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Effect of ring substitution on synthesis of benzimidazolium salts and their silver(I) complexes: characterization, electrochemical studies and evaluation of anticancer potential

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Abstract
A series of bidendate 5,6-dimethyl benzimidazolium-based *N*-heterocyclic carbene (NHC) proligands and their silver complexes were synthesized. The synthetic approaches to the proligands were constrained by the methyl substituents, which impose a significant impact on the reactivity according to their sigma electron-donating abilities. The corresponding silver(I) complexes were obtained by in situ deprotonation of the NHCs and characterized by physicochemical and spectroscopic methods. In addition, a single-crystal X-ray diffraction study of complex **C5** revealed its dinuclear structure. Irreversibility of redox events was observed in electrochemical studies of these complexes. In vitro anticancer studies of the azolium salts and their silver(I) complexes against human breast cancer (MDA-MB-231), colon cancer (HCT-116) and normal endothelial (EA.hy926) cells revealed that all the compounds are more cytotoxic to cancer cells than to normal cells and the complexes are more potent than their corresponding NHC proligands. Increased chain length, the presence of methyl substituents on the benzimidazole ring and aryl linker and two silver centres all enhance the biopotency of these complexes.

Introduction

In contemporary medicinal and material sciences, *N*-heterocyclic carbenes (NHCs) have proved to be valuable species for the design of a broad range of coordination complexes with medicinal and catalytic applications by virtue of their strong σ-donor and weak π-acceptor features and comparatively facile synthesis [1–3]. NHC transition metal complexes can be obtained by various approaches. The simplest strategy involves the in situ deprotonation of the azolium salt in the presence of a basic anion generated from a transition metal precursor [4]. A variety of silver(I) complexes have been synthesized by this method [5–10]. Numerous silver complexes with anticancer potential against various cancer cell lines have been reported [6, 8, 9]. The strong anticancer activity of M-NHC (M = Au, Cu, Ag, Pd) complexes (afforded from azolium salts having an unsubstituted benzene ring) against various cancer cell lines including breast cancer (MB157), renal cancer (Caki-1), ovarian cancer (OVCAR-3) and cervical cancer (HeLa) has been reported [11, 12]. Iqbal and co-workers reported the anticancer potential of dinuclear silver-meta/para-xylyl-linked bis-benzimidazolium complexes (having short Ag–Ag distances) against human leukaemia (HL-60) and colon (HCT-116) cancer cell lines [8, 12]. Their study revealed that metal–metal interactions in both gold(I) and silver(I) complexes (shorter than the sum of their van der Waals radii) are strong enough to dominate the repulsive forces between cationic metal centres, thus leading to substantial cationic NHC networks [13, 14].

The anticancer potential of silver(I) complexes is strongly influenced by their lipophilicity/hydrophilicity which in turn is controlled by the steric and electronic character of the substituents. In contrast to other metal coordination

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complexes which generally target the DNA of cancer cells, Ag/Au–NHC complexes modulate the mitochondria-induced apoptosis by directly targeting the infected cell's mitochondrial membrane [15–18]. A variety of drugs are already available for treatment of cancer, but most of these drugs have severe side effects. Hence, current research is targeted towards the discovery of new metallodrugs with little or no side effects. Previously, we have investigated the anticancer potential of silver(I) complexes derived from unsymmetrical (5-substituted) benzimidazoles [19]. The study revealed that the presence of substituents has a significant impact on synthesis and activity of silver complexes. This article describes a further extension of our previous study. We report the synthesis, characterization, electrochemical and anticancer properties of a series of benzene ring disubstituted benzimidazolium-based silver(I) complexes.

