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Synthesis, Characterization, and Antitumor Activity of Azomethine Derivative of Triazole and Its Complexes with Copper(I) and Zinc(II) Salts

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Abstract—4-[(2-Hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione, a new azomethine derivative of triazole, was synthesized. This Schiff base reacts with the copper(I) and zinc(II) salts in a 2:1 molar ratio to afford complexes. The resulting compounds were characterized by the ^{1}H NMR, IR, UV spectroscopy and by elemental analysis.

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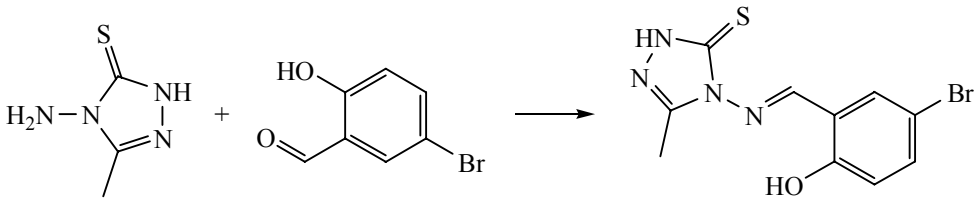
During the last decades of the 20th century it was firmly determined that copper and zinc participate actively in many biological systems and for that reason their normal homeostasis is of a first priority for the normal functioning of the organism [1]. Copper and zinc complexes with nitrogen donor ligands are of considerable interest due to a broad range of biological activities [2–4].

Azomethine compounds are considered to be "privileged ligands" because they are easily obtained by condensation of aldehydes and amines. Azomethine ligands are of great interest in the coordination chemistry due to the fact that they can easily form various kinds of metal complexes [5, 6] and are able to stabilize metals in various oxidation states. Complexes with azomethine ligands are also used as models of biological systems [7, 8]. The structural modification of organic molecules has a considerable biological interest. Furthermore, coordination of a biomolecule to metal ions significantly alters the effectiveness of the biomolecule. It is well known that various derivatives of 1,2,4-triazole and 1,3,4-thiadiazole have a wide range of pharmacological properties including antibacterial and antifungal activity [9,10].

In this work we synthesized 4-[(2-hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione (**L**) and its complexes with copper and zinc, and studied their structure and antitumor activity.

4-[(2-Hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione (**L**) was prepared via the reaction of 4-amino-3-methyl-1,2,4-triazole-5-thione [11] with 5-bromo-2-hydroxybenzaldehyde in ethanol according to the following scheme.



L

The structure of the resulting ligand was confirmed by the IR, UV, and NMR spectroscopy. The main parameters of the IR spectra are shown in Table 1, the parameters of the UV and ^{1}H NMR spectra, in the Experimental.

Schiff bases are known to be able to form stable complexes with metal ions [12, 13]. It was found that **L** is able to form complexes with the transition metal ions. In particular, we have managed to prepare two new complexes with copper(I) and zinc(II) salts that

Table 1. IR spectral parameters of 4-[(2-hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione (**L**) and complexes $[Cu(\mathbf{L})_2Cl]$ and $[Zn(\mathbf{L}_2)\cdot 2H_2O](CH_3COO)_2$

Compound	ν, cm^{-1}
L	3489 m (N–H), 3210 m (O–H), 3078 m (C–H), 2913 s (C–H), 1607 s (C=N), 1586, 777 s (C=N), 1125 w, 1072 s (C=S), 1040 w (Ar–Br)
$Cu(L)_2Cl]$	3215 br (N–H), 1598 s (C=N), 773 m (C–S), 421 w, 334 w (Cu–N), 380 w (Cu–S)
$[Zn(L_2)\cdot 2H_2O](CH_3COO)_2$	3406 br (N–H), 1597 s (C=N), 742 m (C–S), 468 w, 301 w (Zn–N), 370 w (Zn–S)

have not been synthesized and reported so far, by direct reactions with the ligand **L**.

