SEARCH FOR NEW DRUGS

COPPER-CONTAINING COMPLEXES OF NEW THIOSEMICARBAZONES OF SOME AROMATIC ALDEHYDES: SYNTHESIS AND ANTITUMOR ACTIVITY

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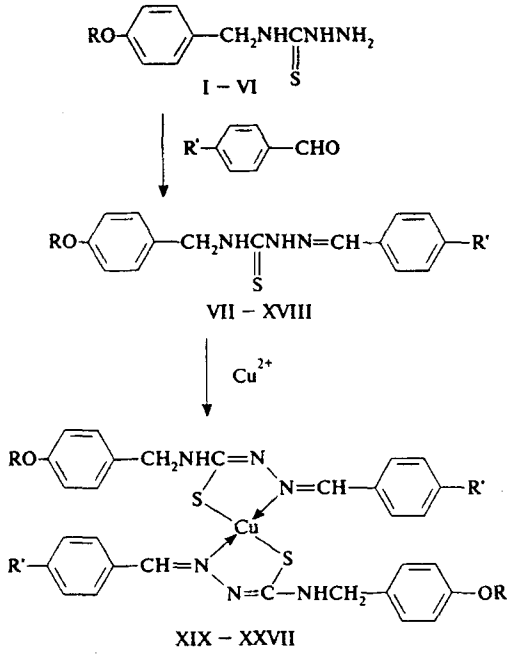
In continuation of our previous investigations [1, 2], we have synthesized 4-alkoxybenzyl-substituted thiosemicarbazones of some aromatic aldehydes containing nitro or heptyloxy substituents, and their chelate complexes with copper(II) ions:



R = Me (I, VII, XIII, XIX, XXIV), Et (II, VIII, XIV, XX, XXV), Pr (III, IX, XV, XXI, XXVI), *i*-Pr (IV, X, XVI, XXII, XXVII), Bu (V, XI, XVII), *i*-Bu (VI, XII, XVIII, XXIII);

R' = NO_2 (VII – XII, XIX – XXIII); OC_7H_{15} (XIII – XVIII, XXIV – XXVII).

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The initial 4-(4-alkoxybenzyl)thiosemicarbazides were synthesized by interaction of 4-alkoxybenzylisothiocyanates with hydrazine hydrate [3] and then reacted with the corresponding aldehydes — 4-nitro- and 4-heptyloxybenzaldehydes. Thiosemicarbazones of 4-nitro- and 4-heptyloxybenzaldehydes were obtained in the form of chromatographically pure substances, either bright-yellow in color or colorless. The proposed structures of thiosemicarbazones VII – XVIII were confirmed by the data of IR and ^1H NMR spectroscopies and elemental analyses.

The interaction of thiosemicarbazones VII – XVIII with copper(II) sulfate in the presence of aqueous ammonium led to complexes XIX – XXVII having the form of chromatographically pure fine-crystalline substances of a red-brown color. For a comparative analysis of the biological activity of chelates obtained with different complex-forming agents (in the case of thiosemicarbazone VII), we have also synthesized an analogous complex (XXVIII) with Ni^{2+} ions.

The formation of complexes was accompanied by significant shifts in the positions of absorption bands in the IR spectra. This, in combination with the elemental analysis data, additionally confirmed the proposed structures of complexes.

The IR spectra of compounds VII – XVIII contain characteristic peaks in the regions of 3360 – 3290 and 3170 – 3130 (–NH–), 1610 – 600 (–C=N–), 1590 – 1550 (–C=C– arom.), and 1250 – 1230 (–C–O–C–) cm^{-1}. Upon formation of the complex, the absorption bands corresponding to –NH– exhibit a large (30 – 40 cm^{-1}) shift toward higher frequencies.

Taking into account the structural similarity between complexes XIX – XXIII and some of the previously synthesized compounds identified by *x*-ray diffraction [4], we suggest that the molecules of complexes XIX – XXVII contain two bidentate thiosemicarbazone ligands capable of chelating

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copper atoms by sulfur and nitrogen atoms at the first position to form a pair of coplanar five-member metallocycles.

EXPERIMENTAL CHEMICAL PART

TLC was performed on Silufol UV-254 plates eluted in the systems A (benzene – ethanol, 9 : 2) and B (hexane – acetone, 3 : 2) and developed by exposure to iodine vapors. The IR spectra were measured on an UR-20 spectrophotometer using samples prepared as nujol mulls. The ^{1}H NMR spectra were recorded on a Varian T-60 spectrometer with TMS as the internal standard. The melting temperatures were determined on a Boetius 72/2064 microscopic heating stage. The data of elemental analyses (C, H, N, S) coincided with the results of calculations according to the empirical formulas.

