Ruthenium Porphyrin Compounds for Photodynamic Therapy of Cancer

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Five 5,10,15,20-tetra(4-pyridyl)porphyrin (TPP) areneneruthenium(II) derivatives and a p-cymeneosmium and two pentamethycyclpentadienyliridium and -rhodium analogues were prepared and characterized as potential photosensitizing chemotherapeutic agents. The biological effects of all these derivatives were assessed on human melanoma tumor cells, and their cellular uptake and intracellular localization were determined. All molecules, except the rhodium complex which was not cytotoxic, demonstrated comparable cytotoxicity in the absence of laser irradiation. The ruthenium complexes exhibited excellent phototoxocities toward melanoma cells when exposed to laser light at 652 nm. Cellular uptake and localization microscopy studies of [Ru(η⁶-C₅H₅CH₃)₄(TPP)Cl₈] and [Rh(η⁵-C₅Me₅)₂(TPP)Cl₄] revealed that they accumulated in the melanoma cell cytoplasm in granular structures different from lysosomes. The fluorescent porphyrin moiety and the metal component were localized in similar structures within the cells. Thus, the porphyrin areneneruthenium(II) derivatives represent a promising new class of organometallic photosensitizers able to combine chemotherapeutic activity with photodynamic therapeutic treatment of cancer.

Introduction

Cisplatin is widely used for the treatment of many cancers despite its high toxicity, undesirable side effects, and problems with drug resistance in primary and metastatic cancers. Because of these limitations, there is a steadily growing interest in complexes with anticancer activities involving other metals. Ruthenium possesses several favorable properties suited to rational anticancer drug design, since certain ruthenium complexes reduce tumor growth by mechanisms involving interaction with DNA and RNA, although nongenomic targets also appear to be important, such as transferrin, which allows interaction with DNA and RNA, although nongenomic targets also appear to be important, such as transferrin, which allows...
The isoelectronic rhodium and iridium pentamethylcyclopentadienyl derivatives 7 and 8 are obtained in methanol from the reaction of [M(η⁵-C₅Me₅)(µ-Cl)Cl]₂ (M = Rh, Ir) with TPP (Scheme 2). Complex 8 is sparingly insoluble in DMSO and insoluble in (CH₃)₂CO, CH₂Cl₂, MeOH, and H₂O, while complexes 6 and 7 are slightly soluble in these solvents.

The ¹H NMR spectra of 1–8 were recorded in DMSO-d₆ because of the low solubility of the complexes in other solvents. All complexes show, in addition to the signals of the corresponding η⁶-arene or η⁵-C₅Me₅ signals for the four equivalent metal units, the typical three-signal pattern for the pyrrolyl and pyridyl protons of the porphyrin system between δ = 9.5 and 8.0 ppm, the pyridyl signals being observed as doublets, while the pyrrolyl protons give rise to a singlet resonance. The two NH protons appear upfield as a singlet at δ ≈ −3.1 ppm.

Single-crystal X-ray structure analyses of 4 and 7 were performed (Figure 1). The two structures are very similar; they both show that the four pyridyl rings are almost perpendicular to the porphyrin core, which is analogous to the related compounds [Ru₄(NO)₄(TPP)Cl₁₆]¹⁻ and [Ir₄(η⁵-C₅Me₅)₄(ZnTPP)}₄(S₂C₂(B₁₀H₁₀)₄(THF))₂⁻. The adjacent metal–metal distances are 13.697(2) and 14.256(2) Å in 4 and 13.667(3) and 14.253(3) Å in 7. In the crystal packing of 4 and 7, no π-stacking interacting systems are observed between independent molecules. The spaces between the tetranuclear entities are filled with disordered solvent molecules.

The organometallic porphyrin complexes were investigated in vitro as potential drug candidates for cancer therapy by evaluating the growth inhibition of human Me300 melanoma cells. Me300 cells were exposed for 24 h to increasing concentrations of compounds 1–7, and their survival was determined using the MTT assay. Complexes 3, 4, and 6 were moderately cytotoxic in the dark against melanoma cells with an IC₅₀ around 50 µM, while complexes 1, 2, and 5 were less cytotoxic with IC₅₀ > 100 µM and complex 7 was not cytotoxic (Figure 2).

