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SYNTHESIS AND CYTOTOXICITY OF METHYL-SUBSTITUTED 8-QUINOLINESELENOLATES OF RUTHENIUM, RHODIUM, OSMIUM, AND IRIDIUM

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A series of 2-methyl, 4-methyl, and 2,4-dimethyl-8-quinolineselenolates of ruthenium, rhodium, osmium, and iridium has been synthesized and their cytotoxicity towards HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) tumor cells studied. It was found that all of the osmium complexes had a high cytotoxicity towards both cell lines. Their toxicity towards the normal mouse embryonic fibroblasts NIH-3T3 depends on the position and number of methyl groups in the quinoline ring and decreases in the order 2-Me > 4-Me > 2,4-Me_2. The greatest selectivity in cytoxic activity is noted for iridium 4-methyl-8-quinolineselenolate and ruthenium 2-methyl-8-quinolineselenolate.

Keywords: methyl-8-quinolineselenolates of iridium, osmium, rhodium, and ruthenium, synthesis, toxicity, cytotoxicity.

The successful use of platinum complexes in tumor chemotherapy together with the presence of unwanted side effects on the one hand and the resistance of several tumors to this preparation on the other [1] has encouraged an extended study of antitumor activity in the complexes of other metals [2].

From the viewpoint of creating novel antitumor agents it has been found that the complexes and organometallic derivatives of ruthenium have low overall toxicity and are selectively accumulated in tumor cells [3-20]. Two of these have already been investigated clinically [2-4] and porphyrin containing ruthenium complexes (which show high phototoxicity towards melanoma cells) are promising agents for the photodynamic chemotherapy of tumors [21].

Similar complexes of rhodium, osmium, and iridium have also shown a photosensitization effect towards melanoma cells [21]. Several rhodium complexes have revealed a greater toxicity towards A549 lung carcinoma and T47D mammary gland cells than their ruthenium analogs [22] and this points to the promise of novel antitumor agents amongst rhodium compounds [23-26]. A series of osmium complexes has also shown comparable cytotoxicity towards various tumor cells [22, 27-31].

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We have found that iridium complexes [32-35] as well as those of ruthenium, rhodium, and osmium show a high cytotoxicity towards human fibrosarcoma HT-1080 and mouse hepatoma MG-22A cells with the use of 8-quinolinethiol [32-34] or 8-quinolineselenol [35] as ligand. Certain polypyridyl complexes of iridium actively inhibit the growth of human MCF-7 (mammary gland cancer) and HT-29 (large intestine cancer) tumor cells [36].

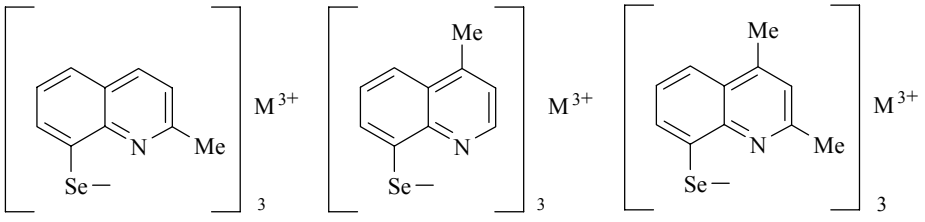
Selenium compounds also inhibit the growth of a series of tumors [35, 37-51] hence we have used the selenium ligands 8-quinolineselenol [35] and its methyl derivatives [51] in the development of the cytotoxic metal complexes. In this way it was found that:

The cytotoxicity of the metal 8-quinolineselenolates depends of the nature of the metal forming the complex;

Complexes having the greatest activity towards tumor cells frequently have the greatest toxicity towards normal cells;

The toxicity of the complexes can be varied by the introduction of a substituent into the quinoline ring [35, 51] but, as in the case of analogous 8-quinolinethiolates [32-34], the selectivity of the substituent is low.

