Probing the antibacterial and anticancer potential of tryptamine based mixed ligand Schiff base Ruthenium(III) complexes

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A B S T R A C T

Development of new chemotherapeutic agents to treat microbial infections and recurrent cancers is of pivotal importance. Metal based drugs particularly ruthenium complexes have the uniqueness and desired properties that make them suitable candidates for the search of potential chemotherapeutic agents. In this study, two mixed ligand Ru(III) complexes [Ru(Cl)\textsubscript{2}(SB)(Phen\textsuperscript{RC-1})] and [Ru(Cl)\textsubscript{2}(SB)(Bipy\textsuperscript{RC-2})] were synthesised and characterized by elemental analysis, IR, UV–Vis, \textsuperscript{1}H, \textsuperscript{13}C NMR spectroscopic techniques and their molecular structure was confirmed by X-ray crystallography. Antibacterial activity evaluation against two Gram-positive strains (S. pneumonia and E. faecalis) and four Gram-negative strains (P. aurogenosa, K. pneumoniae, S. enterica, and E. coli) revealed their moderate antibacterial activity with MIC value of ≥250 μg/mL. Anticancer activity evaluation against a non-small lung cancer cell line (H1299) revealed the tremendous anticancer activity of these complexes which was further validated by DNA binding and docking results. DNA binding profile of the complexes studied by UV–Visible and fluorescence spectroscopy showed an intercalative binding mode with CT-DNA and an intrinsic binding constant in the range of 3.481–1.015×10\textsuperscript{5} M\textsuperscript{−1}. Both the complexes were also found to exert weak toxicity to human erythrocytes by haemolytic assay compared to cisplatin. Potential of these complexes as anticancer agents will be further delineated by \textit{in vivo} studies.

1. Introduction

The increasing incidences of treatment failures due to multiresistance in both microbial infections and cancers necessitate the development of new chemotherapeutic agents and strategies [1–3]. Development of new chemotherapeutic agents based on ruthenium metal has received fascinating interest due to their ability to strongly bind nucleic acids and proteins, ligand exchange kinetics similar to those of their platinum counterparts, the prevalence of two main oxidation states (II and III) and the iron mimicking property when bound to biological molecules [4]. Several Ru(II) ion containing Schiff base complexes of the type [Ru(L)(EPh\textsubscript{3})\textsubscript{2} X\textsubscript{2}] (where L is a bidentate Schiff base ligand and E = P or As; X = Cl or Br) have been shown to inhibit the growth of resistant E. coli (2231) and \textit{Staphylococcus aureus} [5]. Other ruthenium complexes including [ImH][trans-Ru(DMSO)(Im)CL\textsubscript{2}] (NAMIA, Im = imidazole), [IndH][trans-Ru(Ind\textsubscript{2})CL\textsubscript{2}] (KPI1019, Ind = indazole), [(C\textsubscript{6}H\textsubscript{5}Ph) Ru(en)Cl\textsubscript{2}]Pf\textsubscript{2})(RM175, en = ethylenediamine) and [(pi

Pr\textsubscript{C}H\textsubscript{3}Me)Ru(pta)Cl\textsubscript{2}](RAPTA-C, pta = 1,3,5-triaza-7- phosphatricycl[3.3.1.1][3.3.3]decane (Fig. 1) have shown great therapeutic promises with NKP-1339, [6] the first ruthenium-based anticancer drug on the edge to clinical application and more ruthenium complexes waiting in the pipeline [7,8].

Use of labile ligands and auxiliary ligands like 1,10-Phenethroline, 2,2-bipyridyl, imidazole, and pyridine have been demonstrated to show improved biological profile with excellent antibacterial, antitumor, antiviral and antifungal activities already reported [9–13].

Keeping in view the imperative influence of Schiff bases in medicinal chemistry [14], our main rationale of this study was to explore whether the incorporation of a Ruthenium ion, a Schiff base derived from tryptamine and an auxiliary ligand (1,10-phenolhine or 2,2-bipyridyl) would deliver a synergistic effect and thus act as a hopeful stratagem for development of effective ruthenium-based therapeutic agents. Finding rare instances of literature about the tryptamine-based Schiff base ligands and their mixed ligand complexes, we herein report

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The formation of Schiff base (SB) was confirmed by 1H NMR spectra taken in DMSO. Peaks that are in agreement with the calculated structure were assigned to azomethine nitrogen with the metal ion. Another band at 1311 cm\(^{-1}\) attributed to the phenolic C–O stretching vibrations of the Schiff base, displayed a slightly higher frequency shift in the spectra of complexes confirming the involvement of phenolate oxygen in the C–O–M bond formation. Several bands in the low frequency region have been assigned to (M–N) and (M–O) bonds respectively.

