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Ruthenium complexes with phenylterpyridine derivatives target cell membrane and trigger death receptors-mediated apoptosis in cancer cells

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ABSTRACT

Elucidation of the communication between metal complexes and cell membrane may provide useful information for rational design of metal-based anticancer drugs. Herein we synthesized a novel class of ruthenium (Ru) complexes containing phtpy derivatives (phtpy = phenylterpyridine), analyzed their structure-activity relationship and revealed their action mechanisms. The result showed that, the increase in the planarity of hydrophobic Ru complexes significantly enhanced their lipophilicity and cellular uptake. Meanwhile, the introduction of nitro group effectively improved their anticancer efficacy. Further mechanism studies revealed that, complex (**2c**), firstly accumulated on cell membrane and interacted with death receptors to activate extrinsic apoptosis signaling pathway. The complex was then transported into cell cytoplasm through transferrin receptor-mediated endocytosis. Most of the intracellular **2c** accumulated in cell plasma, decreasing the level of cellular ROS, inducing the activation of caspase-9 and thus intensifying the apoptosis. At the same time, the residual **2c** can translocate into cell nucleus to interact with DNA, induce DNA damage, activate p53 pathway and enhance apoptosis. Comparing with cisplatin, **2c** possesses prolonged circulation time in blood, comparable antitumor ability and importantly, much lower toxicity *in vivo*. Taken together, this study uncovers the role of membrane receptors in the anticancer actions of Ru complexes, and provides fundamental information for rational design of membrane receptor targeting anticancer drugs.

1. Introduction

Cisplatin and other platinum (Pt)-based drugs have been wildly used as anticancer agents for decades, more than 50% cancer patients received various treatments with Pt-based drugs [1–3]. However, the serious side effects and drug resistance have limited their further application, which stimulated the research of alternative metal-based drugs [4–6]. Ruthenium (Ru) complexes are wildly considered to be favorable alternatives of Pt complexes [7–9], because of their certain favorable properties suitable for antitumor agent design, such as the rate of ligand exchange, the range of accessible oxidation states, and importantly, their attractive photoactivated biological applications [10–12]. Currently, two Ru-based complexes, NAMI-A and KP1019 are in clinical trials [13].

With increasing numbers of Ru complexes have been developed as potential anticancer drugs, the studies on their action mechanisms have attracted wildly attention in the past years [14–18]. Many researchers have discovered that DNA and some intercellular proteins are main targets of Ru complexes [10,19–22]. For instance, Gasser and co-workers have found polypyridyl Ru(II) compounds can induce cancer cell apoptosis by targeting mitochondria and causing the overproduction of reactive oxygen species (ROS) [10,23,24]. Especially, for the mechanism of apoptosis caused by Ru complexes, the great majority of studies indicated that the activation of mitochondria pathway should take primary responsibility [25–28]. Up to now, most researches are concentrated on the interaction between Ru complexes and intracellular biomacromolecules. However, till now, little information on the roles

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of cell membrane proteins in the action mechanisms of Ru complexes is available. Cell membrane separating the interior of cells from the outside environment is involved in a variety of cellular processes, such as selectively identify, transport certain substances and cell signaling transduction.

Previously, researchers have discovered that the expression levels of cell membrane receptors in cancer and normal cells are very different [29]. Therefore, it is a reasonable and promising strategy for rational design of cancer targeting drugs based on this difference. Thus, revealing the interaction between Ru complexes and membrane receptors is extremely meaningful for future fine-tuning their chemical structure to improve the anticancer efficacy. In recent years, death receptors (DRs) have received increasing attention, as they can selectively trigger cancer cell apoptosis [30]. Death receptors are cell surface receptors of the tumor necrosis factor (TNF) receptor superfamily, a group of cytokines that can bind TNF-related apoptosis-inducing ligand (TRAIL) and mediate apoptosis [31,32]. Many studies have evidenced that, targeting DRs such as TRAIL, Fas and TNF-receptors, can selectively trigger apoptosis in cancer cells while sparing normal cells [33,34]. Thus, targeting DRs can be a promising strategy to develop novel anticancer drugs.

Our previous study has revealed that some Ru complexes can partly induce apoptosis in cancer cells through death receptor signaling pathway [35,36]. Based on these studies, we synthesized a class of novel Ru complexes containing phenylterpyridine derivatives (Fig. 1) to study their structure-activities relationship, identify their target receptors on cell membrane and reveal the interaction mechanism. Our results indicated that the introduction of $-NO_2$ group into phentpy ligand effectively improved cytotoxicity of Ru complexes, and the lipophilicity and solubility of complexes also can determine complexes anticancer ability by affecting their cellular uptake. Among these complexes, **2c** possessed best anticancer activity and selectivity between cancer and normal cells. Death receptors and transferrin receptor (TfR) have been confirmed to be the target of **2c**, controlling the activation of extrinsic apoptosis signaling pathway and the transportation of complexes into cancer cells respectively. Moreover, compared with cisplatin, **2c** possesses improved pharmacokinetic properties and much lower toxicity *in vivo*. Taken together, this study presented fundamental information for further developing membrane receptor target anticancer drugs.

