CHAPTER 5

Synthesis, structural and biological studies of half-sandwich d⁶-metal complexes with pyrimidine-based ligands

5.1 Introduction

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Graphical abstract

Reaction of 2-mercaptopyrimidine with rhodium and iridium dimers yielded four-membered metallacyclic complexes. These complexes exhibited considerable cytotoxicity and antibacterial activity. The selectivity index of these complexes is highest in the case of colorectal cell lines *i.e.* HT-29 and BE. 2-amiono pyrimidine yielded binuclear complexes with rhodium and iridium but potent toxicity has not been found for these complexes. Computational and biophysical studies additionally supported the experimental results.
Abstract
The half-sandwich platinum group metal complexes were prepared by using respective metal precursors of \([\text{Cp}^*\text{MCl}_2]\_2 (\text{M} = \text{Rh, Ir}) / [\text{CpRu(PPh}_3\text{)Cl}]\) and pyrimidine-based ligands. The reaction of metal precursors with 2-aminopyrimidine (L1) gave binuclear complexes as \([\text{(Cp}^*\text{MCl}_2]_2(\mu\text{-L1})\]), where L1 acted as a bridging ligand. While 2-mercaptopyrimidine (L2) with \([\text{Cp}^*\text{MCl}_2]\_2 (\text{M} = \text{Rh(III), Ir(III)})\) formed mononuclear di substituted complexes as \([\text{Cp}^*\text{M(L2)}]_2\), where L2 acted as a chelating as well as a monodentate ligand. The reaction of \([\text{CpRu(PPh}_3\text{)Cl}]\) with 2-mercaptopyrimidine (L2) led to the formation of mononuclear complex as general formula \([\text{CpRu(PPh}_3\text{(L2})]\) in presence of a base. All these neutral complexes were fully characterised by various spectroscopic techniques. 2-Mercaptopyrimidine ligand resulted complexes with strained four-membered metallacycle while 2-aminopyrimidine yielded bridged complexes. The structures of all the complexes were established by single-crystal X-ray diffraction confirming piano-stool geometry. HOMO-LUMO energy gaps and UV-Visible bands were additionally rationalised by the DFT studies. The binding ability of the complexes to the CT-DNA was confirmed using UV-Visible and fluorescence spectroscopy. In vitro antibacterial activity of the complexes was evaluated against human pathogenic bacteria. Cytotoxicity of the complexes was examined by the MTT assay over three cancerous and one non-cancer cell lines viz., BE, HT-29, MIA-Pa-Ca2 and ARPE-19.
5.1. Introduction

Study of pyrimidine and its derivatives has been drawn attention in the last decade due to their interesting coordination modes, biological activities and applications in therapeutic field [1-4]. The pyrimidine analogues, as the most well-known type of the ligands pyrimidine-XH (X= NH and S) bind to metal either in monodentate or bidentate bridging and chelating modes [5, 6]. For instance, Bernhard Lippert et al. reported that the reaction of trans [a2PtCl2] with 2-hydroxyxypyriridine (Hpymo) to form monodentate compound trans [a2Pt(Hpymo-N1)2]X2, \( a = \text{NH}_3, X = \text{NO}_3; a = \text{CH}_3\text{NH}_2, X = \text{NO}_3; a = \text{CH}_3\text{NH}_2, X = \text{ClO}_4 \) where platinum binds through N1 donor atom of the pyrimidine ring. In the case of \([\text{(en)M(H}_2\text{O})_2]_2(\text{NO}_3)_2; \text{en} = \text{ethylenediamine, M} = \text{Pd(II), Pt(II)}\) complex with 2-hydroxyxypyriridine results in self-assembly of forming cyclic complexes of type \( [(\text{en})\text{M(pymo-N1, N3)})_4(\text{NO}_3)_4, \) which are structurally analogous to calix [4] arenes [7, 8]. The complexes of CpRu with heterocyclic thiolates/thiones ligands yielded monodentate complexes, where the ligands coordinated to the metal through sulfur [9, 10]. It is found that the reaction between [Cp*IrCl2]2 and 2,1,3-benzothiadiazole gives binuclear complex \([\text{(Cp*IrCl}_2]_2(\mu-\text{L})\) in which L acts as a bridging ligand connecting two iridium centres [11]. For comparison, \([(\text{HMB})\text{RuCl}_2]_2 (\text{HMB} = \text{hexamethylbenzene})\) with the analogous 2-pyridinethiol and 2-quinolinethiol does not lead to a cationic pyridinium thiolato complexes, but the neutral complexes are observed \([(\text{HMB})\text{Ru(\eta}_2^2-\text{L})(\eta}_1^1-\text{L})\] [1].

Platinum induced inhibition of cell division in E. coli bacterium resulted in a tremendous applicability of metal based complexes in the field of bioinorganic chemistry [12]. Cisplatin, one of the platinum based compound showed antitumor properties on testicular and ovarian cancers, which paved the way for metal based drugs [13-15]. Due to the side effects of cisplatin or oxaliplatin there is a quest for identifying lower toxic metal-based complexes. Recently, complexes of d6 metals have spawned great interest with a different mechanism of action in comparison to the platinum drugs where these metals could target biomolecule such as DNA, protein and enzyme [16-19]. Two of the ruthenium(III) compounds, namely NAMI-A and KP1019 are in phase II clinical trials with their anti-metastatic activity. In particular, half-sandwich organometallic compounds of these metals with various ligands are very interesting and have been widely studied [11, 20]. Among these RM 175 and RAPTA-C compounds are exhibiting anti-metastatic activity [21, 22]. Recently, it was observed that RM 175 is in head to head orientation of guanine bases, which is entirely different from the platinum guanine adduct [23]. Sadler et al. reported that the half-sandwich iridium(III) complexes can bind to nucleobases of DNA as monodentate and could stop the replication of DNA polymerization
and in a few cases it is also observed that inhibition of tumour growth by thioredoxin reductase (Trx-R) [24-26]. Compared to neutral nitrogen donor ligands, negatively charged chelating donors can enhance the antiproliferative activity of the metal complexes [27].

