Synthesis and Anticancer Activity of [RuCl$_2$(η$^6$-arene)(aroylthiourea)]
Complexes—High Activity against the Human Neuroblastoma (IMR-32) Cancer Cell Line

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ABSTRACT: Eight new organometallic Ru(II)—arene complexes of the type [RuCl$_2$(η$^6$-arene)(η$^1$-S-aroylthiourea)] (arene = p-cymene or benzene) were synthesized in order to evaluate the effect of the arene moiety and the substituent of the aroylthiourea ligand on the cytotoxicity of the complexes. The ligands (L1 and L2) and complexes (1–8) were characterized using analytical and spectroscopic (UV–visible, infrared, $^1$H NMR, $^{13}$C NMR, and mass) methods. The structure of the ligands (L1 and L2) and complexes (1 and 3–6) was obtained from single-crystal X-ray diffraction studies. The cytotoxicity of the complexes was evaluated against four different cancer cell lines: MCF-7 (breast), COLO 205 (colon), A549 (lung), and IMR-32 (neuroblastoma). All the complexes showed good cytotoxicity and the highest was in the IMR-32 cancer cell line. The complexes 5, 7, and 8 exhibited remarkable cytotoxicity in the entire cancer cell lines tested, which was comparable with the standard drug, cisplatin. The anticancer mechanism of the complexes 3 and 7 in IMR-32 cells was evaluated by bright-field microscopy, intracellular reactive oxygen species (ROS), mitochondrial membrane potential (MMP), DNA damage, and caspase-3 analyses. The cells treated with the complexes showed upregulated caspase-3 compared to the control, and it was found that ROS and MMP were dose-dependent on analysis. Also, bright-field microscopy and 4′,6-diamidino-2-phenylindole (DAPI) staining have correspondingly shown cellular membrane blebbing and DNA damage, which were morphological hallmarks of apoptosis. The study concluded that the complexes promoted the oxidative stress-mediated apoptotic death of the cancer cells through the generation of intracellular ROS, depletion of MMP, and damage of the nuclear material.

INTRODUCTION

Cancer is described as a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. It is one among the widespread diseases wherein the research toward its complete cure has still not triumphed. In 2015, about 90.5 million people were reported to have cancer. Subsequently, there have been cases of different types of cancers which reached up to a whopping number of 14.1 million a year and the disease has almost claimed about 8.8 million lives (15.7%) till date. There are numerous methods available for the treatment of cancer such as chemotherapy, hormonal therapy, surgery, and palliative care. Chemotherapy, which is known to be quite effective, has been broadly investigated with the usage of organometallic drugs and operates on the principle of the drug interacting with the DNA of the cells that are dividing rapidly and altering it, thus killing them and restricting their growth.

Platinum-based anticancer drugs have been dictating the area till now; cisplatin and its derivatives (carboplatin, oxaliplatin, nedaplatin, etc.) are the most effective. Whereas the chemotherapeutic success of these drugs is undeniable, they by no means provide the perfect cure. There are several cancer cell lines which show drug resistance, which has a deplorable range of side effects including neurotoxicity, hair loss, and nausea. These led scientists to explore the area using other biologically relevant metal ions, particularly ruthenium. Ruthenium comes into play in biological applications as it can mimic iron in binding to transferrin and has similar ligand exchange kinetics to that of platinum. Over the years, attention toward ruthenium has broadened; ruthenium red has been known to inhibit Ca$^{2+}$ uptake, complexes of Ru(II) have been...
documented as nitric oxide scavengers, antimetastatics, immunosuppressants, antibiotics, radiopharmaceuticals, antiparasitics, and also as anticancer agents which have given the metal ion its head start in the field of therapeutics.10,11 The ruthenium-based drug NAMI-A which succeeded in phase I clinical trials got rejected because of its limited efficacy in phase II clinical trials; however, the sodium salt of KP1019 (KP1339) is currently undergoing clinical trials.12 However, organometallic half-sandwich Ru(II) complexes have been established as a separate arena, and their anticancer activity has been well studied recently. Ruthenium(II)—arene RAPTA-type compounds have been widely investigated for their therapeutic properties. These complexes offer numerous ways to tune its structural parameters and electronic property. The change in the arene moiety or any of the other ligands is expected to influence reactions of the complex, thus altering the biological activity and redox property.

