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New water-soluble ruthenium(II) cytotoxic complex: Biological activity and cellular distribution

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ABSTRACT

A novel water soluble organometallic compound, [RuCp(mTPPMSNa)(2,2′-bipy)][CF_3SO_3] (**TM85**, where Cp = η^5-cyclopentadienyl, mTPPMS = diphenylphosphane-benzene-3-sulfonate and 2,2′-bipy = 2,2′-bipyridine) is presented herein. Studies of interactions with relevant proteins were performed to understand the behavior and mode of action of this complex in the biological environment. Electrochemical and fluorescence studies showed that **TM85** strongly binds to albumin. Studies carried out to study the formation of **TM85** which adducts with ubiquitin and cytochrome *c* were performed by electrospray ionization mass spectrometry (ESI-MS). Antitumor activity was evaluated against a variety of human cancer cell lines, namely A2780, A2780cisR, MCF7, MDAMB231, HT29, PC3 and V79 non-tumorigenic cells and compared with the reference drug cisplatin. **TM85** cytotoxic effect was reduced in the presence of endocytosis modulators at low temperatures, suggesting an energy-dependent mechanism consistent with endocytosis. Ultrastructural analysis by transmission electron microscopy (TEM) revealed that **TM85** targets the endomembranar system disrupting the Golgi and also affects the mitochondria. Disruption of plasma membrane observed by flow cytometry could lead to cellular damage and cell death. On the whole, the biological activity evaluated herein combined with the water solubility property suggests that complex **TM85** could be a promising anticancer agent.

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1. Introduction

The discovery of anticancer properties of cisplatin marked a turning point in the medicinal inorganic chemistry. Currently, cisplatin is commonly used to treat numerous cancers, particularly malignant solid tumors like testicular cancer, ovarian cancer, esophageal cancer, bladder cancer, head and neck cancer, and small-cell lung carcinomas [1]. However, the development of resistance to chemotherapy leads to the failure of cisplatin treatment, leading to the search of new effective metallodrugs.

Coordination and organometallic compounds of transition metals have shown great potential for use as therapeutic agents due to their wide structural diversity and binding modes [2,3]. Ruthenium compounds present some features that make them very interesting for drug development, such as low toxicity, good selectivity for tumors, inhibition of the antimetastatic progression and antiangiogenic properties [4,5]. Two ruthenium coordination compounds NAMI-A ([ImH][*trans*-Ru(III)Cl_4Im(Me_2SO)], Im = imidazole) and KP1019 ([Hind][*trans*-Ru(III)Cl_4(Ind)$_2$], Ind = indazole) are under clinical trial against metastatic and colon cancers, respectively [6]. However, coordination compounds present some problems related to their instability and complicated ligand exchange chemistry that have been a problem for their clinical trial evolution. Alternatively, organometallic chemistry appeared as an attractive area of research to provide organoruthenium complexes as suitable drug candidates. The most studied organoruthenium compounds are the ruthenium(II)-(η^6-C_6H_6) derivatives developed by Sadler et al. [7] and Dyson et al. [8]. These complexes were proven to be potent cytotoxic agents against a range of tumor cell lines *in vitro* and *in vivo* [7], and in some cases with anti-metastatic potential [9].

The potentiality of "Ru(η^5-C_5H_5)"derivatives in the field of metallodrugs was reported for [Ru(η^5-C_5H_5)CO'pyridocarbazole'] compounds that revealed strong and selective inhibitors of protein kinases GSK-3 and Pim-1 [10,11]. Also a good activity on TS/A murine

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adenocarcinoma tumor cells was accounted for the water soluble [RuCp*(PTA)$_2$Cl] complex (PTA = 1,3,5-triaza-7-phosphaadamantane) [12].

In our approach to this field, we pioneered the report of strong cytotoxic activity against LoVo and MiaPaca cancer cell lines of two half sandwich cationic complexes derived of "Ru(η^5-C_5H_5)" fragment [13]. The promising results displayed by these two compounds prompted us to the enlargement of this family of compounds which have been revealing a potent cytotoxicity against several human tumor cell lines with IC_{50} values much lower than those found for cisplatin [14–16].

