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Synthesis and Investigation of Copper Complexes with Selected Azomethine Monobenzo Crown Ether Derivatives

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Abstract—A series of novel azomethine derivatives of benzo-18-crown-6, benzo-15-crown-5, and aniline as well as their complexes with copper(II) acetate have been synthesized. Using the MTT assay, the antitumor activity of the obtained compounds has been determined. The effect of individual structural elements on the antitumor activity of the whole compound has been assessed.

Keywords: crown ethers, copper complexes, the Schiff's bases, pyridinecarboxaldehyde, antitumor activity, MTT assay

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Crown ethers are macroheterocyclic compounds which find important applications in different areas of science and technology. The interest to crown ethers is due to their unique ability for selective complexing allowing their use as catalysts of interphase transfer in organic synthesis, selective extracting agent for metals in mineral resource industry, and separation of radionuclides [1]. The investigations of biological and medical potential of crown ethers and their complexes with metals have been numerous. Crown ethers are well-known ionophores and can exhibit antibacterial or antifungal activity [2–4]. Nowadays, the studies on the use of crown ethers derivatives as active compounds for chemotherapy of tumors, carriers of drugs to cancer cell, and photosensitizers have become topical [5–8]. It has been confirmed that copper, cobalt, nickel, palladium, and ruthenium complexes with organic ligands can cause apoptosis of cancer cells [9–12].

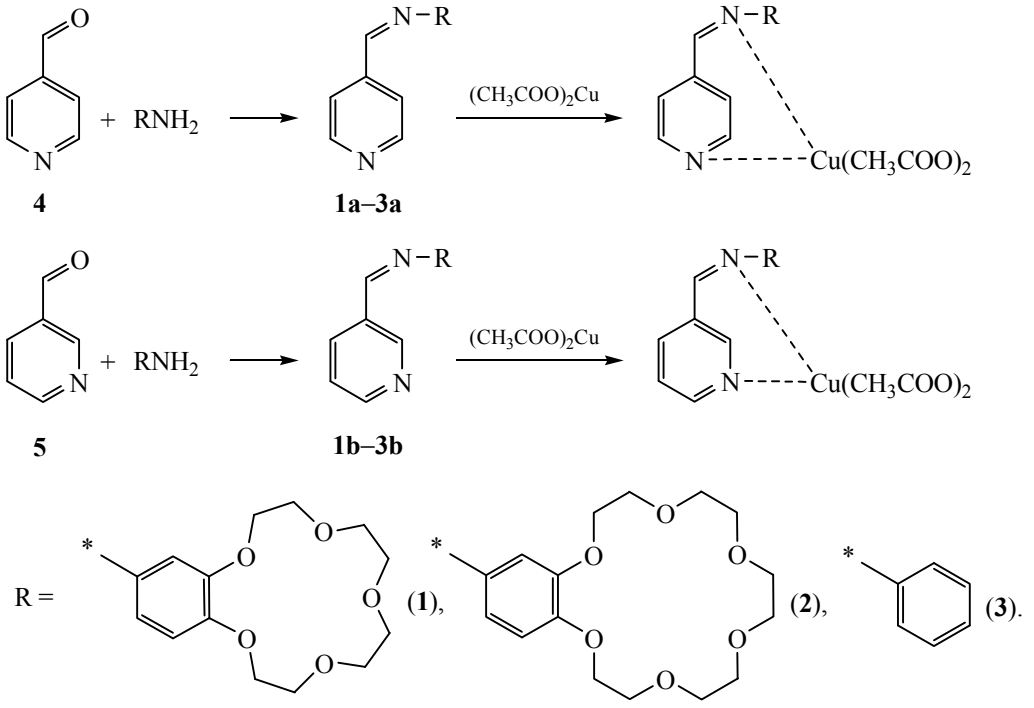
Crown-containing imines are the most interesting, in particular, pyridinylmethyleneaminobenzo crown ethers and their complexes which may be used in medicine, agriculture, and other fields because of their strongly selective sensor properties to metal cations [13].

