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Synthesis and structure–activity relationship of benzylamine supported platinum(IV) complexes†

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A series of benzylamine derivative (BAD) supported platinum(IV) complexes ($PtCl_4(BADs)_2$) have been synthesized. The complexes were tested *in vitro* against the MCF-7 cell line, and the 4-fluoro and 4-chloro containing complexes expressed impressive anticancer activities. Their DNA binding nature for a structure–activity relationship (SAR) study was investigated with physicochemical-indicators (PCI) which categorized them as good intercalators for host–guest chemistry. A mechanism for drug efficacy is proposed by analysis of the resultant viscosity and surface tension of $PtCl_4(BADs)_2$–DNA solutions named as the drug-friccohesity interaction (DFI). The complexes have shown significant antioxidant activity, determined on the basis of free radical scavenging effects due to their terminating action against the reactive species.

Introduction

In the recent past, bioinorganic complexes of platinum have been a fascinating area of research. Advancements in coordination chemistry have emerged from contemporary interests in the fields of agriculture, nanomedicine, MEMS (microelectromechanical systems) and NEMS (nanoelectromechanical systems).[1] Due to increasing interest, cisplatin, a platinum complex (PC), has been used in the treatment of various kinds of human cancer, although side-effects, such as nephrotoxicity and drug resistance, have been a great constraint for its wider uses.[2] Many Pt(II) and Pt(IV) complexes have been synthesized for their anticancer chemistry.[3–6] Pt(II) complexes show several restrictions compared with those of Pt(IV), however, Pt(IV) complexes are also cytotoxic in nature, but have some advantages in comparison to Pt(II) complexes.[7,8] In the higher oxidation state, two extra ligands and the change from planar to octahedral geometry, together with higher kinetic inertness compared to the Pt(II) complexes, make Pt(IV) complexes interesting candidates for the design of new platinum-based anticancer drugs.[9] However, whilst Pt(IV) complexes such as tetraplatin, iproplatin and satraplatin have been in clinical trials, unfortunately, they have not gained clinical approval up to now[3,4] due to high general toxicity, disputed benefit and other factors.[9–11] It could be accepted that Pt(IV) complexes act as prodrugs *via* reductive activation to their reactive Pt(II) complexes.[12,13] Pt(IV) based drugs would have better activity due to extracellular reduction which would lead to deactivation and general toxicity when they are reduced primarily in the cell.[14] Currently, several anticancer drugs have been successfully used to cure cancer diseases,[15] however, such drugs are not effective on solid tumors, for example, breast cancer, whose occurrence has been increasing over the past few years.[16,17] Therefore, cancer chemotherapy against solid tumors is a research thrust area where effective drugs are required. Thus, the selection of benzylamine derivatives in this work as ligands to prepare Pt(IV) complexes and their anticancer screening against the MCF-7 cell line are an endeavour towards obtaining efficient anticancer drugs which may be probed for anticancer activities in order to inhibit cytotoxicity such as is found with other platinum complexes.[3] Also, platinum complexes with benzylamine derivatives as ligands are used for their catalytic or biological applications.[4]

In general, the synthesis of Pt(IV) complexes is a difficult task but their DNA binding activity (DBA) is helpful in the identification of their anticancer nature[8] and is considered a great incentive for their synthesis. DBA has been extensively investigated over the past several decades, due to the extraordinary potentiality in anticancer activities, DNA structural and dependent electron transfer probes, DNA foot-printing, sequence-specific cleaving agents and many others.[18,19] DNA is a primary molecular target of PC based anticancer drugs due to their molecular mechanics and dynamics. Therefore, the PC–DNA interaction ascertains the extent and mode of a drug's chemotherapeutic potential. The DNA targeted PCs act as intercalators

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that can lead to the development of efficient anticancer drugs due to non-covalent binding.[20] Their anticancer activity with DBA is studied with the aim of designing new platinum antitumor agents with low toxicity in the blood stream and non-tumoral tissues.[19,20] Apart from their anticancer activities, transition metal complexes are being widely used as preliminary materials in the development of therapeutic interventions due to their antioxidant potential.[21] Their antioxidant activities are found to prevent ageing and different diseases associated with oxidative damage, due to their terminating action against reactive species such as free radicals.[22] Thus, new anticancer platinum(IV) complexes have been synthesized whose antitumor, DBA and antioxidant studies would be useful in the medicinal field.

