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Short Communication

Novel Ru(III), Rh(III), Pd(II) and Pt(II) Complexes with Ligands Incorporating Azole and Pyrimidine Rings. I. Antiproliferative Activity *in Vitro*

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Abstract. A number of co-ordination compounds of Ru(III), Rh(III), Pd(II) and Pt(II) with ligands incorporating azole and pyrimidine rings has been synthesized. The *in vitro* cell proliferation-inhibitory activity of these compounds was examined against human cancer cell lines: A 549 (lung carcinoma), LS-180 (colon cancer) and MCF-7 (breast cancer), using SRB technique. Six out of 13 compounds studied revealed cytotoxic activity *in vitro*. Inhibitory dose 50% (ID_{50}) was lower than 4 μg/ml, which is an activity criterion accepted in conventional *in vitro* cytotoxic screening tests. Two compounds revealed weak cytotoxic activity with ID_{50} higher than 4 μg/ml and five compounds were inactive.

Key words: azole and pyrimidine complexes; platinum-group metal complexes; cytotoxic activity *in vitro*; human cancer cell lines.

Introduction

Heavy metal complex compounds have been applied in treatment of the patients with various diseases. For instance, they proved effectiveness in the treatment of the patients with cancers, anemia, arthritis, chronic inflammation, bacterial infections and alimentary tract disorders[1, 5, 6, 9]. Studies on antitumor activity of platinum-group metal complexes have markedly been intensified after demonstration of a selective inhibition of cell division by cis-$[Pt(NH_3)_2Cl_2]$ and cis-$[Pt(NH_3)_2Cl_4]$ by ROSENBERG et al[15].

An antitumor effect of these compounds may be explained by their interference with DNA synthesis in the exposed cells, since the structure of the formed complexes resembles an interchain cross-linked framework[7, 10, 12, 19]. In recent studies on antitumor agents, the complexes of the platinum-group metals with ligands incorporating, among others, azole and pyrimidine rings are of particular interest[2, 3, 8, 13, 14, 18, 20].

It was, therefore, interesting to survey the newly synthesized co-ordination compounds of Ru(III), Rh(III), Pd(II) and Pt(II) with 2-mercapto-1-methylimidazole (TMZ), 3,4,5-trimethylpyrazole (3,4,5-TMePz), 1,3,4-trimethylpyrazole (1,3,4-TMePz), 1,3,5-trimethylpyrazole (1,3,5-TMePz), 1,4,5-trimethylpyrazole (1,4,5--TMePz), 1,3,4,5-tetramethylpyrazole (1,3,4,5-TeMePz), 3,4,5,6-tetrahydro-2-pyrimidinethiol (THPT) and 2-mer-

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captopyrimidine (2-MP) for their antiproliferative activity *in vitro* and an expected antitumor properties *in vivo*.

Materials and Methods

Reagents. TMZ (Aldrich, Belgium) was recrystallized from redistilled water. The melting point (m. p.) of the purified compound was 114˚C.

3,4,5-TMePz (m. p. 142˚C), 1,3,4-TMePz (boiling point – b. p. 160˚C), 1,3,5-TMePz (m. p. 26–27˚C), 1,4,5-TMePz (b. p. 175˚C) and 1,3,4,5-TeMePz (m. p. 23˚C) were purchased from the University Medical School, Łódź, Poland. Their purity was checked by the NMR spectroscopy and by TLC.

THPT and 2-MP (Aldrich, Belgium) was crystallized from ethyl alcohol. The m. p. of the purified compounds were 310 and 330˚C, respectively.

The complexes were prepared starting from $RhCl_3$ (Koch-Light Laboratories Ltd., England), $RuCl_3$ (Johnson Mattey Chemical Ltd., England), $PdCl_2$ (Anal. R grade, POCh, Gliwice, Poland) and K_2PtCl_4 by the method of LIVINGSTONE[11].

Synthesis of the complexes. Details concerning synthesis of the complexes, their composition, structure and physico-chemical properties were reported earlier[17, 22–25].

Complexes. The following compounds were examined in *in vitro* screening assay:

- Pd(TMZ) $_4Cl_2 \cdot H_2O$,
- Pt(TMZ) $_2Cl_2$,
- Rh(TMZ) Cl_3,
- Ru(TMZ) Cl_3,
- Pd(1,4,5-TMePz) $_2Cl_2$,
- Pd(1,3,4-TMePz) $_2Cl_2$,
- Pd(1,3,5-TMePz) $_2Cl_2$,
- Pd(3,4,5-TMePz) $_2Cl_2$,
- Pd(1,3,4,5-TeMePz) $_2Cl_2$,
- Pd(THPT) $_4Cl_2 \cdot H_2O$,
- Pt(THPT) $_4Cl_2 \cdot H_2O$,
- Pd(2-MP) $_2$,
- Pt(2-MP) $_2$,
- cisplatin (reference drug).

