Journal of Photochemistry & Photobiology, B: Biology 161 (2016) 295–303

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Synthesis, characterization and anticancer activity studies of ruthenium(II) polypyridyl complexes on A549 cells

Chuan-Chuan Zeng [a], Guang-Bin Jiang [a], Shang-Hai Lai [a], Cheng Zhang [a], Hui Yin [b,*], Bing Tang [a], Dan Wan [a], Yun-Jun Liu [a,*]

[a] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[b] Department of Microbiology and Immunology, Guangdong Pharmaceutical University, Guangzhou 510006, PR China

ARTICLE INFO

Article history:
Received 18 March 2016
Received in revised form 3 June 2016
Accepted 3 June 2016
Available online 05 June 2016

Keywords:
Ru(II) complex
Apoptosis
Cellular uptake
Cell cycle arrest
Western blot analysis

ABSTRACT

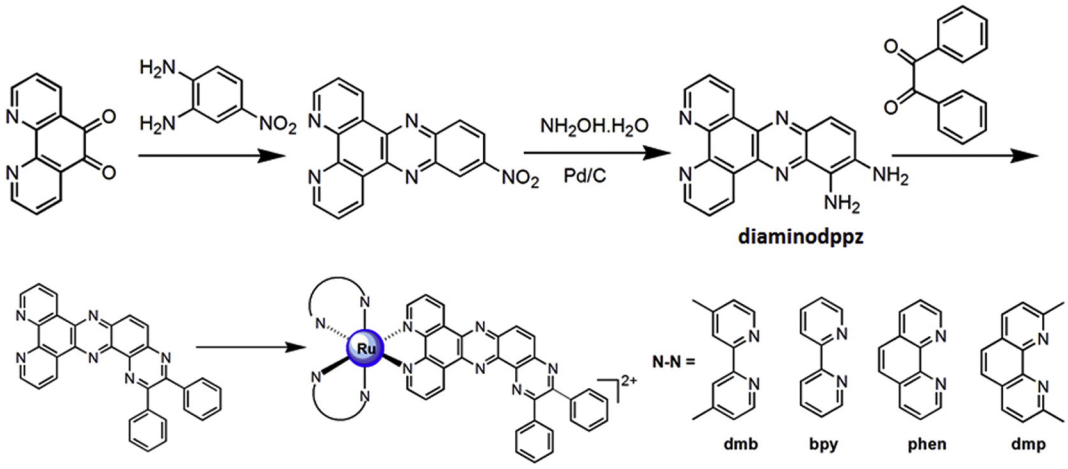
Four new ruthenium(II) polypyridyl complexes $[Ru(N\text{-}N)_2(bddp)](ClO_4)_2$ **1–4** (N-N = dmb: 4,4′-dimethyl-2,2′-bipyridine **1**, bpy: 2,2′-bipyridine **2**, phen: 1,10-phenanthroline **3** and dmp: 2,9-dimethyl-1,10-phenanthroline **4**, bddp = benzilo[2,3-*b*]-1,4-diazabenzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine) were synthesized and characterized by elemental analysis, ESI-MS and ^1H NMR. The cytotoxicity in vitro of the complexes against BEL-7402, HeLa, MG-63 and A549 cell lines was investigated by MTT method. The complexes show high cytotoxic activity toward the selected cell lines with an IC_{50} value ranging from 5.3 ± 0.6 to 15.7 ± 3.6 μM. The apoptosis was studied with acridine orange (AO)/ethdium bromide (EB) and Hoechst 33258 staining methods. The cellular uptake was investigated with DAPI staining method. The reactive oxygen species (ROS) and mitochondrial membrane potential were performed under fluorescent microscope and flow cytometry. The complexes can induce an increase in the ROS levels and a decrease in the mitochondrial membrane potential. The comet assay was studied with fluorescent microscope. The percentage in apoptotic and necrotic cells and cell cycle arrest were assayed by flow cytometry. The effects of the complexes on the expression of caspases and Bcl-2 family proteins were studied by western blot analysis. The results show that the complexes induce apoptosis in A549 cells through an ROS-mediated mitochondrial dysfunction pathway, which was accompanied by regulating the expression of Bcl-2 family proteins.