The electronic spectra furnish quick and reliable information about the arrangement of ligands around the metal atom distinguishing it as a square-planar, octahedral or a tetrahedral geometry. The absorption spectra of SB and its complexes (RC-1 and RC-2) were recorded in DMSO in the range 800–200 nm respectively (Fig. 2). In the spectra of free SB ligand two very sharp bands at 375 and 275 nm regions ascribed to n–π* electronic transitions for the aromatic ring electrons and π–π* transitions for the azomethine, iminic bond, C5 and the intermolecular hydrogen bonded PhOH proton appears as a singlet at 8.5 ppm while as the carbon of azomethinic (–CH=N) group appears in the range 158–160 ppm. Thus, NMR studies back the inferences drawn from other spectroscopic techniques about the formation of the SB.

The important IR spectral data of the ligand and the ruthenium(III) complexes were compared to confirm the binding mode of the ligand to ruthenium ion in the complexes. The IR spectra of the free ligand (SB) showed high intensity bands at 3396 cm\(^{-1}\) (O–H/ν(NH)), 1622 cm\(^{-1}\) (–C=O azomethine), 1311 cm\(^{-1}\) (Ph–CO), and 736 cm\(^{-1}\) (C=O) (Figs. S6–S8, SI). The peaks at 1622 cm\(^{-1}\) attributed to azomethine group (–C=O) of the SB experienced a frequency shift (1618–1598 cm\(^{-1}\)) on complexation, confirming the coordination of azomethine nitrogen with the metal ion. Another band at 1311 cm\(^{-1}\) attributed to the phenolic C–O stretching vibrations of the Schiff base, displayed a slightly higher frequency shift in the complexes confirming the involvement of phenolate oxygen in the C–O–M bond formation. Several bands in the low frequency region have been assigned to (M–N) and (M–O) bonds respectively.

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the tryptamine is nearer to N—C and C=─C bonds lengths, respectively. The unit-cell packing diagram of SB displayed hydrogen bonding interaction between hydroxylhydrogen atom and the nitrogen of azomethinic group (Fig. S9, SI). The single crystal X-ray diffraction study on this Schiff base (SB) displays an imine-ol form. The structural data of SB ligand was employed to get the energy optimised structures of the complexes RC-1 and RC-2 on using quantum calculations with B3LYP/LANL2DZ level of theory for all atoms (Fig. 3b, c, Table S1, Fig. 4) [23]. The frontier orbitals on the atoms were plotted from the electronic charge density distribution. The HOMO for the complexes RC-1 and RC-2 are found to be confined on the ruthenium-Schiff base moiety, while the LUMO is mainly confined on the phenanthroline/bipyridyl core as shown in Fig. 4.

2.3. Biological studies

2.3.1. In vitro antibacterial susceptibility assay

The synthesized compounds (SB, RC-1 and RC-2) were assessed for their antibacterial efficacy against two Gram-positive strains (S. pneumoniae, S. enterica and E. coli) with Ciprofloxacin (CIPRO) as a reference drug. The results have been presented as minimum inhibitory concentration (MIC) in µg/mL and summarized in Table 1. Although none of the compounds was found active against S. enterica and K. pneumoniae, compounds RC-1 and RC-2 showed moderate activity against P. aerogenosa, S. pneumoniae, E. faecalis and E. coli with MIC value of 250 µg/mL. However, SB did not demonstrate any appreciable activity against these bacterial strains.

2.3.2. Growth curve studies

To investigate the effect of the moderately active compounds RC-1 and RC-2 on the growth of E. coli cells, growth kinetics studies were performed. The E. coli cells were exposed to different concentrations of the test compounds (2MIC, MIC and MIC/2). Cells treated with CIPRO acted as positive control while as untreated cells were kept as a negative control.

The E. coli cells did not show any significant growth when treated with 2MIC and MIC concentrations of the compound RC-1 with a continuous lag phase of 12 h and 10 h respectively. While in case of compound RC-2, E. coli cells did not show any significant growth after

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**Fig. 2.** UV–visible spectra of SB (a) ruthenium (III) complexes RC-1 (black) and RC-2 (red) (b) in DMSO. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 3.** ORTEP views of the ligand SB (a) showing thermal ellipsoids at 50% probability level. The energy minimized structures of complexes RC-1 (b) and RC-2 (c) employing B3LYP/LANL2DZ level of theory. Here colour codes are: Ru, red; C, black Cl, Purple; O, blue; N, green and H white color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
exposure to 2MIC and MIC concentrations and showed a continuous lag phase of 10 h and 8 h respectively. Sudden growth was observed in presence of RC-1 after 12 h and 10 h and for RC-2 after 12 h and 9 h in case of 2MIC and MIC respectively. However, at sub-MIC concentrations of both the compounds (RC-1 and RC-2), growth was observed after 10 h and 9 h respectively for standard E. coli strain. The results clearly indicated that compounds RC-1 proved to be better inhibitor of E. coli cell growth than RC-2 (Fig. 5).

