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tris-(Benzimidazol-2-yl-methyl)-amine as a Versatile Building Block in Ru(II) Polypyridyl Chemistry

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Summary. *tris*-(Benzimidazol-2-yl-methyl)-amine, H_3ntb, was prepared and used in the synthesis of dinuclear Ru(II) polypyridyl and polynuclear Ru(II)–Co(III) complexes of the type $[Ru_2(H_2ntb)(bpy)_4]^{3+}$, $[Ru_2(Hntb)(phen)_4]^{2+}$, $[(Ru_2(H_2ntb)(bpy)_4)_2Co(en)_2]^{9+}$, and $[(Ru_2(Hntb)(phen)_4)_2Co(en)_2]^{7+}$ (*bpy* = 2,2′-bipyridine, *phen* = 1,10-phenanthroline, *en* = 1,2-diaminoethane). The complexes were characterized by elemental analysis as well as spectroscopic and redox data. The luminescent properties of the complexes were also studied. The complexes showed significant antitumour and anti-HIV activities.

Keywords. *tris*-(Benzimidazol-2-yl-methyl)-amine; Polynuclear Ru(II) complexes; Polypyridyl chemistry; Redox properties; Luminescence; Antitumour and anti-HIV activities.

Introduction

Multimetallic assemblies of defined architecture have been the object of studies of energy and electron transfer processes [1], the construction of nanostructured materials [2], and the fabrication of molecular devices [3]. In these context, ruthenium polypyridyl complexes have played a key role due to their chemical stability, redox properties, and favourable photophysical characteristics [4].

Among the various synthetic strategies that have been followed for the construction of polynuclear complexes [5–10], the stepwise method based on the preparation of mononuclear building units which are then linked in a desired fashion has shown significant advantages, especially with respect to polydentate ligands.

Two types of bridging ligands (electron-poor and electron-rich) are generally used to assemble polypyridyl ruthenium building blocks [11]. There are some ligands which are electrically neutral but can be charged upon complex formation by deprotonating one or more dissociable NH protons present in the system. Complexes derived from ligands of this type, *e.g.* 2,2′-*bis*-(2-pyridyl)-benzimidazole [12], 2,6-*bis*-(2′-pyridyl) benzimidazole [13], 2,2′-*bis*-(2-benzimidazolyl)-4,4′-bipyridine [14], or 2,6-*bis*-(benzimidazolyl)-pyridine [15, 16], have received

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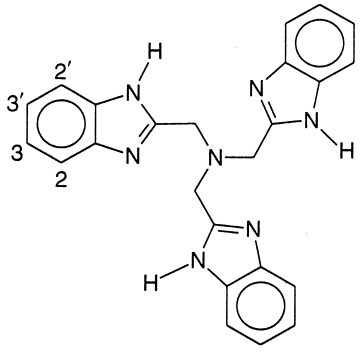
considerable attention. Additionally, compounds with head-to-tail *bis*-benzimidazole units (Hoechst 33258) and several of its derivatives have already been reported [17] as anticancer drugs including *tris*-benzimidazole with an extended recognition site [18].

Thus, in view of above reports, it was found worthwhile to synthesize *tris*-(benzimidazol-2-yl-methyl)-amine (H_3ntb) and to complex it with Ru(II) polypyridyls giving dinuclear complexes which could then be linked with a bridging unit (*trans*-$[Co(en)_2Cl_2]Cl$) to form oligometallic complexes.



tris-(Benzimidazol-2-yl-methyl)-amine

Results and Discussion

The complexes were found to be thermally stable and soluble in acetone, ethanol, methanol, acetonitrile, *DMF*, and *DMSO*. The molecular composition of the complexes was determined on the basis of elemental analysis and MS data. Their molar conductivities in solution (10^{-3} *M* CH_3CN; Table 1) agreed well with the charges present.

The complexes (**1**) and (**2**) gave molecular ion peaks as $[M–PF_6^-]^+$ and $[M–2PF_6^-]^{2+}$ respectively whereas ES mass data for the complexes (**3**) and (**4**) gave molecular ion peak at m/z 3950 and m/z 3848 respectively (Table 1).

