SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTIARRHYTHMIC EFFECT OF 4-ARYL-5-NITRO-6-PHENYL-3,4-DIHYDRO-(1H)-PYRIMIDIN-2-ONES

V. F. Sedova,[1] T. V. Voevoda,[1] T. G. Tolstikova,[1] and O. P. Shkurko[1]

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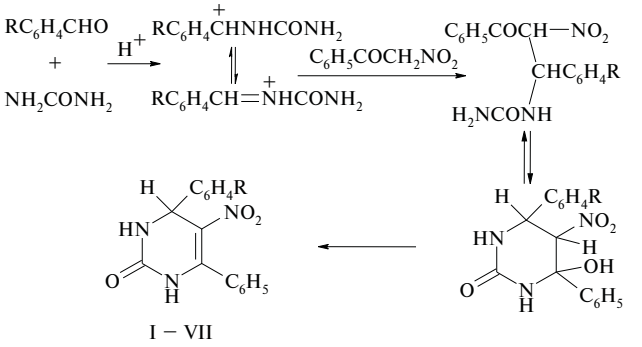
As is known [1, 2], 1,4-dihydropyridine derivatives act as calcium channel blockers. Some compounds of this group (nifedipine, nicardipine, amlodipine, etc.) are used as antihypertensive and antianginal agents [2, 3]. In addition, these compound possess antiarrhythmic activity, which has drawn the interest of clinical chemotherapists [4, 5].

3,4-Dihydro-(1H)-pyrimidin-2-ones, which are close analogs of 1,4-dihydropyridines, possess the same spectrum of pharmacological activity [3]. The hypotensive and spasmolytic properties of dihydropyrimidin-2-ones were originally demonstrated for 5-alkoxycarbonyl-4-phenyl derivatives [6]. Most of the aforementioned dihydropyridine and dihydropyrimidinone derivatives contain ester groups in position 5 of the heterocycle [7, 8]. It was demonstrated that substitution of NO_2 for COOR in the dihydropyridine molecule changes the character of biological action and leads to compounds exhibiting higher toxicity [9, 10].

In the search for new effective cardiovascular drugs among dihydropyrimidines, we have synthesized a series of 4-aryl-5- nitro-6-phenyl-3,4-dihydro-(1H)-pyrimidin-2-ones (I – VII) and studied their pharmacological properties. The interest in these derivatives is due to the generally lower toxicity of pyrimidines as compared to that of pyridines [11, 12]. Below we present data on the antiarrhythmic activity of the synthesized compounds.

The new compounds (II – VII) were obtained via interaction of α-nitroacetophenone, aromatic aldehydes, and urea, by analogy with the synthesis of well-known compound I [13].

The reaction includes the following sequential transformations: (i) acid-catalyzed condensation of aldehyde and urea with the formation of active N-acylimmonium cations, (ii) reaction of these N-acylimmonium cations with nitroacetophenone with the formation of ureido derivatives, and (iii) cyclization of these derivatives into dihydropyrimidinones.



I – VII

R = H (I), *p*-HO (II), *p*-CH_3O (III), *m*-F (IV), *m*-Cl (V), *m*-NO_2 (VI), and *o*-NO_2 (VII).

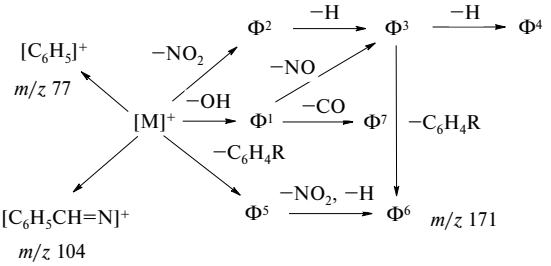
The proposed structures of the new nitrodihydropyrimidinones (II – VII) were confirmed by the data of IR, UV, and ^{1}H NMR spectroscopy and mass spectrometry (Table 1). The UV absorption spectra exhibit two intense bands in the regions of 230 – 250 and 344 – 347 nm. The IR spectra display the characteristic absorption bands of C=O group (1685 – 1712 cm^{-1}) and NO_2 groups featuring antisymmetric (1495 – 1510 cm^{-1}) and symmetric (1323 – 1335 cm^{-1}) vibrations. The ^{1}H NMR spectra of compounds I – VII contain doublet signals from 4-H protons at 6.11 – 6.31 ppm ($J \sim 3$ Hz) and broad doublets from two NH groups in the regions of 7.95 – 7.97 and 8.86 ppm (Table 2).

