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Research Article

Anticancer and antiproliferative activity of ruthenium complex (II) bearing 3,3′-dicarboxy-2,2′-bipyridine ligand

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Abstract

Even though significant progress has been made in cancer treatment, there is always room for improvement. The experimental drug Ruthenium Complex II shows promise as a cancer treatment. In this article, the dichloro-3,3'-dicarboxy-2,2'-bipyridyl bis(dimethylsulphoxide)ruthenium(II) [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$], have been synthesized, characterized, and studied for its anticancer activity against MDA-MB-231 and MRC-5 cell lines, as well as its mechanisms of action and selectivity. According to research, [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$], is highly cytotoxic to the MDA-MB-231 and minimum cytotoxic to MRC-5 cell lines, with IC_{50} values of 5.95 and 579.6 µg/ml, respectively. Ruthenium Complex II is exceptionally effective at destroying cancer cells while causing minimal harm to healthy cells. $RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] caused apoptosis, which was confirmed by the activation of caspase-3. Ruthenium complexes hold great promise as powerful anticancer agents. Their unique mechanisms of action, ability to selectively target cancer cells, and versatility in chemical structure make them attractive candidates for the development of targeted therapies.

Keywords

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

Introduction

Cancer, a complex and devastating disease, often demands innovative treatments due to limitations of traditional drugs that may cause systemic toxicity and drug resistance (Abusamra et al. 2015). In contrast, ruthenium complexes offer promise with targeted mechanisms, tailored design, and potential to overcome resistance, holding potential for more effective and selective cancer treatment (Al-Wahish et al. 2017).

Ruthenium complexes have emerged as intriguing candidates for anticancer therapy due to their unique chemical properties and diverse mechanisms of action (Anitha et al. 2018). These complexes can interact with biomolecules such as DNA, proteins, and enzymes, influencing key cellular processes and leading to apoptosis, or programmed cell death, in cancer cells. Their ability to target specific cancer cells while sparing healthy ones has sparked interest in their potential as selective anticancer agents. Ruthenium complexes can inhibit DNA replication and repair, disrupt cellular signaling pathways, and interfere with angiogenesis, the process by which tumors develop new blood vessels (Anitha et al. 2018). Their structural variability allows for the design of complexes with tailored properties optimized for specific cancer types. However, rigorous research is ongoing to assess their toxicity, bio

distribution, and overall safety profile in order to translate these findings into effective and safe anticancer treatments (Chen et al. 2021; Awwadiet al. 2022).

MDA-MB-231 is a widely studied triple-negative breast cancer cell line known for its aggressive behavior, making it suitable to investigate the potential efficacy of anticancer agents like ruthenium complexes (Csupor-Löffler et al. 2009).

In this study, the dichloro-3,3'-dicarboxy-2,2'-bipyridyl bis(dimethylsulphoxide)ruthenium(II) [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$], was synthesized, characterized using FT-IR and X-ray crystallography. This study aims to synthesize a novel ruthenium complex and characterize it using FT-IR, UV, and NMR techniques. Subsequently, assess the anticancer potential of a ruthenium complex against MDA-MB-231 cells by determining its IC_{50} value and evaluating its impact on caspase 3 activity, shedding light on its cytotoxic and apoptotic effects.

Materials and methods

Materials

According to published procedures, 3,3'-dicarboxy-2,2'-bipyridine and [RuCl2 (DMSO)4] were synthesized. Following procedures outlined in the literature, 1,4-dioxane (Merck) was purified and dried (Armarego W L F, 2017). Ethanol and methanol were redistillated in an atmosphere of nitrogen. All operations were performed in the N2 atmosphere. The tested samples were dissolved using distilled water as the solvent.

