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Research Article

Antiproliferative activity of ruthenium complex II against human cancer cell *in vitro*

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Abstract

Despite significant advancements in cancer treatment, there is a constant need for new and effective therapeutic options. One such potential weapon in the fight against cancer is ruthenium complex II. In this article, we synthesized, characterized, and studied the activity of dithiocyanato-N-bis[8-(diphenylphosphino)quinoline]ruthenium (II) [Ru(N-P)2(NCS)2] against MCF-7 human adenocarcinoma cells and the MRC-5 cell lines from fetal lung fibroblast-like cells as normal cells, as well as the mechanisms of action and selectivity. This study demonstrated that $[Ru(N\text{-}P)_2(NCS)_2]$ has cytotoxic activity against MCF-7 with IC_{50} values of 7.56 µg/ml and cytotoxic activity against MRC-5 cell lines with IC_{50} values of 576.6 µg/ml. $[Ru(N\text{-}P)_2(NCS)_2]$ showed more selective cytotoxic activity against MCF-7 cancer cell lines than MRC-5 normal cell lines. . This study demonstrated the potent apoptotic activity of ruthenium complex II by determining the activation of caspase-3, highlighting its potential as a therapeutic agent in cancer treatment. The $[Ru(N\text{-}P)_2(NCS)_2]$ is considered promising for researchers investigating putative biological activities, particularly antitumor and immune-related activity.

Keywords

Anticancer agents, coordination complexes, cytotoxic activity, ruthenium complex, tumor cell lines

Introduction

Cancer, a devastating disease that affects millions of lives worldwide, requires innovative and effective treatment options (Saadh et al. 2023). In recent years, a unique compound called the Ruthenium Complex has emerged as a promising anticancer agent. Ruthenium, a transition metal, has shown tremendous potential in combating various forms of cancer (Zuba et al. 2020).

The Ruthenium Complex, comprising of a ruthenium atom bonded to organic ligands, possesses exceptional properties that make it an attractive choice for cancer treatment. The use of the Ruthenium Complex in cancer treatment offers several advantages over traditional therapies. Firstly, its selective targeting of cancer cells ensures that healthy cells are spared from unnecessary damage. This targeted approach not only reduces the risk of side effects but also improves the overall quality of life for patients undergoing treatment (Kanaoujiya et al. 2023). Furthermore, the Ruthenium Complex has shown great potential in overcoming drug resistance, a significant challenge in cancer therapy. Many cancer cells develop resistance to traditional chemotherapy drugs, rendering them ineffective. However, studies have shown that the Ruthenium Complex can effectively target and eliminate drug-resistant cancer cells, providing a ray of hope for patients with limited treatment options (Valente et al. 2021; Kanaoujiya et al. 2023). Additionally, the Ruthenium Complex exhibits excellent stability and compatibility with other drugs, making it an ideal candidate for combination therapies. By combining the complex with other anticancer agents, researchers have achieved synergistic effects, enhancing the overall efficacy of the treatment. This

versatility opens up new possibilities for personalized and tailored cancer therapies (Lee et al. 2020; Valente et al. 2021).

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 MCF-7 human brest cancer cell lines and MRC-5 normal cell lines was determined to assess the selectivity of the ruthenium complex for cancer cells. The cytotoxic mechanism of [Ru(N-P)2(NCS)2] was determined by measuring the apoptosis executor caspase3 activity.

Materials and methods

Chemicals and reagents

Silver nitrate, dichloromethane, 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). MCF-7 and MRC-5 cell lines were obtained from the ECACC (Salisbury, UK). 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 synthesized using published procedures (Al-Wahish et al. 2017). Briefly, a suspension of [Ru(N-P)2Cl2] (0.40 g, 0.50 mmol) in acetone (40 mL) was added to a filtered solution of silver nitrate (0.17 g, 1.0 mmol) in distilled water (10.0 mL). Two hours were required for refluxing and stirring under nitrogen gas. A precipitate of white AgCl formed, turning the solution yellow. A crucible made of sintered glass cooled and filtered the mixture. The filtered solution contained 10 mL of 0.10 g (1 mmol) of potassium thiocyanate in acetone. The yellow solution becomes orange. 20 mL of diethyl ether precipitated the product after half the solvent was extracted under reduced pressure. The orange complex was filtered, washed with 210 mL of distilled water and diethyl ether, and then vacuum-dried at 60 °C (Al-Wahish et al. 2017). Yield: 0.31 g (74%), melting point: 205–210 °C (Scheme 1). KBr discs were used by the Nicolet Impact-400 to record the FT-IR spectrum. A dichloromethane solution of the complex evaporated into X-ray-ready crystals. An Oxford Xcalibur diffractometer (Mo-K radiation, 0.7107 A°) collected crystalline complex diffraction at room temperature. The CrysAlispro software generated hkl files (Al-Wahish et al. 2017). The SHELX-TL software package resolved and refined the structure (Al-Wahish et al. 2017). With the exception of hydrogen, which was refined isotropically using a riding model, all atoms were refined anisotropically.