Encouraged by our previous results obtained with compound [RuCp(PPh$_3$)$_2$(2,2′-bipy)][CF_3SO_3] (where Cp = η^5-cyclopentadienyl, mTPPMS = diphenylphosphane-benzene-3-sulfonate and 2,2′-bipy = 2,2′- bipyridine) **TM34** [17] that revealed excellent biological properties, we decided to better explore this type of structure having in mind the great importance of water solubility in chemotherapy. In the present paper, we report the synthesis, characterization and a biological activity of a new water soluble compound [RuCp(mTPPMSNa)(2,2′-bipy)][CF_3SO_3] (**TM85**), which can be considered the water-soluble "version" of **TM34**, to understand how the gain in solubility affects its biologic properties. Considering that cytotoxicity by itself is not exclusive for the evaluation of the metallodrug potential, other studies are considered relevant for the understanding of the mode of action of potential drugs. In this frame, the knowledge of the kind of interaction between drugs and plasma proteins is of major importance to identify the drug pharmacokinetics and pharmacodynamics. The main function of albumin, which is the principal and most abundant drug transport carrier protein of the circulatory system [18,19] is to transport fatty acids as well as a broad range of drug molecules to its targets [20,21]. Remarkably, human serum albumin (HSA) often increases the solubility of hydrophobic drugs in plasma [22]. Thus, to evaluate the role of albumin in the transport of **TM85,** several fluorescence spectroscopic studies and electrochemical experiments were carried out involving two variants of human serum albumin. Considering that enzymes and proteins are relevant targets for the mode of action of ruthenium metallodrugs [23], we found important to evaluate the interaction of **TM85** with ubiquitin and cytochrome *c*, well-known proteins by their importance in the mechanism of cell death. These studies were carried out by mass spectrometry.

The drug distribution within tumor cells, its accumulation in the compartment that houses its target as well as the identification of the mechanisms of uptake into the cell, are fundamental conditions for its activity [24] and are mandatory conditions when evaluating the therapeutic potential of a drug. In the present work, the analysis of cellular distribution was accomplished by analytical (inductively coupled plasma mass spectrometry — ICP-MS) and pharmacological (endocytosis modulators) tools in order to get some insights into the uptake pathways [25].

Finally, attempts to elucidate the cell death mechanisms and to evaluate the value of our potential drug as a therapeutic agent lead us to the examination of the cellular morphological effects induced on MDAMB231 cells treated with **TM85** by transmission electron microscopy (TEM) and the cellular damage by flow cytometry.

2. Materials and methods

2.1. Materials

All chemicals were analytical or reagent grade and were used as received from chemical suppliers, unless otherwise stated. The doubly purified water used in all experiments was from a Millipore® system. Albumin [essentially fatty acid-free HSA and fatty acid HSA], warfarin, horse heart cytochrome *c* and red blood cell ubiquitin were purchased from Sigma Aldrich (A3782, A1653, A2250, C7752 and U6253) and used as received. RPMI, McCoys, DMEM + GlutaMAX™, Trypsin-EDTA, Penicillin/Streptomycin, phosphate buffered saline (PBS) and fetal bovine serum (FBS) were purchased from Invitrogen. Propidium iodide (PI) was purchased from Sigma and Annexin V by BD Biosciences.

