ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2015, Vol. 41, No. 4, pp. 402–408. © Pleiades Publishing, Ltd., 2015.
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Synthesis and Pharmacological Activity of *N*-Hetaryl-3(5)-Nitropyridines

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Received October 31, 2014; in final form, December 9, 2014

Abstract—Previously undescribed 2-, 4- or 6-substituted hetaryl-3(5)-nitropyridines were synthesized by the interaction of a number of chlorosubstituted 3(5)-nitropyridines with some diazoles or 3-chloropyridazin-6-one. In addition, pyrazolyl-3-nitropyridines were prepared by both the above method and cyclization of hydrazinopyridines, which, in turn, were synthesized by the treatment of chlorosubstituted 3-nitropyridines with hydrazine. It has been shown that these compounds have a moderate antibacterial activity against some pathogenic Gram-positive and Gram-negative bacteria (*Staphylococcus aureus* and *Escherichia coli*) and a strong protistocidal effect on protozoa species *Colpoda steinii* surpassing in this respect clinically used reference drugs.

Keywords: N-hetaryl-3(5)-nitropyridines, synthesis, hetarylation, nucleophilic substitution, cyclization, reduction, antimicrobial activity, Staphylococcus aureus, Escherichia coli, protistocidal activity, Colpoda steinii

DOI: 10.1134/S1068162015030048

INTRODUCTION

The creation of new natural and synthetic anti-infectious drugs is known to be one of the urgent problems of modern science because of, first of all, the wide spread of so-called resistant infections (bacterial, protozoal, viral, etc.) insensitive to drugs, which is one of the greatest threats to human health [1–5]. Multiresistant bacterial infection is of particular concern because it is the reason for an annual death of about 25000 patients, even in the European Union. The analogous problems are also typical for veterinary medicine due in particular to the spread of resistant forms of coccidia (parasitic unicellular protozoa species of the *Eimeria* class), which cause Coccidiosis epidemics and mass death of animals, birds, and fish [6–8].

Different heterocyclic compounds, particularly, imidazole, benzimidazole, and pyridine are of great interest in the search for new antimicrobial and antiprotozoal drugs. For example, compounds containing ureide [9] or aryloxyethyl [10–13] substituents, active against Gram-positive bacteria, have been recently found among benzimidazoles. It is also known that some derivatives of imidazole [14] and nitropyridines [15–19] have a pronounced coccidiostatic activity and affect significant pathogens, such as *Eimeria tenella*. It was shown over the last years that some complex substituted derivatives of imidazo[1,2-*a*]pyridine, which combine annelated imidazole and pyridine cycles in their structure, were especially strong coccidiostatics. These compounds were demonstrated to inhibit cGMP-dependent protein kinase (PKG) vital to the micropathogen [20, 21]. The most active among them are, however, potentially genotoxic [22, 23] and have relatively narrow range of actions [24]. Imidazo[1,2-*a*]pyridines without these disadvantages are not very active in vivo; they are only a few times more potent than coccidiostatic salinomycin [25].

In this work, we continued the search of anti-infectious preparations among nitrogen heterocycles and synthesized different previously unknown *N*-hetaryl-3)5-nitro(amino)pyridines (**II**)–(**VIII**), (**X**) containing the imidazole-1-yl, benzimidazol-1-yl, pyrazol-1-yl, or 3-chloropyridazin-6-on-2-yl group as the *N*-hetaryl substituent, and studied the antibacterial and anticoccidal activities of these *N*,*C*-bihetaryls.

RESULTS AND DISCUSSION

The initial compounds for the synthesis of hetarylpyridines were isomeric chloro-3(5)-nitropyridines. Their chlorine atom was subjected to the nucleophilic substitution by the *N*-hetaryl group

