SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 1-SUBSTITUTED 5-ARYL-4-ACYL-3-HYDROXY-3-PYRROLIN-2-ONES

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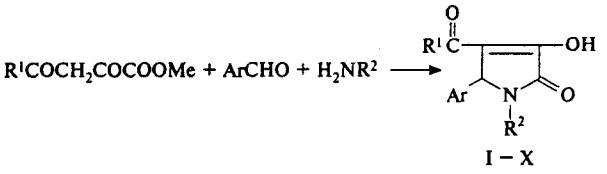
Previously we have reported on the antiinflammatory [1], analgesic [2], antiviral [3], and nootropic [2] properties of 1,4,5-trisubstituted tetrahydropyrrole-2,3-diones.

In continuation of the search for new compounds possessing nootropic and antibacterial activity, we have synthesized a series of tetrahydropyrrole-2,3-diones with various functional groups in position 1 of the heterocycle.

For this purpose we have studied the interaction of methyl ester of acetyl- and aroylpyruvic acids with a mixture of an aromatic aldehyde with aliphatic amines containing variously functionalized groups.

In all cases, the reactions conducted in aqueous – alcohol mixtures with short-time heating led to a good yield of 1-substituted 5-aryl-4-acyl-3-hydroxy-3-pyrrolin-2-ones (I – X):



I – X

R^1 = Me (I – VI, VIII, IX), Ph (VII), 4-ClC_6H_4 (X);
Ar = 4-BrC_6H_4 (I – IV), Ph (V – VII, IX), 4-IC_6H_4 (X), 2-FC_6H_4 (VIII);
R^2 = CH_2CONH_2 (I), CH_2CH_2OH (II), CH_2COOEt (IV, V), CH_2COOH (III, VII, IX, X), $(CH_2)_5ClOOH$ (VI), $CH_2CH(Ph)CH_2COOH$ (VIII).

Compounds I – X are colorless crystalline substances insoluble in water and soluble in the usual organic solvents. Table 1 gives the physicochemical characteristics of these compounds.

The IR spectra of pyrrolinones I – X show the absorption bands due to the stretching vibrations of lactam carbonyl groups (1680 – 1710 cm^{-1}) and ketone side-chain carbonyls (1638 – 1660 cm^{-1}). The spectra of compounds III and VI – X contain additional bands due to carbonyls of the carboxy groups (1710 – 1720 cm^{-1}).

The IR spectra of compounds IV and V show the bands of stretching vibrations of the ester carbonyl (1720 – 1730 cm^{-1}), and the spectrum of compound I exhibits a band due to the amide carbonyl at 1684 cm^{-1} (Table 2).

The ^1H NMR spectra of the synthesized compounds contain signals from the protons of methyl groups of the acetyl substituent (at 2.13 – 2.36 ppm), methylene groups (4.18 – 4.26 ppm), methine protons in position 5 of the heterocycle (4.98 – 5.26 ppm), and aromatic protons (6.88 – 7.58 ppm) (Table 2).

In order to synthesize new biologically active compounds and assess reactivity of the products with respect to nucleophilic agents, we have studied the interactions of compound I with nucleophiles such as *p*-anisidine and thiosemicarbazide.

Our previous data [2] suggested that the nucleophilic agents would predominantly attack carbonyl groups in the side chain of 1-substituted 5-aryl-4-acyl-3-hydroxy-3-pyrrolin-2-ones.

Indeed, the reaction of compound I with thiosemicarbazide led to the formation of 5-(4-bromophenyl)-4-(1-aminothiocarbonylhydrazinoethylidene)-1-(2-aminocarbonylmethyl)tetrahydropyrrole-2,3-dione (XI), while the reaction with *p*-anisidine yielded 5-(4-bromophenyl)-4-[1-(*p*-methoxyphe-

TABLE 1. Physicochemical Characteristics of Synthesized Compounds

Compound	Yield, %	M.p., °C (solvent)	Empirical formula
I	60	234 – 236 (acetonitrile)	$C_{14}H_{13}ÂrN_2O_4$
II	69	234 – 236 (toluene)	$C_{14}H_{14}BrNO_4$
III	75	267 – 269 (isopropanol)	$C_{14}H_{12}BrNO_5$
IV	81	195 – 197 (acetonitrile)	$C_{16}H_{16}BrNO_5$
V	83	145 – 147 (acetonitrile)	$C_{16}H_{17}NO_5$
VI	75	217 – 219 (ethanol)	$C_{18}H_{21}NO_5$
VII	73	232 – 234 (ethanol)	$C_{19}H_{15}NO_5$
VIII	78	232 – 233 (ethanol)	$C_{22}H_{20}FNO_5$
IX	44	240 – 242 (isopropanol)	$C_{14}H_{13}NO_5$
X	78	234 – 236 (ethanol)	$C_{19}H_{13}ClNO_5$
XI	61	194 – 196 (toluene)	$C_{15}H_{17}BrN_5O_3S$
XII	64	168 – 170 (ethanol)	$C_{21}H_{20}BrN_3O_4$

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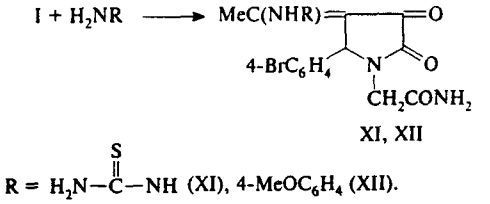
nylamino)ethylidene]-1-(2-aminocarbonylmethyl)tetrahydr opyrrole-2,3-dione (XII).



