Reactions of aminoguanidine and guanidine with 3- and 5-formyl-4-arylaminopyridones

M. I. Medvedeva,[a] N. Z. Tugusheva,[b] L. M. Alekseeva,[b] M. A. Kalinkina,[b] V. A. Parshin,[b] V. V. Chernyshev,[c] V. I. Levina,[b] N. B. Grigor´ev,[b] A. S. Shashkov,[d] and V. G. Granik[b]★

[a]*D. I. Mendeleev University of Chemical Technology of Russia, 9 pl. Miusskaya, 125047 Moscow, Russian Federation. E-mail: marinacasper@gmail.com*
[b]*Moscow Theoretical and Practical Center for Narcology, 37/1 ul. Lyublinskaya, 109390 Moscow, Russian Federation. E-mail: vggranik@mail.ru*
[c]*Department of Chemistry, M. V. Lomonosov Moscow State University, Build. 3, 1, Leninskie Gory, 119992 Moscow, Russian Federation. E-mail: cher@biocryst.phys.msu.su*
[d]*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 47 Leninsky prosp., 119991 Moscow, Russian Federation. Fax: +7 (499)135 5328. E-mail: shash@ioc.ac.ru*

Amidinohydrazones were prepared by the condensation of 4-arylamino-2-oxo-5-formyl-1,2-dihydropyridine-3-carbonitriles and 4-arylamino-2-oxo-1,2-dihydropyridine-3-carbaldehydes with aminoguanidinium carbonate with the purpose to investigate their biological activity as putative NO donors. The reaction of 5- и 3-formylpyridones with diguanidinium carbonate was studied. The formation of stable complexes of 4-arylamino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitriles with aminoguanidine and guanidine was discovered. Amidinohydrazones obtained possess antiinflammatory, antidiabetic, and antihypertensive activities.

Key words: 4-arylamino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitriles, 4-arylamino-2-oxo-1,2-dihydropyridine-3-carbaldehydes, complexes of 4-arylamino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitriles with aminoguanidine and guanidine, 2-oxo-4-anilino-1,2-dihydropyridine-3-carbonitrile, dimethylformamide dimethylacetal, aminoguanidine, guanidine, X-ray diffraction, antiinflammatory, antidiabetic, and antihypertensive activities.

It is well known that nitric oxide (NO) plays a key role in the control of vascular tone, in the maintenance of cardiovascular homeostasis, in the control of breathing, immunity, neurotransmission mechanisms, and at the same time it is a cytotoxic and cytostatic agent. Quite a number of pathological states, *e.g.*, cardiovascular, infectious, inflammatory, and other diseases, can be related to the lack or overproduction of NO in the organism.[1—8]

In this connection, one of the most actively developing line of investigations is currently the search of various compounds which are able to serve as generators or inhibitors of NO formation, *i.e.*, the search of compounds, whose transformation can lead to formation of NO in the organism or inhibition of its production under physiological conditions.

It is known that the guanidine fragment of L-arginine being a target for NO synthases undergoes oxidation to the *N*-hydroxy derivative and then to citrulline and nitric oxide.[1] Binding of substrates to enzyme isoforms involves both the amino acid and, first of all, guanidine fragment (at the special site of «guanidine binding» and interaction with heme).[9] Hence, it was concluded that compounds containing guanidine or guanidine-like groups can be the substrates of NO synthases (NOS) and catalytically transformed with the release of NO. Furthemore, it is known that such compounds as aminoguanidine are selective inhibitors of inducible NO synthase, which accounts for considerable biological activity of this compound or its derivatives under various pathologies.[1]

In the present work, a number of guanidine and aminoguanidine derivatives of 4-amino-1,2-dihydropyridin-2-one was synthesized and some of their parameters of antiinflammatory, antidiabetic, and antihypertensive activities were investigated.

Earlier,[10] we have prepared 4-arylamino-1,2-dihydropyridin-2-ones containing a formyl group in positions 3 or 5. Using HPLC, it was established that 5-formylpyridones are much more reactive in reactions with carbon acids

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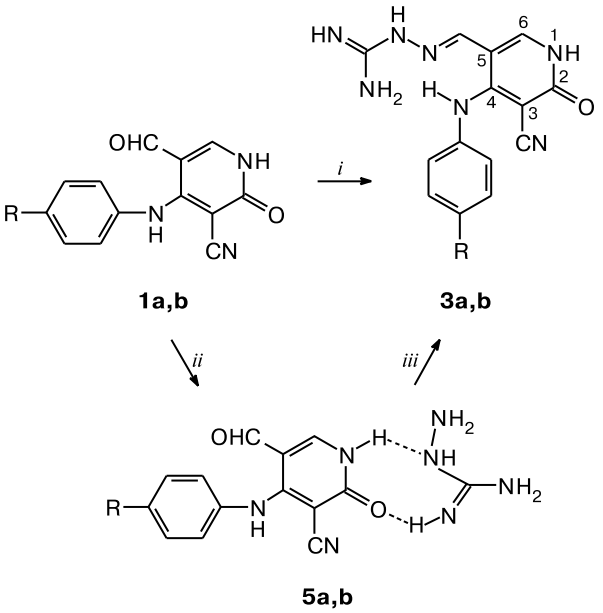
than 3-formylpyridones. It was explained by probable steric hindrance in the first step of the condensation.[11] In continuation of our studies on comparison of the reactivity of the 3- and 5-formyl groups in 4-arylaminopyridones, we describe here reactions of 4-arylamino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitriles (**1a,b**) and 4-arylamino-2-oxo-1,2-dihydropyridine-3-carbaldehydes (**2a,b**) with aminoguanidine and guanidine.

The reaction of aminoguanidinium hydrogencarbonate in 96% ethanol with both 5-formylpyridones **1a,b** and 3-formylpyridones **2a,b** in the presence of an acid results in the expected coupling products **3a,b** and **4a,b** isolated as hydrochlorides in good yields (Schemes 1 and 2). If these reactions are carried out in pyridine in the absence of the acid, the reactions of 5- and 3-formylpyridones follow different routes. White voluminous precipitates were formed upon refluxing of 5-formylpyridones **1a,b** with a twofold excess of aminoguanidinium hydrogencarbonate for 10—30 min. The IR spectra of compounds **5a,b** obtained show signals for the NH, NH_2, CN, CHO, and CO groups (see Experimental). The ^{1}H NMR spectra contain, in addition to the signals corresponding to the structure of the starting 5-formylpyridones but shifted upfield by 0.3 ppm, two broadened signals at δ 4.67 (2 H) and δ 7.30 (4 H) for **5a**, or δ 4.63 (2 H) and δ 7.24 (4 H) for **5b**, which can be assigned to the signals for the NH_2 and NH groups of aminoguanidine. Compound **5a** was investigated in more detail. The overlap of the signals for the protons of CHO, H(6) and phenyl ring was observed in the ^{1}H NMR spectrum of a mixture of the starting 5-formylpyridone **1a** and compound **5a**, while ^{1}H NMR spectrum of a mixture of compounds **3a** as a free base and **5a** shows double set of the signals corresponding to the individual compounds **3a** and **5a**. In the ^{13}C NMR spectra of compounds **1a** and **3a**, the chemical shifts of carbonyl atom C(2)=O virtually coincided (δ 161.4 and 161.5), while in the spectrum of compound **5a** it was shifted downfield (δ 172.9), which can be explained by formation of a hydrogen bond with the H—N group of aminoguanidine. The assignment of the signals in the ^{13}C NMR spectra of compounds **3a** and **5a** was made based on correlation peaks in 2D HMBC spectrum (see Experimental). The ^{13}C NMR spectrum of compound **5a** shows the signal at δ 159.0, which can be assigned to the C atom of the aminoguanidine fragment, *c.f.* the chemical shift of NHC(=NH) in the ^{13}C NMR spectrum of compound **3a**, δ 159.0. According to these data and data from elemental analysis for **5a**, the formation of complexes under short-term refluxing of aminoguanidinium hydrogencarbonate with 5-formylpyridones **1a,b** in pyridine was supposed. This was also confirmed by powder X-ray diffraction analysis of compound **5a** (see Experimental).

It should be noted that the formation of such complexes is an interesting and unusual fact, and in this case it is essential that their structures were established by both spectroscopic and X-ray diffraction analysis.

The appearance of the precipitate changed upon long-term refluxing of compounds **5a,b** (24 h for **5a** and 32 h for **5b**), the isolated products are the expected coupling products **3a,b** as the free bases. The structure of compound **3a** was established by HMBC NMR spectroscopy (see Experimental) and powder X-ray diffraction analysis data.

