2-(N'-Nitroso-N'-2-chloroethylureido)-3-[(4-(N'-nitroso-N'-2'chloroethylureido)phenyl] propionic Acid (XIb). Obtained as for (XIa); yield 0.73 g (64% of theory). IR spectrum, ν, cm^{-1}: 3340 (NH); 1720 (COOH, CONH); 1580 (CNH); 1470 (N—NO); 1000 (N—N).

EXPERIMENTAL BIOLOGY

The antitumor activities of the alkylnitrosourea derivatives of L-phenylalanine (VII), (VIII), and (XI) were examined by a well-known method [2] in C57BL and mongrel mice, using mammary adenocarcinoma AK-755, melanoma B-16, and sarcomas C-37 and C-180. Test doses of the compounds were administered in a single dose intraperitoneally to the animals in the form of suspensions in 3% starch mucilage, using 180 animals. Compounds (VIIb) and (VIIIb) in a dose of 10 mg/kg, (VIIa) in a dose of 200 mg/kg, and (VIII) in a dose of 400 mg/kg were inactive against AK-755, C-37, and C-180 tumors, and melanoma B-16. Compound (VIIIa) in a dose of 200 mg/kg, and (XIa) in a dose of 150 mg/kg inhibited the growth of sarcoma C-37 by 76 and 60% respectively.

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DEPENDENCE OF THE ANTITUMOR ACTIVITY OF SPIROBROMIN ANALOGS ON THEIR STRUCTURE

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Earlier it was reported that N,N"'-di(β-bromopropionyl)-N',N"-dispirotripiperazinium dichloride (I) (spirobromin) is effective in the treatment of acute leukemia, malignant lymphomas, skin reticuloses, and cancer of the larnyx, cervix, and vulva. Spirobromin has been approved for use in medicine [4].

To determine the dependence of the antitumor activity on a chemical structure we conducted a synthesis and biological study of analogs of spirobromin and compounds related to it.

Compounds III and IV, differing from I by the presence of a bromine atom or CH_3 group, respectively, in the α-position of the β-bromopropionyl residue, were produced by the reaction of dispirotripiperazinium chloride (II) with chlorides of α,β-dibromopropionic and α-methyl-β-bromopropionic acids in aqueous medium in the presence of lithium carbonate.

Under analogous conditions, compound V containing no bromine atoms in the acyl residues, was synthesized by the reaction of II with acrylyl chloride. The IR spectra of compounds III-V have the absorption bands of amide CO (1630-1640 cm^{-1}), and the bands of the NH group of the original substance II are absent. In an investigation of the properties of compound V it was noted that the double bond in it possesses high reactivity. Thus, under the action of benzylamine on V in aqueous solution at 20-25°C, two benzylamine residues are added at the double bond, and compound (VI) is formed, the structure of which was confirmed by spectral data. In the PMR spectrum of VI, in addition to the signals of the protons of the dispirotripiperazinium fragment, there are signals of protons of two methylene groups, situated between the carbonyl and the amino groups (δ 2.80 ppm), the signal of the protons of the CH_2

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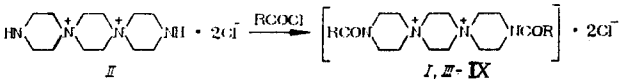
group from the benzyl residue (δ 3.80 ppm), the signal of the protons of the benzene ring (δ 7.44 ppm), and the absence of signals of the acrylyl residue, present in the starting material V. The data of the IR spectrum of VI also correspond to its structure: the absorption bands of the CO groups (1640 cm^{-1}) and NH groups (3350-3450 cm^{-1}) are observed in the spectrum.

For a comparative biological study, it was also of interest to obtain dispirotripiperazinium derivatives containing phenylalkanoic residues at the terminal nitrogen atoms, including the antitumour preparation chlorobutin — $HOOC(CH_2)_3C_6H_4N(CH_2CH_2Cl)_2$-p (X). For this purpose, the reaction of II with chlorides of γ-phenylbutyric, γ-(p-nitrophenyl)butyric, and γ-{p-N-di(2-chloroethyl)aminophenyl}butyric acids in aqueous medium in the presence of lithium hydroxide was used to produce compounds VII, VIII, and IX.



