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Cationic Ru(II), Rh(III) and Ir(III) complexes containing cyclic π-perimeter and 2-aminophenyl benzimidazole ligands: Synthesis, molecular structure, DNA and protein binding, cytotoxicity and anticancer activity

Amit Kumar [a], Ashish Kumar [a], Rakesh Kumar Gupta [a], Rajendra Prasad Paitandi [a], Krishna Beer Singh [b], Surendra Kumar Trigun [b], Maninder Singh Hundal [c], Daya Shankar Pandey [a, *]

[a] Department of Chemistry, Faculty of Science, Banaras Hindu University, Varanasi 221 005 India
[b] Biochemistry Section, Department of Zoology, Banaras Hindu University, Varanasi 221005, India
[c] Department of Chemistry, Guru Nanak Dev University, Amritsar 143 005, Punjab, India

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ABSTRACT

Synthesis, characterization, DNA and protein binding as well as anticancer activity of the organometallic complexes $[(\eta^6\text{-}C_6H_6)RuCl(APBI)]Cl$ (**1**), $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)RuCl(APBI)]Cl$ (**2**), $[(\eta^6\text{-}C_6Me_6)RuCl(APBI)]Cl$ (**3**), $[(\eta^5\text{-}C_5Me_5)RhCl(APBI)]Cl\cdot H_2O$ (**4**) and $[(\eta^5\text{-}C_5Me_5)IrCl(APBI)]Cl\cdot H_2O$ (**5**) containing 2-aminophenyl benzimidazole (APBI) have been described. The complexes **1**–**5** exhibited strong DNA, protein binding and anticancer activity against cervical cancer (SiHa) cell line. Their binding with calf thymus DNA (CT-DNA) and bovine serum albumin (BSA) have been examined by absorption and emission spectral studies. Strong interactions between complexes and CT-DNA have been affirmed by absorption spectral and EthBr displacement studies, while interaction with BSA *via* static quenching explored by fluorescence titration, synchronous and 3D fluorescence spectroscopy. The interactions between **1**–**5** and DNA has also been scrutinized by ^1H NMR spectral studies using guanosine as a model for DNA. These results have been supported by DFT calculations and molecular docking studies. Cytotoxicity, apoptosis and *in vitro* anticancer activity of **1**–**5** toward SiHa cell line have been investigated by MTT assay and acridine (AO)/ethidium bromide (EthBr) fluorescence staining. Overall results revealed that DNA and protein binding, as well as anticancer activity of **1**–**5** follows the order as **5** > **3** > **2** > **1** > **4**.

1. Introduction

Cancer presents a group of most fatal diseases and among these the lung, liver, stomach, colorectal, ovarian and breast cancer cause most of the cancer deaths worldwide [1]. In this regard, designing and development of effective anticancer drugs exhibiting superior cytotoxicity with strong DNA and protein binding are highly sought-after in contemporary medicinal and pharmaceutical research [2]. Presently, platinum based drugs (*cis*-platin, carboplatin, oxaliplatin) are effectively used in the treatment of various types of cancer [3]. However, these drugs exhibit side effects, severe toxicity, drug resistance and selectivity [4]. Considering the limitations of platinum based drugs, various research groups are actively engaged in exploring anticancer properties of metal complexes other than platinum [5–10]. In this context, ruthenium complexes capable of interacting with DNA and exhibiting potent antitumour properties have drawn special attention [11]. Among these, NAMI-A and KP1019 have shown great promise [12]. In addition, it has been clearly shown that half-sandwich Ru(II), Rh(III) and Ir(III) complexes exhibit strong DNA binding and excellent *in vitro*/*in vivo* anticancer activity [13,14]. At present, organometallic arene ruthenium complexes are taken as an alternative to platinum based drugs owing to their greater anticancer activity, lower side effects, and photochemical activity toward DNA under physiological conditions [15]. Cytotoxicity and anticancer activity of the organometallic drugs have also been attributed to their

* Corresponding author.
E-mail address: dspbhu@bhu.ac.in (D.S. Pandey).

