SYNTHESIS AND BIOLOGICAL ACTIVITY OF BIS-THIOSEMICARBAZONES OF 3-ETHOXY-2-OXOBUTYRALDEHYDE AND THEIR COMPLEXES WITH Cu(II)

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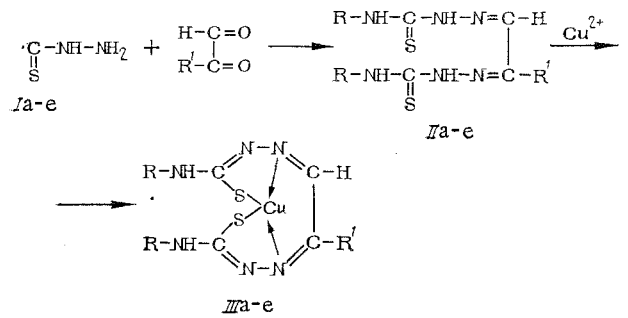
It is known that compounds of the thiosemicarbazone series possess a wide spectrum of biological activity [6, 9]. There is particular interest in bis-thiosemicarbazones (KTS) of 3-ethoxy-2-oxobutyraldehyde which possess marked antitumor activity in relation to a whole series of solid tumors [10, 11]. As has been established recently this compound reacts specifically *in vivo* with Cu(II) ions and in fact the copper complexes of it are active agents [7, 12].

With the aim of obtaining new derivatives of KTS and establishing the influence of structural modifications of KTS on antitumor activity, we have carried out the synthesis and have studied the biological activity of substituted bis-thiosemicarbazones of 3-ethoxy-2-oxobutyraldehyde and their copper complexes.

Synthesis was carried out according to the following scheme.



(I)-(III):R' = β-ethoxyethyl (a-e), R = H (a), 4-methoxybenzyl (b), 3-bromo-4-methoxybenzyl (c), 4-ethoxybenzyl (d), 3-bromo-4-ethoxybenzyl (e).

The initial substituted thiosemicarbazides (Ia-e) were synthesized by the procedure described by us previously in [4]. 3-Ethoxy-2-oxobutyraldehyde was obtained by the oxidation of crotonaldehyde with selenium dioxide in a medium of absolute ethanol [13]. The obtained bis-thiosemicarbazones (IIa-e) were yellow crystalline substances, soluble in organic solvents and insoluble in water.

Copper chelates (IIIa-e) were synthesized by the interaction of equimolar quantities of the corresponding thiosemicarbazone and a copper salt. Their structure was confirmed by studying spectrophotometric titration. Compounds (IIIa-e) were red crystalline substances readily soluble in dimethyl sulfoxide (DMSO), dimethylformamide (DMF), and certain other organic solvents. They were stable in aqueous solution over the pH range 4.0-10.0. Protonation of the ligand occurred in acid media with the formation of charged complexes [2].

All the obtained compounds were identified by elemental analysis and by IR and UV spectroscopy. There were characteristic absorption bands in the IR spectra of these compounds in the region of 1500-1590 cm^{-1} (C=N) and 3130-3350 cm^{-1} (NH). The structures of complexes (IIIa) and (IIIb) were also confirmed by EPR.

Since all these compounds were synthesized with the aim of testing their biological activity, it seemed of interest to us to determine their distribution coefficients in the system octanol—water, since it was considered in [1] that octanol successfully imitated the lipid

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TABLE 1. Bis-thiosemicarbazones of 3-Ethoxy-2-oxobutyraldehyde (IIa-e)

Compound	Yield, %	mp, °C	R_f	Found, %				Empirical formula	Calculated, %			
				C	H	N	S		C	H	N	S
IIa	43,5	210—2*	0,63†	34,58	5,63	30,07	22,81	$C_8H_{16}N_6OS_2$	34,76	5,84	30,41	23,20
IIb	64,5	191—3	0,51	55,91	6,58	15,93	12,69	$C_{24}H_{32}N_6O_3S_2$	55,79	6,24	16,27	12,41
IIc	65,3	71—3	0,57	42,77	4,45	12,07	9,32	$C_{24}H_{30}Br_2N_6O_3S_2$	42,74	4,48	12,46	9,50
IId	54,5	181—3	0,69	56,97	6,79	14,91	11,35	$C_{26}H_{36}N_6O_3S_2$	57,33	6,66	15,43	11,77
IIe	51,4	188—90	0,55	44,61	4,52	12,33	8,89	$C_{26}H_{34}Br_2N_6O_3S_2$	44,45	4,88	11,96	9,13

*From data in [11], mp 202-4°C.
†In the system acetone—methanol 1:2.

