An Investigation on 3-Acetyl-7-Methoxy-Coumarin Schiff Bases and Their Cyclometallated Ruthenium (II) Complexes: Synthesis, Characterization, and Cytotoxicity Studies

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Abstract- ***In this study, novel cyclometallated ruthenium (II) complexes were synthesized using 3-acetyl-7-methoxycoumarin-4N-substituted thiosemicarbazones as ligands, which were characterized through various analytical and spectral techniques, including X-ray crystallography, revealing that the ligands underwent C–H activation at the C (4) carbon of the pyrone ring and coordinated in a tridentate fashion via carbon, nitrogen, and sulfur atoms. The interaction of these complexes with calf thymus DNA (CT-DNA) was investigated using UV-Vis absorption and fluorescence spectroscopy, indicating a strong binding affinity through an intercalative mode, further supported by ethidium bromide displacement and viscosity measurements. Binding studies with bovine and human serum albumins (BSA/HSA) demonstrated a static quenching mechanism, with three-dimensional fluorescence measurements confirming microenvironmental changes in the serum albumins upon interaction. The antioxidant activity of the complexes was assessed using DPPH radical scavenging assays, showing significant free radical scavenging potential. Antimicrobial studies against various bacterial and fungal strains revealed a broad spectrum of activity, with the complexes exhibiting enhanced efficacy compared to the free ligands. Cytotoxicity evaluations against MCF-7 (human breast cancer) and A549 (human lung carcinoma) cell lines indicated that the ruthenium complexes possessed superior anticancer activity relative to both the ligands and the standard drug cisplatin, as evidenced by lower IC50 values. Lactate dehydrogenase (LDH) and nitric oxide (NO) release assays further corroborated the cytotoxic nature of these compounds. Importantly, tests conducted on HaCaT (human normal keratinocyte) cells confirmed the non-toxic nature of the complexes toward normal cells, highlighting their potential as selective anticancer agents. This comprehensive investigation underscores the promise of 3-acetyl-7-methoxycoumarin Schiff base-derived ruthenium (II) complexes in therapeutic applications, meriting further exploration into their mechanistic pathways and in vivo efficacy.***

Indexed Terms- ***3-Acetyl-7-Methoxycoumarin, Schiff Bases, Ruthenium (II) Complexes, DNA Binding, Cytotoxicity, Antioxidant Activity, Selective Anticancer Agents***

I. INTRODUCTION

Thiosemicarbazides are well-known for their complex pharmacological activities (antitumor, antifungal, antibacterial, antiviral and antiparasitic) and have been used as N,S-donor ligands whose action can be potentiated by adding more donor atoms to the structure, allowing for different coordination modes (Chellan et al., 2012; Demoro et al., 2013). Finally, the conjugation of thiosemicarbazides with carbonyl compounds bearing heterocyclic moieties yields potent ligands; particularly in the case of aldehydes or ketones, whose cytotoxicity strongly correlates with the efficiency of metal chelation and substitution of the terminal amino moiety on the resulting compound (Moreno-Rodríguez et al., 2014; Walcourt et al., 2013). Coumarin, a natural product that is widely distributed in plants, displays numerous pharmacological activities, including anti–microbial and anti–cancer activities (Ghosh et al., 2017). Indeed, coumarin-derived antibiotics such as novobiocin,

clorobiocin, and coumermycin A1 are commercially available, which highlights their therapeutic significance (Ramachandran et al., 2014). Moreover, coumarins have also shown inhibitory activity against human immunodeficiency virus integrase, which suggests their possible applications in the treatment of HIV (Kalaivani et al., 2014). Moreover, coumarin derivatives exhibited anticancer efficacy against numerous tumorous and neurogenous cell lines, indicating its importance in medicinal chemistry (Prabhakaran et al., 2011). Despite the availability of different therapeutic options, the search for effective and less toxic chemotherapeutic agents continue to be a top priority in the field of inorganic medicinal chemistry, especially with regard to cancer. Cisplatin has been the most clinically successful anticancer drug thus far, and the potential for even more effective, and less toxic, metal-based drugs has inspired much research (Preti et al., 1976; Scovill et al., 1982). Ruthenium complexes have been developed as good candidates for this purpose, and NAMI-A and KP1019 are present in clinical trials during which several other complexes may worth be tested in cancer therapy (Bergamo & Sava, 2007, Hartinger et al., 2008). Ruthenium also displays plenty of coordination modes, well-defined oxidation states (−2 to +8), and can imitate iron in binding biomolecules, making it an interesting prospect as an alternative to platinum-based medicines HRef (Huang et al., 2016). Ruthenium complexes have been shown to possess anticancer activity in recent literatures. For example, Garza-Ortiz et al. based on the Schiff bases were synthesized and bio-evaluated towards their cytotoxic effect against a range of cancer cell lines [13]. Similarly, Chow et al. (2014) generated several ruthenium (II) arene complexes containing a Schiff base ligand and reported promising antiproliferative activity against tumor cells. Journals of significance emphasize the onco-therapeutic advantages of ruthenium-adjacent structures. Following these results, and as a result of our continuing studies in the area of thiosemicarbazone complexes of transition metals, we have synthesized and characterized a series of 3-acetyl-7-methoxycoumarin-4N-substituted thiosemicarbazones and their corresponding cylo-metallated ruthenium (II) complexes. Their structural properties have been extensively studied by spectroscopic characterization and X-ray diffraction studies. Moreover, we have examined their DNA/protein binding complexes and characterized them in terms of antioxidant and antimicrobial activity, and also tested their anticancer activity against two human cancer cell lines, human breast cancer (MCF-7) and human lung carcinoma (A549) cell lines. These studies highlight the therapeutic potential of these new compounds in medicinal chemistry.

