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Research paper

Visible light-induced cytotoxicity of Ru,Os–polyazine complexes towards rat malignant glioma

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ABSTRACT

Transition metal complexes capable of visible light-triggered cytotoxicity are appealing potential candidates for photodynamic therapy (PDT) of cancer. Two monometallic polyazine complexes, $[(Ph_2phen)_2Ru(dpp)]^{2+}$ (**1**) and $[(Ph_2phen)_2Os(dpp)]^{2+}$ (**2**) (Ph_2phen = 4,7-diphenyl-1,10-phenanthroline; dpp = 2,3-bis(2-pyridyl)pyrazine), were synthesized, characterized and studied as light activated drugs to kill rat malignant glioma F98 cells. Compounds **1** and **2** display strong absorption in visible spectrum, oxygen-mediated DNA and BSA photocleavage and significant photocytotoxicity under blue light irradiation along with negligible activity in the dark. Both compounds show approximately five-fold higher cytotoxicity than the traditional chemotherapeutic drug cisplatin. Furthermore, compound **2** shows promising photocytotoxicity in F98 rat malignant glioma cells within the phototherapeutic window with an IC_{50} value of (86.07 ± 8.48) μM under red light (625 nm) irradiation.

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1. Introduction

Photodynamic therapy (PDT) is a noninvasive treatment that uses a photosensitizer (PS), low energy light, and in most cases $^3O_2$ to produce photocytotoxic reactive oxygen species (ROS) to damage neoplastic cells [1]. The generation of $^1O_2$ is believed to occur via energy transfer from the excited photosensitizer (Fig. 1) [2]. This minimally-invasive, light-activated therapeutic treatment allows for accurate targeting of tumor cells, and reduces side effects associated with systemic chemotherapy [3]. A variety of organic PDT agents have been approved for clinical use or are undergoing clinical trials, including porphyrins, chlorins and phthalocyanines [4]. However, these conventional PDT agents suffer from dark toxicity, prolonged skin sensitivity and hepatotoxicity, greatly limiting their widespread application [5]. Transition metal complexes that have tunable coordination environments and varied spectral and redox properties are promising candidates as the next generation of PDT agents [6]. Among them, ruthenium complexes with polypyridyl ligands, such as derivatives of $[Ru(bpy)_3]^{2+}$ (bpy = 2,2′-bipyridine), have been widely studied due to their active interaction with cellular components (DNA, RNA, and proteins) [7]. Ideally, PDT drugs need to be operative in the phototherapeutic window (600–900 nm), where light can efficiently penetrate bodily tissues [8]. However, few reported metal complexes were able to be photoactivated with low energy light [9]. Thus in this promising research area, it is still highly desirable to find novel PDT agents with enhanced activity within the phototherapeutic window and towards aggressive tumors such as high-grade malignant glioma (MG).

Aggressive, infiltrative MGs make up approximately 1.35% of the total cancer cases reported in 2013 [10]. The survival rate for MG is extremely low, with only 17.7% living beyond 1-year of diagnosis [11]. The lack of external cellular growth control, infiltrative growth pattern, and resistance to host immunologic defences make MG cells a particularly aggressive therapeutic challenge. MGs are resistant to virtually all forms of traditional cancer therapy, including radiation therapy, chemotherapy, and ablative surgical therapy as well as combinations of these procedures. Upon initial diagnosis of glioblastoma multiforme (GBM), standard treatment consists of maximal surgical resection, radiotherapy, and concomitant/adjuvant chemotherapy with temozolomide (TMZ) [12]. However, over 95% of all patients with MG die within two years, regardless of the type of therapy used [13]. TMZ, as the most commonly used

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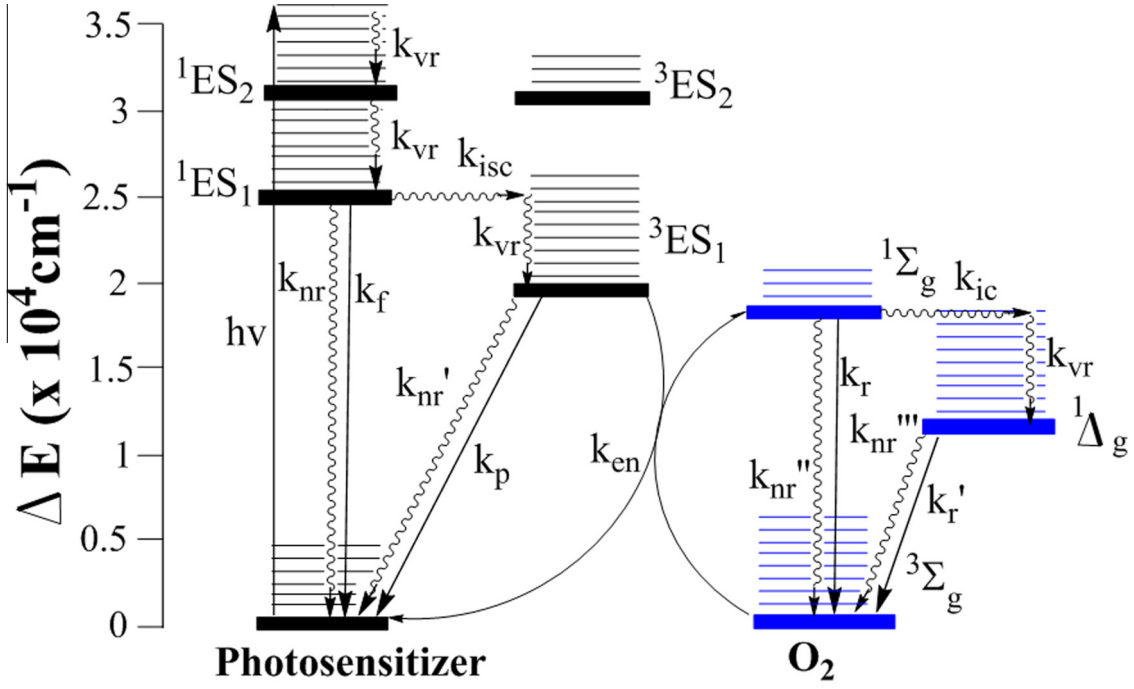

