Journal of Organometallic Chemistry 822 (2016) 74–79

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Rhodium(III) and iridium(III) pentamethylcyclopentadienyl complexes with tris(2-carboxyethyl)phosphine, properties and cytostatic activity

Hanna Pruchnik [a, *], Małgorzata Latocha [b], Aleksandra Zielińska [b], Florian P. Pruchnik [c]

[a] Department of Physics and Biophysics, Wroclaw University of Environmental and Life Sciences, ul. Norwida 25, 50-375, Wrocław, Poland
[b] Faculty of Pharmacy, Medical University of Silesia, ul. Narcyzów 1, 41-200, Sosnowiec, Poland
[c] Faculty of Chemistry, University of Wrocław, ul. Joliot-Curie 14, 50-383, Wrocław, Poland

ARTICLE INFO

Article history:
Received 4 April 2016
Received in revised form
31 July 2016
Accepted 3 August 2016
Available online 9 August 2016

Keywords:
Rhodium
Iridium
Phosphine
Antitumor
DFT calculations

ABSTRACT

New pentamethylcyclopentadienyl complexes $[M(C_5Me_5)Cl_2(TCEP)]$ (M = Rh **1**, M = Ir **2**) with $P(C_2H_4COOH)_3$ (TCEP) were investigated using IR, ^1H, ^{13}C, ^{31}P NMR and ESI-MS spectroscopies. Geometry optimization in the gas phase at the B3LYP/3-21G** level indicated that complex **1** has stable pseudooctahedral structure with large HOMO–LUMO gap. Calculated and experimental IR spectra of **1** agree very well. Cytostatic activity of compounds **1** and **2** was investigated against melanoma and breast tumor cells. Complexes **1** and **2** show very promising activity towards MDA-MB-231 triple negative breast cancer cells.

1. Introduction

The success of cisplatin in chemotherapy has begun a large number of studies of other metal complexes as anti-tumor drugs [1]. To the most intensely recently investigated complexes belong coordination and organometallic compounds of rhodium [2–15], ruthenium [4,5,8,10] and iridium [8–20]. Rhodium and iridium complexes are amongst the most promising class of anti-tumor agents. Cytostatic and anti-cancer activity characterize mainly coordination of rhodium and iridium in oxidation states +1, +2 and + 3 containing ligands with nitrogen, sulfur and oxygen donor atoms. The antitumor activity of these compounds was expected because they have d^8, d^7 and d^6 electronic configuration and are isoelectronic with Pt(II), Pt(III) and Pt(IV) complexes applied as antitumor agents. Cytostatic activity of organometallic complexes of platinum metals was investigated to a lesser extent. Interesting antitumor properties were found in the case of ruthenium(II) half-sandwich compounds $[Ru(arene)X_2(pta)]$ (RAPTA complexes) (pta = 1,3,4-triaza-7-phosphatricyclo[3.3.1.1]decane). These results prompted a switch to investigating cytostatic activity of isoelectronic Rh(III) and Ir(III) half-sandwich pentamethylcyclopentadienyl complexes $[M(C_5Me_5)Cl_2(pta)]$, $[M(C_5Me_5)Cl(pta)_2]^+$ [4,5,9] and compounds $[M(C_5Me_5)Cl(chel)]$ (chel: various bidentate ligands with e.g. N–N, N–C and N–O donor atoms) [6–19]. The cytostatic activity of the Rh(III) and Ir(III) complexes containing pta is comparable with activity of RAPTA compounds, although rather low in comparison with $[M(C_5Me_5)Cl(chel)]$ (M = Rh, Ir) complexes [6,9]. Iridium(III) complexes with (maleimido)pyridocarbazoles show also antiangiogenic properties. They inhibit tumor cell induced angiogenesis [20].