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interaction with proteins, besides DNA. Further, serum albumin is one of the most essential proteins involved in the transportation, distribution, accumulation and excretion of the drugs in tumour cells. Bovine serum albumin (BSA) is used as a model protein to scrutinize binding behaviour and cytotoxicity of the metallo-drugs [16].

Benzimidazole and its derivatives find wide range of applications in pharmaceuticals as antihypertensive, antiinflammatory, antibacterial, antifungal, antiviral, antioxidant, antiulcer, antiproliferative and antitumour activity [17]. Organometallic complexes derived from these ligands exhibit improved antiproliferative activity relative to free ligands and display potent anticancer activity [15a]. These exciting features of the benzimidazole derivatives prompted us to design new organometallic Ru(II), Rh(III) and Ir(III) complexes including it and explore their anticancer activity. During past few years we have been working toward DNA and protein binding, cytotoxicity and *in vitro* antitumour activity of half-sandwich arene Ru(II) and structurally analogous pentamethyl cyclopentadienyl Rh(III) and Ir(III) complexes [18]. In continuation of our earlier work, through this contribution we describe the synthesis, characterization, DNA and protein binding as well as *in vitro* anticancer activity of some cationic arene Ru(II) $[(\eta^6\text{-}C_6H_6)RuCl(APBI)]Cl$ (**1**), $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)RuCl(APBI)]Cl$ (**2**), $[(\eta^6\text{-}C_6Me_6)RuCl(APBI)]Cl$ (**3**) and pentamethylcyclopentadienyl Rh(III) $[(\eta^5\text{-}C_5Me_5)RhCl(APBI)]Cl\cdot H_2O$ (**4**) and Ir(III) $[(\eta^5\text{-}C_5Me_5)IrCl(APBI)]Cl\cdot H_2O$ (**5**) complexes based on 2-(1H-benzo-[d]imidazol-2-yl)aniline. Also, we describe herein their binding behaviour with CT-DNA and BSA, molecular docking study and *in vitro* anticancer activity toward SiHa cell line.

2. Experimental section

2.1. Reagents

Common reagents and solvents were acquired from commercial sources. The solvents were dried and distilled prior to their use following standard literature procedures [19]. Hydrated metal chlorides ($RuCl_3\cdot xH_2O$, $RhCl_3\cdot xH_2O$, $IrCl_3\cdot xH_2O$), hexamethylbenzene, α-terpinene, 1,3-cyclohexadiene, pentamethylcyclo pentadiene, 2-(1H-benzo[d]imidazol-2-yl)-aniline and bovine serum albumin (BSA) have been procured from Sigma Aldrich and used without further purifications. Dulbecco's modified Eagle's medium (DMEM), antibiotics and guanosine were procured from Hi-media, India. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide (MTT), acridine orange and ethidium bromide were obtained from SISCO Research Laboratories, Mumbai, India and CT-DNA from Genei, Bangalore, India. The precursor complexes $[\{(\eta^6\text{-arene})Ru(\mu\text{-}Cl)Cl\}_2$, η^6-arene = C_6H_6, $p\text{-}MeC_6H_4Pr^i$ and $C_6Me_6]$ and $[\{(\eta^5\text{-}C_5Me_5)M(\mu\text{-}Cl)Cl\}_2$, M = Rh(III) and Ir(III)] were synthesized and purified by literature procedures [20].