Scheme 1



R = H (**a**), Cl (**b**)

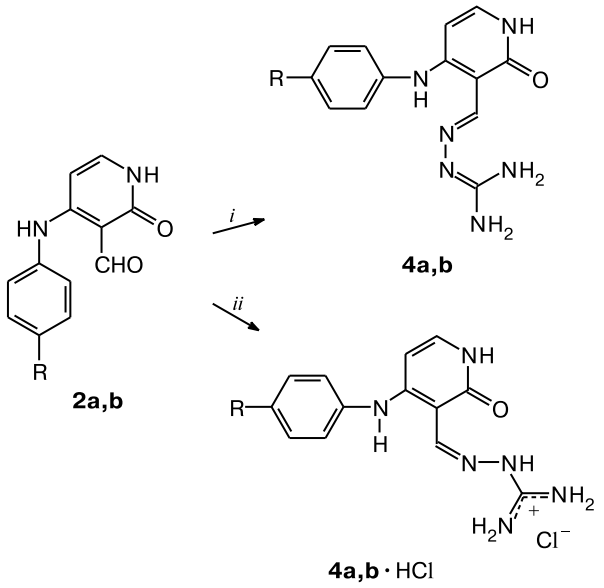
Reagents and conditions: *i*. Aminoguanidinium hydrogencarbonate, Py, 24 h or aminoguanidinium hydrogencarbonate, EtOH, HCl, H_2O; *ii*. Aminoguanidinium hydrogencarbonate, Py, 30 min; *iii*. Long-term refluxing in pyridine.

Coupling of aminoguanidine with 3-formylpyridones **2a,b** in pyridine proceeds much faster (5 h for **2a**, 15 h for **2b**). The expected amidinohydrazones **4a,b** were isolated in good yields (Scheme 2).

The reaction of 5-formylpyridone **1a** with diguanidinium carbonate in pyridine (Scheme 3) also results in complex **6** analogous to complexes **5a,b** (the rection time was 30 min), its structure was confirmed by the data from ^{1}H NMR, IR, and mass spectra and powder X-ray diffraction analysis (see Experimental).

All geometrical characteristics of the molecules in the crystal structures of **3a**, **5a**, **6** (Fig. 1) have values similar to those found in Cambridge Crystallographic Database (CCDC).[12] The crystal packings in all compounds are characterized by the presence of classic intermolecular hydrogen bonds N—H...N and N—H...O (Table 1). The presence of centrosymmetrical tetramers uniting two main

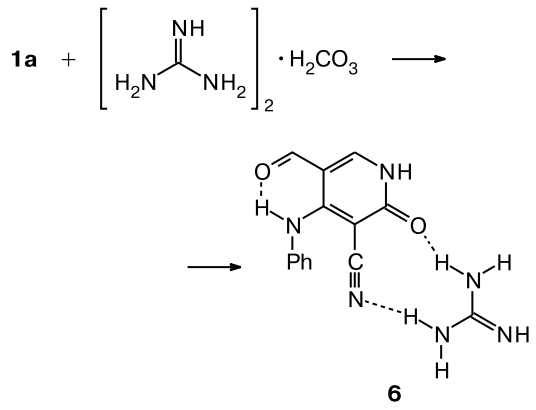
Scheme 2



R = H (**a**), Cl (**b**)

Reagents and conditions: *i*. Aminoguanidinium hydrogencarbonate, Py; *ii*. Aminoguanidinium hydrogencarbonate, 96% EtOH, HCl, H_2O.

Scheme 3



Conditions: Py, 30 min.

molecules and two molecules of guanidines bound by hydrogen bonds can be related as an interesting feature of packing of **6** and **5a** (Fig. 2).

Only the starting 5-formylpyridone **1a** was isolated in quantitative yield under carrying out the reaction of diguanidinium carbonate with 5-formylpyridone in 96% ethanol in the presence of an acid regardless of the reaction time (3.5 h or 35 h), and also upon refluxing complex **6** under the same conditions (the rection time was 11 h).

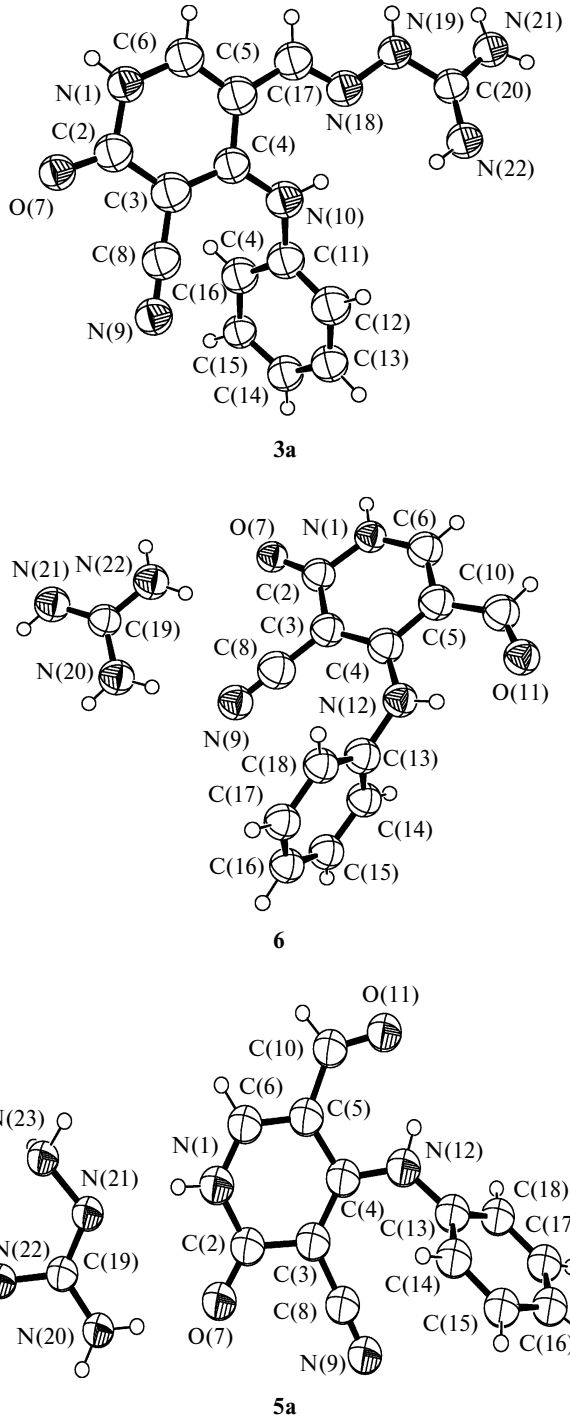


Fig. 1. Molecular structure of compounds **3a**, **6**, and **5a**. Spheres of atom displacements are presented with 50% probability for nonhydrogen atoms.

The reaction of 3-formylpyridone **2a** with diguanidinium carbonate occurs differently. Deformylation product, *viz.* 4-anilinopyridone **7** (Scheme 4), was dominant under long-term refluxing of 3-formylpyridone **2a** in pyridine (22 h), it was also prepared by us from 4-anilino-2-

Table 1. Characteristics of hydrogen bonds in compounds **3a**, **5a**, and **6**

Compound	D—H...A	D—H	H...A	D...A	D—H...A /deg
		Å			
3a	N(1)—H(1)...O(7)^{i}	0.86	1.82	2.676(10)	171
	N(10)—H(10)...N(18)	0.86	2.13	2.728(10)	127
	N(21)—H(21A)...N(9)^{ii}	0.86	2.56	3.411(11)	171
	N(21)—H(21B)...N(22)^{iii}	0.86	2.33	2.930(11)	127
	N(21)—H(21B)...O(7)^{iv}	0.86	2.20	2.747(10)	122
5a	N(1)—H(1)...N(21)	0.86	2.07	2.894(19)	161
	N(12)—H(12)...O(11)	0.86	2.02	2.69(2)	134
	N(20)—H(20A)...O(7)	0.86	1.89	2.743(19)	170
	N(20)—H(20B)...O(7)^{vii}	0.86	2.18	2.918(18)	144
	N(22)—H(22A)...N(23)	0.86	2.28	2.611(18)	103
	N(22)—H(22B)...N(9)^{vii}	0.86	2.13	2.99(2)	175
	N(23)—H(23A)...O(11)^{viii}	0.90	2.47	3.143(18)	131
6	N(1)—H(1)...N(21)v	0.86	2.02	2.872(12)	172
	N(12)—H(12)...O(11)	0.86	2.00	2.714(12)	139
	N(20)—H(20A)...O(11)^{vi}	0.86	2.30	3.058(13)	147
	N(20)—H(20B)...N(9)	0.86	2.17	3.009(14)	164
	N(21)—H(21)...O(11)^{vi}	0.86	2.30	3.066(12)	148
	N(22)—H(22A)...O(7)^{v}	0.86	1.86	2.713(12)	175
	N(22)—H(22B)...O(7)	0.86	2.16	2.869(13)	139

Note. Code of symmetry: (i) $-x, y-1/2, 1/2-z$; (ii) $-x, 1-y, -z$; (iii) $x, y-1, z$; (iv) $x, -y-1/2, z-1/2$; (v) $-x, 1-y, 1-z$; (vi) $x-2, 1/2-y, z-1/2$; (vii) $1-x, 2-y, z$; (viii) $-1-x, 1-y, z$.