$$[Ru(N\text{-}P)_2Cl_2] + 2AgNO_{3(aq)} \longrightarrow [Ru(N\text{-}P)_2(H_2O)_2].2NO_3 + 2AgCl_{(s)}$$
$$\downarrow 2KSCN$$
$$[Ru(N\text{-}P)_2(NCS)_2] + 2KNO_3 + 2H_2O$$

Scheme 1. Ru(II) complex synthesis.

Cell culture

MCF-7 and MRC-5 cells were grown in DMEM with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), and penicillin/streptomycin (100 U/ml, 100 µg/ml) at 37 °C in humidified air with 5% CO_2 (Abusamra et al. 2015).

Cell treatment

After 12 h of attachment, 2.0 ml of fresh medium containing [Ru(N-P)$_2$(NCS)$_2$] (2, 5, 10, 20, 50, 100 µg/ml) was added to six-well plates containing 4×10^4 cells/ml. Cell biochemistry was assessed 24 hours post-treatment.

MTT cytotoxicity assay

Final concentrations of 2, 5, 10, 20, 50, and 100 µg/ml of [Ru(N-P)$_2$(NCS)$_2$] were added to approximately 1×10^4 MCF-7 cells and incubated for 72 hours. After exposure, wells were incubated for four hours with 3-(4,5-dimethylthiazol-2w-yl)-2,5-diphenyl tetrazolium bromide (MTT). After dissolving the MTT crystals in 100 µl of DMSO solution, a multi-well plate reader (Bio-Tek Instrument, USA) measured the optical density (OD) at 570 nm with a reference wavelength of 630 nm (Csupor-Löffler et al. 2009). Positive controls included doxorubicin at 0.1, 0.5, 1.0, 1.5, 10, 25, 50, and 100 µg/ml. Cell growth inhibition percentage was calculated: (Treated OD/Non-treated OD 100) = 100% inhibition (Csupor-Löffler et al. 2009). The IC_{50} concentration of [Ru(N-P)$_2$(NCS)$_2$] that inhibits 50% of cell lines proliferation was determined.

Caspase activity assay

MCF-7 cells were treated for 48 hours with [Ru(N-P)$_2$(NCS)$_2$] (25, 50, and 100 µg/mL) before total protein extraction with RIPA reagent. At 405 nm, caspase-3 activity was measured using a commercial kit (KeyGen Biotechnology, Nanjing, China) and an ELISA reader (ELX800, Promega, US).

Statistical analysis

Data analysis was performed using the SPSS software. Differences among the studied groups were determined based on one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons as post-hoc tests. All of the values are the mean ± SD and are representative of the results of three independent experiments.

Results

Single-crystal X-ray [Ru(N-P)$_2$(NCS)$_2$]

Under nitrogen gas, [Ru(N-P)$_2$(NCS)$_2$] was prepared in dry dioxane. Characterization of [Ru(N-P)(NCS)$_2$] was completed via single crystal X-ray. Fig. 1 shows that each molecule of 8-(diphenylphosphino)quinoline acts as a bivalent chelating ligand, with bonds between the P and N atoms. The thiocyanate anion acts as a monodentate ligand, binding through N-atoms.

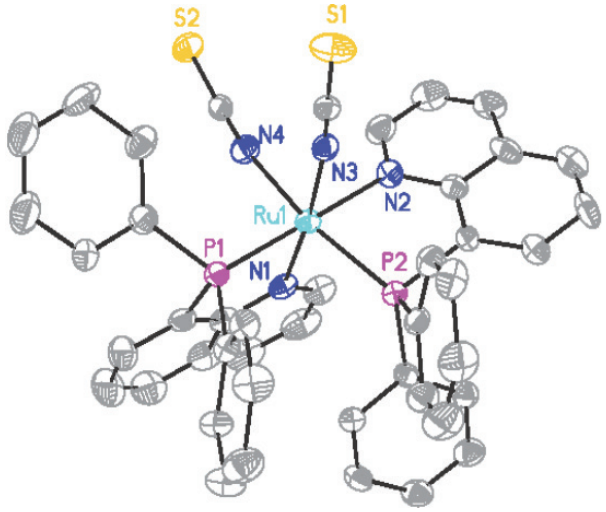

Figure 1. Single crystal X-ray structure of [Ru(N-P)$_2$(NCS)$_2$].