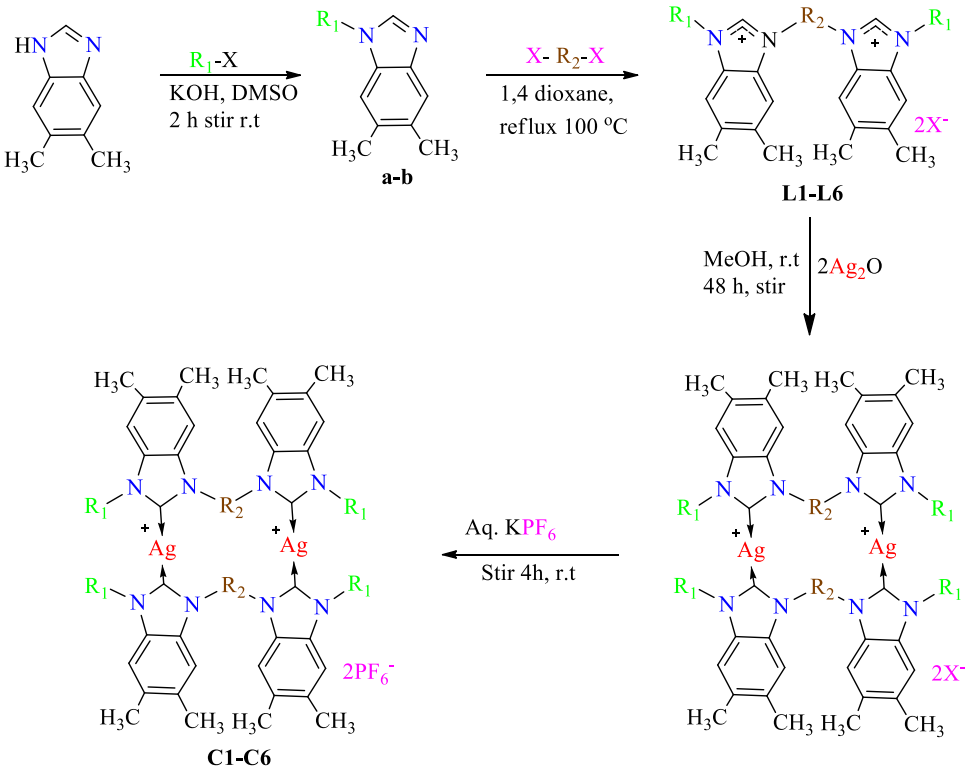
Results and discussion

NHC precursors and their dinuclear silver(I) complexes were synthesized by following a modified literature protocol [7, 9]. This involved the *N*-alkylation of 5,6-dimethyl benzimidazole by reaction with one equivalent of alkyl halide followed by second alkylation with half an equivalent of alkyl halide under reflux for in situ generation of the free carbene, to which two equivalents of silver oxide were added for metallation. For easy handling of the end product, the halide counterions were replaced in situ with hexafluorophosphate by adding two equivalents of aqueous KPF_6. All the silver complexes were purified by washing with water and obtained in powder form. Scheme 1 shows the overall synthesis.

Characterization

FTIR spectra of all the species in Scheme 1 were recorded. A strong band at 3402–3424 cm^{-1} assigned to C_{aliph}–N_{benzim} stretching vibrations was observed in the spectra of compounds **a** and **b** and also of the proligands (**L1**–**L6**), which in some cases appeared broad (see supplementary files Figures S3–S10) due to overlap with residual water involved in H–O–H–X^- interactions (as indicated from their ^{1}H NMR spectra) [7]. A specific pattern of peaks at 1350–1500 cm^{-1} in the spectra of **a** and **b** and **L1**–**L6** can be ascribed to C=N stretching vibrations of the benzimidazole ring, which are less intense for **L1**–**L6** due to the presence of one more electron-donating alkyl group. These bands appear as a strong and specific "four fingers" pattern for the silver complexes (**C1**–**C6**) where conjugation is absent due to Ag(I)–NHC coordination [10, 20] (see supplementary files Figures S11–S16). A very sharp signal at 900–675 cm^{-1} can be ascribed to C–H out of plane (oop) bending vibrations of the aromatic rings, which is a characteristic of methyl substitution to the ring (see supplementary files Figures S11–S16).

NMR studies provided further evidence for the successful synthesis of all the compounds. Synthesis of the NHC



Scheme 1 Synthesis of *N*-alkylated benzimidazoles (**a**–**b**), bis-benzimidazolium salts (**L1**–**L6**) and Ag(I)–NHC complexes (**C1**–**C6**). (**a** R_1 = propyl; **b** R_1 = butyl; **L1**, **C1**, R_1 = propyl, R_2 = butyl; **L2**, **C2**, R_1 = propyl, R_2 = pentyl; **L3**, **C3**, R_1 = propyl, R_2 = m-xylyl; **L4**, **C4**, R_1 = butyl, R_2 = butyl; **L5**, **C5**, R_1 = butyl, R_2 = pentyl; **L1**, **C1**, R_1 = butyl, R_2 = m-xylyl)

proligands (**L1**–**L6**) was confirmed by changes in the chemical shifts compared to **a** and **b** (Figures S16–S17) together with the presence of new signals arising from one more alkyl group and the distinctive signal for the most deshielded proton and carbon (N**C**H**N**) between 9 and 12 ppm in the ^{1}H NMR and 140–145 ppm in ^{13}C NMR spectra, respectively [21] (see supplementary files Figures S19–S30). Similarly, the synthesis of the Ag(I) complexes was confirmed by changes in both the ^{1}H and ^{13}C NMR spectra together with the loss of the signal for the most deshielded proton (NC**H**N) and shift of the carbon (N**C**HN) signal to 180–190 ppm (see supplementary files Figures S31–S42) [22]. Two-dimensional (HSQC) ^{1}H–^{13}C correlation NMR spectra were used to help assign the ^{1}H and ^{13}C signals [23] (see supplementary files Figures S43–S48). Furthermore, the presence of phosphorous and fluorine in the complexes having PF_6^- counter ions was confirmed by the observed heptet and doublet from − 144 to − 145 ppm and from − 71 to − 73 ppm in the ^{31}P and ^{19}F NMR spectra, respectively (see supplementary files Figures S49–S50).