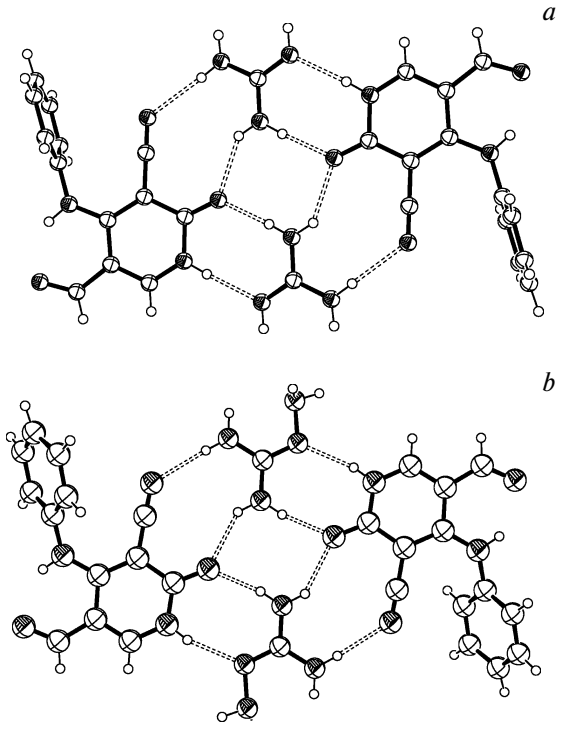
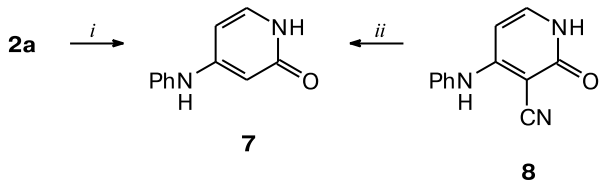


Fig. 2. Centrosymmetrical tetramers in the crystal structure of **6** (*a*) and **5a** (*b*) formed due to intermolecular hydrogen bonds N—H...N and N—H...O (interaction is indicated by dashed lines).

oxo-1,2-dihydropyridine-3-carbonitrile **8** by hydrolysis of the cyano group to the carboxyl group and subsequent decarboxylation of the latter.

Scheme 4



Reagents and conditions: *i*. Diguanidinium carbonate, Py, 22 h or EtOH, HCl, H_2O; *ii*. KOH, ethylene glycol.

Deformylated pyridone **7** is the dominant product too under carrying out the reaction of 3-formylpyridone **2a** with guanidine carbonate in 96% ethanol in the presence of the acid (the reaction time was 20 h).

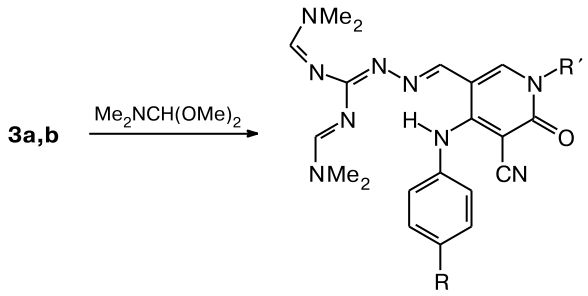
Next we studied the reactions of the condensation products obtained **3a,b** and **4a** with dimethylformamide dimethylacetal. Bis-amidine **9a** (Scheme 5) formed in the reaction of 5-substituted pyridone **3a** as both the free base and hydrochloride with $Me_2NCH(OMe)_2$ under mild conditions (keeping at 20 °C in toluene or in isopropyl alcohol). This compound is unstable and decomposed in attempted recrystallization, therefore we failed to isolate

Table 2. The ability of compounds **3a**,**b**, **4a**, and **9b,c** to release of NO

Compound	The yield of NO_2^- (%)
3a · HCl	7.7±0.8
3b	8.8±0.8
4a	5.8±0.68
9b	4.2±0.4
9c	3.9±0.4
Guanabenz	10.2±1.0

it in pure form. Its structure was established by data from mass spectrometry and ^{1}H NMR spectroscopy (see Experimental). In addition to condensation, alkylation of the pyridine nitrogen atom occurs when conducting the reactions of **3a,b** with $Me_2NCH(OMe)_2$ under more drastic conditions (refluxing in toluene), and as a result analytically pure products **9b,c** were isolated in satisfactory yields.

Scheme 5

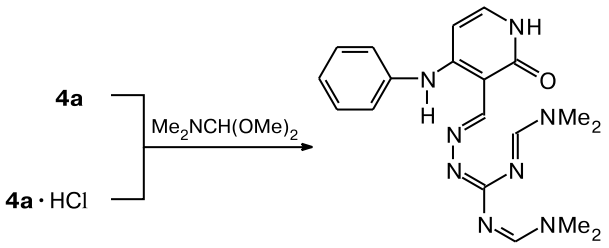


9a—c

R = R′ = H (**a**); R = H, R′ = Me (**b**); R = Cl, R′ = Me (**c**)

3-Substituted pyridone **4a** in contrast to 5-substituted pyridones **3a,b** is less susceptible to alkylation by the acetal. The reaction of **4a** as both the free base and hydrochloride with $Me_2NCH(OMe)_2$ results in amidine **10** under both mild conditions (keeping at 20 °C in toluene or in isopropyl alcohol) and more drastic conditions (refluxing in toluene for 6 h) (Scheme 6). The mass spectrum of the compound isolated after refluxing in toluene suggests the presence of traces of a methylation product. Compound **10** like **9a**, is thermally unstable, and the appearance of 1,2-dihydrobenzo[*b*]-[1,6]-naphthyridin-2-one (obtained from **1b** by us earlier[10]) in the sample was observed.

Scheme 6



10

As was mentioned above, the aim of the present study was the synthesis of a group of new compounds containing the guanidine fragment. This, in turn, was caused by capability of oxidation of these compounds in the organism with release of nitric oxide. Therefore, it was of interest to study the biological activity of the compounds obtained with regard to parameters typical of NO donors.

First, the ability of compounds **3a**,**b**, **4b**, and **9b**,**c** to release NO was studied. The results from Table 2 demonstrate these compounds to be NO donors.

The ability of compounds **3a**,**b**, **4b**, and **9b**,**c** to release NO upon oxidation was determined in their chemical oxidation with a solution of *m*-chloroperbenzoic acid (pH 9.8) (20-fold excess) followed by passing the solution through a column with porous cadmium.

The results obtained were compared with the ability of a hypotensive medicine Guanabenz to release NO under identical conditions. Guanabenz was shown[13] to undergo transformation into nitric oxide under incubation with cytochrome oxidase of liver microsomes and therefore, it can be the exogenous source of NO in the organism.

As a result of biological tests in normotensive and hypertensive rats with ultimate arteriotony corresponding to 180±12.5 Torr, compound **4a** in the dose of 10 mg kg^{-1} was shown to decrease arteriotony. Compound **4a** is com-

Table 3. The influence of compound **4a** under studying upon the system arteriotony for rats

Compound	Dose/mg kg^{-1} (intravenously)	Reduction of arteriotony (Δ) relatively to the initial level Δ/Torr (%)	
		Normotensive rats (n = 5)	Hypertensive rats (n = 5)
4a	10	15.0±0.8 (10.7)	17.0±2.0 (9.4)
5-Mononitrate Isosorbide	10	19.5±0.7 (13.9)	22.5±0.5 (12.5)

Table 4. The influence of compounds **3a** · HCl, **3b** · HCl, **4a**, and **9b** upon pain reaction of mices caused by intraperitoneal introduction of the solution of acetic acid

Compound	The number of animals	Dose/mg kg^{-1} (perorally)	The number of writhes* for mices *m* (%)	Analgesic activity**
Control	30		19.8±2.6 (100)	
3a · HCl	10	100	18.8±1.1 (95.0)	5.0
	10	200	17.5±0.8 (88.4)	11.6
3b · HCl	10	100	17.3±2.0 (87.4)	12.6
	10	200	16.1±1.5 (81.3)	18.7
4a	10	100	17.9±1.4 (90.4)	9.6
	10	200	17.0±1.45 (85.6)	14.4
9b	10	100	18.9±1.2 (95.5)	4.5
	10	200	18.0±1.4 (91.1)	8.9
Indometacin	10	5.0	5.3±0.5 (26.8)	73.2

* Pain reaction upon abdominal membrane sensation. ** Relative to the control (%).

pared with isosorbide-5-mononitrate in the same dose in terms of the hypotensive effect (Table 3).