The mass spectra of nitrodihydropyrimidinones (I – VII) are characterized by low intensity of the peaks of molecular ions (3 – 10 %). There are two possible pathways of dissociative ionization of these compounds: via detachment of NO_2 and OH groups. The main pathway is $[M]^+ \rightarrow \Phi^1$ (I_{rel}, 74 – 100 %) $\rightarrow \Phi^3$ (I_{rel}, 32 – 43 %) $\rightarrow \Phi^4$ (I_{rel}, 64 – 100 %).

Another characteristic feature is the detachment of an aryl substituent occurring in position 4 of the pyrimidine

[1] Vorozhtsov Institute of Organic Chemistry, Siberian Division, Russian Academy of Sciences, Novosibirsk, Russia.

ring, with the formation of the Φ^5 ion with m/z = 218 (I_{rel}, 22 – 100 %). The only exception is presented by the mass spectrum of compound VII containing 4-(*o*-$O_2NC_6H_4$) group, in which maximum intensity is observed for the ion $[M–OH–NO–OH]^+$ with m/z = 276; this is probably explained by spatial proximity of the C_6H_5, NO_2, and *o*-$C_6H_4NO_2$ in the heterocycle.



EXPERIMENTAL PART

The IR absorption spectra were measured on a Bruker Vector 22 spectrophotometer (Germany) using samples pelletized with KBr. The UV spectra of ethanol solution were recorded with a Specord M-40 spectrophotometer (Germany). The mass spectra were obtained with a Finnigan MAT 8200 instrument using direct injection of samples into the ion source. The ^{1}H NMR spectra were measured on a Bruker AC 200 spectrometer using CF_3COOH as a solvent and CH_2Cl_2 as an internal standard. The parameters of IR, UV, ^{1}H NMR, and mass spectra are given in Tables 1 and 2.

The course of reactions was monitored and the purity of the reaction products was checked by TLC on Silufol UV-254 plates eluted with $CHCl_3$. The data of elemental analyses of compounds II – VII for C, H, Cl, F, and N agree with the results of analytical calculations.

4-(*m*-Fluorophenyl)-5-nitro-6-phenyl-3,4-dihydro-(1H)-pyrimidin-2-one (IV). To a solution of 12.0 g (0.2 mole) urea in a mixture of 75 ml isopropanol and 6 ml of concentrated HCl was added 7.4 g (0.06 mole) of m-fluorobenzaldehyde and the reaction mixture was stirred for 1 h and allowed to stand overnight. Then 6.6 g (0.04 mole) of α-nitroacetophenone was added and the mixture was heated to boiling for 12 h. The precipitate was separated, washed sequentially with ethanol, saturated sodium bicarbonate solution, and ethanol again, and crystallized from ethanol to obtain 7.2 g (57%) of compound IV.

Aroyl derivatives I – III and V – VII were obtained using analogous procedures. The products were recrystallized from ethanol (compounds I – III, VI), 60% aqueous ethanol solution (compound V), and an ethanol – dioxane (2 : 1) mixture (compound VII).

EXPERIMENTAL PHARMACOLOGICAL PART

The antiarrhythmic activity of compounds I, II, and IV were studied in a group of mature male rats weighing 170 – 220 g narcotized with thiopental sodium (25 mg/kg, i.p.). The reference drugs were lidocaine and quinidine.

The doses effective in 50% of the test animals (ED_{50}) for the three compounds and reference drugs were determined by probit analysis [14] upon single intraperitoneal administration in rats with model arrhythmias induced by calcium chloride ($CaCl_2$) and epinephrine hydrochloride (EH). Preliminary experiments were used to determine the arrhythmogen doses inducing medium-level heart rhythm violation without lethal ventricular fibrillation. The ED_{50} values were calculated using the experimental data for five doses of each compound (ranging from 3 to 10 mg/kg) and reference drugs (3 to 12 mg/kg). A criterion of the positive action was complete absence of arrhythmia signs.

