SYNTHESIS AND BIOLOGICAL ACTIVITY OF DERIVATIVES OF BENZOPYRAN-2-ONE

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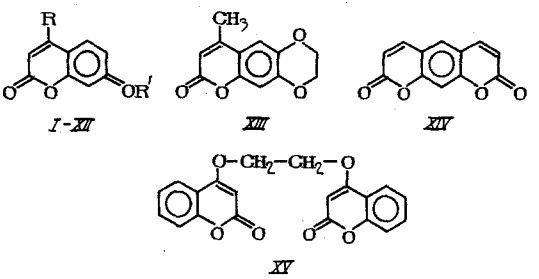
UDC 577.15/17.582.89

We previously studied the biological properties of natural and synthetic derivatives of benzopyran-2-one [1-6] and showed that some of them possess marked spasmolytic, hypotensive, and coronarolytic activity. Later it was discovered during these investigations that several haloalkyl and amino derivatives of benzopyran-2-one also displayed psychotropic properties [7, 8]. These data are the reason for continuing the search for new physiologically active substances in this class of compound.

Results are given in the present work on the synthesis and investigation of the biological activity of certain new derivatives of benzopyran-2-one (Table 1).



EXPERIMENTAL (CHEMICAL)

The IR spectra were taken on a Specord 75 IR instrument in Nujol mulls. The PMR spectra were drawn on a Bruker AC 200 MHz spectrometer, internal standard was TMS. A check on the homogeneity of substances and the course of reactions

TABLE 1. Characteristics of Compounds I-XV

Compound	R	R'	Yield, %	mp, °C	LD_{50}, mg/kg intraperitoneally, mice
I	H	$(CH_2)_2Br$	30,5	135—6	207
II	Me	$(CH_2)_2Br$	46,0	106—6,5	1500
III	H	$(CH_2)_3Cl$	31,0	107—9	367
IV	Me	$(CH_2)_3Cl$	53,0	79—80	4250
V	Me	1-Piperidinylethyl	93,0	90—1	400
VI	Me	Piperidinium ethobromide	88,0	249—51	175
VII	Me	4-Morpholinoethyl	91,0	102—3	398
VIII	H	3-Hydroxyquinuclidinium ethobromide	91,0	246—7	287
IX	Me	3-Hydroxyquinuclidinium ethobromide	88,0	255—6	290
X	Me	(1-Imidazolyl)ethyl	55,0	112—4	1500
XI	H	(1-Benzimidazolyl) ethyl	62,0		1200
XII	Me	(9-Adeninyl)ethyl	91,3	296,0	800
XIII	—		92,5	157—8	2700
XIV	—	—	10,0	344—5	1000
XV	—	—	91,0	175,0	100

St. Petersburg Scientific-Research Institute for Vaccines and Sera. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 27, No. 11, pp. 34-38, November, 1993. Original article submitted December 30, 1992.

0091-150X/93/2711-0766$12.50

was effected chromatographically on Silufol UV 254 plates in benzene—ethyl acetate (2:1) and benzene—acetone (1:1, 1:5, and 10:3). The synthesis of compounds (I)-(IX) is described in [8-10]. The found elemental analysis values corresponded to calculated values.

7-[2-(1-Imidazolyl)ethyloxy]-4-methylbenzopyran-2-one (X). 7-(2-Bromoethyloxy)-4-methylbenzopyran-2-one (II) (1.0 g: 0.003 mole) was added with stirring to a mixture of imidazole (1.6 g: 0.02 mole) and K_2CO_3 (5.0 g) in DMF (50 ml). The reaction mixture was stirred at 110°C for 17 h, then poured into ice—water. The solid was filtered off, dissolved in chloroform, and applied to a column of silica gel L 40/160. The column was eluted with petroleum ether, a mixture of the latter and chloroform (1:5 and 1:1), and with chloroform. Compound (X) (0.52 g: 55.0%) was obtained on eluting with chloroform. IR spectrum, ν_{max}, cm^{-1}: 1700, 1720 (C=O in α-pyrone ring), 1610, 1560, 1510 (CH=CH bond in aromatic ring), 1270, 1280 ($ArOCH_2$). PMR spectrum ($CDCl_3$, δ, ppm: 6.20 (s, H-3), 7.70 and 6.90 (d, J = 8.5 Hz, H-5 and H-6), 7.10 (s, 2H, H-8 in aromatic ring and H- in imidazole ring), 4.40 (m, OCH_2CH_2N), 2.40 (s, CH_3 in position 4 of the α-pyrone ring).