During the condensation of H_3ntb with Ru(II) polypyridyls, initially a mononuclear complex leaving at least one benzimidazole unit uncoordinated was expected. However, complexes of composition $[Ru_2(bpy)_4(H_2ntb)]^{3+}$ and $[Ru_2(phen)_4(Hntb)]^{2+}$ were obtained, excluding the probability of any benzimidazole nitrogen to remain uncoordinated. Therefore, further coordination *via* the amino nitrogen had to be expected.

Infrared spectra

IR peaks due to ν(C=N) observed at 1470 cm^{-1} in the spectrum of free ligand shifted to 1460, 1440, 1460, and 1440 cm^{-1} in the spectra of the complexes **1**–**4**, respectively, indicating coordination with the metal ion. The sharp peak observed at 839–842 cm^{-1} in the spectra of the complexes was assigned to $\nu(PF_6^-)$.

To gain further information on the bonding mode, the ^1H NMR spectra of the complexes were compared with those of the free ligand.

Table 1. Physical and mass spectroscopic data for ligand and complexes

Compound	Λ[a] $(\Omega^{-1} \cdot cm^2 \cdot mol^{-1})$	FAB MS, m/z[b]	ES MS, m/z[b,c]
H_3ntb	10	407 (407) [M]	
$[Ru_2H_2ntb(bpy)_4]^{3+}$ (**1**)	260	1378 (1377) $[M-2PF_6^-]^{2+}$ 1232 (1232) $[M-3PF_6^-]$ 925 (921) $[M-(PF_6^-)_3(bpy)]$ 408 (407) $[M-(PF_6^-)_3(bpy)_4(Ru)_2)]$	
$[Ru_2Hntb(phen)_4]^{2+}$ (**2**)	180	1474 (1472) $[M-PF_6^-]^+$ 1329 (1329) $[M-2PF_6^-]$ 688 (688) $[M-((PF_6^-)_2(phen)_3Ru)]$ 408 (407) $[M-((PF_6^-)_2(phen)_4(Ru)_2)]$	
$[(Ru_2H_2ntb(bpy)_4)_2Co(en)_2]^{9+}$ (**3**)	840	1352 (1352) $[M-((PF_6^-)_9Ru_2H_3ntb(bpy)_4en)]$ 1234 (1232) $[M-((PF_6^-)_9Ru_2H_3ntb(bpy)_4Co(en)_2)]$ 766 (766) $[M-((PF_6^-)_9Ru_2H_3ntb(bpy)_7Co(en)_2)]$	3950 (3949) [M] 2645 (2645) $[M-(PF_6^-)_9]$ 1428 (1427) $[M-((PF_6^-)_9Co(en)_2(bpy)_6Ru)]$ 718 (718) $[M-((PF_6^-)_9Co(en)_2(bpy)_6Ru_4L)]$
$[(Ru_2Hntb(phen)_4)_2Co(en)_2]^{7+}$ (**4**)	690	1474 (1472) $[M-((PF_6^-)_6Ru_2H_3ntb(phen)_4Co(en)_2)]$ 1325 (1327) $[M-((PF_6^-)_7Ru_2H_3ntb(phen)_4Co(en)_2)]$ 585 (586) $[M-((PF_6^-)_7Ru_4H_3ntb(phen)_7Co(en)_2)]$	3848 (3849) [M] 3721 (3723) $[M-PF_5^-]$ 2046 (2047) $[M-((PF_6^-)_7Co(en)_2Ru_2L)]$ 787 (787) $[M-((PF_6^-)_7Co(en)_2(phen)_7Ru_2L)]$

[a] 10^{-3} *M* in acetonitrile at room temperature; [b] calculated values for m/z are given in parentheses; [c] in acetonitrile at room temperature

^{1}H NMR spectra

^{1}H NMR spectra of the ligand and complexes were recorded in *DMSO*–d_6. Peak assignments (see Experimental) were supported from ^{1}H, ^{1}H COSY spectra for **1** and **3**; the spectra of **3** and **4** turned out to be less complex than those of **1** and **2** which is consistent with earlier reports on Ru–Co complexes [19, 20]. The upfield shift of the NH protons from 12.8 ppm in the free ligand to 3.41 and 5.53 ppm in the spectra of **1** and **2** was also found to agree with the [21] literature.

The CH_2 protons of the ligand, observed at $\delta = 4.6$ ppm, did not shift significantly in the spectra of **1** and **2**; in the spectra of **3** and **4**, however, the corresponding δ values are 2.55−2.48 and 3.53 ppm, respectively. The peaks at 2.18 and at 2.10−2.03 ppm in the spectra of **3** and **4** were assigned to the CH_2 and NH_2 protons of ethylenediamine.