Test solutions of the compounds (1 mg/ml) were prepared *ex tempore* for each test by dissolving them either in 100 or 200 µl of DMSO + 900 or 800 µl of water *pro injectione* or, if soluble, only in water. After that, the compounds were diluted in culture medium (described below) to reach final concentrations 100, 10, 1, 0.1 and 0.01 µg/ml.

The anti-proliferative assay in vitro

Cell lines. Cells of the following human cancer lines were used: A 549 (non-small cell lung carcinoma), LS-180 (colon adenocarcinoma) and MCF-7 (breast cancer). All lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and cultured in Cell Culture Collection of Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wrocław, Poland.

Twenty four hours before application of the tested compounds, the cells were plated in 96-well plates (Costar, USA) at density of 10^4 cells per well in 100 µl. The cells were cultured in opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warszawa), (50 µg/ml) streptomycin (Polfa, Jelenia Góra), (50 U/ml) penicillin (Polfa, Jelenia Góra) and 5% fetal calf serum (Gibco, Grand Island, USA). The cells were cultured with the agents for 72 h in 37ºC in humid atmosphere saturated with 5% CO_2.

SRB assay. The cytotoxic effect *in vitro* of all compounds was examined using SRB method as described by SKEHAN et al[16].

The results were calculated as an inhibitory dose 50% (ID50) – the dose of drug which inhibits proliferation rate of the tumor cells by 50% as compared to control untreated cells. Each compound in every concentration was tested in triplicates per experiment. Every experiment was repeated 3 times.

Results and Discussion

The results of experiments, expressed as ID_{50} values, determined for a given cancer cell line for each compound, are summarized in Table 1. The adopted activity criterion of the new compounds in the *in vitro* screening tests was an ID_{50} level not exceeding 4 µg/ml[4].

This criterion was fulfilled by the following compounds: 5, 6, 7, 9, 12 and 13 (Table 1, Fig. 1), i.e. Pd(II) complexes of 1,4,5-TMePz, 1,3,4-TMePz, 1,3,5--TMePz, 1,3,4,5-TeMePz and 2-MP, as well as Pt(II)--2-MP. As shown in Table 1, the highest cytotoxic effect *in vitro* was exhibited by Pt(II)-TMZ and Pd(II)--3,4,5-TMePz. On the other hand, Pd(II), Ru(III) and Rh(III) complexes with TMZ and the Pd(II) and Pt(II) ones with THPT did not reveal any cytotoxic activity. It seems that high cytotoxic effect of Pt(TMZ) $_2Cl_2$ may be explained by its non-electrolyte nature, the *cis* configuration and the planar structure of coordination sphere of the central ion[21].

