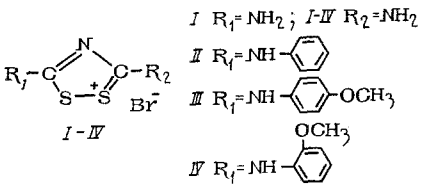
EFFECTS OF DITHIAZOLIUM COMPOUNDS ON RADIORESISTANCE IN ANIMALS

G. N. Krutovskikh, M. B. Kolesova, A. M. Rusanov, and L. P. Vartanyan

UDC 615.849.1.015.25

The discovery of the radioprotective effects of cystamine [1] and its derivatives [2, 3] has stimulated a further search for new, effective, and less toxic compounds from the group of aminothiols.

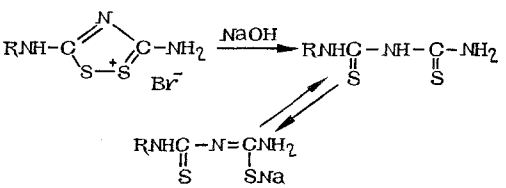
A search for radioprotectants has been carried out among dithiazolium derivatives bearing various substituents in the 3- and 5-positions:



3,5-Diamino-1,2,4-dithiazolium ions are obtained by oxidation of dithiobiurets with halogens, hydrogen peroxide, or ferric chloride in acidic media (Table 1). Treatment of dithiazolium ions with hydride donors affords dithiobiurets (Table 2).

Thus, the acyclic dithiobiurets are conjugated hydro-derivatives of the 3,5-diamino-1, 2,4-dithiazolium ions.

We suggest that a similar ring-opening process may occur in the body in alkaline media to give dithiobiurets, which contain mercapto-groups and may thus be potential radioprotective agents.



Experiments were carried out on white mice weighing 18-22 g. Calculations were carried out by the method of Kerber and Berens. The radioprotective properties of the compounds were investigated in mice which were subjected to x-irradiation in a RUM-3 apparatus in a dose of 700 rad at a dose rate of 60-70 rads/min. The drugs were administered intraperitoneally, ten minutes before irradiation. The effectiveness of the compounds was compared with that of cystamine. The following criteria were adopted for evaluating the radioprotective properties of the compounds: survival of the animals for 30 days after irradiation, longevity, the number of leucocytes in the peripheral blood, the total number of nucleate cells in the femur, and the changes in weight of the spleen at the third and tenth day after irradiation. Examination of the toxicities of the newly synthesized dithiazolium compounds showed them to be more toxic than cystamine. Thus, administration to mice of 3,5-diamino-1,2,4-dithiazolium bromide (I) in a dose of 115 mg/kg resulted in convulsions of a clonic nature, half of the

Central X-Ray Radiological Research Institute, Ministry of Health of the USSR, Leningrad.
Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 9, No. 9, pp. 26-29, September, 1975. Original article submitted June 12, 1974.

TABLE 1. Substituted 3,5-Diamino-1,2,4-dithiazolium Bromides

Compound	Yield (%)	mp (deg)	Found (%)		Molecular formula	Calculated (%)		λ max (nm)	lg ε
			Br	S		Br	S		
I	74	> 300	36,36	—	$C_2H_4BrN_3S_2$	37,37	—	246 278—284	4,22 3,86
II	40	122	26,64	21,33	$C_8H_8BrN_3S_2$	27,58	22,08	244 280	4,3 4,0
III	82,5	204—205	24,70	19,60	$C_9H_{10}BrN_3OS_2$	25,00	20,00	276 318—326	4,24 4,0
IV	90,5	210	24,63	19,59	$C_9H_{10}BrN_3OS_2$	25,00	20,00	274 318—326	4,27 4,13

TABLE 2. Dithiobiurets

$$RNHCNHCNH_2$$
$$\| \quad \|$$
$$S \quad S$$

R	Yield (%)	mp (deg)	λ max (nm)	lg ε	Literature refs.
H	—	180—181	282	4,21	[6]
o-$CH_3OC_6H_4$	41	141—142	290	4,42	[7]
p-$CH_3OC_6H_4$	72	165	254 286—292	4,28 —	[7]
C_6H_5	—	170			[7]

TABLE 3. Radioprotective Effects of Dithiazolium Compounds in Mice Subjected to Irradiation in a Dose of 700 Rad

Compound	LD_{50} (mg/kg)	Dose of drug (mg/kg)	Survival of mice (%)	Mean longevity (days)
I	115	25	30	10,1±1,1
II	55	25	30	11,1±1,0
III	80	25	40	6,2±1,2
IV	70	25	40—50	10,5±1,1
Cystamine	—	200	50—70	11,5±0,8
Control	—	—	0—10	9,7±1,2

animals dying within the first hour. Replacement of the hydrogen atom in the 3-position of the dithiazolium ring by phenyl increased the toxicity twofold (LD_{50} 55 mg/kg). The introduction of a methoxy group into the para- or ortho-position of the benzene ring resulted in a small reduction in the toxicity (Table 3).

Comparison of the structure of the compounds with their radioprotective properties (as judged by the criterion of survival) showed that I and 3-phenylamino-5-amino-1,2,4-dithiazolium bromide (II) exhibit the same radioprotective effects (30% survival of the mice, as compared with the death of all the control animals). Introduction of the phenyl radical into the amino group in the dithiazolium ring, therefore, has no effect on the radioprotective properties.