As discussed above the metal complexes are extensively tested for their anticancer activity. Recent studies on these complexes depicted that they also do possess antibacterial activity, thus making them amenable as double-edged swords with dual functionalities. However, studies elucidating both the antibacterial and anticancer activities of the same compounds, particularly from group 8 and 9 metals are very limited [28-31]. Recently, an iridium(III) complex has been reported with a selective inhibition of S. aureus bacterium as well as potential cytotoxicity against ovarian, cervical and melanoma cells [32]. Photoactivable ruthenium(II) complex cis-[Ru(bpy)₂(INH)]²⁺ with isoniazid was reported as 5.5 times potent towards gram-negative M. smegmatis bacterium in comparison with isoniazid. Moreover, this complex is highly selective for killing mycobacteria [30]. Another ruthenium(II) complex with picolinyldihydrazone was reported with antibacterial activity with a zone of inhibition around 35 mm on eight bacterial species as well as with high antitumor activity on Dalton’s lymphoma (DL) cells versus normal PBMC cells [33]. We have been working for last few years on synthesis of Cp*Rh(III)/ Cp*Ir(III) complexes with various nitrogen donor ligands and their structural and biological studies [33-35]. According to our knowledge, there is no report available in literature with these metal complexes of pyrimidine ligands (Fig. 5.1). Thus, we would like to report here syntheses, structural studies and biological assays of newly synthesized compounds. The structural studies of the complexes are also rationalised by DFT studies.

![Fig. 5.1: Ligands used in the present study](image)

### 5.2. Experimental Section

#### 5.2.1. Materials and methods

2-aminopyrimidine and 2-mercaptopyrimidine were purchased from Sigma-Aldrich and used as received.

#### 5.2.2. Syntheses of metal complexes 1 to 4

A mixture of metal precursor [Cp*MCl₂]₂ (0.1 mmol), 2-aminopyrimidine ligand (0.1 mmol)/2-mercaptopyrimidine (0.4 mmol) were dissolved in dichloromethane (20 mL) and
stirred at room temperature. During the reaction time color of the solution changed to orange red and the mixture was stirred for further 6h (complexes 1 and 2) or overnight (complexes 3 and 4). The reaction mixture was filtered through the celite to remove unreacted materials (mostly ligands) and the filtrate was reduced to 1 mL under reduced pressure, precipitated and washed with diethyl ether (3x10 ml) and air-dried.

5.2.2.1. \([\text{Cp}^*\text{RhCl}_2(\mu-\text{L1})]\) (1)
Yield: 68 mg (95%); IR (KBr, cm\(^{-1}\)): 3144(m), 1593(m), 1545(m), 1420(s), 1122(s), 1054(m), 777(m); \(^1\)H NMR (400 MHz, CDCl\(_3\) and DMSO-d\(_6\)): \(\delta = 8.19\) (d, J = 4.5 Hz, 2H, pyrimidine), 6.49 (t, 1H, pyrimidine), 5.63 (s, 2H, amine), 1.66 (s, 30H, Cp*); \(^{13}\)C NMR (101 MHz, CDCl\(_3\) and DMSO-d\(_6\)) \(\delta 158.16, 110.44, 99.11, 54.90, 9.02\); UV-Vis {Acetonitrile, \(\lambda_{\text{max}}\) nm (Abs)}: 228 (1.34), 282 (0.27) 408 (0.07); Anal. Calc. for C\(_{24}\)H\(_{35}\)N\(_3\)Rh\(_2\)Cl\(_4\) (713.17): C, 40.42; H, 4.95; N, 5.89. Found: C, 40.33; H, 4.91; N, 5.84.

5.2.2.2. \([\text{Cp}^*\text{IrCl}_2(\mu-\text{L1})]\) (2)
Yield: 74 mg (82%); IR (KBr, cm\(^{-1}\)): 3138(m), 2932(m), 1586(m), 1530(m), 1455(m), 1401(s), 1340(w), 1116(s), 765(m); \(^1\)H NMR (400 MHz, CDCl\(_3\) and DMSO-d\(_6\)): \(\delta = 8.19\) (d, J = 4.5 Hz, 2H, pyrimidine), 6.51 (t, 1H, pyrimidine), 5.61 (s, 2H, amine), 1.67 (s, 30H, Cp*); \(^{13}\)C NMR (101 MHz, CDCl\(_3\) and DMSO-d\(_6\)) \(\delta 163.40, 157.65, 109.91, 91.96, 54.30, 8.18\); UV-Vis {Acetonitrile, \(\lambda_{\text{max}}\) nm (Abs)}: 224 (0.51), 294 (0.06); Anal. Calc. for C\(_{24}\)H\(_{35}\)N\(_3\)Ir\(_2\)Cl\(_4\) (891.75): C, 32.32; H, 3.96; N, 4.71. Found: C, 32.28; H, 3.93; N, 4.68.

5.2.2.3. \([\text{Cp}^*\text{Rh}(\text{L2})]\) (3)
Yield: 52 mg (57%); IR (KBr, cm\(^{-1}\)): 1599(m), 1537(m), 1487(s), 1455(w), 1415(s), 1378(m), 1322(w), 1055(m), 777(m), 628(m), 558(s); \(^1\)H NMR (400 MHz, CDCl\(_3\) and DMSO-d\(_6\)): \(\delta = 8.56\) (d, J = 4.8 Hz, 1H, pyrimidine), 8.31 (d, J = 4.8 Hz, 1H, pyrimidine), 8.25 (pseudo doublet, J = 5.7 Hz, 2H, pyrimidine), 6.80 (t, J = 5.1 Hz, 1H, pyrimidine), 6.75 (t, J = 4.7 Hz, 1H, pyrimidine) 1.72 (s, 15H, Cp*); \(^{13}\)C NMR (101 MHz, CDCl\(_3\) and DMSO-d\(_6\)) \(\delta 157.05, 155.61, 155.05, 113.68, 113.58, 95.47, 95.40, 9.07\); UV-Vis {Acetonitrile, \(\lambda_{\text{max}}\) nm (Abs)}: 245 (0.52), 282 (0.52), 339 (0.09); Anal. Calc. for C\(_{18}\)H\(_{21}\)N\(_4\)RhS\(_2\) (460.42): C, 46.96; H, 4.6; N, 12.17. Found: C, 46.85; H, 4.56; N, 12.07.

