Chemistry of Heterocyclic Compounds, Vol. 48, No. 2, May, 2012 (Russian Original Vol. 48, No. 2, February, 2012)

SYNTHESIS AND CYTOTOXICITY OF PYRIDINE AND QUINOLINE OXORHENIUM(V) COMPLEXES WITH TRIDENTATE (NS_2, S_3)/MONODENTATE (S) COORDINATION*

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New oxorhenium complexes with tridentate 3-thia- and 3-methylazapentane-1,5-dithiolate and monodentate pyridine and quinoline derivatives have been synthesized. As a result of investigation of biological activity a high cytotoxicity was found for the synthesized complexes in relation to tumor cells. The specificity of the 2-pyridylthiolato[3-(N-methyl)azapentane-1,5-dithiolato]oxorhenium(V) cytotoxic action towards cells of mouse hepatoma MG-22A on a background of low acute toxicity was established.

Keywords: oxorhenium(V) complexes, pyridine, quinoline, rhenium, cytotoxicity.

Derivatives of quinoline and pyridine are widely used for the synthesis of various medicinal agents including antitumor medications (rogletimide, peldesin, pazelliptine, oxysuran, ledacrine, lurtotecane, emitefur, brequinar, aconiazide, etc.) [1]. As structural fragments they also form part of the structure of the antitumor antibiotics nigrin [1], bruneomycin [2], and antitumor alkaloids camptothecin [3] and meridin [4]. On the other hand, a series of transition metal complexes [5, 6], including quinoline-containing [7-10], and also complexes of rhenium [11-13], display antitumor activity and are potentially biological transporting agents for medications [14, 15].

In the present work, with the aim of studying the effect of the ligand nature on antitumor properties, neutral oxorhenium(V) complexes have been synthesized in which the oxorhenium(V) framework ReO^{3+} is coordinated with the tridentate 3-thia- and 3-methylazapentane-1,5-dithiolate, and also with the monodentate 2-mercaptopyridine and 2-mercaptoquinoline. The formation of the complexes was carried out by "3+1" methodology using two different rhenium precursors **1** and **2** depending on the neutral donor atom of the tridentate ligand.

Chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) (**4**) was obtained from tetra-*n*-butylammonium perrhenate as an intermediate for the synthesis of 2-pyridylthiolato(3-thiapentane-1,5-dithiolato)oxo-rhenium(V) (**6**). As a result of its reaction with pyridine-2-thiol (**5**) in refluxing acetonitrile, the chlorine atom in compound **4** was successfully replaced by a pyridylthiol residue with the formation of the corresponding rhenium complex **6** in high yield. An alternative approach was used for the synthesis of complexes