7-[2-(1-Benzimidazolyl)ethyloxy]benzopyran-2-one (XI) was obtained analogously to (X) from benzimidazole and 7-(2-bromoethyloxy)benzopyran-2-one. The chloroform fractions containing compound (XI) were combined, and evaporated in vacuum to give compound (XI) (0.70 g: 62.0%). IR spectrum, ν_{max}, cm^{-1}: 1700, 1720 (C=O of α-pyrone ring), 1620, 1610, 1550, 1500 (CH=CH bond in aromatic ring), 1250-1300 ($ArOCH_2$). PMR spectrum ($CDCl_3$), δ, ppm: 6.30 and 8.0 (d, J = 10.0 Hz, H-3 and H-4), 7.70 and 6.90 (d, J = 8.5 Hz, H-5 and H-6), 7.0 (d, J = 2.5 Hz, H-8), 7.30 and 7.80 (m, H in benzimidazole ring), 4.50 and 4.70 (m, OCH_2CH_2N).

7-[2-(9-Adeninyl)ethyloxy]-4-methylbenzopyran-2-one (XII). 7-(2-Bromoethyloxy)-4-methylbenzopyran-2-one (II) (2.83 g: 0.01 mole) was added to a solution of adenine sodium salt (1.65 g: 0.01 mole) in absolute DMF (50 ml). The reaction mixture was stirred at room temperature for 1 day. The solid was then filtered off, the filtrate evaporated in vacuum, the residue was washed with water, and dried. Compound (XII) (3.08 g: 91.3%) was obtained after crystallization from chloroform. IR spectrum, ν_{max}, cm^{-1}: 3250-3380 (NH_2 group), 1715 (C=O of α-pyrone ring), 1650, 1530, 1510 (CH=CH bond in aromatic ring), 1280, 1290 ($ArOCH_2$). PMR spectrum (DMSO, δ, ppm): 6.20 (s, H-3), 7.70 (d, J = 8.5 Hz, H-5), 6.95 (d, J = 8.5 Hz, H-6), 7.0 (d, J = 2.5 Hz, H-8), 8.15 and 8.25 (s, H-2 and H-8 of the adenine ring), 4.60 (m, OCH_2CH_2N), 2.40 (s, CH_3 in position 4 of the α-pyrone ring).

6,7-(Ethylenedioxy)-4-methylbenzopyran-2-one (XIII). 1,2-Dibromoethane (20 ml) was added with stirring to a reaction mixture consisting of 4-methylesculetin (2.0 g: 0.01 mole) and K_2CO_3 (2.0 g) in DMF (100 ml). The reaction mixture was heated at 130°C for 5 h, poured into water, the solid filtered off, washed with water, and recrystallized from ethanol. Compound (XIII) (2.1 g: 92.5%) was obtained. IR spectrum, ν_{max}, cm^{-1}: 1710 (C=O of α-pyrone ring), 1620, 1580, 1500 (CH=CH bond in aromatic ring), 1230-1300 ($ArOCH_2$). PMR spectrum ($CDCl_3$, δ, ppm): 6.15 (s, H-3), 7.10 (s, H-5), 6.85 (s, H-8), 4.40 (m, OCH_2CH_2O), 2.40 (s, CH_3 in position 4 of the α-pyrone ring).