1. Introduction

Cancer is one of the most widespread serious diseases. It is characterized by uncontrolled growth of abnormal cells. The growth and metastasis of cancer cells are dependent on angiogenesis; therefore, affecting angiogenesis will be of great importance in inhibition of tumor growth, invasion, and metastasis [1]. Cisplatin has been widely used as antimetastatic drug for treating ovarian and testicular cancers since its discovery over 40 years ago [2]. Dose-limiting side effects, development of resistance after repeated use in treatment has limited the use of the platinum diammolino compounds, cisplatin and carboplatin [3]. To overcome these limitations, the search for anticancer activity among complexes of other metals has received much interest. Presently, ruthenium complexes have been found as an attractive alternate for platinum due to several favorable properties suited to rational anticancer drug design and biological applications [4–6]. Thus, ruthenium metal complexes are considered to be one of the most promising anticancer agents. So far, two ruthenium complexes, NAMI-A ([ImH][*trans*-$RuCl_4$(DMSO)(Im)], where Im = imidazole and DMSO = dimethylsulfoxide) and KP1019 ([IndH][*trans*-$RuCl_4(Ind)_2$], where Ind = indazole) have entered clinical trials. NAMI-A is effective against lung metastases [7,8]. In recent years, a great progress has been made in the anticancer activity of ruthenium(II) polypyridyl complexes, many Ru(II) polypyridyl complexes show interesting anticancer activity [9–18]. $[Ru(phen)_2(biim)](ClO_4)_2$ inhibited the growth of HeLa cells through induction of apoptotic cell death by a mitochondria dysfunction pathway [19]. Ruthenium polypyridyl complex $[Ru(phen)_2(dbtcp)]^{2+}$ possesses high mitochondria-specificity, superior photostability, high resistance to the loss of mitochondrial membrane potential and appreciable tolerance to environmental change, allowing imaging of the mitochondrial morphological changes over long periods of time [20]. In order to obtain more insight into the bioactivity of Ru(II) complexes toward cancer cells, in this report, a new ligand bddp (bddp = benzilo[2,3-*b*]-1,4-diazabenzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine, Scheme 1) and its four Ru(II) polypyridyl complexes: $[Ru(dmb)_2(bddp)](ClO_4)_2$ (**1**) (dmb = 4,4′-dimethyl-2,2′-bipyridine), $[Ru(bpy)_2(bddp)](ClO_4)_2$ (**2**) (bpy = 2,2′-bipyridine), $[Ru(phen)_2(bddp)](ClO_4)_2$ (**3**) (phen = 1,10-phenanthroline) and $[Ru(dmp)_2(bddp)](ClO_4)_2$ (**4**) (dmp = 2,9-dimethyl-1,10-

* Corresponding authors.
E-mail addresses: huiyin0103@163.com (H. Yin), lyjche@163.com (Y.-J. Liu).

http://dx.doi.org/10.1016/j.jphotobiol.2016.06.004
1011-1344/



Scheme 1. The synthetic route of ligand and complexes.

phenanthroline) were synthesized and characterized by elemental analysis, ESI-MS and ^{1}H NMR. We selected dmb, bpy, phen and dmp as ancillary ligand in that it is possible that the ruthenium(II) polypyridyl complexes containing dmb, or bpy, or phen or dmp can show different inhibition effect on the different cancer cell growth or against the same cancer cell. The cytotoxicity in vitro of the ligand and the complexes was evaluated against BEL-7402 (Hepatocellular), A549 (Human lung carcinoma), HeLa (Human cervical cancer) and MG-63 (Human osteosarcoma) cell lines. The apoptosis in A549 cells was investigated with acridine orange (AO), ethidium bromide (EB) and Hoechst 33258 staining methods. The cellular uptake, DNA damage, reactive oxygen species (ROS) and mitochondrial membrane potential were studied by fluorescent microscopy. The percentage in the apoptotic cells and cell cycle arrest were performed with flow cytometry, and the expression levels of Bcl-2 family proteins were investigated by western blotting analysis.

2. Experimental Section

2.1. Materials and Methods

The reagents and solvents we used in the experiments were purchased commercially and used without further purification unless special explanation. Ultrapure MilliQ water was used in all experiments. DMSO and RPMI 1640 (Roswell Park Memorial Institute) were purchased from GIBCO. $RuCl_3 \cdot 3H_2O$ was purchased from the Kunming Institution of Precious Metals. 1,10-Phenanthroline was obtained from the Guangzhou Chemical Reagent Factory. Cell lines of BEL-7402 (human hepatocellular carcinoma cell line), HeLa (human cervical cancer cell line), A549 (human lung cancer cell line), and MG-63 (human osteosarcoma cell line) were purchased from the American Type Culture Collection. All the cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μg/mL) and incubated at 37 °C in a 5% CO_2 incubator.

Microanalyses (C, H, and N) were investigated with a Perkin-Elmer 240Q elemental analyzer. Electrospray ionization mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA) using acetonitrile as mobile phase. 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. ^{1}H NMR spectra were recorded on a Varian-500 spectrometer with DMSO-d_6 as solvent and tetramethylsilane as an internal standard at 500 MHz at room temperature.