Electron spray ionization mass spectroscopy provided further confirmation of successful synthesis of the complexes. ESI–MS of complexes **C2**, **C3** and **C6** displayed peaks at m/z 1247.42, 1315.39 and 1371.45, respectively, which correspond to the molecular weights of the $[M]^+$ cations for these compounds $[C_{58}H_{80}Ag_2N_8PF_6]^+$, $[C_{64}H_{76}Ag_2N_8PF_6]^+$ and $[C_{68}H_{84}Ag_2F_6N_8P]^+$, respectively. This indicates dinuclear structures in which each Ag^+ ion is coordinated by two carbene moieties of two bis-NHC ligands. ESI–MS spectra of the compounds are given in supplementary files Figures S51–S53.

X-ray crystallographic studies

X-ray quality crystals of compound **C5** were grown by slow evaporation of a saturated solution in acetonitrile at room temperature. Single crystals of the compound were obtained as colourless blocks. Crystal refinement data are presented in the experimental part, while selected bond lengths and angles are listed in the supplementary data file (Table 1).

Complex **C5** crystallizes in triclinic space group P-1(2) having one cationic NHC ligand and two hexafluorophosphate counter anions in the unit cell. The bidentate NHC ligand is coordinated to two metal centres in a nonlinear dinuclear structure. The 5,6-dimethyl benzimidazolyl units are located on either side of the central pentyl core. A perspective view of **C5** is shown in Fig. 1. The structure is twinned and symmetry-generated such that each dimer occupies an inversion centre and the two NHC ligands attached to one silver centre are not coplanar, but twisted by 60°. There is no possibility of Ag–Ag interactions since the Ag–Ag distance is greater than 8 Å. The slight differences in bond lengths [e.g. N1–C5 = 1.361(8) Å and N4–C19 = 1.363(7) Å] and bond angles [e.g. N2–C5–N1 = 107.5° and N3–C19–N4 = 106.0(5)°] between similar atoms within two halves of the molecule are the result of twinning. The internal benzimidazole ring angle (N–C–N) at the carbene centre is 107.1(5)° for N1–C5–N2 and 106.0(5)° for N3–C19–N4; these are consistent with reported values [21]. The bond angle between the benzimidazole ring and pentyl linker is 124.5(5)° for C5–N2–C14 and 128.9(2)° for C18–N3–C19, while the dihedral angle N2–C14–C15–C16 between the benzimidazolium ring and pentyl substituent is 179.8(5)° which indicates that they are coplanar. The N–C and P–F bond distances are in the range of 1.341(7)–1.478(9) and 1.592(4)–1.606(4) Å, respectively. The Ag(I) centres have a nonlinear coordination geometry (due to twisting) at 173.6(2)° for C5–Ag1–C19. In the crystal, the hexafluorophosphate anions link the cations into a three-dimensional network via weak electrostatic intermolecular C–F interactions (3.71–5.7 Å). These bond distances and angles are comparable with those reported in similar silver(I) NHC complexes [21].

Table 1 IC_{50} (μM) values of NHC proligands and their silver complexes against MDA-MB-231, HCT-116 and EA.hy926 cell lines

Compound	IC_{50} (μM)		
	MDA-MB-231	HCT-116	EA.hy926
L1	20.51 ± 0.34	18.41 ± 0.45	66.25 ± 1.30
L2	24.30 ± 0.44	21.45 ± 0.54	78.74 ± 1.21
L3	31.21 ± 0.52	27.56 ± 0.31	89.12 ± 1.45
L4	23.40 ± 0.35	20.89 ± 0.62	82.14 ± 1.67
L5	27.60 ± 0.54	25.25 ± 0.53	94.23 ± 2.10
L6	33.15 ± 0.51	29.43 ± 0.22	105.14 ± 2.14
C1	9.12 ± 0.21	12.34 ± 0.24	54.32 ± 1.56
C2	7.91 ± 0.32	10.23 ± 0.87	42.21 ± 1.42
C3	5.22 ± 1.14	8.45 ± 1.02	35.20 ± 1.08
C4	8.14 ± 0.91	11.67 ± 0.93	49.16 ± 2.13
C5	7.42 ± 0.73	10.24 ± 0.82	51.20 ± 2.04
C6	4.41 ± 0.24	7.67 ± 0.51	45.46 ± 1.14
Positive control[a]	7.50 ± 0.12	5.5 ± 0.34	29.31 ± 1.12

[a]Tamoxifen, 5-fluorouracil and betulinic acid were used as positive controls for MDA-MB-231, HCT 116 and EA.hy926 cells, respectively

All attempts to gain single crystal of the other complexes proved unsuccessful. However, ^{1}H, ^{13}C NMR, 2D (HSQC) NMR as well as ESI–MS studies provided strong evidence for the successful synthesis of these complexes.

