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SYNTHESIS AND STUDY OF THE BIOLOGICAL PROPERTIES OF DERIVATIVES OF 5-CARBOXYMETHYLTHIAZOLIDINE-2,4-DIONE AND OF THIOCARBOHYDRAZIDE

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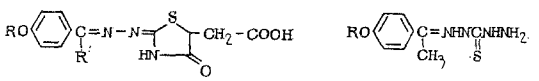
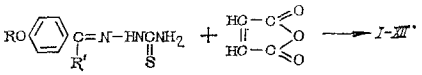
It is known that the thiosemicarbazones of various carbonyl compounds are biologically active substances, and, in particular, possess antiviral properties [1]. The majority of thiosemicarbazones with a high antiviral activity, and also some thiosemicarbazones of α-(N-heteryl)carbaldehydes with antitumoral activity [2], contain free =NNHC(S)NH_2 groupings. It has been established that the introduction of the latter into a cyclic system leads to heterocyclic compounds with a thiourea skeleton which are interesting subjects of pharmacological investigation [3].

On the basis of the above facts, we have found it desirable to use 4-alkoxybenzaldehydes and 4-alkoxyacetophenones in the synthesis of new derivatives of 5-carboxymethylthiazolidine-2,4-dione and of thiocarbohydrazide with the general formulas:



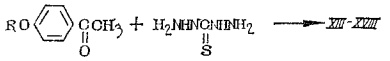
I — XII (for R and R' see Table 2) XIII — XVIII (for R and R' see Table 2)

The main starting materials for the synthesis of compounds (I-XII) were the thiosemicarbazones of 4-alkoxybenzaldehydes and of 4-alkoxyacetophenones obtained by boiling equimolar amounts of the carbonyl compounds with thiosemicarbazide in aqueous alcoholic solution [1, 5]. The latter were caused to react with maleic anhydride in chloroform.



The synthesis of analogous compounds in benzene has been described in the literature [1], but our experiments showed that this always gives mixtures of the initial compounds and the final products. The use of chloroform in place of benzene leads to fairly pure derivatives of 5-carboxymethylthiazolidine-2,4-dione with high yields.

The thiocarbohydrazones of 4-alkoxyacetophenones were obtained by the reaction of the 4-alkoxyacetophenones with thiocarbohydrazide as described in the literature [6].



A. L. Mndzhoyan Institute of Fine Organic Chemistry of the Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 15, No. 5, pp. 62-66, May, 1981. Original article submitted July 9, 1980.

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TABLE 1. 4-Alkoxybenzylidenehydrazones and 4-Alkoxy-α-methylbenzylidenehydrazones of 5-Carboxymethylthiazolidine-2,4-dione

Compound	R	R′	Yield, %	mp, °C	R_f	Found, %		Empirical formula	Calculated, %	
						N	S		N	S
I	CH_3	H	84,3	263—264 (decomp.)	0,53	13,39	10,47	$C_{13}H_{13}N_3O_4S$	13,67	10,43
II	C_2H_5	H	83,1	253—254	0,58	13,47	9,57	$C_{14}H_{15}N_3O_4S$	13,07	9,97
III	C_3H_7	H	82,2	235—236	0,67	12,71	9,20	$C_{15}H_{17}N_3O_4S$	12,52	9,56
IV	iso-C_3H_7	H	75,6	273—274 (decomp.)	0,69	12,57	9,56	$C_{15}H_{17}N_3O_4S$	12,52	9,56
V	C_4H_9	H	87,1	242—243	0,75	11,72	8,83	$C_{16}H_{19}N_3O_4S$	12,02	9,17
VI	iso-C_4H_9	H	72,3	239—240	0,77	12,48	8,86	$C_{16}H_{19}N_3O_4S$	12,02	9,17
VII	CH_3	CH_3	98,9	244—245	0,54	12,65	9,50	$C_{14}H_{15}N_3O_4S$	13,07	9,97
VIII	C_2H_5	CH_3	84,8	243—244	0,62	12,34	10,00	$C_{15}H_{17}N_3O_4S$	12,52	9,56
IX	C_3H_7	CH_3	85,7	233—234	0,66	12,28	9,65	$C_{16}H_{19}N_3O_4S$	12,02	9,17
X	iso-C_3H_7	CH_3	86,9	194—195	0,63	12,34	9,23	$C_{16}H_{19}N_3O_4S$	12,02	9,17
XI	C_4H_9	CH_3	88,8	215—216	0,68	11,30	8,92	$C_{17}H_{21}N_3O_4S$	11,56	8,82
XII	iso-C_4H_9	CH_3	86,0	220—221	0,70	11,74	8,80	$C_{17}H_{21}N_3O_4S$	11,56	8,82

Note. According to the literature [4] is 261°C mp of XII.