2.2. Synthesis of Ligand and Complexes

2.2.1. Synthesis of Bddp

10,11-Diaminodppz (0.156 g, 5 mmol) [21,22] and Benzil (0.105 g, 5 mmol) were dissolved in 30 mL of glacial acetic acid and 30 mL of anhydrous ethanol and refluxed at 130 °C for 6 h. Cooled to room temperature, suction filtered, and dried in vacuo, a pale yellow powder was obtained. Yield: 58%. Anal. calcd for $C_{32}H_{18}N_6$: C, 79.00; H, 3.73; N, 17.27. Found: C, 78.79; H, 3.66; N, 17.41%. ^{1}H NMR (DMSO-d_6): 8.89 (d, 2H, J = 8.0 Hz), 8.83 (d, 1H, J = 8.5 Hz), 8.60 (d, 1H, J = 9.0 Hz), 8.44 (d, 2H, J = 8.0 Hz), 8.38 (d, 1H, J = 6.0 Hz), 8.30 (d, 1H, J = 8.5 Hz), 8.22 (d, 1H, J = 7.0 Hz), 8.13 (d, 1H, J = 5.5 Hz), 7.94 (d, 4H, J = 6.0 Hz), 7.77 (dd, 2H, J = 5.0, J = 8.0 Hz), 7.83 (d, 2H, J = 8.0 Hz). ESI-MS (DMSO): *m/z* 487 [M + H].

2.2.2. Synthesis of $[Ru(dmb)_2(bddp)](ClO_4)_2$ (**1**)

A mixture of *cis*-$[Ru(dmb)_2Cl_2]\cdot 2H_2O$ [23] (0.270 g, 0.50 mmol) and bddp (0.242 g, 0.5 mmol) in ethylene glycol (20 mL) was refluxed under argon for 8 h to give a clear red solution. Upon cooling, a red precipitate was obtained by dropwise addition of saturated aqueous $NaClO_4$ solution. The crude product was purified by column chromatography on neutral alumina with a mixture of CH_3CN–toluene (4:1, v/v) as eluent. The red band was collected. The solvent was removed under reduced pressure and a red powder was obtained. Yield: 78%. Anal. calc. For $C_{56}H_{42}N_{10}Cl_2O_8Ru$: C, 58.24; H, 3.67; N, 12.13%. Found: C, 58.52; H, 3.61; N, 12.41%. ^{1}H NMR (DMSO-d_6): δ 9.62 (d, 2H, J = 8.0 Hz), 9.33 (d, 2H, J = 8.0 Hz), 8.80 (dd, 4H, J = 8.5, J = 8.0 Hz), 8.65 (d, 1H, J = 8.5 Hz), 8.49 (dd, 2H, J = 6.5 Hz, J = 8.5 Hz), 8.40 (d, 1H, J = 6.5 Hz), 8.31 (d, 1H, J = 4.5 Hz), 8.25 (d, 1H, J = 5.0 Hz), 8.19 (d, 4H, J = 8.5 Hz), 8.06 (d, 1H, J = 6.5 Hz), 7.90 (t, 2H, J = 6.0 Hz), 7.76 (d, 2H, J = 7.5 Hz), 7.71 (dd, 2H, J = 5.5 Hz, J = 5.5 Hz), 7.64 (d, 1H, J = 6.0 Hz), 7.47 (d, 2H, J = 6.0 Hz), 7.32 (d, 1H, J = 6.5 Hz), 7.27 (d, 1H, J = 5.5 Hz), 1.93 (s, 6H), 1.85 (s, 6H). ES-MS (CH_3CN): *m/z* 866.5 ([M-2ClO_4-H]$^+$), 433.6([M-2ClO_4]$^{2+}$).

2.2.3. Synthesis of $[Ru(bpy)_2(bddp)](ClO_4)_2$ (**2**)

This complex was synthesized in a manner identical to that described for **1**, with *cis*-$[Ru(bpy)_2Cl_2]2H_2O$ [23] in place of *cis*-$[Ru(dmb)_2Cl_2]\cdot 2H_2O$. Yield: 75%. Anal. calc. For $C_{52}H_{34}N_{10}Cl_2O_8Ru$: C, 56.84; H, 3.12; N, 12.75%. Found: C, 56.77; H, 3.23; N, 12.94%. ^{1}H NMR (DMSO-d_6): δ 9.59 (d, 2H, J = 8.0 Hz), 9.26 (d, 2H, J = 8.0 Hz), 9.05 (d, 4H, J = 8.5 Hz), 8.95 (d, 1H, J = 8.5 Hz), 8.59 (d, 2H, J = 6.5 Hz), 8.60 (d, 1H, J = 8.5 Hz), 8.40 (d, 2H, J = 8.0 Hz), 8.32 (d, 2H, J = 5.5 Hz), 8.28 (d, 2H, J = 8.0 Hz), 8.21 (d, 2H, J = 8.5 Hz), 8.17 (d, 2H,