CHAPTER 1

Introduction

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Introduction

Transition metals possess great advantage by having the several stable oxidation states and various coordination geometries [1-4]. These transition metal complexes are also known to exhibit various oxidation states by the ligand substitutions, dissociations, rearrangements, redox reactions and even catalytic reactions under physiological as well as biological conditions with their structural modifications [5-14]. One of the transition metal complex i.e., cisplatin [15] with the remarkable biological activity over certain cancer cell lines with limited side effects [16-18] offers a great deal of research to the other transition metal complexes [19]. Among the transition metal complexes mainly platinum group d⁶ metal complexes {Ru(II), Rh(III) and Ir(III)} have raised considerable expectations for the treatment of cancer [20]. These d⁶ metal complexes possess the similar ligand (water or bio molecule) exchange kinetics in aqueous solutions or biological environment. These organometallic d⁶ complexes exhibiting less side effects with a different mechanism of action than the platinum compounds [21].

The work presented in this thesis describes about the synthesis of new half sandwich ruthenium, rhodium and iridium metal complexes with some nitrogen and sulphur donor ligands and investigation of their in vitro functional activity towards bacterial strains and cancer cells. The electronic properties of these complexes were also rationalized by the density functional theory and the interaction of the complexes with CT-DNA was observed by the biophysical (UV-Visible and fluorescence) studies.

1.1. d⁶ - Metal complexes

The metals, which have six electrons in their valence d shell, are known as d⁶ metal complexes. Group VIII metals such as Fe²⁺, Ru²⁺, Os²⁺, Co³⁺, Rh³⁺, Ir³⁺ and Pt⁴⁺ are following d⁶ electronic configuration with their stable oxidation states II, III and IV respectively. The d⁶ metal complexes are demonstrating innumerable applications in chemical as well as biological systems. Two naturally occurring and well-known examples of d⁶ metal complexes are metalloproteins i.e., hemoglobin (Fe-3d⁶) and a vitamin B₁₂ (Co-3d⁶). These d⁶ complexes are playing crucial role with their donating and accepting electron property (redox) in the biological systems. The heavier congeners of these 8th and 9th group d⁶ metal complexes have arisen expectations as heavier congeners can mimic the biological properties of the lower congeners. Such an imaginary thought has explored to synthesize and study of their biological
properties of many d\textsuperscript{6} metal complexes. As a result, a few of the complexes either as a prodrug or as a drug have entered and observed for clinical trials with a less side effects and/or catalyzing the biomolecules inside cell.

1.2. Applications of half-sandwich d\textsuperscript{6} metal complexes

Half-sandwich d\textsuperscript{6} {Ru, Rh and Ir} metal complexes have shown some potential applications in the field of classical inorganic chemistry as well as in the field of inorganic chemical biology. Some of the specific applications of these half-sandwich d\textsuperscript{6} metal complexes have been explained precisely in the following sections.

1.2.1. Biological applications of half sandwich d\textsuperscript{6} metal complexes

1.2.1.1. Arene ruthenium(II) complexes

Activation of ruthenium(III) complexes by reduction \textit{viz.} NAMI-A [22-24], KP1019 [22, 25] and NKP-1339 [26, 27] over cancer cell lines have given much attention to the ruthenium(II) complexes biologically [28-31]. These active ruthenium(II) complexes have shown selective toxicity to the cell lines by their higher activity towards the amino acids, nucleobases of biomolecules \textit{viz.} DNA, RNA, protein [32]. Such a property of the ruthenium(II) complexes has drawn much attention to synthesize air stable ruthenium(II) complexes. There are various types of precursors available to synthesize ruthenium(II) complexes but in present study we are describing the arene ruthenium(II) complexes. This category of arene ruthenium complexes is one of the most stable as well most reactive complexes. These arene ruthenium complexes resemble piano stool geometry (Chart 1.1), where arene forms the seat of the piano-stool and the remaining coordinating ligands resemble the legs of stool. Here arene is playing a crucial role as a chemically stabilizing the ruthenium +2 oxidation state and also biologically increasing the lipophilicity of the complexes and is inert towards the displacement reactions under physiological conditions. The variation in ligands in these piano-stool complexes has shown promising \textit{in vitro} as well as \textit{in vivo} anticancer activity [33-36].
Chart 1.1: Piano stool configured arene ruthenium metal complexes.