2H,6H-Benzo[1,2-b:5,5-b]dipyran-2,6-dione (XIV). A mixture of resorcinal (37.0 g: 0.34 mole) and malic acid (57.0 g: 0.4 mole) in concentrated sulfuric acid (150 ml) was heated at 130°C for 30 min, and poured into water. The solid was filtered off, washed with water, and recrystallized from DMF. Compound (XIV) (8.0 g: 10.0%) was obtained. IR spectrum, ν_{max}, cm^{-1}: 1700, 1710 (C=O of α-pyrone ring), 1610, 1570, 1550 (CH=CH bond in aromatic ring). PMR spectrum (DMSO, δ, ppm): 650 and 8.60 (d, J = 10.0 Hz, H-3, H-3′, H-4, and H-4′), 8.0 (s, H-5), 7.5 (s, H-8).

4,4′-Ethylenedioxybisbenzopyrane-2,2′-dione (XV). A mixture consisting of 4-hydroxybenzopyran-2-one (1.62 g: 0.01 mole),K_2CO_3 (4.15 g: 0.03 mole), potassium iodide (0.1 g), 1,2-dibromoethane (11.0 g: 0.06 mole), and dimethylacetamide (65 ml) was heated with stirring on a water bath for 6 h. The solvent was evaporated in vacuum, the residue was washed with water, then with boiling benzene, and was recrystallized from chloroform. Compound (XV) (3.20 g: 91.3%) was obtained. IR spectrum, ν_{max}, cm^{-1}: 1720 (C=O of α-pyrone ring, 1620, 1610, 1580, 1500 (CH=CH bond in aromatic rings), 1250, 1280 (=$COCH_2$). PMR spectrum (DMSO, δ, ppm): 7.30-8.0 (m, 8H, aromatic protons), 5.80 (s, 2H, H-3 and H-3′), 4.60 (s, OCH_2CH_2O).

EXPERIMENTAL (BIOLOGICAL)

Experiments were carried out with 3200 random-bred male mice of weight 18-20 g, and 420 male rats of weight 180-200 g. The substances being studied (see Table 1) were administered to the animals once intraperitoneally and intravenously as an emulsion in Tween-80 in doses containing 1/5 to 1/20 of the LD_{50} at 30-60 min before the investigation. Solvent in appropriate amounts was administered to control animals.

TABLE 2. Neurotropic Activity of Benzopyran Derivatives

Compound	ED_{50} at p = 0.05						
	by test			in relation to convulsive action			
	PTD*	amphetamine hyperactivity in mice $ED_{0.5}$	rotating rod	corazole	thiosemicarbazide	strychnine	maximal electroshock
I	0,5	0,2	7,6	—	91,4	—	71,1
II	2,85	1,2	16,3	8,9	21,1	93,9	62,8
III	31,5	22,8	—	52,5	91,0	—	—
IV	50,0	49,2	117,1	13,6	5,3	89,1	97,2
V	0,1	3,0	13,5	—	—	—	—
VI	0,62	2,7	26,2	3,65	9,9	—	—
VII	0,72	0,5	16,2	—	—	—	57,0
VIII	1,45	1,2	46,9	7,56	16,4	138,8	47,4
IX	2,0	2,3	—	13,7	25,9	85,8	51,5
Chloropromazine	2,3	1,9	0,5	—	—	—	—
Diazepam	2,6	—	2,1	0,53	1,63	7,8	2,79
Chlordiazepoxide	—	—	4,5	4,6	3,67	31,6	9,6

*PTD) Potentiation of the threshold dose of pentothal sodium.

The neurotropic activity of compounds was assessed by generally accepted methods of investigation using the pharmacological test agents chloral hydrate, amobarbital sodium, pentothal sodium, arecoline, nicotine, thiosemicarbazide, strychnine, and amphetamine [11].

