Ruthenium Porphyrin Compounds for Photodynamic Therapy of Cancer

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Five 5,10,15,20-tetra(4-pyridyl)porphyrin (TPP) areneruthenium(II) derivatives and a *p*-cymeneosmium and two pentamethylcyclopentadienyliridium 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 phototoxicities toward melanoma cells when exposed to laser light at 652 nm. Cellular uptake and localization microscopy studies of $[Ru_4(\eta^6\text{-}C_6H_5CH_3)_4(TPP)Cl_8]$ and $[Rh_4(\eta^5\text{-}C_5Me_5)_4(TPP)Cl_8]$ 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 areneruthenium(II) derivatives represent a promising new class of organometallic photosensitizers able to combine chemotherapeutic activity with photodynamic therapeutic treatment of cancer.

Introduction

Cisplatin[1] is widely used for the treatment of many cancers[2,3] despite its high toxicity, undesirable side effects, and problems with drug resistance in primary and metastatic cancers.[4,5] 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,[6–10] 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 them to be selectively transported into cancer cells.[9,11–18] While most research has focused on Ru(III) and to a lesser extent Ru(IV) complexes, recent advances have been made using organometallic Ru(II) arene compounds that show considerable promise for the treatment of cancer and metastasis.[19–26]

One treatment combination modality might be to combine an organometallic agent as a chemotherapeutic agent with a photosensitizing agent and laser destruction of cancer using photodynamic therapy (PDT[a]). Activation of a photosensitizer by light at specific wavelengths leads to the production of singlet oxygen and radical species, resulting in direct tumor cell death, immune response, and damages to tumor vasculature.[27–30] Photosensitizers, mainly porphyric analogues, have been coupled to a wide range of biomolecules in order to improve their cellular uptake and PDT efficiency, including low-density lipoprotein (LDL), epidermal growth factor (EGF), antibodies, hormones, sugars, or vitamins.[31–36] Porphyrin derivatives concentrate in cancer cells[37,38] and are efficient photosensitizing agents.[39–41] Complexes of hematoporphyrin coordinated to platinum were recently developed to combine the cytotoxicity of platinum with the photodynamic action of porphyrin and show some promise.[42–46] One ruthenium porphyrin derivative demonstrated the production of reactive oxygen species necessary to the phototoxic process when activated by light but was not evaluated in experimental biological models.[47]

We were interested to determine if coordinating areneruthenium units to a porphyrin moiety would result in efficient chemotherapeutic phototherapeutic agents against human cancer cells able to combine the photodynamic action of porphyrin with the cytotoxicity of areneruthenium complexes. For comparison, osmium, iridium, and rhodium complexes were also prepared. We report here the synthesis and characterization of five 5,10,15,20-tetra(4-pyridyl)porphyrin (TPP) areneruthenium derivatives coordinated to benzene **1**, toluene **2**, *p*-cymene **3**, hexamethylbenzene **4**, and 1,4-$C_6H_4(COOEt)_2$ **5**. A *p*-cymeneosmium analogue **6** and two pentamethylcyclopentadienyl iridium and -rhodium analogues **7** and **8**, respectively, were also prepared. The effect and the cellular uptake of these complexes have been assessed in human Me300 melanoma cells as a model of human cancer.

Results

The dinuclear areneruthenium complexes $[Ru(\eta^6\text{-arene})(\mu\text{-}Cl)Cl]_2$ (arene = C_6H_6, $C_6H_5CH_3$, *p*-^{i}PrC$_6$H$_4$Me, C_6Me_6, and 1,4-$C_6H_4(COOEt)_2$) react in refluxing methanol with TPP to give, in excellent yield, the corresponding tetranuclear complexes $[Ru_4(\eta^6\text{-arene})_4(TPP)Cl_8]$ (**1**– **5**) (see Scheme 1). Complexes **1** and **5** are slightly soluble in DMSO and insoluble in $(CH_3)_2CO$, CH_2Cl_2, MeOH, and H_2O, while complexes **2**−**4** are soluble in all these solvents.