Ligands derived from thiourea are mostly crystalline and known to be quite thermally stable.20 They can be synthesized in good yields from readily available starting materials using a simple procedure. These compounds have gained importance in the pharmacological sector, in agriculture, and in the synthesis of heterocycles.19–23 Aroylthioureaes are thiourea derivatives that have been recognized for their variable modes of coordination with metal ions.24

Aroylthiourea complexes have had a widespread reach in the fields of therapeutics, agriculture, corrosion, and catalysis.25–28 Furthermore, their complexes have shown excellent biological properties including antibacterial, antiviral, antitubercular, antifungal, and anti-inflammatory.26–28 Our group has been studying the anticancer activity of aroylthiourea and related complexes, obtaining moderate results.24,32 Therefore, in the interest of developing superior drugs than the ones obtained previously, herein, we report the synthesis of new Ru(II)—arene complexes containing diverse aroylthiourea ligands.

■ RESULTS AND DISCUSSION

Formation of the Ligands and Complexes. The aroylthiourea ligands (L1–L4) were synthesized by treating aryl chloride with the corresponding primary amine, after an hour of reflux with potassium thiocyanate.24 The corresponding ruthenium(II) complexes of the type [RuCl2(η8-arene)(η1-S-arylthiourea)] (1–8) were derived from the reactions involving [RuCl(μ-Cl)(η8-arene)]2 (arene = p-cymene or benzene) and aroylthiourea ligands (L1–L4) (Scheme 1). All the complexes were found to be stable in both solid and solution states. The complexes 1–4 were soluble in most of the organic solvents, whereas 5–8 were soluble only in highly polar organic solvents, but both were insoluble in water. The aroylthiourea ligands (L1 and L2) and Ru(II)—arene complexes (1–8) were characterized by elemental analyses and IR, UV–visible, nuclear magnetic resonance (NMR; 1H and 13C), and mass spectroscopic methods. The structure of representative compounds (L1, L2, 1, and 3–6) was confirmed by X-ray diffraction studies.

Characterization by Spectroscopic Methods. The electronic absorption spectra of the ligands (L1 and L2) and complexes (1–8) were recorded in dimethylformamide in the range of 200–800 nm (Figure S1). The ligands showed a band at 267–279 cm−1 because of π → π* transition, whereas the complexes showed the corresponding band at 269–285 cm−1. A band at 306–340 cm−1 corresponding to n → π* transition was shown by the ligands, whereas the complexes showed the same at 331–352 cm−1.

The IR spectra of complexes 1–8 (Figure S2) showed a ν(C=S) stretching at a lower wavenumber than that of the corresponding ligands, whereas the remaining stretching frequencies resided almost in the same region, which signified the neutral coordination of aroylthiourea via sulfur atom (Figures S2–S11).

The 1H NMR spectra (Figures S12–S21) of the ligands (L1 and L2) showed chemical shifts of amide and thioamide N protons, which appeared in the same region as that of the corresponding ligands, whereas the remaining stretching frequencies resided almost in the same region, which signified the neutral coordination of aroylthiourea via sulfur atom (Figures S2–S11). The 1H NMR spectra of the complexes (1–4), signals of amide and thioamide N–H appeared in the same region as that of their corresponding ligands, which substantiated their noninvolvement in the complex formation. The comparative study of NMR spectra of the benzene and p-cymene complexes along with the ligand is shown in Figure 1. After complexation, there was a minor shift in the δ value of N–H protons, which may be attributed to hydrogen bonding of the thioamide and amide N–H protons with carbonyl oxygen and Ru(II)–Cl, respectively. The decrease in the difference of chemical shifts between the two N–H protons in the p-cymene and benzene complexes may be attributed to the presence of electron-donating substituents in the arene moiety of the former complexes. New signals that emerged in the range 5.40–5.26, 3.01–2.72, 2.41–2.13, and 1.28–1.17 ppm for complexes 1–4 showed the occurrence of the p-cymene moiety,24 whereas a singlet at 5.98 ppm in the spectra of complexes 5–8 suggested the presence of benzene moiety.34 In the spectra of complexes 3 and 7, the methyl protons were observed at 2.24 and 2.34 ppm, respectively.

Scheme 1. Synthesis of Aroylthiourea Ligands (L1–L4) and Their Corresponding Ru(II)—Arene Complexes (1–8)
In the $^{13}$C NMR spectra (Figures S22−S31) of the ligands, thiocarbonyl (C=S) and carbonyl (C=O) peaks appeared at 180.9−177.6 and 167.5−165.2 ppm, respectively. The ligands (L1 and L2) displayed signals at 139.1−122.6 ppm, which were credited to the aromatic carbons. The $^{13}$C NMR spectra of the complexes (1−8) did not show any pronounced shift. However, new signals appeared at 103.5−103.2, 99.9−99.8, 84.1−83.8, 82.9−82.7, 30.4−30.2, 22.2−22.0, and 18.3−18.2 ppm, validating the presence of $p$-cymene moiety in complexes 1−4, and a signal at 88.1 ppm for complexes 5−8 signified the presence of a benzene ring. In the spectra of complexes 3 and 7, the methyl carbon signals appeared at 18.3 and 21.4 ppm, respectively.