The activity of these piano stool complexes has started with a hope that biologically active ligands do improve the efficacy of these half-sandwich configured complexes in a synergetic manner. Such a synergetic legacy of the half-sandwich complexes continues from the organic anticancer drug 1-ß-hydroxyethyl-2-methyl-5-nitroimidazole \((\text{metronidazole}; \text{MTZ})\) [37] to sunscreen lotion \(\text{avobenzone}\) [38] (fig. 1.1).

Fig. 1.1: Representation of the first arene ruthenium (A; [Ru(benzene)Cl\(_2\)(metronidazole)]) and recent arene ruthenium complex (B; [(arene)Ru(avobenzone)(PTA)]\(^{2+}\)) with the biologically active ligands.

In this perspective, Tocher et.al, in 1992 observed the cytotoxic activity of the first half-sandwich \(d^6\) ruthenium complex \(i.e., [\text{Ru(benzene)}\text{Cl}_2(\text{metronidazole})]\), by incorporation of
the organic anticancer drug MTZ as ligand [37]. The study suggests that the activity of this complex is much superior than the ligand (MTZ). Moreover, the enhanced activity possessed by the new complex brought some new thoughts/challenges in the half sandwich ruthenium chemistry. Thereafter number of ruthenium metal complexes have been studied for the antiproliferative activity where the metal is coordinated by the biologically active ligands (table 1.1). Due to lack of water solubility of these complexes has limited the biological study so in many cases most of the *in vitro* biological experiments has been carried out in DMSO stock solutions.

Table 1.1: Arene ruthenium complexes with the biologically active ligands.

<table>
<thead>
<tr>
<th>Molecular structure of metal complex</th>
<th>Target enzyme/cell lines/therapeutic indications</th>
<th>Proposed mechanism of action</th>
<th>IC₅₀ value (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>Exhibiting TOPO-II inhibitory activity [39]</td>
<td>Covalent binding to DNA</td>
<td>0.86±0.06 on ovarian (CH1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.8±0.5 on colon (SW480)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.5±0.5 on carcinoma (A549).</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>HIV-1 1N inhibition [40]</td>
<td>Catalytic transfer process</td>
<td></td>
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<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>HIV-1 1N inhibition [40]</td>
<td>Catalytic transfer process</td>
<td></td>
</tr>
</tbody>
</table>
Sadler and his group members have developed arene ruthenium diamine complexes (fig. 1.2) where he suggested that the chelating diamine ligand could help the ligand-exchange kinetics of the complexes. These complexes have exhibited micro molar range cytotoxicity towards cisplatin resistant cancer cell lines. In the nucleotide adducts of these complexes have shown greater selectivity for N7 of guanine that is very similar to cisplatin and little interaction with adenine in contrast to the cisplatin [34, 44]. The cytotoxicity of these ethylenediamine (en) complexes influenced by the size of the arene ring as the size of the arene ring increases the cytotoxicity also increases. Which is following the below order: benzene (ben) < \( p \)-cymene (\( p \)-cym) < biphenyl (bip) < dihydroanthracene (dha) < dihydrophenanthrene (dhpa) \( \approx \) tetrahydroanthracene (tha) [33, 45].

![Mononuclear arene ruthenium ethylenediamine (en) complexes](image)

Fig. 1.2: mononuclear arene ruthenium ethylenediamine (en) complexes.
These arene ruthenium ethylenediamine (en) complexes exhibited interaction with the amino acids [46, 47], peptides [48], proteins [46] and DNA bases [44]. The interaction with the biomolecules of these complexes, first involves the aquation of the complex [36]. In the process of aquation, the ruthenium aqua (H₂O) complex has formed which is more reactive than the ruthenium hydraoxo (OH) as well as chloro complexes; thus lead to the substitution of guanine base (fig. 1.3) [49].