5.2.2.4. \([\text{Cp}^*\text{Ir}(\text{L2})]\) (4)
Yield: 64 mg (58%); IR (KBr, cm\(^{-1}\)): 1598(m), 1565(m), 1536(m), 1487(s), 1454(m), 1417(s), 1394(m), 1339(w), 1054(m), 763(m), 622(w), 559(s); \(^1\)H NMR (400 MHz, CDCl\(_3\) and DMSO-d\(_6\)): \(\delta = 8.7\) (d, J = 4.9 Hz, 1H, pyrimidine), 8.37 (d, J = 5.93 Hz, 1H, pyrimidine), 8.31 (ps d, J = 4.8 Hz, 2H, pyrimidine), 6.99 (t, J = 5.1 Hz, 1H, pyrimidine), 6.80 (t, J = 4.8 Hz, 1H, pyrimidine), 1.79 (s, 15H, Cp*); \(^{13}\)C NMR (101 MHz, CDCl\(_3\) and DMSO-d\(_6\)) \(\delta 184.46, 179.19, 179.19, 179.19\).
155.83, 154.73, 154.59, 114.05, 113.74, 110.16, 88.43, 8.89; UV-Vis \( \lambda_{\text{max}} \) nm (Abs): 250 (0.06), 287 (0.13); Anal. Calc. for C\(_{18}\)H\(_{21}\)N\(_4\)IrS\(_2\) (549.71): C, 39.33; H, 3.85; N, 10.19. Found: C, 39.28; H, 3.83; N, 10.11.

5.2.3. Syntheses of \([\text{CpRu(L2)}(\text{PPh}_3)]\) (5)

A mixture of metal complex \([\text{CpRu(PPh}_3)_2\text{Cl}]\) (0.1 mmol), 2-mercaptopyrimidine (L2) (0.1 mmol) were dissolved in methanol (20 mL) in presence of KOH (0.1 mmol) and stirred at room temperature. During the reaction time, color of the solution changed to red and the mixture stirred for 6 h. The solvent was removed under reduced pressure, and the resultant solid was suspended in dichloromethane and filtered through celite to remove the formed KCl along with the remaining KOH. The solvent of the filtrate was reduced to 1 mL under reduced pressure and addition of diethyl ether gave the precipitate and subsequently the precipitate was washed with hexane to remove impurity such as triphenylphosphine oxide which was formed in the reaction. The resultant product was hygroscopic and it turned to sticky material at room temperature.

5.2.3.1. \([\text{CpRu(L2)}(\text{PPh}_3)]\) (5)

Yield: 42 mg (77%); IR (KBr, cm\(^{-1}\)): 1600(m), 1553(s), 1510(m), 1460(s), 1415(m), 1370(w), 1311(m), 1215(m), 1172(m), 1009(w), 770(m), 701(w), 630(m), 555(s), 496(m); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 7.91 (d, \( J = 4.8 \) Hz, 1H), 7.67 (t, \( J = 7.3 \) Hz, 1H), 7.54 (d, \( J = 6.3 \) Hz, 6H), 7.41 – 7.37 (m, 3H), 7.28 – 7.21 (m, 6H), 7.11 (t, \( J = 7.3 \) Hz, 1H), 4.38 (s, 5H); \(^{13}\)C NMR (101 MHz, CDCl\(_3\)) \( \delta \) 185.64, 159.73, 153.06, 135.76, 135.36, 133.50, 133.38, 132.14, 132.04, 131.98, 131.95, 129.07, 129.05, 128.58, 128.46, 127.66, 127.57, 127.31, 112.12; Anal. Calc. for C\(_{27}\)H\(_{23}\)N\(_2\)PRuS (539.57): C, 60.1; H, 4.3; N, 5.19. Found: C, 60.02; H, 4.26; N, 5.15.

5.3. Results and discussion

5.3.1. Synthesis of the complexes

The reaction of \([\text{Cp*MCI}_2]\) with 2-aminopyrimidine (L1) leads to the formation of binuclear complexes \([\text{(Cp*MCI}_2)(\mu-L_1)]\) (Scheme-5.1) \{where, Rh (1), Ir (2), L1 acts as a bridging ligand\} but these dimers with 2-mercaptopyrimidine (L2) form mononuclear substituted complexes \([\text{Cp*M(L2)}_2]\) (Scheme-1). In similar condition, the reaction of \([\text{CpRu(PPh}_3)_2\text{Cl}]\) with 2-mercaptopyrimidine ligand (L2) leads to the formation of chelating mononuclear complexes as general formula \([\text{CpRu(PPh}_3)(\text{L2})]\) (5) (Scheme-5.2). The effect of the base \{triethylamine or potassium hydroxide\} has been found in complexes 3-5, which permits the reaction to 4-5 h whereas without base reactions have yielded the same product with overnight stirring.
Complexes 1-4 are isolated in good yield and purified by recrystallization method using acetone as a solvent. All these complexes are yellow-orange crystalline solids, resulting as non-hygroscopic, air-stable. They are highly soluble in acetone, acetonitrile and DMSO but they are insoluble in nonpolar solvents and diethyl ether. All these complexes are fully characterised by spectral as well as a single crystal by X-ray analysis.

Scheme 5.1. Preparation of metal complexes 1 to 4.

Scheme 5.2. Preparation of Cp ruthenium complex 5.

5.3.2. Spectral studies of the complexes

The IR spectra of all complexes show sharp bands in range 1600 and 1450 cm\(^{-1}\), which are corresponding to the stretching frequencies of C═N and C═C bonds, respectively. The complexes 3-5 display sharp band around 625 cm\(^{-1}\) is assigned for C─S stretching mode. Upon coordination, the C═N bond stretching frequency of metal complexes shifts to higher values due to ligand coordination to the metal.

\(^{1}\)H NMR spectrum of free ligand L1 exhibited two-resonance signals for protons of the pyrimidine ring at 6.62(d) and 6.53(t) ppm and one broad signal for amine protons of the pyrimidine ring at 4 ppm. The ligand L2 displays doublet and triplet at 8.73 and 7.3 ppm and a singlet at 6.7 ppm for pyrimidine ring proton respectively. Upon coordination with the metal atom, complexes 1 and 2 have indicated three signals in the range around 8.29 to 5.27 ppm similar to free ligand (Fig. 5.2 and 5.3) and complexes 3 and 4 have exhibited four signals in
the range around 8.57 to 6.64 ppm (Fig. 5.4 and 5.5) compared to the free ligand but the coordination of ligand towards the metal is different from \textbf{L1}. Out of the four signals, there are two doublets and two triplets with the absence of singlet. It indicates that the ligand \textbf{L2} bound the metal with the deprotonation as two different ligands are around the metal vicinity. While in the case of complex 5, in addition to 2-mercapto pyrimidine peaks there are triphenyl phosphine peaks in the aromatic region along with the Cp peak at 4.4 ppm (Fig. 5.6). All of these shifts and variations in the signals along with the precursor peaks are indicating the formation of metal coordinated complexes. For all these complexes, the signals of the coordinated pyrimidine protons are shifted to considerable downfield as compared to free ligands due to the consequence of their coordination to metal centers [34]. Besides these signals, complexes 1-4 exhibit a singlet at around 1.65 - 1.75 ppm for the protons of the Cp* ligand. In all these complexes, auxiliary ligand regions are slightly shifted to downfield region in comparison to the precursor materials. The $^{13}$C NMR of the complexes exhibited the expected signals, which correlate with the number of carbon atoms present in the complexes (Fig. 5.7-5.11).

