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SEARCH FOR NEW DRUGS

SYNTHESIS AND ANTITUMOR ACTIVITY OF 2-ARYLINDANE-1,3-DIONE DERIVATIVES WITH AN ALKYLATING FRAGMENT

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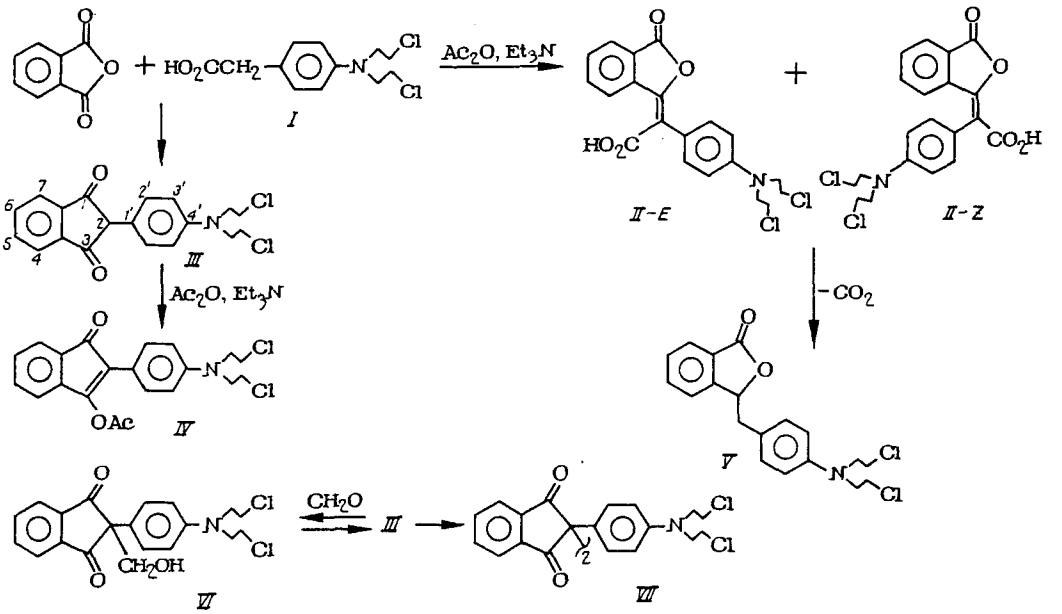
Compounds of the indane-1,3-dione series are known to display a wide spectrum of biological activity. Among them are found blood anticoagulants, analgesics, anticonvulsive preparations, and others [1-5]. Biologically active compounds, containing an indanedione and alkylating fragment in the same molecule, were synthesized and studied because of data previously obtained by us concerning the definite antitumor activity of 2-arcylindane-1,3-diones.

To produce indanedione derivatives with a di(2-chloroethyl)amino group, we studied the reaction of phthalic anhydride with 4-bis(2-choroethyl)aminophenylacetic acid in acetic anhydride, in the presence of triethylamine as a catalyst. By separating the reaction mixture using column chromatography on silica gel, we isolated compounds II-V.



The acetate of enol IV is formed during the reaction as a result of the O-alkylation of indanedione III. Decarboxylation of anion II-E, Z results in the expected amount of phthalate V. Upon heating indanedione III in an alcoholic solution of formalin, 2-hydroxymethyl derivative VI is formed, which easily eliminates formaldehyde on dissolving in alcohol; it is likely that his retroaldehyde reaction takes place *in vivo*. An analogous approach was utilized by Professor G. Ya. Vanag and coworkers in Riga when creating omephine (2-hydroxymethyl-2-phenylindane-1,3-dione), which has prolonged activity as a

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blood anticoagulant compared to fenilin (2-phenylindane-1,3-dione). When compound III is oxidized by atmospheric oxygen on prolonged standing, its dehydrodimer VII is formed.

The structure of compounds synthesized was verified by IR, ^{1}H- and ^{13}C-NMR, and mass spectral data.

EXPERIMENTAL (CHEMICAL)

^{1}H- and ^{13}C-NMR spectra were obtained on a Bruker AC-200 spectrometer (at 200 and 50 MHz, respectively) in $CDCl_3$ with TMS as internal standard. IR spectra were taken on a Specord M80 in KBr tablets, electronic absorption spectra on a Beckman DU-7, and electron-impact mass spectra on a Hitachi M80B, with direct sample injection at an ionization energy of 70 eV. Melting points were measured on a Boetius block.