In a similar manner, the dinuclear *p*-cymeneosmium complex $[Os(\eta^6\text{-}p\text{-}^iPrC_6H_4Me)(\mu\text{-}Cl)Cl]_2$ reacts with TPP to form the tetranuclear areneosmium complex $[Os_4(\eta^6\text{-}p\text{-}^iPrC_6H_4Me)_4$-

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[a] Abbreviations: TPP, 5,10,15,20-tetra(4-pyridyl)porphyrin; PDT, photodynamic therapy; LDL, low-density lipoprotein; EGF, epidermal growth factor; MTT, thiazolyl blue tetrazolium bromide; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; DAPI, 4′,6′-diamidino-2-phenylindolyl hydrochloride.

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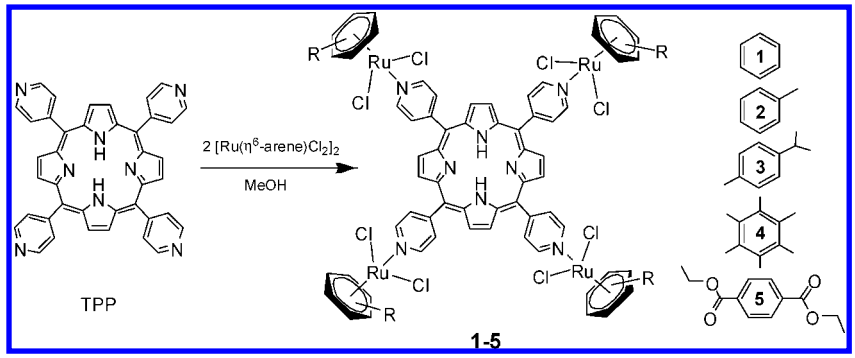
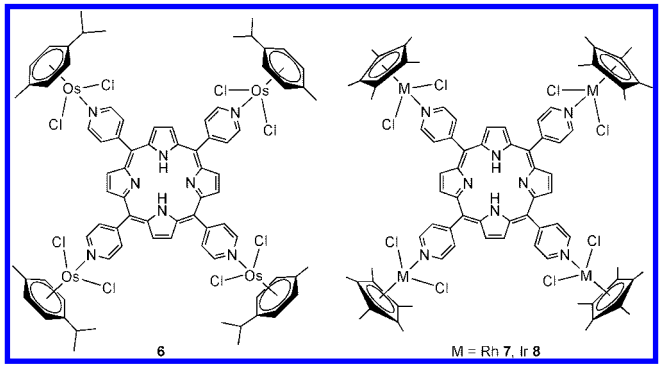
Scheme 1. Synthesis of **1**–**5**



Scheme 2. Structures of **6**–**8**



(TPP)Cl_8] **6**. The isoelectronic rhodium and iridium pentamethylcyclopentadienyl derivatives **7** and **8** are obtained in methanol from the reaction of [M(η^5-C_5Me_5)(μ-Cl)Cl]$_2$ (M = Rh, Ir) with TPP (Scheme 2). Complex **8** is sparingly insoluble in DMSO and insoluble in $(CH_3)_2CO$, CH_2Cl_2, MeOH, and H_2O, while complexes **6** and **7** are slightly soluble in these solvents.

The ^{1}H NMR spectra of **1**–**8** were recorded in DMSO-d_6 because of the low solubility of the complexes in other solvents. All complexes show, in addition to the signals of the corresponding η^6-arene or η^5-C_5Me_5 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 $\delta \approx -3.1$ 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 $[Ru_4(NO)_4(TPP)Cl_{16}]^{4-}$ and $[Ir_4(\eta^5\text{-}C_5Me_5)_4(Zn\text{-}TPP)\{S_2C_2(B_{10}H_{10})\}_4(THF)_2]$.[25] 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 IC_{50} around 50 μM, while complexes **1**, **2**, and **5** were less cytotoxic with $IC_{50} > 100$ 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