Another class of ruthenium compounds developed by Dyson and his group members with an amphiphilic natured PTA (1,3,5-triaza-7-phosphatricyclo[3.3.1.1]decane) ligand [50]. The purpose of choosing such an amphiphilic ligand was to cross the hydrophobic cell membranes as consequently the drug enters into the cancer cells. These ruthenium-arene-PTA complexes (RAPTA) were designed to improve the aqueous solubility of the complex and it has been observed that these complexes have shown promising anticancer activity [51]. Many modifications have done to these RAPTA complexes in order to elucidate the mode of action while some of them depicted in fig. 1.4 [52, 53].

Fig. 1.3: Representation of the MoA of arene Ru ethylenediamine

Fig. 1.4: Structures of RAPTA series complexes, which possess antitumor activity.
The biological activity of these RAPTA complexes is different from the other arene ruthenium complexes with a selective metastasis on primary tumors [54]. Such a different activity over the primary tumors of these RAPTA complexes suggested that the mechanism of action is different from the other promising candidates [55]. The binding studies with the calf thymus DNA (CT-DNA) of these RAPTA complexes shown that the interaction can be thermodynamically driven and highly favorable [50, 51]. Though the major biological target for these arene ruthenium complexes has been observed as DNA but in vitro these complexes shown high affinity towards the proteins and RNA (fig. 1.5) even in the presence of DNA [56-59].

![Diagram](image1.png)

**Fig. 1.5:** Representation of the biological target for the RAPTA complexes.

Apart from these two types of promising arene ruthenium complexes there were many complexes with biological or non-biological relevant ligands, which possess a good amount of cytotoxicity. Many groups have been developed a strategy that if a chelating ligand is biologically active on complexation the activity of the complex may enhance. *Eric Meggers* and his group members have developed some arene ruthenium complexes with an inspiration from the organic anticancer drug staurosporine (fig. 1.6). These staurosporine derived arene ruthenium complexes have exhibited pico molar based drug activity over melanoma cell lines with a kinase (enzyme) inhibition [60, 61]. Another class of kinase (cyclin-dependent kinase (CDK) and glycogen synthase kinase-3) inhibitors are indolo[3,2-d]benzazepines (paullones), the arene ruthenium complexes of these paullones (fig. 1.6) showed highly potent in vitro activity with electron-rich ligands [62] but the mechanism of action of these compound has not elucidated.
Arene ruthenium complexes has developed in a view that cytotoxic arene ruthenium and photosensitizing nature porphyrin unit to generate much more cytotoxic complexes (fig. 1.7). As expected these complexes were low cytotoxic (~ 10 μM) in the dark when they irradiated with laser light at 652 nm the cytotoxicity of the complexes has increased (5 μM) [63, 64] towards melanoma cells.

Fig. 1.6: Arene ruthenium complexes with the biologically active ligand scaffolds.

Fig. 1.7: Tetra nuclear arene ruthenium porphyrin complex.

*Arene rhodium(III) and iridium(III) compounds*

The chemical inertness of the 9th group metal complexes was thought to be more cytotoxic than their labile platinum group or ruthenium counter parts. Surprisingly the rhodium(III) and iridium(III) analogs of NAMI-A and KP-109 [65] were found with no significant biological
activity. The low rate of hydrolysis of the chloride ligands in the chemically inert rhodium and iridium complexes is concluding for low activity [66-68]. Particularly in the case hexahydrate iridium(III) complex, ligand exchange use to happen over hundreds of years [69]. Such a less possibility of ligand exchange rate has been increased as 14 orders of magnitude by the incorporation of negatively charged cyclopentadienyl ligand [70].

Organometallic iridium(III) complexes, are isoelectronic and isostructural to the arene ruthenium(II) dimer, have shown that they undergo hydrolysis with the iridium-chloride bond within a minute’s of time [71]. However, the rate of hydrolysis is also depending on the type of arene (cyclopentadienyl ligands) and the chelating ligand [71-73]. Such a hydrolysis nature of compounds can help to find out the mechanism of action and an interaction with the possible biological targets [74]. As mentioned earlier type of arene and the chelated ligand has played a crucial role to enhance the cytotoxicity of the complexes. These half sandwich iridium(III) complexes have shown promising active over tumor cells with different MoA (fig. 1.8) [71-73].