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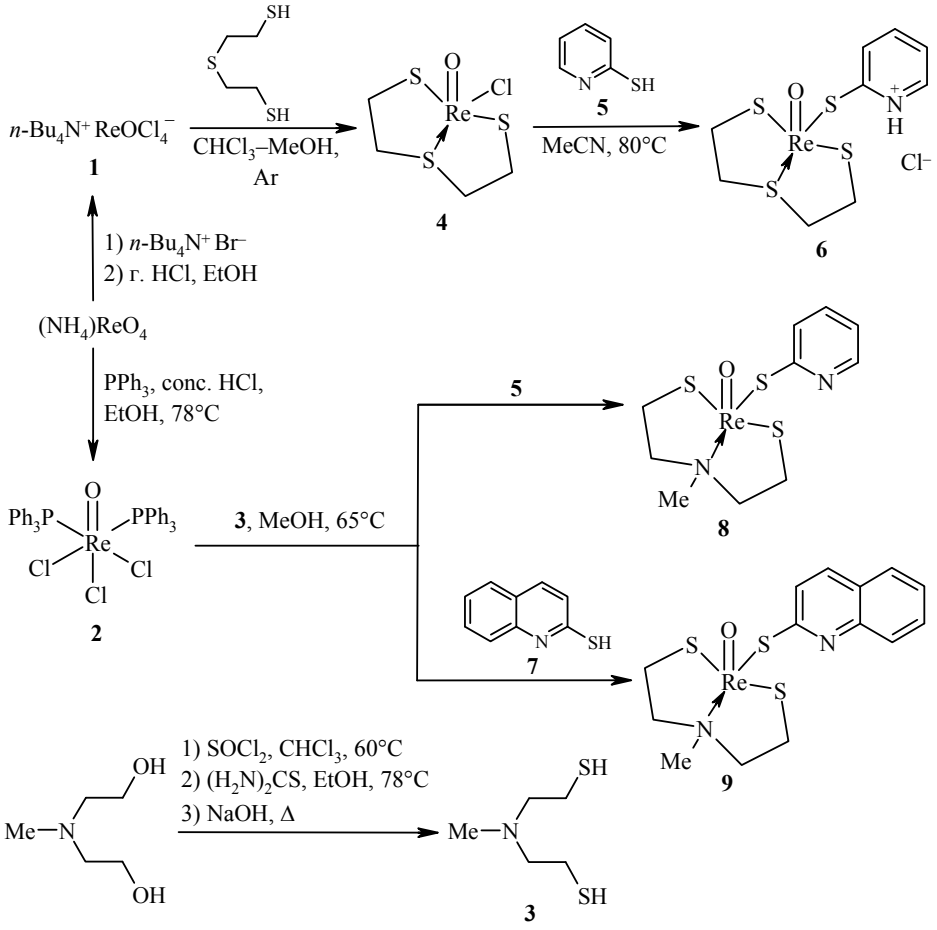
Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 2, pp. 314-319, February, 2012. Original article submitted March 9, 2011.

8 and **9**, which were obtained as a result of the interaction of the previously synthesized phosphine-containing oxorhenium(V) precursor **2** with the tridentate ligand **3** and monodentate thiols **5** or **7** in a ratio 1:1:1.1 in refluxing methanol. The tridentate 3-methylazapentane-1,5-dithiol (**3**) was synthesized from *N*-methyldiethanolamine as a result of a series of sequential conversions. The corresponding dichloro derivative was obtained by the reaction of *N*-methyldiethanolamine with thionyl chloride in chloroform, the interaction of which with thiourea in refluxing ethanol and subsequent alkaline hydrolysis of the resulting isothiouronium salt was completed by the formation of the desired dithiol **3.**



The vibrational frequency $\nu_{(Re=O)}$ in the IR spectra of the synthesized complexes **6, 8**, and **9** depends more on the central donor atom of the tridentate ligand (complexes **6** and **8**) than on the donor properties of the monodentate ligand (complexes **8** and **9**). The signals varied from 960 (**6**) to 956 (**8**) and 953 cm^{-1} (**9**), which is typical for oxorhenium(V) complexes with mixed ligands [16].

The biological properties of the synthesized compounds were studied in four tumor cell lines: HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), B16 (mouse melanoma), and SH-SY-5Y (human neuroblastoma), and also in relation to normal fibroblasts NIH-3T3, which also served for assessment of the compounds' toxicity (alternative method of determining LD_{50} [17]) (Table 1).

The investigations results showed that the cytotoxic activity of 2-pyridyl- and 2-quinolylthiolate complexes of oxorhenium(V) depends on the nature of both the monodentate and tridentate ligands, and in several cases selectivity is displayed in relation to certain cell line. Differences were also revealed in the type of action: the CV test reveals effect on cell membranes, and the MTT test reveals effect on the activity of mitochondrial enzymes in the cell.