Experimental section

Materials and methods

Potassium tetrachloroplatinate (K_2PtCl_4, 99.99%) and BADs (>9.5%) were used (Sigma Aldrich) as received. Structures of the complexes were determined with FTIR (Perkin Elmer) in a KBr palate with polystyrene thin film as a calibration standard. ^1H and ^{13}C NMR spectra were recorded in $(CD_3)_2SO$ (NMR, 99.99%) with a Bruker-Biospin Avance-III 500 MHz FT-NMRspectrometer using TMS as an internal standard for chemical shifts. Mass spectra were obtained with an Agilent Q-TOF LC/MS with ESI+ mode with acetonitrile and water in a 3 : 7 ratio as the mobile phase. Elemental analysis was performed with a Euro vector instrument. UV/Vis spectra were recorded with a Spectro 2060 plus model UV/Vis spectrophotometer over 200–600 nm using a 1 cm path length cuvette in DMSO at 2×10^{-3} M (molar).

Synthesis of platinum complexes

Synthesis of bis(phenylmethanamine)tetrachloroplatinum [MBA]. MBA was prepared by the General procedure given in ESI† with K_2PtCl_4 (0.602 mmol) and benzylamine (1.204 mmol) to give a yellow precipitate. Yield: 0.2676 g, 80%. Elemental analysis, found: C, 30.01; H, 3.0; N, 4.88%. Calcd for $C_{14}H_{18}N_2Cl_4Pt$: C, 30.51; H, 3.29; N, 5.08%. IR (KBr): ν_{max}/cm^{-1} 3259 and 3202 (NH_2), 1496 and 1455 (Ph, C=C), 757.14 (mono substituted Ph), 1079 (C–N), 479.6 (Pt–N), 338 (Pt–Cl). ^1H NMR δH (500 MHz; DMSO-d_6; Me_4Si) 5.273 (2H, s, $PhCH_2NH_2$), 3.577 (2H, s, $PhCH_2NH_2$), 7.383–7.354 (3H, m, PhH), 7.325–7.312 (2H, d, PhH, J = 6.5 MHz). ^{13}C NMR δC (500 MHz; DMSO-d_6; Me_4Si) 50.18 (C1), 138.49 (C2), 129.23 (C4 and C6), 128.95 (C3 and C7) and 128.21 (C5), +ve ESI-MS: m/z 552.1544 [M + 1] (calc. for $[C_{14}H_{18}N_2Cl_4Pt]$ = 551). UV/Vis = λ_{max} (DMSO)/nm 270 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 2432).

Synthesis of bis(((2-chlorophenyl)methanamine)tetrachloroplatinum) [M2CBA]. M2CBA was prepared with K_2PtCl_4 (0.602 mmol) and 2-chlorobenzylamine (1.204 mmol) which gave a yellow precipitate. Yield: 0.2636 g, 70%. Elemental analysis, found: C, 26.95; H, 2.4; N, 4.12%. Calcd for $C_{14}H_{16}N_2Cl_6Pt$: C, 27.12; H, 2.6; N, 4.52%. IR (KBr): ν_{max}/cm^{-1} 238.8 and 3198.0 (NH_2), 1475 and 1447 (Ph, C=C), 753 (mono substituted Ph), 1055 (C–N), 679.6 to 630 (C–Cl), 446.94 (Pt–N), 346 (Pt–Cl). ^1H NMR δH (500 MHz; DMSO-d_6; Me_4Si) 5.373 (2H, s, $PhCH_2NH_2$), 3.890 (2H, s, $PhCH_2N$), 7.629–7.615 (1H, d, PhH, J = 7 MHz), 7.466–7.451 (1H, d, PhH, J = 7.5 MHz), 7.408–7.354 (2H, m, PhH). ^{13}C NMR δC (500 MHz; DMSO-d_6; Me_4Si) 47.77 (C1), 135.72 (C2), 132.91 (C3), 131.05 (C4), 129.77 (C5), 127.82 (C6) and 130.14 (C7). +ve ESI-MS: 622.0361 [M + 2] (calc. for $[C_{14}H_{16}N2Cl_6Pt]$ = 620). UV/Vis = λ_{max} (DMSO)/nm 265 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 1775).

Synthesis of bis(((3-chlorophenyl)methanamine)tetrachloroplatinum) [M3CBA]. Reaction of K_2PtCl_4 (0.602 mmol) and 3-chlorobenzylamine (1.204 mmol) produced a yellow precipitate. Yield: 0.3015 g, 80%. Elemental analysis, found: C, 27.00; H, 2.36; N, 4.20%. Calcd for $C_{14}H_{16}N_2Cl_6Pt$: C, 27.12; H, 2.6; N, 4.52%. IR (KBr): ν_{max}/cm^{-1} 3238.8 and 3193.9 (NH_2), 1475 and 1430 (Ph, C=C), 798.88 (mono substituted Ph), 1083.7 (C–N), 708.16 to 683.67 (C–Cl), 438.78 (Pt–N), 348 (Pt–Cl). ^1H NMR δH (500 MHz; DMSO-d_6; Me_4Si) 5.363 (2H, s, $PhCH_2NH_2$), 3.653 (2H, s, $PhCH_2N$), 7.588–7.393 (4H, m, PhH). ^{13}C NMR δC (500 MHz; DMSO-d_6; Me_4Si) 49.66 (C1), 141.11 (C2), 130.70 (C3), 133.41 (C4), 129.07 (C5), 128.01 (C6) and 127.98 (C7), +ve ESI-MS: m/z 622.0364 [M + 2] (calc. for $[C_{14}H_{16}N_2Cl_6Pt]$ = 620). UV/Vis = λ_{max} (DMSO)/nm 270 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 1987).