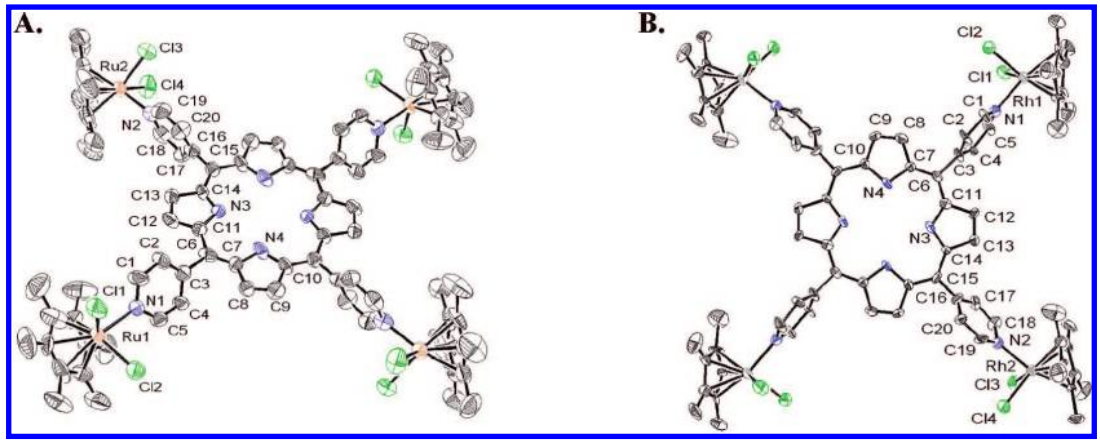


Figure 1. ORTEP representation at 50% probability level and with hydrogen atoms being omitted for clarity. (A) Compound **4**, selected bond lengths (Å) and angles (deg): Ru(1)−Cl(1) 2.416(4), Ru(1)−Cl(2) 2.404(3), Ru(1)−N(1) 2.162(7), Ru(2)−Cl(3) 2.450(4), Ru(2)−Cl(4) 2.395(3), Ru(2)−N(2) 2.100(10); 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) 2.425(4), Rh(1)−N(1) 2.154(9), Rh(2)−Cl(3) 2.428(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).

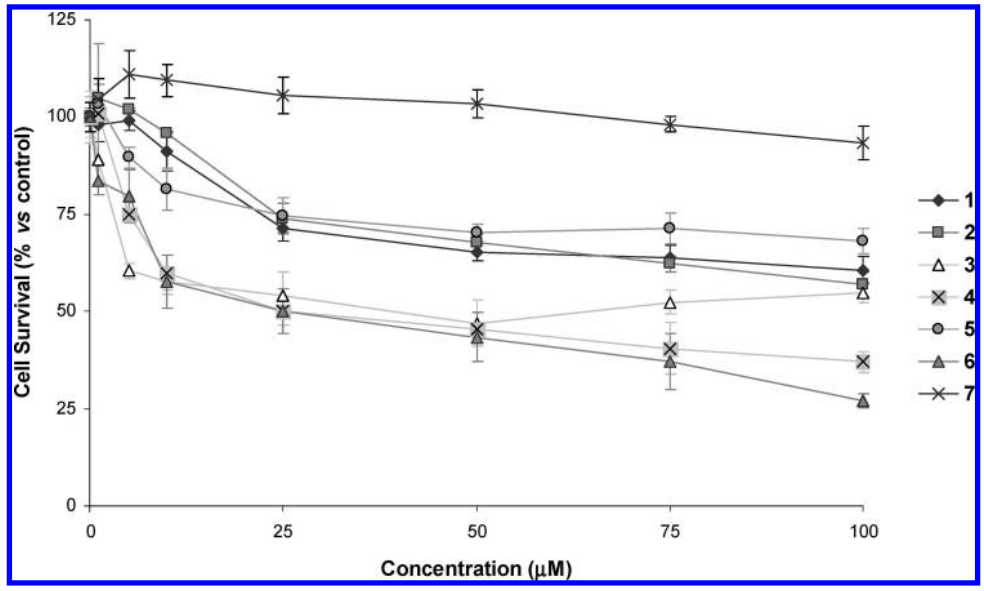


Figure 2. Concentration dependence of the cytotoxicity of organometallic porphyrin complexes **1**–**7** in Me300 melanoma cells after 24 h of exposure in the dark determined by the MTT survival assay.