Preparation of dichloro-3,3′-dicarboxy-2,2′-bipyridyl bis(dimethylsulphoxide)ruthenium(II) [$RuCl_2$(3,3′-dcbpy)$(DMSO)_2$]

A suspension of 3,3'-dcbpy compound (0.121 g, 0.500 mmol) in dry ethanol (20 mL) was added to a suspension of [$RuCl_2$(DMSO)4] (0.242 g, 0.500 mmol) in dry ethanol (20 mL). Two hours were spent heating the reaction mixture to reflux under a nitrogen flow. During this time, the color of the solution changed to a brown-red hue. After allowing the reaction to cool to room temperature, it was filtered. The solvents were eliminated in order to achieve dryness. The residual solid was dissolved in minimal dry methanol and then filtered. Adding 20 mL of diethyl ether produced a brown solid. The product was then filtered, washed with diethyl ether (210 mL), and vacuum-dried at 60 °C for four h (Kostova 2006; He et al. 2019). Yield 89.5%, m.p. 195–200 °C.

The infrared spectra were recorded on KBr discs using a Nicolet Impact-400 FT-IR spectrometer. On a Bruker AVANCE III-500 MHz spectrometer, the 1H and 13C NMR spectra were acquired. The Philip-Harris melting point apparatus was used to determine melting points. Using a Cary 100 Bio UV-Vis spectrophotometer, UV-Visible spectra were generated for 1.0 10-5 M solutions in CH2Cl2 at 25 °C (Kostova 2006; He et al. 2019).

Cell culture

MRC-5 and MDA-MB-231 cells were obtained from ECACC. MCF-7 and MRC-5 cells were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), and penicillin/streptomycin (100 U/ml, 100 g/ml) (Prathima et al. 2023) at 37 °C with 5% CO2.

Cell treatment

After 12 hours of attachment, 2.0 ml of fresh medium containing 2, 5, 10, 20, 50, and 100 g/ml [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] was added to six-well plates containing 4×10^4 cells/ml. The biochemistry of cells was evaluated 24 hours after treatment (Prathima et al. 2023).

MTT cytotoxicity assay

In brief, incubate 1×10^4 cells with Ru(3,3'-dcbpy)(DMSO)2 Cl2] at concentrations of 2, 5, 10, 20, 50, and 100 µg/ml for 72 hours. Wells were incubated with MTT for four hours after exposure. A multi-well plate reader (Bio-Tek Instrument, USA) measured optical density (OD) at 570 nm with a reference wavelength of 630 nm after dissolving MTT crystals in 100 µl of DMSO solution (Prathima et al. 2023; Saadh et al. 2023). Positive controls: doxorubicin (0.1, 0.5, 1.0, 1.5, 10, 25, 50, 100 µg/ml). (Treated OD/Non-treated OD 100) = 100% cell growth inhibition (Prathima et al. 2023; Saadh et al. 2023). The IC_{50} concentration inhibits cell line proliferation 50%.

Caspase activity assay

RIPA reagent was used to extract total protein from MDA-MB-231 cells after 48 hours of treatment with [Ru(3,3'-dcbpy) (DMSO)2 Cl2] (25, 50, and 150 µg/mL). A commercial kit (KeyGen Biotechnology, Nanjing, China) and an ELISA reader (ELX800, Promega, US) measured caspase-3 activity at 405 nm.

Statistical analysis

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

Results

[$RuCl_2$(3,3′-dcbpy)$(DMSO)_2$]

The new ruthenium complex was made by directly reacting $RuCl_2(DMSO)_4$ with 3,3'-dicarboxy-2,2'-bipyridine in

dry ethanol. The [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] is formed by mixing RuCl2(DMSO)4 with one equivalent of ligand (Equation 1). Microelemental analysis confirmed the complex formulation. This complex was characterized by FT-IR and UV-Vis.

The brown complex [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] is soluble in water, methanol, ethanol, acetone, tetrahydrofuran, and dimethylsulphoxide but not in dichloromethane, chloroform, petroleum ether, or diethyl ether.

$$[RuCl_2(DMSO)_4] + 3,3'\text{-dcbpy} \xrightarrow{\text{Dry EtOH/ Reflux}} [RuCl_2(3,3'\text{-dcbpy})(DMSO)_2] \quad ...(1)$$

FT-IR Spectral analysis

Table 1 and Fig. 1 show the characteristic bands in the ligand, Ru-DMSO precursor, and newly synthesized Ru-complex spectra.

