SYNTHESIS AND PHARMACOLOICAL PROPERTIES OF 3-CARBOXYALKYLRHODANINES CONTAINING ALKYLATING MOIETIES

I. A. Frankov, M. V. Kirillov,
T. N. Sokolova, R. V. Skupskaya,
A. N. Kharitonovich, and I. I. Chizhevskaya

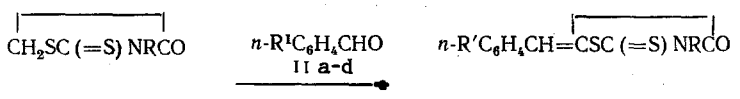
UDC 615.277.3:547.789.3].012.1.07

Thiazolidines are known to possess high bioloigical activity. The naturally occurring penicillic and actithiazinic acids, leucogen, the highly active semisynthetic antibiotics oxacillin, ampicillin, and cloxacillin, and many synthetic drugs with a wide spectrum of therapeutic activity are all thiazolidine derivtives. The new home-produced antitumor drug imifos (2-methylthiazolido-3-phosphoric acid diethyleneimide) has been used clinically for the successful treatment of erythremia [1].

In the search for novel antitumor drugs of the thiazolidine series, some 3-carboxyalkylrhodanines have been synthesized which contain alkylating moieties. Experiments on animals have been used to obtain information on the pharmacological and antitumor properties of the compound obtained since no literature information is available.

The starting rhodanines (Ia-d) were obtained from the amino acids glycine, β-alanine, DL-tryptophan, and DL-glutamic acid, by known methods [2, 5].

Advantage was taken of the nucleophilic activity of the methylene groups in (Ia-d) to introduce alkylating moieties. The aldol condensation of (Ia) with benzaldehyde and with p-di-[2-chloro(bromo)ethyl]amino- and p-(N-methyl- and p-(N-2-chloroethyl)aminobenzaldehydes (IIa-d), affected by heating the reactants in alcohol in the presence of catalytic amounts of piperidine, or in alcohol in the presence of aqueous ammonia and ammonium chloride [4] gave the rhodanines (IIIa-g). Since (IIIa-g) were insoluble in water, salts with inorganic



Ia-d IIIa-g

Ia, IIIa, b, g: R = CH_2COOH; Ib, IIIc, f: R = $(CH_2)_2COOH$;
Ic, IIId: R = 1-carboxy-2-(3-indolyl)ethyl, Id, IIIe: R = $CH(COOH)(CH_2)_2COOH$;
IIa, IIIa: R′ = H; IIb, IIIb-e: R′ = $N(CH_2CH_2Cl)_2$; IIc, IIIf: R′ = $N(CH_2CH_2Br)_2$;
IId, IIIg: R′ = N(Me)$(CH_2)_2Cl$.

and organic bases were prepared [3], namely, salts of (IIIa, b) with ethanolamine (IVa, b), of (IIIb, c, f) with diethanolamine (IVc-e), of (IIIb) with dimethylamine (IVf), the ammonium salts of (IIIb, g) (IVg, h), the sodium salt of (IIId) (IVi), and the disodium salt of (IIIe) (IVj), which were then submitted for biological testing.

The properties and yields of the compounds obtained are given in Tables 1 and 2. The IR spectra of (IIIa-g) showed absorption at ν_{max} 1720-1740 cm^{-1} (C=O), 1580-1600 cm^{-1} and 1485-1520 cm^{-1} (C=C), and those of the salt (IVa-j) absorbed at 1700 cm^{-1} (C=O), 1580 and 1520 cm^{-1} (C=C), and 1620 and 1400 cm^{-1} (COO^-).

The rhodanine derivatives (IVa-j) are of low toxicity in warm-blooded animals, their LD_{50} values being in the range 200-450 mg/kg.