TABLE 2. Copper Complexes of Bis-thiosemicarbazones of 3-Ethoxy-2-oxobutyraldehyde (IIIa-e)

Compound	Yield, %	mp, °C	Found, %					Empirical formula	Calculated, %					UV spectrum, λ_{max}, nm (in DMF)
			C	H	N	S	Cu		C	H	N	S	Cu	
IIIa	59,2	>300	28,21	3,97	24,56	18,63	19,38	$C_8H_{14}N_6OS_2Cu$	28,44	4,18	24,87	18,98	18,80	494,548 sh.
IIIb	75,1	103—5	49,42	5,49	14,25	11,51	11,56	$C_{24}H_{30}N_6O_3S_2Cu$	49,85	5,23	14,54	11,09	10,99	493,548 sh.
IIIc	99,3	91—3	39,19	3,82	10,99	8,55	9,02	$C_{24}H_{28}Br_2N_6O_3S_2Cu$	39,16	3,83	11,42	8,71	8,63	494,550 sh.
IIId	95,8	93—5	51,12	5,78	14,26	10,19	9,91	$C_{26}H_{34}N_6O_3S_2Cu$	51,51	5,65	13,86	10,58	10,48	493,548 sh.
IIIe	75,9	72—4	41,25	4,38	10,67	8,82	7,80	$C_{26}H_{32}Br_2N_6O_3S_2Cu$	40,87	4,22	10,99	8,39	8,32	488, 543 sh.

layer of cell membranes and the solubility of the compound in lipid was an important factor determining its ability to surmount cellular barriers.

It was established by us from the experimental data that the lipophilicity of the substituted bis-thiosemicarbazones and their complexes was far greater than that of the unsubstituted compounds.

EXPERIMENTAL CHEMISTRY

IR spectra were taken on a UR-20 spectrophotometer (East Germany) in the solid state in KBr disks. UV spectra were taken on a Specord UV VIS instrument (West Germany). EPR spectra were taken on a Varian E-12 instrument (USA). Melting points were determined on a Boetius 72/2064 microhot stage. TLC was carried out on Silufol UV-254 plates (Czechoslovakia) in the system benzene–methanol (5:1) with visualization by iodine vapor.

Distribution coefficients were calculated by the procedure of [3] in the system octanol–water at 20 ± 2°C. Initial solutions of concentration $2 \cdot 10^{-5}$ mole/liter were prepared in octanol. Stirring time was 20 min. The concentration of ligands and complexes in the equilibrium octanol solutions was determined spectrophotometrically at several wavelengths in the 250-600-nm region. An octanol phase saturated with water under conditions analogous to the experimental was used as reference solution.

Data on the obtained compounds are given in Tables 1 and 2.

3-Ethoxy-2-oxobutyraldehyde Bis-thiosemicarbazone (IIa). This was obtained according to [11]. UV spectrum λ_{max}, nm (log ε) (DMSO): 335(4.6). $K_{oct/aq}$ = 6.1.

3-Ethoxy-2-oxobutyraldehyde Bis-4-(4-alkoxybenzyl)-(3-bromo-4-alkoxybenzyl)-3-thiosemicarbazones (IIb-e). 3-Ethoxy-2-oxobutyraldehyde (0.65 g:0.015 mole) in ethanol (20 ml) was added with stirring to a solution of (Ib-e) (0.01 mole) in ethanol (20 ml) and the mixture boiled under reflux for 2 h. After cooling the reaction mixture the resulting solid was filtered off and recrystallized from ethanol. Compound (IIb) had UV spectrum, λ_{max}, nm (log ε) (DMSO): 350 (4.4). $K_{oct/aq}$ = 10.7. Compound (IIc) had UV spectrum, λ_{max}, nm (log ε) (C_2H_5OH): 355 (4.3).

3-Ethoxy-2-oxobutyraldehyde Bis-thiosemicarbazonecopper(II) (IIIa). This was obtained according to [8]. EPR spectrum (DMF, t = 20°C): A_{Cu} = 89 G A_N = 15 G, g = 2.04. $K_{oct/aq}$ = 4.9.

