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SYNTHESIS AND CYTOTOXICITY OF METHYL- AND METHOXY-SUBSTITUTED METAL 8-QUINOLINETHIOLATES

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It has been found that the nature of the substituent, its position in the quinoline ring, and the nature of the metal significantly affect the antitumor activity and toxicity of metal 8-quinolinethiolates. The most cytotoxic towards human fibrosarcoma HT-1080 and mouse hepatoma MG-22A tumor cells are the 6-methoxy-8-quinolinethiolates of rhodium, osmium, iridium, indium, antimony, and bismuth, however these are highly toxic towards normal mouse embryonic NIH 3T3 fibroblasts. The iridium 5-methyl-8-quinolinethiolate is somewhat less active to MG-22A cells but shows quite good selectivity of action because of its markedly lower toxicity.

Keywords: metal 3- and 5-methyl-8-quinolinethiolates, metal 2- and 6-methoxy-8-quinolinethiolates, synthesis, toxicity, cytotoxicity.

After more than 40 years, platinum complexes occupy a high profile place in the arsenal of antitumor agents [1-3]. During this time there have appeared problems connected with their use, i.e. high overall toxicity leading to unwanted side effects and resistance to platinum preparations in a series of tumors which can develop during the time of chemotherapy. Attempts have been made to overcome these observations by changing the ligands in the platinum complexes and, more recently, by exchanging the metal [4, 7-11].

In this respect the most promising proved to be ruthenium complexes which have low overall toxicity and are selectively accumulated in the tumor cells [4, 7, 12-18]. Two of them have carried on to clinical investigation [14, 19, 20]. At the same time their rhodium and osmium analogs have shown even greater cytotoxicity towards A549 lung and T47D mammary gland carcinoma cells [21].

We have shown that not only ruthenium, rhodium, and osmium but also iridium complexes [22, 23] with an 8-quinolinethiol [22, 23] or 8-quinolineselenol [24, 25] ligand show high cytotoxicity to HT-1080 human fibrosarcoma and MG-22A mouse hepatoma tumor cells. However, all of these compounds showing high activity towards tumor cells are also toxic to normal NIH 3T3 mouse embryonic fibroblasts [22]. The toxicity of the highly active rhodium and iridium 8-quinolinethiolates can be markedly decreased by the introduction into their molecules of a methyl group at the 4 position of the quinoline ring [23]. It was also found that substituents in different positions of the quinoline ring can increase the selectivity of action of di(8-quinolyl) disulfides [26].

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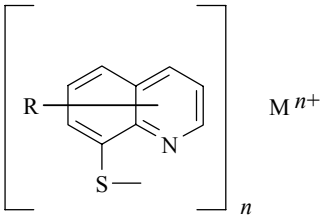
Hence with the aim of decreasing the toxicity and increasing the selectivity of action of cytotoxic 8-quinolinethiolate complexes we have prepared a series of 3-methyl- (**1**), 5-methyl- (**2**), 2-methoxy- (**3**), and 6-methoxy-8-quinolinethiolates (**4**) of rhodium (**a**), osmium (**b**), and iridium (**c**) and also the 6-methoxy-8-quinolinethiolates (**4**) of copper (**d**), cadmium (**e**), indium (**f**), antimony (**g**), bismuth (**h**), ruthenium (**i**), and



1a–c–3a–c, **4 a–j**

1 R = 3-Me, **2** R = 5-Me, **3** R = 2-MeO, **4** R = 6-MeO; **1–4 a** M = Rh, **b** M = Os, **c** M = Ir; **4 d** M = Cu, **e** M = Cd, **f** M = In, **g** M = Sb, **h** M = Bi, **i** M = Ru, **j** M = Pd; **1–4 a–c**, **4 f-i** n = 3; **4 d,e,j** n = 2