The general method of preparation azomethines (imines, the Schiff's bases) is the condensation of aldehydes or ketones with primary amines. Azomethine derivatives of crown ethers, for example, benzo-15-crown-5, have been described earlier [14, 15]. 4-Aminobenzo-15-crown-5 and 2-hydroxy-4-methoxybenzaldehyde or 2-hydroxy-5-methoxybenzaldehyde have been used as the starting compounds. The synthesis of these imines has been carried out in methanol or anhydrous chloroform medium [16].

Herein we synthesized novel derivatives of crown ethers and their copper complexes and investigated cytotoxicity and antitumor activity of the prepared compounds.

At the first stage of the study, we obtained azomethine derivatives of benzo-15-crown-5 **1** [*N*-(pyridin-4-ylmethyleneamino)benzo-15-crown-5 **1a**, *N*-(pyridin-3-ylmethyleneamino)benzo-15-crown-5 **1b**], benzo-18-crown 6 **2** {*N*-(pyridin-4-ylmethyleneamino)benzo-18-crown-6 **2a**, *N*-(pyridin-3-ylmethyleneamino)benzo-18-crown-6 **2b** [17, 18]}, and aniline **3** [*N*-(pyridin-4-ylmethyleneamino)aniline **3a**, *N*-(pyridin-3-ylmethyl-eneamino)aniline **3b**]. Investigations of aniline derivatives which did not contain a crown ring gave an estimation of the influence of that structural fragment on antitumor activity. The Schiff's bases were obtained via condensation of 4-pyridinecar-boxyaldehyde **4** or 3 -pyridinecarboxyaldehyde **5** with the corresponding

Scheme 1.



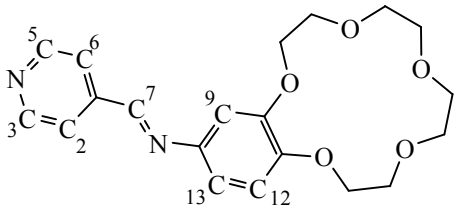
crown ethers **1**, **2** or aniline **3** in ethanolic medium at the aldehyde : crown ether molar ratio = 1.2 : 1.0 (Scheme 1). Copper complexes of the mentioned compounds were prepared via the addition of a methanolic solution of copper acetate to equimolar amount of the corresponding azomethine with further keeping at heating.

The composition and structure of the prepared Schiff's bases were determined using the data of elemental analysis as well as NMR and IR spectroscopy. The assignment of the signals was based on the 1D and 2D spectroscopy data (COSY and ^{1}H–^{13}C HSQC). The ^{1}H NMR spectra of the compounds containing crown ether fragment contained characteristic signals of the CH_2 groups and the aromatic ring protons. For example, the spectrum of compound **1b** contained doublet of doublets at 6.91 ppm corresponding to the proton at the C^9 carbon atom along doublets at 7.00 and 7.04 ppm corresponding to protons at the C^{12} and C^9 carbon atoms, respectively. The ^{1}H NMR spectra of the compounds containing *para*-substituted pyridine fragment showed two weak-field signals corresponding to protons at the C^2, C^6 (multiplet, 7.79–7.85 ppm for compound **1a**) and C^3, C^5 (multiplet, 8.68–8.77 ppm) carbon atoms. According to the data of HSQC spectroscopy, the signal of the proton at the C^7 atom was overlapped with those of protons at the C^3 and C^5 atoms. The assignment of the protons in compound **2a** was performed similarly. As for the *meta*-substituted pyridine fragment, doublet of doublets at 7.54 ppm corresponded to the proton at the C^5 atom, doublet of triplets at 8.28 ppm corresponded to the proton at the C^6 atom, doublet of doublets at 8.68 ppm corresponded to the proton at the C^4 atom, and doublet at 9.03 ppm corresponded to the proton at the C^9 atom. The atoms numbering is given in Scheme 2.