2.2. General methods

Elemental analyses for C, H and N were performed on Euro-E 3000 Elemental Analyzer at Sophisticated Analytical Instrumentation Facility (SAIF), Central Drug Research Institute (CDRI), Lucknow, India. Infrared and electronic absorption spectra were acquired on a PerkinElmer Spectrum Version 10.03.05 FT-IR and Shimadzu UV-1601 spectrophotometer, respectively. ^{1}H (300 MHz) and ^{13}C (75.45 MHz) NMR spectra were obtained on a JEOL AL300 FT-NMR spectrometer using tetramethylsilane (TMS) as an internal reference. Fluorescence spectra were obtained on PerkinElmer LS-55 Fluorescence Spectrometer equipped with a xenon lamp in a 10.0 mm quartz cell with excitation and emission slit widths of 10 and 4 nm, respectively. Electrospray ionization mass spectrometric data were obtained on a JEOL Accu TOF JMS-T100 LC mass spectrometer.

2.3. Synthesis of $[(\eta^6\text{-}C_6H_6)RuCl(APBI)]Cl$ (**1**)

To a stirring solution of 2-(1H-benzo[d]imidazol-2-yl)aniline (0.209 g, 1.0 mmol) in dichloromethane (20 mL), the precursor complex $[\{(\eta^6\text{-}C_6H_6)Ru(\mu\text{-}Cl)Cl\}_2]$ (0.250 g, 0.50 mmol) was added and resulting suspension stirred overnight at room temperature. It afforded brown coloured precipitate which was filtered, washed thrice with diethyl ether and dried under vacuum. Resulting solid was recrystallized from 1:1 MeOH/DCM mixture. Yield: 64% (0.293 g). Anal. Calc. for $C_{19}H_{17}Cl_2N_3Ru$ (458.98), C, 49.68; H, 3.73; N, 9.15. Found: C, 49.56; H, 3.64; N, 9.04%. ^{1}H NMR (DMSO-d_6, δ ppm): 14.44 (s, 1H, −N*H*), 8.85 (d, J = 9.9 Hz, 1H), 7.98 (d, J = 6.9 Hz, 2H), 7.81 (d, J = 7.8 Hz, 1H), 7.63 (m, 1H), 7.46–7.34 (m, 3H), 6.21 (d, J = 9.9, 1H), 5.68 (s, 6H, C_6H_6). ^{13}C NMR (DMSO-d_6, δ ppm): 148.6 (*C*7), 143.6 (*C*1), 142.3 (*C*8), 134.1, 132.0, 128.9, 125.8, 124.2, 123.2, 121.7, 120.7, 120.2, 112.3, 84.1 (C_6H_6). ESI-MS (Calcd, found, *m*/*z*) 424.0154, 424.0157 [M^+ = **1**−Cl]. FT-IR (KBr pellets, cm^{-1}): 3409 (br), 3060 (br), 1620 (s), 1596 (m), 1539 (w), 1487 (s), 1463 (s), 1447 (s), 1434 (s), 1322 (m), 1283 (m), 1231 (m), 1145 (m), 1117 (m), 1007 (w), 838 (s), 751 (s). UV–vis: (c, 10 μM; EtOH:PBS, 1:99, v:v; pH ~7.4; λ_{max} nm, ε M^{-1} cm^{-1}): 302 (1.86×10^4), 238 (2.05×10^4).