4-(4-Alkoxybenzyl)-1-(4-nitrobenzylidene)-3-thiosemicarbazides (VII – XII). To a warm solution of 0.05 mole 4-(4-alkoxybenzyl)-3-thiosemicarbazide in 100 ml of ethanol was added 7.6 g (0.05 mole) of 4-nitrobenzaldehyde, and the mixture was boiled on a water bath with reflux for 2 h. The precipitate was separated by filtration and recrystallized from ethanol (Table 1, TLC in system A).

For compound VII, ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 11.80 (s, 1H, NHN), 9.20 (t, 1H, J 6.0 Hz, NHC), 8.25 and 8.15 (m, 4H, J 8.0 Hz, $C_6H_4NO_2$), 8.20 (s, 1H, CH=N), 7.35 and 6.92 (m, 4H, J 8.1 Hz, $C_6H_4OCH_3$), 4.82 (d, 2H, J 6.0 Hz, CH_2N), 3.72 (s, 3H, OCH_3). For compound XII, ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 11.82 (s, 1H, NHN), 9.18 (t, 1H, J 6.0 Hz, NHC), 8.33 and 8.13 (m, 4H, J 8.0 Hz, $C_6H_4NO_2$), 8.24 (s, 1H, CH=N), 7.36 and 6.92 (m, 4H, J 8.2 Hz, $C_6H_4OCH_3$), 4.82 (d, 2H, J 6.0 Hz, CH_2N), 3.75 (d, 2H, J 6.2 Hz, OCH_2), 1.95 (m, 1H, $\underline{CH}(CH_3)_2$), 0.97 (d, 6H, J 6.1 Hz, CH_3).

4-(4-Alkoxybenzyl)-1-(4-heptyloxybenzylidene)-3-thiosemicarbazides (XIII – XVIII). Compounds XIII – XVIII were obtained by a procedure analogous to that described above using 0.05 mole of I – VI and 11.0 g (0.05 mole) of 4-heptyloxybenzaldehyde (Table 1, TLC in system B).

For compound XIII, ^{1}H NMR spectrum, $CDCl_3$ (δ, ppm): 10.32 (s, 1H, NHN), 7.95 (s, 1H, CH=N), 7.70 (t, 1H, J 6.0 Hz, NHC), 7.57 and 6.90 (m, 4H, J 8.2 Hz, C_6H_4OHep), 7.40 and 6.90 (m, 4H, J 8.2 Hz, $C_6H_4OCH_3$), 4.92 (d, 2H, J 6.0 Hz, CH_2N), 3.97 (t, 2H, J 6.1 Hz, OCH_2), 3.80 (s, 3H, OCH_3), 1.10 – 2.04 (m, 10H, CH_2), 0.88 (m, 3H, CH_3). For compound XVI, ^{1}H NMR spectrum, DMSO-d_6 (δ, ppm): 11.40 (s, 1H, NHN), 8.85 (t, 1H, J 6.0 Hz, NHC), 8.07 (s, 1H, CH=N), 7.76 and 6.95 (m, 4H, J 8.2 Hz, C_6H_4OHep), 7.34 and 6.86 (m, 4H, J 8.0 Hz, C_6H_4OPr-*i*), 4.78 (d, 2H, J 6.0 Hz, CH_2N), 4.63 (m, 1H, OCH), 4.00 (t, 2H, J 6.1 Hz, OCH_2), 1.25 (d, 6H, J 6.0 Hz, $CH(CH_3)_2$), 1.14 – 2.02 (m, 10H, CH_2), 0.86 (m, 3H, CH_3).

Bis[4-(4-alkoxybenzyl)-1-(4-nitrobenzylidene)isothiosemicarbazidato-N,S]copper(II) (XIX – XXIII). To a solution of 0.01 mole of compound VII – XII in 120 ml of ethanol was added 0.005 mole copper(II) sulfate and 8 ml of 25% aqueous ammonia, and the mixture was boiled for 4 h. The precipitate was separated by filtration, washed with hot ethanol, and recrystallized from a dioxane – ethanol (2 : 1) mixture (Table 2, TLC in system A).

Bis[4-(4-alkoxybenzyl)-1-(4-heptyloxybenzylidene)isothiosemicarbazidato-N,S]copper(II) (XXIV – XXVII). Compounds XXIV – XXVII were obtained by a procedure analogous to that described above using 0.01 mole of XIII – XVIII and 0.005 copper(II) sulfate (Table 2, TLC in system B).

Bis[4-(4-alkoxybenzyl)-1-(4-heptyloxybenzylidene)isothiosemicarbazidato-N,S]nickel(II) (XXVIII). Compound XXVIII was obtained by a procedure analogous to that described above using 0.01 mole of compound XIII and 0.005 mole nickel chloride (Table 2, TLC in system B).