The infrared spectrum of [Ru(N-P)$_2$(NCS)$_2$] (Fig. 2) shows characteristic bands at 1092 cm-1 for $\nu_{C\text{-}P}$, 1490 and 1433 cm-1 for $\nu_{C\text{-}C}$ ring stretching vibrations, 2096 and 806 cm-1 for $\nu_{C=N}$ and $\nu_{C=S}$, respectively.

Antiproliferative and cytotoxicity

The MTT assay measured doxorubicin's cytotoxicity. As a positive control, doxorubicin had IC_{50} values of 4.36 and 6.3 µg/ml against MCF-7 and MRC5. [Ru(N-P)2(NCS)2] showed high cytotoxic and antiproliferative activity against MCF-7 cell lines (IC50 = 7.56 µg/ml), but low cytotoxic and antiproliferative activity against normal MRC-5 cell lines (IC50 = 576.6 µg/ml) (Table 1).

Table 1. [Ru(N-P)$_2$(NCS)$_2$] IC_{50} values (mean ± SD µg/ml) from three cytotoxicity assays.

Cytotoxicity assay	Treatment	IC_{50}	
		MCF-7	Fibroblasts (MRC5)
MTT assay	[Ru(N-P)$_2$(NCS)$_2$]	7.56 ± 0.43	576.6 ± 0.47
	Doxorubicin	4.36 ± 0.39	6.3 ± 0.11

Caspase-3 assay

Caspase3 activity, the executor of apoptosis, was measured to determine the cytotoxic mechanism of [Ru(N-P)$_2$(NCS)$_2$]. Caspase 3 is activated by [Ru(N-P)$_2$(NCS)$_2$] ($P < 0.05$), and [Ru(N-P)$_2$(NCS)$_2$] dose-dependently induced caspase-3 (Fig. 3).

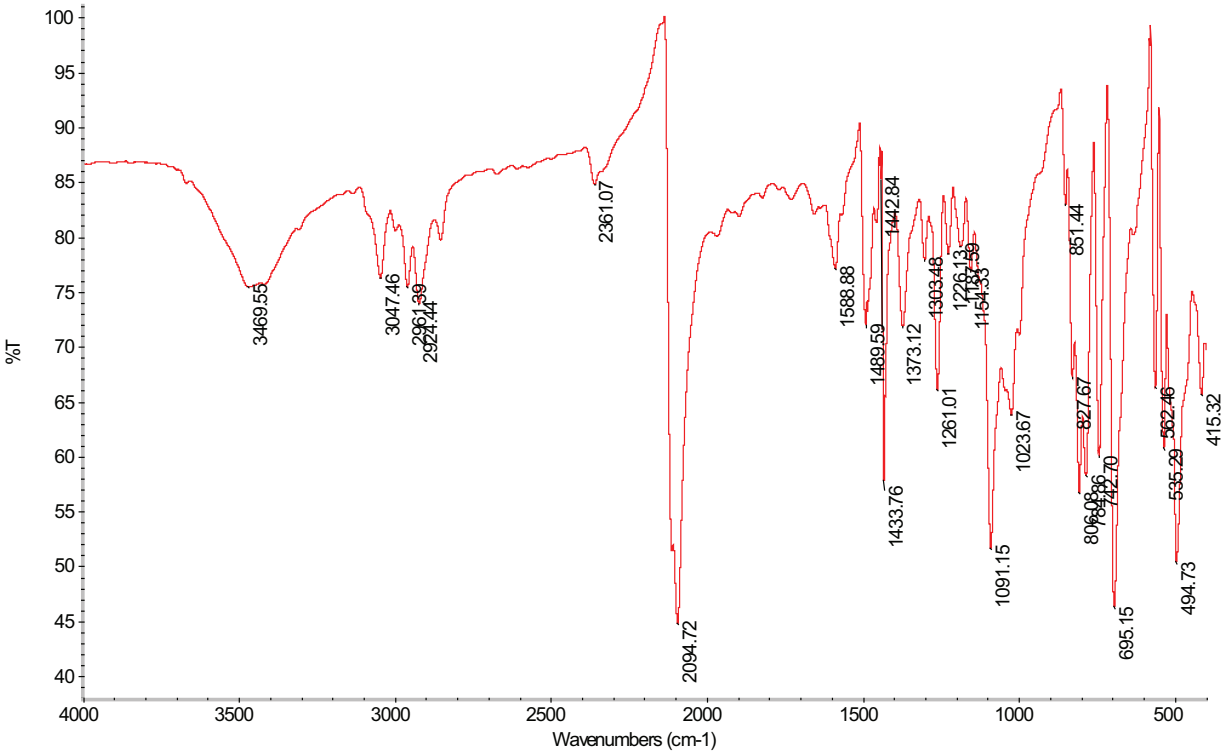

Figure 2. FTIR spectrum of [Ru(N-P)$_2$(NCS)$_2$].