3-Ethoxy-2-oxobutyraldehyde Bis[4-(4-alkoxybenzyl)-(3-bromo-4-alkoxybenzyl)-3-thiosemicarbazonecopper(II) (IIIb-e). A 2 N solution (20 ml) of NaOH was added to a solution of (IIb-e) (0.003 mole) in DMSO (20 ml) and a solution of copper sulfate (0.75 g; 0.003 mole) in water (60 ml) was added dropwise at room temperature with vigorous stirring. The mixture was stirred for 2 h for complete precipitation. The precipitated solid was filtered off, washed several times with water, with alcohol, and dried. Compound (IIIb) had EPR spectrum ($CDCl_3$), t = 20°C: A_{Cu} = 92 G, A_N 16 G, g = 2.06. $K_{oct/aq}$ = 52.8

EXPERIMENTAL PHARMACOLOGY

The investigation of the toxicity and antitumor activity of the synthesized compounds was carried out by the method in [5]. Substances were administered to animals intraperitoneally in a 0.5% solution of carboxymethylcellulose. The acute toxicity was determined in white random-bred mice on single intraperitoneal administration of compounds. The lethal (LD_{100}) and maximum tolerated (MTD) doses were established in this way.

Chemotherapeutic experiments were carried out on sarcoma 45, the Walker carcinosarcoma, and Ehrlich's ascites carcinoma as experimental models. Antitumor effect was assessed as the percentage inhibition of tumor growth (T%) and by the increase in survival (SI) of animals. According to the obtained data (IIa) used as a structural analog possessed marked toxicity and high antitumor activity in relation to the solid animal tumors (Table 3). At therapeutic doses the given compound inhibited growth of sarcoma 45 and the Walker carcinosarcoma by 65-85% having no influence however on the growth of the Ehrlich's ascites carcinoma.

Introduction of an alkoxybenzyl radical into the structure of (IIa) led only to an insignificant drop in toxicity (LD_{100} 500 mg/kg). The alkoxybromobenzyl analog (IIc) was distinguished by weak toxicity or was practically devoid of it (LD_{100} 2500 mg/kg).

TABLE 3. Acute Toxicity and Antitumor Activity of the Studied Compound

Compound	Toxicity		Antitumor activity, T%		
	LD_{100}, mg/kg	MTD, mg/kg	dose, mg/kg	sarcoma 45	Walker carcinosarcoma
IIa	400	300	20	85	65
IIb	500	250	25	58	0
IIc	2500	2000	100	62	18
IId	500	250	25	80	24
IIIa	5	4	0,25	21	0
IIIb	750	500	45	17	50
IIIc	400	300	20	0	28
IIId	>2000	—	100	42	24
IIIe	500	400	25	19	37

The indicated modifications of the (IIa) structure proved to have a significant influence on antitumor activity. Only compound (IId) retained a high therapeutic effect (T 80%) in relation to sarcoma 45 while the activity of the other compounds (IIb, IIc) fell to 58-62%. All the alkoxybenzyl derivatives of KTS proved to have no significant therapeutic action on the Walk : carcinosarcoma and the Ehrlich's ascites carcinoma.

Copper complex (IIIa) possessed significantly higher toxicity in comparison with the ligand (LD_{100} 5 mg/kg) but notwithstanding the literature data of [7, 12] did not display a significant antitumor effect in the experiment (see Table 3). At the same time copper chelates (IIIb-e) were distinguished by relatively weak toxicity (LD_{100} 400-750 mg/kg) and compound (IIId) was completely devoid of toxicity (LD_{100} > 2000 mg/kg).

It follows from Table 3 that the copper chelates with the exception of compound (IIId) displayed antitumor activity in relation to sarcoma 45. Compound (IIId) caused moderate (T 42%) growth inhibition of the tumor in question. Compounds (IIIb) and (IIIe) gave a significant therapeutic effect (T 50 and 37%, respectively) in relation to the Walker carcinosarcoma. All the compounds of this group displayed no antitumor activity in relation to the Ehrlich's ascites carcinoma.

The antibacterial activity of the synthesized compounds (IIc, IIIb-e) was studied *in vitro*, by serial dilution [5] in meat-peptone bouillion at a microbial loading of 2×10^6 microbial cells per ml medium, in relation to the standard test microbes *Staph. aureus* 209P and *Sh. dysenteriae* Flexneri. Activity was assessed as the minimum concentration of compound inhibiting growth. Only (IIIb) inhibited growth of the test cultures at a concentration of 300 µg/ml, the remaining compounds proved to be practically inactive.

The obtained data therefore indicate the expediency of further search for new antitumor agents in the series of bis-thiosemicarbazones.

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