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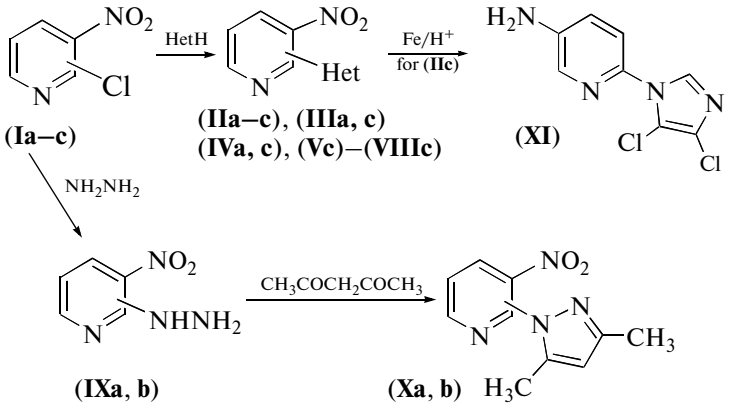
under the action of *N*-anions, which were generated from corresponding 1,2- and 1,3-diazoles or 3-chloropyridazin-6-one in the presence of bases, such as K_2CO_3 (method A) or NaH (method B). The reaction occurs in DMF with yields ranging from 65 to 90% under mild conditions.

Thus, 2-hetaryl-3-nitropyridines (**IIa**)–(**IVa**) were obtained from 3-nitro-2-chloropyridine (**Ia**) and 4-hetaryl-3-nitro- and 2-hetaryl-5-nitropyridines (**IIb, c**), (**IIIc**)–(**VIIIc**), from 4-chloro-3-nitro- and 2-chloro-5-nitropyridines (**Ib**, **c**) (Scheme 1).

In addition to the above one-step method, the synthesis of pyrazolyl nitropyridines was carried out by the two-step method. The first step was the substitution of the chlorine atom in 2-chloro- or 4-chloro-3-nitropyridine (**Ia** or **Ib**, respectively) by the hydrazine group and the second step was cyclization of hydrazine derivatives (**IXa**, **b**) with acetylacetone, with pyrazole ring closure being occurred in the presence of catalytic amounts of acetic acid. Both steps proceed very easily in ethanol with high yields (Scheme 1).

5-Amino-2-(4,5-dichloroimidazol-1-yl)-pyridine (**XI**) was synthesized by reduction of the nitro group in 2-(4,5-dichloroimidazol-1-yl)-nitropyridine (**IIc**) with metallic iron in an ethanol-HCl mixture (Scheme 1).



(**Ia**): 2-Cl; (**Ib**): 4-Cl; (**Ic**): 6-Cl
Het: (**IIa**–**c**): 4,5-dichloroimidazol-1-yl; (**IIIa**, **c**) 4-chloropyrazol-1-yl; (**IVa**, **c**) 4-bromopyrazol-1-yl; (**Vc**) imidazol-1-yl; (**VIc**) 3,5-dimethyl-4-chloropyrazol-1-yl; (**VIIc**) benzimidazol-1-yl; (**VIIIc**) 3-chloropyridzin-6-on-1-yl; (**Xa, b**) 3,5-dimethylpyrazol-1-yl

Scheme 1. Synthesis of 2-, 4-, and 6-hetaryl-substituted derivatives of 3(5)-nitro(amino)-pyridine.

The structure of the resultant hetarylpyridines was confirmed by ^{1}H NMR and IR spectroscopy and element analysis. Taking into account these data and the synthetic methods used, the structure does not give rise to any doubt. Chemical shifts of 2-, 4-, and 6-protons in the pyridine ring of these compounds strongly depends on the electron-acceptor properties of the hetaryl substituent and its position and vary in ranges of 8.69–9.43, 7.62–8.80, and 8.01–8.85 ppm, respectively. As can be expected, the compounds containing especially strong electron-accepting hetaryls, i.e., benzimidazole-2-yl and 3-chloropyridazin-6-on-1-yl, have the maximal shift values. Chemical shifts of protons of the hetaryl groups often significantly exceed those observed for similar protons in corresponding *N*-unsubstituted diazoles and 3-chloropyridazin-6-one. This is, obviously, due to the cumulative and overall deshielding influence of some factors, such as high electronegativity of the present 3(5)-nitropyridyl groups, the anisotropic effect of the lone pair of their ring nitrogen atom, and steric interactions of the hetaryl fragments with the nitro group, if it is in an adjacent position. These factors can significantly impede the conjugation of these groups with the pyridine system. The reduction of nitrocompound (**IIc**) to amino-derivative (**XI**) due to the strong influence of the amino group leads to a significant enhancement of the shielding of protons and the pronounced shift of NMR signals in a strong field, i.e., in the region of $\delta \leq$ 7.85 ppm. In this case, the NMR spectrum contains the additional signal of the primary amino group in the form of a two-proton singlet at 5.57 ppm, and the IR spectrum contains the corresponding two strongly broadened and significantly overlapping bands of symmetric and asymmetric bands of stretching vibrations of the NH bonds at 3295 and 3410 cm^{-1}.