UV/Vis spectra

UV/Vis spectra of $10^{-5}\,M$ solutions of **1**–**4** in *DMSO* were recorded in the region of 200–800 nm. Two intense peaks 284 and 277 nm in the spectrum of the ligand were assigned to intraligand [19] charge-transfer transition; the corresponding transitions in the spectra of **1**–**4** occurred at 285, 286, 291, and 276 nm, respectively. An additional shoulder at 376 nm in **3** and 360 nm in **4** was assigned to MLCT transitions from cobalt to ethylene diamine (π^*). Other peaks arising from *trans*-[Co(*en*)$_2$Cl$_2$]Cl ($10^{-3}M$) were observed at 643 and 647 nm in the spectra of **3** and **4** and are consistent with transitions of cobalt as reported in the literature [22]. An additional intense peak at 448–450 nm in the UV/Vis spectra of **1**–**4** is supposed to arise from $d\pi(Ru) \rightarrow \pi^*$ (ligand) transitions [23].

The structures of the complexes as deduced from IR, ^{1}H NMR, UV/Vis, FAB MS, and elemental analyses are shown in Fig. 1.

Luminescence

Solutions of the complexes in CH_3CN ($10^{-6}\,M$) excited at MLCT wavelengths ($d\pi(Ru) \rightarrow \pi^*$(ligand) *ca.* 450 nm) emitted in the wavelength region of 533–612 nm. From **1** and **2** only one emission peak (612 and 597 nm, respectively) was observed, whereas **3** and **4** showed two emissions each (535, 533 nm; 609, 584 nm). Representative luminescence spectra of the complexes **2** and **4** are given in Figs. 2 and 3. The appearance of an extra emission in **3** and **4** originates either (*i*) from the presence of an impurity or (*ii*) from an excitation level different from those of Ru(II). The first possibility was ruled out on the basis of the spectroscopic data of the complexes. Furthermore, in view of an earlier report [24] it might be considered that the second emission arises from another ruthenium centre. However, these emissions have been reported to occur at longer wavelengths (*ca.* 700 nm), whereas in the present case the emissions were observed at *ca.* 533–535 nm. Therefore, it might be inferred that the additional emissions arise from the Co(*en*)$_2$ unit.