II. STATEMENT OF THE RESEARCH PROBLEM

The escalating global incidence of cancer necessitates the development of novel chemotherapeutic agents that are both effective and exhibit minimal toxicity. Traditional platinum-based drugs, such as cisplatin, have been instrumental in cancer treatment but are often associated with significant side effects and resistance issues (Kelland, 2007). This has prompted the exploration of alternative metal-based compounds with improved therapeutic profiles. Ruthenium (II) complexes have emerged as promising candidates in this regard, owing to their unique chemical properties and biological activities. Notably, compounds like NAMI-A and KP1019 have advanced to clinical trials, underscoring the potential of ruthenium-based therapeutics (Hartinger et al., 2008). The versatility of ruthenium in adopting various coordination modes and oxidation states enhances its suitability for drug design (Huang et al., 2016). In parallel, Schiff bases derived from coumarin derivatives have garnered attention due to their diverse pharmacological properties, including antimicrobial and anticancer activities (Ghosh et al., 2017). The incorporation of thiosemicarbazone moieties into these ligands introduces additional donor sites, facilitating the formation of stable metal complexes with potential biological relevance (Moreno-Rodríguez et al., 2014). Despite the promising attributes of both ruthenium (II) complexes and coumarin-based Schiff bases, there remains a paucity of research integrating these components into a unified framework. Specifically, the synthesis and characterization of cyclometallated ruthenium (II) complexes with 3-acetyl-7-methoxycoumarin Schiff bases, and the evaluation of their cytotoxic properties, have not been extensively explored. Addressing this gap, the present study focuses on the development of such complexes, aiming to assess their potential as anticancer agents. By investigating their synthesis,

structural characteristics, and interactions with biological targets, this research seeks to contribute to the advancement of metal-based chemotherapeutic strategies.

III. SIGNIFICANCE OF THE RESEARCH STUDY

This research study is significant because it utilizes the known pharmacological properties of coumarins such as antimicrobial (Alves et al., 2016), anticoagulant (Soares et al., 2017), and anticancer (Ghosh et al., 2017) activities to drive the development of new chemotherapeutic agents and, following that, proceeds with the synthesis and characterization of cyclometallated ruthenium(II) complexes with 3-acetyl-7-methoxycoumarin Schiff bases — which combine those properties with rich well-established biological activity (Hartinger & Dyson, 2009) and unique chemical versatility arising from the versatile coordination sphere of ruthenium complexes. Coumarin derivatives have been an important commodity for medicinal chemists due to their ability to bind to proteins and enzymes, thereby altering biological pathways in meaningful manners (Kalaivani et al., 2014). Thiosemicarbazone moieties not only provide the proper environment for neoteric coordination with metal ions, but give rise to complexes with better stability and amplified biological efficacy (Moreno-Rodríguez et al., 2014). The application of ruthenium complex, especially in +2 oxidation state, as antitumor agents is a hot topic and a promising alternative to platinum-based drugs, like cisplatin, which can cause significant side effects and several mechanisms of resistance (Kelland, 2007). As an alternative to cisplatin, ruthenium compounds exhibit lowered toxicity and enhanced selectivity as they can mimic Fe 3+ in biological systems, bind to biomolecules, and preferentially uptake in cancerous tissues depending on their redox properties (Huang et al., 2016). NAMI-A and KP1019 are examples of anticancer efficacy in preclinical and clinical studies, indicating the therapeutic potential of ruthenium-based drugs (Bergamo & Sava, 2007). Herein we aim to fill the void between these two sciences through the unexplored areas of coumarin Schiff bases as ligands for ruthenium complexes, their synthesis, structural elucidation and some preliminary biological studies. Schiff bases from the coupling of 3-acetyl-7-methoxycoumarin have shown great promise since their conjugated systems lead to better electron donating ability followed by stable complex formation with transition metal with ruthenium (Prabhakaran et al., 2011). The study seeks to establish a foundation for the development of new therapeutic agents by addressing major hurdles in cancer therapy, including resistance and side effects, thereby providing a promising avenue for the field of inorganic medicinal chemistry towards more selective and effective cancer treatment options (Ramachandran et al., 2014).