The biological properties of synthesized *N*-hetaryl-3(5)-nitropyridines (**II**)–(**VIII**), and (**X**) were studied with on example of their antimicrobial and protistocidal activity. The antimicrobial activity was studied in test cultures of two standard bacterial strains *Staphylococcus aureus* P-209 and *Escherichia coli* 078

Table 1. Antibacterial and protistocidal activity of (*N*-hetaryl)-3(5)-nitropyridines

Compound	Minimal inhibiting concentration (MIC), μg/mL		
	S. aureus P-209	*E. coli* 078 (field strain)	*C. steinii*
(IIa)	100	100	31.25
(IIb)	100	100	62.5
(IIc)	100	100	15.62
(IIIa)	500	500	125
(IIIc)	500	500	500
(IVa)	500	500	500
(IVc)	500	500	500
(Vc)	500	500	500
(VIc)	500	500	500
(VIIc)	100	100	500
(VIIIc)	100	500	500
(Xa)	500	500	500
(Xb)	100	100	500
(XI)	100	100	62.5
Bicocs	–	–	–
Ciprofloxacin	1	10	–

(field strain), which were usually used for testing the antimicrobial activity of hetarylnitropyridines. Ciprofloxacin was used as the reference antibacterial agent. The cultures were grown for a day at 37°C on a Luria-Bertani (LB) agar nutrient medium by the method of two-fold serial dilutions.

The protistocidal activity was studied in vitro against ciliates *Colpoda steinii* (field isolate) by the serial dilution method.

The results presented in Table 1 show that 2-, 4-, and 6-hetaryl-substituted nitropyridines exhibit rather weak, a little variable, and usually almost equal antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. At the same time, the considered compounds possess the protistocidal activity, which varies much more widely depending on their structure. This activity is quite strong for pyridines containing the 4,5-dichloroimidazol-1-yl group, i.e., nitroderivatives (**IIa**–**c**) and aminoderivative (**XI**), with compound (**IIc**) having the strongest effect (MIC = 15.6 μg/mL).

Imidazolyl nitropyridines (**IIa**–**c**) were compared with the best out of currently used coccidiostatics (Baycox, Bayer AG, and amprolium), as well as with furazolidon, sulfadimethoxine, trichopol, and ciprofloxacin (Table 2), which were inactive in the studied range of concentrations (<1000 μg/mL). The comparison of compounds (**IIa**–**c**) with the two above-mentioned specialized and equally effective coccidiostatic drugs allows one to conclude that bihetaryl (**IIa**) is not inferior to them in activity, whereas its isomers (**IIb**) and (**IIc**) show 2–4 times higher activity.

Thus, the results demonstrate that among a number of studied hetaryl-3(5)-nitro(amino)pyridines, the compounds containing the imidazolyl (as the hetaryl) group and having the protistocidal activity may be of practical interest. Their structure should be optimized, and after achieving the required activity, advanced toxicological studies should be performed.

EXPERIMENTAL

We used commercial reactants from Fluka (Germany) and Aldrich-Sigma (United States), and solvents purified by standard methods.

^{1}H NMR spectra of the synthesized bihetaryls were recorded on a Varian Unity-300 spectrometer (300 MHz) (United States) in DMSO-d_6; in case of compound (**IIa**), the spectrum was recorded in $CDCl_3$. Chemical shifts of protons (δ, ppm) are measured relative to the residual proton signals of deuterated dimethylsulfoxide and chloroform (2.49 and 7.25 ppm, respectively). IR spectrum was recorded on the spectrometer "Varian Excalibur 3100 FT-IR" in powder by the method of frustrated total internal reflection. All melting points were determined on a Fisher-Johns melting point apparatus. (Fisher Scientific, United States). The element analysis was performed by the classical microanalytical method [26]. The reactions and the purity of the synthesized compounds were monitored by TLC (Al_2O_3 plates, III degree of activity; eluent, $CHCl_3$; visualization of spots with iodine vapor in a humid chamber).