TABLE 1. Yields and Physicochemical Characteristics of the Synthesized Compounds II – VII

Compound	Empirical formula	M.p., °C	$[M]^+$, m/z found / calcd.	IR spectrum: ν, cm – 1				UV spectrum	Yield, %
				C=O	NO_2(as)	NO_2(s)	NH	λ_{max}, nm (log ε)	
II	$C_{16}H_{13}N_3O_4$	218 – 221	311.0876 / 311.0906	1688	1496	1324	3206, 3408	231 (4.12), 347 (3.81)	57
III	$C_{17}H_{15}N_3O_4$	247 – 249	325.1046 / 325.1062	1696	1510	1335	3207, 3420	230 (4.32), 346 (3.92)	58
IV	$C_{16}H_{12}FN_3O_3$	203 – 205	313.0867 / 313.0863	1686	1502	1326	3208, 3428	242 (3.95), 345 (3.91)	57
V	$C_{16}H_{12}ClN_3O_3$	190 – 192	329.0537 / 329.0567	1688	1500	1323	3204, 3438	242 (3.97), 345 (3.87)	65
VI	$C_{16}H_{12}N_4O_5$	221 – 223	340.0807 / 340.0808	1686	1497, 1530	1326	3201, 3360	250 (4.19), 345 (3.92)	62
VII	$C_{16}H_{12}N_4O_5$	263 – 265	*	1711	1495, 1534	1326	3199, 3386, 3444	245 (3.77), 345 (3.74)	64

* $[M]^+$ peak is absent; for $[C_{16}H_{12}N_4O_5–OH]^+$ fragment, m/z = 323.0734; $C_{16}H_{11}N_4O_4$; calcd., 323.0780.

TABLE 2. Parameters of the ^{1}H NMR Spectra of Compounds I – VII

Compound	Chemical shift: d, ppm (J, Hz)			
	4-H	Ar	N–H	CH_3O
I	6.14 d (3)	7.51 – 7.71 m	7.89 bd, 8.83 s	
II	6.11 d (3)	7.15 d (8), 7.56 – 7.77 m	7.85 bd, 8.79 s	
III	6.09 d (3)	7.20 d (8), 7.50 – 7.72 m	*, 8.62 s	4.07 s
IV	6.12 d (3)	7.20 – 7.65 m	7.75 bd, 8.68 s	
V	6.12 d (3)	7.51 – 7.70 m	7.74 bd, 8.77 s	
VI	6.31 d (3)	7.60 – 7.68 m, 7.82 t (8), 8.11 d (8), 8.45 d (8), 8.60 s	7.98 bd, 8.86 s	
VII	6.67 s	7.62 – 7.85 m, 7.91 m, 8.22 d (8)	8.78 s	

* Signal in the region of aromatic protons.

The therapeutic activity of the compounds studied was characterized by the antiarrhythmic index determined as the ratio of the acute toxicity LD_{50} to ED_{50}. The acute toxicity was studied by single intraperitoneal injections in white mongrel mice and evaluated by the Litchfield – Wilcoxon method [14].

A special series of experiments was devoted to determining the effect of compounds I, II, and IV on the survival of rats with $CaCl_2$ and EH arrhythmia models. These experiments were performed with rats divided into eight groups: group 1, intraperitoneal injection of compounds I, II, and IV (ED_{50}); group 2, $CaCl_2$ control (250 mg/kg, i.v.); group 3, compounds I, II, and IV injected (ED_{50}, i.p.) 10 min before $CaCl_2$ model induction; group 4, EH control (0.3 mg/kg, i.v.); group 5, compounds I, II, and IV injected (ED_{50}, i.p.) 10 min before EH model induction; group 6, intact control (narcotized animals).

The EEGs were recorded at a velocity of 50 mm/sec on an EKIT-03M electrocardiograph (Russia) using subcutaneous needle electrodes in II standard lead. The first record was made immediately after calming of each rat and the dynamics was monitored by EEGs measured 1, 5, 10, and 15 min after drug administration (when possible, up to 45 min). Each test group contained 10 rats. The experimental data were statistically processed using the Student t-criterion [14].