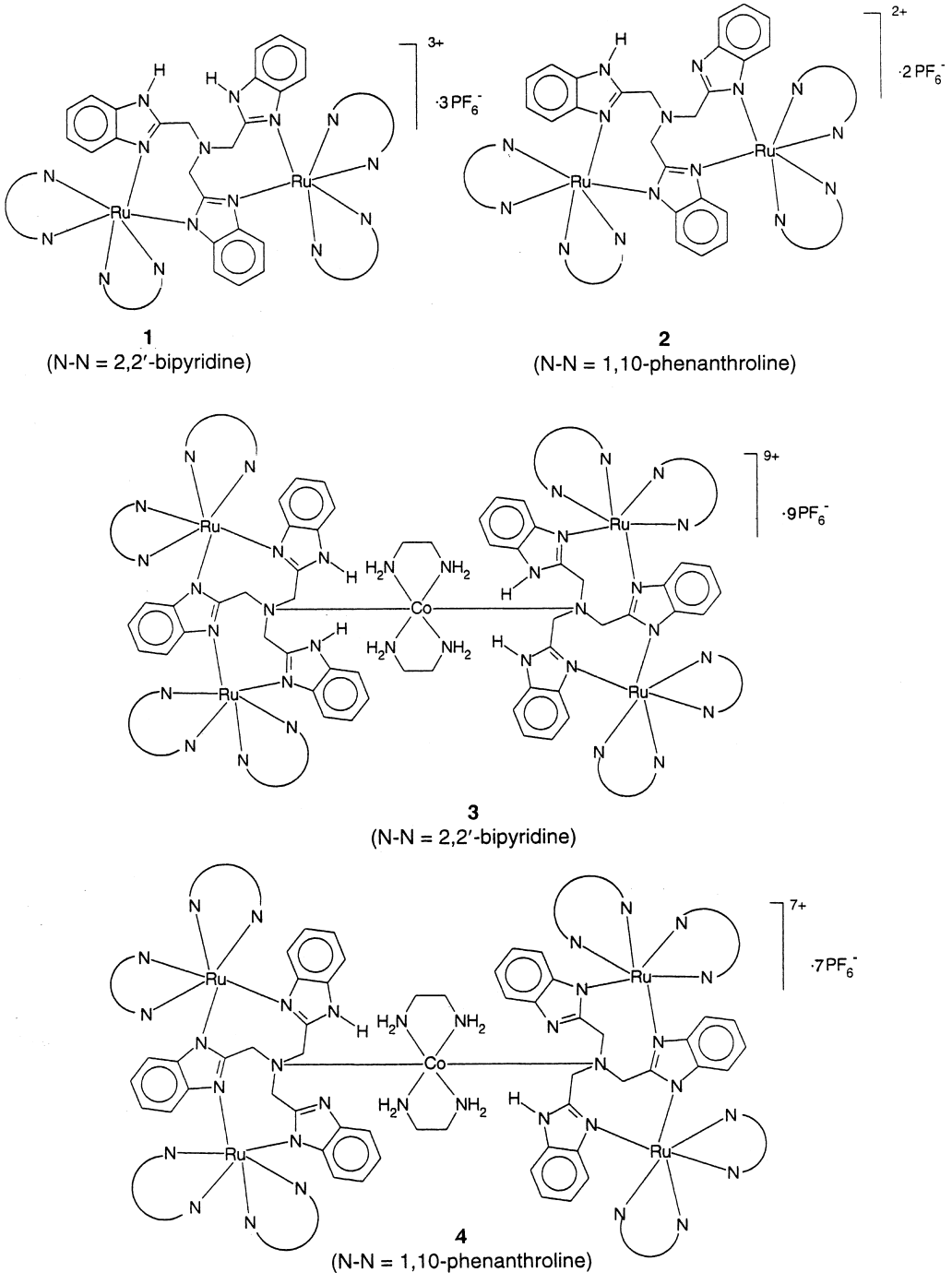


Fig. 1. Proposed structures of the complexes

Electrochemistry

The redox behaviour of the complexes has been studied in acetonitrile solution using cyclic and differential pulse voltammetric techniques with tetrabutyl

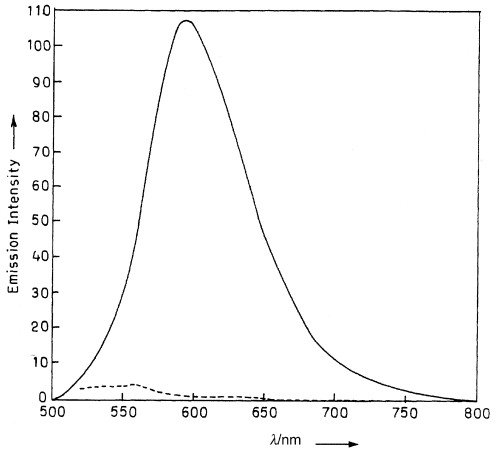


Fig. 2. Room temperature luminescence spectra of $[Ru_2H$*ntb*$(phen)_4]^{2+}$ (**2**, ——) and MeCN (- - - -)

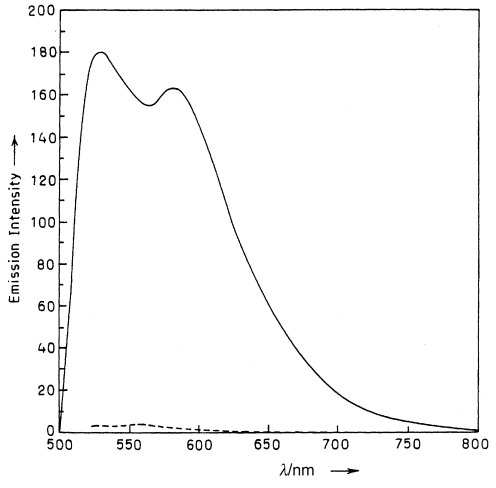


Fig. 3. Room temperature luminescence spectra of $[(Ru_2H$*ntb*$(phen)_4)_2Co(en)_2]^{7+}$ (**4**, ——) and MeCN (- - - -)

ammonium perchlorate (*TBAP*) as supporting electrolyte; a representative voltammogram is shown in Fig. 4.