Anhydride Condensation. A mixture of 27.6 g (0.1 mole) 4-bis(2-chloroethyl)aminophenylacetic acid (I) and 14.8 g (0.1 mole) of phthalic anhydride was heated in 61.2 g (0.6 mole) of acetic anhydride until dissolved and 30.3 g (0.3 mole) triethylamine was then added, followed by heating on a water bath with stirring for 30 min. The reaction mixture was poured onto 300 g of ice and 100 ml concentrated HCl, and neutralized with concentrated Na_2CO_3 solution to pH 5. The oil which formed was extracted with chloroform and dried over Na_2SO_4. The solution was filtered, evaporated, and applied to a column of silica gel. Elution was with 1:3 and 1:1 hexane–benzene, benzene, 3:1 and 1:1 benzene–ethyl acetate, and pure ethyl acetate. Homogeneous fractions were evaporated and crystallized over hexane.

Compounds are listed in order of emergence from the column.

1. Z-3-[4-Bis-(2-chloroethyl) aminobenzylidene] Phthalide (V). Yellow crystals, mp 156-158°C, yield 1.16 g (3%). IR spectrum, cm^{-1}: 1758 (C=O), 1664 (C=C), 1602, 1520. PMR spectrum, ppm: 3.5-3.8 (4H, m, CH_2CH_2), 6.35 (1H, s, CH–), 6.72 (2H, m, H-3), 7.78 (2H, m, H-2′), 7.4-8.0 (4H, m, Ar). ^{13}C spectrum: 40.41 (CH_2Cl), 53.56 (CH_2N), 107.16 (C=), 111.97 (C-3′), 119.36 (C-4), 122.74 (C-1′), 122.89 (C-7a), 125.43 (C-7), 128.90 (C-6), 131.97 (C-2′), 134.21 (C-5), 140.83 (C-3a), 142.39 (C-3), 146.12 (C-4′), 167.24 (C-1). Mass spectrum, m/z: 361 (M^+).

2. 3-Acetoxy-2-[4-bis(2-chloroethyl)aminophenyl]indene-1-one (IV). Wine-colored crystals, mp 115-116°C, yield 3.28 g (8%). IR spectrum, cm^{-1}: 1766 (CO_2), 1708 (C=O), 1608, 1596, 1518. PMR spectrum, ppm: 2.41 (3H, s, Me), 3.5-3.8 (4H, m, CH_2CH_2), 6.70 (2H, m, H-3′), 7.62 (2H, m, H-2′), 7.4-8.0 (4H, m, Ar). ^{13}C spectrum: 20.64 (Me), 40.28 (CH_2Cl), 53.14 (CH_2N), 111.64 (C-3′), 118.13 (C-2), 118.58 (C-4), 121.11 (C-1′), 122.14 (C-7), 128.85 (C-6), 129.97 (C-2′), 130.25 (C-7a), 133.25 (C-5), 139.84 (C-3a), 145.80 (C-4′), 161.38 (C-3), 166.47 (C=O), 193.70 (C-1). Mass spectrum, m/z: 403 (M^+).

3. 2-[4-Bis(2-Chloroethyl)aminophenyl]indane-1,3-dione (III). Yellow crystals, mp 99-101°C [7], yield 6.96 (19%). IR spectrum, cm^{-1}: 1742 (C=O sym), 1708 (C=O antisym), 1610, 1520. PMR spectrum, ppm: 3.5-3.8 (4H, m, CH_2CH_2), 4.18 (1H, s, H-2), 6.64 (2H, m, H-3′), 7.04 (2H, m, H-2′), 7.90 (2H, m, H-5), 8.07 (2H, m, H-4). ^{13}C spectrum: 40.29 (CH_2Cl), 53.43 (CH_2N), 59.06 (C-2), 112.38 (C-3′), 122.02 (C-1′), 123.69 (C-4), 130.06 (C-2′), 135.91 (C-5), 145.59 (C-3a), 146.14 (C-4′), 198.86 (C-1). Mass spectrum, m/z: 361 (M^+).