Table 2. Comparison of protistocidal activity of most active hetaryl-3(5)-nitropyridines (**IIa**–**c**) and clinically used drugs

Preparation	Accounted concentration of preparation, μg/mL								
	1000	500	250	125	62.5	31.25	15.62	0.78	0.39
(IIa)	+	+	+	+	+	–	–	–	–
(IIb)	+	+	+	+	+	+	–	–	–
(IIc)	+	+	+	+	+	+	+	–	–
Furazolidon	–	–	–	–	–	–	–	–	–
Sulfadimethoxine	–	–	–	–	–	–	–	–	–
Trihopol	–	–	–	–	–	–	–	–	–
Ciprofloxacin	–	–	–	–	–	–	–	–	–
Bicocs (toltrazuril)	+	+	+	+	+	–	–	–	–
Amprolium	+	+	+	+	+	–	–	–	–
Water	–	–	–	–	–	–	–	–	–

Designations: (+), all protozoa died; (–) all were alive.

General procedure for the synthesis of hetaryl-3(5)-nitropyridines (IIa–c), (IIIa,c), (IVa,c), (Vc)–(VIIIc). Method A. A mixture of corresponding chloronitropyridine (**Ia–c**) (1.59 g, 10 mmol), diazole or 3-chloropyridazin-6-one (10 mmol), and powdered K_2CO_3 (4.14 g) was thoroughly stirred for several hours at a temperature from 45 to 65°C (the exact time and temperature values are shown for the individual compound). The mixture was then cooled, poured into 100 mL of H_2O, and the formed precipitate was filtered off and washed with water.

Method B. Solution of NH-heterocycle (10 mmol) in dry DMF (12 mL) was cooled to 0°C, followed by the portionwise addition of NaH (2.9 mg, 12 mmol) under stirring. The mixture was stirred for 15 min at 0°C, and corresponding chloronitropyridine (1.59 g, 10 mmol) was portionwise added to the mixture. After 30 min of stirring, the mixture was brought to room temperature, treated with water (100 mL), and the formed precipitate of hetaryl-3(5)-nitropyridine was filtered off.

2-(4,5-Dichloroimidazol-1-yl)-3-nitropyridine (IIa) was synthesized from 2-chloro-3-nitropyridine and 4,5-dichloroimidazole by method A. The reaction was carried out at 50–55°C for 5 h. Yield, 75%; M.p., 114–116°C (from AcOEt). ^{1}H NMR spectrum: 7.75 (1 H, dd, J_1 8.1, J_2 4.8, H5), 7.79 (1 H, s, H2'[2]), 8.58 (1 H, dd, J_1 8.4, J_2 1.5, H4), 8.88 (1 H, dd, J_1 4.8, J_2 1.5, H6). Found, %: C 36.88; H 1.49; Cl 27.00; N 21.40. $C_8H_4Cl_2N_4O_2$. Calculated, %: 37.09; H 1.56; Cl 27.37; N 21.63.

4-(4,5-Dichloroimidazol-1-yl)-3-nitropyridin (IIb) was synthesized from 4-chloro-3-nitropyridin and 4,5-dichloroimidazole by method A at 45°C for 3 h in a yield of 50%. M.p., 106–108°C (from CCl_4). ^{1}H NMR spectrum: 8.01 (1 H, d, J_1 5.1, H5), 8.22 (1 H, s, H2'), 9.19 (1 H, d, J 5.1, H6), 9.51 (1 H, s, H2). Found, %: C 36.63; H 1.40; Cl 27.05; N 21.33. $C_8H_4Cl_2N_4O_2$. Calculated, %: C 37.09; H 1.56; Cl 27.37; N 21.63.

2-(4,5-Dichloroimidazol-1-yl)-3-nitropyridine (IIc) was synthesized from 2-chloro-5-nitropyridin and 4,5-dichloroimidazole by method A at 50–55°C for 4 h in a yield of 85%. M.p., 116–118°C (from AcOEt). ^{1}H NMR spectrum: 8.06 (1 H, d, J 9.0, H3), 8.34 (1 H, s, H2'), 8.85 (1 H, dd, J_1 9.0, J_2 3.0, H4), 9.27 (1 H, d, J 2.7, H6). Found, %: C 36.71; H 1.31; Cl 27.03; N 21.40. $C_8H_4Cl_2N_4O_2$. Calculated, %: C 37.09; H 1.56; Cl 27.37; N 21.63.