The antitumor activity of the compounds was assessed from the leukocyte count in the peripheral blood of the experimental animals. It was found thtat the rhodanines (IVa-j) possess antitumor activity only in relatively high doses of approximately $^1/_5$ of the LD_{50}, inhibiting the growth of tumors by 30-55% ($p < 0.05$). The test compounds were inferior to alkylating drugs in their capactiy to inhibit tumor growth, but unlike all known di-(2-chloro-

Vitebsk Medical Institute, Institute of Bioorganic Chemistry of the Belorussian SSR, Minsk. Translated from Khimiko-farmatsevitichеskii Zhurnal, Vol. 19, No. 8, pp. 943-946, August, 1985. Original article submitted June 30, 1984.

 0091-150X/85/1908-0544$09.50

TABLE 1. 5-Benzylidene-3-carboxyalkylrhodanines (IIIa-g)

Compound	Yield, %	mp, °C	Found, %		Empirical formula	Calc., %	
			C	H		C	H
IIIa	89	186	51,24	3,61	$C_{12}H_9NO_3S_2$	51,61	3,22
IIIb	84	196	45,52	3,89	$C_{16}H_{16}Cl_2N_2O_3S_2$	45,82	3,85
IIIc	92	200	47,03	4,20	$C_{17}H_{18}Cl_2N_2O_3S_2$	47,11	4,19
IIId	78	115—7	54,83	4,70	$C_{25}H_{23}Cl_2N_3O_3S_2$	54,76	4,27
IIIe	87	105—7	46,96	4,20	$C_{19}H_{20}Cl_2N_2O_5S_2$	46,43	4,10
IIIf	76	220	39,11	3,83	$C_{17}H_{18}Br_2N_2O_3S_2$	39,08	3,44
IIIg	87	225	48,88	4,33	$C_{15}H_{15}ClN_2O_3S_2$	48,58	4,84

TABLE 2. Salts of 5-Benzylidene-3-carboxyalkylrhodanines (IVa-j)

Compound	Yield, %	mp, °C	Found, %		Empirical formula	Calc., %	
			C	H		C	H
IVa	83	139	45,92	4,90	$C_{16}H_{20}N_2O_5S_2$	45,79	5,20
IVb	80	168	44,85	4,96	$C_{18}H_{23}Cl_2N_3O_4S_2$	45,00	4,80
IVc	78	159	46,01	5,32	$C_{20}H_{27}Cl_2N_3O_5S_2$	45,79	5,20
IVd	82	153	46,68	5,86	$C_{21}H_{29}Cl_2N_2O_5S_2$	46,83	5,44
IVe	82	145	40,43	5,16	$C_{21}H_{29}Br_2N_3O_5S_2$	40,19	4,67
IVf	81	173	46,26	5,17	$C_{18}H_{23}Cl_2N_3O_3S_2$	46,55	4,95
IVg	84	181—3	44,67	4,55	$C_{15}H_{19}Cl_2N_2O_2S_2$	44,44	4,83
IVh	80	166	46,78	4,25	$C_{15}H_{19}ClN_3O_3S_2$	46,45	4,64
IVi	76	>300 decomp.	50,75	5,05	$C_{25}H_{22}N_2O_3S_2Na \times 1\ H_2O \cdot 1\ C_2H_5OH$	50,60	4,70
IVj	81	>300	41,87	3,84	$C_{19}H_{18}Cl_2N_2O_5S_2Na_2 \times 1\ H_2O \cdot 0{,}5\ C_2H_5OH$	41,60	4,00

TABLE 3. Effect of 5-Benzylidene-3-carboxyalkylrhodanines (IVa, h) on Arterial Pressure

Compound	Dose, mg/kg	Level of hypotension, %	Duration of hypotension, min
IVa	1,5	0	0
	15	20	1,2
IVh	7	0	0
	35	24	3
	70	47	4

TABLE 4. Hypotensive Activity of (IVh) as Compared with Dibasol

Compound	No. of animals	LD_{50}, mg/kg	Dose (administered internally, mg/kg	Arterial pressure (mm Hg, M ± m)		Magnitude of hypotensive region	Reduction in arterial pressure as % of initial value	Duration of hypotensive effect, min (M ± m)
				initial	maximum hypotension			
Dibasol	6	240	5	95±12	84±4	14±2	15±2	1±0,1
			20	97±5	69±2	28±4	29±4	6±3
IVh	6	350	35	100±6	75±4	25±4	24±3,5	3±0,5
			70	95±5	49±4	47±4	47±5,5	4,4±0,3

ethyl)amine derivatives they did not interfere with hemogenesis even following repeated administrtin in doses close to $^1/_5$ of the LD_{50}.