The comparative antiarrhythmic activity of compounds was studied in experiments on mice. Arrhythmogens administered to the animals were potassium chloride (330 mg/kg), aconitine (10 μg/kg), adrenaline (200 mg/kg), pituitrin (1 unit/kg), and strophanthin (60 μg/kg). Arrhythmia caused by the administration of barium chloride (4 mg/kg) was studied in experiments on rabbits. The ECG were recorded with a standard lead II on a RM 6000 polygraph (Japan) with the aid of needle electrodes before administration of arrhythmogens and 5 and 30 min after administration, additional ECG were recorded only when the monitor observations revealed any changes.

The following ECG parameters were subjected to statistical treatment: interval PQ (sec), interval QT (sec), variation amplitude ΔX (sec) determines as the difference of RR_{max}' and RR_{min}'.

The acute toxicity of compounds was determined in experiments in mice on intravenous and intraperitoneal administration of aqueous solutions with Tween 20 (1 drop) calculated as 0.1 ml solution per 10 g mouse body weight. The quantity of solvent was doubled when administering compounds at doses over 1000 mg/kg. Solvent was administered to control animals.

The effect of compounds on erythrocyte membranes was determined from the extent of hemolysis of a suspension of erythrocytes in standard mixtures of isotonic solutions of urea and sodium chloride taken in different ratios.

The experimental data were processed statistically using the Student t criterion (at p = 0.05). Each type of neurotropic activity was calculated as an ED_{50} or $ED_{0.5}$ [12].

RESULTS AND DISCUSSION

Many of the substances shown in Table 2 prolong the hypnotic action of amobarbital sodium, chloral hydrate, and pentothal sodium (30 mg/kg intravenously). We studied the potentiation of the anesthetizing action of a subthreshold dose of pentothal sodium (12 mg/kg intravenously) by the substances in order to exclude the moderating effect of prolongation by the inhibition of liver microsomal enzymes [12]. It was shown that compounds (I)-(IX) gave a clear potentiating effect (see Table 2) which indicated the neurotropic nature of the depriming action of these substances. The compounds investigated had practically no effect on the central and peripheral M-cholinolytic effect of arecoline or on the convulsive component of nicotine hyperkinesia (H-cholinomimetic action of nicotine, 1.2 mg/kg intravenously). Fairly high antiamphetamine activity with marked selectivity was displayed by compounds (I), (II), and (VII) (see Table 2), their selectivity indices (SI) were 2.5, 2.4, and 1.6, respectively. The SI of chlorpromazine is 1.2 and of levomepromazine 1.8. It must be noted that the selective efficiency of the compounds mentioned in the amphetamine hyperativity test is an important prognostic feature enabling these compounds to be regarded as potential neuroleptics. Marked, but nonselective, antiamphetamine action is a characteristic of compounds (V), (VI), (VIII), and (IX) (see Table 2). It must be emphasized that a strengthening of the amphetamine hyperactivity was recorded on administering (I) and (II) at doses of 0.25-1 mg/kg. The piperidine derivatives of phenothiazine display the same effect at low doses [11].

TABLE 3. Effect of Compounds on Erythrocyte Hemolysis (as % of control, n = 10)

Compound	Control	Dose, mg/kg		
		0,1	1,0	10,0
X	100±5,9	82,7±6,6*	94,1±7,8	86,1±8,0*
XI	100±4,8	115,3±4,6*	98,9±6,4	85,9±5,5*
XII	100±4,7	83,6±6,4*	101,3±9,0	125,8±5,1*
XIII	100±7,2	93,7±9,0	87,0±6,8	82,2±7,4*
XIV	100±7,3	89,5±7,5	99,1±8,6	154,3±5,5*
XV	100±7,1	115,1±11,3*	137,0±10,4*	171,8±9,4*

*$p < 0.05$.

In addition, compounds (I) and (II) prevented activation of the EEG caused by the administration of amphetamine (1 mg/kg intravenously).

The anticonvulsant activity of substances was studied in experiments on mice by administration of various anticonvulsants. Compounds (II), (IV), (VI), (VIII), and (IX) displayed high anticonvulsant activity on administration of corazole (110 mg/kg subcutaneously) (see Table 2). However, the antagonism of substances (II), (VI), (VIII), and (IX) toward the convulsant action of corazole is probably determined by the sedative action of these substances. In addition, these compounds surpass the reference preparations in breadth of their therapeutic spectrum [TI of (IV) was 801.9 against 147.2 for diazepam and 45 for chlordiazepoxide].