The ligands (L1 and L2) exhibited the [M + H$^+$]$^+$ ion peak as their molecular ion peak in their mass spectra and the $m/z$ of the [M − 2H$^+$ − 2Cl$^-$ + H$^+$]$^+$ fragment was found to be the value of the molecular ion peak of the complexes (Figures S32−S41).

**Structure Analysis.** The crystal structure of the ligands (L1 and L2) and complexes (1, 3−5 and 6) is shown in Figures S42 and S43 and 2−6. The crystallographic data and refinement parameters for L1, L2, 1, 3−5, and 6 are summarized in Tables S1 and S2. The crystals were obtained from slow evaporation of dichloromethane−acetonitrile (1:1) solutions of the samples. The ligands (L1 and L2) crystallized in the triclinic $P\overline{1}$ space group. The structure of ligand L1 contained two independent molecules in the unit cell, which were closely comparable. The C$_6$H$_4$Cl$_2$ group was found disordered between two positions in the structure of L1, which

![Figure 1. 1H NMR spectra of L1 and its Ru−arene complexes.](image1)

![Figure 2. Single crystal structure for 1.](image2)

![Figure 3. Single crystal structure for 3.](image3)

![Figure 4. Single crystal structure for 4.](image4)

![Figure 5. Single crystal structure for 5.](image5)
was successfully modeled (occupancy ratio of 0.93:0.07). Both the ligands contain one intramolecular hydrogen bond between thioamide N–H and carbonyl oxygen atom [N(2)–H···O(1) = 2.1490 (L1) and 2.1490 Å (L2)]. The bond distances and angles were in the allowed range and were comparable with similar structures.24,34

The X-ray diffraction validated the predicted monodentate coordination of sulfur in Ru(II)–arene complexes. The complexes (1 and 3–6) adopted the characteristic piano stool geometry, wherein the π-bonded arene moiety (p-cymene or benzene) occupied the “seat of the stool”, the two chloride ligands and sulfur from the aroylthioleuare ligand occupied the residual three coordination legs. Complexes 1, 4, and 6 crystallized in triclinic PI whereas the other two complexes (3 and 5) crystallized in monoclinic C2/c space group. The ruthenium to chloride bond distances (2.4040–2.4384 Å) are almost identical, whereas the distances of Ru–C and Ru–S bonds for the complexes were found to be in the range of 2.2195–2.1490 and 2.4120–2.3983 Å, respectively; these distances were usual and were comparable to those of other previously reported Ru–arene complexes.24,34 The bond angles of S–Ru–Cl and Cl–Ru–Cl lay in the range of 94.83°–85.81°. One of the chloride ligands and the carbonyl oxygen were involved in hydrogen bonding with amide N–H [N–H···Cl, 2.19–2.88 Å] and thioamide N–H [N–H···O, 1.88–1.96 Å], respectively. The other bond distances and angles were in the allowed range and were comparable with similar structures.36–39 For 4, elongated thermal ellipsoids on atoms C9–C18, C26, and C27 indicated possible disorder, which was modeled successfully between two positions, each with an occupancy ratio very close to 0.50. Appropriate restraints and/or constraints were used to keep the bond distances, angles, and thermal ellipsoids meaningful.

**Stability Studies.** To evaluate the stability of the complexes in water/dimethyl sulfoxide (DMSO) solutions, the NMR spectra of complexes 3 and 7 were recorded in different time intervals (0, 1, 6, and 24 h) in a D2O/DMSO-d6 (2:8) mixture. Prior to this, the stability of the complexes were tested in DMSO-d6 (0, 6 and 24 h), and they retained all the peaks in the respective regions over a period of 24 h (Figures S44 and S45). Upon the addition of D2O, there was a disappearance of amide N–H and the thioamide N–H protons within an hour in the 1H NMR spectra of the complexes, which may be due to rapid exchange of the two N–H protons with the D2O solvent (Figures S46 and S47). Other peaks of the complexes remained unaltered throughout the time monitored, suggesting their stability.36,37

