Dyes and Pigments 136 (2017) 416–426

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Synthesis, characterization and biological evaluation of ruthenium(II) complexes $[Ru(dtzp)(dppz)Cl]^+$ and $[Ru(dtzp)(dppz)CH_3CN]^{2+}$ for photodynamic therapy

Hui-juan Yu [a], Jiang-ping Liu [b], Zhi-feng Hao [a], Jun He [a], Ming Sun [a], Sheng Hu [a], Lin Yu [a, *], Hui Chao [b, **]

[a] Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, PR China
[b] MOE Laboratory of Bioinorganic and Synthetic Chemistry, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, PR China

ARTICLE INFO

Article history:
Received 2 May 2016
Received in revised form
20 August 2016
Accepted 27 August 2016
Available online 30 August 2016

Keywords:
Photodynamic therapy
Photocytotoxicity
Anticancer drugs
Spectra titration
Ruthenium complex

ABSTRACT

Two new Ru(II) complexes $[Ru(dtzp)(dppz)Cl]^+$ **1** and $[Ru(dtzp)(dppz)CH_3CN]^{2+}$ **2** (dtzp = 2,6-di(thiazo1-2-yl)pyridine; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine) have been synthesized and evaluated as photodynamic anticancer agents. The results of the spectra titration, thermal denaturation and electrophoresis experiments suggest that both complexes could intercalatively bind to DNA and photocleave DNA efficiently by ROS generation and photoinduced electron transfer. When incubated under visible light (470 nm), complex **1** and **2** generate great photocytoxicity towards Hela cells in both 2D cancer cell monolayer and 3D MCTS cancer models, and a much greater photocytotoxicity was observed for complex **1**, which may be associate with its' larger cellular uptake efficiency and stronger absorption at 470 nm. Flow cytometry analysis and immunofluorescence assay revealed that complex **1** inhibited Hela cell proliferation through G2M phase cycle arrest and cell apoptosis and could generate great photodamage to chromatin DNA. Complex **1** may be a prominent PDT candidate used for treating cervical carcinoma.

1. Introduction

Photodynamic therapy (PDT) has emerged as a promising method for treating cancer that offers spatial and temporal selectivity through local interactions between photosensitizer (PS), light, and cellular target [1–4]. The drug in PDT as a photosensitizer is selectively activated at the photoexposed malignant site to generate reactive oxygen species (ROS) destroying tumors and tumor vasculature and leaving the unexposed healthy tissue unaffected. Owning to its high selectivity, minimal side effect and reduced drug resistance, PDT has been thought to be a powerful alternative to traditional chemotherapy and has been utilized in the treatment of esophageal cancer, head and neck tumors, inoperable early central lung cancers, and dermatology [5–8]. Photofrin®, composed of hematoporphyrin and its oligomers, is an FDA approved PDT drugs for treating esophageal, head, and neck tumors [9,10].

Ruthenium complexes with their favorable absorption, photophysical and photochemical properties have been shown to be promising PDT candidates in vitro or in vivo [11–20]. $[Ru(bpy)_2(dppz)]^{2+}$ (bpy = 2,2′bipyridine, dppz = dipyrido[3,2-*a*:2′,3′-*c*] phenazine) possessing a well aromatic planarity, interacts with DNA by intercalative mode and exhibits the “light switch” effect in the presence of DNA [21]. However, due to its short lifetime of triplet excited state and relative low excited redox potential, the quantum yields of ROS of $[Ru(bpy)_2(dppz)]^{2+}$ is very low, resulting in its inefficacy in cleaving DNA [22]. In previous study, we have successfully improved DNA photocleavage ability of $[Ru(bpy)_2(dppz)]^{2+}$ by modification of major ligand or ancillary ligand [23,24]. The success encouraged us to further develop PDT agents based on Ru-dppz complexes. In this paper, we reports the synthesis and characterization of two new Ru-dppz complexes $[Ru(dtzp)(dppz)Cl]^+$ **1** and $[Ru(dtzp)(dppz)CH_3CN]^{2+}$ **2** (dtzp = 2,6-di(thiazo1-2-yl)pyridine). The DNA binding and photocleavage

* Corresponding author.
** Corresponding author.
E-mail addresses: gych@gdut.edu.cn (L. Yu), ceschh@mail.sysu.edu.cn (H. Chao).

http://dx.doi.org/10.1016/j.dyepig.2016.08.059
0143-7208/

activity, in vitro photocytotoxicity and antitumor mechanism of the two complexes were studied (see Scheme 1).