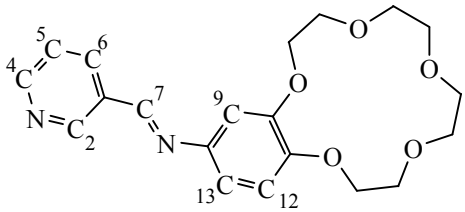
The investigation of cytotoxicity was carried out using five cell lines of human tumors: colon carcinoma HCT-116, prostate carcinoma PC-3, lung carcinoma A549, mammary adenocarcinoma MCF-7, and T cell lymphoblastic leukemia Jurkat, obtained from Cell lines bank of Blokhin National Research Center of Oncology. The MTT assay was used for the estimation of the antitumor activity [19].

Eight compounds of the prepared 12 ones exhibited antitumor activity: **3a**, **2a**, **3a**-$Cu(CH_3COO)_2$, **1a**-$Cu(CH_3COO)_2$, **2a**-$Cu(CH_3COO)_2$, **3b**-$Cu(CH_3COO)_2$, **1b**-$Cu(CH_3COO)_2$, and **2b**-$Cu(CH_3COO)_2$. The degree of inhibition of the cells growth is given in the Table. The obtained data showed that the copper complexes of the prepared ligands exhibited cytotoxic activity, but free ligands (pyridine-containing azomethine crown ethers) were inactive towards the used cell lines, except for the ligand **2a** containing 18-membered crown ether cycle,

Scheme 2.



1a



1b

which was active against T cell lymphoblastic leukemia Jurkat and inhibited the growth of cancer cells by 58%.

The in vitro investigation revealed certain structure–property relationships for the considered crown-containing Schiff's bases and their copper complexes. Generally, the free ligands did not exhibit cytotoxic activity, except for compounds **3a** and **2a** which were active against the Jurkat line. The prepared copper complexes were active, and their activity depended on their structural features. It was shown that *meta* and *para* substitution of the pyridine structural fragment affected the activity of the copper complexes, however, the clear dependence was not observed. In the case of 18-membered cycles, the most active one was the copper complex obtained from 3-pyridinecarboxyaldehyde. In the case of 15-membered cycles, the most active one was the copper complex obtained from 4-pyridincarboxyaldehyde. It was figured out that the copper complexes containing 15-membered crown ring exhibited lower activity than the 18-membered ones. The copper complexes with Schiff's bases (aniline derivatives) were intermediate between the complexes containing 15- and 18-membered crown rings. Hence, the activity of the copper complexes synthesized from azomethine derivatives of amino crown ethers and aniline was increased in the **1** < **3** < **2** series.

Inhibition of cells growth of the human tumors of cell lines by compounds **1a**–**3b** and their copper complexes

Comp. no.	*I*, %				
	PC-3	MCF-7	A549	HCT-116	Jurkat
1a	8	16	14	39	44
1b	20	–2	9	–2	19
2a	1	1	8	10	58
2b	17	2	11	4	24
3a	17	18	25	7	58
3b	8	17	17	13	24
1a-$Cu(CH_3COO)_2$	50	65	54	15	60
1b-$Cu(CH_3COO)_2$	46	53	53	12	35
2a-$Cu(CH_3COO)_2$	68	80	65	26	89
2b-$Cu(CH_3COO)_2$	77	85	84	77	86
3a-$Cu(CH_3COO)_2$	71	77	73	29	87
3b-$Cu(CH_3COO)_2$	50	60	62	18	42

In summary, novel azomethine derivatives of crown ethers and copper complexes based on them which can have wide implications, in particular in medicine were prepared. We showed that the complex based on *N*-(pyridin-3-ylmethynenamino)benzo-18-crown-6 exhibited the highest cytotoxic activity, inhibiting the growth of cell lines of human tumors by 77–86%.

EXPERIMENTAL

The ^{1}H NMR spectra were registered in DMSO-d_6 at 25°C using a Bruker AVANCE III NanoBay device operating at 300.28 MHz. Elemental analysis was performed using an Eurovector EuroEA 3000 CHNS-analyzer. The ATR IR spectra were recorded using a FTIR VERTEX 70 spectrometer (600–3800 cm^{-1} with resolution 4 cm^{-1}; ZnSe crystal).