It has been observed that the introduction of phenyl and biphenyl groups in the arene (Cp*) moiety the cytotoxicity of the complexes has increased in the A2780 human ovarian cancer cell lines. The biological target for these complexes has been observed as guanine residues on plasmid DNA. The accumulation of iridium is also high in the tested cell lines in comparison to the cisplatin which strongly suggesting the interaction of these compound with the biological targets. Such an interaction of these half-sandwich iridium complexes with DNA

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**Fig. 1.8**: Representation of the structural dependence of the cytotoxicity of iridium complexes.

R = CH₃, ph, biph  
Z = Cl or py  
L = N⁺N or C⁺N  
Cytotoxicity increases
either through covalent or non-covalent interaction by enhancing the anticancer activity of these complexes [75, 76]. When it comes to the chelating ligand the negatively charged chelators mostly (C─∩N) are more cytotoxic than the neutral N∩N ligands [74, 77, 78]. The negatively charged ligand enhancing the electron density on the metal center and in turn making the chloro ligand is more labile. These high valent metal complexes with their oxidizing ability through ROS generation triggering cancer cell death.

1.2.2. Catalytic applications of arene d6 metal complexes

The complexation with various organic ligands of these arene d6 metal complexes results the robust stability and the solubility of the complexes in both organic as well as aqueous solutions. Such a characteristic phenomenon makes them use as a catalyst for organic transformations resulting new molecules and also for biomolecules causing cancer cell death. For instance, half sandwich ruthenium complexes were used as a catalyst in the many organic transformation reactions such as C-H bond activation for the construction of C-C and C-heteroatom bonds [79-83], Diels-Alder reactions [84, 85], the asymmetric transfer-hydrogenation of ketones and imines [86-91], C-N bond formation from alcohol and amines [92-94], oxidation of alcohols [95-97], water oxidation [98]. 1-(2H)-phthalazinones are the valuable heterocyclic units which have been found numerous applications in medicinal field such as azelastine, a histamine antagonist, these have been delivered as eye drops or nasal spray in rhinitis indications. In addition, olaparib, an oral drug approved for ovarian cancer, is an inhibitor of poly(ADP-ribose) polymerase [99-101]. Malcolm et.al., studied the alkenylated phthalazinones and exclusively afforded the desired C8-substituted product by using a catalyst [Cp*Rh(Acetonitrile)3](SbF6)2.

1.3. Synthetic aspects of arene d6 metal complexes

The first arene d6 metal complex with the formula [RuCl2(C6H6)]n was proposed by Winkhaus and Singer in 1967 by the reduction of ruthenium trichloride hydrate (RuCl3⋅xH2O) in ethanol in the presence of 1,3-cyclohexadiene [102]. This brown insoluble polymeric structure of the neutral diamagnetic complex later in 1972 showed as a dimeric complex [(C6H6)RuCl2]2 by Zelonka and Baird [103]. Eventually in 1974, Bennett prepared different arene ruthenium complexes from 1,3 and 1,4-cyclohexadiene’s mainly p-cymene ruthenium dimer [104]. Surprisingly this method could not facilitate with the electronically rich dienes such as
hexamethylbenzene (HMB), where he suggested arene exchange reactions could possible with the electron rich dienes by exchange of the para-cymene from the p-cymene ruthenium dimer at around 180°C. The isoelectronic and isostructural chemistry of higher analogous metals i.e., rhodium and iridium has studied by Maitlis in 1969 [105]. They introduced the negatively charged arene i.e., pentamethylcyclopentadiene (Cp*) in place of neutral arenes for the higher analogous metals. Such a negatively charged arene results the metal to follow d⁶ electronic configuration with the dimeric complexes [(Cp*)MCl₂]₂ (M = Ir, Rh) is similar to the case of arene ruthenium dimer.