With the aim of decreasing this toxicity and increasing the selectivity of the cytotoxic activity of metal 8-quinolineselenolates we have prepared series of the 2-methyl- (**1**), 4-methyl- (**2**), and 2,4-dimethyl-8-quinolineselenolates (**3**) of ruthenium (**a**), rhodium (**b**), osmium (**c**), and iridium (**d**) (see Table 1) and we have studied their cytotoxicity to the two tumor cell lines HT-1080 and MG-22A as well as to the normal mouse embrionic fibroblasts NIH 3T3 which were also used for evaluating the compounds toxicity (alternative method of determining LD_{50} [52]).



1 a–d **2 a–d** **3 a–d**

1–3 a M = Ru, **b** M = Rh, **c** M = Os, **d** M = Ir

The results obtained show (Table 2) that the highest cytotoxicity towards the HT-1080 and MG-22A tumor cells in this series is shown by the osmium complexes (LC_{50} 3 μg/ml). Moreover the activity depended little on the nature of the ligand, the compounds with methyl groups in the quinoline ring **1c-3c** being almost the same in cytotoxicity as the complex with the unsubstituted 8-quinolineselenol ligand [35]. At the same time, their toxicity depends markedly on the structure of the ligand and decreases in the series 2-Me > 4-Me > 2,4-Me_2. The toxicity also decreases in the same sequence in the ruthenium, iridium, and rhodium complexes. The rhodium and iridium complexes are the least toxic of all of the compound series studied (LD_{50} > 2000 mg/kg).

By contrast to the osmium complexes the cytotoxicity of the methyl-8-quinolineselenolates of ruthenium, iridium, and rhodium depends significantly on the position and number of methyl groups in the quinoline ring. Hence if the activity of the 2-methyl ruthenium derivative **1a** (LC_{50} 3 μg/ml) is comparable with the activity of the analogous osmium complex **1c** then its 4-methyl- (**2a**) and 2,4-dimethyl derivatives (**3a**) are markedly less active. Their cytotoxicity decreases in the series of ligands 2-Me- > 4-Me > 2,4-Me_2. In the series of iridium complexes the greatest cytotoxicity is seen in the 4-methyl derivative **2d** (LC_{50} 8 μg/ml). The 2-methyl derivative **1d** is less active and the 2,4-dimethyl derivative **3d** proved to be inactive. Amongst the rhodium derivatives showing moderate cytotoxicity the least active towards HT-1080 and MG-22A (CV test) proved to be the 2-methyl derivative **1b**.

TABLE 1. Characteristics of the Metal 8-Quinolineselenolates **1-3**

Compound	Empirical formula	Found, % / Calculated, %			Yield, %
		C	H	N	
1a	$C_{30}H_{24}N_3RuSe_3$	46.83 / 47.13	3.23 / 3.16	5.57 / 5.49	76
1b	$C_{30}H_{24}N_3RhSe_3$	46.60 / 47.02	3.26 / 3.15	5.40 / 5.48	75
1c	$C_{30}H_{24}N_3OsSe_3$	42.50 / 42.21	2.75 / 2.83	4.83 / 4.92	70
1d	$C_{30}H_{24}IrN_3Se_3$	42.20 / 42.11	2.76 / 2.83	4.80 / 4.91	74
2a	$C_{30}H_{24}N_3RuSe_3$	46.79 / 47.13	3.29 / 3.16	5.61 / 5.49	75
2b	$C_{30}H_{24}N_3RhSe_3$	47.30 / 47.02	3.02 / 3.15	5.38 / 5.48	73
2c	$C_{30}H_{24}N_3OsSe_3$	42.52 / 42.21	2.70 / 2.83	4.85 / 4.92	70
2d	$C_{30}H_{24}IrN_3Se_3$	41.76 / 42.11	2.76 / 2.83	4.96 / 4.91	75
3a	$C_{33}H_{30}N_3RuSe_3$	48.66 / 49.14	3.85 / 3.75	5.34 / 5.21	75
3b	$C_{33}H_{30}N_3RhSe_3$	49.50 / 49.03	3.62 / 3.74	5.10 / 5.20	76
3c	$C_{33}H_{30}N_3OsSe_3$	44.70 / 44.25	3.25 / 3.38	4.54 / 4.69	60
3d	$C_{33}H_{30}IrN_3Se_3$	44.53 / 44.15	3.24 / 3.37	4.85 / 4.69	70

All of the studied 4-methyl-8-quinolineselenolates of ruthenium, rhodium, osmium, and iridium **2a-d** were rather less active towards tumor cells than the analogous 4-methyl-8-quinolinethiolates but the latter are 2-3 times more toxic towards normal 3T3 cells [33].