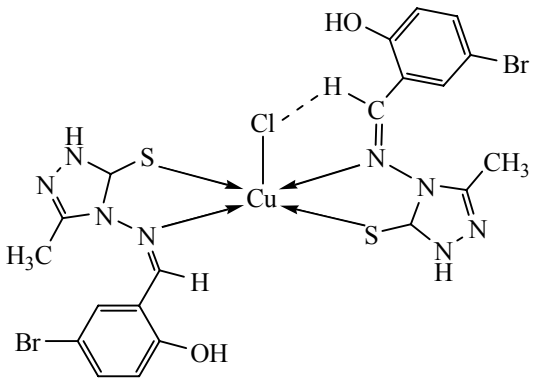
$$2\mathbf{L} + CuCl \rightarrow [Cu(L)_2Cl],$$

$$2\mathbf{L} + Zn(CH_3COO)_2\cdot 2H_2O \rightarrow [Zn(L_2)\cdot 2H_2O](CH_3COO)_2.$$

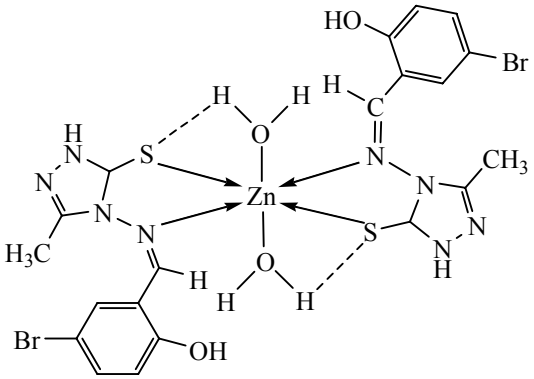
The reaction takes place under mild conditions in a molar ratio ligand–metal salt of 2:1. It is accompanied with a color change of the reaction mixture, which allows visual monitoring the reaction progress.

The structure of the complexes obtained was established by the IR, UV, and NMR spectroscopy (see Figure, Table 1). According to the IR spectroscopy, the metal atoms in the resulting complexes are coordinated with the sulfur and nitrogen atoms (C=N) of 4-[(2-hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione. The structure of the complexes $[Cu(\mathbf{L})_2Cl]$ (a) and $[Zn(\mathbf{L}_2)\cdot 2H_2O](CH_3COO)_2$ (b) can be represented as follows.

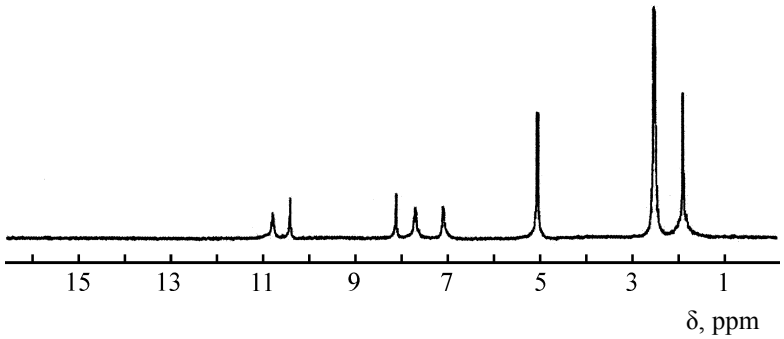
(a)



(b)



Synthesized 4-[(2-hydroxy-5-bromobenzylidene)-amino]-3-methyl-1,2,4-triazole-5-thione and its complexes with the copper(I) and zinc(II) salts were investigated *in vitro* for the cytotoxic activity against the colon cancer cells HT-29 and 742 using the MTT assay. The studies showed that the obtained azomethine base and its complexes $[Cu(\mathbf{L})_2Cl]$ and $Zn(\mathbf{L}_2)\cdot 2H_2O](CH_3COO)_2$ possess a high cytotoxic activity (up to



^{1}H NMR spectrum of $[Zn(\mathbf{L})_2\cdot 2H_2O](CH_3COO)_2$ in DMSO-d_6.

Table 2. Cytotoxic activity (%) of the synthesized compounds toward the HT-29, 742 cell cultures and fibroblasts

Compound	Concentration, M	HT-29	742	Fibroblasts
L	0.1	92.996	87	66.31
	0.01	96.5	93.96	83.57
	0.001	97.85	95.54	87.68
$[Cu(L)_2Cl]$	0.1	94.56	91.46	72.06
	0.01	96.13	95.8	85.76
	0.001	95.44	94.35	89.59
$[Zn(L_2)\cdot 2H_2O](CH_3COO)_2$	0.1	96.57	92.78	80.83
	0.01	97.13	93.96	83.43
	0.001	97.38	94.49	84.25

97.85%) and can be used as the model compounds for the design of new anticancer drugs. The results of the study are shown in Table 2.