cells, observed as red fluorescent spots, but no nucleus-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/cm^2 and light doses from 5 to 30 J/cm^2. 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/cm^2 of light exposition led to 60–80% phototoxicities, whereas this level of phototoxicity was reached with 30 J/cm^2 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 areneruthenium 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/cm^2 and up to 48 J/cm^2 are generally used.[37,38,48] Therefore, the photosensitizing properties of complexes **1**–**5** are quite efficient because 5 J/cm^2 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

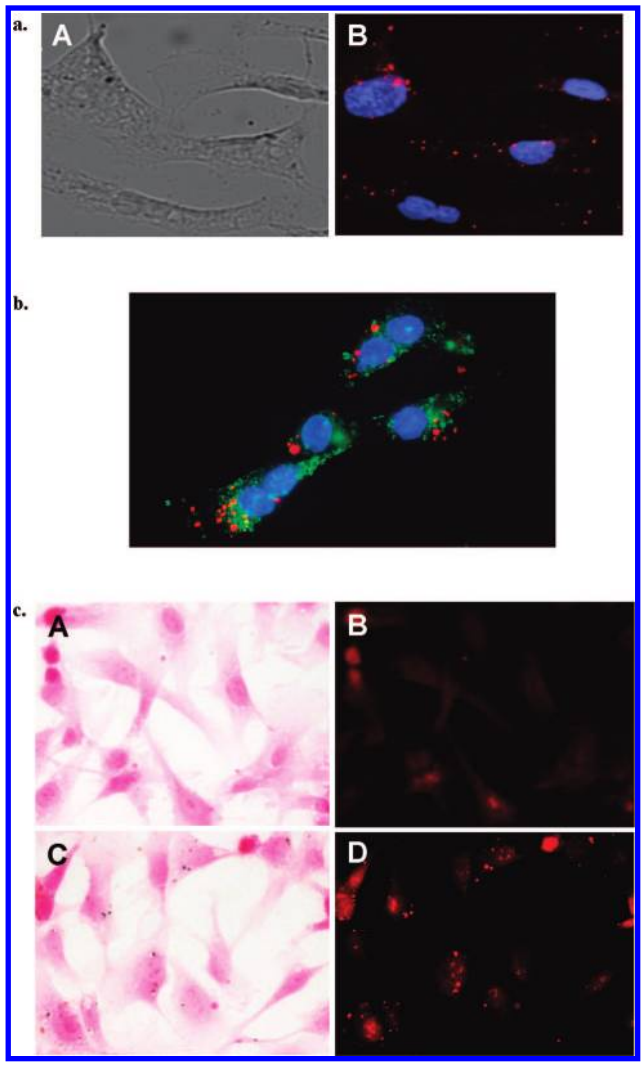


Figure 3. Evaluation of the accumulation of **2** in Me300 human melanoma cells by light and fluorescence microscopy. Me300 melanoma cells were grown on histological glass slides and exposed to **2** (10 μM) for 24 h in the dark. (a) Cells were fixed in 4% buffered formol and treated with DAPI (3 μM, 15 min) and examined. (A) White light source. (B) Overlay of excitation at 365 nm for DAPI staining of cell nuclei (blue) and excitation at 536 nm, indicating accumulation of **2** (red spots) in cells. (b) Cells were incubated with Lysotracker (500 nM, 30 min), then fixed in 4% buffered formol, stained with DAPI (3 μM, 30 min), and examined. Cell nuclei are stained blue with DAPI, lysosomes green with Lysotracker, and accumulation of **2** appears as red spots. (c) Cells were fixed in 4% buffered formol, treated with dithiooxamide, and counterstained with nuclear red. Cells not incubated (A and B) or incubated (C and D) with **2** were analyzed under white light source (A and C) indicating accumulation of **2** (ruthenium, dark-brown spots) in cells and under fluorescent microscopy (B and D) with excitation at 420 nm, indicating accumulation of **2** (porphyrin, red fluorescent spots) in cells.

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 arene ligand. Importantly, this observation indicates that since the arene coordination can be easily modified, a large range of photosensitizing complexes could be prepared with customized arenes bearing, for example, targeting agents, inhibitors of resistance mechanisms, or other cytotoxic/cytostatic agents.