It was established that compounds I, II, and IV belong to the class of low-toxicity substances; none of these compounds produced significant effect on the heart rhythm and frequency in control rats observed for up to 45 min.

RESULTS AND DISCUSSION

Administration of the compounds studied in an effective dose (ED_{50}) before induction of the $CaCl_2$ arrhythmia model significantly reduced the loss of test animals (Table 3). The maximum protection was offered by compounds II and IV. An analysis of the heart rhythm showed that compound IV in all cases prevented the development of dangerous rhythm violations characteristic of this model.

Against the background of compound I, no arrhythmia was developed in 50% of test rats, while the other half of the test animals exhibited cardiac fibrillation at a ventricular rate of about 55 cpm, followed by restoration of the sinus rhythm within 30 – 35 min. Compound II prevented arrhythmia development in 60% of cases and relieved the arrhythmia attack within 2 – 10 min.

The results of tests on the $CaCl_2$ arrhythmia model showed that the average antiarrhythmic activity of compounds I, II, and IV is greater than that of lidocaine and quinidine by a factor of 31 and 15, respectively (Table 4).

The administration of compounds I, II, and IV in effective doses before induction of the epinephrine model showed that the maximum protection of animals from loss was ensured by compound II (Table 3). The average antiarrhythmic activity of compounds I, II, and IV on this model is greater than that of lidocaine and quinidine by a factor of 21 and 8, respectively (Table 4).

TABLE 3. Effect of Compounds I, II, and IV on the Survival of Experimental Animals in the Tests with Calcium Chloride ($CaCl_2$) and Epinephrine Hydrochloride (EH) Induced Arrhythmia Models

Experiment	Dose, mg/kg	Survival, %
$CaCl_2$ (control)	250	40
I + $CaCl_2$	3.85 + 250	70
II + $CaCl_2$	3.75 + 250	90
IV + $CaCl_2$	3.60 + 250	100
EH (control)	0.3	10
I + EH	3.90 + 0.3	40
II + EH	3.75 + 0.3	90
IV + EH	3.75 + 0.3	70

TABLE 4. Comparative Efficacy of Compounds I, II, and IV and Reference Antiarrhythmic Drugs in the Tests with Calcium Chloride ($CaCl_2$) and Epinephrine Hydrochloride (EH) Induced Arrhythmia Models

Compound	ED_{50}, mg/kg		LD_{50}, mg/kg	Antiarrhythmic index (LD_{50}/ED_{50})	
	$CaCl_2$ model	EH model		$CaCl_2$ model	EH model
I	3.85	3.90	> 1500	390	385
II	3.75	3.75	> 1500	400	400
IV	3.60	3.75	> 1500	417	400
Lidocaine	9.80	6.90	128	13.06	18.55
Quinidine	8.30	4.60	220	26.50	47.83

An analysis of the heart rhythm showed that the injection of epinephrine against the background of compound I was typically accompanied by a sinus rhythm, with a sino-auricular (SA) block and a substituting rhythm coming, probably, from the atrioventricular (AV) junction, which was completely restored within 2 – 5 min. Against the background of compound II, the epinephrine injection led to a short-time bradycardia without violation of the sinus rhythm. Epinephrine injection against the background of compound IV led to a short-time sinus bradycardia with SA block and either a substituting rhythm (15%) or a complex (15%) coming, probably, from the AV junction; the sinus rhythm was completely restored within 5 – 7 min.

Thus, the results of our investigation lead to the following conclusions:

(i) The synthesized nitrodihydropyrimidinones belong to the class of low-toxicity substances;

(ii) These compounds prevent or relieve the development of manifestations characteristic of the $CaCl_2$ and epinephrine arrhythmia models;

(iii) The antiarrhythmic activity of these compounds exceeds by one order of magnitude that of the reference antiarrhythmogens such as lidocaine and quinidine;

(iv) The presence of substituents (F, OH) in 4-aryl groups of the compounds studied enhances the antiarrhythmic effect of the 4,6– diphenyl derivative I.

The experimental data obtained call for continuation of the search for effective and low-toxic antiarrhythmic drugs among 5-nitrodihydropyrimidine derivatives.

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