2. Experimental section

2.1. Materials, animals and instrumental details

2-Acetyl pyridine, benzaldehyde, 4-nitrobenzaldehyde, 4-dimethylaminobenzaldehyde, 2,2′-dipyridyl (bpy), 1,10-Phenanthroline monohydrate (phen), imidazo [4,5-f]- [1,10] phenanthroline (ip), (2-phenyl)imidazo [4,5-f]- [1,10] phenanthroline (pip) were purchased from aldrich. Sodium hydroxide (NaOH), sodium perchlorate and Ruthenium trichloride (RuCl3·2H2O) were purchased from aladdin. Thiazolyl blue tetrazolium bromide (MTT) propidium iodide (PI), 4′, 6-Diamidino-2-phenyindole (DAPI), dihydroethidium (DHE), and bicinchoninic acid (BCA) kit for protein determination were purchased from Sigma. Substrate for caspase-3 substrate (Ac-DEVD-AMC), caspase-8 substrate (Ac-IETD-AFC) and caspase-9 substrate (Ac-LEHD-AFC) were purchased from Calbiochem. Caspase-8 inhibitor (Z-IETD-fmk) and caspase-9 inhibitor (Z-IEHD-fmk) were purchased from Merck. DPPH, ABTS and anti-TfR were purchased from Sigma. The cell membrane dye, DIR (1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindotricarbocyanine Iodide) was purchased from ThermoFisher. Terminal transferase dUTP nick end labelling (TUNEL) assay kit. The water used in all experiments was ultrapure by a Milli-Q water purification system from Millipore. Sprague–Dawley (SD) mice (about160–200 g) used in this study were purchased from the Medical Laboratory Animal Center of Guangdong province and they all received good care. The elemental analysis, 1H NMR, ESI-MS, LC-MS, HPLC and UV–Vis spectrum was examined by EA2400 II(PE), Bruker Avance D PX300M Hz, Bruker AVANCE III 600 MHz, Agilent 1100, Thermo Scientific Q Exactive hybrid quadrupole-Orbitrap mass spectrometer, Aglient Technologies 1260 Infinity and Cary 5000 (Varian) respectively. All animal experiments were conducted under the approval of the Animal Experimentation Ethics Committee of Jinan University.

2.2. Synthesis and characterization

The compounds RuIII(phtpy)Cl3 (phtpy = phenyl-2,2′: 6′,2″-terpyridine), RuIII(4-NMe2-phtpy)Cl3 (4-NMe2-phtpy = 4′-(4-dimethylaminophenyl-2,2′:6′,2″-terpyridine), and RuIII(4-NO2-phtpy)Cl3 (4-NO2-phtpy = 4′-(4-nitrophenyl-2,2′:6′,2″-terpyridine) were prepared according to the literature procedures [37,38].

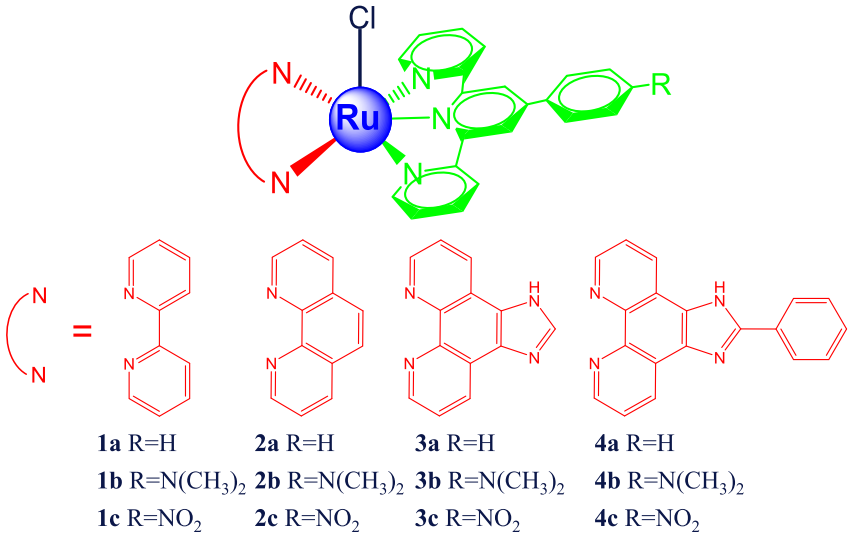

Fig. 1. Chemical structure of Ru complexes studied in this work.