). This compound was obtained as beige powder. IR: 3004, 2986, 2969, 1792, 1717, 1565, 1480, 1436, 1400, 1375, 1317, 1251, 1209, 1171, 1137, 1034, 897, 827, 789, 755 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.28–1.38 (q; 6H, $CH(CH_3)_2$ J_1 6.6 Hz, J_2 7.2 Hz), 1,5 (t; 6H, $CH(CH_3)_2$ *J* 5.6 Hz), 3.49–3.71 (m; 1H; $NCH(CH_3)_2$), 4.06–4.19 (m; 1H; $NCH(CH_3)_2$); 7.45 (m; 1H, 2-pyridyl), 7.82–7.91 (m; 1H, 2-pyridyl); 7.99 (d; 1H, 2-pyridyl, *J* 7.9 Hz), 8.60 (q; 1H, 2-pyridyl, J_1 1.1 Hz, J_2 3.7 Hz) ppm.

4-Diisopropylaminocarbonyl-3-pyridin-3-yl-4H-[1,2,4]oxadiazol-5-one (38). This compound was obtained as beige powder. IR: 2994, 2973, 2938, 1797, 1716, 1588, 1433, 1378, 1309, 1247, 1152, 1029, 898, 852, 793, 755 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 1.38 (d; 12H, $2CH(CH_3)_2$, *J* 6.7 Hz), 4.11–4.25 (m; 2H, $2NCH(CH_3)_2$), 7.43–7.50 (q; 1H, 3-pyridyl, J_1 5.1 Hz, J_2 2.6 Hz), 8.38 (d; 1H, 3-pyridyl, *J* 8.0 Hz), 8.71 (d; 1H, 3-pyridyl, *J* 4.0 Hz), 9.26 (s; 1H, 3-pirydyl).

4-Diisopropylaminocarbonyl-3-pyridin-4-yl-4H-[1,2,4]oxadiazol-5-one (39). This compound was obtained as beige powder. IR: 2983, 1798, 1716, 1534, 1419, 1375, 1308, 1246, 1200, 1027, 995, 901, 827, 794, 755 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.26(d; 3H, CH_3CH, *J* 6.8 Hz), 1.40 (d; 3H, CH_3CH, *J* 6.8 Hz), 1.47 (d; 6H, $CH(CH_3)_2$, *J* 6.8 Hz), 3.63–3.82 (m; 1H, $NCH(CH_3)_2$), 4.07–4.12 (m; 1H, $NCH(CH_3)_2$), 7.56 (d; 2H, 4-pyridyl, *J* 5.4 Hz), 8.82 (d; 2H, 4-pyridyl, *J* 5.4 Hz) ppm.

4-Dimethylaminocarbonyl-3-pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (40). This compound was obtained as small white needles. IR: 2931, 1784, 1725, 1587, 1484, 1450, 1384, 1274, 1193, 1144, 1059, 1019, 910, 868, 794, 765 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.17 (s; 3H, NCH_3), 3.21 (s; 3H, NCH_3), 8.64 (m; 1H, pyrazinyl), 8.77 (d; 1H, pyrazinyl, *J* 2.5 Hz), 9.22 (d; 1H, pyrazinyl, *J* 1.5 Hz) ppm.

4-Dimethylaminocarbonyl-3-pyridin-2-yl-4H-[1,2,4]oxadiazol-5-one (41). Yield: 35%; m.p. 139–141°C (ref. [13], m.p. 142–143°C).

4-Aminocarbonyl-[1,2,4]oxadiazol-5-ones (42–45). In 5 mL of dry pyridine 2.5 mmole of appropriate 1,2,4-oxadiazole-5-one **28–31** was dissolved and 7.5 mmol of carbamoyl chloride was added. The mixture was refluxed for 12–30 h. Reaction progress was monitored by TLC analysis. Pyridine was evaporated. Residue was cooled, filtered, washed, dried and recrystallized.

4-Dimethylaminocarbonyl-3-pyridin-3-yl-4H-[1,2,4]oxadiazol-5-one (42). This compound was obtained as beige powder. IR: 3060, 1772, 1728, 1604 1559, 1512, 1477, 1359, 1260, 1223, 1140, 1124, 1053, 1028, 910, 893, 829, 761 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.01 (s; 3H, NCH_3), 3.20 (s; 3H, NCH_3), 7.59–7.66 (q; 1H, 3-pyridyl, J_1 4.9 Hz, J_2 3.1 Hz), 8.04 (d; 1H, 3-pyridyl, *J* 8.0 Hz), 8.81 (m; 2H, 3-pyridyl) ppm.