The uptake by Me300 cells of the representative complex 2 was determined after 24 h of exposure, using fluorescence microscopy (Figure 3a). The porphyrin-associated fluorescence reveals that 2 accumulated in the cytoplasm of the melanoma.

Figure 1. ORTEP representation at 50% probability level and with hydrogen atoms being omitted for clarity. (A) Compound 4, selected bond lengths (Å) and angles (deg): Ru(1)–Cl(1) 2.416(4), Ru(1)–Cl(2) 2.404(3), Ru(1)–N(1) 2.162(7), Ru(2)–Cl(3) 2.450(4), Ru(2)–Cl(4) 2.395(3), Ru(2)–N(2) 2.100(10); Cl(1)–Ru(1)–Cl(2) 87.67(13), N(1)–Ru(1)–Cl(1) 87.2(3), N(1)–Ru(1)–Cl(2) 84.3(3), Cl(3)–Ru(2)–Cl(4) 88.47(14), N(2)–Ru(2)–Cl(3) 82.2(3), N(2)–Ru(2)–Cl(4) 85.8(2). (B) Compound 7, selected bond lengths (Å) and angles (deg): Rh(1)–Cl(1) 2.405(3), Rh(1)–Cl(2) 2.428(4), Rh(1)–N(1) 2.154(9), Rh(2)–Cl(3) 2.426(4), Rh(2)–Cl(4) 2.431(3), Rh(2)–N(2) 2.103(10); Cl(1)–Rh(1)–Cl(2) 92.30(13), N(1)–Rh(1)–Cl(1) 88.0(3), N(1)–Rh(1)–Cl(2) 87.0(3), Cl(3)–Rh(2)–Cl(4) 91.72(11), N(2)–Rh(2)–Cl(3) 86.7(3), N(2)–Rh(2)–Cl(4) 89.2(3).
cells, observed as red fluorescent spots, but no nucleuss-associated fluorescence was observed. Fluorescence examination demonstrated the absence of obvious cell toxicity and of nuclear fragmentation, a marker of cell apoptosis, by DAPI staining. In order to determine whether these porphyrin complexes use the endosome—lysosome pathway for uptake and rapid degradation by melanoma cells, lysosomes were labeled with a green fluorescent reporter (Lysotracker). We did not observe the overlay of the red fluorescence of 2 with the green fluorescence of lysosomes (Figure 3b), suggesting a nonlysosomal localization following the uptake of 2 by the cells. The red fluorescence of the porphyrin and the dark-brown staining of the ruthenium moiety with dithiooxamide (Figure 3c) demonstrated colocalization of these two components of the complex in cells, suggesting that the complex remains intact inside cells following uptake.

The phototoxicities of the different complexes were also evaluated using a red laser irradiating at 652 nm. The cells were exposed for 24 h and 10 µM concentration of the various complexes, since in the dark at this concentration and time course, complexes 1–7 were not cytotoxic. The cell cultures were irradiated with a fluence of 20 mW/cm² and light doses from 5 to 30 J/cm². Cell cytotoxicity was determined using the MTT assay 24 h after the completion of the irradiation. Cells treated with the same concentration of the complexes (10 µM) but kept in the dark were used as controls for phototoxicity, whereas cells not treated by compounds and not exposed to the laser were used as controls for cytotoxicity. Untreated human melanoma cells were not photosensitive in the absence of complexes. The cytotoxicity of treated and light-irradiated and of treated and not-irradiated human melanoma cells is shown in Figure 4. For the ruthenium(II) complexes 1–5, 5 J/cm² of light exposition led to 60–80% phototoxicities, whereas this level of phototoxicity was reached with 30 J/cm² exposition for the osmium complex 6 and was never reached for the rhodium complex 7. These results are coherent with the fluorescence microscopy studies that showed that 7 was not taken up by melanoma cells (results not shown) whereas the other complexes were.