The stability of complexes 3 and 7 was also evaluated in the biological medium using UV–visible spectroscopy, wherein they displayed the characteristic peaks in the range 200–800 nm (Figures S48 and S49). The absence of significant changes in the spectral characteristics of the tested complexes over the time may suggest that no structural alternations occurred in the biological medium.16,18

**Interaction with Glutathione.** Glutathione (GSH) is known to cause the detoxification of anticancer drugs as the metal center of these drugs gets involved in coordination with it. Hence, the most active complex 7 was studied for its interaction with GSH. The complex bound to GSH after a period of 12 h, which was evident from the appearance of new peaks in the down-field region of the NMR spectrum (Figure S50).17

**Anticancer Activity of the Complexes.** The cytotoxic activity of the complexes (1–8) was evaluated against four different cancer (MCF-7, COLO 205, A549, and IMR-32) cell lines. The percentages of cell viability versus concentration graphs are shown in Figure 7. The half-minimum inhibitory concentration (IC50) values of the complexes are tabulated (Table 1). All the complexes were most active in the IMR-32 [IC50 = 8–47 μM] cell line, which declared the selectivity of the complexes, of which the complexes 7, 5, and 3 showed cytotoxicity which was comparable to that of the standard, cisplatin. However, neither the substituents on the aroyl end nor at the N terminal seem to have a role in the cytotoxicity of the complexes, although we can say that a ligand does play a role in the cytotoxicity as the same ligand exhibited a greater activity in both p-cymene (3) and benzene (7) complexes.

Among the complexes, the most active ones, 7 [IC50 = 8 ± 2 μM] (arene = benzene) and 3 [IC50 = 10 ± 2 μM] (arene = p-cymene), were chosen from each of the complexes to further study their anticancer activity in the IMR-32 cell line at IC50 and IC90 concentrations. The IC50 value on the normal...
being of the cells. However, the cells treated with IC\textsubscript{50} displayed the cell body and dendrites, reflecting the well-being of the cells. DNA damage, and caspase-3 analyses. 

Table 1. \textit{In Vitro} Cytotoxicity of the Complexes (1–8) against Various Cancer Cell Lines

| complex | MCF-7 (μM) | COLO 205 (μM) | A549 (μM) | IMR-32 (μM) |
|---------|-----------|--------------|-----------|-------------|
| 1       | 18 ± 2    | 17 ± 1       | 22 ± 1    | 14 ± 2      |
| 2       | 41 ± 2    | 38 ± 1       | 43 ± 2    | 34 ± 2      |
| 3       | 12 ± 2    | 12 ± 1       | 15 ± 1    | 10 ± 2      |
| 4       | 55 ± 1    | 11 ± 1       | 58 ± 1    | 46 ± 1      |
| 5       | 9 ± 2     | 8 ± 1        | 9 ± 2     | 8 ± 2       |
| 6       | 55 ± 2    | 62 ± 2       | 59 ± 3    | 47 ± 2      |
| 7       | 8 ± 1     | 8 ± 1        | 8 ± 1     | 8 ± 2       |
| 8       | 10 ± 1    | 10 ± 2       | 11 ± 2    | 10 ± 2      |
| cisplatin | 5 ± 1    | 6 ± 2        | 6 ± 1    | 5 ± 1       |

cell line Vero for the active complexes 7 and 3 was found to be 157 ± 3 and 131 ± 2 μM, respectively. The value was much higher when compared to the same in the cancer cell lines and hence it seemed that the complexes were less toxic toward the normal cell line and could be safe and biocompatible.

\textbf{Apoptosis Cell Death Mechanism.} The anticancer mechanism of complexes 3 and 7 in IMR-32 cells was studied using bright-field microscopy, intracellular reactive oxygen species (ROS), mitochondrial membrane potential (MMP), DNA damage, and caspase-3 analyses.

In bright-field microscopic assessment, control cells displayed the cell body and dendrites, reflecting the well-being of the cells. However, the cells treated with IC\textsubscript{50} concentration of complexes 3 and 7 evidently showed cellular membrane damage, leakage of cellular debris, and formation of apoptotic bodies (Figure 8).\textsuperscript{40} The intensity of cellular damage and formation of apoptotic bodies was quite highly evident at IC\textsubscript{50} values.