3-Nitro-2-(4-chloropyrazol-1-yl)pyridine (IIIa) was synthesized by method B from 2-chloro-3-nitropyridine and 4-chloropyrazol in a yield of 65%. M.p., 116–118°C (from EtOH). ^{1}H NMR spectrum: 7.62 (1 H, dd, J_1 8.1, J_2 4.8, H5), 7.76 (1 H, s, H5'), 8.38 (1 H, dd, J_1 8.1, J_2 1.5, H4), 8.55 (1 H, s, H3'), 8.67 (1 H, dd, J_1 4.8, J_2 1.5, H6). Found, %: 42.55; H 2.01; Cl 15.50; N 24.65. $C_8H_5ClN_4O_2$. Calculated, %: C 42.78; H 2.24; Cl 15.78; N 24.94.

5-Nitro-2-(4-chloropyrazol-1-yl)pyridine (IIIc) was synthesized by method B from 2-chloro-5-nitropyridine and 4-chloropyrazol in a yield of 70%. M.p., 147–149°C (from AcOEt). ^{1}H NMR spectrum: 7.85 (1 H, s, H5'), 8.14 (1H, d, J 9.0, H3), 8.71 (1 H, s, H3'), 8.74 (1 H, dd, J_1 9.0, J_2 2.7, H4) 9.23 (1 H, d, J 2.4, H6). Found, %: C 42.48; H 2.12; Cl 15.49; N 24.71. $C_8H_5ClN_4O_2$. Calculated, %: C 42.78; H 2.24; Cl 15.78; N 24.94.

2-(4-Bromopyrazol-1-yl)-3-nitropyridine (IVa) was synthesized by method B from 2-chloro-3-nitropyridine and 4-bromopyrazole in a yield of 70%. M.p., 129–130°C (from EtOH). ^{1}H NMR spectrum: 7.63 (1 H, dd, J_1 7.8, J_2 4.8, H5), 7.72 (1 H, s, H5'), 8.41 (1 H, dd, J_1 8.1, J_2 1.5, H4), 8.57 (1 H, s, H3'), 8.69

[2] Here and below, the numbers with a prime indicate position of protons of nonpyridine hetaryl substitient.

(1 H, dd, J_1 4.8, J_2 1.5, H6). Found, %: C 35.60; H 1.50; Br 29.46; N 20.65. $C_8H_5BrN_4O_2$. Calculated, %: C 35.71; H 1.87; Br 29.70; N 20.85.

2-(4-Bromopyrazol-1-yl)-5-nitropyridine (IVc) was synthesized by method B from 2-chloro-5-nitropyridine and 4-bromopyrazole in a yield of 90%. M.p., 152–154°C (from EtOH). ^{1}H NMR spectrum: 8.09 (1 H, d, *J* 9.3, H3), 8.11 (1 H, s, H5'), 8.76 (1 H, dd, J_1 9.0, J_2 2.7, H4), 8.91 (1 H, s, H3'), 9.27 (1 H, d, *J* 2.4, N6). Found, %: C 35.52; H 1.36; Br 29.61; N 20.61. $C_8H_5BrN_4O_2$. Calculated, %: C 35.71; H 1.87; Br 29.70; N 20.82.

2-(Imidazol-1-yl)-5-nitropyridine (Vc) was synthesized by method B from 2-chloro-5-nitropyridine and imidazole in a yield of 80%. M.p., 217–219°C (from EtOH). ^{1}H NMR spectrum: 7.20 (1H, s, H4'), 8.08 (1 H, s, H2'), 8.09 (1 H, d, *J* 9.3, H3), 8.69 (1 H, s, H5'), 8.78 (1 H, dd, J_1 9.0, J_2 2.7, H4), 9.29 (1 H, d, *J* 2.4, H6). Found, %: C 50.18; H 2.95; N 29.10. $C_8H_6N_4O_2$. Calculated, %: C 50.53; H 3.18; N 29.46.

