European Journal of Medicinal Chemistry 156 (2018) 747–759

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Novel ruthenium(II) triazine complex $[Ru(bdpta)(tpy)]^{2+}$ co-targeting drug resistant GRP78 and subcellular organelles in cancer stem cells

Baskaran Purushothaman [1], Parthasarathy Arumugam [1], Hee Ju, Goutam Kulsi, Annie Agnes Suganya Samson, Joon Myong Song*

College of Pharmacy, Seoul National University, Seoul 151-742, South Korea

ARTICLE INFO

Article history:
Received 9 April 2018
Received in revised form
14 July 2018
Accepted 17 July 2018
Available online 20 July 2018

Keywords:
Ruthenium(II) complex
Cancer stem cells
Glucose regulated protein 78
Mitochondria
Apoptosis

ABSTRACT

Ruthenium(II/III) metal complexes have been widely recognized as the alternative chemotherapeutic agents to overcome the drug resistance and tumor recurrence associated with platinum derivatives. In this work, a novel ruthenium(II) triazine complex namely, **1** ($[Ru(bdpta)(tpy)]^{2+}$) was synthesized and spectroscopically characterized. Drug resistant cancer stem cells (CSCs) were used to evaluate the cytotoxicity of Ru(II) complex **1**. The complex **1** showed a greater cytotoxic potential with IC_{50} values lower than that of cisplatin. The intracellular localization assay confirmed that the complex **1** was effectively distributed into mitochondria as well as endoplasmic reticulum (ER), and executed a ROS-mediated calcium and Bax/Bak dependent intrinsic apoptosis. Interestingly, direct interaction between complex **1** and glucose regulated protein 78 (GRP78), a protein associated with drug resistance caused the ROS-mediated ubiquitination of GRP78. Notably, western blot and confocal microscopy analysis confirmed that complex **1** significantly reduced the protein levels of GRP78. Dose-dependent *in vivo* antitumor efficacy against CD133+HCT-116 CSCs derived tumor xenograft further validated that complex **1** could be an effective chemotherapeutic agent.

1. Introduction

Platinum based cisplatin derivatives such as cisplatin, carboplatin and oxaliplatin are the commonly employed chemotherapeutic drugs against solid tumours such as testicular, head and neck, bladder, ovarian, and lung cancers [1,2]. However, long-term administration of platinum derivatives results in side effects, as well as drug resistance and subsequently relapse of cancer [3–5]. In search of potential alternatives to the platinum derivatives, ruthenium based metal complexes have emerged as a potential contender to platinum derivatives [6,7]. Recent *in-vitro* and *in-vivo* studies on ruthenium(II, III) metal complexes indicated that derivatives of ruthenium metal complexes could resolve the issues such as side effects and drug resistance in cancer [8–14]. Ruthenium(III) complex exhibits a strong affinity towards serum albumin and transferrin, this property ensures the smooth transportation of ruthenium complex across the vascular system [15–17]. Rapidly dividing cells express the elevated levels of transferrin due to the higher requirement of iron. It has previously been reported that the expression of iron transport proteins such as transferrin and ferritin were increased in metastasis cancer [18,19]. Hence, ruthenium(II, III) complexes are more likely to accumulate in cancer tissue. After a successful preclinical studies, NAMI-A, the first ruthenium(III) complex has completed the phase-I/II clinical trials in combination with gemcitabine in non-small cell lung cancer (NSCLC) patients. However, the trial was unsuccessful due to the side effects and less activity of NAMI-A in NSCLC [20,21]. The disappointing results of NAMI-A necessitate the design of new ruthenium complexes in the field of anti-cancer drug discovery. In the present work, we have synthesized a novel ruthenium(II) complex with specific ligand to overcome multidrug resistance in cancer stem cells (CSCs), namely ruthenium(II) triazine complex **1** ($[Ru(bdpta)(tpy)]^{2+}$). The tridentate ligand 4-(4,6-bis(3,5-dimethyl-1H-pyrazole-1-yl)-1,3,5,-triazine-2-yl)-N,N-diethylaniline (**bdpta**) was used in the synthesis of ruthenium(II) complex **1**. In our design, the pyrazolyl-1,3,5-triazine (**bdpta**) was taken as a core component and it acts as chelating ligand to synthesize complex **1**. The 1,3,5-triazine chemical core based compounds have been used as anticancer agents for many groups [22–24]. Few

* Corresponding author.
E-mail address: jmsong@snu.ac.kr (J.M. Song).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejmech.2018.07.048
0223-5234/