![Fig. 5.2: $^1$H NMR spectrum of the complex 1](image)

Pyrimidine based ligands
Fig. 5.3: \(^1\)H NMR spectrum of the complex 2

Fig. 5.4: \(^1\)H NMR spectrum of the complex 3
Fig. 5.5: $^1$H NMR spectrum of the complex 4

Fig. 5.6: $^1$H NMR spectrum of the complex 5
Fig. 5.7: $^{13}$C NMR spectrum of the complex 1

Fig. 5.8: $^{13}$C NMR spectrum of the complex 2
Fig. 5.9: $^{13}$C NMR spectrum of the complex 3

Fig. 5.10: $^{13}$C NMR spectrum of the complex 4
Fig. 5.11: $^{13}$C NMR spectrum of the complex 5

UV-Visible spectra of the complexes were recorded in acetonitrile solutions at $10^{-5}$ M concentration in the range 200-600 nm. Electronic absorption spectra of the complexes 1-4 along with the ligands L1 and L2 are depicted in Fig. 5.12. All the complexes show the high intense absorption band at approximately 220-250 nm, which are tentatively assigned to n-$\pi^*/\pi-\pi^*$ transitions. On complexation, all these high intense bands are exhibiting hypochromicity (Fig. 5.12) [36, 37] compared to free ligands. On the other hand, low-intensity band or broadening of the band at the lower wavelength is observed around 420 nm, which may be due to the weakness of the bands at such low concentrations of the solutions (ca. $10^{-5}$ M) or because they are obscured by the high intense bands. It arises from the excitation of electrons from the metal t$_{2g}$ level to the empty molecular orbitals derived from the $\pi^*$ level of the ligands [38, 39]. The stability of these neutral complexes was investigated by the UV-Visible experiment. The results revealed that the complexes are stable in 10% DMSO of aqueous solutions at room temperature over a period of 48 h (Fig. 5.13).

Fig. 5.12: UV-Visible spectra of the complexes 1-4. Left pictogram represents the complexes 1 and 2 with a comparison of respective ligand L1 whereas right one representing the complexes 3 and 4 with a comparison of respective ligand L2 at a concentration of $10^{-5}$ M acetonitrile solutions.
**Fig. 5.13:** UV-Visible absorption spectra of the complexes 1-5 at a concentration of 20 μM in 10% DMSO of aqueous solutions at 0h and after 4h, 10h, 24h and 48h at room temperature.

### 5.3.3. Molecular structural studies

The molecular structure of mono and binuclear complexes was established by single-crystal X-ray structure analysis. The ORTEP diagram of the complexes including atom numbering has shown in Figs. 5.14-5.16. Crystallographic and structure refinement parameters for complexes have shown in Table 5.1. Complexes 1, 2 and 5 are crystallised in the orthorhombic with the space group Pccn (1, 2) and Pbcn (5), whereas complexes 3 and 4 are in monoclinic with the space group P121/n1. X-ray structure analysis of metal complexes has shown that metal atom coordinated by the Cp/Cp as a spectator ligand, a chloride/triphenylphosphine (PPh3) as one of the auxiliary ligand and the other auxiliary ligand either by bridging nitrogen (N) or by chelated nitrogen-sulfur derivative ligand. The ligand L1 coordinates to the metal through bridging mode as μ-N, N, where ligand plays a bridging role in between the two metal centers. Ligand L2 coordinates to the metal as bisubstituted where ligand possesses dual role around the metal vicinity as κ²-NS by chelation as well as κ¹-S by mono-dentate mode. The ligand atoms are found to bind metal through the chelating bidentate mode to generate a strained four-membered metallacycle. Unlikely, ligand (L1) did not exhibit this kind of binding though the conditions are same for the both ligands. From the single crystal analysis, we observed that 2-aminopyrimidine is generating hydrogen bonding network with one of the chloride ion from each metal and one of the hydrogen atoms from the amino group of the ligand (Fig. 5.17) to form stable dimeric complexes. Whereas, in complexes derived from...
2-mercaptopyrimidine ligand such kind of hydrogen bonding is not found due to the absence of hydrogen on sulfur atom. 2-Mercaptopyrimidine ligand with rhodium and iridium precursors resulted in four-membered metallacyclic complexes by chelation. A similar role has been found in the case of ruthenium metal also, where we could isolate complex 5 with the L2 only and in the case of L1 we could not isolate the complex due to lack of hydrogen bonding network similar to complex 1 and 2.

Table 5.1: Crystallographic and structure refinement parameters for complexes 1-5.

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<td>24100/0/160</td>
<td>2850/0/157</td>
<td>3900/0/231</td>
<td>3860/0/231</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>0.870</td>
<td>1.072</td>
<td>1.070</td>
<td>1.064</td>
</tr>
<tr>
<td>Final R indices (I&gt;2σ(I))</td>
<td>0.0264, wR₂ = 0.0417, wR₂ = 0.0240, wR₂ = 0.0391, wR₂ = 0.0390, wR₂ = 0.0628</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R indices (all data)</td>
<td>0.0338, wR₂ = 0.0510, wR₂ = 0.0294, wR₂ = 0.0540, wR₂ = 0.0480, wR₂ = 0.0386</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max, Min Δρ/e (Å⁻³)</td>
<td>0.208, -0.395</td>
<td>2.028, -1.249</td>
<td>0.482, -0.446</td>
<td>0.891, -1.539</td>
</tr>
<tr>
<td>CCDC No.</td>
<td>1499296</td>
<td>1499297</td>
<td>1499298</td>
<td>1499299</td>
</tr>
</tbody>
</table>

Structures were refined on F₀²: wR₂ = [Σ [w(F₀² - F_c²)²]/Σw(F₀²)²]¹/², where w⁻¹ = [Σ(F₀²)² + (aP)² + bP] and P = [max(F₀², 0) + 2F_c²]/3.

