imidazole-4,5-dicarboxylic acid. The mixture was stirred at 130-140°C for 2 h, then it was poured into cold water and the solid which separated was filtered off.

EXPERIMENTAL (BIOLOGY)

The antiviral activity of the test compounds was determined in tissue culture against variola vaccine virus (VVV), herpes simplex virus (HSV), classical avian plague virus (CAPV), Newcastel disease virus (NDV), vesicular stomatitis virus (VSV), Venezuelan equine encephalomyelitis virus (VEEN), and ECHO-6, using screening tests and reduction of platelets under an agar cover. In the case of ECHO virus, investigations were carried out with monolayer cultures of passivated human embryo musculocutaneous cells, and in the case of the remaining viruses, with primarily trypsinized chicken embryo fibroblasts.

Measures of antiviral activity were provided by the diameter of the zone of suppression of formation of platelets, and the reduction in viral titer in thepresence of the compound in a range of concentrations. The dose of the compound which inhibited platelet formation by 50% (the mean effective dose, ED_{50}) was calculated by the method of Reed and Mensch. The methods of examination and assessment of antiviral activity have been described in detail previously [2].

In this series of compounds, inhibitory activity against the variola vaccine virus is moderate or low (Table 2). Introducing a thiazole or quinazoline moiety into the DBC molecule gives rise to low antiviral activity against vesicular stomatitis virus (IIa) and influenza virus (Va) respectively. The remaining compounds failed to show antiviral activity in these tests.

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RADIOPROTECTIVE PROPERTIES OF PYRROLIDONE-CONTAINING HETEROCYCLIC ANALOGS OF S-AMINOALKYLISOTHIOUREAS

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There have been reports in [1, 2, 4, 5] about the synthesis, radioprotective activity, and some aspects concerning the radioprotective mechanism of aminoalkylthiol derivatives of pyrimidine and quinazoline, being heterocyclic analogs of S-aminoalkylisothioureas. Compounds in this series have radioprotective activity that is related to a reduction in oxygen consumption by the organism. A significant factor in their radioprotective mechanism is hydrolytic decomposition and generation of free aminothiol. It was of interest to study the radioprotective properties of heterocyclic analogs of isothioureas that contain pyrrolidone residues. The distinctive physicochemical properties of the latter – a combination of hydrophilic and lipophilic activity – could increase the biological availability of the compounds and have a positive effect on their biological activity.

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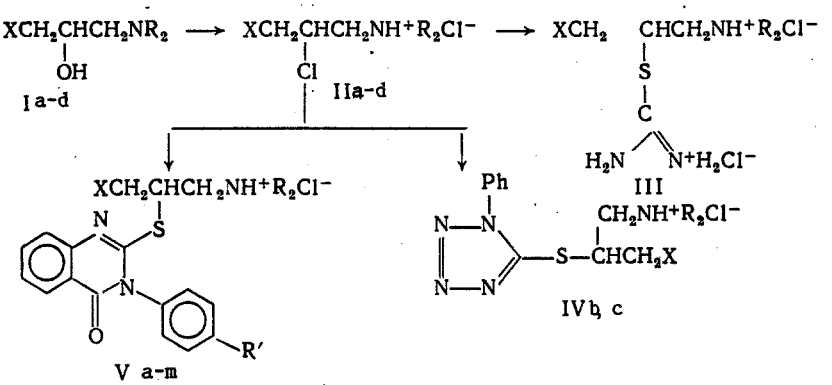
TABLE 1. Compounds IIb d, III, IVb, c, and Va-m

