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Cytotoxicity modulation of ruthenium(II) tris-bathophenanthroline complexes with systematically varied charge

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ABSTRACT

A series of four ruthenium(II) complexes $Ru(bp)_n(bps)_{3-n}$ (n = 0-3; bp = 4,7-diphenyl-1,10-phenanthroline or bathophenanthroline; and bps = disulfonated 4,7-diphenyl-1,10-phenanthroline or bathophenanthroline disulfonate) bearing different charges were synthesized and characterized. In aqueous media, all complexes displayed similar photophysical properties which makes this series ideal to study the effect of charge on the cytotoxicity of Ru(II) complexes. $Ru(bp)_3^{2+}$ (**1**) and $Ru(bps)_3^{4-}$ (**4**) are known photosensitizers that penetrate cancer cells and possess a significant light-induced cytotoxicity. The newly conceived neutral $Ru(bp)_2(bps)^0$ (**2**) and dianionic $Ru(bp)(bps)_2^{2-}$ (**3**) were also found to have significant uptake as well as light-activation properties. Importantly, the cytotoxicity in the dark was successfully tuned upon systematic charge modulation of the complexes. The cationic complex **1** was potent against 5 out of 6 cell lines tested (MDA-MB-231, MCF-7, B16, SF and ML2) and displayed the highest cytotoxicity among the tested complexes with IC_{50} values of 4.0, 3.6, 1.7, 1.0 and 2.9 μM, respectively. The two anionic complexes, **3** and **4**, with respective overall charges of −2 and −4, were not potent against any of the cell lines in the dark ($IC_{50} > 200$ μM), whereas the neutral molecule, complex **2**, was potent against 3 out of 6 cell lines in the dark (B16, SF and ML2) and exhibited an intermediate activity with IC_{50} values of 12.8, 3.0 and 10.3 μM, respectively. All complexes presented significant phototoxicity when activated at 6 h post-incubation, which is consistent with their fast uptake and ability to produce singlet oxygen. The localization of complexes **1–4** within the cells was found to be dependent on the charge and photoexcitation conditions. The charge-activity relationship elucidated in this work facilitates the development of new sensitizers for photodynamic therapy.

1. Introduction

Cancer is typically detected at a late stage leading to high mortality rates among patients [1,2], and is often correlated with the formation of tumors due to uncontrolled malignant cell growth in undesired areas of the body [3]. To date, there is a lack of cancer-curing drugs that are efficient, specific and possess little to no side effects. Cisplatin and its analogues are common chemotherapeutic agents used to treat cancer patients [2,4,5]. These Pt(II) transition metal complexes are highly potent and are commonly associated with severe side effects and/or drug resistance which compromise their effectiveness in therapy [6]. The mechanism of action of cisplatin was extensively investigated and summarized in detailed review articles [7,8]. It was found that the active species leading to cross-reactivity of cisplatin with DNA is the Pt(II)-aqua complex that results from chloride substitution by water [9,10]. In order for the drug to have minimal side effects, the most intuitive strategy is to specifically target cancer cells through a distinct molecular mechanism, which is often exclusive to the type of cancer targeted and incurs high production and treatment costs. Photodynamic therapy (PDT), a process that utilizes light to drive therapeutic processes, is known for several decades and is clinically applied to treat certain types of diseases such as psoriasis, vitiligo and skin cancer [11–13]. The commonly investigated mechanisms of PDT focus on formation of singlet oxygen ($^1O_2$) and subsequently reactive oxygen species (ROS) as well as photobinding and cleavage of DNA [12]. In addition, photoactivatable chemotherapy (PACT), the photochemical transformation of prodrugs to active potent molecules, has been shown to be another effective mechanism that is worth investigating [14]. Based on these proposed mechanisms, several transition-metal complexes bearing varied ligand

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frameworks were designed and tested for their potency against human cancer cell lines [14–24]. Octahedral Ru(II) polypyridyl complexes are typically luminescent, which facilitates the visualization of tumors through fluorescence imaging [12]. In addition, their absorption can be readily tuned through ligand modification to match the therapeutic window [25,26]. Importantly, Ru(II) octahedral complexes are active even in the presence of large quantities of glutathione, a molecule known to deactivate square planar Pt(II) complexes such as cisplatin [7,14,27]. The utilization of Ru(II) for photodynamic therapy of cancer was recently reviewed by Gasser et al. [26]. Different mechanisms of cancer cells death are plausible depending on the physical and chemical properties of the therapeutic agent. The production of reactive oxygen species (ROS) via quenching of triplet metal-to-ligand charge transfer (^{3*}MLCT) excited state is of prime importance in PDT [28–31]. A large amount of phenanthroline-based ligands [32] and ruthenium complexes [25,26,33–36] coordinated to nitrogen and carbon based ligands were tested for their ability to target cancer cells. Our research effort was focused on the synthesis and characterization of series of Ru(II) complexes bearing varied ligand frameworks. In previous studies, the dicationic Ru(II) tris(bathophenanthroline) [$Ru(bp)_3^{2+}$ (**1**)] was found to intercalate with DNA [37–39] and was significantly toxic towards cancer cell lines [30]. However, the sulfonated tetraanionic counter-part, Ru(II) tris(bathophenanthroline disulfonate) [$Ru(bps)_3^{4-}$ (**4**)], was found to bind to proteins [40,41] and lacked any cytotoxicity in the dark [30]. Interestingly, even anionic Ru(II) and Pt(II) complexes bearing bps ligands possessed significant cellular uptake. [30,42] Here, we sought the synthesis and characterization of two new complexes, $Ru(bp)_2(bps)^0$ (**2**) and $Ru(bp)(bps)_2^{2-}$ (**3**), that together with **1** and **4** elucidate the effect of charge on the different chemical, physical and biological properties of these complexes.