Examination of the effects of (Va-j) on arterial pressure in acute experiments in rats showed that (IVb, c, e, f), which contain the di-[2-chloro(bromo)ethyl]amino group, have no effect on the arterial pressure, whereas (IVa, h) have moderate hypotensive activity (Table 3).

In its hypotensive activity (IVh) was approximately as active as the clinical drug dibasol, but it was somewhat inferior to the latter in the duration of its hypotensive effect (Table 4).

EXPERIMENTAL (CHEMISTRY)

IR spectra were recorded on an IR-20 instrument (East Germany) in KBr disks.

Compounds (IIIa-g) (Table 1) were finely crystalline compounds of a red color, which were insoluble in water but soluble in alcohol, acetone, and chloroform.

Compounds (IIIa-c, f, and g) were purified by recrystallization from alcohol, acetone, or a mixture of the two, and (IIId, e) were purified by column chromatography on silica gel (L 100/160 μ) using as eluents the mixtures: chloroform–acetone (1:4) for (IIId) and chloroform–ethyl acetate (1:1) for (IIIe). The purities of (IIIa-g) were checked by TLC on Silufol UV-254 plates.

3-(Carboxymethyl)-5-[p-di(2-chloroethyl)aminobenzylidene]rhodanine (IIIb). A mixture of 3.82 g (0.02 mole) of 3-carboxymethylrhodanine (Ia) and 4.92 g (0.02 mole) of p-di-(2-chloroethyl)aminobenzaldehyde (IIb) in 20 ml of alcohol was boiled with three drops of piperidine for 6 h. Partial evaporation of the alcohol resulted in the separation of a crystalline solid, which was filtered off and recrystallized from a mixture of alcohol and acetone (2:1) to give 7.1 g of (IIIb). Compounds (IIIa, b, f, and g) were obtained similarly.

3-(α,γ-Dicarboxypropyl)-5-[p-di(2-chloroethyl)aminobenzylidene]rhodanine (IIIe). A mixture of 2.63 g (0.01 mole) of 3-(α,γ-dicarboxypropyl)rhodanine (Id), 2.46 g (0.01 mole) of (IIb), 1.6 ml of 25% aqueous ammonia, and 1 g of ammonium chloride was dissolved in 15 ml of ethanol, and stirred at 50°C for 15 h. The mixture was cooled and neutralized with 10% HCl, and the oily solid which separated was filtered off. Purification was carried out on a chromatographic column of silica gel, the compound being eluted with a mixture of chloroform and ethyl acetate (1:1). The mixed solvents were removed under a water pump vacuum, and the residue crystallized by triturating with light petroleum to give a finely crystalline red solid, weight 4.2 g.

Compound (IIId) was obtained similarly.

Disodium Salt of 3-(α,γ-Dicarboxypropyl(-5-[p-di-(2-chloroethyl)aminobenzylidene]-rhodanine (IVj). Metallic sodium (0.46 g, 0.02 mole) was dissolved in 10 ml of absolute alcohol and added dropwise to a solution of 4.9 g (0.01 mole) of (IIIe) in 40 ml of dry acetone. The precipitated disodium salt was filtered off, washed with ether, and crystallized from alcohol or purified by reprecipitation from its alcohol solution with ether, to give 4.3 g of (IVj).

Compound (IVi) was obtained similarly.

Diethanolamine Salt of 3-(Carboxymethyl)-5-[p-di(2-chloroethyl)aminobenzylidene]rhodanine (IVc). A mixture of 4.2 g (0.01 mole) of (IIIb) and 1 g (0.01 mole) of diethanolamine was dissolved in 10 ml of alcohol, and the mixture kept at 50°C for 0.5 h. When no more solid separated, the solution was kept in the refrigerator for 6-10 h. The solid was filtered off and recrystallized from methanol to give 4.1 g of (IVc).