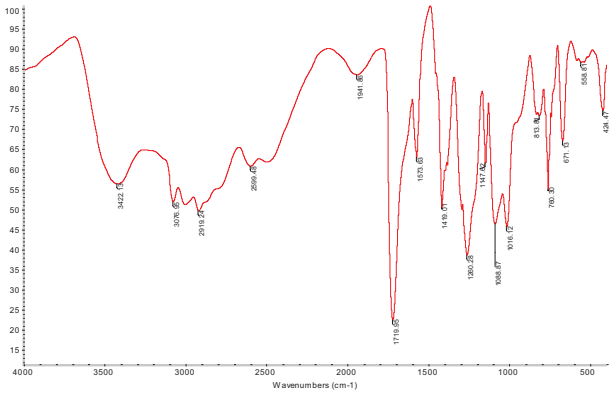

Figure 1. FTIR spectrum of [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$].

Table 1. (N-P), [$RuCl_2(DMSO)_4$], and [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] complexes infrared spectral data.

Mode	Compounds		
	[$RuCl_2(DMSO)_4$]	(3,3'-dcbpy)	[$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$]
n_{O-H}	–	3392	3422
n_{C-H} (Aromatic)	–	3073	3076
n_{C-H} (Aliphatic)	3002, 2920	–	2919, 2599
n_{C-C} (Aromatic)	–	1578. 1433	1573, 1419
$n_{C=O}$	–	1717	1719
$n_{S=O}$ (S-bonded)	1100, 1021	–	1088, 1016
$n_{S=O}$ (O-bonded)	927	–	–

UV-Vis spectroscopy.

Ru-complex [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] UV-visible spectrum was measured in MeOH solution. Fig. 2 shows three absorption bands at 381, 302, and 204 nm for the complex.

Antiproliferative and cytotoxicity

The MTT assay assessed doxorubicin's cytotoxicity. As a positive control, doxorubicin showed IC50 values of 5.15 and 7.45 µg/m against MDA-MB-231 and MRC5. The [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] exhibits IC50 values of 5.95 and 579.6 µg/ml against MDA-MB-231 and MRC5, respectively (Table 2).

Table 2. [$RuCl_2$(3,3'-dcbpy) $(DMSO)_2$] IC_{50} values (mean ± SD µg/ml) from three cytotoxicity assays.

Cytotoxicity assay	Treatment	IC_{50}	
		MDA-MB-231	Fibroblasts (MRC5)
MTT assay	[$RuCl_2$(3,3'-dcbpy) $(DMSO)_2$]	5.95 ± 0.39	579.6 ± 0.41
	Doxorubicin	5.15 ± 0.35	7.45 ± 0.17

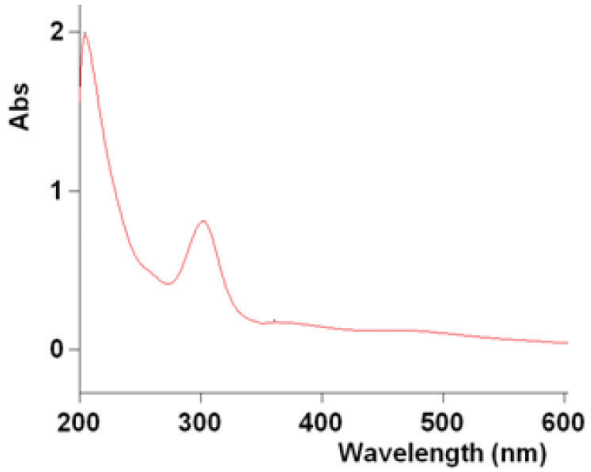

Figure 2. UV-Visible spectrum of [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$].