2-(3,5-Dimethyl-4-chloropyrazol-1-yl)-5-nitropyridine (VIc) was synthesized by method B from 2-chloro-5-nitropyridine and 3,5-dimethyl-4-chloropyrazole in a yield of 65%. M.p., 140–142°C (from AcOEt). ^{1}H NMR spectrum: 2.26 (3 H, s, 5'CH_3), 2.65 (3 H, s, 3'CH_3), 8.05 (1 H, dd, J_1 9.0, J_2 6.0, H3), 8.70 (1 H, d, J_1 9.0, J_2 3.0, H4), 9.26 (1 H, d, *J* 2.7, H6). Found, %: C 47.40; H 3.25; Cl 13.70, N 22.00. $C_{10}H_9ClN_4O_2$. Calculated, %: C 47.54; H 3.59; Cl 14.03, N 22.17.

2-(Benzimidazol-1-yl)-5-nitropyridine (VIIc) was synthesized by method A from 2-chloro-5-nitropyridine and benzimidazole at 50–60°C for 2 h in a yield of 90%. M.p., 206–208°C (from EtOH). ^{1}H NMR spectrum: ^{1}H NMR spectrum: 7.37–7.47 (2 H, m, H5', 6'), 7.79–7.81 [1 H, m, H7' (or H4')], 8.25 [1 H, d, *J* 9.0, H4' (or H7')], 8.48 (1 H, dd, J_1 7.8, J_2 1.6, H3), 8.82 (1 H, dd, J_1 9.0, J_2 3.0, H4), 9.17 (1 H, s, H2'), 9.43 (1H, d, *J* 3.0, H6). Found, %: C 59.68; H 2.98; N 23.00. $C_{12}H_8N_4O_2$. Calculated, %: C 60.00; H 3.36; N 23.32.

5-Nitro-2-[(3-chloropyridazin-6-on)-1-yl]-pyridine (VIIIc) was synthesized by method A from 2-chloro-5-nitropyridine and 3-chloropyridin-6-one at 60–65°C for 1.5 h in a yield of 70%. M.p., 147–149°C (from AcOEt). ^{1}H NMR spectrum: 7.25 (1 H, d, *J* 9.9, H4'), 7.71 (1 H, d, *J* 9.9, H5'), 8.01 (1 H, d, *J* 8.7, H3), 8.80 (1 H, dd, J_1 9.0, J_2 2.7, H4), 9.40 (1 H, d, *J* 2.4, H6). Found, %: C 42.55; H 1.68; Cl 13.69; N 21.79. $C_9H_5ClN_4O_3$. Calculated, %: C 42.79; H 2.00; Cl 14.03; N 22.18.

General procedure of two-step synthesis of pyrazolyl-3-nitropyridines (Xa, b). Hydrazine hydrate (0.65 mL, 20 mmol) was dropwise added to 2- or 4-chloro-3-nitropyridine (1.4 g, 10 mmol) in 15 mL of EtOH and boiled for 30 min. The reaction mixture was cooled, followed by the addition of water (20 mL). The formed precipitate of hydrazine pyridine (**IXa**, **b**) was filtered and thoroughly dried. The mixture of resultant compound (**IXa**, **b**) (1.54 g, 10 mmol), acetylacetone (1.51 mg, 15 mmol), and acetic acid (0.1 mL) in 20 mL of EtOH was boiled for 3 h and cooled, followed by the addition of water (100 mL). The formed precipitate of pyrazolyl-3-nitropyridine was filtered off.

2-(3,5-Dimethylpyrazol-1-yl)-3-nitropyridine (Xa) was synthesized from 3-nitro-2-chloropyridine by the above method in a yield of 90%. M.p., 107–109°C (from AcOEt). ^{1}H NMR spectrum: 2.10 (3 H, s, 5'CH_3), 2.50 (3 H, s, 3'CH_3), 6.16 (1 H, s, H4'), 7.67 (1 H, dd, J_1 7.8, J_2 4.8, H5), 8.50 (1 H, dd, J_1 8.1, J_2 1.5, H4), 8.77 (1 H, dd, J_1 4.8, J_2 1.5, H6). Found, %: C 54.66; H 4.28; N 25.41. $C_{10}H_{10}N_4O_2$. Calculated, %: C 55.04; H 4.62; N 25.67.

4-(3,5-Dimethylpyrazol-1-yl)-3-nitropyridine (Xb) was synthesized from 4-chloro-3-nitropyridine similarly to compound (Xa) in a yield of 88%. M.p., 155–158°C (from AcOEt). ^{1}H NMR spectrum: 2.12 (3 H, s, 5'CH_3), 2.34 (3 H, s, 3'CH_3), 6.21 (1 H, s, H4'), 7.86 (1 H, d, *J* 5.4, H5), 8.95 (1 H, d, *J* 5.4, H6), 9.19 (1 H, s, H2). Found, %: C 54.70; H 4.00; N 25.21. $C_{10}H_{10}N_4O_2$. Calculated, %: C 55.04; H 4.62; N 25.67.