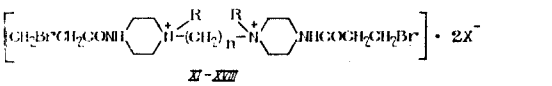
II — I, III-IX

I:R = CH_2CH_2Br; III:R = $CHBrCH_2Br$; IV:R = $CH(CH_3)CH_2Br$;
V:R = $CH{=}CH_2$; VI:R = $CH_2CH_2NHCH_2C_6H_5$; VII:R = $(CH_2)_3C_6H_5$;
VIII:R = $(CH_2)_3C_6H_4NO_2$-p; IX:R = $(CH_2)_3C_6H_4N(CH_2CH_2Cl)_2$-p

Compounds III-IX were studied in experiments on animals with transplantable Jensen Sarcoma in comparison with I and X (Table 1).

From the data obtained it follows that the introduction of a bromine atom or methyl group into the α-position of the β-propionyl residues causes a loss of antitumor activity. In the presence of an acrylic acid residue in the molecule (V), a tendency is manifested for stimulation of tumor growth. The addition of benzylamine residues at the double bond of the acryloyl groups (VI) leads to the appearance of weak antitumor activity. Replacement of the haloalkanoic acid residues by phenylalkanoic residues leads to the formation of compounds whose antitumor activity either is very weak compound IX or is absent (compound VIII). The introduction of di(2-chloroethyl)amine groups into the γ-phenylbutyric acid residues (compound IX does not promote any intensification of the delayed increase in the effect, whereas the presence of such a group in γ-phenylbutyric acid itself provides for high antitumor activity (compound X).

Then, in comparison with spirobromin, we studied substances XI-XVIII with an open central piperazine ring, possessing β-bromopropionyl residues at the terminal nitrogen atoms, just as in spirobromin; moreover, in the molecule of compounds XI-XIV, like I, two quaternary nitrogen atoms are preserved [3] (Table 2).



XI-XVIII

XI:$n = 2$, R = CH_3, X = Cl; XII:$n = 3$, R = CH_3, X = Cl; XIII:$n = 6$, R = CH_3, X = Br; XIV:$n = 10$, R = CH_3, X = Br; XV:$n = 2$, R = H, X = Cl; XVI:$n = 3$, R = H, X = Cl; XVII:$n = 6$, R = H, X = Cl; XVIII:$n = 10$, R = H, X = Cl.

It was found that compounds XI, possessing two methylene groups between piperazinium rings, possesses substantial antitumor activity (I_T = 85%) with low toxicity (LD_{95} = 550 kg/ml). The antitumor effect is maintained at a high level when the number of methylene links is increased to three and six (compounds XII and XIII), and it is virtually absent if this number reaches 10 (compound XIV). It should be noted that replacement of a fluoride anion in compound XII by a bromide anion (compound XIII) has no significant effect on the antitumor action, but it led to a certain increase in the toxicity (LD_{95} = 550 mg/kg for compound XII and LD_{95} = 450 mg/kg for XIII, see Table 2).

At the same time, analogous derivatives of dipiperazinyl alkanes XV-XVIII, differing from the compounds XI-XIV discussed above by the absence of quaternary nitrogen atoms in the molecule (R = H), did not exhibit any antitumor action (see Table 2).

TABLE 1. Activity of Dispirotripiperazinium Derivatives I, III-IX, and X

Compound	LD_{95}, mg/kg	I_T, %
I (spirobromin)	2240	99
III	550	0
IV	550	0
V	35	stimulation
VI	78	30—40
VII	550	30—40
VIII	550	0
IX	550	30
X (chlorbutin)	80	94

TABLE 2. Activity of Dipiperazinylalkane Derivatives XI-XVIII

Compound	LD_{95}, mg/kg	I_T, %
XI	550	85
XII	550	73
XIII	450	78
XIV	10	0
XV	550	0
XVI	550	0
XVII	432	0
XVIII	50	0

Note. Chlorbutin was administered internally to rats.