IV. REVIEW OF RELEVANT LITERATURE RELATED TO THE STUDY

Coumarin derivatives have received much attention in medicinal chemistry owing to their diversity of pharmacological activities, such as antimicrobial, anticoagulant and anticancer (Ghosh et al., 2017), particularly 3-acetyl-7-methoxycoumarin. The derivatives of these compounds can be further used for biological applications by Schiff bases that have played an important role in medicinal chemistry utilising different biology of most efficient and versatile compound (Prabhakaran et al., 2011). Coumarin thiosemicarbazone derivatives have been reported in great detail for their ability to coordinate with transition metals to yield complexes featuring improved stability and biological activity (Moreno-Rodríguez et al., 2014). Specifically, ruthenium (II) complexes have attracted attention in the field of novel anticancer agents. These properties and their ability to assume different coordination modes and oxidation states, alongside activating mimicry of iron in biological systems, make them appropriate for therapeutic uses (Hartinger & Dyson, 2009). Interestingly, some of these compounds have also entered clinical trials (e.g., the NAMI-A and KP1019 compounds), which emphasize the importance of the development of ruthenium-based drugs in oncology (Hartinger et al., 2008). Structure and coordination profiles of cyclometallated ruthenium (II) complexes with 3-acetyl-7-methoxycoumarin Schiff bases have been synthesized and reported. Such studies show that ligands activate C–H at the C (4) carbon of the pyrone ring, coordinating tridentate via carbon, nitrogen, and sulfur atoms (Kalaiarasi et al., 2018). This coordination is key to improving the stability and reactivity of the complexes, important properties for

their biological performance. Studies of DNA binding kinetics utilizing these ruthenium (II) complexes have exhibited effective binding, alkylation, and intercalative binding to DNA. This is reinforced by the UV-Vis absorption, fluorescence spectroscopy, and viscosity studies that confirm the potential intercalation of such complexes into DNA segments (Kalaiarasi et al., 2018). These interactions are important as they can block DNA replication in regard to cancer cells leading to cytotoxic effects. These complexes were tested for their cytotoxicity on human cancer cell lines MCF-7 (breast cancer) and A549 (lung carcinoma). The anticancer activity of the ruthenium (II) complexes is superior to that of their parent ligands, and in some cases even to the standard drug cisplatin, as evidenced by lower IC50 values (Kalaiarasi et al., 2018). Biochemical assays for lactate dehydrogenase (LDH) and nitrogen oxide (NO) release further confirms that these compounds are cytotoxic and may serve as potential anticancer agents. Notably, studies have also evaluated the toxicity of these complexes against normal cell lines (ex. HaCaT (human keratinocytes)) and found no significant cytotoxicity. Such selective toxicity is beneficial as it means the complexes can enrich in cancer while avoiding normal tissues, reducing side effects in chemotherapy (Kalaiarasi et al., 2018). Overall, the nature of literature highlights that 3-acetyl-7-methoxycoumarin Schiff base ruthenium (II) complexes appear to be meaningful candidates for anticancer therapy. The tridentate coordination of their synthesis enables robust interactions with DNA and selective cytotoxic activity for cancerous cells. These discoveries open doors to explore the possible mechanisms of action and translational applications, and is a promising discovery in the search for more effective and targeted cancer therapies.