In conclusion, we have prepared a series of organometallic-modified porphyrin compounds and shown that the ruthenium

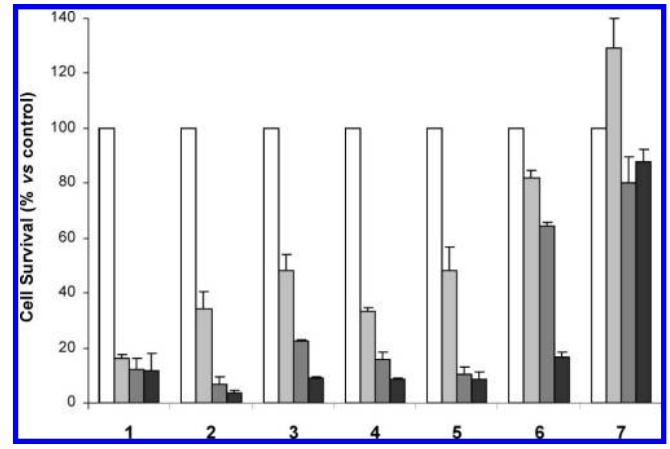


Figure 4. Photodynamic sensitivities for compounds **1**–**7** in Me300 melanoma cells. Survival by MTT test was assessed for cells exposed to increasing doses of light at 652 nm wavelength (0 J/cm^2 in white, 5 J/cm^2 in light gray, 15 J/cm^2 in dark gray, and 30 J/cm^2 in black). Cells were incubated with photosensitizers (10 μM) for 24 h before light treatment.

Table 1. UV–Visible Absorption Maxima and Molar Extinction Coefficients [λ nm ($\epsilon \times 10^{-3}$ M^{-1}·cm^{-1})] Determined in CH_2Cl_2 and Fluorescence Quantum Yields (φ) of Photosensitizers in MeOH at 648 nm after 410 nm Excitation

	Soret band	Q band IV	Q band III	Q band II	Q band I	φ_f (%)
TPP	417 (212.9)	512 (24.2)	545 (9.4)	587 (9.8)	642 (5.7)	10.4
1	427 (198.5)	515 (21.7)	550 (8.6)	590 (9.5)	645 (7.1)	6.1
2	423 (156.5)	516 (13.6)	550 (7.7)	590 (6.7)	645 (4.4)	7.4
3	422 (188.2)	515 (19.8)	550 (10.1)	590 (6.9)	645 (4.8)	7.9
4	422 (200.0)	515 (18.7)	550 (8.0)	589 (6.2)	645 (2.7)	7.3
5	423 (208.8)	517 (30.3)	551 (18.6)	590 (15.8)	647 (10.3)	7.2
6	418 (183.4)	514 (25.5)	548 (11.1)	588 (9.7)	643 (6.6)	7.0
7	423 (216.2)	514 (26.7)	549 (12.1)	589 (10.6)	648 (5.7)	7.9
8	418 (203.9)	513 (15.8)	547 (11.7)	588 (8.3)	644 (7.3)	4.5

facilitates uptake and results in highly active photosensitizing drugs under a light dose irradiation of only 5 J/cm^2. 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(η^6-C_6H_6)(μ-Cl)Cl]$_2$, [Ru(η^6-$C_6H_5CH_3$)(μ-Cl)Cl]$_2$, [Ru(η^6-*p*-^{i}PrC_6H_4Me)(μ-Cl)Cl]$_2$, [Ru(η^6-C_6Me_6)(μ-Cl)Cl]$_2$, [Ru(η^6-1,4-C_6H_4(COOEt)$_2$)(μ-Cl)Cl]$_2$, [Os(η^6-*p*-^{i}PrC_6H_4Me)(μ-Cl)Cl]$_2$, [Rh(η^5-Cp*)(μ-Cl)Cl]$_2$, and [Ir(η^5-Cp*)(μ-Cl)Cl]$_2$ were prepared according to published methods.[49–54] ^{1}H 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 an Uvikon 930 spectrophotometer and fluorescence spectra on a Perkin-Elmer LS50 spectrofluorometer.