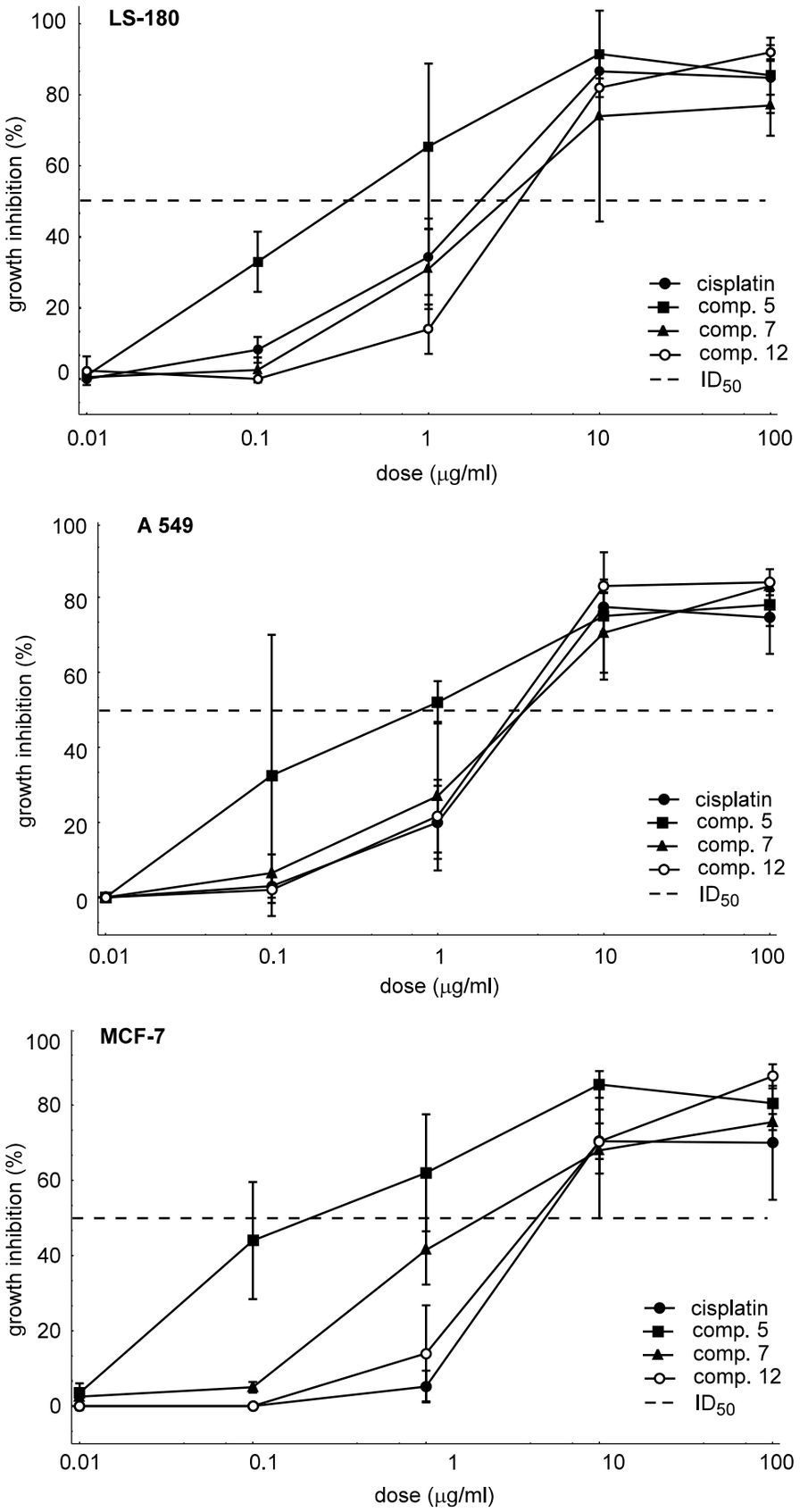

Fig. 1. The cytotoxic activity *in vitro* of selected compounds against human cancer cell lines

Table 1. The cytotoxic activity *in vitro* (ID_{50} in μg/ml) of the tested compounds against human tumor cell lines

No.	Compound	Cell line (ID_{50} μg/ml)		
		A 549	MCF-7	LS-180
1	$Pd(TMZ)_4Cl_2 \cdot H_2O$	–	–	–
2	$Pt(TMZ)_2Cl_2$	26	24.5	28
3	$Rh(TMZ)Cl_3$	–	–	–
4	$Ru(TMZ)Cl_3$	–	–	–
5	$Pd(1,4,5\text{-}TMePz)_2Cl_2$	0.8	0.218	0.334
6	$Pd(1,3,4\text{-}TMePz)_2Cl_2$	6.0	0.554	1.32
7	$Pd(1,3,5\text{-}TMePz)_2Cl_2$	3.36	2.10	2.81
8	$Pd(3,4,5\text{-}TMePz)_2Cl_2$	75	72	35
9	$Pd(1,3,4,5\text{-}TeMePz)_2Cl_2$	1.05	1.91	1.51
10	$Pd(THPT)_4Cl_2 \cdot H_2O$	–	–	–
11	$Pt(THPT)_4Cl_2 \cdot H_2O$	–	–	–
12	$Pd(2\text{-}MP)_2$	2.88	4.40	3.40
13	$Pt(2\text{-}MP)_2$	3.0	1.49	2.14
14	Cisplatinum	3.38	4.86	4.0

Inactive: –

On the other hand, the high activity of $Pd(3,4,5\text{-}TMeP_2)_2Cl_2$ may be explained by its possible interaction with a receptor site via hydrogen bonding, as is the case of compounds carrying the $-NH_2$ group[10].

The lack of cytotoxic effect of Pt(II) -TMZ, Pd(II)--TMZ and Pt-THPT complexes is likely due to their inert electrolytic nature, whereas Ru(III) and Rh(III) complexes form inert dimeric and/or polymeric structures contributing to suppression of their cytotoxic activity *in vitro*. An important feature of these compounds is the sulfur atom of the thiocarbonyl group functioning as an electron-donating center[24].

Again, Pd(II) methylpyrazole complexes meeting activity criterion ($ID_{50} < 4$ μg/ml) are non-electrolytes with *trans* configuration, labile Cl^- ions, and azomethine nitrogen atoms of the heterocyclic ring as a electron-donating centers. On the other hand, Pd(II) and Pt(II) 2-MP chelates are non-electrolytes, the chelate rings being formed by involving electron pairs located both on the nitrogen and sulfur atoms[16].

Generally, it seems that during investigations on the correlation between the structure and cytotoxic activity *in vitro*, restriction to only certain fragment of compound is insufficient. Biological activity should be related to physico-chemical properties of a whole molecule[10].

Some of our complexes tested could be selected for further advanced studies *in vitro* using larger panel of human cancer cell lines of different tissue origin, and *in vivo* using experimental mouse tumor model.

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