Tris(2-carboxyethyl)phosphine $P(CH_2CH_2COOH)_3$ (TCEP) is readily soluble in water and stable in air. Thus it can be used for preparation phosphine complexes reasonably soluble in water. It is frequently used in biochemistry as an efficient reducing agent to break disulfide bond in peptides, proteins and other compounds containing S–S bond [21–24]. TCEP is now often used instead of dithiothreitol (DTT), which is not stable in the reduced form for a long times. Application of TCEP is very convenient, because it is stable in aqueous solutions, odourless and stoichiometrically and irreversibly reduces disulfides:

$$RSSR + P(CH_2CH_2COOH)_3 + H_2O \rightarrow 2RSH + OP(CH_2CH_2COOH)_3$$

TCEP forms complexes with transition metals. Coordination compounds with Zn(II) [25], Ni(II), Cu(II), Zn(II), Cd(II), Pb(II) [26], Co(III) [27] and Fe(I) [28] were investigated and X-ray structures of Zn(II), Cd(II), Co(III) and Fe(I) complexes were determined. In all

* Corresponding author.
E-mail address: hanna.pruchnik@up.wroc.pl (H. Pruchnik).

http://dx.doi.org/10.1016/j.jorganchem.2016.08.005
0022-328X/

complexes TCEP is coordinated via P atom and COO- groups except Fe(I) compound, in which it is bonded via P atom. In platinum(II) compound with 3-{di(2-methoxyphenyl)phosphanyl}propionate *trans*-[PtCl{P(C_6H_4OMe)$_2$(C_2H_4COOH)-κ*P*}{P(C_6H_4O-Me)$_2$(C_2H_4COO)-κ*COO*,κ*P*}] one phosphine is a chelating ligand bound via P atom and COO- group, while the other is terminal ligand coordinated via phosphorus [29]. We have found that in *trans*-[$PtCl_2$(TCEP)$_2$] and *cis*-[$PtCl_2$(TCEP)$_2$] Pt–P bonds are formed, however, in aqueous solutions trans complex isomerizes to the cis compound and TCEP-s coordinate as chelate ligands via P atom and COO- group [30]. In palladium(II) complexes *trans*-[$PdCl_2$(TCEP)$_2$] and *trans*-[Pd_2(μ-Cl)$_2$$Cl_2$(TCEP)$_2$] phospine ligands are coordinated via P atom. In aqueous solution dinuclear complex gives polymeric compound with bridging phosphine ligand [PdCl{P(RCOO-κ*O*-μ-*O*′)(RCOOH)$_2$-κ*P*}] [31]. Recently TCEP was used for reactivity studies between cisplatin, oxaliplatin and model proteins. Due to the presence of redox active cysteine residue, investigations of interaction of proteins with metal complexes are typically performed in the presence of TCEP as reducing agent. It was found that cisplatin interacts with Cu(I) transporters ATP7B, Atox1 [32–34]. Recently discovered that TCEP significantly promoted the reaction of cisplatin with Sp1 zinc finger protein [35]. In Pt(Atox1)(TCEP), the Pt(II) atom has square-planar coordination with two S atoms of Cys in trans coordinating sites and amide N atom of Cys and TCEP coordinated via P atom [34]. However, rhodium and iridium complexes with TCEP were not obtained and investigated. Here we report complexes [Rh(C_5Me_5)Cl_2(TCEP)] and [Ir(C_5Me_5)Cl_2(TCEP)] and their properties and cytostatic activity against tumor cells.

2. Experimental

2.1. Materials and measurements on physical and chemical properties

Reagents and solvents (analytical grade) were purchased from the Polish company POCH, Sigma-Aldrich and ABCR Gmbh and were used as received. Infrared spectra (KBr pellets and nujol) were recorded with a Bruker IFS 113v and Bruker 66/s spectrometers, ^{1}H, ^{13}C and ^{31}P NMR spectra on a Bruker AMX 300 and Bruker Avance 500 spectrometers. Proton chemical shifts (δ) were reported with reference to the residual protons in D_2O, DMSO-d_6 and CD_3OD; ^{13}C chemical shifts were given with respect to the natural contents of ^{13}C in DMSO-d_6, ^{31}P chemical shifts were reported with reference to the external 85% H_3PO_4. The mass spectra were recorded on a Bruker MicrOTOF-Q spectrometer (Bruker Daltonik, Bremen, Germany), equipped with an Apollo II electrospray ionization source with an ion funnel. The mass spectrometer was operated in the negative and positive ion modes. The instrumental parameters were as follows: scan range *m*/*z* 250–2000, dry gas–nitrogen, temperature 200 °C, ion source voltage 4500 V. The spectra of compounds were recorded for H_2O, H_2O/DMF, H_2O/CH_3OH and CH_3OH solutions. Before analysis the instrument was calibrated externally with the Tunemix™ mixture (Bruker Daltonik, Germany) in the quadratic regression mode. The stoichiometry of the analyzed ions was confirmed by the isotopic patterns. All elemental analyses were performed with a Vario EL3 CHN analyzer. The IR, NMR and ESI-MS data are given in Appendix A.