Synthesis of bis(((4-chlorophenyl)methanamine)tetrachloroplatinum) [M4CBA]. M4CBA was prepared with K_2PtCl_4 (0.602 mmol) and 4-chlorobenzylamine (1.204 mmol) to give a yellow precipitate. Yield: 0.3088 g, 82%. Elemental analysis, found: C, 26.98; H, 2.41; N, 4.40%. Calcd for $C_{14}H_{16}N_2Cl_6Pt$: C, 27.12; H, 2.6; N, 4.52%. IR (KBr): ν_{max}/cm^{-1} 3230.6 and 3193.9 (NH_2), 1491 and 1447 (Ph, C=C), 802.04 (mono substituted Ph), 1096–1018.4 (C–N), 842.46 (C–Cl), 495.92 (Pt–N), 352 (Pt–Cl). ^1H NMR δH (500 MHz; DMSO-d_6; Me_4Si) 5.313 (2H, s, $PhCH_2NH_2$), 3.623 (2H, s, $PhCH_2N$), 7.518–7.505 (2H, d, PhH, J = 6.5 MHz), 7.452–7.433 (2H, d, PhH, J = 9.5 MHz). ^{13}C NMR δC (500 MHz; DMSO-d_6; Me_4Si) 49.42 (C1), 137.44 (C2), 132.81 (C5), 131.16 (C3 and C7), 128.81 (C4 and C6). +ve ESI-MS: m/z 622.0362 [M + 2] (calc. for $[C_{14}H_{16}N_2Cl_6Pt]$ = 620). UV/Vis = λ_{max} (DMSO)/nm 265 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 1836).

Synthesis of bis(((4-fluorophenyl)methanamine)tetrachloroplatinum) [M4FBA]. K_2PtCl_4 (0.602 mmol) and 4-fluorobenzylamine (1.204 mmol) gave a yellow precipitate. Yield: 0.2571 g, 72%. Elemental analysis, found: C, 28.54; H, 2.55; N, 4.56%. Calcd for $C_{14}H_{16}N_2Cl_4F_2Pt$: C, 28.64; H, 2.75; N, 4.77%. IR (KBr): ν_{max}/cm^{-1} 3259 and 3202 (NH_2), 1496 and 1455 (Ph, C=C), 757.14 (mono substituted Ph), 1079.6 (C–N), 834.1 (C–F), 475.51 (Pt–N), 349 (Pt–Cl). ^1H NMR δH (500 MHz; DMSO-d_6; Me_4Si) 5.301 (2H, s, $PhCH^2NH^2$), 3.625 (2H, s, $PhCH^2N$), 7.449–7.438 (2H, d, PhH, J = 5.5 MHz), 7.23–7.195 (2H, t, PhH, J = 17.5 MHz). ^{13}C NMR δC (500 MHz; DMSO-d_6; Me_4Si) 49.41 (C1), 134.81 (C2), 131.39 (C3 and C7), 115.53 (C4 and C6), 161.09 (C5). +ve ESI-MS: m/z 590.097 [M + 3] (calc. for $[C_{14}H_{16}N_2Cl_4F_2Pt]$ = 587.183). UV/Vis = λ_{max} (DMSO)/nm 265 ($\varepsilon/dm^3\ mol^{-1}\ cm^{-1}$ 2229).

In vitro anticancer activity

Cell viability was estimated colorimetrically using 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfobenzenesulfonate, SRB assay as standard.[23]

Cell lines and culture conditions

Human breast cancer cell lines (MCF-7) were obtained from NCI, USA, and grown in minimal essential medium (MEM). Eagles media were supplemented with 10% heat inactivated fetal bovine serum (FBS, Sigma-Aldrich), 2 mM L-glutamine and 1 mM sodium pyruvate (Hyclone) in humidified CO_2 incubators.

Assay of cytotoxicity in cancer cell lines

The cytotoxicities of platinum complexes were determined by SRB assay where $\approx$5000 cells were seeded into each well of a 96 well clear flat bottom polystyrene tissue culture plate and incubated for 2 h in MEM. An additional 190 μL cell suspension was added in each well containing 10 μL test sample in 10% DMSO with 10 μL Adriamycin (doxorubicin) as a positive drug control. Each experiment was carried out in 3 replicate wells. After an incubation of 48 h, 100 μL of 0.057% SRB solution (w/v) was added into each well. Then 200 μL of 10 mM Tris base solution (pH 10.5) was added into each well and the wells shaken smoothly. The cell viability was assayed by absorption at 510 nm with a microplate reader. The experiments were repeated thrice with 5 replicates each time and 99% reproducibility was obtained.