Compound **4a**, as well as **3a** · HCl, **3b** · HCl, and **9b** in doses 100 and 200 mg · kg^{-1} with oral administration possesses slight analgesic effect in mice in the test of writhes caused by intraperitoneal injection of a solution of acetic acid (Table 4).

Compounds **3a** · HCl, **3b** · HCl, **4a**, and **9b** in doses 100 and 200 mg kg^{-1} under oral administration possess antiinflammatory action in mice on the peritonitis models produced by LPS and carrageenan. This effect of compounds under study is much less expressed than that of indometacin (Table 5).

Compound **4a** in doses 100 and 400 mg kg^{-1} possesses hypoglycemic action on the model of srteptozocin diabetes in mice, but it yield in activity of metformin (Table 6).

In studies of overall toxic properties, compounds **3a** · HCl, **3b** · HCl, **4a**, and **9b** were shown to be low toxic compounds, LD_{50} for these compounds is more than 1000 mg kg^{-1} under oral administration.

Thus, the investigation of pharmacological activity of guanidine derivatives, *viz.*, compounds **3a** · HCl, **3b** · HCl, **4a**, and **9b** as regards their antihypertensive, antiinflammatory, analgesic, and hypoglycemic activities recognized all compounds to possess weak antiinflammatory activity.

Compound **4a** possessing antiinflammatory, hypoglycemic, and antihypertensive activities and compound **3a** · HCl possessing antiinflammatory and antihypertensive activities have the most significant spectrum of activity. Their activity is weaker in comparison with known drugs.

The analogous spectrum of activity is noted for the NO donor aminoguanidine (antiinflammatory, hypoglycemic, and hypertensive activities), which is used in the treatment of diabetic retinopathies and nephropathies. Aminoguanidine increases arteriotony (probably, it is important for the treatment of microangiopathies), while compound **4a** decreases arteriotony, which could be promising for the development of new peripheral vasorelaxants in this class of compounds.

Table 5. Antiinflammatory activity of compounds **3a**·HCl, **3b**·HCl, **4a**, and **9b** on the peritonitis models produced by introduction of lipopolysaccharide (LPS) for mices

Compound	Dose/mg kg^{-1} (perorally)	LPS-peritonitis		Carrageenan-peritonitis	
		V/mL[a]	Inflammation (%)	*V*/mL[a]	Inflammation (%)
3a · HCl	100	3.5±0.35	7.9	0.4±0.1	88.2
	200	2.85±0.45[b]	25.0	3.4±0.5	0
3b · HCl	100	3.5±0.5	7.9	2.8±0.35[b]	17.6
	200	2.9±0.45[b]	23.7	2.8±0.35[b]	17.7
4a	100	3.15±0.35	17.1	2.45±0.3[b]	27.9
	200	2.65±0.40[b]	30.3	2.7±0.3[b]	20.6
9b	100	3.7±0.3	2.6	2.45±0.3[b]	27.9
	200	3.0±0.3	21.1	2.7±0.3[b]	20.6
Indometacin	5.0	1.0±0.12	73.7	2.8±0.35[b]	17.6
Control (LPS)		3.8±0.5	0	2.8±0.35[b]	17.7

[a] *V* — the volume (size) of the substrate. *$P < 0.05$ under comparision with control.

Table 6. Intensity and continuance of hypoglycemic activity of compounds **3a** · HCl, **3b** · HCl, **4a**, and **9b**

Compound	Dose/mg · kg^{-1} (perorally)	The mean level of glucose in mices´ blood/mol L^{-1}		Reduction of the level glucose to control 2 ΔM (%)	Sugar-lowering effect, N/h
		Initial level	After compounds		
3a · HCl	400.0	14.5±1.2	14.4±1.4	Non active	—
3b · HCl	400.0	14.0±1.3*	14.0±1.5	Non active	—
4a	100.0	14.4±1.5*	12.0±1.5**	16.7	2.0
	400.0	14.3±1.5*	11.1±1.0**	23.1	2.0
9b	400.0	13.5±1.2	13.4±1.3	Non active	—
Metformin	100	13.7±1.4	8.4±1.4**	38.7	3.0
	200	13.8±1.5	7.7±1.0**	44.2	3.0
	400	14.7±1.4	7.1±1.5**	51.7	4.0
Control 1 (mices without diabete)	—	6.3±1.0	—	100	—
Control 2 (mices with diabete)	—	14.3±1.5	—	0	—

* Differences with control I at $P < 0.05$. ** Differences with control II at $P < 0.05$.

Furthermore, the combination of three various types of activity for compound **4a** (antiinflammatory, hypoglycemic, and antihypertensive activities) can be promising for the treatment of diabetic angiopathies (angiopathy is injury of the blood vessels of various size, from capillaries to large vessels, caused by the nervous regulation failure), when not only vascular and metabolic components, but elements of inflammatory process are also involved.

Experimental

The IR-spectra were recorded on an FSM-1201 instrument in Nujol mulls. The electrospray ionization (ESI) mass spectra were recorded on a Waters ZQ-2000 mass spectrometer using a direct inlet system without a chromatographic column. The ^{1}H NMR spectra were recorded on a Bruker AC-300 spectrometer in DMSO-d_6 and DMSO-d_6—CCl_4. The ^{13}C NMR spectra were recorded on a Varian Unity+400 (operating frequency 100 MHz) in DMSO-d_6. The numeration shown in the Schemes was used for the description of the NMR spectra. The course of the reactions was monitored, and the purity of the compounds was checked, by TLC on Silica gel 60 F_{254} plates (Merck) (chloroform; chloroform—ethanol, 10 : 1; ethyl acetate—ethanol 10 : 1; acetone). The melting points were determined on an Electrotermal 9100 instrument (UK). *N*,*N*-dimethylformamide dimethylacetal (purity 97%, Lancaster) was used.

Examination of the ability of compounds 3a,b, 4a, and 9b,c to release NO upon oxidation (general procedure). Exact amounts ($5 \cdot 10^{-6}$ mol) of compounds **3a,b**, **4a**, and **9b,c** were dissolved in 0.02 *M* solution of *m*-chloroperbenzoic acid (5 mL) in a 1 : 1 mixture of 96% ethanol and aqueous borate buffer solution (pH 9.8). The solutions obtained were kept at 27±1 °C for 2 days. Then aliquots withdrawn from the solutions were diluted with a buffer solution made of ammonia buffer solution (pH 9.6, State Standard Specification 29270-95, Products of processing of fruits and vegetables. Methods for nitrate test), 96% ethanol and water (2 : 1 : 7, 4 volumes). The solutions obtained were passed through columns with porous cadmium prepared according to the same State Standard Specification. The content of the NO_2^- anion in the effluents was determined by the Griess reaction (diazo component is sulfanilic acid, azo component is 1-naphthylethylenediamine). It should be noted that the nitrite anion is an accepted marker of nitric oxide. The determination was carried out in 0.1 *M* hydrochloric acid by the method of additions. The accuracy of determination was 10%.

Biological studies

1. The influence upon the arteriotony for rats. The investigations were carried out using pedigreeless rat-males (250—270 g each) which were normotensive with initial arteriotony of 40±25 Torr and hypertensive animals.[14]

Hypertension was produced by inhibitor of NO synthase (nitro-L-arginine). Inhibitor of NO synthase was injected to the rat's stomach in the form of 1% solution during 21 days on the basis of 3.0 mg per one animal daily. In 3 weeks stable increasing of the arteriotony to 180—185 Torr was became.

System arteriotony was monitored in the left carotid of the animals narcotized by urethane (1.5 g kg^{-1} intraperitoneally) by means of ADInstruments complex (Australia) consisted of the pressure sensor joined with signal reduction system and displaying on computer monitor by means of Chart v. 4.2.3 program. The compounds under studying were dissolved in 20—50% ethanol and injected into jugular vein *via* catheter. Each compound was tested on 3—4 animals.