Compound	Yield, %	mp, °C	Found, %					Empirical formula	Calculated, %				
			C	H	N	S	Cl		C	H	N	S	Cl
II b	75	198—200	51,3	7,9	10,2	—	25,2	$C_{12}H_{22}Cl_2N_2O$	51,3	7,8	10,0	—	25,3
II d	65	152—3	52,7	8,3	9,2	—	24,3	$C_{13}H_{24}Cl_2N_2O$	52,9	8,1	9,5	—	24,1
III	80	151—2	43,5	7,4	—	9,1	19,9	$C_{13}H_{26}Cl_2N_4OS$	43,7	7,3	—	9,0	19,9
IV b	50	177—8	54,4	6,5	—	7,7	9,0	$C_{19}H_{27}ClN_6OS$	54,0	6,4	19,9	7,6	8,4
IV c	45	194—6	50,7	6,1	19,4	7,6	8,8	$C_{18}H_{25}ClN_6O_2S$	50,9	5,9	19,8	7,5	8,4
V a	40	182—3	61,6	6,2	11,4	6,3	7,7	$C_{25}H_{31}ClN_4O_2S$	61,7	6,4	11,5	6,6	7,3
V b	45	138—9	60,3	6,6	11,0	6,2	7,2	$C_{26}H_{35}ClN_4O_3$	60,2	6,7	10,8	6,2	6,9
V c	40	180—1	57,7	5,7	10,7	6,0	13,2	$C_{25}H_{30}Cl_2N_4O_2S$	57,6	5,8	10,8	6,1	13,6
V d	55	218—9	62,4	6,2	11,1	6,3	7,2	$C_{26}H_{31}ClN_4O_2S$	62,6	6,2	11,2	6,4	7,2
V e	55	223—4	62,5	6,4	10,7	6,3	7,3	$C_{27}H_{33}ClN_4O_2S$	63,0	6,4	10,9	6,2	7,0
V f	60	210—11	58,2	5,7	10,4	6,0	13,3	$C_{26}H_{30}Cl_2N_4O_2S$	58,5	5,6	10,5	6,0	13,3
V g	40	153—5	61,7	6,5	10,6	6,0	7,0	$C_{27}H_{33}ClN_4O_3S$	61,8	6,2	10,6	6,0	6,7
V h	60	217—8	59,9	5,9	11,4	6,4	7,2	$C_{25}H_{29}ClN_4O_3S$	59,9	5,8	11,2	6,4	7,1
V i	60	208—10	60,4	6,1	10,8	6,3	7,2	$C_{26}H_{31}ClN_4O_3S$	60,6	6,0	10,9	6,2	6,9
V j	55	208—9	56,3	5,5	—	5,9	13,1	$C_{25}H_{28}Cl_2N_4O_3S$	56,1	5,3	10,5	6,0	13,3
V k	45	211—3	59,1	6,0	10,3	5,9	6,9	$C_{26}H_{31}ClN_4O_4S$	58,8	5,8	10,6	6,0	6,7
V l	55	221—3	63,5	6,3	11,1	6,1	7,2	$C_{27}H_{33}ClN_4O_2S$	63,2	6,4	10,9	6,2	6,9
V m	60	222—4	63,3	6,5	10,5	5,9	6,9	$C_{28}H_{35}ClN_4O_2S$	63,8	6,5	10,6	6,1	6,7

When pyrrolidone-containing aminoalcohols I are treated with thionyl chloride, 1-N-pyrrolidonyl-2-chloro-3-dialkylaminopropane hydrochlorides (II) are obtained. The latter are used for the alkylation of thioamides. When IIb is boiled with thiourea in ethanol, isothiouronium salt (III) is isolated; and when compounds II are reacted with heterocyclic thioamides, pyrrolidone-containing dialkylaminopropylthio derivatives of tetrazole (IV) and of quinazoline (V) are obtained.

Compounds (V) can be hydrolyzed, as was demonstrated by compound Vd as an example When it was boliled in an aqueous soluton of HCl, 3-phenylquinazoline-2,4-dione was isolated. The second hydrolysis product — 1-N-pyrrolidonyl-2-mercapto-3-piperidinopropane — was detected in the reaction mixture from the color reaction on the mercapto group. The composition of the hydrolysis products demonstrates the S-alkyl structuure of compound Vd, and the analogous structure of all compounds V is confirmed by the similarity of their UV spectra.



R = Et (Ia, IIa, Va, Vb, Vc), NR_2 = piperidino (Ib, IIb, III, IVb, Vd, Ve, Vf, Vg), NR_2 = morpholino (Ic, IIc, IVc, Vh, Vi, Vj, Vk), NR_2 = hexamethylenimino (Id, IId, Vℓ, Vm), R' = H (Va, Vd, Vh, Vℓ), R' = Me (Vb, Ve, Vi, Vm), R' = Cℓ (Vc, Vf, Vj), R' = OMe (Vg, Vk), X = N-2-pyrrolidonyl.