DNA binding

CT-DNA (Sigma) was used as received (analytical grade). Tris–HCl buffer (10 M, pH = 7.2) was prepared in Milli-Q water, and used for DNA stock solution preparation, absorption titration, viscosity, surface tension and zeta potential measurements. The DNA concentration was determined using an absorption spectrophotometer as a molar absorptivity (6600 M^{-1} cm^{-1}) at 260 nm.[24,25] The CT-DNA in buffer gave a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9, indicating the DNA was free of protein.[26–29] Absorption titrations in Tris-buffer were performed at 10, 30, 50, 70 and 90 μM, to which DNA stock solutions (50 μM) were added, (ri = [complex]/[DNA] = 0.2, 0.6, 1, 1.4 and 1.8). The $PtCl_4(BADs)_2$–DNA solutions were incubated at room temperature for 15 min before recording the absorption spectra. To elucidate their binding strength, an intrinsic binding constant (K_b) with CT-DNA was obtained by monitoring a change in absorbance of the DNA with increasing amounts of $PtCl_4(BADs)_2$,[30] and calculated with the following equation:

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_b} = \frac{[DNA]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b(\varepsilon_b - \varepsilon_f)}$$

ε_a, ε_f and ε_b are the apparent, free and bound complex extinction coefficients, respectively. ε_f was determined using a calibration curve of an isolated metal complex, with the Beer–Lambert law. ε_a was determined as the ratio between the measured absorbance and $PtCl_4(BADs)_2$ concentration such as A_{obs}/[Pt]. A plot of [DNA]/($\varepsilon_a - \varepsilon_f$) *vs.* [complex] produced a slope of 1/($\varepsilon_b - \varepsilon_f$), and a Y intercept equal to 1/K_b($\varepsilon_b - \varepsilon_f$); K_b is the ratio of the slope to the Y intercept.[30]

Viscosity and surface tension measurements were conducted with a Borosil Mansingh survismeter, BMS.[31,32] For an estimation of their relevance in inducing structural changes in medium, the temperature was controlled at 298.15 K with ±0.01 °C by an auto temperature control LAUDA ALPHA RA 8 thermostat. About 15 to 20 measurements for each composition were made to assure high precision. Flow time and pendent drop were measured with a digital stopwatch and drop counter, respectively, and were repeated five times to obtain average flow times and numbers of pendent drops. The data are presented as $(\eta/\eta^0)^{1/3}$ *versus* binding ratio; η is the dynamic viscosity of DNA with complexes while the η^0 is the viscosity of the DNA mixture in buffer.[33]

Conductance and zeta potentials of DNA solutions with and without complexes were measured with a LABINDIA, PICO+ conductivity and Microtrac Zetatrac, U2771, DLS, respectively at 250 °C. 0.1, 0.01 and 0.001 M aqueous KCl solutions having conductances 12.88, 1.413 and 147 μS cm^{-1} respectively, were used for calibration of the conductivity meter. An auto suspended solution of alumina suspension (400-206-100) was used as a zeta potential standard. Initially, for DMSO + Tris buffer a set-zero was made for the zeta potential. For both the measurements, the DNA concentration was kept constant while the concentration of the $PtCl_4(BADs)_2$ was varied from 50 to 400 μM with 50 μM intervals.

DPPH free radical antioxidant activity

Antioxidant or scavenging activities were determined on the basis of a free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effect.[34] Stock solutions of 1000 μM of the tested samples and DPPH$^\bullet$ (0.002%) were prepared with DMSO–water (1 : 1). To prepare samples, 1 mL of 0.002% DPPH$^\bullet$ solution was added into 1 mL of complex of 50, 100, 150, 200, 250, 300, 350 and 400 μM separately. The reaction mixtures were thoroughly mixed by shaking the test tubes vigorously and incubated at 25 °C for 60 min keeping in a water bath in the dark. Absorbance was measured at 517 nm in a spectro 2060 plus modeled UV/Vis spectrophotometer. The radical scavenging activity was determined as a result of the decrease in absorbance of DPPH,[35] and calculated with the following formula:

$$\text{Scavenging activity (\%)} = (A_0 - A_S/A_0) \times 100$$

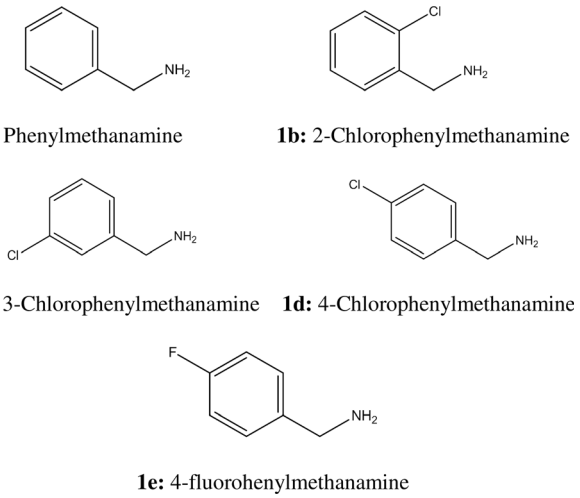
A_S is the absorbance of DPPH with a tested compound and A_0 is the absorbance of DPPH without a tested compound (control). Radical scavenging potential was expressed as an EC_{50} value and represents the test compound concentration at which 50% of the DPPH radicals are scavenged. The data calculated for antioxidation are presented as means ±SD of three determinations.

Results and discussion

Synthesis

The synthesis of $PtCl_4(BADs)_2$ was achieved by reaction of K_2PtCl_4 with different BADs (Fig. 1a–e) in ethanol–aqueous solution in a 1 : 2 ratio over 24 h (reaction, Scheme 1).

For their synthesis, K_2PtCl_4 and BADs were reacted in a ratio of 0.602 mmol to 1.204 mmol respectively and the complexes

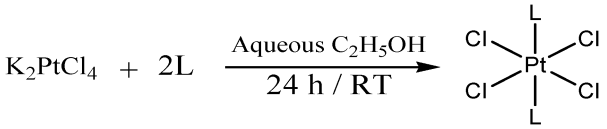


1a: Phenylmethanamine **1b:** 2-Chlorophenylmethanamine

1c: 3-Chlorophenylmethanamine **1d:** 4-Chlorophenylmethanamine

1e: 4-fluorohenylmethanamine

Fig. 1 (a–e) Structures of the ligands (L = BADs).



Scheme 1 Synthesis of Pt(IV) complexes where L = BADs (Fig. 1a–e).

were formed as yellow powders. The reaction of tetrachloroplatinate (a compound of platinum +2) with a simple primary amine, to give a compound of platinum +4, is an oxidation process which is carried out in the presence of an oxidant, but many coloured complexes of Pt(IV) have been synthesized in aqueous organic solvent without adding any oxidant.[5] For structure determination, elemental analysis, FTIR, ^{1}H and ^{13}C NMR, LC-MS and UV/Vis were used and data are given in experimental section. The platinum nitrogen coordinate bond vibrations were from 495.92 to 438.78 cm^{-1}.[36,37] The ESI +ve mass spectra had peaks of M + 1 for MBA, M + 2 for M2CBA, M3CBA, M4CBA and M + 3 for M4FBA, confirming their molecular mass. The UV/Vis absorption from 265 to 270 nm and 1H NMR coupling constant within 5 to 9 MHz confirmed their *trans* and octahedral geometry.[5,6,38] The elemental analysis, FTIR, ^{1}H and ^{13}C NMR, LC-MS and UV/Vis data for structural illustration were in close agreement.

Anticancer activity

The complexes were tested *in vitro* against the MCF-7 human breast tumor cell line in comparison to Adriamycin (ADR) by colorimetric micro culture 2-(3-diethylamino-6-diethylazaniumylidene-xanthen-9-yl)-5-sulfo-benzenesulfonate (SRB) assay.[23] The data were also compared with cisplatin whose data were taken from the literature.[38] This assay has several advantages over the tetrazolium assays, being able to distinguish between cytostatic effects because the drug decreases the rate of cell proliferation and a cytotoxic effect is a true decrease in the number of viable cells.[39] The MCF-7 interaction activity for 10, 20, 40 and 80 μg mL^{-1} $PtCl_4(BADs)_2$ was plotted (Fig. 2).

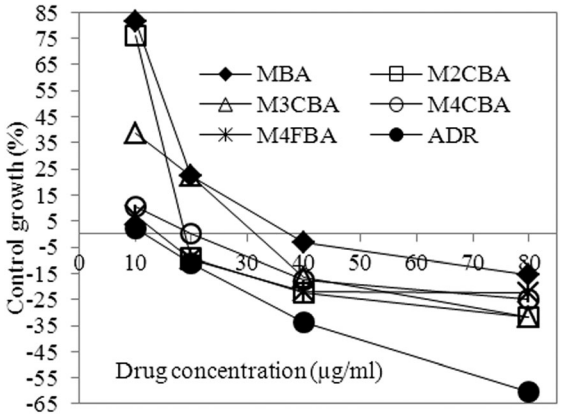


Fig. 2 Growth curves for a human breast cancer cell line (MCF-7).

We estimated the GI_{50}, TGI and LC_{50} in μg mL^{-1} as a response parameter (Table 1) which inferred a 50% growth inhibition, resultant total growth inhibition and a net loss of 50% cells after treatment respectively.