Thus, a comparative biological study of spirobromin and compounds related to it permitted the detection of a number of interesting relationships between the structure and the antitumor action. It was found that of the substances studied, the greatest activity is possessed by the dispirotripiperazinium derivative that contains β-bromopropionic acid residues at the terminal nitrogen atoms. The introduction of a second bromide atom or a methyl group into the α-position causes a loss of antitumor activity. Replacement of the β-bromopropionyl groups by β-N-benzylaminopropionic and γ-phenylbutyric acids had an analogous effect. The antiblastic activity was also absent when residues of the antitumor preparation chlorobutin were introduced into the dispirotripiperazinium molecule. Opening of the central piperazine ring with conservation of the quaternary nitrogen and the β-bromopropionic acid residues should provide for preservation of a substantial antitumor effect. However, these substances too are less active than spirobromin. An analogous pattern was noted earlier [2] for prospidin and its analogs with an open piperazinium ring, containing β-hydroxy-γ-chloropropyl groups on the terminal nitrogen atoms. For this series of substances, just as in the case of spirobromin, the highest activity was observed for dispiropiperazinium derivatives.

EXPERIMENTAL CHEMISTRY

N,N'''-Di(α,β-dibromopropionyl)-N',N''-dispirotripiperaninium Dichloride (III). To a solution of 1.7 g N',N''-dispirotripiperazinium dichloride (II) in 15 ml of water, 4 g of α,β-dibromopropionyl chloride was added at 0-5°C in the presence of 1.1 g lithium carbonate. It was mixed for 2 h, and the precipitate was filtered off and washed with 10 ml of ethanol, 20 ml of acetone, and 10 ml of ether. To a suspension of the precipitate obtained in 50 ml of water, 4 ml of acetic acid was added at 55-60°C, mixed for 15 min, the undissolved precipitate filtered off, and 40 ml of ethanol, 120 ml of acetone, and 100 ml of ether were added to the filtrate, cooled to 20°C. After cooling at −10°C for 14 h, the precipitate formed was filtered off, washed with 20 ml of methanol, 20 ml of ether, and dried at 100°C under vacuum. We obtained 2 g of III (yield 47.7%, mp 320-325°C. IR spectrum: 1640; 3400 cm^{-1} (C = O, H_2O). Found, %: C 28.92; H 3.92; Br 43.49; Cl 9.65; N 7.48; H_2O 1.24. $C_{18}H_{30}Br_4Cl_2N_4O_2.0.5\ H_2O$. Calculated, %: C 29.43; H 4.22; Br 43.6; Cl 9.67; N 7.63; H_2O 1.23.

N,N'''-Di(α-methyl-β-bromopropionyl)-N',N''-dispirotripiperazinium Dichloride (IV). To a solution of 2 g of compound II in 10 ml of water, 3.71 g α-methyl-β-bromopropionyl chloride was added slowly with mixing at 0-5°C in a presence of 1.72 g lithium carbonate. The mixture was mixed at this temperature for 2.5 h, filtered, 1 ml of acetic acid, 30 ml methanol, and 100 ml of ether were added to the filtrate, and the mixture was cooled at −5°C for 2 h. The precipitate filtered off, washed successively with 30 ml of acetone, 10 ml of methanol, and 20 ml of ether, and dried at 80°C under vacuum. We obtained 2.5 g IV (yield 56.1%), mp 307-310°C (dec.). IR spectrum: 1640; 3400 cm^{-1} (C = O, H_2O). Found, %: C 39.23 H 6.3; Br 26.26; Cl 11.64; N 9.18; H_2O 2.35. $C_{20}H_{36}Br_2Cl_2N_4O_2.H_2O$. Calculated, %: C 39.15; H 6.20; Br 26.1; Cl 11.58; N 9.14; H_2O 2.94.

N,N'''-Diacryloyl-N',N''-dispirotripiperazinium Dichloride (V). To a solution of 1 g (0,00335 mole) of compound II in 4 ml we added 1 g (0.0135 mole) lithium carbonate, and then we added 1.1 g (0.011 mole) acrylyl chloride at 0-5°C over a period of 30 min with mixing.

The mixture was mixed for 15 min at 0°C, for 30 min at 15°C, filtered, 1 ml of acetic acid and 50 ml of methanol were added to the filtrate, and filtered. Then 50 ml of isopropanol was added to the filtrate and cooled for 16 h at −5°C. The precipitate formed was filtered off, washed with 20 ml of isopropanol, with 20 ml of ether, and dried under vacuum at 80°C for 3 h. We obtained 1.1 g V (81%), mp 320-330°C (dec.). IR spectrum 1640, 3400 cm^{-1} (C=O, H_2O). Found, %: C 52.19; H 7.50; Cl 17.07; N 13.45; H_2O 1.8. $C_{18}H_{30}Cl_2N_4O_2 \cdot 0.5\ H_2O$. Calculated, %: C 52.17; H 7.49; Cl 17.15; N 13.53; H_2O 2.17.