2.2. Synthesis of the compounds

2.2.1. Dichlorido(pentamethylcyclopentadienyl)[(2-carboxyethyl) phosphine]rhodium(III), [Rh(C_5Me_5)Cl_2{P(C_2H_4COOH)$_3$}], **1**

The mixture of Rh_2(C_5Me_5)$_2Cl_4$ (0.5 mmol, 0.309 g) and P(C_2H_4COOH)$_3$·HCl (1 mmol, 0.287 g) in dioxane (10 ml) was stirred at room temperature for 3 h. The mixture was evaporated to dryness and the red product was washed three times with propan-2-ol and dried in air at room temperature. Yield 0.475 g, 85%. Anal. Calc. for $C_{19}H_{30}O_6PRhCl_2$: C 40.81, H 5.41, Cl 12.68. Found: C 40.88, H 5.54, Cl 12.95.

2.2.2. Dichlorido(pentamethylcyclopentadienyl)[(2-carboxyethyl) phosphine]iridium(III), [Ir(C_5Me_5)Cl_2{P(C_2H_4COOH)$_3$}], **2**

The mixture of Ir_2(C_5Me_5)$_2Cl_4$ (0.5 mmol, 0.398 g) and P(C_2H_4COOH)$_3$·HCl (1 mmol, 0.287 g) in dioxane (20 ml) was stirred at room temperature for 3 h. The mixture was evaporated to dryness and the red product was washed three times with propan-2-ol and dried in air at room temperature. Yield 0.525 g, 81%. Anal. Calc. for $C_{19}H_{30}O_6PIrCl_2$: C 35.19, H 4.66, Cl 10.93. Found: C 35.58, H 4.33, Cl 10.61.

2.3. Cytostatic activity

The melanoma cell lines: SK-mel (human, Caucasian, skin, melanoma), SH-4 (melanotic melanoma), Colo-829 (human, umbilical metastatis, melanoma) and C-32 (amelanotic melanoma) and breast cell lines: MCF7, T-47D and MDA-MB-231 were used for the proliferation assay. The experiments were repeated in triplicate for each tested compound concentration. Statistical significance was determined using Student's t-test ($p < 0.05$ was considered statistically significant). The cell concentration was 6000 cells/well on the 96-well microplate. The basic culture medium for tumor cells consisted of RPMI-1640 medium with 10% fetal calf serum (FCS) and 1% antibiotic–antimycotic solution. The cultured cells were maintained at 37 °C in humidified air containing 5% CO_2, for 24 h. The next day the media were changed to RPMI with 0.2% FCS and cells were incubated for a further 24 h. The concentration of FCS was changed two times to get synchronized cell cultures. After the preincubation the experimental media composed with RPMI, 5% FCS and solution of investigated complex was added. The concentrations of rhodium and iridium compounds in the cultures were μM - mM. The stock solution of coordination compound was prepared in DMSO. The tested compound was diluted with culture medium to reach the final concentrations of the complex. The final concentration of DMSO in the cultured cells was 0.1–1% (v/v). The control cell culture was incubated in the standard media enriched with 5% FCS and adequate concentration of DMSO (0.1–1%, v/v). After 72 h of incubation, cell viability was quantified by a cell proliferation assay (WST-1; Roche, Basel, Switzerland). The amount of WST-1-formazan produced was measured at 450 nm and appropriate calculations were performed as described previously [31,36–38]. The results of cytotoxic activity in vitro were expressed as ID_{50} – the dose of compound that inhibits proliferation rate of the tumor cells by 50% as compared to control untreated cells.

2.4. Computational details

DFT calculations were performed using the Gaussian 03 package of programs [39] with B3LYP functional and 3-21G** basis set. The numerical calculations have been performed in part at Wrocław Center for Networking and Supercomputing. Computed results were analyzed using Chemcraft program (www.chemcraftprog.com).

3. Results and discussion

3.1. Properties and structures of compounds

Complexes [Rh(C_5Me_5)Cl_2{P(C_2H_4COOH)$_3$}] (**1**) and [Ir(C_5Me_5)Cl_2{P(C_2H_4COOH)$_3$}] (**2**) were obtained in reactions of [Rh_2(C_5Me_5)$_2Cl_4$] and [Ir_2(C_5Me_5)$_2Cl_4$] with tris(2-carboxyethyl)