4. [4-Bis-(2-chloroethyl)aminophenyl]phthalidylideneacetic Acid (II-E, Z), a 1:1 mixture of isomers. Bright yellow crystals, mp 176-178°C, yield 11.24 g (28%). IR spectrum, cm^{-1}: 3300-2400 (OH), 1786 (C=O lact), 1688 and 1682 (COOH), 1608, 1520. PMR spectrum (E), ppm: 3.5-3.8 (4H, m, CH_2CH_2), 6.73 (2H, m, H-3′), 7.50 (2H, m, H-2′), 6.7-8.0 (4H, m, Ar). ^{13}C spectrum (E): 40.33 (CH_2Cl), 53.39 (CH_2N), 111.60 (C-3′), 116.37 (C-1′), 121.53 (C=), 124.97 (C-4), 125.50 (C-7a), 125.62 (C-7), 130.47 (C-6), 131.44 (C-2′), 134.49 (C-5), 137.61 (C-3a), 145.65 (C-3), 146.97 (C-4′), 165.80 (C-1), 170.33 (C=O). PMR spectrum (Z), ppm: 3.5-3.8 (4H, m, CH_2CH_2), 6.79 (2H, H-3′), 7.34 (2H, H-2′), 6.6-8.2 (4H, m, Ar). ^{13}C spectrum (Z): 40.33 (CH_2Cl), 53.43 (CH_2N), 112.24 (C-3′), 114.47 (C-1′), 121.02 (C=), 124.32 (C-4), 124.84 (C-7a), 125.77 (C-7), 131.40 (C-6), 131.77 (C-2′), 134.49 (C-5), 138.35 (C-3a), 145.65 (C-3), 146.27 (C-4′), 165.80 (C-1), 163.38 (C=O). Mass spectrum, m/z: 405 (M^+).

5. 2-[4-Bis(2-chloroethyl)aminophenyl]-2-hydroxymethylindane-1,3-dione (VI). A solution of 1.81 g (0.005 mole) of indanedione III and 20 ml formalin in 100 ml of ethanol was heated 30 min. The solvent was distilled off and water added, followed by extraction with chloroform and drying over Na_2SO_4. The solution was filtered, evaporated, and the residue recrystallized from a mixture of benzene–hexane to give 1.21 g (62%) of yellow crystals, mp 67-69°C. IR spectrum, cm^{-1}: 1740 (C=O sym), 1702 (C=O antisym), 1608, 1520, 1258, 1058 (C–O). PMR spectrum, ppm: 3.5-3.8 (4H, m, CH_2CH_2), 4.32 (2H, s, CH_2), 6.61 (2H, m, H-3′), 7.32 (2H, m, H-2′), 7.86 (2H, m, H-5), 8.04 (2H, m, H-4). ^{13}C spectrum: 40.25

TABLE 1. Antitumor and Antileukemic Activity of Compounds

Compound	LD_{50}, mg	MTD, mg/kg	Strain	Therapeutic dose, mg/interval between doses × amount given	ILS, %	% Animals surviving	Inhibition of tumor growth, % inhibition in number of metastases, %*	Change in weight of animals, %
II	300	200	L-1210	120/48×5	22	0	—	—1,7
			P-388	150/24×5	155	0	—	—2,2
			La	60/24×5	29	0	—	0,0
			Ca-755	60/48×5	8	0	11/—	+1,5
III	360	300	L-1210	120/48×5	165	0	—	—3,3
			P-388	120/24×5	384	20	—	—3,0
			La	50/24×5	70	0	—	—1,6
			Ca-755	100/48×4	17	0	40/—	—0,5
IV	465	300	L-1210	150/48×5	165	30	—	—3,3
			P-388	150/48×5	329	30	—	—0,7
			La	120/24×5	80	0	—	—2,6
			Ca-755	100/48×4	0	0	50/—	—1,0
			МелВ16	150/96×4	23	0	40/72	—1,0
				100/96×4	7	0	36/100	—3,3
VI	300	200	L-1210	120/48×5	35	0	—	—0,7
			P-388	100/48×5	191	10	—	—4,1
			La	60/24×5	87	0	—	—2,5
			Ca-755	60/48×5	10	0/—	0/—	+0,3
VII	2400	2400	P-388	1000/один	13	0	—	—0,7
Sarcolysin	17—23	10	L-1210	5/24×5	145	20	—	—3,7
			P-388	10/96×2	100	0	—	—1,8
			La	7/96×2	144	0	—	—
			Ca-755	7/96×2	56	0	91/—	—
			МелВ16	10/96×2	0	0	35/100	—1,5

*Significance of results assessed according to Mann–Whitney criterion ($p < 0.005$).