Fig. 1. State diagram illustrating the mechanisms of action for drugs displaying oxygen-dependent photodynamic action with *k*'s (rate constants) for f (fluorescence), p (phosphorescence), r (radiative), nr (nonradiative), ic (internal conversion), isc (intersystem crossing), en (energy transfer) and vr (vibration). The electronic state $^1\Delta_g$ is observed at 1270 nm or 0.97 V, $^1\sum_g$ is estimated at 1.6–1.8 V but is short-lived.

chemotherapeutic agent in the therapy of GBM, has an IC_{50} (concentration resulting in cell viability of 50% of control) of approx. 200 μM towards U87-MG malignant glioma cells [14]. Thus, new treatments to high-grade MG are urgently needed to address and alleviate the limitations of current therapeutic approaches and improve patient survival rates.

Transition metal complexes are actively being developed as PDT agents [15]. Monometallic compounds are very attractive due to their simple synthetic approach and concise structure [16]. Common approaches employed in the design of monometallic PDT agents include ligand variation/modification and metal center variation. Metal center/ligand variation allows for tuning of redox, spectroscopic and photophysical properties with ligand modification allowing for added targeting ability [9a,17]. Herein, we report the design of a new set of $[(Ph_2phen)_2M(dpp)]^{2+}$ (Ph_2phen = 4,7-diphenyl-1,10-phenanthroline; dpp = 2,3-bis(2-pyridyl)pyrazine; M = Ru/Os) polyazine complexes with light-promoted cytotoxicity in rat malignant glioma F98 cells. To the best of our knowledge, compounds **1** and **2** are the first reported transition metal complexes that display PDT activity towards gliomas cells. These compounds show approximately five-fold higher cytotoxicity than the traditional chemotherapeutic drug cisplatin. Furthermore, compound **2** is the first complex to show photocytotoxicity in malignant gliomas within the phototherapeutic window. Photoactivity of the compounds was studied in DNA and bovine serum albumin (BSA) as model biomolecules, which are potential targets for these phototoxic species (See Fig. 2).

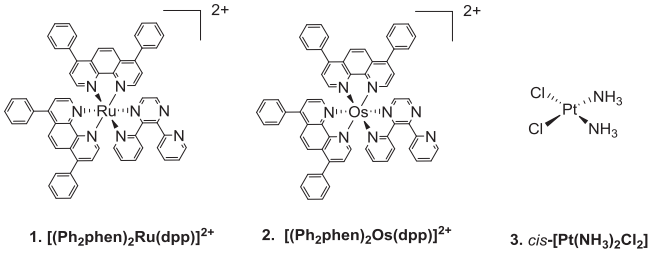

Fig. 2. Structural representations of metal compounds in this study. Ph_2phen = 4,7-diphenyl-1,10-phenanthroline;dpp = 2,3-bis(2-pyridyl)pyrazine.

2. Experimental

2.1. Materials and methods

$[(Ph_2phen)_2RuCl_2]$ and $[(Ph_2phen)_2OsCl_2]$ were synthesized as previously reported [18]. Ph_2phen, dpp, $RuCl_3{\cdot}3H_2O$, Bu_4NPF_6, NH_4PF_6 and cisplatin, *cis*-$[Pt(NH_3)_2Cl_2]$, were purchased from Alfa Aesar. Solvents were HPLC grade. All materials were used as received without further purification unless otherwise noted. Sephadex LH-20 was purchased from GE healthcare Biosciences Corporation. Supercoiled pUC19 plasmid DNA was purchased from Bayou Biolabs. Lamba/Hind III markers was purchased from Promega Corporation. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich. NuPAGE™ Novex™ 4–12% Bis–Tris Protein Gels (1.0 mm, 15-well) was purchased from Invitrogen. F98 malignant glioma rat cancer cells were purchased from American Type Culture Collection (ATCC® CRL-2397™) and used in toxicity studies at passage 7. Cells were seeded in a Thermo Scientific 1300 Series A2 sterile hood and incubated at 37 °C with 5% CO_2. Dulbecco's Modified Eagle's Medium (DMEM), Fetal Bovine Serum (FBS), penicillin–streptomycin antibiotic solutions were obtained from ATCC®. 0.05% Trypsin–EDTA and phosphate buffer solutions (PBS) were purchased from Life Technologies, while 75 cm^2 tissue flask, serological pipettes and 15 mL/50 mL centrifuge tubes were obtained from Fisher Scientific. Cells were collected with a Thermo Scientific Sorvall ST 8R refrigerated centrifuge and counted in a Beckman Coulter Vi-Cell cell viability analyzer. Samples were submitted to Galbraith Laboratories, Inc., for elemental analysis.