Study of the antagonism of the benzopyran-2-one derivatives by strychnine enabled their effect on the loss of nervous impulses in the spinal cord and brain stem to be assessed. The anticonvulsant activity of the compounds studied proved to be low in this model. However, substance (VIII) surpassed chlordiazepoxide in anticonvulsant action. Its ED_{50} was 17.1 mg/kg while that of chlordiazepoxide was 31.6 mg/kg.

Investigation of the antiarrhythmic activity of compounds in the calcium and barium chloride models showed that in these models compounds (VI) and (XV) were the most active at doses of 0.1-1 mg/kg, although they proved to have no cutting action on barium chloride arrhythmia. On prophylactic administration at a dose of 1-5 mg/kg these substances completely prevented ventricular arrhythmia in individual cases and in the remaining cases the duration of the arrhythmia was curtailed two- to threefold. In the adrenaline model the compounds investigated proved to be practically ineffective.

Compounds (X), (XI), and (XV) eliminated the mixed form of arrhythmia of the atrioventricular type caused by aconitine. Compound (XI) displayed the most marked action and at a dose of 0.5 mg/kg completely protected animals from death, and also prevented disorders of cardiac rhythm and conduction in the majority. The frequency of cardiac contractions in animals of this group was practically no different from the background.

The compounds differed sharply in effect in the strophanthin model. Compound (VI) reduced the number of mice with arrhythmia and eliminated conduction disorders. Compounds (XII) and (XV) had a negative effect on the course of strophanthin arrhythmia, killing 70% of the animals, though no animal deaths were observed on administering strophanthin to the control group.

In the pituitrin model of arrhythmia the same compounds (XII) and (XV) reduced the number of animals with disturbances of cardiac conduction but at the same time the bradycardia caused by pituitrin was enhanced. The other compounds either showed no effect or aggravated its course, causing the death of some animals.

Thus compounds (X), (XII), and (XV) of those studied displayed moderate antiarrhythmic activity in the various arrhythmia models linked with the slow calcium and fast sodium channels.

When studying hemodynamic parameters in normo-tensive rats only compounds (VII) and (XI) reduced the indicators of arterial pressure (AP). At a dose of 5 mg/kg compound (VII) reduced AP_{syst} by 10-13%, AP_{diast} by 20-22%, and had no effect on AP_{precap}. Compound (XI) (at 5 mg/kg and to a larger extent at 10 mg/kg), proved to have a hypotensive action. The AP_{syst} was reduced on average by 25% and AP_{diast} by 28% after intravenous administration, and an unreliable fall in AP_{precap} was noted. The duration of the hypotensive effect was 60-90 min.

The membranotropic activity of the compounds was also studied (Table 3). As is evident from Table 3, some are active membrane modulators; however, the character of their action proved to be varied.

Compounds (X) and (XII) at all the doses studied enhanced the resistance of erythrocytes toward hemolysis, which indicates a stabilizing action on erythrocyte membranes. Compounds (XII) and (XIV) at 0.1 mg/kg enhanced the resistance of erythrocytes toward hemolysis but on increasing the dose to 10 mg/kg the resistance of erythrocytes toward hemolysis was reduced. It must

be noted that compound (XV) of those studied showed a clearly marked potentiating action on erythrocyte hemolysis. The other compounds showed no effect on membrane permeability.

Substances have been discovered among derivatives of benzopyran-2-one which possess a marked central effect, moderate antiarrhythmic, and membrane-stimulating action. This is particularly of practical interest for introducing them into clinical use. Furthermore, analysis of the data on structure—activity relationships of the compounds published previously [1-10] and of those mentioned here shows that hydrophobic molecules such as (IV), (VII), and (XIII)-(XV) possess the most marked biological activity.

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