2. Hypoglycemic activity. Hypoglycemic activity was investigated using white pedigreeless mice-males (24—25 g each) with experimental pancreatic diabete.[15]

For the simulation of pancreatic diabete of the second type (non-insulin-dependent type of pancreatic diabete) streptozocin was injected intraperitoneally in dose 30.0 mg kg^{-1} during three days. Blood for glucose test was taken from tail vein, glucose testing was carried out by means of test strips of blood glucose meter Accu-Chek Active (Germany).

The compounds under investigations were tested orally in doses 100.0 and 400.0 mg·kg^{-1}. At present work metformin was the medicine of comparision in doses 200.0—400.0 mg kg^{-1} (orally).

3. Analgesic activity. Analgesic activity of compounds was investigated using mice-males (20—22 g each, in groups of 10 animals) under chemical painful stimulation.[16]

Painful stimulation was caused by intraperitoneal injection of 0.75% acetic acid (0.1 mL·10 g^{-1} of body weight). During 15 min the number of writhes (it is a pain reaction caused by irritation of peritoneum) was counted for the animals placed into individual cage. The compounds under studying in 100 and 200 mg kg^{-1} doses were injected into the stomach by pump an hour before injection of acetic acid. Indometacin in 5.0 mg kg^{-1} (orally) was the medicine of comparision.

4. Antiinflammatory activity. Antiinflammatory activity was estimated on mices by antiexudative action on the on the peritonitis models produced by lipopolysaccharide (LPS)[17] and carrageenan.[18]

Experiments were carried out using mice-males (22.0—23.0 g each, in groups of eight animals). Peritonitis was caused by intraperitoneal injection of lipopolysaccharide (LPS) isolated from *Escherichia coli* (Sigma) in 1.0 mg·kg^{-1} or 0.2 mL of 1% X-carrageenan.

In 4 h after injection of LPS animals were slaughtered (by means of CO inhalation), abdominal cavity was dissected and exudate volume (mL) was measured. Compounds under investigation were injected orally (per os) in 100 and 200 mg kg^{-1} doses an hour before LPS. The control animals were given 0.3 mL of physiologic saline orally. Indometacin in 5.0 mg kg^{-1} (orally) was the medicine of comparision.

The results of the experiments were processed by the methods of variational statistics for biological investigations (detection of arithmetic average, standard error, Student criterion t was applyed under comparison of average).

5. The determination of the acute toxicity of the compounds investigated under single dose for mice. The experiments were carried out using mice-males (18—20 g each). The compounds under studying were injected as a suspension in water with twin-80 into stomach. Each dose was injected to five animals. Behaviour and state of health was looked at during 5 days. LD_{30} was estimated acording to the Kerber's method.[19]

Synthesis and physicochemical characteristics of compounds

4-Anilino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (1a)[10]. ^{1}H NMR (DMSO-d_6), δ: 7.20—7.50 (m, 2 H, Ph); 8.42 (s, 1 H, H(6)); 9.60 (s, 1 H, CHO); 10.60 (br.s, 1 H, N(1)H); 12.46 (s, 1 H, C(4)NH). ^{13}C NMR (DMSO-d_6), δ: 78.0 (C(3)); 107.4 (C(5)); 113.9(C≡N); 124.7, 125.9, 128.6, 137.6 (Ph); 152.6 (C(6)); 155.6 (C(4)); 161.4 (C(2)); 190.7 (CHO).

Synthesis of compounds 3a,b and 4a,b (general procedure). Method ***A.*** A mixture of compound **1a,b** or **2a,b** (2 mmol) and aminoguanidinium hydrogencarbonate (4 mmol) in pyridine (10 mL) was refluxed for 24 h (for **1a**), the necessary reaction time was determined using TLC. After cooling the reaction mixture to 20 °C, the precipitate was filtered off, washed with isopropyl alcohol and petroleum ether.

B. Concentrated HCl (0.2 mL) in water (0.2 mL) was added to a mixture of compound **1a,b** or **2a,b** (0.2 g, 0.84 mmol) and aminoguanidinium hydrogencarbonate (0.2 g, 1.50 mmol) in 96% ethanol (4 mL) stirred under reflux. The reaction mixture was stirred under reflux for 7 h (the precipitate was not dissolved under these conditions), and cooled to 20 °C. The precipitate was filtered off and washed with 96% ethanol. Compounds **3a,b** and **4a,b** were obtained as hydrochlorides.

2-[(4-Anilino-3-cyano-2-oxo-1,2-dihydropyridin-5-yl)-methylidene]aminoguanidine (3a) was prepared from compound **1a** according to the method ***A***. M.p. 298—300 °C (DMF : Pr^{i}OH, 1 : 1), the yield was 78%. ESI MS, m/z: 296 $[M + H]^+$, 591 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 5.49, 5.66 (both br.s, 2 H each, NHC(=NH)NH_2); 7.22 (t, 1 H, Ph, J_o = 6.9 Hz); 7.30—7.41 (m, 4 H, Ph); 7.74 (s, 1 H, H(6)); 8.04 (s, 1 H, C(5)CH); 11.58 (s, 1 H, C(4)NH). ^{13}C NMR (DMSO-d_6), δ: 79.5 (C(3)); 106.05 (C(5)); 115(C≡N); 124.7, 125.9, 128.6, 137.6 (Ph); 141.0 (C(6)); 144.4 (C(5)CH); 155.3 (C(4)); 159.0 (NHC(=NH)); 161.5 (C(2)). The assignment of the signals in the ^{13}C NMR spectrum of compound **3a** was made based on the HMBC technique. Characteristic correlation peaks in the HMBC spectrum of compound **3a** are as follows: 7.74/106.0 H(6)/C(5), 7.74/144.4 H(6)/C(5)CH, 7.74/155.3 H(6)/C(4), 7.74/161.5 H(6)/C(2), 8.04/106.0 C(5)CH/C(5), 8.04/141.0 C(5)CH/C(6), 8.04/155.3 C(5)CH/C(4) (the numeration shown on Scheme 1 was used).

2-[(4-Anilino-3-cyano-2-oxo-1,2-dihydropyridin-5-yl)-methylidene]aminoguanidine hydrochloride (3a·HCl) was prepared from compound **1a** according to the method ***B***. M.p. 310—311 °C (DMF : Pr^{i}OH, 1 : 1), the yield was 50%. ESI MS, m/z: 296 $[M + H]^+$, 591 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 7.22—7.30 (m, 3 H, Ph); 7.35—7.40 (m, 2 H, Ph); 7.7 (br.s, 4 H, NHC(=NH)NH_2); 8.07 (s, 1 H, H(6)); 8.11 (d, 1 H, C(5)CH, J_m = 2.9 Hz); 9.8 (br.s, 1 H, C(4)NH); 11.47 (br.s, 1 H, N(1)H). Found (%): C, 50.77; H, 4.54; N, 29.50. $C_{14}H_{13}N_7OCl$. Calculated (%): C, 50.68; H, 4.25; N, 29.55.

C. Concentrated HCl (0.05 mL) in water (0.05 mL) was added to a solution of compound **5a** (0.05 g, 0.12 mmol) in 96% ethanol (1 mL) with stirring under reflux. The reaction mixture obtained was refluxed with stirring for 2 h (the precipitate was not dissolved), and was cooled to 20 °C. The precipitate was filtered off and washed with 96% ethanol. Compound **3a** was obtained as hydrochloride in the yield of 0.013 g (26%).

2-{[4-(4-Chloroanilino)-3-cyano-2-oxo-1,2-dihydropyridin-5-yl)methylidene}aminoguanidine (3b) was prepared from compound **1b** according to the method ***A***. The reaction time was 32 h, m.p. 335 °C (Pr^{i}OH), the yield was 67%. ESI MS, m/z: 330 $[M + H]^+$, 659 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 5.49, 5.72 (both br.s, 2 H each, NHC(=NH) NH_2); 7.37 (d, 2 H, Ar, J_o = 8.6 Hz); 7.42 (d, 2 H, Ar, J_o = 8.6 Hz); 7.77, 8.04 (both s, 1 H each, H(6), C(5)CH); 11.47 (br.s, 1 H, C(4)NH). Found (%): N, 30.05. $C_{14}H_{12}N_7OCl$. Calculated (%): N, 29.73.

2-{[4-(4-Chloroanilino)-3-cyano-2-oxo-1,2-dihydropyridin-5-yl)methylidene}aminoguanidine hydrochloride (3b·HCl) was prepared from compound **1b** according to the method ***B***. M.p. 328 °C (DMF), the yield was 90%. ESI MS, m/z: 330 $[M + H]^+$, 659 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 7.29, 7.37 (both d, 2 H each, ClC_6H_4); 7.69 (br.s, 4 H, NHC(=NH)NH_2); 8.10, 8.12 (both s, 1 H each, H(6), C(5)CH); 9.78 (br.s, 1 H, C(4)NH); 12.00 (br.s, 1 H, N(1)H). Found (%): C, 45.77; H, 3.13; N, 26.78. $C_{14}H_{13}N_7OCl_2$. Calculated (%): C, 45.92; H, 3.58; N, 26.77.