Cyclic voltammetry and chemical oxidation experiments

In cyclic voltammetry experiments, these complexes showed similar redox behaviour in line with their common metal ion,

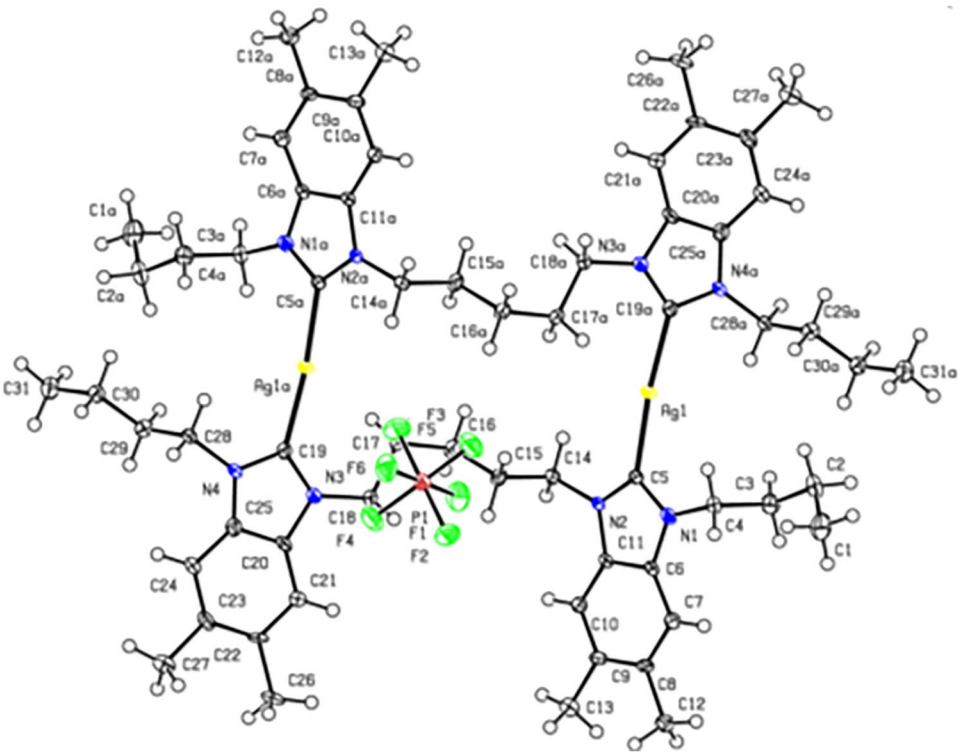


Fig. 1 ORTEP view of **C5** (50% probability level). The other PF_6^- counter anion is omitted for clarity

i.e. Ag^+. Therefore, the electrochemistry of complex **C6** will be discussed as representative.

Cyclic voltammograms for complex **C6** are shown in Fig. 2a–c. Three scan cycles were performed, and the direction of the scans did not affect the voltammograms. An irreversible redox process was observed for the complex in both the reduction and oxidation cycles [24]. It can be seen from Fig. 2a that during the reduction half cycle (− 1.2 to 1.4 V), an irreversible one-electron reduction process Ag(I)/Ag(0) occurs at E_{pc} − 750 mV (at scan rates of 50 and 100 mV/s) and the irreversibility indicates that the reduced complex is unstable and decomposes before the Ag(0)/Ag(I) oxidation. The presence of a very small bump at 250 mV may be attributed to a small impurity which appeared after the initial scans. The irreversibility of the reduction event was confirmed from three scan cycles (Fig. 2b). During the oxidation half cycles, oxidation events were observed at E_{pa} 950, 1600 and 1750 mV which may be most simply attributed to Ag(0)/Ag(I) redox event of species other than the original silver complex, i.e. during reduction, the silver complex decomposed and the oxidation occurred in the decomposed species (Fig. 2c). These oxidation processes cannot be attributed to Ag(II)/Ag(I) as it has been reported at $E_{1/2} > 2500$ mV depending on the pH of the solution [25].

Anticancer studies

Over the last few years, several groups have tested a number of structurally diverse silver(I) complexes against various cancer cell lines [9, 26–28]. All of these silver complexes were based on NHC ligands containing unsubstituted benzimidazole rings. However, to the best of our knowledge, the anticancer potential of silver(I) complexes with NHC ligands containing disubstituted benzimidazole rings has not yet been reported. We have therefore explored the activities of the present complexes against human breast cancer (MDA-MB-231), colon cancer (HCT 116) and normal endothelial (EA.hy926) cell lines by obtaining minimum inhibitory concentration (IC_{50}) values.

The IC_{50} values of the free NHC proligands (**L1**–**L6**) and their Ag(I) complexes (**C1**–**C6**) are given in Table 1. All of these compounds are less cytotoxic to normal cells (EA.hy926) compared to cancer cells (MDA-MB-23 and HCT 116). The free NHC salts exhibited pronounced antiproliferative behaviour against MDA-MB-231 and HTC-116 cells (with IC_{50} values in the range 20.51–33.15 μM against MDA-MB-231 and 18.41–29.43 μM against HCT-116 cells). Similarly, their Ag(I) complexes exhibited strong cytotoxic activities against both cancer cell lines (with IC_{50} values 4.41–9.12 μM against MDA-MB-231