Caspase-3 assay

To determine [$RuCl_2$(3,3'-dcbpy)$(DMSO)_2$] cytotoxic mechanism, caspase3 activity, the apoptosis executor, was measured. Activation of caspase 3 by [$RuCl_2$(3,3'-dcbpy) $(DMSO)_2$] ($P < 0.05$) is dose-dependent (Fig. 3).

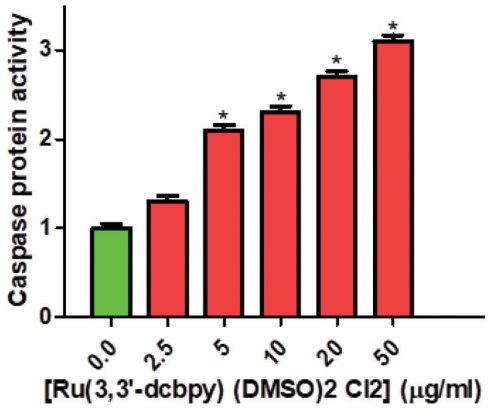

Figure 3. Caspase protein activity of MDA-MB-231cells treated with different concentrations of [$RuCl_2$(3,3'-dcbpy) $(DMSO)_2$]. Values were significantly different compared with the control group. *$P < 0.01$.

Discussion

Ruthenium complexes exhibit unique chemical properties that enable targeted interactions with cancer cells, resulting in effective cytotoxicity (Chen et al. 2021; Awwadiet al. 2022). Their versatile coordination chemistry allows for tailored modifications, enhancing selectivity and reducing off-target effects. This specificity, coupled with their diverse mechanisms of action and potential to overcome drug resistance, underscores their promise as valuable candidates in the development of innovative and potent anticancer agents (Chen et al. 2021; Awwadiet al. 2022).

Ruthenium exhibits cancer cell specificity, minimizing impact on normal cells. Its selective behavior holds promise for targeted therapies with reduced side effects (Sha et al. 2015). This agreement with our study which indicate the $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$ has been shown to highly cytotoxicity against MDA-MB-231 cells in a dose-dependent manner with IC_{50} ~ 5.95 ± 0.39µg/ml, indicating its potential as an effective anti-proliferative agent against cancer cells, while exhibiting minimal impact on normal cells. 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 (Shabani et al. 2023). Also, Ru (II) complexes inhibit HeLa cells while having minimal effects on normal cells (Valente 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 (van Rijt and Sadler 2009; Sha et al. 2015). The caspase-3 assay was utilized to elucidate the cytotoxic mechanism of $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$. Caspase 3, a pivotal mediator of apoptosis, exhibited dose-dependent activation triggered by the ruthenium complex. similarly, ruthenium complex displays a potential to induce apoptosis in MDA-MB-231 cells, reinforcing its role as an anticancer agent by initiating programmed cell death pathways. 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 ruthenium complex a promising candidate for cancer treatment (Yu et al. 2014). Moreover, the ruthenium complex displays inhibition against lung cancer (A549) by instigating apoptosis, DNA damage, and oxidative stress, showcasing its therapeutic potential (Zeng et al. 2017). Similarly, it inhibits colon cancer (HCT116) cells through apoptosis induction, DNA damage, and modulation of cellular signaling pathways, highlighting its promise in colon cancer treatment (Zhang et al. 2019). Additionally, the complex hinders HeLa cells by prompting apoptosis, potentially impairing DNA, and influencing cellular signaling pathways, underscoring its anticancer capabilities.

Conclusion

In conclusion, $[RuCl_2(3,3'\text{-dcbpy})(DMSO)_2]$ demonstrated significant cytotoxicity against MDA-MB-231. Ruthenium exhibits cancer cell specificity, minimizing impact on normal cells. Moreover, the fact that it activates caspase-3 in a dose-dependent manner suggests an apoptotic mechanism of action, showing that it could be a promising anticancer agent. However, we need to do more research to fully understand its mechanism of action.

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