Besides these, all the metal complexes have normal bond distances and bond angles but slight variation in bond distances and bond angles of 2-aminopyrimidine to the metal. Whereas, bond distances and strain bond angles of 2-mercaptopyrimidine to the metals are in almost comparison to the reported strained ruthenium complex [10]. The bite angle of metal complexes in 3-5 is around ~ 67° which is the strain angle thus, leads to a pseudo-octahedral arrangement of piano stool half sandwich complexes. The bond distance of metal to the nitrogen atom in all
the complexes is within the range, i.e., ~2.088(3) to 2.198(5) Å (Table-5.2). The complexes contain 2-aminopyrimidine are showing M-N and M-Cl bond lengths longer than the complexes containing 2-mercaptopyrimidine ligand which is identical to the earlier values reported in literature \([(\text{arene/Cp}^*)_2\text{M}_2(\mu-L1)\text{Cl}_3]^+\) where, \(L1 =\) pyridine-2-carbaldehyde nicotinoyl-hydrazone 2.152(6);[40] \([\text{Cp}^*\text{MLCl}]^+\) where, \(L =\) benzoyl(3-picolyl)thiourea/1,3-bis(3-picolyl)thiourea 2.335(2)-2.4027(11);[35]. The metal to chloride ion of complexes (1, 2) is 2.3983(19) to 2.434(2) Å which is the normal distance in the metal chloride complexes [35, 40, 41]. In complex 5, the ruthenium-sulfur bond is 2.45 Å, which is longer than that of the arene metal complexes [42-45].

**Table 5.2: Selected bond lengths and bond angles for complexes 1-5**

<table>
<thead>
<tr>
<th>Bond Lengths (Å)</th>
<th>Complex-1</th>
<th>Complex-2</th>
<th>Complex-3</th>
<th>Complex-4</th>
<th>Complex-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>M-N</td>
<td>2.198</td>
<td>2.198</td>
<td>2.180(5)</td>
<td>2.183</td>
<td>2.093(18)</td>
</tr>
<tr>
<td>M-S1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.4128(6)</td>
<td>2.462</td>
</tr>
<tr>
<td>M-S2/P</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.3677(6)</td>
<td>2.409</td>
</tr>
<tr>
<td>M-Cl1/Cl2</td>
<td>2.398/</td>
<td>2.433/</td>
<td>2.3983(19)/</td>
<td>2.485/</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2.434</td>
<td>2.481</td>
<td>2.428(2)</td>
<td>2.446</td>
<td>-</td>
</tr>
<tr>
<td>M-Cp*/Cp*</td>
<td>1.768</td>
<td>1.851</td>
<td>1.772</td>
<td>1.825</td>
<td>1.799</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bond Angles (°)</th>
<th>Complex-1</th>
<th>Complex-2</th>
<th>Complex-3</th>
<th>Complex-4</th>
<th>Complex-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-M-Cl1</td>
<td>86.32</td>
<td>87.76</td>
<td>85.23(17)</td>
<td>86.41</td>
<td>-</td>
</tr>
<tr>
<td>N-M-Cl2</td>
<td>92.62</td>
<td>90.36</td>
<td>91.32(17)</td>
<td>88.48</td>
<td>-</td>
</tr>
<tr>
<td>S1-M-S2/P</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>88.28(2)</td>
</tr>
<tr>
<td>S1-M-N1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>67.46(6)</td>
</tr>
<tr>
<td>S2/P-M-N1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>91.13(5)</td>
</tr>
</tbody>
</table>

M is rhodium in complex 1 and 3, Iridium in complex 2 and 4 and Ruthenium in complex 5, \(^a\) Calculated centroid to metal distances (\(\eta^5\)-C\(_5\) coordinated aromatic ring).
Fig. 5.14: ORTEP diagram of complexes \([\text{Cp}^*\text{RhCl}_2(\mu-L_1)]^2 (1)\) and \([\text{Cp}^*\text{IrCl}_2(\mu-L_1)]^2 (2)\), with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

Fig. 5.15: ORTEP diagram of complexes \([\text{Cp}^*\text{Rh}(L_2)_2] (3)\) and \([\text{Cp}^*\text{Ir}(L_2)_2] (4)\), with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.
**Fig. 5.16:** ORTEP diagram of complex [CpRu(L2)(PPh3)] (5) with 50% probability thermal ellipsoids. Hydrogen atoms are omitted for clarity.

**Fig. 5.17:** Hydrogen bonding network of complex 1 expressing the soft interactions as N-H…Cl and C-H…Cl (Green-Gray colored dashed bond) along b axis.

### 5.3.4. Geometry optimisation, charge distribution and electronic spectrum analysis

The input files of the complexes were prepared from the crystallographic coordinates obtained from X-ray measurements. The important bond lengths and bond angles of these complexes are tabulated in Table-5.2. The calculated bond lengths and bond angles are in good agreement with the experimental crystal X-ray data measurements.

The d-orbital occupancy of rhodium (Rh) metal atoms in complexes 1 and 3 are 8.10 (4d) and 8.23 (4d) whereas for iridium (Ir) metal atoms in complexes 2 and 4 are 7.89 (5d) and 8.01 (5d), respectively. On complexation, the d orbital occupancy increases in the respective metal atoms compared to their free states where d orbital occupancy is 6.00 (4d) and 6.00 (5d), respectively. The increase in the d orbital occupancy is attributed to the transfer of negative charge from chloride and Cp* ligands to the respective metal atoms in complexes 1 and 2, whereas in complexes 3 and 4 it is from L2 and Cp* ligands.

HOMO-LUMO excitation is the lowest possible electronic transition occurring in a molecule. The HOMO-LUMO energy difference governs the kinetic stability, chemical reactivity and the colour of the transition metal complexes in solution. The molecular orbital diagrams of the complexes along with their HOMO (H), LUMO (L) energies and energy gaps are shown in Fig. 5.18. The HOMO-LUMO energy differences for complexes 1-4 are 3.54, 3.77, 3.36 and 3.76 eV respectively. The calculated absorption bands are in good agreement
with the experimental absorption bands and their corresponding electronic transitions could be predicted from Table-5.3. Mulliken population analysis is used for calculating the percentage contributions of various groups to each molecular orbital. The HOMO is located over Rh1 and Cl1 for complex 1, Ir1 and Cl1 for complex 2. In the case of complexes 3 and 4, the HOMO is located on L2 (mono-dentate ligand). LUMO is located over the Rh2, Cp* for complex 1, on L1 for complex 2, Rh, Cp* and L2 (chelated ligand) for complex 3 and L2 (chelated ligand) for complex 4, respectively. The experimental absorption bands observed for complexes 1-3 around the 408-444 nm respectively, correspond to MLCT/LLCT for complex 1 and 2, LMCT/LLCT/ILCT for complex 3. The dominant excitations are H-2→L+1 (13%) (for complex 1), H→L+1 (35%) (for complex 2), and H-2→L (56%) (for complex 3), respectively. Moreover, for complexes 2 and 3 another absorption band observed around 339 nm corresponds to MLCT/LLCT/ILCT and the dominant excitations leading to charge transfers are H-2→L+3 (18%) for complex 2 and H-5→L (54%) for complex 3, respectively.