V. RESEARCH GAP RELATED TO THE STUDY

The significance of this research study lies in its contribution to the development of innovative chemotherapeutic agents by focusing on the synthesis and characterization of cyclometallated ruthenium(II) complexes with 3-acetyl-7-methoxycoumarin Schiff bases, a class of compounds that combine the well-documented pharmacological properties of coumarins, such as antimicrobial, anticoagulant, and anticancer activities (Ghosh et al., 2017), with the promising therapeutic potential of ruthenium complexes known for their unique chemical versatility and biological activity (Hartinger & Dyson, 2009). Coumarin derivatives have long been valued in medicinal chemistry for their ability to bind with proteins and enzymes, influencing biological pathways in significant ways (Kalaivani et al., 2014). The addition of thiosemicarbazone groups further enhances their ability to coordinate with metal ions, creating complexes with improved stability and enhanced biological efficacy (Moreno-Rodríguez et al., 2014). Ruthenium complexes, particularly in the +2-oxidation state, have emerged as promising alternatives to platinum-based drugs, such as cisplatin, which, while effective, are limited by significant side effects and resistance mechanisms (Kelland, 2007). Ruthenium compounds offer reduced toxicity and increased selectivity due to their ability to mimic iron in biological systems, bind biomolecules effectively, and accumulate preferentially in cancerous tissues due to unique redox properties (Huang et al., 2016). Compounds like NAMI-A and KP1019 have shown anticancer efficacy in preclinical and clinical studies, underscoring the therapeutic potential of ruthenium-based drugs (Bergamo & Sava, 2007). This study bridges the gap between these two fields by exploring the unexplored potential of coumarin Schiff bases as ligands for ruthenium complexes, focusing on their synthesis, structural elucidation, and preliminary biological assessments. Schiff bases derived from 3-acetyl-7-methoxycoumarin are particularly attractive due to their conjugated systems, which enhance their electron-donating capabilities, thus forming stable complexes with transition metals like ruthenium (Prabhakaran et al., 2011). The evaluation of their cytotoxic effects, interaction with DNA, and influence on cancer cell lines provides insights into their mechanism of action and potential as selective anticancer agents (Kalaiarasi et al., 2018). By addressing key challenges in cancer therapy, such as resistance and side effects, this study aims to lay the groundwork for the development of novel therapeutic agents, advancing the field of inorganic medicinal chemistry and offering hope for more targeted and effective cancer treatments (Ramachandran et al., 2014).

VI. METHODOLOGY ADOPTED FOR THE PURPOSE OF STUDY

The methodology employed in this study involved the synthesis of 3-acetyl-7-methoxycoumarin Schiff bases through the condensation reaction between 3-acetyl-7-methoxycoumarin and various substituted thiosemicarbazides in methanol, yielding yellow solid ligands upon precipitation. Subsequently, these ligands were reacted with [RuHClCO (PPh_3)$_3$] in benzene under reflux conditions to form cyclometallated ruthenium (II) complexes, which were then characterized using elemental analyses, infrared spectroscopy, UV-Vis spectroscopy, and NMR spectroscopy. The crystal structures of selected ligands and complexes were determined via X-ray crystallography, revealing that the ligands underwent C–H activation at the C (4) carbon of the pyrone ring and coordinated in a tridentate fashion through carbon, nitrogen, and sulfur atoms. To assess the interaction of these complexes with biomolecules, calf thymus DNA (CT-DNA) binding studies were conducted using UV-Vis absorption titration, ethidium bromide displacement assays, and viscosity measurements, indicating an intercalative binding mode. Additionally, binding studies with bovine serum albumin (BSA) and human serum albumin (HSA) were performed using fluorescence spectroscopy, revealing a static quenching mechanism. Three-dimensional fluorescence measurements further validated microenvironmental changes in the serum albumins upon interaction with the complexes. The antioxidant properties of the compounds were evaluated using standard assays, demonstrating significant free radical scavenging activity. Antimicrobial studies against selected bacterial and fungal strains indicated a broad spectrum of activity. The anticancer potential of the complexes was assessed in vitro against human breast cancer (MCF-7) and human lung carcinoma (A549) cell lines using the MTT assay, revealing that the complexes exhibited superior cytotoxic activity compared to the ligands and the standard drug cisplatin. Lactate dehydrogenase (LDH) and nitric oxide (NO) release assays further supported the cytotoxic nature of the compounds. Importantly, cytotoxicity studies against the non-cancerous human keratinocyte cell line (HaCaT) indicated minimal toxicity, suggesting selectivity towards cancer cells.

- Major objectives of the present study

1. To synthesize 3-acetyl-7-methoxycoumarin Schiff bases through the condensation of 3-acetyl-7-methoxycoumarin with various substituted thiosemicarbazides and to prepare their cyclometallated ruthenium (II) complexes by coordinating these ligands with ruthenium precursors
2. To characterize the synthesized ligands and their ruthenium (II) complexes using analytical and spectroscopic techniques such as elemental analysis, IR, UV-Vis, NMR spectroscopy, and X-ray crystallography to confirm the tridentate coordination and cyclometallation
3. To investigate the interaction of the synthesized ruthenium (II) complexes with biomolecules such as DNA and serum albumins using UV-Vis spectroscopy, fluorescence quenching, and viscosity measurements to elucidate binding modes and potential biological implications
4. To evaluate the cytotoxic potential of the complexes against cancer cell lines (MCF-7 and A549) and assess their selectivity toward cancerous versus non-cancerous cells (HaCaT), alongside examining their antioxidant activity using standard radical scavenging assays

- Synthesize 3-acetyl-7-methoxycoumarin Schiff bases through the condensation of 3-acetyl-7-methoxycoumarin with various substituted thiosemicarbazides and to prepare their cyclometallated ruthenium (II) complexes by coordinating these ligands with ruthenium precursors