2. Experimental

2.1. Physical measurements

Microanalysis (C, H, and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. ^{1}H NMR spectra were recorded on a Varian-500 spectrometer and all chemical shifts are given relative to tetramethylsilane (TMS). Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA). The spray voltage, tube lens offset, capillary voltage and capillary temperature were set at 4.50 KV, 30.00 V, 23.00 V and 200 °C, respectively, and the quoted *m*/*z* values are for the major peaks in the isotope distribution. UV–Vis spectra were recorded on a Perkin-Elmer Lambda 850 spectrophotometer. Cyclic voltammetric measurements were performed on a CHI 660A Electrochemical Workstation at room temperature. The supporting electrolyte was 0.1 M TBAP (tetrabutylammonium perchlorate, Fluka, electrometric grade) in acetonitrile freshly distilled from phosphorus pentaoxide. All samples were purged with argon prior to measurements. The inductively coupled plasma mass spectrometry (ICP-MS) experiments were performed on an Agilent's 7700× instrument. Confocal microscopy was performed on a Carl Zeiss LSM 710 (Göttingen, Germany) confocal laser scanning microscope.

2.2. Materials

All reagents and chemicals were purchased from commercial sources and used without further purifications. Supercoiled pBR322 DNA, Calf Thymus DNA (CT-DNA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), propidium iodide (PI), 4′,6-diamidino-2-phenyindole (DAPI), Dulbecco's modified eagle medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), fetal bovine serum (FBS), penicillin G and streptomycin were purchased from Sigma Aldrich. The tested complexes were dissolved in DMSO just before the experiments, and the concentration of DMSO was 1% (v/v). Tris-HCl buffer (5 mM Tris-HCl, 50 mM NaCl, pH = 7.2) solution was prepared using doubly distilled water. A solution of Calf Thymus DNA in the buffer gave a ratio of UV absorbance at 260 and 280 nm of about 1.8–1.9:1, indicating that the DNA was sufficiently free of protein [25]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [26].

2.3. Synthesis

The compounds dtzp [27] and dppz [28] were synthesized according to literature methods.

2.3.1. Synthesis of Ru(dtzp)Cl_3

A mixture of $RuCl_3 \cdot 3H_2O$ (0.262 g, 1.0 mmol), dtzp (0.245 g, 1.0 mmol) and ethanol (125 mL) were refluxed for 3 h. After cooling to room temperature, the dark product precipitate was obtained by filtration. The precipitate was washed with three 30 mL portions of ethanol and diethyl ether, and dried in vacuum. Yield: 0.32 g, 70%. Anal. Calc. for $C_{11}H_7Cl_3N_3RuS_2$: C, 29.18; H, 1.56; N, 9.28. Found: C, 29.12; H, 1.59; N, 9.37. ES-MS (CH_3CN): 418 ($[M-Cl]^+$).