Table 1
In vitro antibacterial activity of SB and its complexes (RC-1 and RC-2) against some bacterial strains.

<table>
<thead>
<tr>
<th>Compound</th>
<th>P. aeruginosa</th>
<th>S. enterica</th>
<th>S. pneumoniae</th>
<th>E. faecalis</th>
<th>K. pneumoniae</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC-1</td>
<td>250</td>
<td>1000</td>
<td>250</td>
<td>250</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>RC-2</td>
<td>250</td>
<td>1000</td>
<td>&gt;1000</td>
<td>250</td>
<td>1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>SB</td>
<td>1000</td>
<td>1000</td>
<td>&lt; 7.5</td>
<td>1000</td>
<td>1000</td>
<td>&lt; 7.5</td>
</tr>
<tr>
<td>CIPRO</td>
<td>&lt; 7.5</td>
<td>&lt; 7.5</td>
<td>&lt; 7.5</td>
<td>&lt; 7.5</td>
<td>&lt; 7.5</td>
<td>&lt; 7.5</td>
</tr>
</tbody>
</table>

Table 2
In vitro cytotoxicity and haemolytic activities of SB, RC-1 and RC-2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC_{50} (μg/mL)</th>
<th>HD_{50} (μg/mL)</th>
<th>Safety index (SI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1299 Cell line</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td>100 ± 5.0</td>
<td>ND</td>
<td>NC</td>
</tr>
<tr>
<td>RC-1</td>
<td>10–12.5 ± 0.5</td>
<td>&gt; 100</td>
<td>&gt; 800</td>
</tr>
<tr>
<td>RC-2</td>
<td>15–20 ± 0.5</td>
<td>&gt; 100</td>
<td>&gt; 665</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>6.0–7.5 ± 1.1</td>
<td>≥ 50</td>
<td>&gt; 665</td>
</tr>
</tbody>
</table>

ND: Not done. 
NC: Not calculated. 

Concentration inhibiting fifty percent of cell growth for 24 h exposure period of tested samples. Data represent mean values ± standard deviation for three independent experiments.

HD_{50} is the concentration inducing fifty percent of erythrocytes haemolysis.

exposure to 2MIC and MIC concentrations and showed a continuous lag phase of 10 h and 8 h respectively. Sudden growth was observed in presence of RC-1 after 12 h and 10 h and for RC-2 after 12 h and 9 h in case of 2MIC and MIC respectively. However, at sub-MIC concentrations of both the compounds (RC-1 and RC-2), growth was observed after 10 h and 9 h respectively for standard E. coli strain. The results clearly indicated that compounds RC-1 proved to be better inhibitor of E. coli cell growth than RC-2 (Fig. 5).

Fig. 5. (a and b) Dose dependent growth curve of E. coli in presence of RC-1 and RC-2.
2.3.3. Anticancer activity evaluation

2.3.3.1. Cell viability test. Since the complexes showed moderate to negligible antibacterial activity, we further evaluated the complexes for their anticancer activity. Ruthenium complexes are well known for their excellent anticancer activity and are considered to replace or augment platinum-based chemotherapy. The compounds (SB, RC-1 and RC-2) were therefore subjected to cell viability assay performed on H1299 cancer cells using various concentrations (3.12–400 µg/mL) as shown in Fig. 6 the ligand SB showed less cytotoxicity compared to the two ruthenium complexes RC-1 and RC-2.

A sub-confluent population of H1299 cells was treated with increasing concentration of these compounds and the number of viable cells was measured after 48 h by MTT cell viability assay. The concentration range of all the compounds was 3.12–400 µg/mL. The cell viability (%) obtained with continuous exposure for 48 h are depicted in Fig. 6. The cytotoxicity of all the tested compounds was found to be concentration-dependent. Fig. 6 depicts that all the compounds including the positive control showed viability ranging from 80 to 100% at the concentration of 3.13 µg/mL and with increase in concentration all the tested compounds displayed increasing cytotoxicity which was observed with a decrease in percentage viability. The IC$_{50}$ values obtained for the test compounds are given in Table 2 and the %viability against increasing concentration is given in Fig. 6. From the results it is clear that the complexes RC-1 and RC-2 show significant cytotoxicity with IC$_{50}$ value of 10–12.5 ± 0.5 and 15–20 ± 0.5 µg/mL, respectively compared to cisplatin with IC$_{50}$ value of 6.0–7.5 ± 1.1 µg/mL.