TABLE 2. Thiocarbohydrazones of 4-Alkoxyacetophenones

Compound	R	Yield, %	mp, °C	R_f	Found, %		Empirical formula	Calculated, %	
					N	S		N	S
XIII	CH_3	99,1	173—174	0,61	23,10	13,35	$C_{10}H_{14}N_4OS$	23,51	13,45
XIV	C_2H_5	97,6	186—188	0,63	21,59	12,40	$C_{11}H_{16}N_4OS$	22,20	12,70
XV	C_3H_7	94,1	174—175	0,64	20,40	12,46	$C_{12}H_{18}N_4OS$	21,03	12,40
XVI	iso-C_3H_7	89,5	142—144	0,67	20,62	12,37	$C_{12}H_{18}N_4OS$	21,03	12,40
XVII	C_4H_9	57,1	182—183	0,68	19,62	11,77	$C_{13}H_{20}N_4OS$	19,98	11,43
XVIII	iso-C_4H_9	71,4	179—180	0,69	19,60	11,56	$C_{13}H_{20}N_4OS$	19,98	11,43

The purities and individualities of the compounds (I-XVIII) synthesized were checked by chromatography, and their structures were shown by mass spectrometry. In the mass spectra fairly strong peaks of the molecular ions and of a number of fragmentary ions the origin of which confirmed their structure were found.

EXPERIMENTAL (BIOLOGICAL)

In the chemotherapeutic experiments performed in order to determine the antibacterial and antiviral activities of the compounds synthesized, we used 1050 mice and 340 rats. The antibacterial action of compounds (I-XVIII) was studied by the serial dilution method in a dense nutrient medium (meat-peptone agar, pH 7.2-7.4) with the subsequent seeding of a staphylococcus or Flexner dysentry bacillus (microbial load 20 million microbial bodies per 1 ml of medium). The compounds exhibited no antibacterial activity *in vitro* in relation to these organisms.

The chemotherapeutic activity was studied on a model of the generalized staphylococcal infection of white mice caused by intraperitoneal infection with a lethal dose of the culture ($4 \cdot 10^8$ microbial bodies). The experiments were performed with strains of the cultures sensitive to sulfanilamides, of which two were laboratory strains (Smith and 4-0 strains) and one was a recently-isolated strain (strain 12), and also with one resistant strain (strain 18b). The compound studied was administered simultaneously with the infection. As a control drug we used sulfathiazole (series 130275). The significance of the differences in the survival time of the treated animals as compared with controls was established by means of the alternative form of estimating the reaction with the calculation of the criterion χ^2 [7].

Of the 5-carboxymethylthiazolidine-2,4-dione derivatives we studied compounds (III, V, IX, and XI) *in vivo* in chemotherapeutic experiments. When administered to random-bred mice in doses of 2500 and 4000 mg/kg, these compounds caused no changes in the behavior and state of the animals; they were not tolerated in larger doses.

On infection with strain 4-0 of the staphylococcus, all the compounds studied had a therapeutic effect (Table 3). On treatment with compound (V), as with sulfathiazole, a therapeutic effect was observed at all the doses used. At a dose of 1000 mg/kg, the survival rate of the animals was 61%; at this dose, the compound was also active in the infection caused by another laboratory strain — Smith (Table 4). Compounds (III, V, and IX) were active also in the treatment of the infection caused by a recently-isolated staphylococcal strain (strain 12; see Table 2). So far as concerns the infection caused by the resistant

TABLE 3. Chemotherapeutic Action of Thiazolidine-2,4-dione Derivatives in a Staphylococcal Infection of Mice Caused by *Staphylococcus aureus*, Strain 4-0

Compound	Dose, mg/kg, once per os	Number of animals	Survived	Died	Total survival time of the animals		
					absolute, days	% of control	*P*
III	500	5	0	5	0/50	0	—
	1000	10	5	5	52/100	52	0.01
	1500	10	1	9	11/100	11	—
V	500	10	4	6	41/100	41	0,05
	1000	20	12	8	122/200	61	<0,001
	1500	25	7	18	70/250	28	<0,1
IX	500	5	1	4	10/50	20	—
	1000	15	7	8	71/150	47	<0,01
	1500	10	1	9	10/100	10	—
XI	500	5	0	5	0/50	0	—
	1000	15	7	8	71/150	47	<0,01
	1500	10	2	8	21/100	21	>0,1
Sulfathiazole	500	5	2	3	20/50	40	<0,1
	1000	10	7	3	70/100	70	<0,001
	15000	10	6	4	60/100	60	<0,01
Control (untreated animals)	—	15	0	15	0/150	0	—

Note. Here and in Table 4 the numerator is the number of mouse-days in the given group and the denominator is the maximum possible number of mouse-days on observation for 10 days.

