International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 6, 2023

Original Article

EVALUATION OF THE POTENTIAL CYTOTOXICITY OF RUTHENIUM COMPLEX II AGAINST U-373 GLIOBLASTOMA CELLS

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Received: 27 Jul 2023, Revised and Accepted: 28 Aug 2023

ABSTRACT

Objective: The potential of ruthenium complexes as anticancer agents has gained significant attention in the scientific community. The aim of this study was to investigate the effect of dithiocyanato-N-bis[8(diphenylphosphino)quinoline]ruthenium (II), [Ru(N-P)$_2$(NCS)$_2$] on the glioblastoma U-373 tumor cells and apoptosis.

Methods: Ru(N-P)$_2$(NCS)$_2$] was synthesized and characterized using FTIR, and X-ray crystallography. The cytotoxic effects of [Ru(N-P)$_2$(NCS)$_2$] on glioblastoma U-373 tumor cells were evaluated using both the trypan blue assay and the activity of caspase-3 to detect apoptosis. A DPPH scavenging assay was used to evaluate the antioxidant activity.

Results: The [Ru(N-P)$_2$(NCS)$_2$] complex effectively inhibited the glioblastoma U-373 tumor cells with an IC_{50} of ~ 23 µg/ml. Similar to the majority of chemotherapeutic agents that kill via the intrinsic pathway, [Ru(N-P)$_2$(NCS)$_2$] induces apoptosis, which was confirmed by the activation of caspase-3, and these effects were dose-dependent. Ruthenium has antioxidant properties, so ruthenium Complex II exhibits lower toxicity towards normal cells while effectively targeting and eliminating cancer cells.

Conclusion: [Ru(N-P)$_2$(NCS)$_2$] is considered promising for researchers investigating putative biological activities, particularly antitumor and immune-related activity.

Keywords: Anticancer agents, Coordination complexes, Cytotoxic activity, Ruthenium, Tumor cell lines

DOI: https://dx.doi.org/10.22159/ijap.2023v15i6.48940. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Ruthenium belongs to the platinum group of the periodic table. Ruthenium complexes are compounds composed of ruthenium atoms that are coordinated to ligands. These complexes have numerous applications in the field of chemistry, such as catalysis, electronics, and materials science. In the past decade, ruthenium complexes have emerged as a promising new class of anticancer drugs [1].

In a variety of cancer cell lines, ruthenium complexes have demonstrated potent anticancer activity. Ruthenium (II) complexes with N-heterocyclic carbene (NHC) ligands have been shown to inhibit breast cancer and colon cancer cells [2]. These complexes inhibit the activity of thiol-and selenol-containing biomolecules, and Ruthenium complexes induce cell death by binding to DNA [3]. In addition, it has been discovered that Ruthenium complexes induce apoptosis in cancer cells via a variety of mechanisms.

For decades, chemotherapy drugs like cisplatin and oxaliplatin have been used to treat cancer [4]. However, these medications have significant limitations, such as toxicity and the emergence of drug resistance [4]. Ruthenium complexes have several advantages over conventional chemotherapy drugs, including lower toxicity, selective targeting of cancer cells while avoiding healthy cells, and different mechanisms of action that can aid in overcoming drug resistance [5, 6]. Therefore, in this study, the dithiocyanato-N-bis[8(diphenylphosphino)quinoline]ruthenium (II), [Ru(N-P)$_2$(NCS)$_2$], was synthesized, characterized using FT-IR and X-ray crystallography, and its putative cytotoxicity against the glioblastoma U-373 cell line was evaluated.

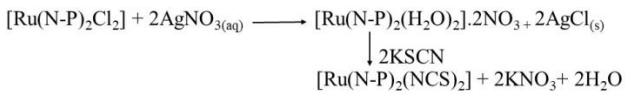
MATERIALS AND METHODS

Chemicals and reagents

Silver nitrate, acetone, potassium thiocyanate, heat-inactivated fetal bovine serum, L-Glutamine trypan blue dye, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The U-373 human glioblastoma cell line was obtained from ATCC (Rockville, Maryland). The caspase-3 colorimetric assay kit was obtained from *(*KeyGen Biotech, Nanjing, China*)*

Preparation of di(thiocyanato-N-)bis[8-(diphenylphosphino) quinoline]ruthenium(II) [Ru(N-P)$_2$(NCS)$_2$]

[Ru(N-P)2Cl2] was prepared using literature methods [7]. In brief, a [Ru(N-P)$_2$Cl$_2$] suspension (0.4 g, 0.5 mmol) in acetone (40 ml) was added to a filtered silver nitrate solution (0.17 g, 1.0 mmol) in distilled water (10 ml). Refluxing and stirring under nitrogen gas took two hours. A white AgCl precipitate formed, turning the solution yellow. A sintered-glass crucible cooled and filtered the mixture. The filtered solution contained 10 ml of potassium thiocyanate (0.10 g, 1.0 mmol) in acetone. The yellow solution turns orange. After extracting half the solvent under reduced pressure, 20 ml of diethyl ether precipitated the product. The orange complex was filtered, washed with 210 ml of distilled water and diethyl ether, and vacuum-dried at 60 °C. Yield: 0.31 g (74%), m. p. 205-210 °C (scheme 1) [8].