2-[4-Anilino-(2-oxo-1,2-dihydropyridin-3-yl)methylidene]-aminoguanidine (4a) was prepared from compound **2a** accordingly to the method ***A***. The reaction time was 5 h. M.p. 280—282 °C

(DMF), the yield was 65%. IR, ν/cm^{-1}: 3325, 3169, 3147 (NH, NH_2), 1640 (CO). ESI MS, *m/z*: 271 $[M + H]^+$, 541 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 5.31, 5.49 (both br.s, 2 H each, NHC(=NH)NH_2); 6.08 (d, 1 H, H(5), J_o = 7.5 Hz); 7.07 (d, 1 H, H(6), J_o = 7.5 Hz); 7.14 (t, 1 H, Ph, J_o = 8.1 Hz); 7.28 (d, 2 H, Ph, J_o = 8.1 Hz); 7.38 (t, 2 H, Ph, J_o = 8.1 Hz); 8.47 (s, 1 H, C(3)CH); 10.89 (br.s, 1 H, N(1)H); 11.46 (br.s, 1 H, C(4)NH).

2-[4-Anilino-(2-oxo-1,2-dihydropyridin-3-yl)methylidene]-aminoguanidine hydrochloride (4a · HCl) was prepared from compound **2a** according to the method ***B***. The reaction time was 1 h. M.p. 306—308 °C (DMF), the yield was 62%. ESI MS, *m/z*: 271 $[M + H]^+$, 541 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 5.82 (d, 1 H, H(5), J_o = 7.5 Hz); 7.11 (d, 1 H, H(6), J_o = 7.5 Hz); 7.30, 7.41 (both m, 5 H, Ph); 7.70 (br.s, 3 H, C(=NH)NH_2); 8.63 (s, 1 H, C(3)CH); 9.77, 11.67 (both br.s, 1 H each, C(4)NH, NH_2C(=NH)N<u>H</u>—N=); 11.06 (br.s, 1 H, N(1)H). Found (%): C, 51.12; H, 5.14; N, 27.53. $C_{13}H_{15}N_6OCl$. Calculated (%): C, 50.90; H, 4.93; N, 27.40.

2-{[4-(4-Chloroanilino)-2-oxo-1,2-dihydropyridin-3-yl)-methylidene}aminoguanidine (4b) was prepared from compound **2b** according to the method ***A***. The reaction time was 15 h. M.p. 320 °C (Pr^iOH), the yield was 41%. IR, ν/cm^{-1}: 3325, 3169, 3147 (NH, NH_2), 1640 (CO). ESI MS, *m/z*: 305 $[M + H]^+$, 609 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 5.34, 5.50 (both br.s, 2 H each, NHC(=NH)NH_2); 6.06 (d, 1 H, H(5), J_o = 7.5 Hz); 7.06 (d, 1 H, H(6), J_o = 7.5 Hz); 7.29 (d, 2 H, Ar, J_o = 8.8 Hz); 7.33 (d, 2 H, Ar, J_o = 8.8 Hz); 8.45 (s, 1 H, C(3)CH); 10.89 (br.s, 1 H, N(1)H); 11.43 (br.s, 1 H, C(4)NH).

Complex of 4-anilino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitrile with aminoguanidine (5a). ***A***. A mixture of compound **1a** (0.1 g, 0.42 mmol) and aminoguanidinium hydrogencarbonate (0.11 g, 0.84 mmol) in pyridine (2 mL) was refluxed until formation of white voluminous precipitate (the reaction time was 30 min). After cooling the reaction mass to 20 °C, the precipitate was filtered off and washed carefully with isopropyl alcohol and water. Compound **5a** was obtained in a yield of 0.11 g. M.p. 314—315 °C (acetonitrile), the yield after recrystallization was 45%. IR, ν/cm^{-1}: 3452, 3288, 3142 (NH, NH_2); 2206 (CN); 1681 (CHO); 1637 (CO). ^{1}H NMR (DMSO-d_6), δ: 4.67 (br.s, 2 H, NH_2); 7.13—7.18 (m, 2 H, Ph); 7.32 (t, 3 H, Ph, J_o = 7.7 Hz); 7.30 (br.s, 4 H, NHC(=NH)NH_2); 8.13 (s, 1 H, H(6)); 9.34 (s, 1 H, CHO); 10.41 (br.s, 1 H, N(1)H). ^{13}C NMR (DMSO-d_6), δ: 79.5 (C(3)); 106.6 (C(5)); 117.5 (C≡N); 124.3, 125.1, 128.5, 138.2 (Ph); 155.2 (C(4)); 162.9 (C(6)), 172.9 (C(2)); 188.6 (CHO). The assignment of the signals in the ^{13}C NMR spectrum of compound **5a** was made based on the HMBC technique. Characteristic correlation peaks in the HMBC spectrum of compound **5a** are as follows: 8.13/106.6 H(6)/C(5), 8.13/155.2 H(6)/C(4), 8.13/172.9 H(6)/C(2), 8.13/188.6 H(6)/CHO, 9.35/106.6 CHO/C(5), 9.35/155.2 CHO/C(4) 9.35/162.9 CHO/C(6) (the numeration shown in Scheme 1 was used). Found (%): C, 53.34; H, 4.76; N, 31.28. $C_{14}H_{15}N_7O_2$. Calculated (%): C, 53.67; H, 4.83; N, 31.29.

B. A mixture of compound **1a** (0.1 g, 0.42 mmol) and aminoguanidinium hydrogencarbonate (0.057 g, 0.42 mmol) in pyridine (2 mL) was refluxed for 2 h. The precipitate was filtered off after cooling the reaction mass to 20 °C and washed carefully with isopropyl alcohol. Compound **5a** was obtained in a yield of 0.076 g (58%). The sample for powder X-ray diffraction analysis was obtained in the following way: compound **5a** (0.05 g) in acetonitrile (100 mL) was refluxed and filtered twice, the filtrate was kept for 10 days at 20 °C. Powder X-ray diffraction analysis of the precipitate that formed was carried out.

Complex of 4-(4-chloroanilino)-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitrile with aminoguanidine (5b) was prepared analogously from compound **1b** according to the variant ***A***. M.p. 310—312 °C. The yield was 69%. IR, ν/cm^{-1}: 3435, 3335, 3285, 3263 (NH, NH_2); 2198 (CN); 1672 (CHO); 1630 (CO). ^{1}H NMR (DMSO-d_6), δ: 4.63 (br.s, 2 H, NH_2); 7.17, 7,36 (both d, 2 H each, ClC_6H_4, J_o = 8.6 Hz); 7.24 (br.s, 4 H, NHC(=NH)NH_2); 8.26 (s, 1 H, H(6)); 9.33 (s, 1 H, CHO); 10.41 (br.s, 1 H, C(4)NH).

Complex of 4-anilino-5-formyl-2-oxo-1,2-dihydropyridine-3-carbonitrile with guanidine (6). ***A***. A mixture of compound **1a** (0.1 g, 0.48 mmol) and diguanidinium carbonate (0.08 g, 0.43 mmol) in pyridine (2 mL) was refluxed until formation of white voluminous precipitate (the reaction time was 30 min). After cooling the reaction mass to 20 °C, the precipitate was filtered off and washed carefully with isopropyl alcohol. Compound **6** with admixture of diguanidinium carbonate was obtained in a yield of 0.15 g. This precipitate was refluxed in acetonitrile (130 mL), filtered off and compound **6** was obtained in a yield 0.1 g (45%). M.p. 235—236 °C (acetonitrile). IR, ν/cm^{-1}: 3425, 3311, 3232 (NH, NH_2); 2204 (CN), 1664, 1657 (CO). ^{1}H NMR (DMSO-d_6), δ: 7.13—7.18 (m, 2 H, Ph); 7.32 (t, 3 H, Ph, J_o = 7.7 Hz); 7.30 (br.s, 5 H, NH_2C(=NH)NH_2); 8.10 (s, 1 H, H(6)); 9.34 (s, 1 H, CHO); 10.40 (br.s, 1 H, C(4)NH). ^{1}H NMR (DMSO-d_6+CD_3OD), δ: 7.15 (d, 2 H, Ph, J_o = 7.7 Hz); 7.30 (t, 3 H, Ph, J_o = 7.7 Hz); 8.06 (s, 1 H, H(6)); 9.32 (s, 1 H, CHO).