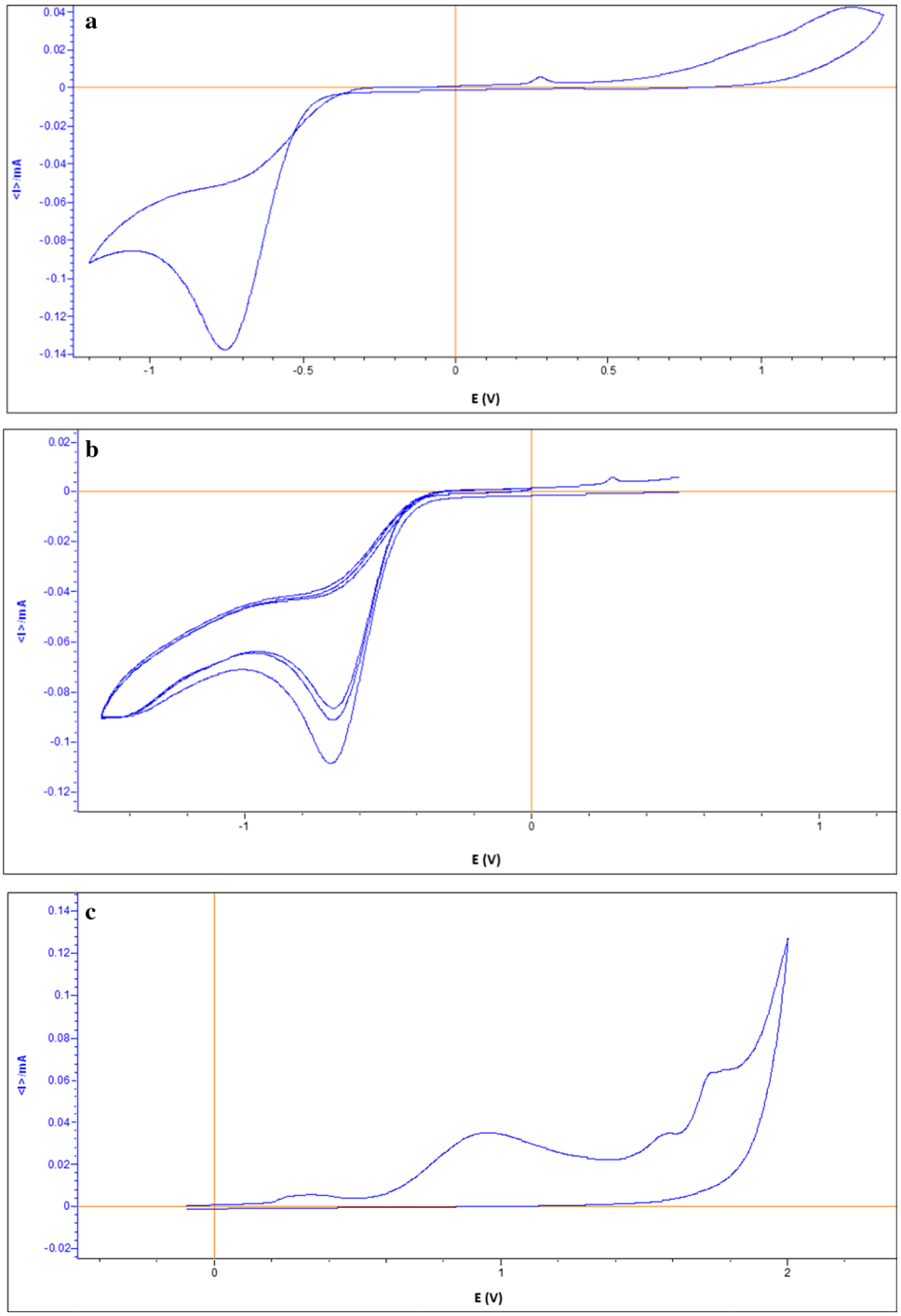


Fig. 2 Cyclic voltammograms for complex **C6** indicating various events of oxidation and reduction. The measurements were taken at 298 K on acetonitrile solutions containing 0.1 M of $[Bu_4N]$ $[PF_6]$ and 1 mM of the complex

and 7.67–12.34 µM against HCT-116 cells). All of the NHC proligands **L1**–**L6** are less active than their silver complexes **C1**–**C6**. Hence, it seems that coordination of silver to these NHCs results in enhanced antiproliferative effects, perhaps due to participation of silver ions in the cell death mechanism. It is also evident that the aryl group linker decreases the cytotoxicity of proligands **L3** and **L6** but increases that of silver complexes **C3** and **C6**. This effect can be attributed to increased lipophilicity aiding the passage of silver cations through the cell membrane and into the cell organelles, resulting in inhibition of metabolic and respiratory mechanisms. Hence, increased chain length, presence of methyl substituents on the benzimidazole ring and aryl linker and two silver centres all enhance the biopotencies of these complexes. Figures 3, 4, 5 and 6 show the dose-dependent antiproliferative effects of the proligands **L1-L6** and complexes **C1**–**C6** on MDA-MB-231 and HCT-116 cells. All of these compounds