(CH_2Cl), 53.25 (CH_2N), 65.78 (CH_2), 67.42 (C-2), 112.14 (C-3'), 121.92 (C-1'), 123.49 (C-4), 128.27 (C-2'), 135.78 (C-5'), 142.34 (C-3a), 145.76 (C-4'), 200.87 (C-1). Mass spectrum, m/z: 391 (M^+).

6. 2,2-Bis{[4-bis(2-chloroethyl)aminophenyl]indane-1,3-dione} (VIII). Obtained by letting a benzene solution of starting indanedione III stand in air for 1 month, with quantitative yield, analogously to a previous method [6]. Yellow crystals, 201-202°C. IR spectrum, cm^{-1}: 1733 (C=O sym), 1707 (C=O antisym), 1608, 1517. PMR spectrum, cm^{-1}: 1733 (C=O system), 1707 (C=O antisym), 1608, 1517. PMR spectrum, ppm: 3.5-3.8 (4H, m, CH_2CH_2), 6.55 (2H, m, H-3'), 7.08 (2H, m, H-2'), 7.73 (2H, m, H-5), 7.87 (2H, m, H-4). ^{13}C spectrum: 40.35 (CH_2Cl), 53.36 (CH_2N), 63.70 (C-2), 110.72 (C-3'), 118.51 (C-1'), 123.63 (C-4), 131.77 (C-2'), 135.40 (C-5), 140.96 (C-3a), 146.19 (C-4'), 197.61 (C-1).

EXPERIMENTAL (BIOLOGICAL)

Maximal tolerated dose (MTD) and toxic dose (LD_{50}) were determined by the method of Behrens–Kerber in CBF_1 mice of both sexes, weighing 20-22 g. All preparations were injected intraperitoneally in a volume of 0.2 ml as a suspension on a mixture of Tween-20 with water (1:9 by volume).

Animals were observed daily for 60 days following injection of compounds; we recorded their external appearance, weight loss, and day of death.

The antitumor activity of compounds was studied using the following experimental systems: leukemias La, L-1210, and P-388; Lewis lung carcinoma LL; adenocarcinoma Ca-755; and melanoma B-16. Passage of tumors was carried out according to standard methods [7]. All studies were performed on purebred mice of both sexes, of total body weight 20-22 g. Compounds were injected intraperitoneally. Criteria for assessing antitumor activity included increase in average lifespan of treated animals compared with controls (ILS), percent of animals surviving to 60 days, and percent inhibition of tumor growth and in the number of metastases (for strains LL and B16 melanoma) at day 27 of tumor development. Data obtained are presented in Table 1. In all experiments, sarcolysin was used for comparison, as the most structurally similar clinically used preparation.

Comparison of overall toxicity showed that compounds II-VII are considerably less toxic than sarcolysin. The following conclusions may be drawn concerning the relative antitumor activity of the compounds studied.

Compound III, and corresponding acetate IV, are comparable in activity to sarcolysin against leukmias. One can detect slightly higher antileukemic activity of III and IV against leukemia P-388. Increase in lifespan on administration of these compounds was more than 3 times greater than when treating with sarcolysin. And what is more revealing is that 20-30% of animals survived when given compounds III and IV, whereas on treatment with sarcolysin, this survival is not seen. The significant antimetastatic activity of IV should also be noted.

By analogy with known data on the metabolism of the structurally similar omephine, it may be supposed that during its metabolism, compound VI is converted to indaneone III. The antileukemic activity of VI is actually high, though lower than III. Dimerization of indaneone III results in loss of antileukemic activity and absence of a toxic effect, for Dimer VII. Apparently, not just the alkylating fragment, but the presence of, or possibility of generating an H-2 hydrogen in the indaneone portion of the molecule during metabolism, plays a role on the realization of an antitumor effect. We have previously observed a similar loss of antitumor activity with bromination of 2-arylindane-1,3-diones at position 2.

Acid II, a reaction by-product, is considerably less active. It is interesting to note that leukemia P-388 was the most sensitive to the action of indaneones with an alkylating fragment.

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