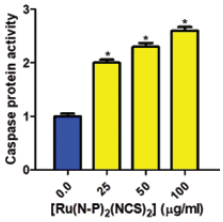

Figure 3. Caspase protein activity of MCF-7cells treated with different concentrations of [Ru(N-P)$_2$(NCS)$_2$]. Values were significantly different compared with the control group. *P < 0.01.

Discussion

Ruthenium complexes offer several advantages over platinum-based drugs. Firstly, ruthenium complexes can exhibit a broader range of coordination geometries, allowing for a more precise and tailored design of drug molecules (Valente et al. 2021; Prathima et al. 2023). This flexibility enables the customization of ruthenium complexes to specifically target cancer cells, minimizing the impact on healthy tissues. Additionally, ruthenium complexes have shown excellent stability, ensuring their efficacy throughout the treatment process. Furthermore, ruthenium complexes have a lower propensity for causing drug resistance, making them more effective in combating cancer (Valente et al. 2021; Prathima et al. 2023).

Targeted cancer therapy focuses on specific molecular targets in cancer cells, thus minimizing damage to normal cells and increasing treatment effectiveness (Kostova 2006). In agreement with our study, [Ru(N-P)2(NCS)2] has been shown to exhibit high cytotoxicity and anti-proliferative activity against MCF-7 cells in a dose-dependent manner with an IC_{50} of 7.56 µg/ml.. However, [Ru(N-P)$_2$(NCS)$_2$] has low cytotoxicity against MRC-5 normal cell lines. The in vitro anticancer activity of Ru(II) complexes have demonstrated potent cytotoxic effects against cancer cells, while exhibiting minimal impact on normal cells (Anitha et al. 2018; Zhang et al. 2019; Chen et al. 2021). For example, Ru(bpy)$_2$(dtdpq)$_2$ exhibits potent cytotoxicity against MCF-7 cells and has the ability to inhibit their proliferation and induce apoptosis, with an IC_{50} value of 2.3 ± 0.3 µM against MCF-7 cells (Zhang et al. 2019). Also, Ru (II) complexes inhibit HeLa cells while having minimal effects on normal cells (Chen et al. 2021). Ru (II) complexes have multiple mechanisms to inhibit cancer cells by generating reactive oxygen species (ROS), inducing apoptosis, inhibiting DNA repair enzymes, and causing DNA damage which can damage cancer cells and cause cell death (Kostova 2006; Anitha et al. 2018).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 (Zhang et al. 2019). For instance, HB324 interacts with specific proteins within leukemia cells, disrupting their normal function and signaling pathways. This disruption leads to cell cycle arrest and ultimately apoptosis. At a concentration of 1 µM, HB324 caused 100% proliferation inhibition (Wilke et al. 2023). The ruthenium complex II used in this study had a similar effect by stimulating the process of apoptotic cell death through caspase stimulation. Recent studies have shown that ruthenium complex can act as a potent activator of caspase 3, promoting apoptosis in cancer cells (Li et al. 2012). The unique properties of ruthenium complex, such as its ability to interact with DNA and proteins, make it an ideal candidate for targeting cancer cells specifically. When ruthenium complex is introduced to cancer cells, it interacts with cellular components, triggering a cascade of events that ultimately leads to caspase 3 activation and apoptosis. This targeted approach minimizes damage to healthy cells, making the ruthenium complex a promising candidate for cancer treatment (Li et al. 2012). In this article, we proved the potential of the ruthenium complex in inducing apoptosis, presumably by activating caspase 3, which plays an important role in cancer treatment. The anticancer activity was shown of the Ru(II) complexes against HepG2 cells (Hu et al. 2023). Also, the ruthenium (II) complexes exhibited a pronounced cytotoxic effect on B16 cells with a low IC_{50} value of 1.2 ± 0.2 µM (Huang et al. 2023).

Conclusion

[Ru(N-P)$_2$(NCS)$_2$] represents a promising solution for combating MCF-7 human breast cancer cell lines. [Ru(N-P)$_2$(NCS)$_2$] has ability to selectively target cancer cells, induce apoptosis, and overcome drug resistance makes it a valuable addition to the field of cancer therapy. Therefore, [Ru(N-P)2(NCS)2] is a promising chemotherapeutic agent for the prevention and treatment of human breast cancer. However, further research is needed to fully understand its mechanism of action, optimize its delivery, and ensure its long-term safety.

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