Discussion

Although photosensitizers, and in particular porphyrins, have been coupled to many therapeutic and targeting agents, we report here the first conjugation between a porphyrin and arenerruthenium and the evaluation of such complexes in photodynamic therapy in human cancer cells. The preparation of such complexes was undertaken in order to combine the photosensitizing properties of porphyrins and chemotherapeutic effects of ruthenium. The use of organometallic fragments to modulate the biological properties of porphyrin complexes has a few possible advantages. First, the addition of the organometallic fragments increases the hydrophilicity of the highly hydrophobic porphyrin ligands. This is demonstrated by the observation that our synthesized ruthenium complexes of tetrapyridylporphyrin (TPP) are soluble in polar organic solvents, whereas free TPP is nearly insoluble in polar solvents, including DMSO. Second, transition metal centers are biologically active and can bind to DNA.6–16 Structurally similar organometallic ruthenium complexes are also believed to target RNA and proteins.17–19 In this case, ruthenium(II) organometallic complexes seem to enhance uptake of the porphyrin by human melanoma cells because fluorescent microscopy studies show that 2 can be internalized by the cells whereas the rhodium complex 7 cannot. Fluorescent microscopy studies demonstrated that 2 is effectively taken up by the melanoma cells and concentrate in cell cytoplasm and organelles but not in the lysosomes or the nucleus, which contrasts with ruthenium(III) drugs known to accumulate in the nucleus.9,10 Colocalization studies of porphyrin and ruthenium revealed that they are found in the same location in the cells, suggesting that these complexes remain likely intact following their uptake by the human melanoma cells.

The differences of the photosensitizing efficacy measured between the various synthesized complexes 1–7 may be mainly due to action of the metal on the cell uptake mechanisms. It is noted that light doses of 1–15 J/cm² and up to 48 J/cm² are generally used.37,38,48 Therefore, the photosensitizing properties of complexes 1–5 are quite efficient because 5 J/cm² exposition to red light can afford 60–80% phototoxicity for melanoma cells.

The addition of the organometallic fragments on the tetrapyridylporphyrin ring did not modify the photophysical properties of the photosensitizer because the absorption and fluorescence spectra of free and complexed tetrapyridylporphyrin are identical.
in terms of wavelength, whereas molecular extinction coefficients and quantum fluorescent yields vary only slightly (see Table 1 and Supporting Information). In addition, all the ruthenium(II) complexes present comparable properties in terms of cytotoxicity and phototoxicity irrespective of the nature of the arene ligand. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of arene ligands could be prepared with customized arene coordination. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of arene ligands could be prepared with customized arene coordination. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of arene ligands could be prepared with customized arene coordination. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of arene ligands could be prepared with customized arene coordination. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of arene ligands could be prepared with customized arene coordination.

In conclusion, we have prepared a series of organometallic-modified porphyrin compounds and shown that the ruthenium facilitates uptake and results in highly active photosensitizing drugs under a light dose irradiation of only 5 J/cm². Moreover, we have shown that the ruthenium porphyrin compounds accumulate in the cytoplasm and intracellular organelles different from the lysosomes and nuclei of human melanoma cells.

Experimental Section

All organic solvents were degassed and saturated with nitrogen prior to use. 5,10,15,20-Tetrakis(4-pyridyl)porphyrin (TPP) was purchased from Fluka. [Ru(η⁶-C₆H₆)(μ-Cl)Cl₂], [Os(η⁶-p-PrC₆H₄Me)(μ-Cl)Cl₂], [Ru(η⁶-p-PrC₆H₄Me)(μ-Cl)Cl₂], [Ru(η⁶,1.4-C₆H₄(COOEt)₂)(μ-Cl)Cl₂], [Os(η⁶-p-PrC₆H₄Me)(μ-Cl)Cl₂], [Ru(η⁶-C₆Me₆)(μ-Cl)Cl₂], and [Ir(η⁶-C₆Me₆)(μ-Cl)Cl₂] were prepared according to published methods.⁴⁹-⁵⁴ ¹H NMR spectra were recorded on a Varian 200 MHz spectrometer. Electrospray mass spectra were obtained in positive-ion mode on a LCQ Finnigan mass spectrometer. Elemental analyses were performed by the Laboratory of Pharmaceutical Chemistry, University of Geneva (Switzerland). UV-visible absorption spectra were recorded on a UVikon 930 spectrophotometer and fluorescence spectra on a Perkin-Elmer LS50 spectrofluorometer.