4-Dimethylaminocarbonyl-3-pyridin-4-yl-4H-[1,2,4]oxadiazol-5-one (43). This compound was obtained as beige powder. IR: 2943, 1785, 1718, 1586, 1548, 1492, 1417, 1382, 1269, 1140, 1060, 1000, 903, 835, 759 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.18 (s; 3H, CH_3), 3.25 (s; 3H, CH_3), 7.56 (d, 2H, 4-pyridyl, *J* 6.2 Hz), 8.83 (d; 2H, 4-pyridyl, *J* 6.1 Hz) ppm.

4-(Morpholine-4-carbonyl)-3-pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (44). This compound was obtained as white powder. IR: 2922, 2867, 1808, 1735, 1584, 1421, 1377, 1263, 1233, 1178, 1114, 1017, 905, 840, 760 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.64–3.74 (m; 8H, morpholine), 8.88–8.95 (m; 2H, pyrazinyl), 9.26 (d; 1H, pyrazinyl, *J* 1.4 Hz) ppm.

4-(Morpholine-4-carbonyl)-3-pyrazin-2-yl-4H-[1,2,4]oxadiazol-5-one (45). This compound was obtained as white powder. IR: 2965, 2928, 1799, 1774, 1711, 1591, 1564, 1432, 1406, 1392, 1292, 1262, 1109, 1020, 1000, 900, 797, 755 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 3.61 (t, 2H, NCH_2, *J* 4.4 Hz), 3.76 (d; 2H, NCH_2, *J* 4.9 Hz), 3.85 (d; 4H, 2 OCH_2, *J* 3.3 Hz), 7.50 (t; 1H, 2-pyridyl, *J* 4.9 Hz); 7.85–8.02 (m; 2H, 2-pyridyl), 8.65 (d; 1H, 2-pyridyl, *J* 4.7 Hz) ppm.

5-Alkylamino-[1,2,4]oxadiazoles (46, 47). 3 mmole of compound **34** or **37** was heated in pressure vessel in silicon bath gradually to 215°C. Then vessel was cooled slowly. Decarboxylation product was separated from substrate and purified by column chromatography on silica gel using chloroform–ethyl acetate as liquid phase.

5-Diethylamino-3-pyridin-3-yl-[1,2,4]oxadiazole (46). This compound was obtained as beige powder. IR: 2972, 1636, 1594, 1579, 1519, 1439, 1355, 1261, 1214, 1137, 1083, 1022,

963, 883, 821, 759 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.20 (t; 6H, $2CH_2\underline{CH_3}$, *J* 7.0 Hz), 3.48–3.59 (q; 4H, $2N\underline{CH_2}CH_3$, J_1 7.3 Hz, J_2 7.0 Hz), 7.51–7.58 (q; 1H, 3-pyridyl, J_1 4,84 Hz, J_2 3,14 Hz,), 8.23 (d; 1H, 3-pyridyl, *J* 7.9 Hz); 8.72 (d; 1H, 3-pyridyl, *J* 4.8 Hz), 9.05 (s; 1H, 3-pyridyl) ppm. MS: m/z 219 ($100MH^+$).

5-Diisopropylamino-3-pyridin-2-yl-[1,2,4]oxadiazole (47). This compound was obtained as beige powder. IR: 2976, 1610, 1523, 1410, 1391, 1368, 1298, 1197, 1160, 1123, 1024, 990, 925, 807, 757 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 1.37 (d; 12H, $2CH(\underline{CH_3})_2$, *J* 6.8 Hz), 4.26 (t; 2H, $2NC\underline{H}(CH_3)_2$, *J* 6.5 Hz); 7.39 (q; 1H, 2-pyridyl, J_1 5.4 Hz, J_2 1.5 Hz), 7.82 (t; 1H, 2-pyridyl, *J* 7.1 Hz), 8.07 (d; 1H, 2-pyridyl, *J* 7.8 Hz), 8.80 (d; 1H, 2-pyridyl, *J* 4.4 Hz) ppm.

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: *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 [20,21]. 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.

Mycobacterium tuberculosis. The compounds were examined for their tuberculostatic activity towards *Mycobacterium tuberculosis* $H_{37}Rv$ strain and two ''wild'' strains isolated from tuberculotic patients: one (Spec. 210) resistant to p-aminosalicic acid (PAS), isonicotinic acid hydrazide (INH), etambutol (ETB) and rifampicine (RFP), another (Spec. 192) fully sensitive to the administrated 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 [22].

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