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DNA binding and photocleavage properties and apoptosis-inducing activities of a ruthenium porphyrin complex [(Py-3′)TPP-Ru(phen)$_2$Cl]Cl and its heterometallic derivatives

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ABSTRACT

The interactions of a ruthenium porphyrin complex [(Py-3′)TPP-Ru(phen)$_2$Cl]Cl (phen = 1,10-phenanthroline, (Py-3′)TPP = 5-(3′-pyridyl-10,15,20-triphenylporphyrin) (**1**) and its heterometallic derivatives, [Ni(Py-3′)TPP-Ru(phen)$_2$Cl][PF$_6$] (**2**) and [Cu(Py-3′)TPP-Ru(phen)$_2$Cl][PF$_6$] (**3**), with calf thymus DNA have been investigated by spectroscopic and viscosity measurements in this study. The results showed that these synthetic complexes can bind to double strand helix DNA in groove binding mode, and the intrinsic binding constants of complexes **1**, **2** and **3**, as calculated according to the decay of the Soret absorption, are $(1.35 \pm 0.5) \times 10^5\ M^{-1}$ ($s = 4.2$), $(1.29 \pm 0.5) \times 10^5\ M^{-1}$ ($s = 5.6$) and $(1.22 \pm 0.5) \times 10^5\ M^{-1}$ ($s = 6.2$) (s is the binding-site size), respectively, which are consistent with those obtained from ethidium bromide-quenching experiments. Further investigations on the photocleavage properties of these complexes on plasmid pBR 322 DNA showed that complexes **1**, **2** and **3** could cleave single chain DNA and convert DNA molecules from supercoiled form to the nicked form. As determined by MTT assay, the complexes were also identified as potent antiproliferative agents against A375 human melanoma cells, MCF-7 human breast adrenocarcinoma cells, Colo201 human colon adenocarcinoma cells and HepG2 human liver cancer cells. Complex **1** inhibits the growth of A375 cells through induction of apoptotic cell death and G0/G1 cell cycle arrest. Further investigation on intracellular mechanisms indicated that Complex **1** induced depletion of mitochondrial membrane potential ($\Delta\Psi_m$) in A375 cells through regulating the expression of pro-survival and pro-apoptotic Bcl-2 family members. Our results suggest that ruthenium porphyrin complexes could be candidates for further evaluation as chemopreventive and chemotherapeutic agents for human cancers.

1. Introduction

Porphyrins and their metalloporphyrins have been widely studied due to their spectroscopic and electrochemical properties, and novel biological activities. Ruthenium (Ru), a rare transition metal of the platinum group, possesses several favorable properties suited to rational anticancer drug design, such as the higher coordination number by comparing with platinum, which could provide additional coordination sites that could potentially be used to fine-tune the properties of the complexes [1]. Ru porphyrin complexes have attracted much attention over the last decade due to their application potential as anticancer drugs [1–5] and novel DNA-binding abilities [6–8]. This kind of complexes could bind to DNA by intercalating, groove binding and electrostatic modes, and those ones with larger aromatic intercalating ligands often show higher binding abilities [9–11]. For instance, complexes with porphyrin coordinated to two [Ru (bipy)$_2$Cl]$^+$ (bipy = 2,2-bipyridine) groups, could convert plasmid pBR 322 DNA from the supercoiled to the nicked form, and even to the linear form with the presence of light [12]. Ru complexes attached to asymmetric porphyrins, such as [Ru(L)$_2$MPyTPPCl]Cl (L = bpy, phen or pip; bpy = 2,2-bipyridine; phen = 1,10-phenanthroline; pip = 2-Phenyl-1*H*-1,3,7,8-tetraazacyclopenta[*l*]phenanthrene; MPyTPP = 5-(4′-pyridine)-10,15,20-triphenyl porphyrin), exhibited high affinity to nucleic acids,

