CONSTRUCTION OF Ru(II) POLYPYRIDYL BASED MACROCYCLES:
SYNTHESIS, CHARACTERIZATION, ELECTROCHEMICAL, Li⁺ BINDING,
ANTITUMOUR AND ANTI-HIV PROPERTIES

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Abstract
Some ruthenium (II) polypyridyl complexes with a bis-chalcone (obtained by the condensation of 3-methyl-
thiophene-2-carboxaldehyde and 4-acetyl pyridine) have been synthesized and characterized
spectroscopically (IR, NMR, UV/Vis), conductimetric, elemental analysis and FAB mass data. Their
luminescent, redox and Li⁺ binding properties have been studied. The anti-HIV and antitumour activities
have also been reported.

Introduction
Studies of ruthenium (II) polypyridyl complexes have been interesting owing to their unique photophysical
and redox characteristics [1]. In fact, these properties have led to the development of new ruthenium
polypyridyl systems either by incorporating the desired groups within the bipyridine moiety itself or by
using other type of donor sites along with the \([\text{Ru(bpy/phen})_2]\) core to form mixed ligand tris-chelates
modulating the photo-redox activity of this class of complexes.
Furthermore, it has been reported that Ru(II) complexes bind DNA and a correlation between their binding
affinity and activities have already been established [2,3]. Recently a detailed accounts of such properties
of ruthenium complexes have been reviewed by Clarke et al.[4]. Based on antiviral [5], antifungal [6],
antibacterial [7] and antitumour [8] activities of chalcones very recently, several Ru(II) complexes bearing
bis-chalcones synthesized by us [9] have been evaluated for their antitumour and anti-HIV activities [10] and
some of them have shown quite interesting properties. It was therefore considered worthwhile to explore
some new Ru(II) complexes bearing chalcones for the evaluation of their antitumour and anti-HIV properties.
The Li⁺ binding properties of newly synthesized complexes containing 14-membered ring are also studied.

Materials and Methods
All the solvents purchased from Merck were distilled using standard procedures prior to use. 3-Methyl-
thiophene-2-carboxaldehyde, 4-acetyl pyridine, 2,2'-bipyridine and 1,10-phenanthroline purchased from
Sigma-Aldrich were used as supplied whereas the cis-[Ru(bpy)_2Cl_2] and cis-[Ru(phen)_2Cl_2] were prepared by
a reported [11] procedure. Tetra-n-butylammonium bromide supplied by Merck was converted into pure
tetrabutyl ammonium perchlorate (TBAP) by an available procedure [12]. Caution: TBAP could
be explosive so use the size of small amounts of it is recommended. Neutral alumina for column chromatography
was supplied by Merck and used as such. All the reactions were carried out under N₂ atmosphere
Microanalysis (C, H and N) performed on a Carlo Ebra Elemental Analyzer 1108 and FAB mass data using a
JEOL SX-102 mass spectrometer were carried out at the Central Drug Research Institute, Lucknow, India. IR
(KBr Pellets) and UV/Vis data were obtained using a JASCO FT IR 5300 spectrometer and a Shimadzu UV-
1601 spectrophotometer respectively, \(^1\)H and \(^{13}\)C NMR (DMSO-d₆) were recorded on a JEOL FX 90Q
spectrophotometer at B.H.U., Varanasi, India and \(^1\)H-\(^{13}\)H COSY (DMSO-d₆) spectra was recorded at TIFR,
Mumbai, India. Electrochemical measurements were made using an electrochemical interface SI 1287
potentiostat, using a graphite disc as working electrode, a platinum wire auxiliary electrode and Ag/AgCl
reference electrode in a three electrode configuration. All electrochemical experiments were performed after
bubbling the solution with dimethane for 0.25 h. Solution (CH₃CN) emission spectra were measured at
University of Bristol, UK. The antitumour and anti-HIV activities were evaluated at the School of Pharmacy,
University of North Carolina, USA.

Synthesis of Ligand (L)
The ligand was prepared by addition of a solution of 4-acetyl pyridine (0.02 M, 2.2 mL) to an
ethanolic solution (10 mL) of 3-methyl-thiophene-2-carboxaldehyde (0.02 M, 2.2 mL) containing aqueous
NaOH (12 mL, 50%) following reported procedure [5]. After 72 h of stirring distilled water (20 mL) was
added to the reaction mixture and then neutralised with dil HCl (10%). The brick-red precipitate formed was
filtered and washed several times with H₂O followed by EtOH and was purified by column chromatography
on alumina with CH₃CN as eluent. Two dark red and brown fractions were collected. Minor dark red fraction
was discarded while the major brown fraction was concentrated by evaporation in vacuo. The solid (L) was
crystallized from ethanol and dried in vacuo, M.P. 90 ± 1°C.
Synthesis of complexes