2.3.4. DNA binding studies

2.3.4.1. Electronic absorption study. DNA, a pharmacological target of several anticancer drugs, particularly cisplatin, has proved to be of premier importance in the successful development of active metal containing chemotherapeutic drugs [24]. Therefore, the possible binding modes of SB, RC-1 and RC-2 to CT-DNA were studied by UV–Visible spectroscopy. The electronic absorption spectra of SB, RC-1 and RC-2 in the presence and absence of CT-DNA at varying concentrations are given in (Fig. S10a–S10c, SI). The peaks at 275 nm and 380 nm were ascribed to intra ligand π–π$^*$ transition and ligand to metal charge transfer transitions (LMCTs). On increasing the CT-DNA concentration, the bands were slightly changed, displaying slight longer wavelength shifts accompanied with hypochromism, which suggested the interaction of the synthesized complexes (RC-1 and RC-2) with CT-DNA. A hypochromic shift with a slight bathochromic (redshift) was observed for SB and the complexes RC-1 and RC-2, respectively [25]. The range of the hypochromism is generally reliable with the strength of intercalative interaction [26].

2.3.4.2. Fluorescence study. Further support for the binding of SB, RC-1 and RC-2 with CT-DNA was obtained through the fluorescence quenching study. A mixture of the compounds (SB, RC-1 and RC-2) (50 µM) was subjected to competitive binding with increasing amount of CT-DNA. The fluorescence gets efficiently quenched upon binding to CT-DNA and fluorescence intensity decreases with increasing concentration of CT-DNA, which indicated binding interaction with CT-DNA. A result of emission titration for compounds SB, RC-1 and RC-2 with CT-DNA is shown in Fig. 7a. All stock solutions were made in double distilled water and the quenching data was analysed in accordance with the Stern–Volmer equation used for determining the fluorescent quenching mechanism:

$$\frac{I_0}{I} = 1 + K_{SV} [Q]$$

(1)

where $I_0$ and $I$ are fluorescence intensities in the presence and absence of the quencher, ($Q$ = CT-DNA) respectively. $K_{SV}$ is Stern-Volmer quenching constant. The plots of $I_0/I$ versus [Quencher] appear to be linear.

The calculated Stern-Volmer constant ($K_{SV}$) values give an implication about the extent of interaction of the compounds with CT-DNA. The corresponding $K_{SV}$ values obtained for the compounds (SB, RC-1, RC-2) were found to be $3.951 \times 10^8$, $1.229 \times 10^8$ and $7.642 \times 10^{10}$M$^{-1}$ respectively. These values indicated static quenching complex formation. The binding constant values ($K_b$) and number of binding sites ($n$) of compound (SB, RC-1 and RC-2) with CT-DNA were determined from the data of fluorescence titrations using following equation [27]

$$\log \frac{I_0 - I}{I} = \log K_b = n \log [Q]$$

(2)

where [Q] is the concentration of the quencher, $K_b$ is the binding constant and $n$ is the number of binding sites. The values of $K_b$ and $n$ are obtained from the intercept and slope by the linear plots of $\log(I_0 - I)/I$ against [DNA].
The binding constant values $K_b$ of the compounds were found to be $3.481 \times 10^5$, $1.229 \times 10^5$ and $1.015 \times 10^5 \text{M}^{-1}$ at $298 \text{K}$, respectively which indicates the interaction of compounds with CT-DNA.

### 2.3.5. Haemolytic assay

The two ruthenium complexes RC-1 and RC-2 displayed significant cytotoxicity against H1299 lung cancer cell line and showed moderate antibacterial activity. To exclude any possible toxicity, haemolytic activity of both the complexes human red blood cells (hRBCs) was determined and the HD$_{50}$ values are given in Table 2. The results clearly showed that the two complexes displayed weak haemolytic activity compared to the positive control. The two complexes had HD$_{50}$ value of $>100 \mu\text{g/mL}$ compared to $\geq 50 \mu\text{g/mL}$ for Cisplatin. These complexes have a safety index higher than the positive control as shown in Table 2.

### 2.3.6. Physicochemical properties

The importance of physicochemical properties in designing bioavailable drugs has been widely recognized and increased efforts have been spent on assessing the drug “developability” based on calculated and measured physicochemical parameters [28–32]. The $pKa$, solubility, and lipophilicity are among the most fundamental physicochemical properties of a drug candidate, and their measurements are essential for both in silico and in vitro evaluation of drug-like properties [28–36]. They are also the fundamental parameters for assessing ADMET properties of drug candidates, whose deficiencies account for 50–60% of compound failures during development [35]. Although poor physicochemical properties should not be the only reason to reject a promising lead compound with great in vitro receptor affinity and selectivity, the challenges and risks are much greater when the compound is developed at a later stage. A considerable number of studies have been conducted in this area and it has been accepted that the optimal range of lipophilicity for good drug candidates lies in a narrow range of logD between −1 and 3 [32] or $\leq 5$ (Lipinski’s Ro5) [33], and to achieve a solubility of $>100 \mu\text{M}$, a typical desirable value for drug molecules [34], it requires a logP value $< 3.25$. In general, a lower logP means increased compound solubility [35,36]. Good intestinal absorption reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area (PSA) or total hydrogen bond count (sum of donors, HBDs and acceptors, HBAs), are also important predictors of good oral bioavailability. Molecular properties such as membrane permeability and bioavailability are associated with logP, molecular weight (MW), or hydrogen bond acceptors and donors count in a molecule. The rule states that most molecules with good membrane permeability have logP $< 5$, molecular weight $< 500$, number of hydrogen bond acceptors $< 10$, and number of hydrogen bond donors $< 5$. This rule is widely used as a filter for drug-like properties. The ligand SB and the complexes RC-1 and RC-2 in this study obeyed the ‘Rule of Five’ with logP values $< 5$ and HBA $< 10$, hydrogen bond donor’s $< 5$, except that the molecular weight of the complexes was higher than 500 as shown in Table 3. The complexes RC-1 and RC-2 have desired drug likeness properties comparable to that of the cytotoxic drug cisplatin. Cytotoxicity of the complexes increases with an increase in lipophilicity and is consistent with the results obtained from MTT assay.