![HOMO-LUMO energy gap](image)

**Fig. 5.18:** The HOMO-LUMO energy gap of the complexes 1-4 from left to right respectively, which are obtained from the DFT optimised structures and the band gaps are expressed in electron volts (eV).

Rhodium and iridium complexes also exhibit two other absorptions bands in the range of 282-294 nm and 224-250 nm. The absorption band around 282 nm corresponds to ILCT and the dominant excitations leading to this charge transfer is H-5→L+4 (67%) for complex 1 and H→L+5 (44%) for complex 3. The absorption band around 294 nm due to H→L+4 (32%) excitation for complex 2 and 287 nm due to H-5→L+1 (35%) excitation for complex 4 corresponds to MLCT/LLCT, respectively.
Table 5.3: Calculated major orbital excitation contributions (%), dominant excitation character, oscillator strength ($f$), energy difference (in eV), theoretical and experimental wavelengths ($\lambda$ in nm) obtained from electronic transitions analysis with TDDFT (B3LYP) method.

<table>
<thead>
<tr>
<th>C. No.</th>
<th>Major Orbital Excitations</th>
<th>Oscillator strengths</th>
<th>Dominant Excitation Character</th>
<th>Energy Gap (eV)</th>
<th>Calc. $\lambda$ (nm)</th>
<th>Expt. $\lambda$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H-2 $\rightarrow$ L+1 (13%)</td>
<td>0.0014</td>
<td>Rh2/C1/C2 $\rightarrow$ Cp2* (MLCT/LLCT)</td>
<td>3.87</td>
<td>414</td>
<td>408</td>
</tr>
<tr>
<td>1</td>
<td>H-5 $\rightarrow$ L+4 (67%)</td>
<td>0.0101</td>
<td>C1/C2 $\rightarrow$ L1 (ILCT)</td>
<td>4.63</td>
<td>284</td>
<td>282</td>
</tr>
<tr>
<td>1</td>
<td>H-17 $\rightarrow$ L (15%)</td>
<td>0.4479</td>
<td>Rh1 $\rightarrow$ Cp1*/L1 (MLCT)</td>
<td>5.97</td>
<td>234</td>
<td>228</td>
</tr>
<tr>
<td>2</td>
<td>H-2 $\rightarrow$ L+3 (18%)</td>
<td>0.0054</td>
<td>Ir1/C2/C1 $\rightarrow$ L1/Cp1* (MLCT/LLCT)</td>
<td>4.30</td>
<td>408</td>
<td>432</td>
</tr>
<tr>
<td>2</td>
<td>H-2 $\rightarrow$ L+3 (18%)</td>
<td>0.0246</td>
<td>Ir1/C2/C1 $\rightarrow$ L1/Cp1* (MLCT/LLCT)</td>
<td>4.59</td>
<td>333</td>
<td>339</td>
</tr>
<tr>
<td>2</td>
<td>H-2 $\rightarrow$ L+3 (18%)</td>
<td>0.0021</td>
<td>Ir1/C2/C1 $\rightarrow$ L1/Cp1* (MLCT/LLCT)</td>
<td>4.48</td>
<td>294</td>
<td>294</td>
</tr>
<tr>
<td>2</td>
<td>H-16 $\rightarrow$ L (47%)</td>
<td>0.0269</td>
<td>Ir2/Cp2 $\rightarrow$ L1 (MLCT/LLCT)</td>
<td>6.13</td>
<td>226</td>
<td>224</td>
</tr>
<tr>
<td>3</td>
<td>H-2 $\rightarrow$ L (56%)</td>
<td>0.0211</td>
<td>L2(M)/Cp $\rightarrow$ L2(M)/Cp2/C1 $\rightarrow$ L1 (MLCT/LLCT)</td>
<td>4.04</td>
<td>432</td>
<td>444</td>
</tr>
<tr>
<td>3</td>
<td>H-5 $\rightarrow$ L (54%)</td>
<td>0.0054</td>
<td>Rh/L2(C) $\rightarrow$ Cp2/L2(C) (MLCT/ILCT)</td>
<td>5.32</td>
<td>341</td>
<td>339</td>
</tr>
<tr>
<td>3</td>
<td>H-5 $\rightarrow$ L (54%)</td>
<td>0.3195</td>
<td>L2(M) $\rightarrow$ L2(M) (ILCT)</td>
<td>4.91</td>
<td>276</td>
<td>282</td>
</tr>
<tr>
<td>4</td>
<td>H-5 $\rightarrow$ L+1 (35%)</td>
<td>0.0065</td>
<td>Ir $\rightarrow$ L2(C)/Cp* (MLCT)</td>
<td>5.81</td>
<td>286</td>
<td>287</td>
</tr>
<tr>
<td>4</td>
<td>H-3 $\rightarrow$ L+2 (56%)</td>
<td>0.0852</td>
<td>L2(C) $\rightarrow$ L2(M)/L2(C) (LLCT/ILCT)</td>
<td>5.40</td>
<td>250</td>
<td>250</td>
</tr>
</tbody>
</table>

Where L2(M) indicates that the ligand L2 involved as mono-dentate binding and L2(C) indicates that the ligand L2 involved as chelation mode of bonding.

The absorption band around 228 nm due to H-17 $\rightarrow$ L (15%) excitation for complex 1 and 224 nm due to H-16 $\rightarrow$ L (47%) excitation for complex 2 corresponds to MLCT/LLCT, respectively whereas absorption bands at 245 and 250 nm for complexes 3 and 4 corresponds to LLCT/ILCT due to H-4 $\rightarrow$ L+5 (29%) and H-3 $\rightarrow$ L+2 (56%) excitations, respectively.