B. A mixture of compound **1a** (0.10 g, 0.42 mmol) and diguanidinium carbonate (0.05 g, 0.25 mmol) in pyridine (2 mL) was refluxed for 0.5 h. After cooling the reaction mass to 20 °C, the precipitate was filtered off and washed carefully with isopropyl alcohol. Compound **6** was obtained in a yield of 0.11 g (58%). The sample for powder X-ray diffraction analysis was obtained in the following way: compound **6** (0.09 g) in acetonitrile (130 mL) was refluxed and filtered twice, the filtrate was kept for 10 days at 20 °C. The crystals that formed were filtered off (the yield was 0.012 g) and powder X-ray diffraction analysis of the precipitate was carried out.

4-Anilino-2-oxo-1,2-dihydropyridine (7). ***A.*** A mixture of compound **2a** (0.15 g, 0.7 mmol) and diguanidinium carbonate (0.13 g, 0.72 mmol) in pyridine (3 mL) was refluxed for 22 h. After cooling the reaction mass to 20 °C, the precipitate was filtered off and washed carefully with isopropyl alcohol. The crude product **7** was obtained in a yield of 0.08 g. The mother liquor was concentrated *in vacuo*, the crystalline residue was triturated with isopropyl alcohol, filtered off, and washed with isopropyl alcohol. The crude product **7** was additionally obtained in a yield of 0.11 g. The yield of recrystallized product was 33%. M.p. 215—218 °C (acetonitrile). ESI MS, *m/z*: 187 $[M + H]^+$, 209 $[M + Na]^+$, 395 $[2 M + Na]^+$, 581[3 M +Na]$^+$. ^{1}H NMR (DMSO-d_6), δ: 5.65 (d, 1 H, H(3), ^4J = 2.0 Hz); 5.86 (d.d, 1 H, H(5), ^3J = 7.2 Hz, J_m = 2.0 Hz); 7.01 (t, 1 H, Ph, J_o = 7.7 Hz); 7.03 (d, 1 H, H(6), ^3J = 7.2 Hz); 7.15 (d, 2 H, Ph, J_o = 7.7 Hz); 7.31 (t, 2 H, Ph, J_o = 7.7 Hz); 8.47 (br.s, 1 H, C(4)NH); 10.56 (br.s, 1 H, N(1)H). Found (%): C, 70.63; H, 5.68; N, 15.09. $C_{11}H_{10}N_2O$. Calculated (%): C, 70.97; H, 5.38; N, 15.05.

B. KOH (1 g, 18 mmol) and ethylene glycol (10 mL) were added to pyridone **8** (1 g, 4.7 mmol), and the mixture obtained was refluxed for 6 h. Then it was cooled, diluted with water (40 mL) and kept at 20 °C, the precipitate was filtered off and washed carefully with water. The crude product **7** was obtained

in a yield of 0.75 g. The yield of recrystallization product was 41%. M.p. 228—230 °C (ethanol).

1,3-Bis[(dimethylamino)methylidene]-2-[(4-anilino-3-cyano-2-oxo-1,2-dihydropyridin-5-yl)methylidene]aminoguanidine (9a). ***A.*** A solution of compound **3a** as a free base (0.02 g, 0.68 mmol) and dimethylformamide dimethylacetal (0.35 g, 3.0 mmol) in isopropyl alcohol (2 mL) was kept at 20 °C for 3 days. The solvent and excess of the acetal were evaporated *in vacuo*, the residue was triturated with petroleum ether, the precipitate that formed was filtered off and washed with petroleum ether. Compound **9a** was obtained in a yield of 0.02 g (74%). M.p. 238—240 °C. ESI MS, *m/z*: 406 $[M + H]^+$, 428 $[M + Na]^+$, 811 $[2 M+ H]^+$. ^{1}H NMR (DMSO-d_6), δ: 2.34, 2.87, 2.94, 3.04 (all s, 3 H each, 4 CH_3); 7.24—7.30, 7.35—7.39 (both m, 5 H, Ph); 7.75, 8.27 (both s, 1 H each, H(6); C(5)CH); 8.20, 8,21 (both s, 1 H each, 2 ($(CH_3)_2NC\underline{H}$(=N)); 11.50 (br.s, 1 H, N(1)H); 12.94 (br.s, 1 H, C(4)NH).

B. It was prepared from hydrochloride of compound **3a** according to the method ***A***. A suspension was kept at 20 °C for 13 days. The precipitate was filtered off and washed with isopropyl alcohol. Compound **9a** was obtained in a yield of 0.014 g (50%), m.p. 238—240 °C.

C. It was prepared from hydrochloride of compound **3a** according to the method ***A***. A suspension was kept at 20 °C in toluene for 48 h. The precipitate was filtered off and washed with toluene and petroleum ether. The crude product **9a** was obtained in a yield of 0.02 g (79%), m.p. 190 °C (decomp.). The precipitate with admixture of inorganic impurities decomposed upon attempted recrystallization.

1,3-Bis[(dimethylamino)methylidene]-2-[(4-anilino-3-cyano-1-methyl-2-oxo-1,2-dihydropyridin-5-yl)methylidene]aminoguanidine (9b). ***A.*** $Me_2NCH(OMe)_2$ (0.45 g, 3.8 mmol) was added to a suspension of compound **3a** as a free base (0.2 g, 0.68 mmol) in dry toluene (15 mL). The reaction mixture was refluxed with stirring for 6 h, cooled to 20 °C, the precipitate was filtered off, triturated with isopropyl alcohol, filtered off, and washed with petroleum ether. Analytically pure compound **9b** was obtained in a yield of 0.09 g (34%). M.p. 221—222 °C. ESI MS, *m/z*: 420 $[M + H]^+$, 442 $[M + Na]^+$, 839 $[2 M + H]^+$, 861 $[2 M + Na]^+$. ^{1}H NMR (DMSO-d_6), δ: 2.37, 2.93, 3.00, 3.11, 3.43 (all s, 3 H each, 5 CH_3); 7.24—7.30, 7.33—7,39 (both m, 5 H, Ph); 7.99, 8.16 (both s, 1 H each, H(6), C(5)CH); 8.22, 8,24 (both s, 1 H each, 2 ($(CH_3)_2NC\underline{H}$(=N)); 12.89 (br.s, 1 H, C(4)NH). Found (%): C, 59.64; H, 5.80; N, 29.44. $C_{21}H_{25}N_9O$. Calculated (%): C, 60.13; H, 6.01; N, 30.05.

B. Triethylamine (0.067 g, 0.7 mmol) was added to a suspension of hydrochloride of compound **3a** (0.02 g, 0.07 mmol) in dry toluene (2 mL) and the reaction mixture was stirred for 10 min. Then $Me_2NCH(OMe)_2$ (0.22 g, 1.9 mmol) was added dropwise and the reaction mass was refluxed with stirring for 2 h. The solvent and excess of the acetal were evaporated *in vacuo*, the residue was triturated with isopropyl alcohol, the precipitate that formed was filtered off and washed with isopropyl alcohol; analytically pure compound **9b** was obtained in a yield of 0.01 g

Table 7. Crystallographic characteristics of compounds **3a**, **5a**, and **6**

Parameter	**3a**	**5a**	**6**
Molecular formula	$C_{14}H_{13}N_7O$	$C_{13}H_9N_3O_2 \cdot CH_6N_4$	$C_{13}H_9N_3O_2 \cdot CH_5N_3$
Crystal system	Моноклинная	Моноклинная	Моноклинная
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$
a/Å	13.9356(12))	6.2161(11)	5.4448(5)
b/Å	4.8199(4)	8.2131(13)	17.4721(19)
c/Å	21.6685(19)	30.309(4)	15.3241(18)
α/deg	90	90	90
β/deg	100.96(3)	104.72(3)	103.14(3)
γ/deg	90	90	90
V/Å^3	1428.9(3)	1496.4(4)	1419.5(3)
M_{20}*	27	21	30
F_{30}*	33 (0.010, 47)	34 (0.011, 51)	41 (0.009, 53)
Z	4	4	4
D_x/g cm^{-3}	1.373	1.391	1.396
Radiation	$CuKa_1$	$CuKa_1$	$CuKa_1$
(wave-length/Å)	(1.5406)	(1.5406)	(1.5406)
Powder diagram: $2\theta_{min} - 2\theta_{max}$	4.00—80.00	4.00—75.00	5.00—80.00
(step of measurement/deg)	0.01	0.01	0.01
R_p*	0.0236	0.0142	0.0259
R_{wp}*	0.0308	0.0177	0.0334
R_{exp}*	0.0130	0.0142	0.0185
χ^2*	5.176	1.604	4.252

* Indicators of indication quality M_{20} (see Ref. 26) and F_{30} (see Ref. 27) were determined previously, R_p, R_{wp}, R_{exp} and χ^2 — see Ref. 28.

