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Original article

# Synthesis, characterization, and anticancer activity of ruthenium(II)-β-carboline complex

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ABSTRACT

Four [Ru(tpy)(N–N)(L)] type complexes: $[Ru(tpy)(bpy)(Nh)]^{2+}$ (**Ru1**, tpy = 2,2′;6′,2″-terpyridine, bpy = 2′2-bipyridine, Nh = Norharman), $[Ru(tpy)(phen)(Nh)]^{2+}$ (**Ru2**, phen = 1,10-phenanthroline), $[Ru(tpy)(dpa)(Nh)]^{2+}$ (**Ru3**, dpa = 2,2′-dipyridylamine) and $[Ru(tpy)(dip)(Nh)]^{2+}$ (**Ru4**, dip = 4,7-diphenyl-1,10-phenanthroline) were presented as anticancer drugs. *In vitro* cytotoxicity assays indicated that these complexes showed anticancer activity against various cancer cells. Flow cytometry and signaling pathways analysis demonstrated that these complexes induced apoptosis *via* the mitochondrial pathway, as evidenced by the loss of mitochondrial membrane potential and the release of cytochrome *c*. The resulting accumulation of p53 proteins from phosphorylation at serine-15 and serine-392 was correlated with an increase in p21 and caspase activation. Taken together, these findings suggested that **Ru1**–**Ru4** may contribute to the future development of improved chemotherapeutics against human cancers.



## 1. Introduction

Since cisplatin, one of the foremost and widely used metal-based anticancer drugs for cancer therapy that discovered in 1969 [1], the therapeutic value of metal-based drugs has long been established. DNA is generally accepted to be the main target of cisplatin, which has been demonstrated to bind most frequently to two adjacent guanine residues *via* their N7 position, thereby generating a kink in the DNA structure [2–4]. However, serious side-effects, toxicity, targeting, delivery, acquired resistance displayed by certain tumors and cancer specificity have limited its clinical applications [5–7]. Therefore, in efforts to improve the efficacy and overcome the side effects, numerous transition metal complexes have been synthesized and tested for their anticancer activities [8,9]. Ruthenium complexes are regarded as the most promising alternatives to cisplatin as anticancer drugs because of their rich synthetic chemistry, variable oxidation states that are accessible under physiological conditions, selective antimetastatic properties, multiple mechanisms of action that are distinct from those of platinum-based drugs and low systemic toxicity [10–13].

Ruthenium anticancer chemistry has already yielded many promising results. Several compounds have been described which display an activity comparable to that of cisplatin, and in some cases activities are even better [14–20]. Ruthenium complexes currently in clinical trials are complexes with dimethyl sulfoxide (DMSO) as monodentate ligand (NAMI-A and KP1019) [21,22]. Here we are interested in the chemistry of [Ru(tpy)(N–N)(L)] type of complexes (tpy = 2,2′;6′,2″-terpyridine, N–N = bpy (2′2-bipyridine), phen (1,10-phenanthroline) or other N–N bidentate ligand) which contain only one available coordination site. Recently, Brabec [23] found that despite a high cell uptake, [Ru(tpy)(bpy)Cl]Cl exhibited only a slight *in vitro* cytotoxicity. Grover [24,25] proved the DNA binding and cleavage activity based on aquaruthenium complex $[Ru(tpy)(N–N)H_2O]^{2+}$. Chattopadhyaya [26] have developed a Ru(II) complex containing acetonitrile leads to the formation of a cross-link between the metallocomplex tether and the G base. Corral [18] using toxic 2,2′-azobispyridine as the bidentate ligand to improve the antitumor activity. However, it is clear that causing cytotoxicity through aqua or chloro leaving groups, such complexes lack selectivity [27].