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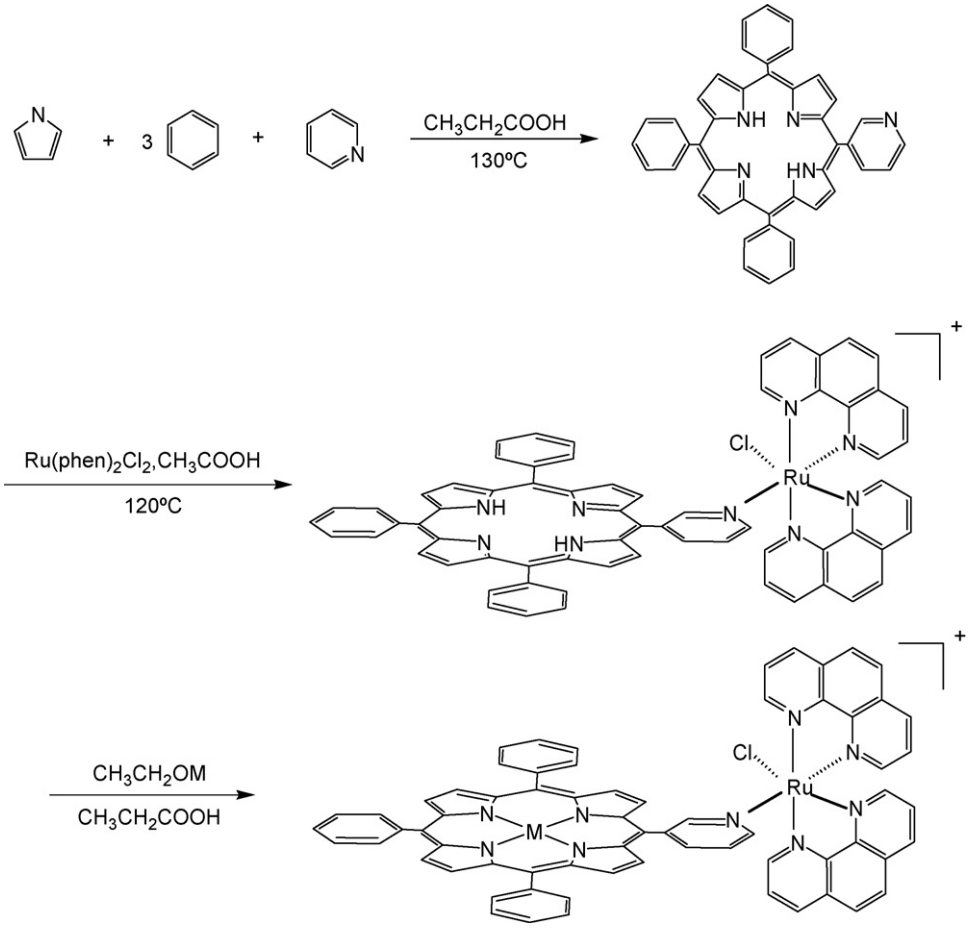

Scheme 1. Schematic routes for synthesis of Ru porphyrin complexes **1** (M = 2H); **2** (M = Ni); **3** (M = Cu).

including calf thymus DNA (CT-DNA), RNA and G-quadruplex, indicating that this type of complexes has potential in chemotherapy [13–15].

However, studies also reported that the DNA-binding abilities of Ru complexes were not positively related to their antitumor activities. One of the reasons should be their difference in the solubility, which may affect their effective crossing of the cell membrane [16]. Thus, during the past years, a lot of potent Ru porphyrin complexes have been designed to achieve better solubility and greater chemopreventive efficacy by structural modifications. Studies have revealed that the antitumor activity of Ru complexes can be significantly improved by introducing hydrophobic porphyrin molecules [17–19]. For example, increase in lipid/water partition and membrane binding has been found in Ru complexes with cationic porphyrin groups [20]. Schmitt and co-workers have shown that ruthenated porphyrin, $[Ru_4(\eta^6\text{-}C_6H_5CH_3)_4(TPP)Cl_8]$ (TPP = 5,10,15,20-tetra(4-pyrid-yl) porphyrin), could effectively cross the cell membrane and accumulate in the granular structures of melanoma cells [21]. In the present study, the synthesis of a Ru porphyrin complex, [(Py-3′)TPP-Ru(phen)$_2$Cl]Cl ((Py-3′)TPP = 5-(3′-pyridyl-10,15,20-triphenylporphyrin) and its heterometallic derivatives (Scheme 1), and their interaction with CT-DNA were investigated. The photocleavage properties of these complexes against the plasmid PBR 322 DNA were also examined by gel electrophotolysis experiments. Moreover, we screened their antiproliferative and apoptosis-inducing activities and elucidated the molecular mechanisms of apoptotic cell death induced by the complexes in selected cancer cells.

2. Materials and methods

2.1. Materials

Pyrrole (CP, Fluka), benzaldehyde (AR, Fluka) and 3-aldehyde pyridine (AR, Acros) were purchased commercially and used without further purification unless specially noted. Calf thymus DNA (CT-DNA) was purchased from the Sino-American Biotechnology Company and the plasmid DNA, pBR322 DNA, was from the Sangon Biotechnology Company (Canada). DNA Solutions in 5 mM Tris–HCl buffer (pH 7.2), 50 mM NaCl gave a ratio of UV absorbance (260/280 nm) of 1.8–1.9:1, indicating that the DNA was sufficiently free of protein. The concentration of CT-DNA was determined spectrophotometrically by using the molar absorption coefficient of 6600 M^{-1} cm^{-1} (260 nm). Thiazolyl blue tetrazolium bromide (MTT) and propidium iodide (PI) were obtained from Sigma. Dulbecco's modified Eagle's medium (DMEM), RPMI-1640 medium, bovine calf serum, and the antibiotic mixture (penicillin–streptomycin) were purchased from Invitrogen (Carlsbad, CA). Milli-Q water was used to prepare buffer solutions.

2.2. Physical measurements

Elemental analyses for C, H and N were carried out with a PerkinElmer 240C elemental analyser. Electrospray ionisation mass spectra (ESI–MS) were acquired on a Thermo Finnigan LCQ DECA XP ion trap mass spectrometer, equipped with an ESI source. The concentrations of Ru, Ni and Cu were determined by ICP–AES analysis. The compounds (5 mg) were digested with 3 mL concentrated