Next, the level of intracellular ROS was measured by 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) staining and it is one of the most commonly used techniques for qualitative analysis of oxidative stress. DCFH-DA readily diffuses into the cell and gets deacetylated to a nonfluorescent compound by cellular esterases, which is later oxidized to fluorescent 2′,7′-dichlorofluorescein by intracellular ROS molecules. The intensity of fluorescence is directly proportional to the level of ROS molecules.\textsuperscript{41–44} In the present study, cells treated with complexes 3 and 7 showed higher ROS levels compared to the control cells and ROS was found to be dose-dependent (Figures 9 and 12). The cells treated with IC\textsubscript{50} and IC\textsubscript{90} concentration of complexes 3 and 7 showed bright green fluorescence, which was found to be higher at IC\textsubscript{90}.

Further, the MMP of the cells was measured by rhodamine 123 staining. The rhodamine 123 is a cell-permeant, cationic, green-fluorescent dye and is used in the measure of membrane polarization.\textsuperscript{45,46} The intensity of fluorescence directly reflects the MMP of the cell. Here, control cells showed bright green fluorescence, which was found to be higher at IC\textsubscript{90}.
fluorescence, which appealed to the well-being of the cell (Figures 10 and 12). In contrast, the MMP of cells depleted on treatment with complexes 3 and 7 and was dose-dependent. The greater MMP depletion was noticed at IC90 value of the complexes.

**Figure 10.** Assessment of MMP by rhodamine 123 staining. (A) Control cells. (B,C) were cells treated with IC50 (5 ± 1 μM) and IC90 (9 ± 0.2 μM) of cisplatin, respectively. (D,E) were cells treated with IC50 (10 ± 2 μM) and IC90 (19 ± 0.1 μM) of complex 3, respectively. (F,G) were cells treated with IC50 (8 ± 2 μM) and IC90 (14 ± 1 μM) of complex 7, respectively. The bright green fluorescence under the GFP filter directly proportionate to the MMP.

In the next study, the effect of complexes 3 and 7 on the nuclear material of the cell was demonstrated by 4’,6-diamidino-2-phenylindole (DAPI) staining (Figure 11). The DAPI dye can pass through an intact cell membrane of both live and dead cells. However, DAPI passes less efficiently through the membrane of live cells and generates lower fluorescence compared to that in dead cells.46,47 In the present study, control cells have shown a blue fluorescent nucleus at the center of the cell and the nucleus was found intact and unbroken. However, the cells treated with complexes 3 and 7 showed higher intensity of fluorescence compared to the control and displayed dispersion or leakage of nuclear fragments from the cell, which concluded as DNA damage.

Finally, the role of the complexes in the promotion of the apoptotic process was assessed by caspase-3 analysis. The caspases are decisive mediators and their upregulation promotes the death of the cell by apoptosis.46–48 The caspases are activated in apoptotic process by extrinsic (death receptor) or intrinsic (mitochondrial) pathways. The morphological hallmarks of apoptosis include fragmentation of DNA and blebbing of cellular membrane.49 In the present study, the cells treated with complexes showed upregulated caspase-3 compared to control and were found dose-dependent in ROS and MMP analyses (Figure 12). Also, bright-field microscopy and DAPI staining have correspondingly shown cellular membrane blebbing and DNA damage, which were morphological hallmarks of apoptosis. Overall, the study expresses that the complexes are probable anticancer agents and may promote the oxidative stress-mediated apoptotic death of the cancer cells through generation of intracellular ROS, depletion of the MMP, and damage of the nuclear material.

The biological relevance of ruthenium was very first seen in the 1950s by Dwyer and his co-workers.50–52 However, discovery of cisplatin by Rosenberg and co-workers12–54 created a blueprint for the effect of metal complexes on the anticancer activity, which led to the development of ruthenium anticancer drugs and the arena has been ever flourishing.55 Ru–arene complexes seem to have an improved activity when compared to the organometallic anticancer compounds which are currently on clinical trial or in use.56 These complexes are stable and their scaffolds offer a significant scope for augmenting the biological activity and minimizing the side effects by altering the arene and other coordinated ligands.14–16 The added advantages of Ru complexes are their biomolecular interactions with albumin and transferrin in blood plasma, actins on the cell surface, or collagens of the extracellular matrix, regulatory enzymes within the cell membrane/in the cytoplasm, and DNA in the cell’s nucleus. Schmitt et al. reported 4-aryl-4H-naphthopyran-based Ru–arene complexes with a cytotoxicity ranging from 0.5 to 966 μM in various cancer cell lines.57 Meier-Menches et al. summarized structure–activity relationships for ruthenium and osmium metallo drugs with respect to in vitro antiproliferative and in vivo tumor-inhibiting properties.58 In recent times alone, there have been many publications on the biological applications of Ru–arene complexes.59–63 However, still Ru–arene complexes containing aroyliourea ligands have not been extensively researched in the past for their biological activities though Ru–arene and aroyliourea compounds were independently known for their biological potentials. Among the available Ru–arene complexes with thiourea ligands, our complexes exhibited better cytotoxicity. In the past, Ru–pycyme complexes bearing monodentate (S) aroyliourea ligand showed IC50 value of 55–102 (AS49) and 52–500 μM (MCF-7) in cancer cell lines,64 whereas similar complexes with benzene as the arene moiety showed an IC50 value of 96–250 and 151–163 μM in AS49 and MCF-7 cell lines.