Scheme 1: The synthetic procedures for preparing Ru (II) complexes

Nicolet Impact-400 recorded the FTIR spectrum as KBr discs. Evaporating a dichloromethane solution of the complex yielded X-ray-ready crystals. At room temperature, an Oxford Xcalibur diffractometer (Mo-Kα radiation, 0.7107 A°) collected crystalline complex diffraction. CrysAlispro software created *hkl* files [7]. The SHELXTL program package solved and refined the structure [7]. All atoms were refined anisotropically except hydrogen, which was refined isotropically using a riding model.

Cell culture

ATCC (Rockville, Maryland) provided the U-373 human glioblastoma cell line. The cells were grown in DMEM with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mmol), and penicillin/streptomycin (100 U/ml, 100g/ml) at 37 °C in humidified air with 5% CO_2 [9].

Cell treatment

4 x 10^4 cells/ml were seeded in six-well plates, and after 12 h of attachment (time 0), the culture medium was replaced with 2.0 ml of fresh medium containing $[Ru(N\text{-}P)_2(NCS)_2]$ (2, 5, 10, 20, 50, 100 µg/ml). Cells were biochemically assessed 24 h after treatment.

Cytotoxicity assay

The trypan blue assay is one of the oldest and most widely used methods for counting cells and determining proliferation [9, 10]. Under a light microscope, nonviable cells with ruptured membranes are stained blue, while viable cells with intact plasma membranes are transparent. To allow cell attachment, 96-well plates were seeded at 1.0 x 10^4 cells/ml and incubated at 37 °C. Each well received 100 µl of drug-containing medium and was incubated for 72 h. After 72 h, trypsinization emptied the wells and mixed 25 µl of cell suspension with 25 µl of trypan blue dye. After 30 seconds, 25 µl of solution was added to a hemocytometer and examined under a microscope. Total and stained cells were counted. The following calculated the IC_{50}, indicating a net cell loss after treatment:

$$\text{Cytotoxicity (\%)} = \frac{\text{Number of dead cells}}{\text{Number of total cells (dead and viable)}} \text{X100}$$

The average cytotoxicity percentage from three trials was plotted against the concentration of the extract. The IC_{50} values have been determined.

Caspase activity assay

Human glioblastoma U-373 cells were treated with [Ru(N-P)2(NCS)2] at concentrations of 25, 50, and 150 g/ml for 48 h before total protein extraction with the RIPA reagent. A commercial kit (KeyGen Biotechnology, Nanjing, China) and an ELISA reader (ELX800, Promega, US) measured caspase-3 activity at 405 nm.

Antioxidant activity

DPPH scavenging activity measures antioxidant activity [10]. In brief, DPPH can measure the antioxidant activity of the [Ru(N-P)2(NCS)2] complex because it is a stable free radical that reacts with hydrogen-donating substances. 200 µl of 200, 100, 50, 20, 10, and 5 µg/ml Ru(II) complex or 100, 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/ml ascorbic acid (standard) was prepared in methanol and added to 2 ml of DPPH (0.21 mmol in 95% ethanol). After 60 min of shaking at room temperature in the dark, a spectrophotometer measured absorbance at 517 nm [10]. The sample reaction absorbs as, while the control reaction absorbs Ac. The IC_{50} was estimated by plotting percentages of inhibition against sample concentrations in 1 ml of the reaction mixture.

Statistical analysis

SPSS 19.0 performed an unpaired Student's t-test on mean, standard deviation and P value data. A $P<0.05$ was considered statistically significant.

RESULTS

Single-crystal X-ray $[Ru(N\text{-}P)_2(NCS)_2]$

$[Ru(N\text{-}P)_2(NCS)_2]$ was prepared in dry dioxane under nitrogen gas. $[Ru(N\text{-}P)_2(NCS)_2]$ characterization was completed by single crystal X-ray (fig. 1).