Syntheses. [Ru$_4$(η^6-C_6H_6)$_4$(TPP)Cl$_8$] (1). A mixture of [Ru(η^6-C_6H_6)(μ-Cl)Cl]$_2$ (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%. ^{1}H NMR (DMSO-d_6, 200 MHz): δ (ppm) = 9.07 (d, 8H, $^3J_{H\text{-}H}$ = 5.88 Hz, H$_\alpha$, pyridine), 8.92 (s, 8H, CH, pyrrole), 8.29 (d, 8H, H$_\beta$, pyridine), 6.52 (s, 24H, C_6H_6), −3.05 (s, 2H, NH). ESI-MS (CH_3CN): m/z = 1619 [Ru$_4$(η^6-C_6H_6)$_4$(TPP)Cl$_8$]. Anal. ($C_{64}H_{50}N_8Cl_8Ru_4$) 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 = 0.710 73 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.[55] The refinement and all further calculations were carried out using SHELXL-97.[56] In all cases, the H-atoms were included in calculated positions and treated as riding atoms using the SHELXL default parameters. Examination of the structures with PLATON[57] reveals voids between the areneruthenium porphyrin molecules. Indeed, voids corresponding to solvent molecules were found. Therefore, new data sets corresponding to omission of the missing solvents were generated with the SQUEEZE algorithm[58] and the two structures were refined to convergence. However, in both cases the non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Crystallographic details are summarized in Table S1 (see Supporting Information). **4** and **7** were presented using ORTEP representation[59] at the 50% probability level and with hydrogen atoms being omitted for clarity.

Cells and Cell Treatment. Human Me300 melanoma cells were provided by Dr. D. Rimoldi, Ludwig Institute of Cancer Research, Lausanne branch. All cell culture reagents were obtained from Gibco-BRL (Basel, Switzerland). The cells were grown in RPMI 1640 medium containing 10% fetal calf serum (FCS) and antibiotics. The organometallic complexes were dissolved in DMSO as 10 mM stock solution and then diluted in complete medium to the required concentration. DMSO at comparable concentrations did not show any effects on cell cytotoxicity (results not shown).

Determination of Cytotoxicity. Cells were grown in 48-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.[60] 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 ^{i}PrOH/0.04 N HCl, and absorbance at 540 nm was measured in a 96-well multiwell-plate reader (iEMS Reader MF, Labsystems, Bioconcept, Switzerland) and compared to the values of control cells incubated without complexes. Experiments were conducted in triplicate wells and repeated at least twice.

Determination of Phototoxicity. Cells were grown in 96-well cell culture plates (Corning, NY) until 75% confluent. The culture media were replaced with fresh medium containing complexes **1**–**7** at 10 μM concentration, and the cells were exposed to the complexes for 24 h. Thereafter, the media were replaced with RPMI without phenol red containing 5% FCS and cells were irradiated at 652 nm using a diode laser (Applied Optronics, South Plainfield, NJ) coupled to a frontal diffuser (Medlight SA, Ecublens, Switzerland), at an irradiance of 20 mW/cm^2 and light doses ranging between 5 and 30 J/cm^2. Experiments were conducted in triplicate. Analysis of cell phototoxicity using the MTT assay as described above was performed after a further incubation of 24 h after irradiation and compared to the values of control cells without laser irradiation.

Microscopy Experiments. Cells were grown on histological slides in complete medium until 25% confluent and exposed to compound **2** (10 μM) for 24 h in the dark. At the end of incubation, Lysotracker (Invitrogen, Paisley, U.K.) was added at 500 nM concentrations for a further 30 min of incubation at 37 °C. Slides were fixed for 10 min in buffered formol, and nuclei were stained with 4′,6′-diamidino-2-phenylindolyl hydrochloride (DAPI, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Then slides were mounted with 20% PBS−glycerol and analyzed under a fluorescence microscope (Axioplan2, Carl Zeiss, Feldbach, Switzerland) with filters set at 365 ± 5 nm excitation light (BP 365/12, FT 395, LP 397) for DAPI, 470 ± 20 nm excitation light (BP 450–490, FT 510, BP 515–565) for Lysotracker, and 535 ± 25 nm excitation light (BP 510–560, FT 580, LP 590) for porphyrins.

For colocalization studies of porphyrins and ruthenium, cells were grown on histological slides in complete medium until 25% confluent, exposed to compound **2** (10 μM) for 24 h in the dark, and fixed for 10 min in buffered formol. Slides were incubated overnight 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).

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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.

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