*Synthesis of [(p-cymene)RuCl₂]*

The [(p-cymene)RuCl₂]₂ was prepared according to well established synthetic procedure. A solution of hydrated ruthenium trichloride RuCl₃·xH₂O containing 38-39% Ru (1g, 3.85 mmol) in 80 ml absolute ethanol was treated with 5 ml of α-phellandrene / α-terpinene and heated under reflux for 4 hours. The solution was filtered through normal filter paper to remove the black material. The solution was kept in refrigerator overnight at 4 ºC and the resulted crystalline product was filtered off and washed with diethyl ether and collected. The additional product was collected by reducing the orange filtrate to approximately half of its volume and keeping in refrigerator overnight at 4 ºC. The resulting crystalline compound was washed with diethyl ether, air dried and collected.

Yield: 0.98 g (81%). ¹H NMR (400 MHz, CDCl₃): 5.48 (d, 2H, J = 6.40 Hz, Ph(cym)), 5.34 (d, 2H, J = 6.40 Hz, Ph(cym)), 2.95 (sept, 1H, CH(CH₃)₂), 2.15 (s, 3H, CH₃), 1.28 (d, 6H, J = 6.40 Hz, CH(CH₃)₂).
Synthesis of [(benzene)RuCl$_2$]$_2$

Hydrated RuCl$_3$·xH$_2$O (1g, 3.85 mmol) in 80 ml of absolute ethanol was heated under reflux with 5 ml of 1,4-cyclohexadiene for 4 hours. The brown precipitate was filtered off and washed with diethyl ether and air-dried. Yield: (1.10g, 95%).

$^1$H NMR (400 MHz, CDCl$_3$): 6.07 (S, 6h, C$_6$H$_6$).

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Synthesis of [Cp*RhCl$_2$]$_2$ [Cp*IrCl$_2$]$_2$

A solution of hydrated MCl$_3$·xH$_2$O [M = Rh and Ir] (1 g) in 30 ml methanol was treated with 0.8 ml of pentamethylcyclopentadiene and heated under reflux for 48 hours. The solution was allowed to cool to room temperature and reddish-brown crystalline product was filtered off. The crystalline product was filtered and washed with diethyl ether and air-dried. Yield: 0.9 g for rhodium dimer and 1.1 g for iridium dimer.

M = Rh; $^1$H NMR (400 MHz, CDCl$_3$): 1.62 (s, 15H, Cp*), M = Ir; $^1$H NMR (400 MHz, CDCl$_3$): 1.58 (s, 15H, Cp*).
1.4. Features of ligands under study

Transition metal complexes based on polypyridyl ligands have been established as good DNA binders with their remarkable property _i.e._, charge transfer with in DNA [106]. These extended polypyridyl ligands such as dpq, dppz and dppn with the arene systems may strengthen the mode of binding which may consequently result the cellular uptake of the complexes [107]. As expected these are well correlation with the observed IC$_{50}$ values over MCF-7 cell lines. The polypyridyl hexadentate ligand based on dipyridyl amine (DPA) has been designed and the flexibility of the DPA moieties can furnish the mono- to hexa- nuclear complexes. Such a flexible DPA moieties could promote for DNA intercalation, which may enhance the biological activity of the metal complex (for example see fig. 1.9) [108].

![Figure 1.9](image)

Fig. 1.9: Representative complex of multi-nuclear ligand with platinum metal

Most of the organometallic complexes are water insoluble due to that many complexes are disqualifying for the clinical trials. To avoid such a difficulty, one has to develop water-soluble complexes, which can offer a wide range of potential applications. Dipyridyl ligands well-studied systems that can readily offer the six-membered chelating metal complexes, however if an additional coordinating sites are available they provide a serendipitous bonding modes (fig. 1.10). Particularly when they have imine nitrogen that contains the lone pair on the imine nitrogen can regulate bonding modes of the metal complexes that results the diverse structural motifs [109-111]. We combined such a versatile dipyridyl unit with the hydrazides to increase the aqueous solubility of the complexes [112-115].
Complexes with planar heterocyclic aromatic ligands are always been fascinating in the field of supramolecular and biological inorganic chemistry with their flexible bonding modes. These small ligands can intercalate with the biomolecules that may result in the biological activity of the complexes apart from intercalation the heterocyclic moiety can mimic with the DNA bases.