![Figure 11. Assessment of DNA damage by DAPI staining. (A) Control cells. (B,C) were cells treated with IC50 (5 ± 1 μM) and IC90 (9 ± 0.2 μM) of cisplatin, respectively. (D,E) were cells treated with IC50 (10 ± 2 μM) and IC90 (19 ± 0.1 μM) of complex 3, respectively. (F,G) are cells treated with IC50 (8 ± 2 μM) and IC90 (14 ± 1 μM) of complex 7, respectively. The arrows indicate the dispersed and damaged nuclear material of the cell.](image-url)
respectively. Cationic Ru–p-cymene complexes of bidentate (N, S) thiourea derivatives have displayed IC₅₀ values of 32–40 μM in the A549 cell line. Ru–p-cymene complexes with the monodentate (S) arylthiourea ligand showed an IC₅₀ value of 23–44 μM, whereas the bidentate coordination (O, S) of the same ligand with Ru–p-cymene yielded an IC₅₀ value of 10–17 μM in the A549 cancer cell line (Figures 13 and 14).

It is obvious that IC₅₀ values of our complexes are well comparable to that of reported Ru–arene complexes. Although the comparison with well-known anticancer drug, cisplatin, proved less effective, it is a persuasive factor that there are evidences for complexes which had higher IC₅₀ values than cisplatin in vitro exhibited better activity in vivo.

CONCLUSIONS

In summary, a series of Ru–arene complexes containing the arylthiourea ligand as an anticancer agent have been designed, synthesized, and characterized. The molecular structure of the ligands (L1 and L2) and complexes (1 and 3–6) was solved using single-crystal X-ray diffraction studies, with the complexes showing the typical piano-stool geometry adopted by Ru–arene complexes. The cytotoxic activity of the complexes was evaluated in four different (MCF-7, COLO 205, A549, and IMR-32) cancer cell lines. Complexes 5, 7, and 8 exhibited cytotoxicity in close range with the standard...
cisplatin in four cell lines (MCF-7, COLO 205, A549, and IMR-32) tested, which ascertains the potential activity of the complexes. All the complexes showed highest activity (IC₅₀ = 7–46 μM) against human neuroblastoma (IMR-32) cancer cells, warranting them their selective nature. The active complexes 3 and 7 showed less toxicity on normal cell line Vero compared to the cancer cell line. The anticancer mechanism of these two complexes was studied further by bright-field microscopy, intracellular ROS, MMP, DNA damage, and caspase-3 analyses. The studies revealed dose-dependent ROS and MMP and upregulation of caspase-3. On summation of the studies, it may be said that the complexes promote the oxidative stress-mediated apoptotic death of the cancer cells through generation of intracellular ROS, depletion of MMP, and damage of the nuclear material.

## EXPERIMENTAL SECTION

### Synthesis of the Ligands. 2,4-Dichloro-N-(phenylcarbamothioyl)benzamide (L1).

Yield: 89%. White solid. mp 160 °C. Anal. Calc'd for C₁₈H₁₂Cl₂N₂O: C, 57.61; H, 3.22; N, 13.74, 13.74, 13.22, 131.3, 130.8, 130.5, 128.0, 127.0, 124.1 (aromatic carbons). ESI-MS (m/z): calcd for C₁₈H₁₂Cl₂N₂O, 323.9890; found, 324.9960 [M + H]+.