The toxicity and radioprotective action of compounds III, IV, and V were studied. They do not exhibit uniform toxicity. When III is administered to an animal, a brief period of agitation is produced and there is an increase in motor activity, IV produces adynamia, while V causes tonic convulsions (excluding Vd and Vh, where toxic doses were not reached, and the animals only showed depression). A toxicity curve was not obtained for Ve and Vf because of the small range between the maximum tolerable and absolutely lethal doses.

Isothiouronium derivative III did not exhibit radioprotective activity, but its heterocyclic analogs IV and V, which contain an amidine group as part of the heterocyclic ring system, gave more significant results. Tetrazole derivatives IV, with lower toxicity than III (0.6-0.9 mmole/kg compared to 0.4 mmole/kg) give a distinct radioprotective effect (25-

TABLE 2. Radioprotective Activity of Compounds III, IV, and V

Compound	LD_{50} toxicity, mg/kg	Dose of agent, mg/kg	Administration prior to irradiation, min	Survival rate, %	Average life period, days	No. of animals per test
III	152,4	47,5	15	5,0	11,3	40
		47,5	30	0	11,1	41
		96,6*	30	5,0	10,1	20
IVb	252,2	110,0	30	26,3	12,8	19
		110,0	60	40,0	14,3	20
IVc	391,8	174,2	15	40,0	9,0	40
		174,2	30	37,5	9,5	40
		174,2	60	25,0	16,3	20
V a	102,3	45,8	10	10,5	11,3	19
		45,8	15	12,5	13,4	16
		45,8	30	20,0	11,4	40
		45,8	60	0	9,2	20
V b	137,3	54,1	15	0	7,5	16
		54,1	30	0	8,3	20
V c	230,0	96,5	15	5,3	9,8	19
		96,5	30	5,0	10,9	20
V d	150**	150,0	15	75,0	11,4	40
		150,0	30	42,5	9,8	40
		150,0*	30	5,0	8,5	20
Ve	$128>LD_{50}>103$	51,3	15	22,8	14,1	39
		51,3	30	0	12,2	39
		102,6*	30	0	9,4	20
V f	$160>LD_{50}>133$	53,3	15	32,1	11,4	39
		53,3	30	25,0	11,5	40
		53,3	60	0	13,1	20
		106,6*	30	5,0	9,7	20
V g	214,0	79,4	15	0	10,5	20
		79,4	30	9,5	12,4	42
		79,4	60	9,5	10,8	21
V h	150,1**	50,1	15	0	9,5	17
		50,1	30	0	11,0	9
		75,2	15	20,0	11,5	20
		75,2	30	0	10,7	20
		751,6*	15	10,5	9,8	10
		751,6	30	0	11,6	19
V i	882,8*	381,2*	30	1,7	7,9	58
		381,2	45	0	10,1	19
		381,2	60	8,5	9,3	47
V j	1412,4*	535,5*	15	20,0	6,6	20
		535,5	30	0	7,4	20
V k	676,6*	185,9*	15	5,3	8,0	19
		185,9	30	16,7	12,8	18
		95,1*	30	0	9,3	20
V l	224,7	89,8	15	0	10,4	15
		89,8	30	0	12,1	19
V m	228,8	105,4	15	5,0	7,7	20
		105,4	30	0	7,1	20
		527,1*	50	0		18
Control	—	Physiological solution		3,9	7,2	648

*Administered po.
**Toxic doses not attained.

40% animal survival). Compounds V, all administered intraperitoneally (except for Vi-k) have toxic doses in the range 0.2-0.4 mmole/kg. They can be arranged in the following order of decreasing toxicity: NR_2 = piperidino > diethylamino > hexamethylenimino; R' = H > CH_3 > Cl > OCH_3. Compound Vd is prominant for the magnitude of its radioprotective activity; it has a marked effect – about 75% survival of irradiated animals. The activity of the remaining compounds V is small, and by comparing the different derivatives it may be concluded that in relation to the structure of the compound and its radioprotective activity optimum results are obtained when the substitutents NR_2 = piperidino and R' = H.

Compounds V and 2-dialkylaminoethylthio-3-aryl-4-quinazolones (analogs of V that have no pyrrolidone residue) [1] on average have similar values for toxic doses and radioprotective activity.