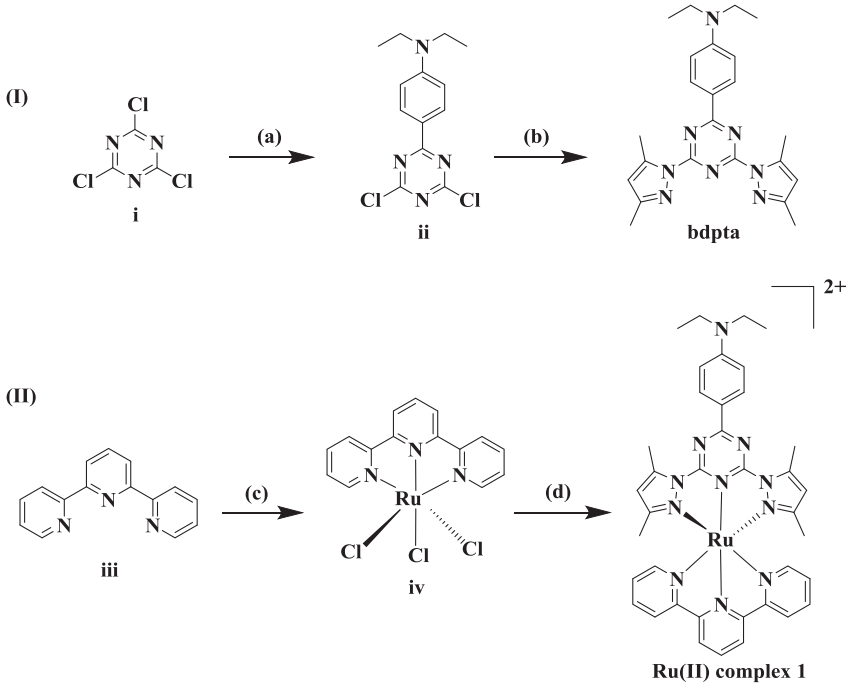
ruthenium complexes containing 1,3,5-triazine ligand were reported and their redox behaviour and luminescence properties were studied [25,26]. However, no attention has been focused on the ruthenium(II) complexes containing a pyrazolyl-1,3,5-triazine core as an anticancer agent, targeting subcellular organelles as well as drug resistant proteins. Inspired by these findings our attention was focused on the synthesis of novel Ru(II) complexes with ligands targeting subcellular organelles. The complex **1** containing *N,N*-diethylaniline moiety in 1,3,5-triazine core targets mitochondria in cancer stem cells. Similarly, a polypyridyl group of the complex **1** helps to accumulate in endoplasmic reticulum (ER) due to its lipophilic nature [27].

The newly synthesized Ru(II) complex **1** was characterized by spectroscopic methods and their cytotoxicity was evaluated using a cancer cell lines as well as CSCs. The CSCs was chosen as an *in vitro* model to study the cytotoxicity, sub-cellular localization, drug resistance and other molecular mechanisms pertaining to the ruthenium(II) complex. For this purpose, CD133+ MCF-7 cells and CD133+ HCT-116 cells were used. Cancer cells which expressing CD44 and CD133 markers are widely recognized as CSCs. A number of reports indicated that cisplatin resistance cancer cell lines expressed significant levels of stem cell markers such as CD133 and CD44 [28]. In addition, CSCs are widely considered for the recurrence of cancer since they express high levels of proteins associated with drug resistance and cell survival mechanism [29]. Hence, the CSCs model is an ideal model to evaluate the efficacy of drug pertaining to drug resistance. It has been reported that the ROS induced oxidation process can bring conformational changes to protein and its side chain amino acids, which leads to the ubiquitination of modified protein [30,31]. Due to the ROS generating ability of the complex **1**, its ROS mediated direct effect on the proteins associated with drug resistance mechanism such as GRP-78, clusterin (CLU), and ataxia telangiectasia and Rad3-related protein (ATR) were analysed. GRP78, a major molecular chaperon in the ER, was demonstrated in the drug resistance, stemness characteristics of cancer cells and the recurrence of cancer [32,33]. Clusterin is an anti-apoptotic protein, elevated expression of clusterin has been reported in the paclitaxel/docetaxel resistance in prostate, breast and lung cancer [34,35]. ATR is a cell cycle regulating protein, it has been demonstrated that suppression of ATR stimulates the malignant cells to apoptosis [36]. In addition, the *in vivo* efficacy of complex **1** was evaluated using CD133+HCT-116 CSCs induced tumor xenograft mice model. This work, to the best of our knowledge, presents a detailed biological evaluation of a multi-targeting ruthenium(II) triazine complex **1** on CSCs by *in vitro*, as well as *in vivo* model.

2. Results and discussion

2.1. Synthesis and characterization of Ru(II) complex 1

The ligand 4-(4,6-bis(3,5-dimethyl-1H-pyrazole-1-yl)-1,3,5,-triazine-2-yl)-N,N-diethylaniline (**bdpta**) was prepared using a previously described method and it is explained in detail in the supporting information [37,38]. The ruthenium(II) complex **1** was synthesized with their corresponding ligand (bdpta). The synthetic protocol for Ru(II) complex **1** is represented in Scheme 1. The $[Ru(tpy)Cl_3]$ (**iv**) was prepared by refluxing the equivalent molar ratio of 2,2′:6′,2″-terpyridine (**iii**) and $RuCl_3 \cdot 3H_2O$ in ethanol at 80 °C for 3 h. The complex **1** was synthesized from an equal molar ratio of $[Ru(tpy)Cl_3]$ and **bdpta** in ethylene glycol at 170 °C overnight under nitrogen protection. This was followed by anion exchange with $NaClO_4$ and purification by column chromatography with CH_3CN and 10% KNO_3 as an eluent. The complex **1** was isolated as brown solid. The stability of the synthesized Ru(II) complex **1** was checked by dissolving the compound in PBS buffer and kept at room temperature for 24 h. No obvious change was observed, and this was further confirmed by using UV-Vis absorption spectroscopy. The UV-Vis absorption and emission spectrum of ruthenium(II) complex **1** were measured in acetonitrile (See Fig. S1). The electronic absorption spectrum for the Ru(II) complex **1** display high energy bands at 396 nm, due to ligand-centered π-π*



Scheme 1. Synthesis of Ru(II) complex **1**; Reagents and conditions: (a) *N,N*-diethylaniline, 75 °C, 8 h; (b) 3,5-dimethylpyrazole, K metal, THF, reflux, 8 h; (c) $RuCl_3 \cdot 3H_2O$, ethanol, reflux, 3 h; (d) bdpta, ethylene glycol, N_2, reflux, 16 h.