5.3.5. Antibacterial studies

The antibacterial potency of the synthesized complexes 1-4 has been explored by an agar well diffusion method towards the different gram-positive (S. aureus, B. thuringensis), and gram-negative (E. coli, P. aeruginosa) bacterial strains. Among the synthesized complexes, biological studies (antibacterial antiproliferative and DNA binding studies) were performed for complexes 1-4. Complex 5 was not amenable due to hygroscopic nature and such complex would not produce reliable assays. Among these strains, S. aureus, B. theringensis and P. aeruginosa are pathogenic strains. E. coli, a non-pathogenic strain was used as a reference to compare the activity of complexes against the pathogenic bacteria. The MIC of the compounds was found to be around $\sim 0.5$ mg mL$^{-1}$. The current antibacterial studies were performed using a standard 2X MIC value i.e., at 1 mg mL$^{-1}$. The histogram of the resultant zone of inhibition are presented in Fig. 5.19, clear zone of inhibition obtained by the complexes 1-4 on chosen
pathogens are depicted in Fig. 5.20 and the results are measured to the nearest millimeter, which is tabulated in Table 5.4.

![Graphical representation of zone of inhibition](image)

**Fig. 5.19:** Graphical representation of zone of inhibition in mm obtained by the standard gentamycin and complexes 1-4 against four bacterial strains *S. aureus* (Green Bar), *B. thuringiensis* (Orange Bar), *E. coli* (Dark cyan Bar) and *P. aeruginosa* (Wine Bar). “*” Represents no zone of inhibition was observed for the complexes against the *S. aureus* strain.

![Pictorial representation of zone of inhibition](image)

**Fig. 5.20:** Pictorial representation of zone of inhibition obtained by the complexes 1-4 against the four bacterial strains (a) *S. aureus*, (b) *B. thuringiensis*, (c) *E. coli* and (d) *P. aeruginosa*. Where clear area surrounded by the well indicates the effective inhibition produced by the corresponding complexes 1-4.

**Table 5.4:** Zone of inhibition values in mm of complexes 1-4 and standard gentamycin against four bacterial strains *S. aureus*, *B. thuringiensis*, *E. coli* and *P. aeruginosa*. All the values are within the ±1 range.

<table>
<thead>
<tr>
<th>Complex</th>
<th>S. aureus</th>
<th>B. thuringiensis</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>18</td>
<td>22</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>17</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>18</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>18</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>23</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>
From the zone of inhibition, it is clear that the complexes containing rhodium are found to be active on all chosen pathogens. The best antibacterial activity is observed against gram-negative \textit{E. coli} bacterium (~21 mm). Whereas in the case of iridium complexes, almost no antibacterial activity is observed on \textit{S. aureus} bacterium and medium to high antibacterial activity is found on other chosen bacteria \textit{B. thuringensis}, \textit{E. coli} and \textit{P. aeruginosa}. Such a differential behavior of antibacterial activity of these complexes upon changing the metal reflects the facts that, the size of the metal ion can also influence the antimicrobial behavior and further, it is clear that lower its size, better its activity. In general, metal complexes are profoundly known to be antibacterial against the gram-positive to that of the gram-negative bacteria \cite{46}. However, we observed that our complexes are active against both gram-positive and gram-negative bacteria, evidencing them as robust antibacterial agents.

Overall antibacterial activity index of the synthesised metal complexes is in the following order:

\textbf{Complex 1} > \textbf{Complex 3} > \textbf{Complex 4} > \textbf{Complex 2}

\subsection*{5.3.6. Antiproliferative Activity}

The cytotoxic activity of the complexes \textbf{1-4} and cisplatin have been evaluated on a selection of three human tumour cell lines \{HT-29 (human colorectal cancer), BE (human colorectal cancer) and MIA-Pa-Ca2 (human pancreatic cancer)\} and one non-cancer cell line \{ARPE-19 (human retinal epithelial cells)\} by the MTT assay. The IC\textsubscript{50} values are tabulated in Table\textsuperscript{5.5}, the median cytotoxic concentrations have been determined after 96 h of the complex incubation. All complexes are expressing less potent than cisplatin, particularly against the colorectal cancer lines where cisplatin has been found particularly potent (IC\textsubscript{50} values of 0.66 ± 0.33 and 0.25 ± 0.11 μM against BE and HT-29 respectively). A comparison of the relative response of non-cancer to tumour cell lines is presented in Fig. \textbf{5.21}. The selectivity index (SI) which is defined as the ratio of IC\textsubscript{50} values in ARPE19 cells divided by the IC\textsubscript{50} value in each cancer cell line demonstrates that complexes \textbf{3} and \textbf{4} are more effective against cancer compared to non-cancer cell lines with SI values ranging from 1.2 to 3.31. In comparison to cisplatin, SI values for colorectal lines treated with complexes \textbf{3} and \textbf{4} are significantly lower than cisplatin where SI values are 9.71 and 25.64 for BE and HT-29 respectively (Fig. \textbf{5.21a}). In contrast, SI values for MIA-Pa-Ca2 cells (Fig. \textbf{5.21b}) are comparable to cisplatin (for complexes \textbf{3} and \textbf{4}) but are almost higher than cisplatin (SI = 2.77) for complex \textbf{3} (SI = 2.31) and complex \textbf{4} (SI = 3.48).
**Table 5.5**: IC$_{50}$ values obtained by the complexes 1-4 and cisplatin by MTT assay against three human cancerous and one human normal cell lines after 96h complex exposure.

<table>
<thead>
<tr>
<th>Complex</th>
<th>BE (μM) ± Standard deviation</th>
<th>HT-29 (μM) ± Standard deviation</th>
<th>MIA-Pa-Ca2 (μM) ± Standard deviation</th>
<th>ARPE-19 (μM) ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>2</td>
<td>23.13 ± 3.83</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>3</td>
<td>11.91 ± 2.14</td>
<td>20.32 ± 2.80</td>
<td>17.07 ± 4.59</td>
<td>39.45 ± 4.88</td>
</tr>
<tr>
<td>4</td>
<td>36.29 ± 8.68</td>
<td>49.55 ± 2.97</td>
<td>17.12 ± 4.58</td>
<td>59.71 ± 1.75</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>0.66 ± 0.33</td>
<td>0.25 ± 0.11</td>
<td>2.10 ± 2.26</td>
<td>6.41 ± 0.95</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation from three independent experiments. IC$_{50}$ values > 100 indicate that the compound did not induce an IC$_{50}$ at the highest dose tested (100 μM).