EXPERIMENTAL CHEMICAL

The purity of the agents was monitored by TLC on Silufol plates using the system butanol–water–AcOH (2:2:1). UV spectra were recorded on a Specord UV-VIS instrument (GDR) in aqueous solutions of concentration $1 \cdot 10^{-3}$ M.

1-N-Pyrrolidonyl-2-chloro-3-dialkylaminopropane Hydrochlorides (IIa-d). Compounds Ia-d (35 mmole), obtained according to [3], were dissolved in 50 ml of $CHCl_3$, and 4 ml of $SOCl_2$ was added over a period of 15 min at 20°C. The mixtures were kept at 20°C for 24 h and the $CHCl_3$ was distilled off to give products II in the residue. Compounds IIc, d were solid precipitates, which were crystallized from acetone (Table 1); compounds IIa, c were viscous liquids that did not crystallize and they were used without further purification.

(1-N-Pyrrolidonyl-3-piperidino)propyl-2-isothiouronium Dihydrochloride (III). Thiourea (0.8 g, 10 mmole) and IIb (1.4 g, 10 mmole) were boiled in 30 ml of absolute ethanol for 3 h. The precipitate of III which formed after cooling was filtered off and recrystallized from absolute ethanol (see Table 1).

1-Phenyl-5-(1 N-pyrrolidonyl-3-cycloalkylenimino-2-propyl)thiotetrazole Hydrochlorides (IVb, c). To a solution of 3.5 g (20 mmole) of 1-phenyltetrazoline-5-thione and 3 g (60 mmole) of NaOH in 50 ml of water was added a solution of 20 mmole of IIb, c in 50 ml of water, and the mixture was agitated at 20°C for 12 h. The precipitate of base IV that formed was filtered off, dried, dissolved in 75 ml of benzene, and gaseous HCl was passed through the solution until it was saturated. The precipitate of IVb, c that formed was filtered off and crystallized from ethanol (see Table 1).

2-(1-N-Pyrrolidonyl-3-dialkylamino-2-propyl)thio-3-aryl-4-quinazolone Hydrochlorides (Va-m). 2-Thio-3-aryl-4-quinazolone (20 mmole), obtained according to [1], was mixed with 16 g (160 mmole) of anhydrous Na_2CO_3, and 200 ml of acetone and a solution of 20 mmole of IIa-d in 25 ml of water were added. The reaction mixture was boiled for 12 h, filtered, and acetone was distilled off. The residue (base V) was crystallized from a benzene–petroleum ether (1:4) mixture, dissolved in 50 ml of benzene, and gaseous HCl was passed through the solution. The precipitate of Va-m that formed was filtered off and crystallized from ethanol (see Table 1). UV spectrum, λ_{max}, nm ($\varepsilon \cdot 10^{-3}$): 228-232, 29.4-36.9; 276-278, 12.0-14.0; 316, 3.75-4.35.

EXPERIMENTAL BIOLOGICAL

Tests for toxicity and radioprotective activity of the compounds were carried out on 3-4 month-old male mice of BALB strain. The agents were administered ip or po on the basis of 0.2 ml of liquid per 20 g weight of animal. When there was limited solubility (Vd, h), the maximum possible amount of agent was taken by increasing the volume of solution. Insoluble compounds (Vi-k) were administered only po, a suspension being mixed on a magnetic stirrer in order to produce a uniform concentration.

The toxicity was determined from the reaction of the animals after administration of the agent and from their deaths over a period of 3 days (observations carried out for 7 days). The data obtained were processed by the Probit method, and LD_{16}, LD_{50}, and LD_{84} values were calculated.

The radioprotective activity was determined from the 30-day survival rate of the animals, which were irradiated with a minimum lethal dose (208.98 mC/kg) of Cs-137 gamma-radiation (magnitude of dose 0.51 mA/kg) on an "Igur" apparatus. The agents were administered 10-60 min before irradiation at a dose level of half the LD_{16} or half the maximum tolerable dose for those agents whose toxicity curves were not obtained. For each time period 20-40 mice were used. Irradiation of the test animals and control animals was carried out at the same setting. The control animals received a physiological solution. The control data were combined for all agents and amounted to 3.9% survival (Table 2).

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