[RuL(bpy)$_2$]$_2$(PF$_6$)$_2$, (I): An ethanolic solution (10 mL) of cis-[Ru(bpy)$_2$Cl$_2$] (bpy = 2,2'-bipyridine) (1 mM, 0.520 g) was mixed to an ethanolic solution (10 mL) of (L) (1 mM, 0.350 g) in a 1:1 molar ratio while stirring and the resulting mixture was refluxed for 24 h then cooled to room temperature and filtered. To the filtrate, a saturated methanolic solution of NH$_4$PF$_6$ was added. Solid thus obtained was filtered and washed with H$_2$O and MeOH, and purified on an alumina column with MeCN-saturated aqueous KNO$_3$-water (7:1.0.5 v/v) as eluent [13]. The eluate was then concentrated to dryness and the residue obtained was dissolves in minimum volume of acetone followed by the excess addition of methanolic solution of NH$_4$PF$_6$. The solid complex (I) was recrystallized (by the addition of diethyl ether to a solution of complex in acetone) as crystalline material, M.P. > 250°C, yield: 40%.

[RuL(phen)$_2$]$_2$(PF$_6$)$_2$, (2) was also prepared as reported above by refluxing an ethanolic solution of cis-[Ru(phen)$_2$Cl$_2$] (where phen = 1,10-phenanthroline) (1 mM, 0.532 g) with an ethanolic solution (10 mL) of (L) (1 mM, 0.350 g).

The elemental analysis and FAB-mass data along with other properties of ligand and complexes are shown in Table I.

Results and Discussion

The synthesis of ligand has been considered in view of Michael addition [14] (Scheme-I).

Table I: Physical, analytical and FAB Mass data of ligand and Ru(II) complexes

| Compound | Analysis found (Calc.) | m/z Found (Calcld.) | $\Lambda$ (1 cm$^2$ mol$^{-1}$) |
|----------|------------------------|---------------------|-------------------------------|
| L        | %C 68.03 %H 4.63 %N 7.95 | 350 (350)           |                              |
| [RuL(bpy)$_2$]$_2$(PF$_6$)$_2$.2Me$_2$CO | (68.96) (4.59) (8.04) | [M] |                              |
| [RuL(phen)$_2$.2Me$_2$CO | (47.21) (3.93) (7.18) | [M-PF$_6$]$^+$ | 250                           |

The microcrystalline complexes were found thermally stable at room temperature and soluble in acetone, acetonitrile, methanol, ethanol, DMF and DMSO. The molar conductances of the complexes (CH$_3$CN, 10$^{-3}$ M) shown in Table I is in agreement with the number of counteranions present in the complexes [15]. However, the presence of two moles of acetone in the complex has been considered in view of the fitting of their elemental analysis and observation of a band at ~ 1720 cm$^{-1}$ in their IR spectra.

IR Spectra: In the IR Spectra (KBr) of the complexes a band at 1676 cm$^{-1}$ due to the v(C=O) was found to be similar compared to free ligand (1680 cm$^{-1}$) which indicates that the $>$C=O group did not participate in the bonding with ruthenium. However, peak observed at 1570 cm$^{-1}$ in the spectrum of the free ligand due to pyridyl moiety shifted to 1545 cm$^{-1}$ and 1500 cm$^{-1}$ in the spectrum of complexes (1) and (2) respectively, indicating that pyridyl group coordinate to ruthenium.

$^1$H NMR spectra: To get further structural support $^1$H NMR spectra of the ligand and its complexes were recorded in DMSO-$d_6$. The $^1$H NMR shows (complex) patterns at $\delta$ 9.01 - 8.53, 7.53 - 7.3, 7.06, 6.86, 2.4, 2.00 ppm due to ortho and meta-protons of both pyridine rings, HC=CH (thiophene), -C-H, -CH$_2$ and -CH$_3$ protons respectively. Its $^{13}$C NMR spectrum also showed peaks at $\delta$ 185.2 ($>$C=O), 150.3 (C$_2$ C$_2$'-py), 149.8 (C$_6$, C$_6$'-py), 129.8 (C$_4$, C$_4$'-py), 128.2 (C$_3$, C$_3$'-py), 126.0 (C$_5$-thio), 123.8 (C$_2$-thio), 121.8 (C$_3$-thio), 50.6 (-CH$_2$), 48.9 (-CH), 33.4 (-CH$_3$). The $^1$H NMR spectra of the complex shows resonances at $\delta$ 8.75 to 8.6 ppm due to pyridyl protons. Peaks observed in the spectrum of complex (I) at $\delta$ 8.34 - 8.19, $\delta$
8.09 - 8.02, δ 7.9 - 7.89 and δ 7.61 - 7.42 ppm were assigned to 3,3', 4,4', 5,5' and 6,6' protons of the bipyridine ring and the bands at δ 9.2, 8.6, 8.37, 8.2 - 7.86 and 7.6 ppm in the spectrum of complex (2) were assigned to H2/Hb, H6/H2, H5/H6 and H3/H8 protons of the phenanthroline ring in agreement with earlier report [15].