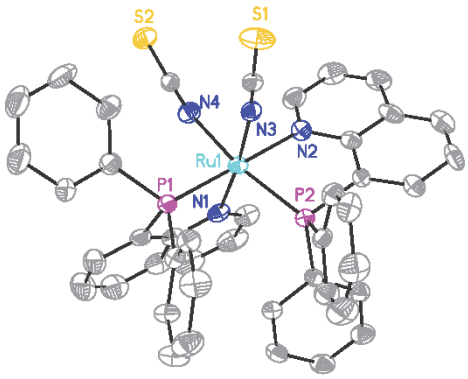


Fig. 1: Single crystal X-ray structure of $[Ru(N\text{-}P)_2(NCS)_2]$

The infrared spectrum (fig. 2) for the $[Ru(N\text{-}P)_2(NCS)_2]$ exhibits characteristic bands; 1092 cm^{-1} for $\nu_{C\text{-}P}$, 1490 and 1433 cm^{-1} were assigned for the $\nu_{C\text{-}C}$ of N-P ligand and 2096 and 806 cm^{-1} were assigned for $\nu_{C=N}$ and $\nu_{C=S}$ of NCS ligand, respectively.

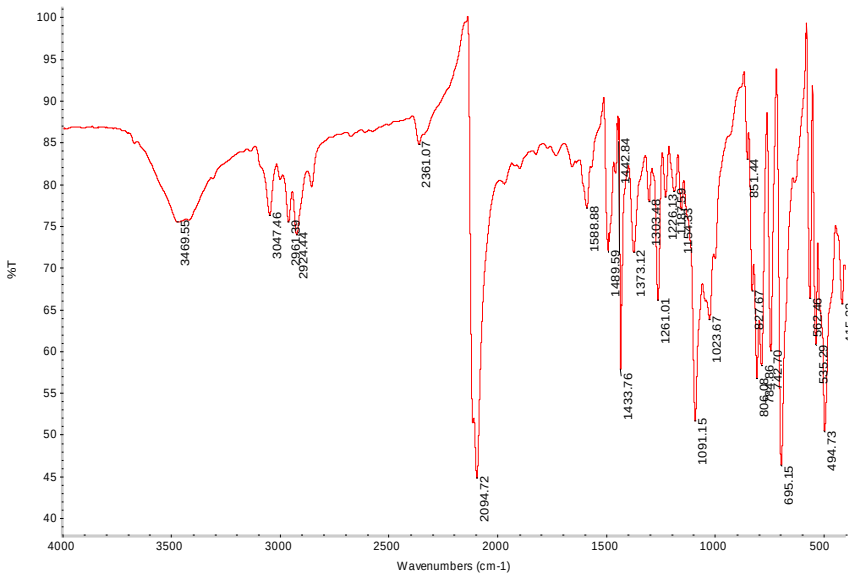


Fig. 2: FTIR spectrum of $[Ru(N\text{-}P)_2(NCS)_2]$

Cytotoxicity (trypan blue exclusion test)

Using the trypan blue exclusion assay for cell viability, the cytotoxic effects of $[Ru(N\text{-}P)_2(NCS)_2$ were quantified. The cytotoxicity of $[Ru(N\text{-}P)_2(NCS)_2]$ differed significantly from that of the control ($P < 0.001$). The estimated IC_{50} for $[Ru(N\text{-}P)_2(NCS)_2]$ is 23 g/ml (fig. 3).

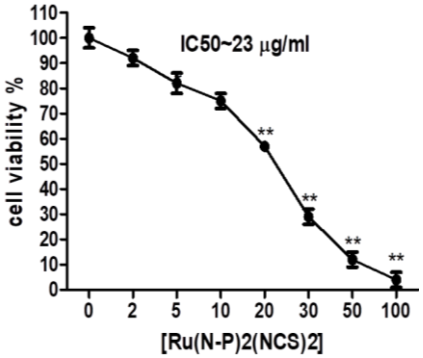


Fig. 3: IC_{50} values (mean±SD µg/ml) of $[Ru(N\text{-}P)_2(NCS)_2]$ from three cytotoxicity assays. The data are presented as the mean±standard deviation (SD) of triplet samples

Caspase-3 assay

Caspase3 activity—the apoptotic process's executioner—was measured to determine [Ru(N-P)2(NCS)2's cytotoxicity mechanism. $Ru(N\text{-}P)_2(NCS)_2$ activates Caspase 3 ($P<0.05$), suggesting caspase-independent programmed cell death. $[Ru(N\text{-}P)_2(NCS)_2]$ dose-dependently induced caspase-3 (fig. 4).