1.5 Materials and methods

1.5.1. Physical methods and materials

All the experiments were performed without using any inert conditions. All reagents were purchased from commercial sources and used as received. RuCl$_3$$\cdot$H$_2$O, RhCl$_3$$\cdot$H$_2$O and IrCl$_3$$\cdot$H$_2$O were purchased from Arora Matthey limited, while α-phellandrene (Merck), α-terpinene (Sigma-Aldrich) 1,4-cyclohexadiene (Sigma-Aldrich), pentamethylcyclopentadiene (Sigma-Aldrich) were used as received without further purification. The solvents were purified and dried according to standard procedures [116]. The metal precursor complexes [(arene)RuCl$_2$]$_2$ (arene = $p$-cymene and benzene), [Cp*MCl$_2$]$_2$ (M= Rh and Ir) and [CpRu(PPh$_3$)$_2$Cl] were prepared according to literature methods [103, 104, 117-119].

NMR spectra were recorded on a Bruker Avance II 400 MHz spectrometer. Infrared spectra were recorded as KBr pellets on a Perkin-Elmer 983 spectrophotometer. Elemental analyses were performed on a Perkin-Elmer-2400 CHN analyzer. Absorption spectra were obtained at room temperature using a Perkin-Elmer Lambda 25 UV-Visible spectrophotometer.
1.5.2. Single-crystal X-ray structures analyses

Single crystal X-ray diffraction measurements were carried out using an Oxford Diffraction Xcalibur Eos Gemini diffractometer. Crystal data were collected at 300 K using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). The strategy for the data collection was evaluated using the CrysAlisPro CCD software. Crystal data were collected by standard “phi-omega scan” techniques and were scaled and reduced using CrysAlisPro RED software [120]. The structures were solved by direct methods using SHELXS-97 and refined by full matrix least squares with SHELXL-2013 refining on F² [121, 122]. The positions of all the atoms were obtained by direct methods. All non-hydrogen atoms were refined anisotropically. The hydrogen atoms were placed in geometrically constrained positions and refined with isotropic temperature factors, generally 1.2 Ueq of their parent atoms. The molecular structures were drawn with ORTEP-3 [123], packing pattern and interactions like hydrogen bonding were drawn with MERCURY [124].

The crystallographic data of the complexes can be obtained free of charge by the corresponding CCDC numbers via www.ccdc.cam.ac.uk/data_request/cif, by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

1.5.3. Computational Methodology

The gas phase geometries of complexes were fully optimized without any geometrical constraints in singlet ground states with the density functional theory (DFT) based B3LYP [125] hybrid exchange-correlation functional method implemented in GAUSSIAN-09 [126] package. The calculations were performed using ECP LANL2DZ [127] basis set for post third-row atoms and the standard 6-31G(d,p) for other atoms. All calculated harmonic vibrational frequencies of complexes were found to be real indicating that the optimized structures of the complexes are at the minimum in the potential energy surface. Natural Bond Orbital analysis (NBO) [128] was used to obtain the natural electronic configurations, charges on the individual atoms and the d-orbital occupancies of the rhodium and iridium metal atoms present in the complexes. The electronic spectra of complexes were calculated using the time-dependent density functional theory (TDDFT) [129, 130] with the same density functional. The solvent effect (acetonitrile as solvent) was simulated using the polarizable continuum
solvent-effect model (PCM) [131]. Gauss Sum 3.0 [132] was used for the analysis of UV-vis spectra, oscillator-strengths, HOMO-LUMO energy gap, transitions between various states and the fractional contributions of various groups to each molecular orbital.