N,N'''-Di[β-(N-benzyl)aminopropionyl]-N',N''-dispirotripiperazinium Dichloride (VI). To a solution of 2 g (0.005 mole) of compound II in 2 ml of water we added 10 g (0.093 mole) benzylamine and left it at 20-25°C for 43 h. The reaction mixture was filtered, and 10 ml of ethanol and 100 ml ether were added to the filtrate. The oily substance that precipitated was removed and dissolved with a mixture of 10 ml of ether and 10 ml of methanol. The precipitate formed was filtered off, washed with 10 ml of ether, and dried. We obtained 2.17 g VI (yield 70%), mp 317-320°C (dec.). IR spectrum: 1650; 3350 cm^{-1} (C-O, NH). Found, %: Cl 11.7; N 13.73; $C_{32}H_{48}Cl_2N_6O_2$. Calculated, %: Cl 11.47; N 13.57.

N,N''-Di(γ-phenylbutyryl)dispirotripiperazinium Dichloride (VII). We mixed 15 ml of water, 1.5 ml glacial acetic acid, 0.38 g lithium hydroxide, and 2.25 g of compound II in such a way that the temperature did not exceed 30°C. Then 3 g of γ-phenylbutyryl chloride was added to the solution at 3-5°C in such a way that the temperature did not exceed 10°C. It was mixed at 3-5°C for 30 min and at room temperature for 3 h. The precipitate formed was filtered off, washed with water, and dried. We obtained 2.7 g VII (yield 60%), mp 320-325°C (from water). Found, %: C 65.40; H 7.81; N 9.58; Cl 12.16. $C_{32}H_{46}N_4O_2Cl_2$. Calculated, %: C 65.19; H 7.81; N 9.51; Cl 12.0.

N,N'''-Di[γ-(p-nitrophenyl)butyryl]dispirotripiperazinium Dichloride (VIII). We mixed 8 ml of water, 1.8 g glacial acetic acid, 0.06 g lithium hydroxide, and 3.57 g compound II in such a way that the temperature did not exceed 30°C. Then 6 g of γ-nitrophenylbutyryl chloride was added to the solution at 3-5°C. It was mixed for 30 min at 3-5°C and for 2 h at 20°C. The precipitate was filtered off, washed with water, with alcohol, and dried. We obtained 5.3 g VIII (yield 65%), mp 285°C (dec.). Found, %: C 56.37; H 6.49; N 12.26; Cl 10.18; $C_{32}H_{44}N_6O_6Cl_2$. Calculated, %: C 56.55; H 6.48; N 12.37; Cl 10.46.

N,N'''-Di{γ-[p-N-di(2-chloroethyl)aminophenyl]butyryl}dispirotripiperazinium Dichloride (IX). We mixed 7 ml of water, 0.6 g glacial acetic acid, 0.2 g lithium hydroxide, and 1.19 g of compound II. Then 2.4 g of the acid chloride of chlorobutyne was added to the solution at 3-5°C in such a way that the temperature did not exceed 10°C. It was mixed at 3-5°C for 30 min and at 18-20°C for 2 h. The precipitate was filtered off, washed with water and with alcohol. Yield 2.1 g (60%). Found, %: C 54.95; H 7; N 9.77; Cl 24.56; $C_{40}H_{60}N_6O_2Cl_6$. Calculated, %: C 55.23; H 6.9; N 9.67; Cl 24.51.

EXPERIMENTAL BIOLOGY

The experiments were conducted on noninbred rats weighing 110-120 g with transplantable Jensen sarcoma. The substances were injected intraperitoneally on the fifth day after transplantation of the tumor, once a day, daily for a period of 6-7 days. One day after the last injection the animals were sacrificed, and the index of inhibition of tumor growth (I_T) was determined [5]. The general toxic action of the substances was determined on noninbred white mice with a single intraperitoneal injection, and LD_{95} was determined according to the Kerber method [1].

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