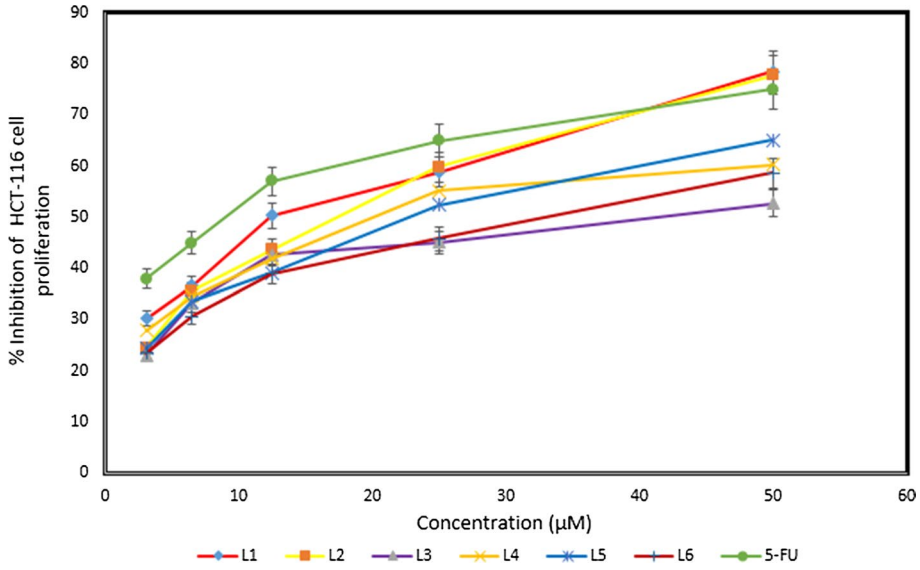


Fig. 3 Dose-dependent antiproliferative effects of proligands **L1**–**L6** on HCT-116 cells

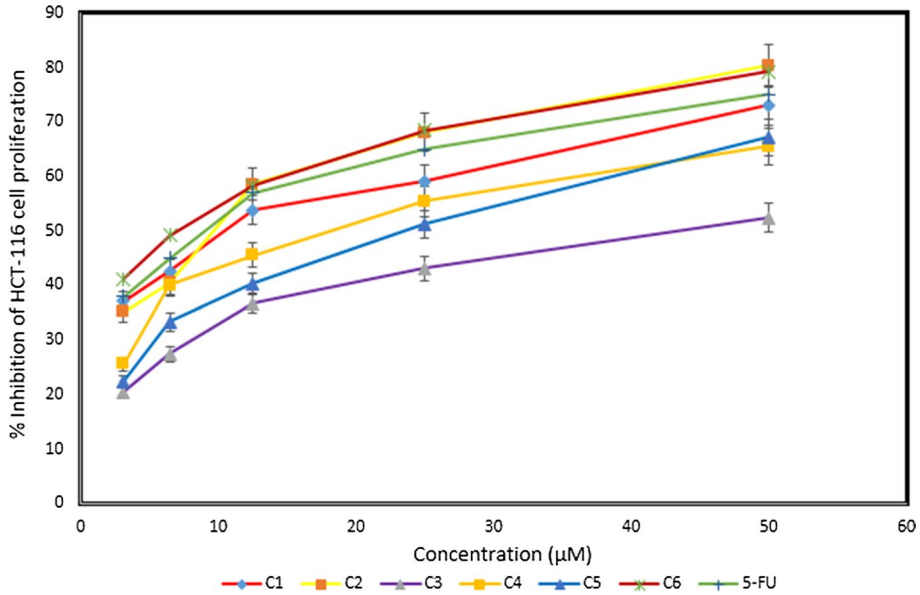


Fig. 4 Dose-dependent antiproliferative effects of complexes **C1**–**C6** on HCT-116 cells

displayed dose-dependent cytotoxic activities against both cancer cells lines.

Selectivity index

The selectivity index (SI) provides an indication of the cytotoxic selectivity of a compound against cancer cells compared to normal cells [29]. The SI values were calculated from the ratio of the IC_{50} values for normal cells (EA.hy926) versus those for cancer cells. A compound with SI > 3 is considered highly selective for that cell line [29]. SI values for the present compounds are shown in Table 2. The complexes displayed greater selectivities than the free proligands. In particular, complex **C6** showed high SI values against both cancer cell lines.

Mechanism of action and structure–activity relationships

It is known that, in general, free silver is inert towards cancer cells; however, in its cationic form its activity depends on its bioavailability. Thus, solubility, delivery approaches and ionization of the silver sources all are important factors that influence the cytotoxicities of silver compounds [30]. In this study, we have found that complexation of Ag(I) with NHC ligands gives higher anticancer activity than the NHC salts alone. It has been established that the