*Abbreviations:* tpy, 2,2′:6′,2″-terpyridine; Nh, Norharman; bpy, 2,2′-bipyridine; phen, 1,10-phenanthroline; dpa, 2,2′-dipyridylamine; dip, 4,7-dibenzyl-1,10-phenanthroline; PI, propidium iodide; DCFH-DA, 2′,7′-dichlorofluorescein-diacetate; tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt; NAC, *N*-acetylcysteine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

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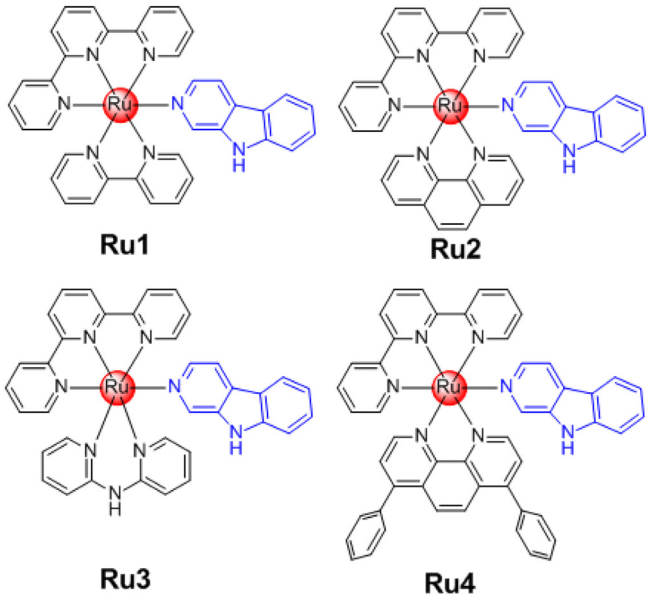
In this work, we present four new [Ru(tpy)(N–N)(L)] type complexes: $[Ru(tpy)(bpy)(Nh)]^{2+}$ (**Ru1**, tpy = 2,2′;6′,2″-terpyridine, bpy = 2′2-bipyridine, Nh = Norharman), $[Ru(tpy)(phen)(Nh)]^{2+}$ (**Ru2**, phen = 1,10-phenanthroline), $[Ru(tpy)(dpa)(Nh)]^{2+}$ (**Ru3**, dpa = 2,2′-dipyridylamine) and $[Ru(tpy)(dip)(Nh)]^{2+}$ (**Ru4**, dip = 4,7-diphenyl-1,10-phenanthroline) (Scheme 1). In contrast to the aqua or chloro leaving ligands, a kinetically more stable β-carboline group was introduced and the complexes have no free positions available for coordination to a DNA base. The choice of Norharman as a co-ligand is based on the known anticancer activity of some β-carboline derivatives [28]. The antitumor activity and cellular uptake, as well as cell-cycle arrest and apoptosis signaling pathways were studied. The unique structures and characteristics of Ru(II) complexes designed in this investigation may contribute to the future development of improved chemotherapeutics.

## 2. Chemistry

The free ligand Norharman was easily prepared using the Pictet–Spengler condensation of L-tryptophan with formaldehyde following the method described in the literature [29]. **Ru1**–**Ru4** were then obtained by refluxing the relative precursor with Norharman in ethanol, respectively. The synthesized complexes were characterized *via* $^1$H NMR spectroscopy, ESI–MS, infrared spectrum and elemental analysis (Supporting information – Fig. S1–S4). Single peaks between 10.65 ppm and 11.67 ppm in $^1$H NMR spectroscopy and peaks between 3000 cm and 1 to 3500 $cm^{-1}$ from infrared spectrum are characters of imino group. These results indicated that Norharman are coordinated with ruthenium *via* 2-N atom.