2.3.2. Synthesis of [Ru(dtzp)(dppz)Cl](ClO_4) **1**

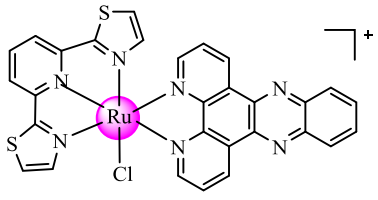
A mixture of Ru(dtzp)Cl_3 (0.226 g, 0.5 mmol), dppz (0.141 g, 0.5 mmol) and triethylamine (1 mL) in 70 mL ethanol was refluxed for 6 h under argon, during which time the solution turned dark purple. After being cooled to room temperature, a dark purple precipitate was obtained by addition of aqueous $NaClO_4$ solution. The product was purified by column chromatography on alumina with acetonitrile as eluent. Yield: 0.292 g, 75%. Anal. Calc. for $C_{29}H_{17}Cl_2N_7O_4RuS_2$: C, 45.61; H, 2.24; N, 12.84. Found: C, 45.52; H, 2.35; N, 12.76. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.23(d, J = 3.6 Hz, 2H); 7.59(t, $J_1 = J_2$ = 4.0 Hz, 1H); 7.83 (d, J = 6.0 Hz, 1H); 8.10 (d, J = 4.0 Hz, 2H); 8.14–8.22 (m, 3H); 8.48 (d, J = 5.9 Hz, 1H); 8.53–8.57 (m, 2H); 8.69 (d, J = 8.0 Hz, 2H); 9.29 (d, J = 7.8 Hz, 1H); 9.81 (d, J = 8.0 Hz, 1H); 10.46 (d, J = 6.8 Hz, 1H), ES-MS (CH_3CN): 664 ($[M\text{-}ClO_4]^+$).

2.3.3. Synthesis of [Ru(dtzp)(dppz)CH_3CN](ClO_4)$_2$ **2**

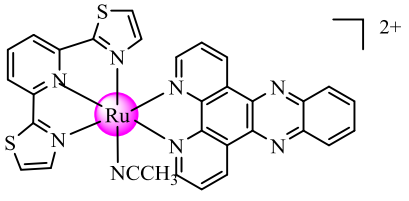
A mixture of [Ru(dtzp)(dppz)Cl] (ClO_4) **1** (0.38 g, 0.5 mmol) and $AgClO_4$ (0.81 g) in 40 mL acetonitrile was refluxed for 5 h under argon. Upon cooling, an orange solution was obtained by filtration of AgCl. After concentration under vacuum, the red precipitation was obtained by the addition of cold water. The product was purified by column chromatography on alumina with acetonitrie-toluene (1:1, v/v) as eluent. Yield: 0.283 g, 65%. Anal. Calc. for $C_{31}H_{20}Cl_2N_8O_8RuS_2$: C, 42.86; H, 2.32; N, 12.90. Found: C, 42.72; H, 2.39; N, 12.82. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.23 (s, 3H); 7.35 (d, J = 3.6 Hz, 2H); 7.72(t, $J_1 = J_2$ = 5.6 Hz, 1H); 7.89 (d, J = 8.0 Hz, 1H); 8.18 (d, J = 4.5 Hz, 2H); 8.20–8.22 (m, 2H); 8.42–8.52 (m, 3H); 8.59 (d, J = 7.8 Hz, 1H); 8.82 (d, J = 8.0 Hz, 2H); 9.43 (d, J = 9.2 Hz, 1H); 9.89 (d, J = 8.5 Hz, 1H); 10.14 (d, J = 8.0 Hz, 1H), ES-MS (CH_3CN): 335 ($[M\text{-}2ClO_4]^{2+}$).

2.4. Log Po/w measurements

Log Po/w is the partition coefficient between octanol and water which is determined using the flask-shaking method [29]. An aliquot of aqueous solution of Ru was added to an equal volume of octanol. The mixture was shaken overnight at 60 rpm at 298 K to allow partitioning. After the sample was centrifuged at 3000 rpm for 10 min, the aqueous layer was carefully separated from the octanol layer for ruthenium analysis. The Ru concentration in the aqueous phase was determined using ICP-MS and used to calculate the [Ru]o/[Ru]w ratio.



[Ru(dtzp)(dppz)Cl]$^+$ **1**



[Ru(dtzp)(dppz)CH_3CN]$^{2+}$ **2**

Scheme 1. Chemical structures of complexes [Ru(dtzp)(dppz)Cl]$^+$ **1** and [Ru(dtzp)(dppz)CH_3CN]$^{2+}$ **2**.