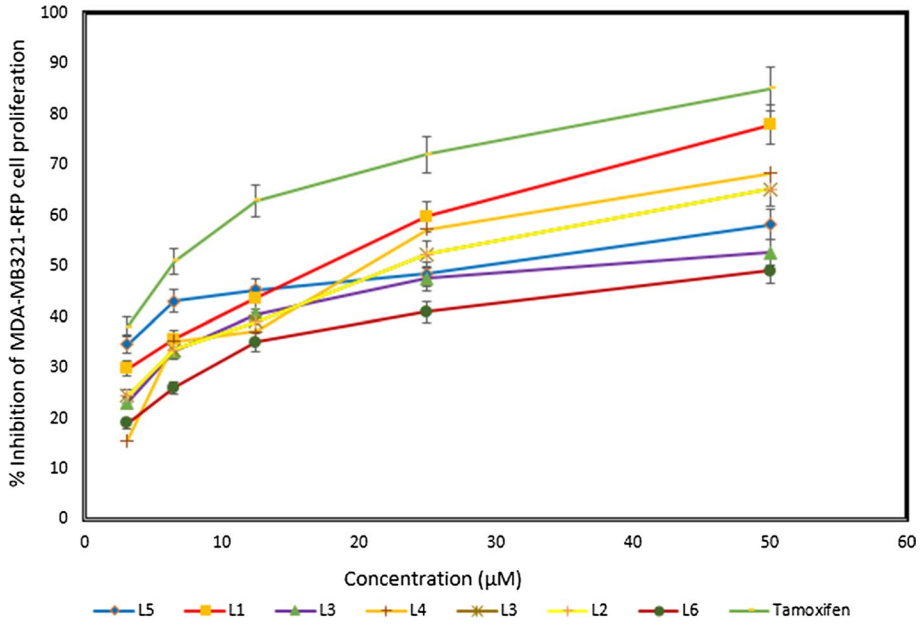


Fig. 5 Dose-dependent antiproliferative effects of proligands **L1**–**L6** on MDA-MB-231 cells

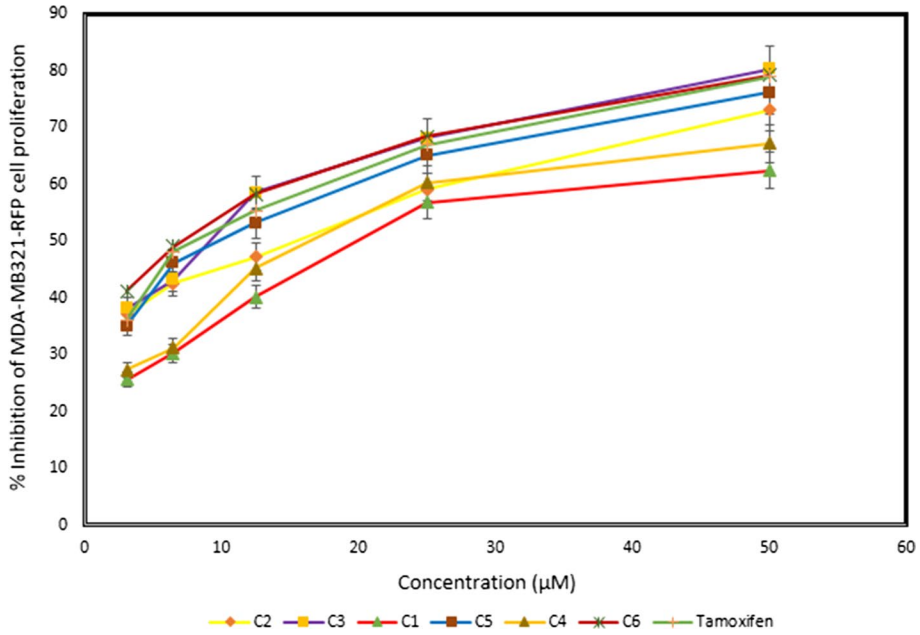


Fig. 6 Dose-dependent antiproliferative effects of complexes **C1**–**C6** on MDA-MB-231 cells

anticancer potential of drugs is generally decreased by fast release of silver cations [27]. To overcome this problem, silver(I) complexes are of interest. Due to their weak π-accepting and strong σ-donating features, NHC ligands are slow to release silver ions [27, 31]. Previous studies have shown that anticancer properties of silver NHC complexes against human colon cancer (HCT 116) and breast cancer (MDA-MB-231) cells may proceed through apoptosis (caspase independent) by external/internal stress factors [32], which can be verified from the observation of apoptotic bodies and chromatin condensation in the cytoplasm of cancer cells [6, 7, 10]. Additionally, it has been shown that silver ions hinder cell functions by getting deposited in the cytosol, where they may interact with proteins and enzymes [7, 30, 33]. Thus, the outcomes of this study are in agreement with previously reported work in which black deposits (which may be of silver oxide) in treated cancer cells have been observed [34]. Lipophilicity depends on alkyl chain length and influences the ability of compounds to penetrate cell membranes, interact with cell organelles and disturb their functions. Hence, it may be inferred that longer side chains, the presence of aryl linker and greater number of silver centres all enhance the biopotencies of silver(I)-complexes.