1.5.4. In vitro antimicrobial assay

The synthesized complexes were evaluated for their antibacterial activity by the agar well diffusion method [133, 134]. The bacterial strains used in the present study are: gram-positive strains (Staphylococcus aureus; Bacillus thuringiensis); and gram-negative strains (Escherichia coli; Pseudomonas aeruginosa). The required nutrient broth was prepared and sterilized at 121 ºC. The bacterial strains were inoculated onto nutrient broth (10\(^8\) cells per mL) and incubated overnight. The overnight grown bacterial cultures were inoculated into 30 mL of sterilized agar medium, which was transferred aseptically into each sterilized petri plate. The plates were left at room temperature for solidification. After solidification of media; a well of 5 mm diameter was made using a sterile cork borer. The antibacterial assay plates were incubated at 37±2 ºC for 24 h and after the incubation period, the diameter of the inhibition zone was measured as an indicator of the activity of the complexes. The minimum inhibitory concentration (MIC) for the complexes was obtained as 0.5 mg mL\(^{-1}\) using a standard spectrophotometric assay. For the well diffusion experiments, the complexes were used at a concentration of 1 mg mL\(^{-1}\). Gentamycin was used as positive control drug; dimethylsulphoxide (DMSO) was used both as a solvent and as a control. No inhibition zone was observed in the control (i.e. for DMSO). Each experiment was performed in triplicate.

1.5.5. Chemo sensitivity studies

All compounds and cisplatin were tested against a panel of three tumour cell lines consisting of HT-29 (human colorectal cancer), BE (human colorectal cancer) and MIA-PaCa2 (human pancreatic cancer) and one non-cancer cell line ARPE-19 (human retinal epithelial cells). Cells were seeded into 96 well plates at 1 x 10\(^3\) cells per well and incubated at 37 ºC in a CO\(_2\) enriched (5%), humidified atmosphere overnight to adhere. Media were removed and replaced with fresh media containing a range of drug concentrations and cells were incubated for four days before cell survival was determined using the MTT assay as described elsewhere [135]. Results were expressed in terms of IC\(_{50}\) values and all studies were performed in triplicate. The results were also expressed in terms of a ‘selectivity index’
defined as the IC$_{50}$ of the non-cancer cell line ARPE divided by the IC$_{50}$ of cancer cell lines [136]. Values greater than 1 demonstrate that the compound is preferentially active against tumor compared to normal cell lines.

1.5.6. Spectroscopic studies on DNA interaction

The DNA binding experiments were performed at room temperature. The DNA concentration per nucleotide was determined by electronic absorption spectroscopy using 1 cm path length cuvettes. DNA solutions in 5 mM Tris–HCl/50 mM NaCl buffer, pH 7.4 gave the ratio of UV absorbance at 260 nm and 280 nm, $A_{260}/A_{280}$, of 1.9, indicating that the DNA was sufficiently free from protein [137]. The DNA concentration was determined by measuring the UV absorption at 260 nm, taking the molar absorption coefficient ($\varepsilon_{260}$) of CT-DNA as 6600 M$^{-1}$ cm$^{-1}$ [138, 139]. Absorption titration experiments of the complex in Tris-HCl buffer was performed by using a fixed complex concentration to which increments of the DNA stock solutions were added. Complex solutions were allowed to incubate for 10 min before the absorption spectra were recorded. The absorption titration experiments were performed with a fixed concentration of the compounds (20 μM) with a concentration of DNA (5 – 20 μM). While measuring the absorption spectra, an equal amount of DNA (5 μM) was added to both the test solution and the reference solution, to eliminate the absorbance of DNA itself. Fluorescence experiments with complexes 1 and 2 were performed with 20 μM complex and 50 μM CT-DNA. The emission spectra were recorded at 300-500 nm by exciting the sample at 220 nm. Ethidium bromide fluorescence quenching experiments were carried out by the metal complexes (25 μM) to the samples containing 5 μM EB, 50 μM DNA, and tris buffer. The emission was recorded at 530-750 nm by exciting the sample at 522 nm. All the samples were incubated at room temperature for 15 min to attain thermodynamic equilibrium before performing absorption and fluorescence experiments.
1.6. References


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