Ligand oxidation was not observed in the region from 0 to $+2$ V. However, complexes **1** and **2** showed a single reversible two-electron oxidation (Ru^{III}/Ru^{II}) at 1.34 and 1.35 V, whereas **3** and **4** exhibited a single reversible four-electron oxidation at 1.37 and 1.36 V, indicating that in the former case two ruthenium centres are oxidized (each by one electron), whereas in the latter reaction the four ruthenium centres are oxidized by one electron each.

Additionally, H_3*ntb* and its complexes exhibited three reversible reductive processes. As reported earlier [20], a Co(III)–Co(II) reduction process could not be

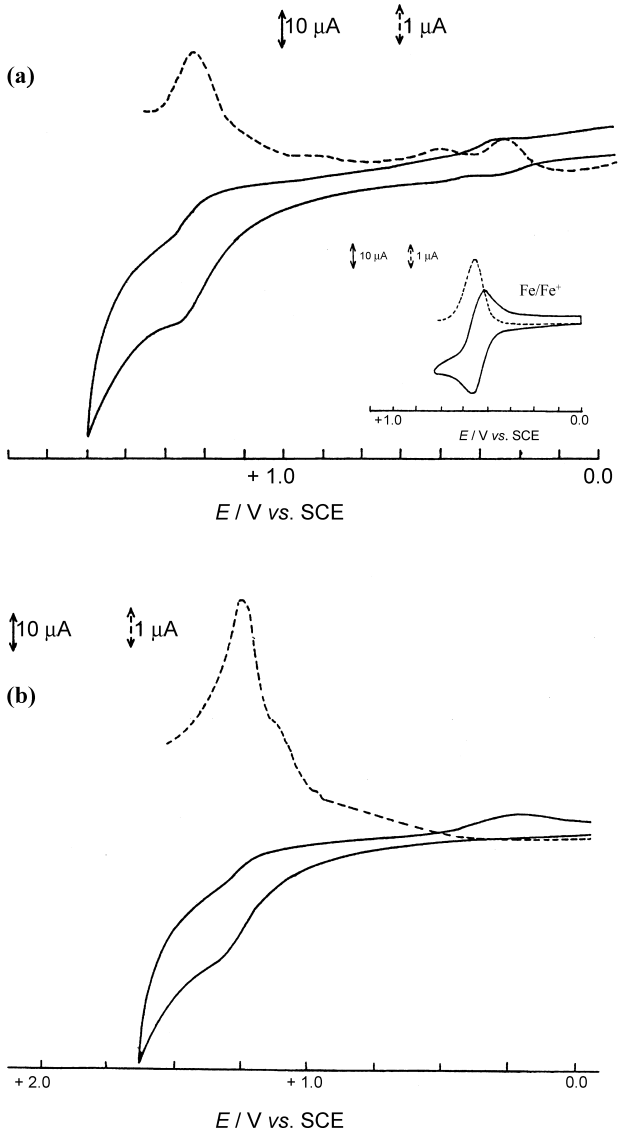


Fig. 4. Cyclic and differential pulse voltammograms of (a) $[Ru_2Hntb(phen)_4]^{2+}$ (**2**) and (b) $[(Ru_2Hntb(phen)_4)_2Co(en)_2]^{7+}$ (**4**) in MeCN (10^{-3} *M*) using *TBAP* (10^{-1} *M*) as supporting electrolyte and Fc/Fc^+ as internal reference at a scan rate of $100\,mV \cdot s^{-1}$

observed in present system, but Co(II)–Co(I) reduction was observed at a potential overlapping with the reduction potential [25, 26] for *bpy* and *phen* ligands. Therefore, these different redox processes could not be distinguished.

Cytotoxic and anti-HIV activities

The cytotoxicity (IC_{50}) of the free ligand and its Ru(II) polypyridyl complexes tested on different tumour cells are shown in Table 2. Significant activity of the free ligand against HCT-8, IA9, HOS, KB, KB-VIN, MCF-7, A549, and U87-MG has

Table 2. Cytotoxicity[a] and anti-HIV[b] (IC_{50}, μg/cm^3) activity against different tumour cells and HIV