XI, XII

R = $H_2N-\overset{\overset{S}{\|}}{C}-NH$ (XI), 4-$MeOC_6H_4$ (XII).

Compounds XI, XII (Table 1) are yellow crystalline substances insoluble in water and soluble in most of the common organic solvents. The characteristics of these compounds are given in Table 1.

The IR spectrum of compound XI displays absorption bands due to the stretching vibrations of lactam carbonyls (1710 cm^{-1}), amide carbonyls (1690 cm^{-1}), and double bonds (1632 cm^{-1}).

The ^1H NMR spectrum of compound XI contains signals from the protons of acetyl group (1.73 ppm), methine protons in position 5 of the heterocycle (5.35 ppm), two doublets of the enantiotope protons of a methylene group in position 1 of the heterocycle (3.18 and 4.15 ppm, spin – spin coupling constant 18 Hz), a multiplet of aromatic protons centered at 7.18 ppm, and signals due to the NH-proton (12.55 ppm) and three protons of the methoxy group (3.71 ppm). A shift of the signal of methyl protons toward stronger field (by 0.52 ppm) as compared to the signal of this group observed in the spectrum of initial 5-(*p*-bromophenyl)-4-acetyl-3-hydroxy-1-aminocarbonylmethyl-3-pyrrolin-2-one is evidence of the oxygen displacement in the side chain.

The IR spectrum of compound XII displays absorption bands due to the stretching vibrations of lactam carbonyls (1694 cm^{-1}), amide carbonyls (1678 cm^{-1}), and double bonds (1630 cm^{-1}).

The ^1H NMR spectrum of compound XII contains signals from the protons of acetyl group (2.11 ppm), methine protons in position 5 of the heterocycle (5.08 ppm), doublets of the enantiotope protons of a methylene group in position 1 of the heterocycle (3.18 and 4.05 ppm, spin – spin coupling constant 18 Hz), and signals of aromatic protons in position 5 of the heterocycle, centered at 7.25 ppm.

EXPERIMENTAL CHEMICAL PART

The IR absorption spectra of the synthesized compounds were recorded on a UR-20 spectrophotometer using samples prepared as nujol mulls. The ^1H and ^{13}C NMR spectra were measured on an RS-60 spectrometer operated at a working frequency of 60 MHz, using DMSO-d_6 as the solvent and hexamethyldisiloxane as the internal standard.

The course of the reactions was monitored and the purity of the synthesized compounds was checked by TLC on Silufol UV-254 plates eluted in the ether – benzene – acetone (10 : 9 : 1) system. The spots of 5-aryl-4-acetyl-1-carboxyalkyltetrahydropyrrole-2,3-diones were developed in iodine vapors.

The data of elemental analyses agree with the results of analytical calculations according to the empirical formulas.

General method of obtaining 1-substituted 5-aryl-4-acyl-3-hydroxy-3-pyrrolin-2-ones (I – X). To a mixture of 0.01 mole methyl ester of acylpyruvic acid and 0.01 mole aromatic aldehyde in 10 ml of ethyl alcohol was added 0.01 mole carboxy- or hydroxyalkylamine in 5 ml water. The mixture was heated until dissolution of the reagents and then allowed to stand for 12 h at room temperature. The precipitate was filtered and crystallized from ethyl alcohol. Reaction mixtures for the synthesis of compounds IV, V, and VIII contained additionally 0.01 mole of sodium bicarbonate in 5 ml water.

5-(*p*-Bromophenyl)-4-(1-aminothiocarbonylhydrazin oethylidene)-1-(2-aminocarbonylmethyl)-tetrahydropyrrole-2,3-dione (XI). A mixture of 1 g 5-(*p*-bromophenyl)-4-acetyl-3-hydroxy-1-(2-aminocarbonylmethyl)–3-pyrrolin-2 –