### 2.3.7. Molecular docking studies

Molecular docking study of the newly synthesized complexes and the ligand SB was performed to further validate the experimental results. 2D structure of the synthesized ligand molecules were converted to energy minimized 3D structures and used for docking studies. DNA duplex of sequence d (CGCGAATTCGCG)2 dodecamer was taken as the target receptor. The Lamarckian genetic algorithm, inculcated in the docking program AutoDock 4.2, was employed to satisfy the purpose. Crystal structure was obtained from Protein Data Bank (https://www.rcsb.org) with the PDB ID 1BNA. Molecular docking calculations were carried out with Auto dock vina [37]. The conformation with the lowest binding free energy was used for analysis. All molecular docked models were prepared using PyMOL viewer. Docked images are shown in Fig. 8. On analysing the docked structures, it is evident that the metal complexes fit well into the A-T rich region of target DNA. The Schiff base and the ruthenium complexes RC-1 and RC-2 are stabilized in the A-T rich region through various interactions, such as van der Waals and hydrogen bonding.

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Table 3

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.W</th>
<th>LogP</th>
<th>LogD</th>
<th>No. of HBA$^a$</th>
<th>No. of HBD$^a$</th>
<th>PSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>264.32</td>
<td>3.73</td>
<td>3.53</td>
<td>2</td>
<td>3</td>
<td>48.38</td>
</tr>
<tr>
<td>RC-1</td>
<td>615.50</td>
<td>1.34</td>
<td>−1.32</td>
<td>2</td>
<td>1</td>
<td>35.9</td>
</tr>
<tr>
<td>RC-2</td>
<td>591.47</td>
<td>2.31</td>
<td>−2.31</td>
<td>2</td>
<td>1</td>
<td>35.79</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>331.34</td>
<td>1.32</td>
<td>−1.13</td>
<td>6</td>
<td>2</td>
<td>72.88</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>300.05</td>
<td>−2.27</td>
<td>0.04</td>
<td>2</td>
<td>2</td>
<td>52.04</td>
</tr>
</tbody>
</table>

$^a$ Values adopted from referred sources.

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Fig. 8. Docked images of SB, RC-1 and RC-2 with 1BNA. Images were generated using PyMol.

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Fig. 7b. Plot of log [CTDNA] versus log (I$_0$-I)/I for the estimation of drug–DNA binding constant ($K_b$) for SB, RC-1 and RC-2.
hydrophobic interactions. Binding affinity values of the docked target compound were found to be in the range of $-12.6$ to $-8.1$ kcal mol$^{-1}$.

Since lung cancer is one of the most common and serious types of cancers, the only treatment includes surgical resection followed by chemo or radio-therapy. Non-small-cell lung cancer is the most common type, accounting for more than 80% of cases. Each year, more people die of lung cancer than of colon, breast, and prostate cancers combined. Due to recurrence and chemoresistance the disease often becomes lethal and is therefore one of the most prevalent cause of cancer mortality [38]. Therapies targeting tumor recurrence and resistance mechanisms are therefore needed to effectively treat lung cancer. Cisplatin, a platinum based metallogrug has been a drug of choice and is believed to kill cancer cells by binding to DNA and interfering with its repair mechanism, eventually leading to cell death [39]. A crystal structure of cisplatin bound to DNA, obtained from PDB entry 1A84 is shown in Fig. 9(a). The cisplatin-DNA complex attracts the attention of HMG (high mobility group)-1 and other DNA repair proteins which become irreversibly bound as shown in Fig. 9(b) obtained from PDB entry 1CKT. The resulting distortion to the shape of the DNA prevents effective repair [40].