TABLE 1. Characteristics of the Metal 8-Quinolinethiolates **1-4**

Compound	Empirical formula	Found, % / Calculated, %			Yield, %
		C	H	N	
1a	$C_{30}H_{24}N_3RhS_3$	57.01 / 57.59	3.79 / 3.87	6.60 / 6.72	75
1b	$C_{30}H_{24}N_3OsS_3$	51.04 / 50.54	3.48 / 3.39	6.02 / 5.89	73
1c	$C_{30}H_{24}IrN_3S_3$	50.02 / 50.40	3.48 / 3.38	5.99 / 5.88	74
2a	$C_{30}H_{24}N_3RhS_3$	57.98 / 57.59	3.74 / 3.87	6.80 / 6.72	77
2b	$C_{30}H_{24}N_3OsS_3$	50.02 / 50.54	3.79 / 3.39	5.81 / 5.89	75
2c	$C_{30}H_{24}IrN_3S_3$	50.85 / 50.40	3.49 / 3.38	5.96 / 5.88	75
3a	$C_{30}H_{24}N_3O_3RhS_3$	53.95 / 53.49	3.67 / 3.59	6.13 / 6.24	76
3b	$C_{30}H_{24}N_3O_3OsS_3$	47.76 / 47.35	3.26 / 3.18	5.65 / 5.52	75
3c	$C_{30}H_{24}IrN_3O_3S_3$	47.65 / 47.23	3.10 / 3.17	5.65 / 5.51	77
4a	$C_{30}H_{24}N_3O_3RhS_3$	53.98 / 53.49	3.70 / 3.59	6.35 / 6.24	75
4b	$C_{30}H_{24}N_3O_3OsS_3$	47.12 / 47.35	3.27 / 3.18	5.65 / 5.52	75
4c	$C_{30}H_{24}IrN_3O_3S_3$	47.65 / 47.23	3.25 / 3.17	5.63 / 5.51	75
4d	$C_{20}H_{16}CuN_2O_2S_2$	54.46 / 54.10	3.71 / 3.63	6.20 / 6.31	87
4e	$C_{20}H_{16}CdN_2O_2S_2$	48.98 / 48.73	3.17 / 3.27	5.79 / 5.68	84
4f	$C_{30}H_{24}InN_3O_3S_3$	52.95 / 52.56	3.43 / 3.53	6.25 / 6.13	85
4g	$C_{30}H_{24}N_3O_3S_3Sb$	52.35 / 52.03	3.58 / 3.49	6.19 / 6.07	85
4h	$C_{30}H_{24}BiN_3O_3S_3$	46.53 / 46.21	3.19 / 3.10	5.48 / 5.39	85
4i	$C_{30}H_{24}N_3O_3RuS_3$	53.96 / 53.63	3.51 / 3.60	6.34 / 6.25	74
4j	$C_{20}H_{16}N_2O_2PdS_2$	49.61 / 49.30	3.44 / 3.31	5.62 / 5.75	88

palladium (**j**) (Table 1) and studied their cytotoxicity on two tumor cell lines (HT-1080 and MG-22A) and on normal NIH 3T3 fibroblasts which also served to estimate the toxicity of the compounds (alternative method of determination (LD_{50} [27]).

The results given in Table 2 show the introduction of a methyl group into the quinoline ring of the rhodium, osmium, and iridium complexes decrease their toxicity when compared to their unsubstituted quinoline ligands [22]. This is most markedly seen in the toxicity of the iridium complex **2c** with a methyl group at position 5 (LC_{50} 220 μg/ml, LD_{50} 1785 mg/kg). The toxicity of the iridium and rhodium complexes with a methyl group at position 4 (LC_{50} 85 and 78 μg/ml respectively) are also an order less than toxicity of the complexes with the unsubstituted ligand [23]. The 5-methyl derivatives of iridium and osmium proved to be the least toxic while the 4-isomer was the least toxic of the rhodium derivatives. All of the 3-methyl derivatives **1** are significantly more toxic, even the iridium complex **1c** being more toxic for normal than for tumor cells.