Compound	Tumour cells								HIV
	HCT-8[c]	IA9	HOS	KB	KB-VIN	MCF-7	A549	U87-MG	IC_{50} (μg/cm^3)
H_3ntb	< 5.2 (52)	1.5	5	4.3	6.2	3.5	< 2.5 (57)	> 20 (34)	1.99
1	4.2	2.3	4.9	4.9	6.5	4.8	3.9	> 20 (48)	2.16
2	7.5	3.8	10	9.2	19	> 20 (38)	5	> 20 (48)	2.33
3	NA	NA	NA	NA	NA	NA	15.5	17	21.0
4	NA	NA	NA	NA	NA	NA	> 20 (5.9)	NA	1.95
AZT	–	–	–	–	–	–	–	–	500

[a] NA = not active (inhibition ≤ 5% at 20 μg per cm^3) during prescreen test, value in parentheses is the percent inhibition; tumour/tissue type: HCT-8: ileoceca, IA9: ovarian, HOS: bone, KB: nasopharynx, MCF-7: breast, A549: lung, U87-MG: glioblastoma; [b] *AZT* = azidothymidine; IC_{50} = inhibitory concentration for 50% growth inhibition; EC_{50} = effective concentration for 50% growth inhibition; [c] ED_{50}(μg per cm^3) is less than 50% at highest test concentration

been observed with ligands bearing similar functional groups [17]. As reported [17], the inner ring nitrogen atom of a benzimidazole ring might coordinate with *DNA*; therefore, the ligand containing three NH groups was found to be very active. Among the complexes **1** and **2**, **1** was found to be more active since it contains two NH groups in addition to the flexibility associated with bipyridine as compared to the rigid nature of 1,10-phenanthroline. Complexes **3** and **4** were inactive against HCT-8, 1A9, HOS, KB, KB-VIN, and MCF-7, but showed some activity against A-549 and U87-MG. The decrease in the activity of the Ru–Co complexes may be due to their bulkier nature.

The anti-HIV activity of the complexes is also given in Table 2. Although the free ligand showed slightly higher anti-HIV activity as compared to its complexes **1** and **2**, all of them were found to be very significant anti-HIV active as compared to standard *AZT*.

The steric effects of terminal ligands (polypyridyl) on the activity of the complexes is quite apparent. Ru(II) complexes containing a 1,10-phenanthroline ring as the terminal ligand were found to be less active compared to the complexes bearing a 2,2′-bipyridine ring [27, 28]. However, this surmise calls for deeper investigation.

Experimental

Solvents and starting material were purchased from Sigma-Aldrich and used without further purification except for ethanol which was used after distillation. All reactions were carried out under a nitrogen atmosphere. Aluminum oxide (Merck) was used for column chromatography. Commercial tetrabutylammonium bromide (Fluka) was converted to pure tetrabutylammonium perchlorate (*TBAP*) according to Ref. [29].

Microanalytical, FAB mass, and ES mass data were acquired with a Carlo Erba Elemental Analyzer 1108, a JEOL SX-102/DA-6000 mass spectrometer, and a JEOL D-300(El/Cl) mass spectrometer at C.D.R.I., Lucknow, India. The results of elemental analyses agreed with the calculated values within experimental error. IR (KBr pellets) and UV/Vis spectra (CH_3CN) were recorded on Jasco FTIR 5300 and Shimadzu UV-1601 spectrophotometers at the Department of Chemistry, B.H.U., Varanasi, India. Luminescence spectra were measured in MeCN using a Perkin-Elmer 50B

luminescence spectrometer at the University of Bristol, UK, whereas electrochemical measurements were performed at the University of Hyderabad, India (working and auxiliary electrodes: platinum, reference electrode: SCE, reference: ferrocene.

Synthesis of the ligand

tris-(Benzimidazol-2-yl-methyl)-amine (H_3ntb) was prepared by a reported procedure [32]. *o*-Phenylenediamine (3.24 g, 0.03 mol) and tricarboxyamine (1.92 g, 0.01 mol) were dissolved in 4 *N* HCI and refluxed for 8 h. After cooling to room temperature, the content was diluted with water (10 cm^3) and neutralized with 10% aqueous Na_2CO_3 solution. The violet coloured solid thus obtained was filtered and recrystallized from EtOH followed by purification by column chromatography using alumina as adsorbent and CH_3CN as eluent. Evaporation of the solvent *in vacuo* yielded a solid which was recrystallized from CH_3CN and Et_2O to afford colourless shiny crystals.