Introduction of a methoxy group into the o- or p-position of the benzene ring increases the radioprotective properties. Thus, both 3-p-methoxyphenylamino- and 3-o-methoxyphenyl-5-amino-1,2,4-dithiazolium bromide (III and IV) maintain the survival of the animals at the 40-50% level, as compared with a mortality of 90-100% in the controls (see Table 3).

In addition to increasing the survival of the irradiated animals, III and IV prevent the development of radiation leucopenia, megakaryocytosis, and loss in weight of the spleen (Table 4).

Thus it has been shown that, of the dithiazolium compounds tested, III and IV exhibit radioprotective properties. However, these compounds only decrease the radiosensitivity of the animals at the maximum tolerated doses. Treatment with these compounds in small doses (10 mg/kg) halves their radioprotective effects.

TABLE 4. Effect of Dithiazolium Compounds on the Number of Leucocytes in the Peripheral Blood, Karyocytes in the Femur, and Weight of the Spleen in Irradiated Mice (700 rad; % of Initial Level)

Compound	No. of leucocytes		No. of karyocytes		Wt. of spleen	
	3rd day	10th day	3rd day	10th day	3rd day	10th day
I	9,7 (6,3—13,1)	4,3 (3,1—5,5)	9,4 (6,8—12,0)	6,0 (2,5—9,4)	33,2 (25,9—40,5)	37,0 (32,2—43,2)
II	30,1 (24,8—35,4)	7,0 (5,8—8,2)	9,2 (8,0—10,2)	10,7 (8,0—13,3)	29,0 (26,5—32,0)	32,0 (27,0—38,0)
III	40,8 (37,8—43,8)	15,7 (11,6—19,8)	10,9 (8,6—13,2)	10,1 (6,1—14,0)	31,0 (25,3—38,0)	30,6 (27,6—33,6)
IV	39,9 (33,6—46,2)	15,0 (10,0—20,0)	9,5 (9,2—9,8)	10,5 (8,9—12,1)	23,0 (20,1—26,5)	30,6 (26,5—34,7)
Control	6,2 (5,5—6,9)	4,3 (3,8—4,8)	7,9 (6,59—3)	4,7 (3,9—5,5)	25,0 (23,9—26,1)	26,2 (22,6—30,0)

Note. Confidence limits given in parthenses.

EXPERIMENTAL METHODS

Xanthane Hydride (isopersulfocyanic acid) [5], yellow, finely crystalline powder, mp > 300°C, λ_{max} 286 and 355 nm; log ε 4.35, 3.89 (alcohol).

Dithiobiuret [6], pale yellow crystals, sparingly soluble in cold, readily soluble in boiling water. Mp 180-181°, λ_{max} 282 nm, log ε 4.21 (alcohol).

Methoxyphenyldithiobiurets. Obtained by the general method used for aryldithiobiurets [7]. A mixture of xanthane hydride (5 g), 7.5 ml of o-anisidine, and 10 ml of alcohol was boiled under reflux for 20 min. After cooling, 40 ml of 1 N hydrochloric acid was added, and the solid which separated was filtered off and washed with water. It was then suspended in 5N sodium hydroxide, and diluted with 200 ml of water. The solution was filtered, and the filtrate neutralized to pH 7 with acetic acid, to give a white crystalline powder. Yield 3.5 g (43.5%), mp 135-136°. Crystallized from a mixture of 10 ml of alcohol and 15 ml of water, mp 139-140°.

3-o-Methoxyphenylamino-5-amino-1,2,4-dithiazolium Bromide (IV). o-Methoxyphenyldithiobiuret (1 g) was dissolved in 10 ml of alcohol, and 0,3 ml of bromine was added dropwise with stirring. All the solid dissolved. Kept for one day at room temperature. Solution evaporated to half its volume. Cooled, and the precipitate filtered off. Yield 1.2 g (90.5%), mp 207-209° (decomp.). Crystallized from 10 ml of alcohol, mp 210°. Bromides I-III were obtained similarly.

LITERATURE CITED

1. Z. Bacq, A. Herve, J. Lecomte, et al., "Protection against x-irradiation by β-mercaptoethylamine" [in French], Arch. int. Physiol., 1951, 59, 442-451.
2. A. S. Mozzhukhin and F. Yu. Rachinskii, Chemical Prophylaxis of Radiation Injury [in Russian], Atomizdat, Moscow (1964), p. 242.
3. E. F. Romantsev, Radiation and Chemical Protection [in Russian], Atomizdat, Moscow (1968), p. 207.
4. M. B. Kolesova, L. I. Maksimova, and A. V. El'tsov, "3,5-Diamino-1,2,4-dithiazolium ions in hydride transfer reactions' [in Russian], Zh. Org. Khim., 1971 (7), pp. 2227-2232.
5. P. Klasson, "Iso-perfulfocyanic acid" [in German], J. Prakt. Chem., 1888, 38, 368-369.
6. F. Kurtser, Guanylthiourea, in: Organic Syntheses [in Russian], Moscow, 1956, 7, 27-30.
7. A. E. S. Fairfull and D. A. Peak, "Dithiobiurets. Part I. Some 1- and 1,5-substituted derivatives," J. Chem. Soc., 1955, pp. 796-802.