Whilst the chemotherapeutic success of cisplatin is undeniable, it is by no means the perfect drug. It is not effective against many common types of cancer, drug resistance is common, and it has a deplorable range of side effects, which can include nerve damage, hair loss and nausea [41]. To overcome these limitations, some compounds based on ruthenium have been developed and tested against cancer cell lines. These compounds tend to cause fewer (and less severe) side effects compared to platinum drugs [42]. Ruthenium (Ru) complexes are reflected as potential alternatives for platinum compounds and have been proved as encouraging anticancer drugs with high efficacy and low side effects [42]. The mechanism of DNA binding has been probed and certain ruthenium complexes form cross-links between DNA strands - possibly favoured due to the steric restrictions imposed by the octahedral geometry of the complexes. This binding mechanism differs from the intrastrand cross-links favoured by cisplatin, and consequently the cancer cell lines that have developed resistance to cisplatin by accelerating the rate of repair of intrastrand cross-links is still susceptible to ruthenium anticancer drugs [43]. In this study we evaluated two new ruthenium complexes against a non-small lung cancer cell line and the results were encouraging. We further tried to validate the results by performing DNA binding and docking studies. DNA binding studies revealed that the complexes show intercalative binding modes with the DNA probably mimicking cisplatin in their modes of action. Inside a cell, cisplatin loses its two chloride ions, creating a reactive species that forms bonds with DNA bases. The chloride ions in cisplatin are particularly important in its action as a drug. They are relatively stable when the drug is outside the cell, where the chloride concentration is normally high. But when the drug gets inside the cell and the chloride concentration drops, cisplatin loses its two chlorides, and they are replaced by water molecules. These water molecules are loosely bound and fall off easily, allowing the platinum to attack other molecules, such as DNA [44]. Resistance is a significant problem with cisplatin treatment: cells use all the mechanisms at their disposal to fight poisoning by cisplatin. They reduce the amount of cisplatin reaching the nucleus, perhaps through the use of the mechanisms involved in the maintenance of copper levels. Once the platinum is bound to DNA, cells use their powerful nucleotide excision repair system to fix the problems. Since many ruthenium compounds are known to have high selectivity for binding to DNA. There are data suggesting that NAMI-A[ImH] [trans-RuCl4(DMSO)(Im)] (Im = imidazole, DMSO = dimethylsulfoxide, the first approved ruthenium complex in clinical trials is capable of binding to DNA and RNA. The electron-deficient metal atoms in these complexes might act as electron acceptors for electron-rich DNA nucleophiles by the hydrolysis of ligands. Furthermore, Ru(II) complexes can bind to DNA via interaction with aromatic ligands. There are two main categories of binding modes between DNA and Ru compounds: covalent and noncovalent binding. The covalent binding is irreversible and forms adducts consisting of DNA and Ru(II) complexes. The covalent mode of binding in Ru–DNA distorts the DNA backbone, which impairs DNA replication and transcription. The non-covalent binding of Ru(II) complexes is usually reversible and occurs as electrostatic binding, intercalation, and groove binding, amongst which intercalation has received the most attention. The docking studies revealed the intercalative binding modes of the two ruthenium metal complexes under study. Intercalation usually occurs when planar aromatic compounds are inserted between adjacent base pairs in the DNA double helix. It has also been found that some ruthenium complexes are active against cancers that are not responsive to platinum drugs. This feature suggests that ruthenium complexes exert their effect through a route different from that of platinum, and, importantly, their spectrum of action is expected to be broader [42].

3. Conclusion

Ruthenium complexes have received growing interest mainly due to their excellent cytotoxicity and target specificity and efficacy were platinum-based drugs have failed. Schiff base ligands have been attractively employed to fine tune the biological properties of complexes. Enthused by the amazing potential of ruthenium complexes, we synthesized a simple tryptamine based ligand SB and its mononuclear octahedral ruthenium(III) complexes (RC-1 and RC-2). The structures were validated by different spectroscopic techniques and X-ray crystallographic analysis. Antibacterial activity evaluation against a panel of Gram-positive (S. pneumoniae and E. faecalis) and Gram-negative strains (P. aeruginosa, S. enterica, K. pneumoniae and E. coli) showed their moderate antibacterial potential. Because of inherent anticancer potential of ruthenium complexes, we evaluated the complexes and the ligand for their potential anticancer activity and validated the results by performing DNA binding and docking studies. The binding strength of the SB, RC-1 and RC-2 with CT-DNA calculated with fluorescence spectroscopic titration has shown $K_b = 3.481 \times 10^5$, $1.229 \times 10^5$ and $1.015 \times 10^5$ M$^{-1}$ which indicate an intercalation mode of binding.

Fig. 9. (a) Cisplatin bound to DNA; (b) cisplatin bound to DNA, along with High Mobility Group 1 Protein.
which was further validated by docking studies. The drug-likeness properties of the complexes were also studied, which strongly supported the results. Overall the complexes show a strong anticancer potential which can be further delineated by screening these complexes using other cell lines and further in vivo studies. We are currently working on the project and the results will be reported in due course of time.