5-Amino-2-(4,5-dichloroimidazol-1-yl)-pyridine (XI). The mixture of iron (33.6 g, 0.6 g-atom), EtOH (150 mL), and conc. HCl (2 mL) was boiled under stirring for two hours. After the portionwise addition of nitropyridine (**IIc**) (25.9 g, 0.1 mol), the mixture was boiled for another three hours. K_2CO_3 (5 g) was then added by portions of 0.2–0.3 g, and the reaction mixture was boiled for 30 min. The mixture was filtered hot and washed with hot ethanol (3 × 20 mL). Ethanol (100 mL) was evaporated, water (100 mL) was added, and the precipitate of amine (**XI**) was filtered off. The yield, 20.6 g (90%). M.p., 179–181°C (from EtOH). ^{1}H NMR spectrum: 5.57 (2 H, s, NH_2), 7.06–7.17 (2 H, m, H3,4), 7.77 (1 H, s, H2'), 7.85 (1 H, d, J_1 4.8, H6). Found, %: C 41.66; H 2.25; Cl 30.69; N 24.02. $C_8H_6Cl_2N_4$. Calculated, %: C 41.95; H 2.64; Cl 30.95; N 24.46.

Study of antimicrobial properties of prepared compounds. Solutions of the studied compounds (2 mL) of different concentrations obtained by two-fold serial dilutions in a liquid medium [27–30] were added to the prepared suspensions of bacteria (2 mL). The accounted microbial load was 250000 microbial bodies in 1 mL. The resultant solutions were incubated for 18 h at 37°C. The nutrient medium containing bacteria at the concentration of 250000 microbial bodies in 1 mL was used as the positive control. The nutrient medium without bacteria was used as the negative control. The experiments were carried out using the standard bacterial strains, i.e., *S. aureus* P-209, *E. coli* 078 (field strain).

Study of protistocidal activity was performed by the method [31, 32] on protozoa of the *C. stenii* species (field isolate, collection of the Laboratory of Parasitology of North Caucasian Zonal Scientific Research

Veterinary Institute, Russia). Experiments were carried out in the enzyme immunoassay microplates. A mixture of boiled tap water and sterile distilled water in equal volumes was used as the medium for the exposure of protozoa. The substances were initially diluted by distilled water. The serial dilutions of the substances were performed as follows.

Solution 1. The compound (5 mg) was added to 70% aqueous DMSO (50 μL), followed by the addition of distilled water (5 mL). The final concentration was 1000 μg/mL.

Solutions 2–12. A mixture of boiled tap water and sterile distilled water (1 : 1) was added to wells 2–12 (150 μL/well) with an automatic 8-channel pipette; 150 μL of solution 1 was added to well 2 and after stirring, 150 μL of the resultant solution was added to well 3, and so on until the end of the row; 150 μL from well 12 was removed after stirring. Three-day culture of *C. steinii* (30 μL) was added to each well. Well 1 was filled with solution 1 (150 μL) and of protozoa suspension (30 μL). The protozoa suspension was prepared so that 10–15 active species were observed in each field of view at low magnification. After addition of protozoa, the plate was covered with a lid and allowed to stay at room temperature (20–22°C) for 18–20 h.

Results were accounted for as follows. The mixture of well 12 (30 μL) was applied onto a clean glass slide and examined under a microscope at low magnification (10 × 15). The presence or absence of living protozoa was noted. The view was performed from right to left. Well 1 without living species was considered as that containing minimal protistocidal concentration of the studied compound.

The following solutions were used as controls:

—control of the medium (boiled tap water + sterile distilled water), 5 wells;

—control of the solvent (50 μL of 70% DMSO + 5 μL of distilled water and all serial dilutions as in the case of the studied compounds), 12 wells;

—reference drug, Baycox.

ACKNOWLEDGMENTS

The work was supported as part of the State project in the field of scientific activity (project no. 4.196.2014/K) and performed using the equipment of the Center of Collective Use "Molecular spectroscopy" of South Federal University.

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Translated by A. Levina