Salts (IVa, b, and d-f) were obtained similarly.

Ammonium Salt of 3-(Carboxymethyl)-5-[p-di(2-chloroethyl)aminobenzylidene]rhodanine (IVg). Compound IIIb (4.2 g) was dissolved in 50 ml of dry acetone, and the solution was saturated with dry gaseous ammonia until no more solid separated. The solid was filtered off and recrystallized from alcohol to give 3.8g of (IVg).

Compound (IVh) was obtained similarly.

EXPERIMENTAL (PHARMACOLOGY)

The toxicities of the rhodanine derivatives were assessed in short-term tests on mice of both sexes, weighing 18-22 g, by the intraperitoneal route. The test compounds were administered to the experimental animals as solutions, obtained using isotonic sodium chloride solution. The experimental animals were observed for a period of 10 days following dosing. Each dose was tested in five mice, and the results were evaluated by the Behrens frequency accumulation method. The LD_{50}'s were determined graphically.

The effects of the test compounds on tumor growth were assessed in rats with Pliss' lymphosarcoma. The tumor was grafted subcutaneously. Grafting was carried out with a suspension of tumor cells obtained as follows. The tumor nucleus was extracted from a donor rat with lymphosarcoma, and the tissue of the former was comminuted and mixed with physiological saline. The suspension was filtered through a layer of gauze, diluted with physiological saline to the required concentration, and placed in a closed vessel, into which had previously been introduced the rod of a magnetic stirrer and a long needle, through which the suspension was withdrawn at the instant of transplantation into the experimental animal. While the mixture was being withdrawn, it was stirred with a magnetic stirrer. This procedure enabled the tumor to be grafted under aseptic conditions, and approximately the same numbers ($3.2 \cdot 10^7$) of cells to be introduced into each mouse.

The test compounds were administered from the third day following grafting of the tumor, and were given once daily for ten days. The control animals were treated at the same times with 1.0 ml of physiological saline. On the sixth day following grafting, the animals were killed, and the tumor nodes extracted and weighed. The percentage inhibition of tumor growth was calculated using the equation:

$$\frac{M_C - M_0}{M_C} \cdot 100,$$

where M_C is the mean weight of the tumor in the control mice, and M_0 the mean weight of the tumor in the experimental group.

The effects of the test compounds on hemogenesis were assessed by measuring the regular elements in the peripheral blood in the same animals used for the investigation of the antitumor activity of these compounds. Blood for examination was taken from the coccygeal vessels. The numbers of regular elements per 1.0 mm^3 of blood were obtained using a Goryaev chamber.

The effects of the test compounds on arterial pressure were examined in short-term experiments in rats of both sexes, weighing 230-250 g, under urethane narcosis. The urethane was administered intraperitoneally in a dose of 1.0-1.2 g/kg. On the right, the common jugular vein was revealed and a needle introduced into it which was subsequently used to introduce the solution of the test compound. On the left, the common carotid artery was separated, and a cannula introduced which was connected to a mercury manometer. The system was filled with 10% sodium nitrate solution. The solutions of the test compounds were also made using physiological saline.

LITERATURE CITED

1. S. A. Giller, M. Yu. Lidak, and E. Ya. Markova, [in Russian], Institute of Medicine and Physiology (IMIFOS), (1968), pp. 11-23.*
2. B. M. Turkevich, in: The Synthesis of Naturally Occurring Compounds, and Analogs and Fragments [in Russian], Moscow–Leningrad (1965), pp. 205-208.
3. S. M. Berge, L. D. Bichley, and D. C. Monkhouse, J. Pharm. Sci., 66, 1-19 (1977).
4. M. Girard, Ann. Chim., 16, 326-394 (1941).
5. A. Miolati, Justus Liebigs Ann. Chem., 262, 84-88 (1891).

*Russian text omitted the title of the work – Editor.