TABLE 1. Cytotoxicity (LC_{50}) and Ability to Generate NO within Cells by Oxorhenium(V) Complexes **6**, **8**, and **9***

Compound	HT-1080			MG-22A			B16		SH-SY-5Y		NIH-3T3	
	LC_{50}, μg/ml		NO, %	LC_{50}, μg/ml		NO, %	LC_{50}, μg/ml		LC_{50}, μg/ml		LC_{50}	LD_{50},
	CV	MTT		CV	MTT		CV	MTT	CV	MTT	NR	mg/kg
6	4	3	100	2	3	133	3	3	3	2	52	801
8	12	23	86	2	3	250	91	63	15	10	445	1877
9	3	2	167	3	3	250	3	3	2	2	4	256

*CV – crystal violet; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; NR – neutral red; NO – degree of NO generation, determined and calculated by the procedure of [22].

Rhenium(V) complexes **6** and **9** possess high cytotoxicity (2-3 μg/ml) in relation to each tumor cell line studied in each test. In turn, complex **8** displayed high tissue specificity. Cells of hepatoma MG-22A proved to be the most sensitive towards compound **8**. Its inhibiting action was moderate in relation to HT-1080 and SH-SY-5Y, low relative to B-16, and very low towards normal NIH-3T3 fibroblasts (Table 1).

All of the studied complexes were nontoxic compounds. The least toxic proved to be 2-pyridylthiolato-[3-(*N*-methyl)azapentane-1,5-dithiolato]oxorhenium(V) (**8**). Replacement of the monodentate ligand in complex **8** by quinolylthiolate (complex **9**) led to a more than sevenfold increase in toxicity. In turn, replacement of the tridentate ligand, namely 3-(*N*-methyl)azapentane-1,5-dithiolate by 3-thio-1,5-dithiolate (complex **6**), increased the toxicity of the compound more than twice (Table 1).

It has therefore been established that the studied compounds possess a selective cytotoxic action in relation to the studied tumor cells. A specificity of the cytotoxic effect towards line MG-22A was displayed by compound **8**.

EXPERIMENTAL

The IR spectra were recorded on a Perkin Elmer FTIR Specord 2000 spectrometer using KBr pellets. The ^1H and ^{13}C NMR spectra were recorded on a Varian Inova 400 (at 400 and 100 MHz, respectively), internal standard was the signal of the residual solvent protons ($CDCl_3$ δ_H 7.26 ppm, δ_C 77.0 ppm, DMSO-d_6 δ_H 2.5 ppm, δ_C 39.5 ppm). Elemental analyses were carried out on a LECO CHNS 932 analyzer. Reactions progress and the purity of the obtained compounds were monitored by TLC on Machery-Nagel plates in the system $CHCl_3$–MeOH. Merck type silica gel (0.040-0.063 mm) was used for column chromatography.

Tetra-*n*-butylammonium tetrachlorooxorhenate(V) (1) was obtained by the procedure of [18] with minor amendments. *n*-$Bu_4N \cdot ReOCl_4$ (4.51 g, 9.17 mmol), obtained from an aqueous NH_4ReO_4 solution by precipitation with *n*-$Bu_4N^+Br^-$, was dissolved in abs. EtOH (70 ml). The obtained solution was saturated with gaseous HCl. The reaction mixture was then stirred for 3 h, the solvent was partially evaporated, and the reaction mixture was cooled to -18°C. The precipitated solid was filtered off and washed with ether, then dried in vacuum for 4 h. Yield 5.05 g (94%); mp 143-145°C (decomp.). The IR spectral data agreed with those described in the literature [18].

***trans*-Monooxotrichlorobis(triphenylphosphine)rhenium(V) (2)** was obtained by the procedure of [19]. NH_4ReO_4 (1.61 g, 6 mmol) and PPh_3 (9.00 g, 34 mmol) were dissolved in abs. EtOH (100 ml) and conc. HCl (10 ml) was added. The obtained reaction mixture was refluxed for 1 h, then cooled, and filtered. The solid was washed with hot EtOH. The yield of crude product was 4.92 g (98%). Product **2** was used for the synthesis of compounds **8** and **9** without further purification.