The less than 10 μg mL^{-1} of GI_{50} depicts anticancer activity with respect to ADR and cisplatin.[38] Data analysis for M4CBA and M4FBA ($GI_{50} < 10$) showed impressive anticancer activity as compared to standard ADR and cisplatin which have GI_{50} values <10 (Table 1). The GI_{50} = 13.4 and 11.9 values of M2CBA and M3CBA respectively, were near to standard against the MCF cell line while MBA, having GI_{50} = 21.9, is far from anticancer activity. This data analysis led us to infer that the anticancer nature of $PtCl_4(BADs)_2$ is associated with the *o*-, *m*- and *p*-halide substitution of the benzylamine ligands as MBA < M2CBA < M3CBA < M4CBA = M4FBA. Several platinum complexes have been synthesized for the curing of the cancer but our focus was to investigate the anticancer activity of synthesized platinum(IV) complexes with benzylamine derivatives as ligands. However, cytotoxicity studies of the ligands only are being pursued in our laboratory and will soon be communicated in the next paper of the series on benzylamine derivatives. Thus, the complexes most probably will have bright prospects as effective anticancer drugs, especially against solid tumors like other Pt(IV) complexes.[4,9]

DNA binding activities

DBA is a recent mechanism for SAR studies of metallic compounds, depicted in absorption spectra. Thus, the $PtCl_4(BADs)_2$ were mixed with CT-DNA which resulted in changes in absorbance. Generally, the extent of hypochromism reveals the intercalative binding strength of complexes can be attributed to the interaction with DNA bases, as a characteristic for intercalation.[40,41] Similarly, a hyperchromic effect might be ascribed to an external contact or to a partial uncoiling of DNA structure, exposing more bases of the DNA due to electrostatic binding.[42,43] We observed a significant hypochromic effect in absorption titrations of interacting DNA with MBA, M2CBA, M3CBA, M4CBA and M4FBA which exposes their intercalation[40,41] with the base pairs of DNA (Fig. S1–S5, ESI†). With UV spectrophotometric titration, the $PtCl_4(BADs)_2$–DNA

Table 1 LC_{50}, TGI and GI_{50} in μg mL^{-1} against the MCF-7 cell line and antioxidant EC_{50} values for $PtCl_4(BADs)_2$

Entry	Complex	LC_{50}	TGI	GI_{50}	EC_{50} (μM)
1	MBA	>80	57.3	21.9	80
2	M2CBA	77.6	45.5	13.4	—
3	M3CBA	>80	46.7	11.9	—
4	M4CBA	>80	42.7	<10	150
5	M4FBA	>80	40.8	<10	195
6	ADR	62.4	30.6	<10	
7	Cisplatin[38]	>30	>30	<10	

binding constants (K_b) were 1×10^4, 1.5×10^4, 1×10^4, 1×10^4 and 1.33×10^4 M^2 cm for MBA, M2CBA, M3CBA, M4CBA and M4FBA respectively. The K_b values predict the stronger interactions caused by M2CBA and M4FBA whose intercalating strengths depend on the size and electron densities of interacting aromatic rings including an amine group, due to a combined effect of hydrophobic and hydrophilic interactions.[40–43] The DBA of a ligand such as benzylamine was investigated (Fig. S6, ESI†) and the absorption attributed interaction with DNA was lower in comparison to its complex (Fig. S1, ESI†). Thus the complex is more strongly intercalated with DNA than the free ligand. Table S1 (ESI†) presents a comparative study of λ_{max} for MBA, M2CBA, M3CBA and M4CBA which is 240 nm and M4FBA which is 245 nm without DNA, while with DNA they showed an almost negligible absorbance at these wavelengths. Thus, their nature is changed entirely on binding with DNA for the development of a concept of SAR for the understanding of their anticancer mechanism.

Viscosity

For detailed DNA binding patterns, spectrophotometric studies are supported by fluid dynamics in the form of viscosity data (Fig. 3) as the most critical test for a classical intercalation model. The viscosity is a classical intercalative mode in the case of a DNA solution which is increased due to an increase in overall DNA length[44] with an expression of their molecular mechanics and dynamics expressed as:

$$E_{covalent} = E_{bond} + E_{angle} + E_{dihedral}$$

The DNA and complexes have their specific covalent bond lengths, bond angles and dihedral energies which develop a critical DFI model as is given in the above equation and discussed below. Thus, a partial non-classical intercalation produces a bend or kink in the DNA helix that reduces its effective length concomitantly. It is well known that with cisplatin, a decrease in relative viscosity is explained due to covalent binding which causes shortening in an axial length of the double helix DNA.[45] A partial intercalator decreases the relative viscosity by reducing an axial length, whereas a classical organic intercalator such as ethidium bromide increases the relative viscosity by increasing an axial length of the DNA.[46,47] To observe covalent binding or classical organic interaction, their relative specific viscosities $(\eta/\eta^0)^{1/3}$ (η^0 and η are specific viscosity contributions of DNA with and without $PtCl_4(BADs)_2$,