Synthesis of the Schiff's bases. 0.012 mol of the corresponding aldehyde **5** or **4** was added dropwise to a solution of 0.01 mol of aniline **3** or crown ether **1** or **2** in 10 mL of ethanol. The obtained mixture was kept at 40°C for 3 h and then evaporated off. The precipitate was filtered off and dried in air at room temperature.

***N*-(Pyridin-4-ylmethylenamino)benzo-15-crown-5 (1a).** Yield 86%, light-green powder, mp 75.1–76.0°C. IR spectrum, ν, cm^{-1}: 627 w, 814 w, 851 w, 934 w, 989 w, 1050 m, 1091 m, 1131 s (C–O–C), 1230 s, 1266 s, 1316 m, 1363 w, 1414 w, 1453 m, 1513 s, 1555 w, 1588 m, 1598 m, 1626 w (C=N), 2868–2922

br. s (C–H), 3030–3060 br. w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 3.62 br. s (8H, CH_2), 3.75–3.82 m (4H, CH_2), 4.05–4.16 m (4H, CH_2), 6.96 d. d (1H, CH, *J* = 8.5, 2.2 Hz), 7.01 d (1H, CH, *J* = 8.5 Hz), 7.08 d (1H, CH, *J* = 2.2 Hz), 7.79–7.85 m (2H, CH), 8.68–8.77 m (3H, CH + HC=N). Found, %: C 64.69; H 6.57; N 7.58. $C_{20}H_{24}N_2O_5$. Calculated, %: C 64.50; H 6.53; N 7.52.

***N*-(Pyridin-3-ylmethylenamino)benzo-15-crown-5 (1b).** Yield 88%, light-green powder, mp 87.3–88.0°C. IR spectrum, ν, cm^{-1}: 623 w, 705 w, 811 w, 844 w, 918 w, 939 w, 991 w, 1050 w, 1091 w, 1129 s (C–O–C), 1142 s, 1237 m, 1262 s, 1331 w, 1373 w, 1421 w, 1449 w, 1510 s, 1568 w, 1586 w, 1625 w (C=N), 2873–2930 br. s (C–H), 3030–3060 br. w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 3.63 br. s (8H, CH_2), 3.75–3.82 m (4H, CH_2), 4.04–4.15 m (4H, CH_2), 6.91 d. d (1H, CH, *J* = 8.4, 2.3 Hz), 7.00 d (1H, CH, *J* = 8.4 Hz), 7.04 d (1H, CH, *J* = 2.3 Hz), 7.54 d. d (1H, CH, *J* = 7.9, 4.8 Hz), 8.28 d. t (1H, CH, *J* = 7.9, 1.7 Hz), 8.68 d. d (1H, CH, *J* = 4.8, 1.7 Hz), 8.75 s (1H, HC=N), 9.03 d (1H, CH, *J* = 1.7 Hz). Found, %: C 64.51; H 6.57; N 7.61. $C_{20}H_{24}N_2O_5$. Calculated, %: C 64.50; H 6.53; N 7.52.

***N*-(Pyridin-4-ylmethylenamino)benzo-18-crown-6 (2a).** Yield 89%, light-green powder, mp 63.9–64.7°C. IR spectrum, ν, cm^{-1}: 629 w, 713 m, 802 m, 829 m, 849 m, 899 w, 933 m, 949 m, 984 m, 1032 m, 1056 m, 1116 s (C–O–C), 1189 w, 1222 m, 1258 m (C–H), 1319 w, 1359 w, 1414 w, 1457 w (Py), 1482 w (Py), 1509 s (CH_2), 1587 w (Py), 1624 w (C=N), 2855 br, 2925 w (C–H), 2932 w (C–H), 3030 w (C–H_{Ar}), 3055 w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 3.52 s (4H, CH_2), 3.55–3.64 m (8H, CH_2), 3.73–3.80 m (4H, CH_2), 4.07–4.18 m (4H, CH_2), 6.95 d. d (1H, CH, *J* = 8.5, 2.0 Hz), 7.01 d (1H, CH, *J* = 8.5 Hz), 7.08 d (1H, CH, *J* = 2.0 Hz), 7.82 d (2H, CH, *J* = 5.8 Hz), 8.68–8.77 m (3H, CH + HC=N). Found, %: C 63.31; H 6.81; N 6.58. $C_{22}H_{28}N_2O_6$. Calculated, %: C 63.45; H 6.77; N 6.73.