Yield: 80% m.p.: 180 ± 1°C; MS: $m/z = 407$; IR (KBr): $\nu = 3450$ (ν_{NH}), 1570 (ν_{ar}), 1470 ($\nu_{C=N}$) cm^{-1}; ^1H NMR (*DMSO*-d_6, 90 MHz): $\delta = 12.8$ (3H, s, NH), 7.8 (6H, d, $PhH^{2,2'}$), 7.5 (6H, t, $PhH^{3,3'}$), 4.6 (6H, s, CH_2) ppm.

Synthesis of metal complexes

[Ru(*bpy*)$_2$Cl$_2$], [Ru(*phen*)$_2$Cl$_2$], and *trans*-[Co(*en*)$_2$Cl$_2$]Cl were prepared according to the literature [22, 33].

[$Ru_2(H_2ntb)(bpy)_4$][$(PF_6)_3$] (**1**; $C_{69}H_{62}F_{18}N_{15}P_3Ru_2$) and *[$Ru_2(Hntb)(phen)_4$][$(PF_6)_2$]* (**2**; $C_{72}H_{51}F_{12}N_{15}P_2Ru_2$)

To an ethanolic solution (10 cm^3) of Ru(*bpy/phen*)$_2$Cl$_2$ (1.04/1.06 g, 0.002 mol), H_3ntb (0.407 g, 0.001 mol) in EtOH (5 cm^3) was added under stirring. Stirring was continued for 2 h, followed by heating at reflux for 24 h. Subsequently, precipitation was effected by addition of an excess of a methanolic solution of NH_4PF_6. Light orange (**1**) or light brown (**2**) solids were obtained which were purified by column chromatography using alumina as adsorbent and MeCN:saturated aqueous KNO_3:water = 7:1:0.5 as eluent [34]. The eluates were evaporated to dryness, and the residues obtained were dissolved in a minimum volume of acetone followed by the addition of an excess of a methanolic solution of NH_4PF_6. The solids were recrystallized from ethanol.

1: Yield: 60%; m.p.: >250°C; MS: $m/z = 1378$; IR (KBr): $\nu = 3428$ (ν_{NH}), 1640 (ν_{py}), 1541 (ν_{ar}), 1460 ($\nu_{C=N}$); 860 ($\nu_{PF_6^-}$) cm^{-1}; ^1H NMR (*DMSO*-d_6, 90 MHz): $\delta = 8.84$ (8H, d, $H^{3,3'}$), 8.17 (8H, t, $H^{4,4'}$), 7.74 (6H, d, $PhH^{2,2'}$), 7.57 (6H, t, $H^{5,5}$ + 2H, d, $H^{66''}$), 7.54 (6H, t, $PhH^{3,3'}$), 7.19 (2H, m, $H^{5,5'}$), 7.17 (6H, d, $H^{6,6'}$), 4.20 (6H, s, CH_2), 3.41 (2H, s, NH) ppm.

2: Yield: 55%; MS: $m/z = 1474$; IR (KBr): $\nu = 2975$ (ν_{NH}), 1640 (ν_{py}), 1485 (ν_{ar}), 1440 ($\nu_{C=N}$), 860 ($\nu_{PF_6^-}$) cm^{-1}; ^1H NMR (*DMSO*-d_6, 90 MHz): $\delta = 9.40$ (2H, d, $H^{2,9}$), 9.06 (6H, d, $H^{2,9}$), 8.66 (8H, s, $H^{5,6}$), 8.06 (8H, d, $H^{4,7}$), 8.33 (8H, t, $H^{3,8}$), 7.80 (6H, d, $PhH^{2,2'}$), 7.5 (6H, t $PhH^{3,3'}$), 5.53 (IH, s, NH), 4.30 (6H, s, CH_2) ppm.

[$(Ru_2(H_2ntb)(bpy)_4)_2Co(en)_2$][$(PF_6)_9$] (**3**; $C_{132}CoH_{120}F_{54}N_{34}P_9Ru_4$)

To a solution of *trans*-[Co(*en*)$_2$Cl$_2$]Cl (0.032 g, 0.125 mmol) in 10 cm^3 *DMSO*, a solution of **1** (0.438 g, 0.25 mmol) in 5 cm^3 *DMSO* was added under stirring; then, the mixture was refluxed for 24 h. After filtration, an excess of a methanolic solution of NH_4PF_6 was added. The crude greenish-brown solid obtained was filtered and purified as described for **1** and finally crystallized from EtOH.