The geometries of complexes **Ru1**–**Ru4** were optimized by DFT method. Some ruthenium related geometric parameters are listed in Table S1 (Supporting information). The metallic center coordinated atomic arrangements include N(1) in Nh, N(2)–N(4) from group tpy and N(5), N(6) in substituent group N–N (N–N = bpy, phen, dpa and dip respectively for **Ru1**–**Ru4**). The optimized molecular structures show the ruthenium atoms are octahedral coordinated. The ruthenium coordination sphere deviates from the ideal octahedron with the small bite angles of tpy (N(2)–Ru–N(3), N(3)–Ru–N(4), 78.86°–79.18°). The distances between ruthenium atom and group Nh, as well as tpy, are pretty similar (deviations within 0.024 Å) in these complexes. Unlike other complexes, the two pyridine rings in **Ru3** are non-conjugated bonded to amido leading to larger angle of N(1)–Ru–N(5)/N(3)–Ru–N(6) and smaller angle of N(2)–Ru–N(5)/N(1)–Ru–N(6). The bond lengths of Ru–N(5) and Ru–N(6) are thus slightly longer in **Ru3** due to the weaken bond strengths between pyridines and ruthenium.




**Scheme 1.** Chemical structure of complex **Ru1**, **Ru2**, **Ru3** and **Ru4**.

The properties of frontier molecular orbital for the calculated complexes are given in Table S2 (Supporting information). The diagrams of highest-occupied molecular orbitals (HOMOs) and the lowest-unoccupied molecular orbitals (LUMOs) are presented in Fig. S5 (Supporting information). It is found that these compounds have similar frontier molecular orbital energies except HOMO of **Ru3**, which reveals a higher energy than other HOMOs. Calculated group compositions figure that HOMOs are delocalized on group N–N, while LUMOs are localized on tpy in general. As seen from Table S2, delocalizations of HOMO on dip for **Ru4** and LUMO on bpy for **Ru1** are not so obvious compared with others. It is worthwhile to note that electrons on the LUMO localize most on tpy (91.60%) but least on N–N (dpa) (0.52%) for **Ru3**, but its deviation between components of these two groups on HOMO (5.46%) is the smallest in all compounds.

The natural bond orbitals (NBO) analysis was performed for **Ru1**–**Ru4** to give the bonding properties. The occupancies and hybridizations of Ru–N bonding are listed in Table S3 (Supporting information). All the Ru–N bond orbitals are polarized towards the nitrogen atoms, which contribute more than 80% electrons. The values of bond order expose that bonding of ruthenium and tpy is stronger than others. The charge distribution with ruthenium and its combined groups are summarized in Table S4 (Supporting information). Charge donation from ligands towards metallic center is observed for the calculated natural charges on ruthenium are lower than theirs formal charge of +2. Results show tpy is the most positive charged ligand. It donates more charge for **Ru3** (0.8749) and less charge for **Ru2** (0.6858). On the other hand, Nh group in **Ru2** has more positive charges than other complexes (0.5242).

**Ru1**–**Ru4** exhibited intense spin-allowed intraligand ($^1$IL) absorption bands in the UV region at approximately 250–400 nm and less intense spin-allowed metal-to-ligand charge transfer ($^1$MLCT) absorption bands at approximately 450–550 nm (Supporting information – Fig. S6); these absorption properties are typical of Ru(II) polypyridine complexes. **Ru1**–**Ru4** displayed intense photoluminescence signal (380–550 nm) at 298 K upon excitation at 350 nm, which was contributed from Norharman (Supporting information – Fig. S7). When irradiated at 488 nm, no emission was observed. This is caused by the steric strain resulting from tridentate analogs compared to those of the corresponding bidentate bpy analogs; consequently, thermal access to a metal-centered (MC), non-emissive state is enhanced at room temperature [30].

In order to assess the possibility of hydrolysis in aqueous solution, the continuous irradiation experiments were carried out [31]. No change was observed in the absorption spectra of ruthenium(II) complexes under visible irradiation (Supporting information – Fig. S8).

## 3. Results and discussion

### 3.1. Cellular uptake properties

The cellular uptake characteristics of transition metal-based anticancer drugs are important influences on their cytotoxicity. For platinum complexes, the rates of cellular uptake and cytotoxic activities are proportional to their lipophilicities [32,33]. Thus, the uptake of **Ru1**–**Ru4** by the HeLa cancer cell line was studied to investigate a possible relationship between the cellular uptake and