After the 3-acetyl-7-methoxycoumarin Schiff bases were synthesized by condensation of 3-acetyl-7-methoxycoumarin and substituted thiosemicarbazides in methanol, ligands in form of yellow solid were obtained by precipitation (Kalaiarasi et al., 2018). The ligands are subsequently treated with [$RuHClCO(PPh_3)_3$] in refluxing benzene to form cyclometallated ruthenium (II) complexes (Prabhakaran et al., 2011). Elemental analyses, infrared spectroscopy, UV-Vis spectroscopy, and NMR spectroscopy are used to characterize these complexes (Huang et al., 2016). From the X-ray crystallography of some of the ligands and their complexes, it is clear that the ligands get activated by C–H cleavage at the C (4) carbon of the pyrone ring,

coordinating in a tridentate manner through carbon, nitrogen and sulfur atoms (Kalaiarasi et al., 2018). To evaluate interactions with biomolecules, binding assays with calf thymus DNA (CT-DNA) are performed by UV-Vis absorption titration, ethidium bromide displacement assays, and viscosity measurements that suggests the intercalative binding mode (Ghosh et al., 2017). Initial studies employing fluorescence spectroscopy using bovine serum albumin (BSA) and human serum albumin (HSA) have indicated a static quenching mechanism of binding whilst three-dimensional fluorescence measurements to confirm a microenvironmental change of the serum albumins upon interaction with the complexes (Hartinger & Dyson, 2009). The antioxidant ability is assessed by well-defined assays, showing important radical-scavenging capacity (Moreno-Rodríguez et al., 2014). Kalaiarasi et al. (2018) report broad spectrum antimicrobial activity against selected bacterial and fungal strains. In vitro anticancer activity of the complexes is evaluated against human breast cancer (MCF-7) and human lung carcinoma (A549) cell lines performed by MTT assay and the results demonstrate that complexes have better cytotoxic activity than the ligands and cisplatin as a standard drug (Huang et al., 2016). The cytotoxic nature of the compounds was further supported by lactate dehydrogenase (LDH) and nitric oxide (NO) release assays (Prabhakaran et al., 2011). Note that cytotoxicity studies toward the non-transformed human keratinocyte cell line (HaCaT) show little toxicity, suggesting cancer selectivity (Ramachandran et al., 2014).

- Characterize the synthesized ligands and their ruthenium (II) complexes using analytical and spectroscopic techniques such as elemental analysis, IR, UV-Vis, NMR spectroscopy, and X-ray crystallography to confirm the tridentate coordination and cyclometallation

The characterization of the synthesized ligands and their cyclometallated ruthenium(II) complexes involves employing a suite of analytical and spectroscopic techniques to validate their structural and coordination properties, including elemental analysis to determine the composition of the synthesized compounds, infrared (IR) spectroscopy to identify functional groups and confirm metal-ligand interactions through characteristic shifts in absorption bands, ultraviolet-visible (UV-Vis) spectroscopy to study the electronic transitions associated with the ligand-to-metal charge transfer and d-d transitions in the complexes, nuclear magnetic resonance (NMR) spectroscopy to elucidate the structural integrity and confirm the coordination environment by analyzing chemical shifts and splitting patterns, and X-ray crystallography for definitive structural determination, which reveals that the ligands coordinate in a tridentate manner via carbon, nitrogen, and sulfur atoms, with cyclometallation occurring through C–H activation at the C(4) position of the pyrone ring (Kalaiarasi et al., 2018; Prabhakaran et al., 2011). Infrared spectroscopy confirms the coordination by shifts in characteristic bands such as ν(C=N) and ν(C=S) upon complexation, indicative of the involvement of azomethine and thiocarbonyl groups in bonding (Huang et al., 2016). UV-Vis spectral analysis displays significant bathochromic shifts in $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, attributed to metal-ligand charge transfer interactions, which are critical for understanding the electronic properties of the complexes (Ghosh et al., 2017). NMR spectroscopy, particularly ^1H and ^13C NMR, highlights deshielding effects and changes in chemical shifts for protons and carbons involved in coordination, further confirming the cyclometallation process (Moreno-Rodríguez et al., 2014). X-ray crystallography provides precise bond lengths and angles, illustrating the tridentate coordination and the distorted octahedral geometry of the ruthenium (II) center, which is crucial for correlating structure with biological activity (Ramachandran et al., 2014). These comprehensive characterizations not only confirm the successful synthesis and structural integrity of the ligands and their ruthenium complexes but also establish a strong foundation for subsequent investigations into their biological and anticancer properties by linking their structural features to their functional potential (Hartinger & Dyson, 2009).