Yield: 50%; m.p.: >250°C; MS: $m/z = 3950$; IR (KBr): $\nu = 3422$ (ν_{NH}), 1620 (ν_{py}), 1540 (ν_{ar}), 1460 ($\nu_{C=N}$), 860 ($\nu_{PF_6^-}$) cm^{-1}; ^1H NMR (*DMSO*-d_6, 90 MHz): $\delta = 8.85$ (16H, d, $H^{3,3'}$), 8.83 (4H, d, $H^{6,6'}$), 8.17 (16H, t, $H^{4,4'}$), 8.16 (4H, t, $H^{5,5'}$), 7.74 (12H, d, $PhH^{2,2'}$), 7.73 (12H, d, $H^{6,6'}$), 7.55 (12H,

t, $PhH^{3,3'}$), 7.53 (12H, t, $H^{5,5'}$), 3.36 (4H, s, NH), 2.25 (12H, 5, CH_2), 2.48 (8H, s, CH_2^{en}), 2.18 (8H, s, NH_2^{en}) ppm.

$[(Ru_2(Hntb)(phen)_4)_2Co(en)_2][(PF_6)_7]$ (**4**; $C_{144}CoH_{118}F_{42}N_{34}P_7\ Ru_4$)

4 was prepared and purified similar to the procedure employed for **3** except that **2** (0.267 g, 0.25 mmol) was used as an educt instead of **1**.

Yield: 40%; m.p.: >250°C; MS: $m/z = 3848$; IR (KBr): $\nu = 3360$ (ν_{NH}) 1660 (ν_{py}), 1500 (ν_{ar}), 1440 ($\nu_{C=N}$), 860 ($\nu_{PF_6^-}$) cm^{-1}; ^1H NMR (*DMSO*-d_6, 90 MHz): $\delta = 8.73$ (16H, d, $H^{2,9}$), 8.30 (16H, 8, $H^{5,6}$), 8.00 (12H, m, $H^{4,7}$), 7.73 (16H, m, $PhH^{2,2'}$ and $H^{4,7}$), 7.50 (12H, m, $PhH^{3,3'}$), 7.33 (16H, m, $H^{3,8}$), 5.53 (2H, s, NH), 3.53 (20H, s, CH_2 and CH_2^{en}), 2.18 (8H, s, NH_2^{en}) ppm.

Assay of cytotoxic and anti-HIV activities

The free ligand and its complexes were assayed for their cytotoxicity and anti-HIV activities at the School of Pharmacy, University of North Carolina, USA adopting reported procedures [30]. Stock cultures were grown in T-75 flasks containing 50 cm^3 of RPMI-1640 medium with glutamine, bicarbonate, and 5% fetal calf serum. Cells were dissociated with 0.25% trypsin and 3 m*M* 1,2-cyclohexanediamine tetraacetic acid in *NKT* buffer (137 m*M*, NaCl, 5.4 m*M* KCl and 10 m*M tris*, *pH* 7.4). Experimental cultures were plotted in microtiter plates containing 0.2 cm^3 of growth medium per well at densities of 1000–200000 cells per well.

The assay of cytotoxicity of the free ligand and its complexes against different tumour cells was performed in 96-well microtiter plates based on metabolic reduction of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (*MTT*) by the mitochondrial dehydrogenase of viable cells to a blue formazon product which was estimated spectrophotometrically.

The procedure used in the National Institute's test [31] was adopted to evaluate the activity of free ligand and its complexes against HIV to detect agents acting at any stage of the virus reproductive cycle. Compounds that degenerate or are rapidly metabolized in the culture conditions may not show activity in the screen. All tests were compared with at least one positive (*e.g. AZT* treated) control performed at the same time under identical conditions. In this procedure, the compounds were initially dissolved in *DMSO* and then diluted (1:100) in a cell culture medium before preparing serial half-logarithmic dilutions. T_4 lymphocytes (CEM cell line) were added, and after a brief interval HIV-1 was added resulting into 1:200 final dilution of the compound. Uninfected cells served as basic controls. *MTT* was added to all wells, and cultures were incubated at 37°C in 5% CO_2 atmosphere for 6 days to allow the development of formazon colour by viable cells which were then analyzed spectrophotometrically for quantitative formazon production.

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