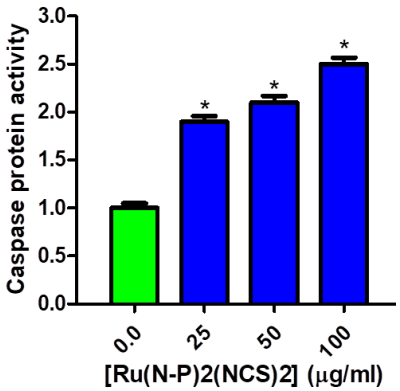


Fig. 4: Caspase protein activity of human glioblastoma U-373 cells treated with different concentrations of $[Ru(N\text{-}P)_2(NCS)_2]$. Values were significantly different compared with the control group. *P<0.01. The data are presented as the mean±SD of triplet samples

Antioxidant activity

DPPH assay was used to evaluate the antioxidant activity of $[Ru(N\text{-}P)_2Cl_2]$ (table 1). Ascorbic acid served as the positive control and had an IC_{50} of 4.1 µg/ml, whereas $[Ru(N\text{-}P)_2(NCS)_2]$ had an IC_{50} of 19.3 µg/ml.

Table 1: Antioxidant activity of $[Ru(N\text{-}P)_2(NCS)_2]$. The data are presented as the mean±standard deviation (SD) of triplet samples

Sample	IC_{50} (µg/ml)
$[Ru(N\text{-}P)_2(NCS)_2]$	19.3±0.26
Ascorbic acid	4.1±0.15

DISCUSSION

Ruthenium complexes have many advantages over platinum-based drugs, including lower toxicity, potent efficacy, and decreased drug resistance [11-13]. This study utilized X-ray crystallography, a powerful technique that provides a three-dimensional structure of the ruthenium (II) complex. Researchers can comprehend the arrangement of atoms and ligands, as well as any potential interactions with human glioblastoma U-373 cells, by determining the crystal structure of the complex.

In this study, $[Ru(N\text{-}P)_2(NCS)_2]$ has been shown to have highly cytotoxic activity against human glioblastoma U-373 cells in a dose-dependent manner with $IC_{50} \sim 23$ µg/ml, indicating its potential as an effective anti-proliferative agent. Ruthenium (II) complexes have shown low cytotoxicity but exhibit high cytotoxic activity against cancer cells [14]. Selective uptake of ruthenium complexes by cancer cells, such as human cervical carcinoma cells, has been observed, leading to autophagy-dependent cell apoptosis through mitochondrial dysfunction and reactive oxygen species (ROS) accumulation [14]. For instance, indazolium bis-indazole tetra chlororuthenate (KP1019) has demonstrated encouraging activity against colorectal tumors [15]. Ruthenium complex HB324 has been shown to effectively inhibit the proliferation of leukemia cells in a dose-dependent manner. At a concentration of 1 µM, HB324 caused 100% proliferation inhibition, indicating its potential as an effective anti-proliferative agent [16].

The anticancer activity of the four Ru(II) complexes, including 4,4'-dimethyl-2,2'-bipyridine (dmbpy), 2,2'-bipyridine (bpy), 1,10-phenanthroline (phen), and 2,9-dimethyl-1,10-phenanthroline (dmp), was shown against HepG2 cells [11]. TCPIP (2-(2,3,5-trichlorophenyl)-1H-imidazo[4,5-f][1,10]phenanthroline) exhibited a pronounced cytotoxic effect on B16 cells with a low IC_{50} value of 1.2±0.2 µM [17].

The *in vitro* anticancer activity of Ru(II) complexes has demonstrated potent cytotoxic effects against cancer cells while exhibiting minimal impact on normal cells [18-22]. For example, Wang *et al.* demonstrated that Ru(II) complexes induced apoptosis in cancer cells, leading to their cytotoxicity. a water-soluble Ru(II) complex, $Ru(bpy)_2(dtdpq)_2$, exhibited low cytotoxicity but showed high activity upon irradiation, with an IC_{50} value of 2.3±0.3 µM against MCF-7 cells [18]. This highlights the potential of Ru(II) complexes in photodynamic therapy. This is compatible with our study, where $[Ru(N\text{-}P)_2(NCS)_2]$ induces apoptosis through the induction of caspases depending on increasing ruthenium concentration.

Chen *et al.* investigated the uptake and cytotoxicity of Ru(II) complexes in human cervical carcinoma (HeLa) cells. They found that these complexes selectively killed cancer cells while having minimal effects on normal cells. The complexes induced autophagy-dependent cell apoptosis through mitochondrial dysfunction and reactive oxygen species accumulation [19].

CONCLUSION

Ruthenium (II) complexes have emerged as promising anticancer agents with unique biochemical properties and versatile applications. They offer several advantages over traditional chemotherapy agents, including greater selectivity and reduced toxicity. However, their mechanisms of action are still not fully understood, and more research is needed to fully realize their potential. With continued research and development, ruthenium (II) complexes could become a crucial tool in the fight against cancer.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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