4. Experimental

4.1. Materials and instrumentation

All chemicals were used without further purification throughout the course of experimental work. 2-Hydroxybenzaldehyde, Tryptamine and RuCl₃·nH₂O were bought from Sigma Aldrich. CT-DNA was obtained operating in the range 4000–400 cm⁻¹. CHN analyses were carried out on a Thermo Multiskan GOMicroplate Spectrophotometer [46].

4.2. Methods

4.2.1. Synthesis of tryptamine based ligand (SB)

The Schiff base ligand, (E)-2-(((2-(1H-indol-3-yl)ethyl)imino)methyl)phenol (SB) resulted from the condensation of equimolar salicylaldehyde and tryptamine in around 20 mL of methanol at room temperature for 2 h. The reaction mixture was kept overnight at room temperature which resulted in the formation of a solid product which was filtered, washed with cold ethanol and then dried in a desiccator over CaCl₂.

4.2.2. Synthesis of [Ru(Cl)₂(SB)(Phen)] (RC-1)

To a 1 mmol solution of Schiff base (SB) in methanol was added triethylamine (1 mmol), there action mixture was stirred for few minutes. A solution of ruthenium chloride hydrate (1 mmol) was added to the deprotonated Schiff base solution. After stirring the solution for half an hour another solution of 1,10-Phenanthroline (Phen) (1 mmol) in the same solvent was added dropwise. Refluxing the reaction mixture while stirring for about 6 h resulted in the precipitation of a solid mass. The solid mass obtained was filtered, washed with cold ethanol and diethyl ether several times to remove any unreacted compounds. Finally, the residue was collected and placed in a desiccator over CaCl₂. Yield 54%; Color: Black; M.P: 229°C. Anal. Cal. for C₂₉H₂₃N₄Cl₂ORu: C, 56.59; H, 3.77; N, 9.10. Found: C, 56.53, H, 3.72; N, 9.07. ESI-Mass (m/z): Calc.: 615.46: Found: 615.74 as [M + H]⁺ (Fig. S5, SI).

4.2.3. Synthesis of [Ru(Cl)₂(SB)(Bipy)] (RC-2)

The complex (RC-2) was prepared by following a method adopted for RC-1 except 2,2-Bipyridyl was used in place of 1,10-Phenanthroline as an ancillary ligand. The solid product obtained was filtered, washed with cold methanol and diethyl ether several times to remove any unreacted compounds. Finally, the residue was collected and placed in a desiccator over CaCl₂. Yield 54%; Color: Black; M.P: 226°C. Anal. Cal. for C₂₉H₂₃N₄Cl₂ORu: C, 56.59; H, 3.77; N, 9.10. Found: C, 56.53, H, 3.72; N, 9.07. ESI-Mass (m/z): Calc.: 591.47: Found: 592.23 as [M + H]+ (Fig. S3, SI).

4.3. Crystal structure determination

A single crystal of the Schiff base (SB) was obtained by the slow evaporation of a methanolic solution of the compound. The X-ray analysis of the Schiff base ligand was done on a Bruker SMART APEX II CCD diffractometer at 273 K and diffraction data was collected using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å) by u and v scans. X-ray data reduction structure solution and refinement were performed using the SHELXL-2013/1 package [45]. The crystal structure was resolved by direct methods and the structural information of the compound (SB) has been deposited at the Cambridge Crystallographic Data Center (CCDC 1867350).

4.4. In vitro antibacterial activity

All the compounds, (SB, RC-1 and RC-2) were screened for their in vitro antibacterial action against two Gram-positive bacterial strains (S. pneumoniae, E. faecalis) and four Gram-negative bacterial strains (P. aerogenosa, S. enterica, K. pneumoniae, E. coli) using broth dilution technique in accordance with the standard antibacterial assessment protocol NCCLS. Ciprofloxacin was selected as a positive control for antibacterial study. The compounds were dissolved in DMSO and serially diluted in broth medium to achieve the final concentration of DMSO less than 4%. Varying concentrations (7.8–1000 µg/mL) of compounds were dispensed into a 96-well plate in Nutrient broth medium in a final volume of 100 µL. Then, 100 µL of bacterial cells (approximate 2.5×10⁵ cells/mL) were dispensed into the 96-well plate (Taron) and incubated at 37°C for 24 h. After incubation period each well was analyzed for the presence or absence of visual growth of bacterial cells. The lowest concentration of the test compound at which no visible growth occurs represents its MIC value. Moreover, after incubation, the growth was measured turbidimetrically at 600 nm using a Thermo MultiskanGOMicroplate Spectrophotometer [46].