2.2. General procedures

Synthesis was carried out under dinitrogen atmosphere using current Schlenk techniques and the solvents used were dried by standard methods [26]. Starting material [RuCp(mTPPMSNa)$_2$Cl] was prepared following the method described in the literature [27]. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance 400 spectrometer at probe temperature. The ^{1}H and ^{13}C chemical shift (s = singlet; d = duplet; t = triplet; m = multiplet for ^{1}H) are reported in parts per million (ppm) downfield from internal Me_4Si and the ^{31}P NMR spectra are reported in ppm downfield from external standard 85% H_3PO_4. A FT-IR spectrum was recorded in a Thermo Nicolet 6700 spectrophotometer with KBr; only significant bands are cited in the text w = weak, vw = very weak, m = medium, s = sharp, vs = very sharp. ESI-HRMS spectra were acquired in an Apex Ultra FTICR Mass Spectrometer equipped with an Apollo II Dual ESI/MALDI (electrospray ionization/matrix-assisted laser desorption/ionization) ion source, from Bruker Daltonics, and a 7 T actively shielded magnet from Magnex Scientific. Electronic spectra were recorded at room temperature on a Jasco V-660 spectrometer in the range of 220–900 nm.

2.3. Synthesis of [RuCp(mTPPMSNa)(2,2′-bipy)][CF_3SO_2] (TM85)

A stirred solution of [RuCp(mTPPMSNa)$_2$Cl] (0.47 g, 0.5 mmol) in methanol (25 mL) was added 2,2′-bipyridine (0.08 g, 0.5 mmol) and $AgCF_3SO_3$ (0.13 g, 0.5 mmol). After refluxing for 4.5 h the solution turned from orange to red. The solution was separated from the AgCl precipitate by cannula-filtration and the solvent evaporated under vacuum. The residue was washed with n-hexane (3 × 10 mL). Yield: 92%. ^{1}H NMR [CD_3OD, Me_4Si, δ/ppm]: 9.33 [d, 2, H4, $^3J_{HH}$ = 6.10 Hz], 8.33 [d, 2, H1, $^3J_{HH}$ = 6.56 Hz], 8.03 [dd, 2, H2, $^3J_{HH}$ = 7.24 Hz], 8.05–6.99 [m, 14, mTPPMS], 7.78 [dd, 2, H3, $^3J_{HH}$ = 5,98 Hz], 4.82 [s, 5, C_5H_5]. ^{13}C NMR [CD_3OD, δ/ppm]: 157.07 (C4, 2,2′-bipy), 151.98 (C1, 2,2′-bipy), 137.48 (C5, 2,2′-bipy), 124.60 (C3, 2,2′-bipy), 124.21 (C2, 2,2′-bipy), 140.27–126.04 (all singlets, aromatic mTPPMS), 79.77 (C_5H_5). ^{31}P NMR [CD_3OD, δ/ppm]: 52.35 [s, mTPPMS]. FTIR [KBr, cm^{-1}]: 3100–3040 cm^{-1} (ν_{C-H}, Cp and phenyl rings), 1435 cm^{-1} ($\nu_{C=C}$, phenyl rings), 1264 cm^{-1} ($\nu(CF_3SO_3)$), 1195 cm^{-1} ($\nu(SO_3)$). ESI-HRMS (HRMS = high resolution mass spectrometry): calc. for [M$^+$] 681.044819, found 681.04571.

2.4. Stability tests towards aqueous medium and air

The stability of **TM85** in water was tested by ^{1}H and ^{31}P NMR and UV–visible (UV–vis) spectroscopies. Stability of **TM85** was also studied in buffered aqueous media (Hepes buffer, 10 mM, pH 7.4) and RPMI 1640 medium (cells medium) by UV–vis spectroscopy.

A 5-mm NMR tube was charged in the air with the 10 mg of **TM85** and D_2O (0.8 mL). ^{1}H NMR and ^{31}P NMR were monitored during four days in order to evaluate if there was any decomposition product. Decomposition/instability of the compound can be assessed by the disappearance of the NMR signals and/or by appearance of new signals that could not assigned to the known complex beyond the free mTPPMS phosphane.

Any eventual changes in the charge transfer bands between ruthenium and 2,2′-bipyridine ligand were followed by UV–visible in the range 300–900 nm.

2.5. Electrochemical studies

Cyclic voltammograms were obtained using an EG&G Princeton Applied Research Potentiostat/Galvanostat Model 273A equipped with Electrochemical PowerSuite v2.51 software for electrochemical analysis,

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