3-(*N*-Methyl)azapentane-1,5-dithiol (3) was obtained by the procedure of [20] with modifications. A solution of $SOCl_2$ (26.7 ml, 369.2 mmol) in $CHCl_3$ (10 ml) was added dropwise with stirring to a solution of *N*-methyldiethanolamine (20.0 g, 167.8 mmol) in $CHCl_3$ (100 ml) cooled to 0°C. The reaction mixture was refluxed for 24 h. The lighter fractions were then removed by evaporation of the mixture on a rotary evaporator. Ethanol (50 ml) and thiourea (28.3 g, 369.2 mmol) were then added to the residue. The obtained mixture was refluxed for 6 h, cooled, and the alcohol was removed by evaporation on a rotary evaporator. A 3 N NaOH solution (280 ml) was added to the residue, and the mixture was refluxed with stirring in an atmosphere of argon for 2 h. After cooling to room temperature, the reaction mixture was neutralized with 2 N HCl solution, the product was extracted with ether (3×200 ml), the ether extract was dried over $MgSO_4$, then evaporated on the rotary evaporator, and the residue was distilled in vacuum. Yield 10.64 g (48%); bp 65-67°C ($4.1 \cdot 10^{-1}$ mm Hg), which corresponded to the literature data of [20].

Chloro(3-thiapentane-1,5-dithiolato)oxorhenium(V) (4) was obtained by the procedure of [21] with minor amendments. *n*-$Bu_4N \cdot ReOCl_4$ (**1**) (1.17 g, 2 mmol) was dissolved in a mixture of $CHCl_3$–MeOH, 10:1 (100 ml), and cooled to 0°C. A solution of bis(2-mercaptoethyl) sulfide (0.31 g, 2 mmol) in $CHCl_3$ (50 ml) was added dropwise with stirring to the obtained solution in an atmosphere of argon. Afterwards the reaction mixture was brought to room temperature; the precipitated solid was filtered off, washed with $CHCl_3$ and with ether, and dried. The yield of product was 0.61 g (78%). The physicochemical properties corresponded to literature values [21].

2-Pyridylthiolato(3-thiapentane-1,5-dithiolato)oxorhenium(V) Hydrochloride (6). Compound **4** (85.6 mg, 0.22 mmol) was dissolved with stirring in hot acetonitrile (5 ml), and a solution of pyridyl-2-thiol (**5**) (48.9 mg, 0.22 mmol) in acetonitrile (5 ml) was carefully added dropwise to the refluxing solution. The reaction mixture was refluxed for an additional 30 min, cooled, and filtered. The solid was washed with ether (5×20 ml) and dried. Yield 105.6 mg (96%); mp 190-192°C. IR spectrum, ν, cm^{-1}: 960 (Re=O). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.42 (2H, m), 3.12 (2H, td, *J* = 14.0, *J* = 4.3), 4.17 (2H, dd, *J* = 10.8, *J* = 3.0), and 4.27 (2H, dd, *J* = 13.0, *J* = 4.5, $2SCH_2CH_2S$); 7.21 (1H, m, H-5); 7.65 (1H, d, *J* = 7.6, H-3); 7.74 (1H, td, *J* = 7.6, *J* = 1.8, H-4); 8.50 (1H, dd, *J* = 4.7, *J* = 0.9, H-6); 13.46 (1H, br. s, NH). ^{13}C NMR spectrum (DMSO-d_6), δ, ppm: 43.2 (SCH_2); 46.1 (SCH_2); 121.3; 129.7; 135.9; 149.2 (C Ar); 167.6 (C-2). Found, %: C 21.56; H 2.58; N 2.75; Cl 7.02; S 25.36. $C_9H_{13}ClNOReS_4$. Calculated, %: C 21.57; H 2.61; N 2.80; Cl 7.07; S 25.59.