***N*-(Pyridin-3-ylmethylenamino)benzo-18-crown-6 (2b).** Yield 89%, light-green powder, mp 73.9–74.2°C. IR spectrum, ν, cm^{-1}: 629 w, 713 m, 802 m, 829 m, 849 m, 899 w, 933 m, 949 m, 984 m, 1032 m, 1056 m, 1116 s (C–O–C), 1189 w, 1222 m, 1258 m (C–H), 1319 w, 1359 w, 1414 w, 1457 w (Py), 1482 w (Py), 1509 s (CH_2), 1587 w (Py), 1624 w (C=N), 2855 br, 2925 w (C–H), 2932 w (C–H), 3030 w (C–H_{Ar}), 3055 w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 3.53 br. s (4H, CH_2), 3.55–3.61 m (8H, CH_2), 3.74–3.80 m (4H, CH_2), 4.07–4.18 m (4H, CH_2), 6.91 d. d (1H, CH, *J* = 8.5, 2.3 Hz), 7.00 d (1H, CH, *J* = 8.5 Hz), 7.04 d (1H, CH, *J* = 2.4 Hz), 7.53 d. d (1H, CH, *J* = 7.9, 4.7 Hz), 8.28 d. t (1H, CH, *J* = 7.9, 1.9 Hz), 8.68 d. d (1H, CH, *J* = 4.7, 1.4 Hz), 8.75 s (1H, HC=N), 9.03 d (1H, CH, *J* = 1.0 Hz). Found, %: C 63.61; H 6.62; N 6.32. $C_{22}H_{28}O_6N_2$. Calculated, %: C 63.45; H 6.77; N 6.27.

***N*-(Pyridin-4-ylmethylenamino)aniline (3a).** Yield 92%, white powder, mp 67.8–69.2°C. IR spectrum, ν, cm^{-1}: 649 m, 690 s, 734 m, 764 s, 819 s, 881 w, 913 m, 961 w, 977 w, 987 w, 1022 w, 1059 w, 1074 w, 1165 w, 1186 w, 1209 w, 1226 w, 1236 w, 1284 w, 1325 m, 1364 w, 1411 s (Py), 1449 w, 1482 m (Py), 1554 w, 1579 w (Py), 1593 m (Py), 1620 m (C=N), 1671 m, 1711 m, 1799 w, 1881 w, 1953 w, 2001 w, 2884 w (C–H), 2991 w (C–H), 3025 w (C–H_{Ar}), 3054 w (C–H_{Ar}), 3072 w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 7.29–7.37 m (3H, CH), 7.41–7.50 m (2H, CH), 7.83–7.88 m (2H, CH), 8.69 s (1H, HC=N), 8.76 d (1H, CH, *J* = 3.4 Hz). Found, %: C 78.99; H 5.57; N 15.58. $C_{12}H_{10}N_2$. Calculated, %: C 79.10; H 5.53; N 15.37.