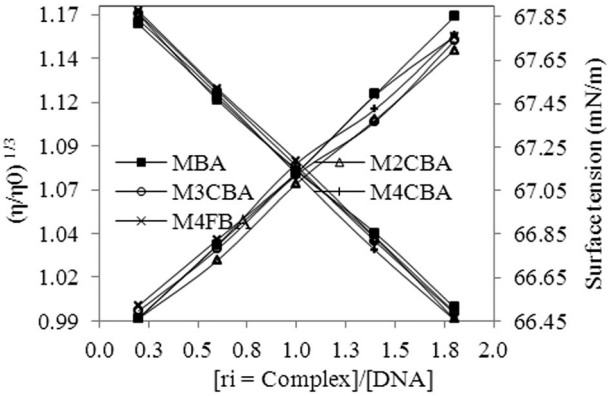


Fig. 3 Effect of increasing amounts of $PtCl_4(BADs)_2$ on viscosity and surface tension of CT-DNA (5×10^{-5} M), a drug efficacy study.

respectively) were plotted against $1/R$ (R = [DNA]/[complex] = 0.2, 0.6, 1.0, 1.4, 1.8) (Fig. 3).

An increase in concentration of $PtCl_4(BADs)_2$ increased the viscosity of DNA (Fig. 3) which is explained in terms of the behaviour of the $PtCl_4(BADs)_2$ as DNA intercalators. For ethidium bromide, the relative viscosity[48] of DNA is increased with a slope varying from 0 to 0.9448 whereas with 0.0351, 0.0317, 0.0312 and 0.0327 and 0.0318 slopes, the relative viscosity of DNA increased for MBA, M2CBA, M3CBA, M4CBA and M4FBA respectively. A decrease in slope value, maybe due to an interaction of $PtCl_4(BADs)_2$ with DNA, made DNA longer, which is reasonably explained as due to another interaction occurring between DNA and $PtCl_4(BADs)_2$. The measured DNA binding constant for $PtCl_4(BADs)_2$ is lower than that of ethidium bromide. The viscosity of free ligand (benzylamine) with DNA was measured and found to be lower than that of the complex, from which it was inferred that interaction of DNA with free ligand is weaker as compared to that with complex. Therefore, a higher increase in viscosity of $PtCl_4(BADs)_2$–DNA as compared to ethidium bromide and free ligand could have produced a lower binding constant which clearly ascertained intercalation.

Surface tension

Apart from the viscosity, the surface tension is an interaction probing thermodynamic indicator to depict the disruption of intramolecular forces existing in DNA, and acts as evidence for stronger interactions with higher viscosity. This force disruption is measured as a decrease in surface tension, and therefore the surface tension values of a DNA solution with increasing amounts of $PtCl_4(BADs)_2$ ($1/R$ = 0.2, 0.6, 1.0, 1.4, 1.8) (Fig. 3) have been determined. The resultant surface tension of $PtCl_4(BADs)_2$–DNA decreased which confirmed that cohesivity or intramolecular interaction of DNA is lost on interaction with the $PtCl_4(BADs)_2$.

Conductivity and zeta potential

The conductivity and zeta potential analyses for $PtCl_4(BADs)_2$–DNA interactive complexes were key factors for the conformational

Table 2 Conductivities (μS) of interacting and pure DNA[a]

Entry	Conc. (μM)	MBA	M2CBA	M3CBA	M4CBA	M4FBA
1	50	772	689	678	586	617
2	100	648	654	605	555	594
3	150	601	594	555	518	569
4	200	547	547	522	480	525
5	250	510	523	480	411	487
6	300	471	469	446	420	444
7	350	446	434	413	392	406
8	400	404	399	366	355	354

[a] DNA solution of 50 μM with 878 μS cm^{-1} conductance.

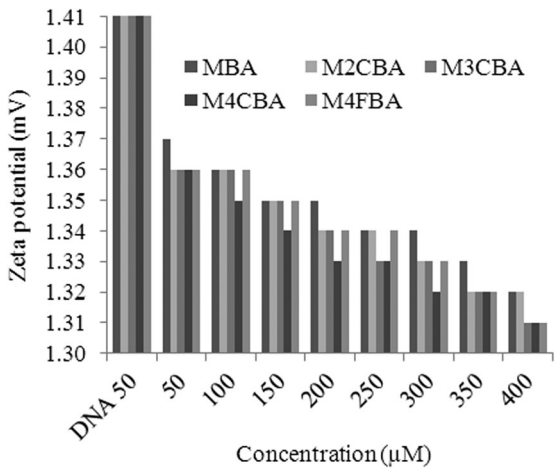


Fig. 4 Zeta potential of DNA (5×10^{-5} M) in the absence and presence of increasing amounts of $PtCl_4(BADs)_2$.

behaviour of an isolated DNA chain with the complexes. A DNA solution of 50 μM had an 878 μS cm^{-1} conductivity while the addition of increasing amounts of $PtCl_4(BADs)_2$ decreased the conductivities (Table 2). DNA molecules are negatively charged due to phosphate groups, but by interaction with $PtCl_4(BADs)_2$, the negative charge density is decreased due to the positively charged metal which balances the charge density.