2-Pyridylthiolato[3-(*N*-methyl)azapentane-1,5-dithiolato]oxorhenium(V) (8). Compound **2** (1.25 g, 1.50 mmol) was dissolved in MeOH (100 ml), the solution was stirred at room temperature in an argon atmosphere, and solutions of pyridyl-2-thiol (**5**) (0.18 g, 1.65 mmol) in MeOH (5 ml) and 3-(*N*-methyl)-azapentane-1,5-dithiol (**3**) in MeOH (2 ml) were added dropwise. The reaction mixture was refluxed with stirring for 2 h, and after cooling, the solvent was evaporated. The product was isolated by column chromatography, eluent was $CHCl_3$–MeOH, 10:1. Yield 0.39 g (56%); mp 268-269°C. IR spectrum, ν, cm^{-1}: 956 (Re=O). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.74 (2H, m), 2.93 (2H, m), 3.19 (2H, m), and 3.57 (2H, m, $2SCH_2CH_2N$); 3.36 (3H, s, NCH_3); 7.15 (1H, m, H-5); 7.53 (1H, d, *J* = 7.7, H-3); 7.73 (1H, td, *J* = 6.3, *J* = 1.1, H-4); 8.44 (1H, dd, *J* = 4.9, *J* = 0.9, H-6). ^{13}C NMR (DMSO-d_6), δ, ppm: 40.5 (SCH_2); 53.9 (NCH_3); 67.1 (NCH_2); 120.7; 128.3; 135.8; 149.0 (C Ar); 175.0 (C-2). Found, %: C 26.00; H 3.26; N 5.99; S 20.87. $C_{10}H_{15}N_2OReS_3$. Calculated, %: C 26.02; H 3.28; N 6.07; S 20.84.

2-Quinolylthiolato[3-(*N*-methyl)azapentane-1,5-dithiolato]oxorhenium(V) (9). Compound **2** (1.13 g, 1.4 mmol) was dissolved in MeOH (100 ml), and the solution was stirred at room temperature in an atmosphere of argon. Solutions of quinolyl-2-thiol (**7**) (0.24 g, 1.5 mmol) in MeOH (5 ml) and 3-(*N*-methyl)-azapentane-1,5-dithiol (**3**) in MeOH (5 ml) were added dropwise. The reaction mixture was refluxed with stirring for 2 h, cooled, and then the solvent was evaporated. The product was isolated by column chromatography, eluent was $CHCl_3$–MeOH, 5:1. Yield 0.33 g (48%); mp 220-221°C. IR spectrum, ν, cm^{-1}: 953 (Re=O). ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 2.75 (2H, m), 2.94 (2H, m), 3.22 (2H, m), and 3.57 (2H, m, $2SCH_2CH_2N$); 3.35 (3H, s, NCH_3); 7.54 (1H, m, H-6); 7.65 (1H, d, *J* = 8.5, H-3); 7.74 (1H, m, H-5); 7.91 (2H, m, H-4,7); 8.26 (1H, d, *J* = 8.7, H-8). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 41.6 (SCH_2); 54.2 (NCH_3);

68.2 (NCH_2); 126.0; 126.9; 127.5; 129.1; 129.5; 135.1; 148.1 (C Ar). Found, %: C 32.84; H 3.36; N 5.43; S 18.74. $C_{14}H_{17}N_2OReS_3$. Calculated, %: C 32.86; H 3.35; N 5.47; S 18.80.

Cytotoxicity of compounds 6, 8, and 9 (Table 1) *in vitro* in relation to monolayers of tumor cells HT-1080, MG-22A, B16, SH-SY-5Y, and normal NIH-3T cells was determined in 96-hole plastic plates using dyestuffs CV, MTT, and NR according to the procedures of [22, 23]. The concentration of compounds causing death of 50% cells (LC_{50}, μg/ml) is given in Table 1. The expected acute toxicity (LD_{50}, mg/kg) was calculated by the procedure of [17].

The work was carried out with the financial support of the Latvian Council of Science (projects 09.1321 and 10.0030).

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