TABLE 2. Spectral Characteristics of Compounds I – XII

Compound	IR spectrum: ν, cm^{-1}				^1H NMR chemical shift δ, ppm				
	OH (NH)	COR	CON	CO	ArH	5-CH, s	CH_2 (J, Hz)	CH_3, s	Other protons
I	3168 (3428)	1684	1696	1638	7.05 d 7.35 d	5.15	2.95 d (18), 4.05 d (18)	2.25	
II	3154 (3544)		1690	1660	7.38 q	5.21	3.41 m	2.18	
III	3110	1710	1680	1650	7.21 m	5.18	3.18 d (18), 4.21 d (18)	2.35	
IV	3150	1720	1690	1642	7.21 m	5.15	3.31 d (18), 4.33 d (18)	2.23	1.05 t, 3.78 q (OC_2H_5)
V	3164	1730	1700	1650	7.38 m	5.21	3.45 d (18), 4.43 d (18)	2.25	1.08 t, 3.90 q (OC_2H_5)
VI	3110	1710	1680	1650	7.28 m	5.15		2.21	1.18 – 3.38 m ($(CH_2)_5$)
VII	3100	1710	1660	1647	6.88 m	4.98	3.06 d (18), 4.15 d (18)		
VIII	3150	1720	1670	1650	7.58 m	5.10	3.65 – 3.71 m	2.15	4.45 m (CHPh), 7.31 m (Ph)
IX	3150	1720	1690	1660	7.38 m	5.26	3.36 d (18), 4.26 d	2.36	
X	3080	1710	1670	1645	7.25 m	5.65	3.55 d (18), 4.15 d (18)		
XI	3120 (3252, 3404)	1690	1710		7.18 m	5.35	3.18 d (18), 4.15 d (18)	1.73	3.71 s (OCH_3), 12.55 s (NH)
XII	(3232, 3280, 3322)	1678	1694		7.25 m	5.08	3.18 d (18), 4.05 d (18)	2.11	

TABLE 3. Antimicrobial Activity Compounds I – XII

Compound	MIC, μg / ml	
	St. aureus	*E. coli*
I	1000	1000
II	1000	1000
III	1000	500
IV	1000	1000
V	1000	1000
VI	500	125
VII	125	1000
VIII	125	500
IX	500	1000
X	125	500
XI	500	500
XII	250	250
Aethacridine lactate	2000	500
Mercury dichloride	1000	1000

TABLE 4. Acute Toxicity and Antiamnesic Effect of Compounds I – XII

Compound	LD_{50}, mg / kg	Time of stay in dark compartment, sec	Number of animals visiting dark compartment, %
I	890.0	17.5	10
II	325.0	28.5	20
III	> 1000	65.0	50
IV	650.0	103.3	60
V	282.0	21.9	10
VI	> 500	35.0	20
VII	> 500	41	40
VIII	> 500	45.0	40
IX	> 500	56	80
X	> 500	71.0	60
XI	43.3	101.2	60
XII	> 500	68.6	80
Control	–	133.9	90
Piracetam	> 8000	41.2	40

0.25 g thiosemicarbazide was dissolved in 5 ml of ethyl alcohol. The reaction mixture was heated for 2 h on a water bath and cooled. The precipitate was filtered to obtain 1.16 g of compound XI.

5-(*p*-Bromophenyl)-4-[1-(*p*-methoxyphenylamino)-ethylidene]-1-(2-aminocarbonylmethyl)tetra-hydropyrrole-2,3-dione (XII). A mixture of 1 g 5-(*p*-bromophenyl)-4-acetyl-3-hydroxy-1-(2-aminocarbonylmethyl)-3-pyrrolin-2 – 0.35 g *p*-anisidine was dissolved in in 5 ml of ethyl alcohol. The reaction mixture was heated for 2 h on a water bath and cooled. The precipitate was filtered to obtain 1.10 g of compound XII.

EXPERIMENTAL PHARMACOLOGICAL PART

Bacteriostatic properties of the synthesized compounds with respect to standard strains of *Staphylococcus aureus* and *Escherichia coli* were studied by the method of double serial dilutions in a beef-infusion broth using a bacterial load of 250 × 10^3 microbial cells per ml solution [4]. The acting dose was determined as a minimum inhibiting concentration of the compound (MIC, μg / ml) suppressing the growth of test microbes.

Taking into account the structural similarity between the compounds studied and piracetam, the antiamnesic activity was studied as described in [5].

The tests were performed on a group of white mongrel rats weighing 180 – 220 g. The synthesized compounds were intraperitoneally injected at a dose of 0.1 LD_{50} as suspensions in a 2% starch jelly 24 h after the psychogenic amnesia induction. Animals in the control group were injected with the same volume of pure starch jelly. The testing procedures began after another 24 h. The antiamnesic effect was assessed by the percentage of rats visiting the dark compartment and the duration of the stay in this compartment. Each compound was studied in a group of ten animals.

It was established that the antimicrobial activity of most compounds is comparable to that of aethacridine lactate and mercury dichloride (Table 3). For example, compounds VII, VIII, and X suppressed the growth of *St. aureus* at a concentration about 1 / 8 and 1 / 16 of MIC for mercury dichloride and aethacridine lactate, respectively. The maximum activity with respect to *E. coli* was observed for compound VI, whose MIC was 1 / 8 and 1 / 4 of that for mercury dichloride and aethacridine lactate, respectively.

Table 4 presents experimental data on the effect of the synthesized compounds upon psychogenic amnesia in the test animals. As is seen, most of the animals (90%) in the control group visited the dark (penalized) compartment and stayed there on the average for 133.9 sec (prior to the psychogenic amnesia, these characteristics had close values of 100% and 160.7 sec).

All the synthesized compounds favored recovery of the memory trace in the test rats, as evidence by a drop in the number of animals visiting the dark compartment and the time of stay there. The most pronounced antiamnesic action (comparable to that of piracetam) was observed for compounds I, II, V.

The nootropic properties observed in the compounds studied justify the further search for effective antiamnesic drugs.

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