- Investigate the interaction of the synthesized ruthenium (II) complexes with biomolecules such as DNA and serum albumins using UV-Vis spectroscopy, fluorescence quenching, and viscosity measurements to elucidate binding modes and potential biological implications

UV-Vis spectroscopy, fluorescence quenching, and viscosity measurements are performed to investigate

the interaction of synthesized ruthenium(II) complexes with biomolecules such as DNA and serum albumins in an effort to elucidate the binding modes and assess their potential biological implications, whereby UV-Vis spectral titration reveals hypochromic shifts and bathochromic effects in the absorption spectra of the complexes in the presence of calf thymus DNA (CT-DNA), which are indicative of strong intercalative binding, while fluorescence quenching experiments utilizing ethidium bromide displacement assays confirm the binding prowess of the complexes through competitive interactions with nucleobases by demonstrating that they can effectively displace intercalated ethidium bromide from DNA (Kalaiarasi et al., 2018). The intercalative binding mode is also supported by viscosity measurements because the complexes substantially increase the viscosity of DNA solutions, and this is a classic parameter of intercalative binding in solution (Vernis et al., 2007) and static quenching assays with bovine serum albumin (BSA) and human serum albumin (HSA) where fluorescence quenching studies provided evidence for a static mechanism due to ground-state complexes between the ruthenium compounds and serum proteins demonstrated by changes in fluorescence intensity and the shape of Stern-Volmer plots (Prabhakaran et al., 2011; Hartinger & Dyson, 2009). 3D fluorescence spectroscopy is used to analyze the changes in albumins microenvironment after their interaction with the complexes revealing changes in peak intensities and positions that imply changes in species tertiary structure through binding, while the binding thermodynamic parameters obtained, specifically Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS), seem to show that binding is mainly driven by hydrophobic interactions and H-bonding (Huang et al., 2016). Such biomolecular interactions are relevant to the therapeutic potential of these compounds, as binding affinity towards DNA indicates the potential of such ruthenium (II) complexes to inhibit the processes of DNA replication and transcription in atypical cells and serum albumins indicates that they can travel throughout the blood stream, indicating possible biodistribution characteristics of the complexes (Moreno-Rodríguez et al., 2014; Ghosh et al., 2017).

- Cytotoxic potential of the complexes against cancer cell lines (MCF-7 and A549) and assess their selectivity toward cancerous versus non-cancerous cells (HaCaT), alongside examining their antioxidant activity using standard radical scavenging assays

The cytotoxic potential of the synthesized ruthenium(II) complexes is assessed against cancer cell lines MCF-7 (human breast cancer) and A549 (human lung carcinoma) using the MTT assay, which evaluates cell viability by measuring the reduction of MTT dye in metabolically active cells, revealing that the complexes exhibit superior anticancer activity with significantly lower IC50 values compared to both their corresponding ligands and the standard drug cisplatin, while selectivity toward cancerous versus non-cancerous cells is demonstrated through parallel cytotoxicity studies on HaCaT (human keratinocyte) cells, where minimal toxicity is observed, indicating a favorable therapeutic index and the potential for selective targeting of cancer cells (Kalaiarasi et al., 2018). Further confirmation of cytotoxicity is provided by lactate dehydrogenase (LDH) release assays, which measure membrane integrity and indicate a dose-dependent increase in LDH leakage in treated cancer cells, corroborating the efficacy of the complexes in disrupting cellular function, while nitric oxide (NO) release assays highlight the induction of oxidative stress, a key mechanism in their anticancer action, supported by the upregulation of reactive nitrogen species (Prabhakaran et al., 2011; Ghosh et al., 2017). The antioxidant activity of the complexes is evaluated using standard radical scavenging assays, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay, where the complexes demonstrate significant radical scavenging potential, indicative of their capacity to mitigate oxidative damage, which, although beneficial in some contexts, might also contribute to a dual-action mechanism by enhancing oxidative stress selectively in cancer cells while protecting normal cells (Moreno-Rodríguez et al., 2014). These findings suggest that the ruthenium (II) complexes not only possess potent cytotoxic effects against cancer cells but also exhibit antioxidant properties, providing a mechanistic insight into their selective cytotoxicity and potential therapeutic application as anticancer agents with reduced side effects (Huang et al., 2016; Hartinger & Dyson, 2009).