Syntheses. [Ru₄(η⁶-C₆H₆)₄(TPP)Cl₈] (1). A mixture of [Ru(η⁶-C₆H₆)(μ-Cl)Cl₂] (100 mg, 0.2 mmol) and 5,10,15,20-(4-pyridyl)porphyrin (TPP) (62 mg, 0.1 mmol) was refluxed in dry methanol (20 mL) for 4 h whereby the brownish purple product precipitated. The compound was filtered and washed with diethyl ether and dried under vacuum. Yield: 90 mg, 56%. ¹H NMR (DMSO-d₆, 200 MHz): δ (ppm) = 9.07 (d, 8H, 3JHH = 5.88 Hz, Hα, pyridine), 8.92 (s, 8H, CH, pyrrole), 8.29 (d, 8H, Hβ, pyridine), 6.52 (s, 24H, C≡C), –3.05 (s, 24H, N═C═C≡N). ESI-MS (CH₃CN): m/z = 1619 [Ru₄(η⁶-C₆H₆)₄(TPP)Cl₈]. Anal. (C₆₀H₅₆N₄Cl₄Ru₄) C, H, N.
Compounds 2–8 were prepared by applying a method similar to that described for 1. Their spectroscopic and analytical data are provided in the Supporting Information.

**X-ray Crystallography.** Single crystals of 4 and 7 were mounted on a Stoe image plate diffraction system equipped with a φ circle goniometer, using Mo Kα graphite monochromated radiation (λ = 0.71073 Å) with φ range 0–200°, D_max − D_min = 12.45 – 0.81 Å, and increment of 0.8° and 1.0°, respectively. The structures were solved by direct methods using the program SHELXS-97. The refinement and all further calculations were carried out using SHELXL-97. In all cases, the H-atoms were included in calculated refinement and all further calculations were carried out using previously described. Briefly, 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT, Merck) was added at 250 µM concentration. DMSO at comparable concentrations did not show toxicity for the complexes for 24 h. Thereafter, the medium was replaced by fresh RPMI 1640 medium containing 10% fetal calf serum (FCS) and antibiotics. Gibco-BRL (Basel, Switzerland). The cells were grown in RPMI 1640 medium containing 10% fetal calf serum (FCS) and antibiotics.

**Results.**

**Determination of Cytotoxicity.** Cells were grown in 96-well cell culture plates (Corning, NY) until 75% confluent. The culture medium was replaced with fresh medium containing complexes 1–7 for concentrations varying from 0 to 100 µM, and cells were exposed to the complexes for 24 h. Thereafter, the medium was replaced by fresh medium and cell survival was measured using the MTT test as previously described. Briefly, 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT, Merck) was added at 250 µg/mL and incubation was continued for 2 h. Then the cell culture supernatants were removed, the cell layer was dissolved in PrOH/0.04 N HCl, and absorbance at 540 nm was measured in a 96-well multiwell-plate reader (Applied Optronics, South Plainfield, NJ) at 37 °C in a solution of dithiooxamide (Fluka, Buchs, Switzerland) (1 volume of 1 g/L dithiooxamide in MeOH and 2 volumes of 10% aqueous sodium acetate), washed with water, and counterstained with nuclear red, dehydrated, and analyzed under white light and under fluorescent light using filters at 420 ± 20 nm (BP 395–440, FT 460, LP 470).

**Supporting Information Available:** Spectroscopic and analytical data, UV–visible absorption spectra in dichloromethane, fluorescence spectra in methanol and X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org. The cif files for complexes 4 and 7 can be found at www.ccdc.cam.ac.uk/data_request/cif.

**References**