***N*-(Pyridin-3-ylmethylenamino)aniline (3b).** Yield 93%, yellow oil. IR spectrum, ν, cm^{-1}: 620 w, 667 w, 693 s, 760 s, 804 m, 830 w, 874 m, 910 w, 977 w, 1023 w, 1074 w, 1092 v.w, 1114 v.w, 1186 w, 1204 m, 1236 w, 1324 m, 1365 w, 1386 v.w, 1418 m (Py), 1450 w, 1485 m (Py), 1570 m (Py), 1587 m (Py), 1626 m (C=N), 1703 m, 1943, 2857 w (C–H), 2886 w (C–H), 3001 w (C–H_{Ar}), 3031 w (C–H_{Ar}), 3054 w (C–H_{Ar}), 3080 w (C–H_{Ar}). ^{1}H NMR spectrum, δ, ppm: 7.26–7.33 m (3H, CH), 7.39–7.48 m (2H, CH), 7.54 d. d (1H, CH, *J* = 7.9, 4.6 Hz), 8.31 d. t (1H, CH, *J* = 7.9, 1.9 Hz), 8.67–8.73 m (2H, HC=N + CH), 9.06 d (1H, CH, *J* = 1.9 Hz). Found, %: C 78.89; H 5.56; N 15.58. $C_{12}H_{10}N_2$. Calculated, %: C 79.10; H 5.53; N 15.37.

General procedure for preparation of complexes with Schiff bases 1a–3b. A solution of 0.001 mol of the corresponding Schiff's base in 10 mL of methanol was added to a solution of 0.001 mol of copper(II) acetate in 15 mL of methanol. The obtained mixture was kept at 45°C for 4 h. The precipitate was filtered off and dried in air at room temperature.

Complex 1a-Cu(CH_3COO)$_2$. Yield 94%, green powder, mp 203.2–203.8°C (decomp.). IR spectrum (KBr), ν, cm^{-1}: 617 s, 628 s, 680 s, 792 w, 831 w, 890 s, 942 m, 974 w, 1012 w, 1026 m, 1053 m, 1084 w, 1120 s (C–O–C), 1142 s, 1202 w, 1276 m (C–H_{Ar}), 1301 m, 1350 w, 1418 s, 1432 s, 1508 s, 1608 s (COOCu), 1628 s (C=N), 2869–2930 br. m (C–H).

Found, %: C 52.12; H 5.51; N 5.14. $C_{24}H_{30}N_2O_9Cu$. Calculated, %: C 52.02; H 5.46; N 5.06.

Complex 1b-Cu(CH$_3$COO)$_2$. Yield 91%, green powder, mp 208.9–210.0°C (decomp.). IR spectrum, ν, cm^{-1}: 628 s, 641 s, 682 s, 713 m, 803 m, 822 m, 841 m, 887 m, 929 w, 980 m, 1031 w, 1052 m, 1080 m, 1137 s (C–O–C), 1191 w, 1237 m, 1262 m, 1316 w, 1428 s, 1513 s, 1610 s (COOCu), 1629 s (C=N), 2870–2950 br. w (C–H). Found, %: C 53.06; H 5.51; N 5.12. $C_{24}H_{30}N_2O_9Cu$. Calculated, %: C 53.03; H 5.46; N 5.06.

Complex 2a-Cu(CH$_3$COO)$_2$. Yield 88%, green powder, mp 223.2–223.9°C (decomp.). IR spectrum, ν, cm^{-1}: 627 w, 793 m, 819 m, 839 w, 862 w, 879 w, 945 m, 987 m, 1049 m, 1107 s, 1127 s (C–O–C), 1225 s, 1245 m, 1265 s, 1315 w, 1326 w, 1354 w, 1412 w, 1427 w, 1449 w (Py), 1463 w (Py), 1511 s (CH_2), 1552 v.w, 1582 m (Py), 1598 m (C=N), 2868 m (C–H), 2923 w (C–H), 3030 w (C–H_{Ar}), 3056 w (C–H_{Ar}). Found, %: C 52.30; H 5.81; N 4.57. $C_{26}H_{34}N_2O_{10}Cu$. Calculated, %: C 52.22; H 5.73; N 4.69.