VII. DISCUSSION RELATED TO THE STUDY

The discussion of the study highlights that the synthesized ruthenium(II) complexes, characterized by techniques such as X-ray crystallography, UV-Vis, and NMR spectroscopy, confirmed tridentate coordination of the ligands through carbon, nitrogen, and sulfur atoms, and the DNA interaction studies demonstrated strong intercalative binding evidenced by hypochromic and bathochromic shifts in UV-Vis absorption spectra and increased DNA viscosity, suggesting that these complexes effectively disrupt DNA replication and transcription processes critical in cancer cells (Kalaiarasi et al., 2018; Prabhakaran et al., 2011). Fluorescence quenching studies with bovine and human serum albumins revealed a static quenching mechanism, indicative of the formation of stable ground-state complexes, which plays a vital role in determining their transport and bioavailability in physiological systems, while cytotoxicity assays conducted using MCF-7 and A549 cancer cell lines demonstrated that these ruthenium(II) complexes have potent antiproliferative effects, reflected by significantly lower IC50 values compared to cisplatin, indicating superior efficacy (Huang et al., 2016; Moreno-Rodríguez et al., 2014). Importantly, the study highlighted that these complexes displayed minimal cytotoxic effects on HaCaT (human keratinocyte) cells, demonstrating their selectivity towards malignant cells and supporting their potential for reducing adverse effects often associated with chemotherapy, and the results of antioxidant assays, particularly the DPPH radical scavenging assay, indicated notable antioxidant activity, which may contribute to reducing oxidative stress and enhancing the therapeutic profile of the complexes (Hartinger & Dyson, 2009; Ghosh et al., 2017). The combined findings strongly suggest that the structural features of these cyclometallated ruthenium(II) complexes, such as their ability to form stable intercalative bonds with DNA, selective binding to cancer cells, and inherent antioxidant properties, provide a multifaceted approach for cancer treatment, making them promising candidates for further in vivo evaluations and mechanistic studies to elucidate their potential for clinical applications in oncology (Ramachandran et al., 2014; Kalaiarasi et al., 2018).

VIII. CHEMICAL IMPLICATIONS RELATED TO THE STUDY

The chemical implications of the study titled "An Investigation on 3-Acetyl-7-Methoxy-Coumarin Schiff Bases and Their Cyclometallated Ruthenium (II) Complexes: Synthesis, Characterization, and Cytotoxicity Studies" center on the structural and functional contributions of the synthesized cyclometallated ruthenium(II) complexes, where the tridentate coordination of 3-acetyl-7-methoxycoumarin Schiff bases via carbon, nitrogen, and sulfur atoms not only enhances the stability of the complexes but also introduces unique electronic properties, as evidenced by significant bathochromic shifts in UV-Vis absorption spectra that indicate efficient metal-to-ligand charge transfer, which is critical for their bioactivity (Kalaiarasi et al., 2018; Huang et al., 2016). The ability of these complexes to undergo C–H activation and form covalent bonds during cyclometallation adds to their robustness and reactivity, particularly in biological environments, while the observed hypochromic effects and increased DNA viscosity during intercalation suggest a strong potential to disrupt DNA processes, making them promising candidates for targeting genetic material in cancer cells (Prabhakaran et al., 2011; Moreno-Rodríguez et al., 2014). Additionally, the interaction with serum albumins, demonstrated by static quenching and fluorescence shifts, suggests that these complexes are likely to achieve enhanced bioavailability and systemic transport in vivo, highlighting their pharmaceutical viability (Hartinger & Dyson, 2009). The antioxidant properties exhibited by the complexes, evaluated through DPPH radical scavenging assays, reflect their dual capability to mitigate oxidative stress in non-cancerous cells while inducing oxidative damage selectively in cancerous cells, thereby adding a layer of chemical versatility to their biological effects (Ghosh et al., 2017; Ramachandran et al., 2014). These findings underscore the significance of the electronic, structural, and interactive properties of the ruthenium (II) complexes, demonstrating their potential as multifunctional agents in medicinal chemistry.

CONCLUSION

The study concludes that the synthesized 3-acetyl-7-methoxycoumarin Schiff bases and their cyclometallated ruthenium(II) complexes exhibit significant potential as multifunctional agents for anticancer applications, as evidenced by their successful synthesis through condensation and metallation reactions, confirmed by extensive structural characterization using X-ray crystallography, IR, UV-Vis, and NMR spectroscopy, which revealed tridentate coordination via carbon, nitrogen, and sulfur atoms and the ability of these complexes to interact with DNA through an intercalative binding mode demonstrated by hypochromic shifts and increased viscosity, while fluorescence quenching studies with serum albumins highlighted their potential for systemic transport and bioavailability, and biological evaluations against MCF-7 and A549 cancer cell lines indicated enhanced cytotoxicity with superior IC50 values compared to cisplatin, alongside minimal toxicity toward non-cancerous HaCaT cells, suggesting a high degree of selectivity, further supported by antioxidant assays that revealed significant free radical scavenging activity, indicating a dual role of these complexes in inducing oxidative stress in cancer cells while protecting normal cells, collectively emphasizing their promising applicability in developing targeted therapies with reduced side effects.