With complexes, the zeta potential of the resultant $PtCl_4(BADs)_2$–DNA also decreased (Fig. 4), from which it could be inferred that an increase in Coulombic interaction and the disassociation of counterions in DNA was limited.

The decrease in conductivity and zeta potential confirmed strong interactions where the DNA could structurally be modified as a result of this interaction.

Drug efficacy studies or DFI with DNA binding

The intrinsic structural domain of an activity of a drug is related to its therapeutic effect noted as the relative ability of a drug–receptor complex with a maximum functional response. Viscosity data in monitoring drug efficacy are gaining momentum for a drug intake for trajectory areas because of their functional domains. Initially, an effective anticancer drug breaks cohesivity of DNA (distortion of intermolecular forces) and decreases surface tension; secondly, the nitrogen atoms of adenine and thymine or guanine and cytosine are co-ordinately or covalently bonded with platinum through intrastrand or interstrand binding,[49–52] producing higher viscosities. Thus, the drug efficacy or intrinsic activity is dependent on its disruptive activity as well as fluidity, and both should be in an inverse manner because at first, the drug breaks DNA cohesivity and then it interacts. Such behaviour of the $PtCl_4(BADs)_2$–DNA interaction has shown the higher viscosities and lower surface tension of a $PtCl_4(BADs)_2$–DNA solution (Fig. 3). Thus, disruption of cohesivity, and the development of interaction bonding or intercalation can be retrieved from the DNA–drug interacting mechanism[49–52] leading to the development of a new model, referred to as DFI. DFI has inferred a decrease in cohesivity as well as an increase in interaction (Fig. S6, ESI†) which is supported by the anticancer activities of $PtCl_4(BADs)_2$ (Table 1). To our best understanding, in DNA–complex interactions, such a comparative study of surface tension and viscosity as DFI has never been reported before; this could be beneficial and useful in identifying the anticancer nature of such metal complexes.

Antioxidant activities

Antioxidant activities were analysed by the scavenging effect of a stable free radical di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH) as per standard procedure,[34,35] with a slight modification. The percentage scavenging activity of $PtCl_4(BADs)_2$ was determined in a concentration-dependent mode with a comparison with the DPPH free radical's absorption at 517 nm.[34,35] The DPPH free radical's absorption at 517 nm in DMSO–water was 0.440 to 0.445, a maximum. The complexes from 50 to 400 μM at an interval of 50 μM, showed a decrease in absorption, from which their antioxidant activities could be inferred[34] (Fig. 5).

The EC_{50} values for MBA, M4CBA and M4FBA (Table 1) and M2CBA and M3CBA showed a 35.31 and 29.61% maximum scavenging effect at 50 and 150 μM respectively.

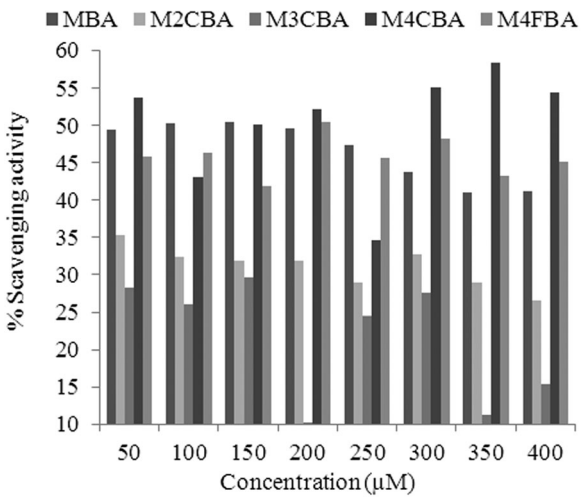


Fig. 5 Free radical scavenging activities of $PtCl_4(BADs)_2$.

Conclusions

The synthesized platinum(IV) complexes have shown effective activities against an MCF cell line. Especially, the complexes have exhibited prominent anticancer activity against a solid tumor cell line which correlates with their CT-DNA interaction analysis. Comparative studies of their viscosity and surface tension for DBA have suggested the stronger intercalating nature of the complexes. Their isotropic zeta potential and conductance data have revealed stronger DNA interactions as a great support in favour of SAR. The complexes have also shown significant free radical scavenging activities behaving as antioxidants. Further investigations into diffusion coefficients, structural modifications and other biological studies to determine their role in apoptotic and proliferation pathways in tumor cell lines are in progress.

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