EXPERIMENTAL

In this work copper(I) chloride and zinc acetate (dihydrate) manufactured by Merck were used. The solvents were purified by the standard techniques.

The IR spectra were recorded on a Bruker Tensor 27 Model 420 spectrometer from pellets with CsI or KBr. The UV-Vis spectra were registered on a Shimadzu 2100 spectrophotometer. The ^{1}H NMR spectra were taken on a Bruker Avance spectrometer.

For *in vitro* studies on the anticancer activity 190 ml of a cell suspension ($5\cdot10^4$ cells ml^{-1}) were exposed to the ligand solutions of different concentrations (0.1–0.001 M.) and its copper(I) or zinc(II) complexes in a sterile DMSO. The incubation period was 72 h for all cell lines. Cytotoxic activity (%) was calculated from the results of six series of the experiments.

Synthesis of 4-[(2-hydroxy-5-bromobenzylidene)-amino]-3-methyl-1,2,4-triazole-5-thione. To a solution of 1 g (7.7 mmol) of 4-amino-1,4-dihydro-3-methyl-1,2,4-triazole-5-thione in ethanol was added a solution of 1.54 g (7.7 mmol) of 5-bromo-2-hydroxybenzaldehyde in ethanol. The reaction mixture was refluxed for 4 h. The resulting yellow precipitate was filtered off, washed with ethanol, and dried at a room temperature. Yield 75%, mp 215–217°C. The ligand is soluble in DMSO, DMF, acetonitrile, water, and acetone, but insoluble in hexane. IR spectrum (KBr), ν, cm^{-}: 3489, 3210, 3078, 2913, 1607, 1586, 777, 1072. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 13.47, 10.91, 10.31, 7.97–6.94, 2.36. UV spectrum (DMSO), λ, nm: 275, 345. Found, %: C 38.31; H 3.89; N 17.85. $C_{10}H_9BrN_4OS$. Calculated, %: C 38.35; H 3.87; N 17.89.

Synthesis of $[Cu(L)_2Cl]$. To a solution of 0.626 g (2 mmol) of 4-[(2-hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione in a methanol–chloroform mixture (1:2) was added 0.11 g (1.1 mmol) of CuCl under stirring at room temperature. After 3 h, the resulting green precipitate was filtered off, washed with methanol, and dried at room temperature. Yield 70%, mp 290–293°C. The complex is soluble in DMSO, DMF, and ethanol, but insoluble in hexane and water. IR spectrum (CsI), ν, cm^{-1}: 3215, 1598, 773, 421, 334, 380. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.81, 10.57, 6.9–7.9, 2.33. UV spectrum (DMSO) λ, nm: 279, 343, 403. Found, %: C 33.22; H 2.46; N 15.52. $C_{20}H_{18}Br_2ClCuN_8O_2S_2$. Calculated, %: C 33.11; H 2.48; N 15.45.

Synthesis of $[Zn(L)_2\cdot 2H_2O](CH_3COO)_2$. To a solution of 0.626 g (2 mmol) of 4-[(2-hydroxy-5-bromobenzylidene)amino]-3-methyl-1,2,4-triazole-5-thione in ethanol was added a hot solution of 0.21 g (1 mmol) of $Zn(CH_3COO)_2\cdot 2H_2O$ in ethanol under stirring at room temperature. After 3 h, the resulting yellow pre0cipitate was filtered off, washed with methanol, and dried at room temperature. Yield 70%, mp > 300°C. The complex is soluble in DMSO, DMF, but insoluble in hexane, acetone and water. IR spectrum (CsI), ν, cm^{-1}: 3406, 1597, 742, 468, 301, 370. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 10.81, 10.57, 7.97–6.94,

1.82. UV spectrum (DMSO), λ, nm: 289, 387. Found, %: C 34.15; H 3.34; N 13.30. $C_{24}H_{28}Br_2N_8O_8S_2Zn$. Calculated, %: C 34.07; H 3.31; N 13.25.

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