4.5. Growth curve studies

The Bacterial cells were freshly revived by subculture on the nutrient broth (NB) agar plate. A loop full of inoculum was introduced into the NB broth and cells were grown for 16 h at 37°C before use. Approximately 2×10⁵ cells/mL were then inoculated into the freshly prepared 50 mL sterile NB medium. Different concentrations, equivalent to 2MIC, MIC, MIC/2, of compounds were added separately into the conical flasks containing inoculated medium and incubated at 37°C and 160 rpm. Strain specific concentration of CIPRO was used as positive control viz. 7.5 µg/mL for bacterial strains. At predetermined time periods (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 after incubation with agitation at 37°C), 1 mL aliquot to each sample was removed from the conical flask and growth was measured turbidimetrically at 600 nm using spectrophotometer. Optical density was recorded for each concentration against time (h) [47].

4.6. DNA binding assay

Absorption spectral titrations were performed in aqueous solutions at room temperature to study the binding affinity of CT-DNA with SB,
RC-1 and RC-2. The concentration of the SB, RC-1 and RC-2 were kept constant (50 µM) while the concentration of CT-DNA was gradually increased. Firstly, λmax and absorbance of the compounds (SB, RC-1 and RC-2) were kept constant (50µM) while the concentration of CT-DNA was gradually changed. The concentration of the SB, RC-1 and RC-2 were varied in the range of 1000–7.8 µg/mL of test compounds in microplates (150µL/well) in triplicates which were allowed to attach and grow for 24 h as reported previously [48]. After attachment the cells were treated with varying concentrations of the test compounds ranging from 3.12 to 400 µg/mL. After 48 h of treatment the medium was removed, and cells were incubated with MTT (0.5 mg/mL) in fresh medium for 4 h at 37°C. Formazan crystals formed by mitochondrial reduction of MTT, were solubilized in DMSO (150 µL/well) and absorbance was measured at 570 nm using a Mark Microplate Reader (Bio-Rad).

4.7. Cell viability assay

Cell viability assay was performed using MTT assay on H1299 lung cancer cell line. 1 × 10^5 cells/well were seeded into flat bottom 96-well plates (150 µL/well) in triplicates which were allowed to attach and grow for 24 h as reported previously [48]. After attachment the cells were treated with varying concentrations of the test compounds ranging from 3.12 to 400 µg/mL. After 48 h of treatment the medium was removed, and cells were incubated with MTT (0.5 mg/mL) in fresh medium for 4 h at 37°C. Formazan crystals formed by mitochondrial reduction of MTT, were solubilized in DMSO (150 µL/well) and absorbance was measured at 570 nm using iMark Microplate Reader (Bio-Rad).

4.8. Haemolytic assay

The haemolytic activity of the compounds (RC-1 and RC-2) was determined on human red blood cells (hRBCs) [49]. Human erythrocytes from healthy individuals were collected in tubes containing EDTA as an anticoagulant. The erythrocytes were harvested by centrifugation for 10 min at 2000 rpm and 20°C and washed three times in PBS. To the pellet, PBS was added to yield a 10% (v/v) erythrocytes/PBS suspension. The 10% suspension of erythrocytes was then further diluted with PBS at a 1:10 ratio. 100 µL of the final diluted erythrocytes was added to 100 µL of PBS having a previously determined concentration gradient (1000–7.8 µg/mL) of test compounds in microcentrifuge tubes. Total haemolysis was achieved with 1% Triton X-100. The tubes were incubated for 1 h at 37°C and then centrifuged for 10 min at 2000 rpm and at room temperature. From the supernatant fluid, 150 µL was transferred to a flat-bottomed microtiter plate (Tarson), and the absorbance was measured spectrophotometrically at 450 nm by using a Thermo MultiSkans spectrophotometer. The hemolysis percentage was calculated by following the equation:

\[
\text{% Hemolysis} = \left( \frac{A_{450} \text{ of test compound treated sample} - A_{450} \text{ of 1% TritonX treated sample}}{A_{450} \text{ of buffer treated sample}} \right) \times 100\%.
\]

where \(A_{450}\) is absorbance at 450 nm.

4.9. Docking studies

The ligands (SB, RC-1 and RC-2) were drawn in ChemDraw Ultra 6.0 (ChemOffice package) assigned with proper 2D orientation and analyzed for connection error in bond order. OSIRIS, an ADMET based Java library layer that provides reusable cheminformatics functionality which is an entirely in-house developed drug discovery informatics system was used to predict the total drug score. Dundee PRODRG2 server was used to minimize the energy of the molecules [50,51]. The energy minimized compounds were then read as input for AutoDock4.2, in order to carry out the docking simulation. Auto Dock used the local search to search for the optimum binding site of small molecules to the protein. The active site was defined by a grid box of 85 × 80 × 90 points and spacing of 0.375 Å with the ligand binding site as the center. The final structure was then saved in pdbqt format. During the docking process, a maximum of 10 conformers were considered for each compound. AutoDock 4.2 and AutoDock vina was compiled and run under Windows 7 operating system [37].

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Appendix A. Supplementary material

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC 1867350. Whole information can be attained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/deposit). Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.03.080.

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