The 1H - 1H COSY spectrum of one of a representative complex (I) showed that the ortho and meta protons of both pyridine rings couple with the 6,6'-protons of bipyridine. The COSY spectrum also showed weak coupling between -CH3 and -CH2 protons supporting the quartet and triplet observed at δ 2.20 and 2.5 ppm respectively for these protons.

**UV-Vis spectra:** UV-Vis spectra of the complexes in CH3CN solution (10⁻³ M) were recorded in the range 200 - 800 nm and showed intra-ligand charge-transfer peaks at λ 264, 290 (I) and λ 246 nm (2) whereas broad peaks observed in the respective spectra at λmax 485 and 417 nm were assigned to the t₅g(Ru)→π*(ligand) transition.

Thus, on the basis of spectroscopic (IR, ¹H NMR, UV/Vis) and conductimetric data along with elemental analysis and FAB mass data, the proposed general structure of the complexes is shown in Figure 1.

**Figure 1:** Proposed structures of Ru(II) complexes

**Room temperature emission spectra**

Emission properties of the complexes have been studied in acetonitrile solution (10⁻⁶ M) at room temperature (excitation at 450 nm). Complexes did not luminesce most likely due to the efficient migration of the electron/energy [16-18] from the triplet excited state of Ru°° to the ligand, as probability of non-radiative emission was discarded since complex (2) weakly emitted.

**Redox Properties:** Redox properties of the ruthenium complexes have been studied in acetonitrile solution (10⁻³ M) in the potential range ± 2 V using Ag/Ag⁺ as reference and graphite disc as working electrodes.

**Oxidation**

Redox potential (E°) data are reported in Table II. Cyclic voltammogram of the complex (I) showed two oxidation peaks at 0.88 V and 1.45 V, the former peak being considered to arise from a Ru(II) → Ru(III) oxidation as the latter peak lies in the potential range of ligand oxidation in view of the fact that upon coordination peaks could be shifted towards less positive potentials. Similarly, in the cyclic voltammogram (CV) of complex (2) these oxidations occurred at 0.92 V and 1.43 V.

**Reduction**

During the reduction of the free ligand two peaks were observed at -0.85 and -1.2 V. However, four reduction peaks were observed at -0.8, -1.1, -1.35 and -1.55 V in the cyclic voltammogram of complex (I). The reduction peaks at -0.8 and -1.1 V were assigned to arise from ligand reduction whereas the peaks at -1.35 and -1.55 V could be considered to arise from bipyridine reduction in view of earlier report [19]. Similarly four reduction peaks at -0.45, -1.2, -1.35 and -1.8 V also arise upon reduction of the complex (2) which were in consistence with the earlier report [20].

**Li⁺ binding studies:** Due to the presence of a 14-membered ring cavity in the complexes, their binding with Li⁺ on a preliminary level was studied using UV/Vis and electrochemical techniques. The presence of LiCl (10⁻⁴ M, CH₃CN) in the solution of the complexes in acetonitrile (10⁻⁵ M) shifted both the MLCT and intra-ligand transitions towards higher energies along with the increase in their absorbances (Table III).
Table II: Electrochemical data* of ligand and Ru(II) complexes in MeCN at 298 K

| Ligand            | Redox potential | Oxidations | Reductions |
|-------------------|-----------------|------------|------------|
|                   | E° 298 / V vs Ag/AgCl | Ru³⁺ / Ru²⁺ | Ligand     |
| [Ru.L(bpy)₂]²⁺    | 0.88            | 1.55       | -0.85, -1.2 |
| [Ru.L(phen)₂]²⁺   | 0.92            | 1.40       | -0.45, -1.2, -1.35, -1.8 |
| [Ru.L(bpy)₂]²⁺ + 10⁻⁴ mol dm⁻³ LiCl | 1.15 | 1.72 | -0.5, -1.08, -1.65 |
| [Ru.L(phen)₂]²⁺ + 10⁻⁴ mol dm⁻³ LiCl | 0.95 | 1.43 | -0.5, -1.45, -1.8 |

* Conditions: solvent, MeCN, supporting electrolyte TBAP (0.1 M); working electrode, graphite disc; auxiliary electrode, platinum; reference electrode Ag/Ag⁺. Solute concentration -10⁻³ M, E½ = (Epa + Epc) / 2 where Epa and Epc are anodic and cathodic peak.