### 2,4-Dichloro-N-(naphthalen-2-ylcarbamothioyl)benzamide (L2).

Yield: 84%. White solid. mp 223 °C. Anal. Calc'd for C₂₅H₂₈Cl₂N₂O₄S: C, 52.08; H, 4.90; N, 4.86; S, 5.56. Found: C, 52.17; H, 4.98; N, 4.75; S, 5.64. FT-IR (KBr, cm⁻¹): 3368 (m, ν(thioamide N–H)), 3183 (s, ν(amide–N–H)), 1687 (s, ν(C=O)), 1257 (s, ν(C=S)). 1H NMR (500 MHz, CDCl₃): δ ppm 12.28 (s, 1H, OC–NH), 9.42 (s, 1H, SC–NH), 7.69 (d, J = 8.1 Hz, 3H, aromatic-H), 7.50 (d, J = 0.8 Hz, 1H, aromatic-H), 7.41 (dd, J = 16.7, 8.4 Hz, 3H, aromatic-H), 7.29 (t, J = 7.3 Hz, 1H, aromatic-H). 13C NMR (125 MHz, CDCl₃): δ ppm 176.6 (C=O), 165.2 (C=O), 139.1, 137.4, 132.2, 131.3, 130.8, 130.5, 128.0, 127.0, 124.1 (aromatic carbons). ESI-MS (m/z): calcd for C₂₅H₂₈Cl₂N₂O₄S, 576.0342; found, 576.0342 [M + H]+.

### Synthesis of the Ru–Arene Complexes. [Dichloro(p-cymene)ruthenium(II)] (1A).

L1 (130 mg, 0.4 mmol) was used. Yield: 93%. Orange solid. mp 295 °C. Anal. Calc'd for C₂₅H₂₈Cl₂N₂O₄S: C, 52.08; H, 4.90; N, 4.86; S, 5.56. Found: C, 52.17; H, 4.98; N, 4.75; S, 5.64. FT-IR (KBr, cm⁻¹): 3368 (m, ν(thioamide N–H)), 3183 (s, ν(amide–N–H)), 1687 (s, ν(C=O)), 1257 (s, ν(C=S)). 1H NMR (500 MHz, CDCl₃): δ ppm 12.28 (s, 1H, OC–NH), 9.42 (s, 1H, SC–NH), 7.69 (d, J = 8.1 Hz, 3H, aromatic-H), 7.50 (d, J = 0.8 Hz, 1H, aromatic-H), 7.41 (dd, J = 16.7, 8.4 Hz, 3H, aromatic-H), 7.29 (t, J = 7.3 Hz, 1H, aromatic-H). 13C NMR (125 MHz, CDCl₃): δ ppm 176.6 (C=O), 165.2 (C=O), 139.1, 137.4, 132.2, 131.3, 130.8, 130.5, 128.0, 127.0, 124.1 (aromatic carbons). ESI-MS (m/z): calcd for C₂₅H₂₈Cl₂N₂O₄S, 576.0342; found, 576.0342 [M + H]+.

### [Dichloro(p-cymene)ruthenium(II)] (1B).

L1 (150 mg, 0.4 mmol) was used. Yield: 87%. Orange solid. mp 318 °C. Anal. Calc'd for C₂₅H₂₈Cl₂N₂O₄S: C, 52.08; H, 4.90; N, 4.86; S, 5.56. Found: C, 52.17; H, 4.98; N, 4.75; S, 5.64. FT-IR (KBr, cm⁻¹): 3368 (m, ν(thioamide N–H)), 3183 (s, ν(amide–N–H)), 1687 (s, ν(C=O)), 1257 (s, ν(C=S)). 1H NMR (500 MHz, CDCl₃): δ ppm 12.28 (s, 1H, OC–NH), 9.42 (s, 1H, SC–NH), 7.69 (d, J = 8.1 Hz, 3H, aromatic-H), 7.50 (d, J = 0.8 Hz, 1H, aromatic-H), 7.41 (dd, J = 16.7, 8.4 Hz, 3H, aromatic-H), 7.29 (t, J = 7.3 Hz, 1H, aromatic-H). 13C NMR (125 MHz, CDCl₃): δ ppm 176.6 (C=O), 165.2 (C=O), 139.1, 137.4, 132.2, 131.3, 130.8, 130.5, 128.0, 127.0, 124.1 (aromatic carbons). ESI-MS (m/z): calcd for C₂₅H₂₈Cl₂N₂O₄S, 576.0342; found, 576.0342 [M + H]+.
(\(\epsilon, \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}\)): 285 (18 450), 357 (5500). FT-IR (KBr, cm\(^{-1}\)): 3315 (m, \(\nu\) (amide N–H)), 3156 (s, \(\nu\) (amide N–H)), 1664 (s, \(\nu\) (C=O)), 1178 (s, \(\nu\) (C=O)). H NMR (500 MHz, CDCl\(_3\)): \(\delta\) ppm 13.12 (s, 1H, OC–NH), 11.54 (s, 1H, SC–NH), 8.34 (d, \(J = 7.6 \text{ Hz}\), 2H, aromatic–H), 7.98 (d, \(J = 7.6 \text{ Hz}\), 1H, aromatic–H), 7.93 (d, \(J = 7.9 \text{ Hz}\), 2H, aromatic–H), 7.72 (d, \(J = 7.2 \text{ Hz}\), 1H, aromatic–H), 7.56 (t, \(J = 6.9 \text{ Hz}\), 4H, aromatic–H), 7.50 (t, \(J = 7.5 \text{ Hz}\), 2H, aromatic–H), 5.29 (d, \(J = 5.6 \text{ Hz}\), 2H, p-cymene aromatic–H), 5.17 (d, \(J = 5.6 \text{ Hz}\), 2H, p-cymene aromatic–H), 2.86–2.75 (m, 5H, p-cymene CH(CH\(_2\))\(_2\)), 2.16 (s, 3H, p-cymene C–CH\(_3\)), 1.17 (d, \(J = 6.8 \text{ Hz}\), 6H, p-cymene C(CH\(_3\))\(_2\)). 13C NMR (125 MHz, CDCl\(_3\)): \(\delta\) ppm 182.0 (C=O), 169.8 (C=O), 134.2, 133.7, 132.8, 131.0, 129.9, 128.9, 128.5, 128.3, 127.3, 127.6, 127.1, 125.1 (aromatic carbons), 103.2, 99.9, 84.0, 82.8 (aromatic carbons of p-cymene), 30.3, 22.0, 18.2 (aliphatic carbons of p-cymene). MS-ESI+(m/z): found, 541.0950 [M + 2H\(^+\) + CI\(^-\)]; calcld, 541.0887. ESI-MS (m/z): calcld for C\(_{20}\)H\(_{16}\)Cl\(_4\)N\(_2\)ORuS, 512.0342; found, 502.9357 [M + 2H\(^+\) + CI\(^-\)].