2.4. Synthesis of $[(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)RuCl(APBI)]Cl$ (**2**)

It was prepared following the above procedure for **1** except that $[\{(\eta^6\text{-}p\text{-}MeC_6H_4Pr^i)Ru(\mu\text{-}Cl)Cl\}_2]$ (0.306 g, 0.50 mmol) was used in place of $[\{(\eta^6\text{-}C_6H_6)Ru(\mu\text{-}Cl)Cl\}_2]$. Yield: 68% (0.351 g). Anal. Calc. for $C_{23}H_{25}Cl_2N_3Ru$, (515.04): C, 53.59; H, 4.89; N, 8.15. Found: C, 53.47; H, 4.76; N, 8.04%. ^{1}H NMR (DMSO-d_6, δ ppm): 14.62 (s, 1H, −N*H*), 8.73 (d, J = 9.9 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.66 (s, 1H), 7.61 (d, J = 7.5 Hz, 1H), 7.46–7.41 (m, 4H), 6.08 (d, J = 9.9 Hz, 1H), 5.68, 5.63, 5.53 (d, J = 5.7 Hz, 4H, C_6H_4), 2.18 (q, J = 6.9 Hz, 1H, $(CH_3)_2CH$), 1.60 (s, 3H, CH_3), 0.94 and 0.86 (d, J = 6.9 Hz, 3H, $(CH_3)_2CH$). ^{13}C NMR (DMSO-d_6, δ ppm): 148.1 (*C*7), 143.0 (*C*1), 142.4 (*C*8), 134.1, 131.9, 129.0, 125.8, 124.2, 123.2, 121.6, 121.3, 120.3, 112.4, 83.7, 83.1, 80.8, 78.9 (C_6H_4), 30.1$(CH_3)_2CH$), 22.6 (CH_3), 20.4 $(CH_3)_2CH$), 17.3 $(CH_3)_2CH$). ESI-MS (Calcd, found, *m*/*z*) 480.0780, 480.0750 [M^+ = **2**−Cl]. IR (KBr pellets, cm^{-1}): 3403 (br), 3233 (br), 2963 (s), 1618 (m), 1596 (m), 1534 (w), 1487 (s), 1461 (s), 1444 (s), 1418 (s), 1378 (m), 1324 (m), 1228 (m), 11147 (m), 1105 (s), 878 (s), 761 (s) 748 (s). UV–vis: (c, 10 μM; EtOH:PBS, 1:99, v:v; pH ~7.4; λ_{max} nm, ε; M^{-1} cm^{-1}): 301 (2.01×10^4), 239 (2.44×10^4).

2.5. Synthesis of $[(\eta^6\text{-}C_6Me_6)RuCl(APBI)]Cl$ (**3**)

It was synthesized following the procedure adopted for **1** using $[\{(\eta^6\text{-}C_6Me_6)Ru(\mu\text{-}Cl)Cl\}_2]$ (0.334 g, 0.50 mmol) in place of $[\{(\eta^6\text{-}C_6H_6)Ru(\mu\text{-}Cl)Cl\}_2]$. Yield: 63% (0.342 g). Anal. Calc. for $C_{25}H_{29}Cl_2N_3Ru$ (543.07); C, 55.25; H, 5.38; N, 7.73. Found; C, 55.13; H, 5.27; N, 7.61%. ^{1}H NMR (DMSO-d_6, δ ppm): 13.26 (s, 1H, −N*H*), 7.26 (s, 1H), 7.12 (d, J = 6.4 Hz, 2H), 6.82 (s, 1H), 6.75 (t, J = 6.3 Hz, 2H), 6.62–6.42 (m, 3H), 5.14 (d, 1H), 0.86 (s, 18H, CH_3, C_6Me_6). ^{13}C NMR (DMSO-d_6, δ ppm): 146.1 (*C*7), 140.4 (*C*1), 139.0 (*C*8), 132.6, 129.3, 126.7, 123.9, 122.3, 120.9, 120.5, 119.6, 118.1, 110.7, 90.1 (C_6Me_6), 13.41 (CH_3, C_6Me_6). ESI-MS (Calcd, found, *m*/*z*) 508.1093, 508.1093 [M^+ = **3**−Cl]. FT-IR (KBr pellets, cm^{-1}): 3465 (br), 3246 (m), 3003 (br), 1616 (s), 1594 (m), 1538 (m), 1485 (s), 1462 (s), 1447 (s), 1416 (s), 1323 (m), 1279 (m), 1229 (m), 1101 (m), 1070 (m), 748 (s). UV–vis: (*c*, 10 μM; EtOH:PBS, v:v; 1:99, pH ~7.4; λ_{max} nm, ε M^{-1} cm^{-1}): 304 (1.54×10^4), 238 (2.28×10^4).