TABLE 2. Cytotoxicity of the 8-Quinolineselenolates **1-3***

Compound	LC_{50}, μg/ml							LD_{50}, mg/kg
	HT-1080			MG-22A			3T3	
	CV	MTT	NO	CV	MTT	NO	NR	
1a	3	4	58	3	3	47	37	841
1b	100	32	10	100	33	10	*[2]	>2000
1c	3	3	233	3	3	133	10	512
1d	28	20	275	27	29	275	300	2255
2a	20	25	150	10	27	233	34	826
2b	36	28	67	66	90	16	1000	3549
2c	3	3	114	3	3	400	20	682
2d	8	9	150	9	8	128	494	2822
3a	27	24	54	36	23	67	90	1291
3b	60	40	10	38	36	50	*[2]	>2000
3c	4	3	200	5	5	160	40	895
3d	*[2]	*[2]	8	*[2]	*[2]	11	532	3050

* LC_{50} – the concentration causing 50% cell death; CV = Crystal Violet (action on cell membranes); MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- 2H-tetrazolium bromide (effect on mitochondrial enzyme activity in the cell); NR – Neutral Red; NO – degree of NO generation determined and calculated by method [53]; LD_{50} – acute toxicity

*[2] Cytotoxic effect absent.

It should be noted that the compounds of osmium **1c-3c** and iridium **1d, 2d** show high cytotoxicity towards tumor cells, markedly inducing the formation of nitric oxide in them (Table 2).

Hence it has been shown that osmium 8-quinolineselenolate [35] and its 2-methyl, 4-methyl, and 2,4-dimethyl derivatives possess high cytotoxicity towards HT-1080 and MG-22A tumor cells but the ruthenium 2-methyl-8-quinolineselenolate **1a** and iridium 4-methyl-8-quinolineselenolate stand out in their selectivity of cytotoxic activity.

EXPERIMENTAL

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

Synthesis of metal 2-methyl-8-quinolineselenolates 1 (General Method). Di(2-methyl-8-quinolyl)-diselenide (0.1 g, 0.23 mmol) was dissolved in 3M hydrochloric acid (1 ml), ethanol (5 ml) and 50% H_3PO_2 (0.5 ml) were added, and left for 5 min. A saturated solution of sodium acetate (3 ml) was added to the obtained solution of 2-methyl-8-quinolineselenol followed, with stirring, by a solution of the metal salt in water (3 ml) as follows: $K_2[Ru(H_2O)Cl_5]$ (0.05 g, 0.14 mmol), $(NH_4)_3[RhCl_6]\cdot H_2O$ (0.05 g, 0.13 mmol), K_3OsBr_6 (0.12 g, 0.16 mmol), or $(NH_4)_3[IrCl_6]\cdot H_2O$ (0.06 g, 0.12 mmol). The reaction mixture was heated for 10 min on a water bath. The precipitated 2-methyl-8-quinolineselenolates **1a-d** were filtered off, washed with water, dried in air, and recrystallized from chloroform (Table 1).

4-Methyl-8-quinolineselenolates 2a-d were prepared from di(4-methyl-8-quinolyl)diselenide as described in the method above. The yields and results of elemental analysis for the complexes obtained are given in Table 1.

2,4-Dimethyl-8-quinolineselenolates 3a-d were prepared from di(2,4-dimethyl-8-quinolyl)diselenide as described in the method above (Table 1).

Cytotoxicity of compounds 1-3 (Table 2) *in vitro* towards monolayers of the HT-1080 (human fibrosarcoma) and MG-22A (mouse hepatoma) tumor cells and normal NIH 3T3 cells (mouse embryonic fibroblasts) was determined on 96 well panels using the dyes CV, MTT, and NR and the method reported in [54]. The median acute toxicity (LD_{50}, mg/kg) was calculated by method [52] using data obtained on the 3T3 cell culture.

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