(34%). M.p. 221—222 °C. ES MS, *m/z*: 420 $[M + H]^+$, 442 $[M + Na]^+$, 839 $[2 M + H]^+$, 861 $[2 M + Na]^+$.

1,3-Bis[(dimethylamino)methylidene]-2-{[4-(4-chloroanilino)-3-cyano-1-methyl-2-oxo-1,2-dihydropyridin-5-yl]methyliden}aminoguanidine (9c) was prepared analogously from compound **9a** according to the method ***A***. The yield was 83%. M.p. 260 °C (decomp.). ESI MS, *m/z*: 454 $[M + H]^+$, 476 $[M + Na]^+$, 907 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 2.42, 2.93, 2.97, 3.07, 3.41 (all s, 3 H each, 5 CH_3); 7.27 (d, 2 H, Ar, J_o = 8.5 Hz); 7.41 (d, 2 H, Ar, J_o = 8.5 Hz); 8.05, 8.43 (both s, 1 H each, H(6), C(5)CH); 8.21, 8,25 (both s, 1 H each, 2 ($(CH_3)_2NC\underline{H}$(=N)); 12.91 (br.s, 1 H, C(4)NH). Found (%): C, 55.70; H, 5.55; N, 27.67. $C_{21}H_{24}N_9OCl$. Calculated (%): C, 55.57; H, 5.33; N, 27.77.

1,3-Bis[((dimethylamino)methylidene]-2-[(4-anilino-2-oxo-1,2-dihydropyridin-3-yl)methylidene]aminoguanidine (10). ***A.*** $Me_2NCH(OMe)_2$ (0.26 g, 2.2 mmol) was added to a suspension of compound **4a** as a free base (0.06 g, 0.22 mmol) in Pr^iOH (3 mL). The reaction mass was kept at 20 °C for 5 days. The precipitate was filtered off and washed with Pr^iOH. The crude product **12** was obtained in a yield of 0.05 g (61%). M.p. 231—234 °C. ESI MS, *m/z*: 381 $[M + H]^+$, 761 $[2 M + H]^+$. ^{1}H NMR (DMSO-d_6), δ: 2.86, 2.94 (both s, 3 H each, 2 CH_3); 3.05, (s, 6 H, 2 CH_3); 5.87 (d, 1 H, H(5), J_o = 7.5 Hz); 7.19 (d, 1 H, H(6), J_o = 7.5 Hz); 7.21, 7.41 (both m, 5 H, Ph); 8.21 (s, 2 H, $(CH_3)_2NC\underline{H}$(=N); 8.70 (s, 1 H, C(3)CH); 10.80 (br.s, 1 H, N(1)H); 12.80 (br.s, 1 H, C(4)NH).

B. It was prepared from compound **4a** as a free base according to the method ***A***. The solution in dry toluene was refluxed for 6 h. The precipitate that formed after keeping the reaction mixture at 20 °C for 48 h was filtered off and washed with toluene. The crude product **10** was obtained in a yield of 0.024 g (29%), m.p. 240—241 °C. The mass spectrum of this sample points to the presence of traces of *N*-methylated (at the pyridine nitrogen atom) product. ESI MS, *m/z*: 395 $[M + H]^+$.

C. A mixture of hydrochloride of compound **4a** (0.022 g, 0.07 mmol) and $Me_2NCH(OMe)_2$ (0.35 g, 3 mmol) in dry toluene (1 mL) was kept at 20 °C for 22 h. The precipitate was filtered off, washed with toluene and petroleum ether. The crude product **10a** was obtained in a yield of 0.026 g (95%), m.p. 212—214 °C. The precipitate was contaminated with inorganic impurities and decomposed upon attempted recrystallization.

X-Ray diffraction analysis of compounds 3a, 5a, and 6. The structures of compounds **3a**, **5a**, and **6** were established by powder X-ray diffraction.[20] Powder diagram was measured in the Guinier camera «Huber G670» containing bent germanium monochromator. The positions of first 30 peaks were refined on the powder diagrams. Using these positions, indicating in the triclinic cell was carried out with the TREOR90 program.[21] The crystal structures of compounds **3a**, **5a**, and **6** were solved by the method of simulated annealing.[22] Three-dimensional molecule model obtained as a result of the optimization by the density functional method using the PRIRODA program[23] was used. Then the solution obtained was refined by the Rietveld method using the MRIA program,[24] peak profiles were described by the modified Voight function.[25] In the refinement, restriction to the permissible deflections of the interatomic distances in the molecule and to the planarity of the rings were applied. Parameters of thermal fluctuations (U_{iso}) of nonhydrogen atoms in **3a** and **6** were refined in an isotropic approximation. For only two general parameters U_{iso} were refined, *viz.*, one for the main molecule and one for the molecule of 2-aminoguanidine. Hydrogen atoms were placed at the calculated positions and were not refined. The principal crystallographic characteristics and experimental parameters of compounds **3a**, **5a**, and **6** are given in

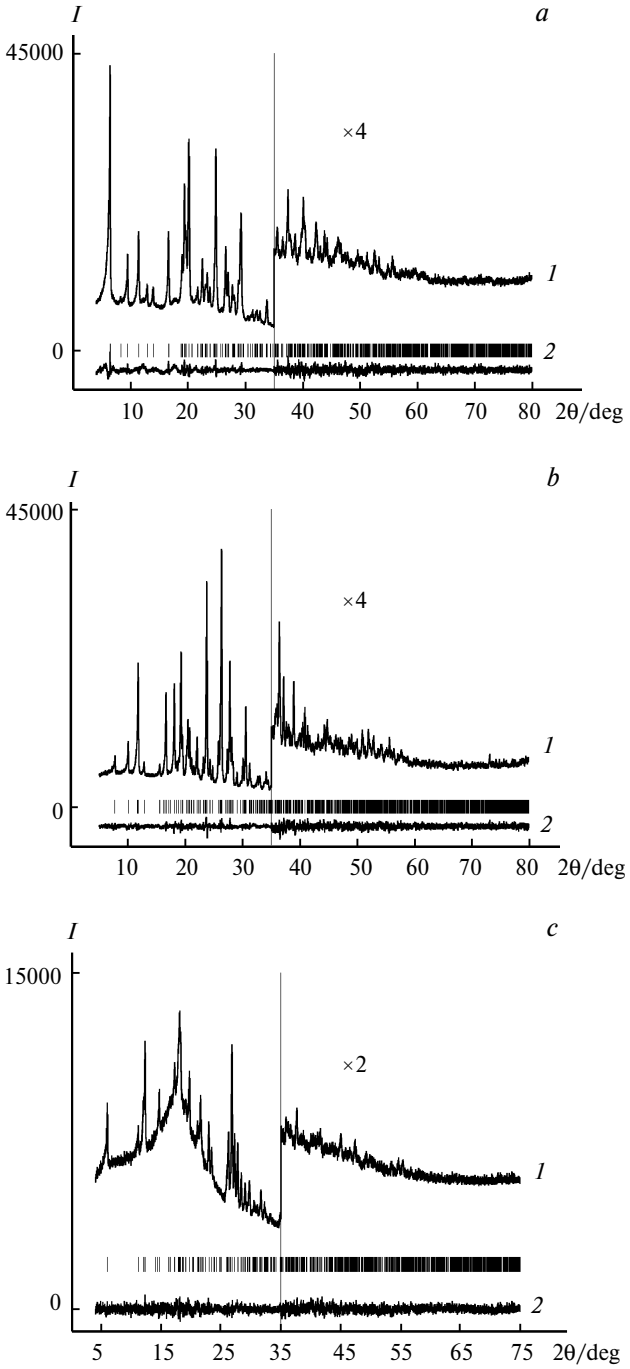


Fig. 3. The result of elucidation of crystal structures of compounds **3a** (*a*), **6** (*b*), and **5a** (*c*) by the Rietveld method: experimental curve (*1*), the difference between the experimental and calculated curves as a result of refinement (*2*); high-angle area (2θ > 35°) is presented on a large scale. The calculated positions of reflexes are indicated as vertical segments.

Table 7. The experimental powder diagram and the difference curve as a result of refinement using the Rietveld method are shown in Fig. 3.

All structural data were deposited with the Cambridge Crystallographic Database (CCDC 785159—785161)*.

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