EXPERIMENTAL BIOLOGICAL PART

The acute toxicities of the synthesized compounds were determined by single intraperitoneal injections to white mongrel mice. The antitumor properties were studied on rats and mice inoculated with sarcomas 45 and 180, the Pliss lymphosarcoma (PLS), or the Walker carcinosarcoma (WCS). The therapeutic effect was evaluated by the percentage tumor

TABLE 1. Physicochemical Characteristics of Substituted Thiosemicarbazones VII – XVIII

Compounds	Yield, %	M.p., °C	R_f	Empirical formula
VII	91	242 – 244	0.62	$C_{16}H_{16}N_4O_3S$
VIII	89	194 – 196	0.63	$C_{17}H_{18}N_4O_3S$
IX	89	171 – 173	0.64	$C_{18}H_{20}N_4O_3S$
X	90	185 – 187	0.65	$C_{18}H_{20}N_4O_3S$
XI	91	184 – 186	0.67	$C_{19}H_{22}N_4O_3S$
XII	94	189 – 190	0.66	$C_{19}H_{22}N_4O_3S$
XIII	96	168 – 170	0.51	$C_{23}H_{31}N_3O_2S$
XIV	92	177 – 178	0.52	$C_{24}H_{33}N_3O_2S$
XV	96	183 – 185	0.54	$C_{25}H_{35}N_3O_2S$
XVI	93	190 – 191	0.56	$C_{25}H_{35}N_3O_2S$
XVII	92	173 – 174	0.66	$C_{26}H_{37}N_3O_2S$
XVIII	89	175 – 176	0.67	$C_{26}H_{37}N_3O_2S$

TABLE 2. Physicochemical Characteristics of Cu_2+ and Ni^{2+}Complexes XIX – XXVIII with Substituted Thiosemicarbazones

Compounds	Yield, %	M.p., °C	R_f	Empirical formula
XIX	68	228 – 230	0.41	$C_{32}H_{30}CuN_8O_6S_2$
XX	66	216 – 218	0.45	$C_{34}H_{34}CuN_8O_6S_2$
XXI	72	190 – 192	0.46	$C_{36}H_{38}CuN_8O_6S_2$
XXII	60	196 – 198	0.46	$C_{36}H_{38}CuN_8O_6S_2$
XXIII	54	189 – 190	0.47	$C_{38}H_{42}CuN_8O_6S_2$
XXIV	56	121 – 123	0.48	$C_{46}H_{60}CuN_8O_4S_2$
XXV	52	131 – 133	0.50	$C_{48}H_{64}CuN_6O_4S_2$
XXVI	59	142 – 144	0.53	$C_{50}H_{68}CuN_6O_4S_2$
XXVII	61	135 – 137	0.52	$C_{50}H_{68}CuN_6O_4S_2$
XXVIII	72	100 – 102	0.50	$C_{46}H_{60}N_6NiO_4S_2$

growth inhibition. Because of poor solubility, all compounds were tested as suspensions in 0.5% carboxymethylcellulose solution.

It was established that 4-alkoxysubstituted thiosemicarbazones with nitro and heptyloxy substituents (VII – XVIII) and their copper complexes (XIX – XXVII) exhibited low acute toxicity. The maximum tolerated doses of all these compounds exceeded 2500 mg/kg. For this reason, the chemotherapeutic tests were performed using drug doses in the range 100 – 150 mg/kg for sarcoma 45, WCS, and PLS, and 200 – 250 mg/kg for sarcoma 180.

Similarly to the thiosemicarbazones of some aromatic aldehydes tested previously [5], the structural analogs with 4-nitrobenzylidene group (VII – XII) produce weak inhibition (30 – 40%, $p = 0.05$) of the growth of WCS and PLS tumors. A somewhat greater antitumor activity of these compounds is observed with respect to sarcomas 45 and 180. For example, the analogs with propoxy-(IX), isopropoxy-(X), and butoxy-(XI) radicals inhibited the growth of sarcoma 45 by 58 – 65% ($p < 0.05$). Substitution of the heptyloxybenzylidene group for the nitrobenzylidene one does not significantly change the properties of products XIII – XVIII. All these compound produce, besides a weak effect on the WCS and PLS tumors (inhibition by 30 – 42%, $p = 0.05$), a pronounced inhibition (up to 63%, $p < 0.05$) of sarcomas 45 and 180.

The results of our investigations of the antitumor activity of the copper-containing complexes XIX – XXVII showed that all these compounds exhibit the same spectrum of therapeutic action as the ligands. The effect of chelates, like that of the corresponding ligands, is less pronounced for WCS and PLS (tumor growth inhibition below 40%, $p = 0.05$). At the same time, most of the compounds showed a therapeutic effect exceeding 55% ($p < 0.05$) with respect to sarcoma models.

According to our data, the complex with Ni^{2+} ions has the same antitumor activity as the corresponding copper chelate.

Thus, the group of 4-alkoxysubstituted thisemicarbazones of 4-nitro- and 4-heptyloxybenzaldehydes and their copper complexes exhibit the same character and extent of antitumor activity as the thiosemicarbazones of some aromatic aldehydes studied previously [3, 5].

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