Complex 2b-Cu(CH$_3$COO)$_2$. Yield 88%, green powder, mp 230.1–230.9°C (decomp.). IR spectrum (KBr), ν, cm^{-1}: 628 w, 642 w, 680 s, 702 m, 811 m, 855 w, 948 m, 993 m, 1031 w, 1052 m, 1120 s, 1223 m, 1257 m, 1297 w, 1333 w, 1354 w, 1371 w, 1426 s, 1443 m, 1510 m, 1587 w, 1616 s, 2866 br, 2923 br, 3032 w, 3050 w. Found, %: C 52.33; H 5.61; N 4.53. $C_{26}H_{34}N_2O_{10}Cu$. Calculated, %: C 52.22; H 5.73; N 4.69.

Complex 3a-Cu(CH$_3$COO)$_2$. Yield 91%, green powder, mp 236.2–236.8°C (decomp.). IR spectrum (KBr), ν, cm^{-1}: 631 m, 660 m, 683 s, 735 w, 768 m, 824 m, 883 w, 913 w, 961 w, 976 w, 1013 w, 1033 w, 1056 w, 1185 w, 1211 w, 1244 w, 1320 w, 1350 w, 1418 s, 1433 s, 1484 w, 1559 w, 1607 s, 1624 s, 2885 w, 3000 w, 3061 w. Found, %: C 52.61; H 4.51; N 7.57. $C_{16}H_{16}N_2O_4Cu$. Calculated, %: C 52.82; H 4.43; N 7.70.

Complex 3b-Cu(CH$_3$COO)$_2$. Yield 89%, green powder, mp 239.1–240.0°C (decomp.). IR spectrum, ν, cm^{-1}: 627 w, 643 w, 681 s, 699 m, 761 w, 800 w, 892 w, 988 w, 1029 w, 1049 w, 1074 v.w, 1100 v.w, 1124 v.w, 1186 w, 1197 w, 1244 w, 1327 w, 1384 w, 1374 w, 1417 s, 1427 s, 1471 w, 1488 w, 1578 m, 1617 s, 2930 w, 3007 w, 3071 w. Found, %: C 52.73; H 4.51; N 7.62. $C_{16}H_{16}N_2O_4Cu$. Calculated, %: C 52.82; H 4.43; N 7.70.

Biological experiments. The cell lines were cultivated in RPMI-1640 medium containing 10% of fetal bovine serum, 10 mM HEPES (Sigma, USA), 2 mM of L-glutamine (Sigma, USA), 40 ng/mL of gentamicin (ICN, USA), amino acids, sodium pyruvate, and vitamin solution (PanEko, Russia) at 37°C under atmosphere containing 5% of CO_2. The cells were kept in logarithmic growth phase via continuous passage every 3–4 days. Versene solution was used for detachment of the cultures from plastic.

For the MTT assay, the cells were distributed in 96-well flat-bottom plates (Costar, USA). The investigated compounds in concentration 100 μM were added to each well after one day and incubated with cells for 72 h in 5% CO_2 at 37°C. Each compound was tested in triplicate. The compounds were dissolved in DMSO so that the DMSO concentration in a well did not exceed 1%. The wells with cells and 1% of DMSO in solid growth medium were used as reference. 20 μM of the MTT solution (final concentration 1 μg/mL) was added to each well after 72 h and incubated for 4 h at 37°C in 5% CO_2. After the formation of formazan, the supernatant was removed, and the precipitate was dissolved in 150 μL of DMSO. Then the plates were placed in a thermostat for 10 min at 37°C and then shaken for uniform dissolution of the formazan crystals. Then the intensity of medium coloration was measured using an AIFR-01 Uniplan photometrical analyzer of immunoenzymatic reactions (Pikon) at λ = 540 nm. The absorption was directly proportional to the number of alive cells.

The degree of inhibition of the cell growth (I, %) was determined as follows:

$$I = \left(1 - \frac{D_0}{D_r} \right) \times 100\%,$$

where D_r is absorbance in reference wells, D_0 is absorbance in the specimen wells. A compound was considered cytostatically active if it caused the inhibition of cell growth by more than 50%.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

1. Glagolev, Yu.V., Logunov, M.V., Mamakin, I.V., Polosin, V.M., Rovnyi, S.I., Starchenko, V.A., Shishelov, Yu.P., and Yakovlev, N.G, RU Patent no. 2318258, 2008.