2. Experimental section

2.1. Instrumentation

Absorption data were acquired on a Shimadzu UV-1650PC spectrophotometer. Photoluminescence was measured on a FL/FS920 spectrofluorimeter from Edinburgh Instruments equipped with a 450 W Xe arc lamp and a Peltier cooled, red sensitive PMT (R2658P, Hamamatsu). Lifetimes and transient absorption data were collected on an LP920 laser flash photolysis system from Edinburgh Instruments. The excitation pump source was a Vibrant LD 355 II Nd:YAG/OPO system (OPOTEK), kinetic traces were collected with a PMT (R928 Hamamatsu) and transient absorption difference spectra using an iStar ICCD camera (Andor Technology). Origin 8.1 was used for fitting kinetic traces. Real-time absorbance was measured using an ocean optic spectrometer (HR2000+) coupled to a deuterium/halogen light sources (DT-MINI-2-GS, Ocean Optics). Transmittance was acquired at a right angle of an incident He/Cd laser excitation (λ_{exc} = 442 nm and $P_{incident}$ = 30 mW). ^{1}H spectra were recorded on an AC500 Bruker spectrometer operating at 500 MHz. Chemical shifts are reported in delta (δ) units, expressed in parts per million (ppm) using the residual protonated solvent as an internal standard DMSO-d_6 2.50 ppm. The multiplicity of signals is designated by the following abbreviations: s, singlet; d, doublet; dd, doublet of doublets; m, multiplet. HPLC data were acquired on a Shimadzu quaternary pump coupled with a PDA detector and equipped with a C18 column (Column Technologies Inc.) and fitted with a Phenomenex guard column using a published method [14]. High-resolution ESI–MS measurements were performed at Michigan State University Mass Spectrometry Core Facility; MALDI-TOF MS at the Lebanese American University proteomics facility (4800 PLUS MALDI TOF/TOF Analyzer from AB Sciex); and elemental analyses at Atlantic Microlab facilities, USA.

2.2. Materials

LiCl, $RuCl_3 \cdot 3H_2O$, bathophenanthrolinedisulfonic acid disodium salt trihydrate ($Na_2bps \cdot 3H_2O$), bathophenanthroline (bp), tetrabutyl ammonium chloride (TBACl), 4′,6-Diamidino-2-phenylindole dihydrochloride (DAPI nucleic acid stain), and all other chemicals and solvents were obtained from Sigma-Aldrich and used without any further purification.

2.3. Syntheses

$Ru(bps)_2Cl_2$ and $Ru(bp)_2Cl_2$ were prepared in near quantitative yields by literature methods [43,44]. According to NMR studies [45], the predominate isomer for sulfonate is the meta substituted as depicted by Fig. 1.

2.3.1. $Ru(bp)_3Cl_2$ (1)

This complex was prepared by modified literature methods [44,46]. $RuCl_3 \cdot 3H_2O$ (0.191 mmol) and bp (0.75 mmol) were added to 10 mL of N_2 degassed ethylene glycol. The mixture was refluxed for 4 h under nitrogen. After cooling to room temperature, a saturated aq. KPF_6 solution was added, which produced a dark orange precipitate that was collected by vacuum filtration. The product was dissolved in the minimum amount of acetone and saturated aq. tetrabutylammonium chloride solution was then added to produce an orange precipitate that was collected by vacuum filtration and purified by column chromatography (LH-20 sephadex column; eluent: EtOH). The orange band was collected and evaporated to afford the desired product as orange solid (yield 67%). ^{1}H NMR (dmso-d_6, 500 MHz): δ 8.37 (d, J = 5.5 Hz, 6H), 8.29 (s, 6H), 7.85 (d, J = 5.5 Hz, 6H), 7.72-7.61 (m, 30H). MALDI-TOF MS (m/z, amu): 1098.08 $[M]^+$.

2.3.2. $Ru(bp)_2(bps)$ (2)

$Ru(bp)_2Cl_2$ (0.090 mmol) and bps ligand (0.099 mmol) were added to 10 mL of N_2-degassed 1:1 ethanol/water mixture. The

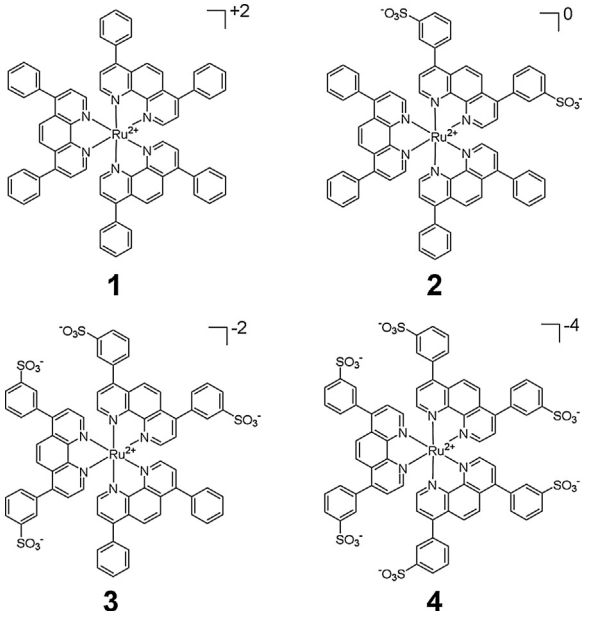

Fig. 1. Chemical structures of a series of ruthenium complexes bearing different overall charges: $Ru(bp)_3^{2+}$ (**1**); $Ru(bp)_2(bps)^0$ (**2**); $Ru(bp)(bps)_2^{2-}$ (**3**) and $Ru(bps)_3^{4-}$ (**4**).