The cytotoxicity of the methyl derivatives depends little on the position of the substituent (some decrease observed in the series 4- > 3- > 5-) and the nature of the metal (4-methyl derivatives of osmium somewhat more active). On the other hand, the selective action of the 5-methyl iridium derivative **2c** significantly exceeds the unsubstituted complexes which, for this index, are also inferior for the 4-methyl rhodium and iridium derivatives.

Considering that in the series the derivatives of di(8-quinolyl) disulfides, having a substituent at position 2 of the quinoline ring are assigned as a group of low toxicity compounds and the 2-methoxy derivative shows quite good selectivity [26] we have examined a series of 2-methoxy-8-quinolinethiolate derivatives of rhodium (**3a**), osmium (**3b**), and iridium (**3c**). All of the complexes proved low toxicity (LD_{50} ~ 3300 mg/kg) but at the same time were also inactive or showed little cytotoxicity towards HT-1080 tumor cells (LC_{50} of the osmium complex **3b** 30 μg/ml).

TABLE 2. Cytotoxicity of the 4-Methyl-8-quinolinethiolates **1-4**

Compound	LC_{50}, μg/ml							LD_{50}, mg/kg
	HT-1080			MG-22A			3T3	
	CV	MTT	NO	CV	MTT	NO	NR	
1a	4	4	91	4	5	122	1.4	187
1b	3	3	250	3	3	86	2	214
1c	3	3	450	3	2	400	0.7	143
2a	5	7	100	5	7	43	9	438
2b	3	3	467	4	4	111	20	641
2c	9	9	450	5	7	133	220	1785
3a	100	53	11	*[2]	*[2]	6	1000	3365
3b	30	34	167	100	*[2]	22	789	3268
3c	100	85	8	*[2]	*[2]	5	830	3353
4a	<1	<1	550	2	2	133	0.8	135
4b	<1	<1	350	<1	<1	83	<0.3	<76
4c	<1	<1	160	<1	<1	67	0.9	152
4d	3	2	50	1	1	50	0.3	89
4e	3	2	50	3	3	38	2	197
4f	<1	<1	150	<1	<1	100	0.4	109
4g	<1	<1	750	<1	<1	450	<0.3	<69
4h	<1	<1	550	<1	<1	333	<0.3	<78
4i	1	1	433	<1	<1	550	0.5	121
4j	3	3	500	3	3	600	1.5	146

* CV = crystal violet (effect on cell membrane); MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (effect on mitochondrial enzymes activity in cell); NR = neutral red; NO = degree of generation of NO, determined and calculated by the method [33].

*[2] Cytotoxic effect absent.

Amongst the methoxy derivatives of the di(8-quinolyl) disulfide the 6-isomer also showed good antitumor activity and selectivity [26]. The rhodium (**4a**), osmium (**4b**), iridium (**4c**), and ruthenium (**4i**) complexes with 6-methoxy-8-quinolinethiol also revealed high cytotoxicity towards the HT-1080 (LC_{50} > 1 μg/ml) and MG-22A (LC_{50} 1-2 μg/ml) tumor cells but they were all highly toxic to normal cells. Exchange of the metal in the complex did not improve this situation, the high cytotoxicity (LC_{50} < 1 μg/ml) was also preset in the indium (**4f**), antimony (**4g**), and bismuth (**4h**) complexes, the copper (**4d**), cadmium (**4e**), and palladium (**4j**) complexes being somewhat less (LC_{50} < 1-3 μg/ml) but they all caused the death of normal cells at low concentration (LC_{50} ~ 0.3 μg/ml).

It was interesting to note that the antimony **4g**, bismuth **4h**, palladium **4j**, rhodium **4a**, iridium **1c** and **2c**, ruthenium **4i**, and osmium (**2b** > **1b** > **4b**) complexes showed high cytotoxic activity to tumor cells, strongly inducing the formation of nitric oxide in them (Table 2).