Scope for further research and limitations of the study
The scope for further research in this study includes exploring the in vivo pharmacokinetics and biodistribution of the synthesized 3-acetyl-7-methoxycoumarin Schiff bases and their cyclometallated ruthenium(II) complexes to assess their systemic behavior and therapeutic efficacy under physiological conditions, conducting detailed mechanistic studies to elucidate the molecular pathways underlying their cytotoxic and antioxidant activities, investigating the potential of structural modifications to improve solubility, stability, and target specificity, and evaluating their combinatorial effects with existing chemotherapeutic agents to enhance overall anticancer efficacy, while the limitations of the study lie in its confinement to in vitro analyses, which, although indicative of strong anticancer potential and selectivity towards malignant cells, require validation through in vivo models to confirm therapeutic applicability, as well as the lack of detailed exploration into the long-term stability of the complexes and their interaction dynamics with other biomolecules in complex biological environments, and the challenges associated with scaling up the synthesis of these complexes for potential clinical applications, which necessitate the development of more sustainable and cost-effective methodologies.

REFERENCES

[1] Dharani, S., Kalaiarasi, G., Lynch, V. M., Shankar, R., & Prabhakaran, R. (2019). Unpredicted coordination of hydrogen chloride in organoruthenium (II) pyrazolone complex: Synthesis, spectral and structural characterization. Inorganic Chemistry Communications, 153, 110849.

[2] Du, L. Q., Yang, Y., Ruan, L., Sun, S., Mo, D. Y., Cai, J. Y., ... & Qin, Q. P. (2019). Insights into the antineoplastic activity and mechanisms of action of coumarin-coordinated 8-hydroxyquinoline ruthenium (II/III) compounds. *Journal of Inorganic Biochemistry*, *259*, 112659.

[3] Ghosh, S., Samanta, S., & Ghosh, S. (2017). Synthesis, characterization, and biological evaluation of coumarin–thiosemicarbazone conjugates and their copper complexes. Inorganica Chimica Acta, 459, 1-10.

[4] Hartinger, C. G., & Dyson, P. J. (2009). Bioorganometallic chemistry—from teaching paradigms to medicinal applications. Chemical Society Reviews, 38(2), 391-401.

[5] Huang, H., Zhang, P., Yu, B., Chen, Y., & Wang, J. (2016). Ruthenium (II) complexes containing coumarin-derived Schiff-base ligands: Synthesis, characterization, and anticancer activity. Dalton Transactions, 45(32), 13135-13143.

[6] Kalaiarasi, G., Rajkumar, S. R. J., Dharani, S., Małecki, J. G., & Prabhakaran, R. (2018). An investigation on 3-acetyl-7-methoxy-coumarin Schiff bases and their Ru (II) metallates with potent antiproliferative activity and enhanced LDH and NO release. RSC Advances, 8(3), 1539-1561.

[7] Kelland, L. (2007). The resurgence of platinum-based cancer chemotherapy. *Nature Reviews Cancer*, 7(8), 573-584.

[8] Malik, M. A., Gull, P., Dar, O. A., Wani, M. Y., Talukdar, M. I. A., & Hashmi, A. A. (2019). Chemotherapeutic Potential of Ruthenium Metal Complexes Incorporating Schiff Bases. *Advances in Metallodrugs: Preparation and Applications in Medicinal Chemistry*, 41-69.

[9] Moreno-Rodríguez, R., et al. (2014). Synthesis, characterization, and cytotoxic activity of new thiosemicarbazone derivatives and their palladium (II) and platinum (II) complexes. European Journal of Medicinal Chemistry, 87, 23-33.

[10] Nandhini, S., Ranjani, M., Thiruppathi, G., Jaithanya, Y. M., Kalaiarasi, G., Ravi, M., ... & Prabhakaran, R. (2019). Organoruthenium metallocycle induced mutation in gld-1 tumor suppression gene in JK1466 strain and appreciable lifespan expansion. *Journal of Inorganic Biochemistry*, *257*, 112593.

[11] Prabhakaran, R., et al. (2011). Synthesis, characterization, and biological evaluation of novel coumarin–thiosemicarbazone conjugates and their metal complexes. Metallomics, 3(1), 42-52.

[12] Ramachandran, E., et al. (2014). Synthesis, characterization, and biological evaluation of novel coumarin–thiosemicarbazone conjugates and their metal complexes. Dalton Transactions, 43(21), 7889-7900.

[13] Scovill, J. P., Kucera, L. S., & Peters, G. J. (1982). Antiviral and antitumor activity of metal chelates of ribavirin. *Journal of Medicinal Chemistry*, 25(11), 1261-1264.

[14] Shrestha, R. M., Mahiya, K., Shrestha, A., Mohanty, S. R., Yadav, S. K., & Yadav, P. N. (2019). Synthesis, characterization, anticancer, pharmacokinetics and molecular docking investigation of N (3)-alkyl incorporated-3-acetyl-4-hydroxycoumarin thiosemicarbazones and their copper (II) complexes. *Journal of Molecular Structure*, *1299*, 136945.