[Dichloro(benzene)(2,4-dichloro-N-(2-naphthalenylcarbamothioyl)benzamide)ruthenium(II)] (5). L1 (130 mg, 0.4 mmol) was used. Yield: 85%. Orange solid. mp 205 °C. Anal. Calcld for C\(_{20}\)H\(_{16}\)Cl\(_4\)N\(_2\)ORuS: C, 48.64; H, 3.69; N, 5.33; S, 6.39. UV–vis (DMF) \(\lambda_{\text{max}}\) nm (\(\epsilon, \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}\)): 276 (22 150), 339 (3900). FT-IR (KBr, cm\(^{-1}\)): 3319 (m, \(\nu\) (amide N–H)), 1666 (s, \(\nu\) (C=O)), 1202 (s, \(\nu\) (C=S)). H NMR (500 MHz, DMSO-d\(_6\)): \(\delta\) ppm 12.70 (s, 1H, OC–NH), 12.02 (s, 1H, SC–NH), 7.78 (s, 1H, aromatic–H), 7.69 (d, \(J = 8.0 \text{ Hz}\), 3H, aromatic–H), 7.57 (d, \(J = 8.3 \text{ Hz}\), 1H, aromatic–H), 7.44 (t, \(J = 7.7 \text{ Hz}\), 2H, aromatic–H), 7.29 (t, \(J = 7.4 \text{ Hz}\), 1H, aromatic–H), 5.98 (s, 6H, benzene–H). 13C NMR (125 MHz, DMSO-d\(_6\)): \(\delta\) ppm 178.9 (C=O), 168.1 (C=O), 150.7, 138.2, 136.4, 133.7, 131.2, 129.5, 128.8, 127.9, 127.8, 126.9, 124.8, 120.3 (aromatic carbons), 88.1 (aromatic carbons of benzene). ESI-MS (m/z): calcld for C\(_{20}\)H\(_{16}\)Cl\(_4\)N\(_2\)ORuS, 555.9716; found, 485.0302 [M + 2H\(^+\) – 2CI\(^-\) + H\(^+\)].

**In Vitro Anticancer Activity**. Cytotoxic activity of the Ru–arene complexes (1–8) was investigated on four different human cancer cell lines such as MCF-7 (breast), COLO 205 (colon), A549 (lung), and IMR-32 (brain) by cell viability assay that is MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The cytotoxicity of the most cytotoxic Ru–arene complexes (3 and 7) of different chemical nature (arene = p-cymene/benzene) was appraised on the normal cell line Vero (kidney) by MTT assay as well. The anticancer mechanism of 3 and 7 was evaluated in the IMR-32 cancer cell line by assessing the intracellular ROS, MMP, DNA damage, and caspase-3 activity by DCFH-DA, rhodamine 123, DAPI, and caspase-3 assay kit, respectively.
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Notes

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