Table III: UV-Vis data in MeCN (10⁻³ M) at 298 K for Ru(II) complexes in presence and absence of LiCl

| Complex           | Transitions | λ max, nm (ε, d m⁻³ mol⁻¹ cm⁻¹) +LiCl (5 × 10⁻⁴ mol dm⁻³ LiCl) |
|-------------------|-------------|---------------------------------------------------------------|
| [Ru.L(bpy)₂]²⁺    | π - π* (2)²⁺| 246 (7.75) 242 (8.81)                                         |
|                   | π - π* (1)²⁺| 290 (6.65) 286 (7.06)                                         |
|                   | m.l.c.t.    | 485 (0.83) 476 (0.97)                                         |
| [Ru.L(phen)₂]²⁺   | π - π* (2)²⁺| 230 (10.16) 226 (13.95)                                       |
|                   | π - π* (1)²⁺| 264 (10.69) 263 (12.04)                                       |
|                   | m.l.c.t.    | 417 (0.75) 349 (2.98)                                         |

* (1) and (2) used to denote different ligand orbitals.

Electrochemical studies of the complexes (1 and 2) in the presence of Li⁺: As shown in Table III the addition of a CH₃CN solution of LiCl (10⁻⁴ M) to the complexes (10⁻³ M, CH₃CN) shifted metal-based oxidation peak towards a more positive potential by 230 mV in complex (1) whereas in complex (2) shift was 40 mV compared to LiCl unbound complexes. The larger shift observed for complex (1) as compared to complex (2) may be considered in view of more flexibility of the bipyridine ring.

Thus, in view of these observations and earlier reports [21] binding of Li⁺ inside the cavity of the macrocycle could be considered on a preliminary level.

Antitumour and anti-HIV activities

The antitumour and anti-HIV activities were evaluated using standard procedure [22,23]. The cytotoxicity data of free ligand along with their ruthenium complexes are shown in Table IV. The free ligand showed potential activity against IA9 and A549 which could be considered [24] in view of the significant bioactivities shown by an earlier ligand system bearing similar functional (N-C=O) group. However, the complexes showed a better activity as compared to the free ligand against almost all tumour cells. This inhibitory behaviour of the complexes may be considered in view of their interaction with DNA as reported [25,26] earlier. Complex (2) however showed better activity against IA9, KB-VIN and A-549 as compared to complex (1).

From the anti-HIV data of the ligand and complexes as shown in Table IV, it could be seen that complex (1) show a smaller IC₅₀ value than the free ligand. However the EC₅₀ value of the free ligand (13.3 µM) showed that ligand is more active than the complex (1) which may be understood in terms of steric factors [27]. Complex (2) bearing a bulkier group (1,10-phenanthroline) did not show activities (NA = inhibition < or = 5% at 20 g/mL). The activity of these compounds has been compared with the standard AZT (azidothymidine) treated as control under the similar experimental conditions.
Table IV: Antitumour* and anti-HIV activity of free ligand and its Ru(II) complexes

| Compound | Tumour cells | Anti-HIV
|----------|--------------|----------|
|          | HCT-8 | IA9 | HOS | KB | KB-VIN | MCF-7 | A549 | U87-MG | IC50 (μM) |
| L        | 41.4  | 37.1 | >20 (114) | 46.2 | 50 | 50 | 29.1 | >20 (60) | 48.0 |
| (I)      | >20 (33.3) | 10.3 | >20 (23.9) | 14.5 | >20 (13.6) | 16.6 | 14.9 | >20 (39.3) | 17.7 |
| (2)      | 13.9  | 9.7  | 12.3 | 13.9 | 7.2 | 14.8 | 9.0  | >20 (36.1) | -  |
| AZT**    | -     | -    | -   | -   | -   | -   | -    | 1870   | -    |

* Values are IC50 concentration in μM. Note, if inhibition < 50% at 20 microgram per mL, percent inhibition observed is the value in brackets.

Turnout/tissue type: HCT-8 - Coloereal, IA9 - Ovarian, HOS - Bone, KB - Nasopharynx, MCF - Breast, A549 - Lung, U87-MG - Glioblastoma.

o ED50 in microgram per mL is less than 50% at highest test concentration.

** AZT = Azido-thymidine; m/z = 267.25

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