Hence we have shown a marked effect for the nature of the substituent, its position in the quinoline ring, and also the nature of the metal on the antitumor activity and toxicity of metal 8-quinolinethiolates. All of the 6-methoxy derivatives **4** proved not only highly active towards tumor cells but also highly toxic to normal cells, the 2-methoxy derivatives on the other hand being of low toxicity and low activity. The 3-methyl derivatives **1** possess good activity but they are very toxic. The 5-methyl derivatives **2** are somewhat less active but their lower toxicity can lead to better selectivity. Thus in the iridium complex **2c** the LC_{50} for normal cells is 24-44 times greater than the mean lethal concentration for tumor cells.

EXPERIMENTAL

Elemental analysis was carried out using a CHN Analyser (Czechoslovakia).

Synthesis of Metal 6-Methoxy-8-quinolinethiolates 4 (General Method). 6-Methoxy-8-quinolinethiolate hydrochloride [28] (0.1 g, 0.39 mmol) was dissolved in 80% ethanol (15 ml), buffered acetate solution (pH 5, 5 ml) was added and then with stirring a solution of the metal salt in water (5 ml) as follows: $(NH_4)_3[RhCl_6]\cdot H_2O$ (0.04 g, 0.1 mmol); K_2OsBr_6 (0.08 g, 0.11 mmol); $(NH_4)_3[IrCl_6]\cdot H_2O$ (0.05 g, 0.1 mmol); $CuCl_2\cdot 2H_2O$ (0.03 g, 0.18 mmol); $CdSO_4$ (0.03 g, 0.14 mmol); $In_2(SO_4)_3$ (0.07 g, 0.14 mmol); $K(SbO)C_4H_4O_6\cdot 0.5H_2O$ (0.04 g, 0.12 mmol); $Bi(NO_3)_3\cdot 5H_2O$ (0.05 g, 0.11 mmol); $K_2[Ru(H_2O)Cl_5]$ (0.04 g, 0.11 mmol); or $PdCl_2$, (0.03 g, 0.17 mmol). In the case of the Rh, Os. Ir, and Ru salts the reaction mixture was heated for 10 min on a water bath. The precipitated 6-methoxy-8-quinolinethiolates **4** were filtered off, washed with water, dried in air, and recrystallized from chloroform (Table 1).

Metal 5-Methyl-8-quinolinethiolates (General Method). The di(5-methyl-8-quinolyl) disulfide [29] (0.12 g, 0.28 mmol) was dissolved in conc. HCl (2 ml), a solution of 50% H_3PO_2 (2 ml) was added, and the product was heated for 20 min on a water bath. The solution obtained was diluted with ethanol (10 ml), a saturated solution of sodium acetate (4 ml) was added, and then with stirring a solution of the metal salt in water (5 ml): $(NH_4)_3[RhCl_6]\cdot H_2O$ (0.06 g, 0.15 mmol); K_2OsBr_6 (0.12 g, 0.16 mmol), or $(NH_4)_3[IrCl_6]\cdot H_2O$, (0.08 g, 0.16 mmol). The reaction mixture was then heated on a water bath for 10 min. The precipitated 4-methyl-8-quinolinethiolate **2** was filtered off, washed with water, dried in air, and recrystallized from chloroform.

Metal 3-Methyl-8-quinolinethiolates 1 were prepared from the di(3-methyl-8-quinolyl) disulfide [30] as described in the method given above.

Metal 2-Methoxy-8-quinolinethiolates 3 were obtained from di(2-methoxy-8-quinolyl) disulfide [31] using the same method.

The cytotoxicity of compounds 1-4 (Table 2) *in vitro* relative to HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) tumor cells and normal NIH 3T3 mouse embryonic fibroblasts was measured using 96 well panels using CV, MTT, and NR dyes by the method described in [32]. The expected acute toxicity (LD_{50}, mg/kg) was calculated by method [27] using data obtained on the 3T3 cell culture.

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