2. Deshmukh, M.B., Alasundkar, K.N., Salunkhe, D.K., and Sankpal, S.A., *J. Chem. Pharm. Res*., 2010, vol. 2, no. 4, p. 437. doi 10.1007/s10847-005
3. Alfonso, I. and Quesada, R., *Chem. Sci*., 2013, vol. 4, no. 8, p. 3009. doi 10.1039/C3SC50882J
4. Huang Sh.-T., Kuo, H.-S., Hsiao Ch.-L., and Lin, Yu.-L., *Bioorg. Med. Chem*., 2002, vol. 10, no. 6, p. 1947. doi 10.1016/S0968-0896(02)00004-4
5. Supek, F., Ramljak, T.S., Marjanovic, M., Buljubasic, M., Kragol, G., Llic, N., Smuc, T., Zahradka, D., Mlinaric-Majerski, K., and Kralj, M., *Eur. J. Med. Chem*., 2011, vol. 46, no. 8, p. 3444. doi 10.1016/j.ejmech.2011.05.009
6. Karawajew, L., Glibin, E.N., Maleev, V.Y., Czerwony, G., Dörken, B., Davies, D.B., and Veselkov, A.N., *Anti-Cancer Drug Des*., 2000, vol. 15, no. 5, p. 331.
7. Jansen, B.A.J.,Wielaard, P., den Dulk, H., Brouwer, J., and Reedijk, J., *Eur. J. Inorg. Chem*., 2002, no. 9, p. 2375. doi 10.1002/1099-0682(200209) 2002:9<2375::AID-EJIC2375>3.0.CO;2-B
8. Yoo, J., Sohn, Y.S., and Do, Y., *J. Inorg. Biochem*., 1999, vol. 73, no. 3, p. 187. doi 10.1016/S0162-0134 (99)00016-1
9. Majouga, A.G., Zvereva, M.I., Rubtsova, M.P., Skvortsov, D.A., Mironov, A.V., Azhibek, D.M., Krasnovskaya, O.O., Gerasimov, V.M., Udina, A.V., Vorozhtsov, N.I., Beloglazkina, E.K., Agron, L., Mikhina, L.V., Tretyakova, A.V., Zyk, N.V., Zefirov, N.S., Kabanov, A.V., and Dontsova, O.A., *J. Med. Chem*., 2014, vol. 57, no. 14, p. 6252. doi 10.1021/jm500154f
10. Iakovidis, I., Delimaris, I., and Piperakis, S.M., *Mol. Bio. Int*., 2011, vol. 2011, p. 1. doi 10.4061/ 2011/594529
11. Marzano, C., Pellei, M., Tisato, F., and Santini, C., *Anti-Cancer Agents Med.Chem*., 2009, vol. 9, no. 2, p. 185. doi 10.2174/187152009787313837
12. Plotek, M., Dudek, K., and Kyziol, A., *Chemik*., 2013, vol. 67, no. 12, p. 1181.
13. Usacheva, T.R., *Cand. Sci. (Chem.) Dissertation*, Ivanovo, 2016.
14. Dorokhov, A.V., *Cand. Sci. (Chem.) Dissertation*, Moscow, 2007.
15. Timonin, A.N., *Cand. Sci. (Chem.) Dissertation*, Moscow, 2011.
16. Guler, H., Hayvali, Z., Dal, H., and Hokelek, T., *Polyhedron*, 2012, vol. 31, no. 1, p. 688. doi 10.1016/ j.poly.2011.10.034
17. Glushko, V.N. and Sadovskaya, N.Yu., RU Patent no. 2655166, 2018.
18. Glushko, V.N. and Sadovskaya, N.Yu., RU Patent no. 2661871, 2018.
19. Mossman, T., *J. Immunol. Meth*., 1983, vol. 65, nos. 1–2, p. 55. doi 10.1016/0022-1759(83)90303-4