TABLE 4. Chemotherapeutic Action of Compounds (III, V, IX, and XI) in a Dose of 1000 mg/kg on a Staphylococcal Infection

Strain of Staph. aureus	Compound	Number of animals	Survived	Died	Total survival time of the animals		
					absolute, days	% of control	P
Smith	III	15	1	14	11/150	7,3	—
	V	15	7	8	70/150	47	<0,01
	IX	15	1	14	10/150	7	—
	XI	15	2	13	22/150	15	—
	Sulfathiazole	15	12	3	120/150	80	<0,001
	Control (untreated animals)	15	0	15	0/150	0	—
12	III	15	10	5	101/150	68	<0,001
	V	10	4	6	40/100	40	<0,05
	IX	15	5	10	52/150	35	0,05
	XI	15	3	12	32/150	21	—
	Sulfathiazole	10	9	1	90/100	90	<0,001
	Control (untreated animals)	15	0	15	0/150	0	—
18b	III	5	0	5	0/50	0	—
	V	10	0	10	0/100	0	—
	IX	5	0	5	0/50	0	—
	XI	5	0	5	0/50	0	—
	Sulfathiazole	10	0	10	0/100	0	—
	Control (untreated animals)	10	0	10	0/100	0	—

staphylococcus, the compounds studied, like sulfathiazole, had no therapeutic action. A study of compounds (XIII-XVIII) showed that they were more toxic and less active than (I-XII), i.e., the presence of a free =NNHC(S)–NH_2 grouping in the molecule leads to deterioration of the therapeutic action in relation to a staphylococcal infection. Thus, 5-carboxymethylthiazolidine-2,4-dione derivatives have a low toxicity and under the conditions of integrity of the organism in the treatment of a staphylococcal infection of mice exhibit pronounced chemotherapeutic activity the degree of which is determined by the nature of the compound and of the causative agent. A further search for active compounds in this series is desirable.

The antitumoral activity of the compounds synthesized was determined on two mouse tumors (sarcoma 180 and Ehrlich's ascitic tumor) and two rat tumors (sarcoma 45 and Walker's carcinosarcoma 256). The treatment of the animals after the transplantation of the tumors into them was begun after 24 h (in the case of Ehrlich's ascitic carcinoma) or after 5-6 days (in the case of the solid tumors). The drugs were used in the maximum tolerated doses intraperitoneally (six times for the mice and eight times for the rats). The antiblastic efficiency of the substances tested was evaluated by a known method [8]. It was established that the majority of the compounds studied inhibited sarcoma 45 moderately (40-45%), and compounds (VII-XII) prolonged the life of mice with Ehrlich's ascitic carcinoma by a factor of 1.5-2. The substances possessed no antiblastic activity in relation to sarcoma 180 and Walker's carcinosarcoma.

EXPERIMENTAL (CHEMICAL)

Mass spectra were taken on an MKh-1303 instrument with direct introduction of the sample into the ionization region at a temperature 40-50°C below the melting point; the energy of the ionizing electrons was 30 eV. Chromatography was performed on Silufol UV-254 plates in ether–ethanol systems [20:1 for (I) and (II); 40:1 for (III) and (IV)]; the ether–acetone

systems (40:1) for (V) and (VI); ether–methanol systems [40:1 for (VII) and (VIII) and 80:1 for (IX, X, XI, and XII)]; and the acetone–benzene (1:1) system for (XIII-XVIII). The spots were revealed with the aid of UI-1 ultrachemiscope.

4-Alkoxybenzylidenehydrazones and 4-Alkoxy-α-methylbenzylidenehydrazones of 5-Carboxymethylthiazolidine-2,4-dione (I-XII). The appropriate 4-alkoxybenzylidene thiosemicarbazone or 4-alkoxy-α-methylbenzylidenethiosemicarbazone was added to a solution of 1.96 g (0.02 mole) of maleic anhydride in 100 ml of chloroform, and the mixture was boiled in a water bath for 3-4 h. The precipitate formed was filtered off, washed with chloroform, and recrystallized from ethanol (Table 1).

Thiocarbohydrazones of 4-Alkoxyacetophenones (XIII-XVIII). With stirring, 0.05 mole of the appropriate 4-alkoxyacetophenone in 15 ml of methanol was added to a solution of 5.3 g (0.05 mole) of thiocarbohydrazide in 100 ml of hot water, and stirring was continued at 20°C for 12 h. The resulting precipitate was filtered off, washed, and recrystallized from absolute ethanol (Table 2).

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SYNTHESIS AND PHARMACOLOGICAL STUDY OF 2-[(3'-INDOLYL)METHYL]QUINUCLIDINES

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In preceding investigations we have shown that in their action on choline- and histaminergic systems, the quinuclidine compounds are much more active than the analogous derivatives of aliphatic or monocyclic amines [1]. In contrast, in the case of adrenergic systems, the transition to quinuclidine derivatives induces a decrease in the pharmacological effect, or even its complete disappearance [2]. It is clear that the above features of the biological activity of the quinuclidine compounds are largely due to the presence in their molecules of a bridgehead nitrogen atom with a descreened free electron pair, which readily interacts with electrophilic centers of choline- and histaminergic receptors, but the bridgehead nitrogen atom is uncapable of entering into similar reactions with the electrophilic centers of adrenoreceptors, which is apparently due to the steric structure features of sections of protein molecule adjoining these centers.

A practical result of the investigations related to the influence of the quinuclidine compounds on the choline- and histaminergic systems was the production and introduction into medicinal practice of original medicinal preparations aceclidine, oxylidine, temequine, phencarol, etc.

From the theoretical and practical points of view, it was very interesting to study the interaction of quinuclidine derivatives with serotoninergic systems. Because of the previously discovered [3] high anti-serotonin activity of certain (3'-quinuclidyl) substituted derivatives of diphenylcarbinols, it could also be assumed that in other series of quinuclidine

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