**Fig. 5.21**: Selectivity index of complexes and cisplatin in colorectal cell lines (a) and the MIA-Pa-Ca2 pancreatic cell line (b). The selectivity index is defined as the IC$_{50}$ of ARPE19 cells divided by the IC$_{50}$ of each tumor cell line. SI is determined from the mean IC$_{50}$ values in table-5.5 and therefore error bars cannot be included in the figure.

The IC$_{50}$ values ranging from 12-60 μM in the chosen tumour and non-tumor cell lines. Complexes 3 and 4 are active towards the all chosen cell lines. Iridium complex 2 is active only on BE cell line and inactive on other chosen cell lines. Surprisingly, rhodium complex 1 is inactive on all chosen cell lines. The mode of bonding and variation of pyrimidine ligand resulted in the difference in activity of complexes 1-4. Among the complexes 3 or 4 of 2-mercaptopyrimidine, rhodium complex is exhibiting more potent IC$_{50}$ value towards the colorectal cancer cell lines. The best activity ~12 μM (not exceed cisplatin) has been noted on BE cell line for complex 3. In the case of pancreatic cancer cell line, such a metal effect has not been found; both the complexes 3 and 4 are showing almost equipotent (~17 μM) cytotoxicity.

5.3.7. **DNA interaction studies**

DNA is a crucial cellular target of many metallodrugs for the treatment of multiple pathological conditions [47, 48]. The nucleotide bases and/or phosphate backbone of the DNA...
double helix is the potential site of a target for these metallodrugs. Earlier studies have shown that half sandwich ruthenium, rhodium and iridium metal complexes exhibit antiproliferative property by interacting with the nucleobases and amino acids of biomolecules like DNA, RNA and protein [49-51].

In order to study the DNA binding properties of the synthesised complexes, we monitored the absorption features of the complexes 1-4 by titrating the CT-DNA (5 μM-20 μM) to a fixed concentration (20 μM) of the complex. From the UV-Visible absorption studies, it is clear that there is a noticeable change in the spectral features of the complexes on the gradual addition of CT-DNA but the observed spectral changes are not adequate to deduce the binding constant of the complexes (Fig. 5.22). In the case of complexes 1 (C1), 3 (C3) and 4 (C4) evidenced for a hypochromic shift, whereas complex 2 (C2) indicated a hyperchromic shift. Moreover, all the complexes resulted in a hypsochromic shift. Such changes in the intensity and the wavelength of the complex absorption properties suggest binding interactions with CT-DNA. However, as the observed hyper/hypo/hypsochromic shifts are considerably small, it is difficult to derive reliable binding constants and/or differentiating the mode of binding i.e, intercalation vs electrostatic/groove binding on the basis of these small spectral changes [47, 48, 52-56].

![Fig. 5.22](image)

**Fig. 5.22:** The absorption spectra at fixed concentration (20 μM) of the complexes 1-4 (Black plot) with increasing concentrations (5-20 μM) of CT-DNA (Tris-HCl buffer, pH 7.2) {Red (5 μM), Green (10 μM), Yellow (15 μM) and Blue (20 μM) plots}.

We then analysed the fluorescence properties of the compounds in order to probe the binding interactions through fluorescence spectroscopy. We observed that among the complexes, Complex 1 and 2 are fluorescent. It is evident from Fig. 5.23 that addition of CT-DNA to these complexes resulted in fluorescence quenching of the compound fluorescence thus
justifying the physical interaction of these compounds with CT-DNA. The quenching of the fluorescence can be attributed to the change in the hydrophobic nature and/or alternation of their excited states [57].

![Fig. 5.23](image)

**Fig. 5.23:** The emission spectra of apo (Red) and CT-DNA bound (green) at fixed concentrations (20 μM, complex, 50 μM, CT-DNA) of the complexes 1, 2 (C1, C2) respectively.

As we established a binding interaction between the complexes 1-4 with DNA, we tried to establish the nature of the interaction (such as intercalation, groove binding/electrostatic interactions) between the complexes and CT-DNA. We performed the EtBr based competitive quenching experiments with CT-DNA in the presence of complexes 1-4 (Fig. 5.24).

![Fig. 5.24](image)

**Fig. 5.24:** The emission spectra of the DNA-EB system ($\lambda_{exc} = 522$ nm, $\lambda_{em} = 530-750$ nm), in the presence of complexes 1-4. (Red DNA + EtBr, Green DNA + EtBr + complex) [DNA] = 50 μM, [Complex] = 25 μM, [EB] = 5 μM.

EtBr is a well-known planar non-fluorescent organic molecule and a good intercalator of the DNA molecule, this intercalation of EtBr with DNA makes EtBr fluorescent upon complex formation. In a competitive binding experiment, if the other molecule also binds to
DNA via intercalation, EtBr will be released from DNA and the fluorescence intensity of EtBr will be greatly quenched [58-60]. In our experiments, we did not observe such a noticeable effect of EtBr fluorescence quenching upon addition of 5 times excess of complexes 1-4 to the EtBr-DNA complex. The EtBr quenching experiments taken together with the UV-Vis properties suggested that the complexes are not strong intercalators of DNA, thus leaving the possibility that either they are partial intercalators or bind to DNA through electrostatic interactions.

5.4. Conclusions

In this work, we have observed the effects of pyrimidine ligands containing d⁶ {(rhodium(III), iridium(III) and ruthenium(II))} metal complexes on structural studies and their biological activities. We successfully synthesized and characterized the strained four-membered metallacycle complexes of rhodium(III), iridium(III) and ruthenium(II) with 2-mercaptopyrimidine ligand. However, the reaction of Cp ruthenium complex with 2-aminopyrimidine remained unsuccessful. The synthesized complexes 1-4 interacted with the CT-DNA via partial intercalation or through electrostatic interactions. Antibacterial activity measurements suggested that the rhodium(III) metal complexes (C-1 and C-3) are more active than the iridium(III) complexes (C-2 and C-4) and best activity has been noted against E. coli bacterium among the chosen bacterial strains. The antiproliferative activity of the complexes strongly depends on the type of pyrimidine and the nature of binding mode to the metal. Rhodium complex (C-3) has exhibited moderate antibacterial activity on all the chosen strains as well as low micro molar dose dependent anticancer activity on the chosen cancer cells. The chelation effect has enhanced the cytotoxic activity of complexes, a common phenomenon in metal complexes.

5.5. References