Table 2 Selectivity index values of the compounds towards the tested cancer cells

Compound	SI	
	MDA-MB-231	HCT-116
L1	3.23	3.59
L2	3.24	3.67
L3	2.86	3.23
L4	3.50	3.93
L5	3.41	3.73
L6	3.17	3.57
C1	5.95	4.40
C2	5.33	4.12
C3	6.74	4.16
C4	6.0	4.21
C5	6.9	5.0
C6	10.3	5.9
Positive control[a]	3.90	5.32

[a]Tamoxifen, 5-fluorouracil and betulinic acid were used as positive controls for MDA-MB-231, HCT 116 and EA.hy926 cells, respectively

Experimental

Materials and methods

Analytical-grade starting materials were obtained from commercial sources and used without further purification. 5,6-Dimethyl benzimidazole, 1-bromopropane, 1-bromobutane, 1,5-dibromopentane, 1,3-dichloro metaxylene, 1,4-dibromobutane and Ag_2O were purchased from Sigma-Aldrich. A TECAN multimode microplate reader was obtained from the USA. Phosphate-buffered saline (PBS), penicillin/streptomycin (PS) solution, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent, 5-fluorouracil, tamoxifen and betulinic acid were purchased from Sigma-Aldrich. Human breast cancer (MDA-MB-231), colon cancer (HCT-116) and human endothelial normal cells (EA.hy926) were obtained from the American type culture collection (Rockville, MD, USA). The cells were kept in RPMI 1640 media supplemented with 10% heat-inactivated foetal bovine serum (HIFBS) and 1% phosphatidylserine (PS) and cultured in a 5% CO_2 humidified atmosphere at 37 °C.

Melting points were obtained with a Stuart Scientific SMP-1 (UK) instrument. The solvents were evaporated using an EYELA 1L Rotary Evaporator N-1001V-WD. FTIR spectra of the compounds were recorded with between 4000 and 250 cm^{-1} with an ALPHA-P compact FTIR spectrometer equipped with a universal attenuated total reflectance (UATR) accessory. ^{1}H, $^{13}C\{^{1}H\}$, HSQC, ^{19}F and $^{31}P\{^{1}H\}$ NMR spectra were recorded on Bruker Avance-300, Avance-400 or Varian Inova-300 spectrometers in $CDCl_3$, acetone-d_6, C_6D_6 and DMSO-d_6 as solvents at chemical shift (δ) ranges of 0–12 ppm for ^{1}H NMR, 0–200 ppm for ^{13}C NMR and −200 to 0 ppm for both ^{19}F and ^{31}P NMR studies. Chemical shifts are reported in ppm relative to tetramethylsilane, and coupling constants (J) are given in Hertz. The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were referenced using the residual solvent signal as internal standard, while ^{19}F and $^{31}P\{^{1}H\}$ spectra were referenced to $CFCl_3$ and H_3PO_4 (85%), respectively. Spectral assignments were made with the help of ^{1}H–^{13}C heteronuclear single quantum correlation (HSQC) experiments. The *N*-alkylation reactions were monitored by chromatography using an Agilent Technologies 7890A GC/MS. C, H, N elemental analyses were carried out using a Fisons EA1108 instrument. ESI mass spectra were recorded with an ESI-POS-DI-TOF 6224 spectrometer.

Electrochemical measurements were taken with a BAS Epsilon potentiostat. Crystallographic data for complex **C5** were collected on a Bruker APEX II diffractometer equipped with an Incoatec I\muS Microsource and graphite-monochromated Mo Kα radiation ($\lambda = 0.71073$ Å). Cell refinement and data reduction were done using SAINT software [35]. An empirical absorption correction, based on the multiple measurements of equivalent reflections, was applied using the SADABS program [36]. The space group was confirmed by the XPREP routine [37] in the program SHELXTL [38]. The structure was solved by direct methods and refined by full-matrix least-squares and difference Fourier techniques with SHELX-97 [39]. The crystal appeared as a two-component twin. The HKLF4 reflection file was generated by TWINABS to treat the twinning. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were set in calculated positions and refined as riding atoms with a common thermal parameter.

Preparation of 5,6-dimethyl-1-propyl-1H-benzo[d] imidazole (a)

A solution of 5,6-dimethyl benzimidazole (2.92 g, 20 mmol), 1-bromopropane (1.92 mL, 20 mmol) and KOH (1.68 g, 30 mmol) in DMSO (20 mL) was stirred for 3 h at room temperature, while monitoring the reaction by GC–MS. Upon completion of the reaction, the mixture was poured into ice-cold distilled water (200 mL). The white precipitates were filtered off, washed with water and dried. Yield 3.31 g (87%); M.P 40–43 °C. FTIR (cm^{-1}): 3376(m) (C_{aliph}–$N_{benzimid}$), 3077(m), 3037(w) (C_{arom}–H str), 2964(m), 2932(m), 2873(m) (C_{aliph}–H str), 1361(s), 1330(s), 1292(s) (C_{arom}–C_{arom} str), 880(s), 864(s), 838(s), 823(s), 625(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 0.95 (3H, t, 1 × CH_3, $J = 14$ Hz), 1.91 (2H, m, 1 × CH_2), 2.32 (3H, s, 1 × CH_3–Ar), 2.41 (3H, s, CH_3–Ar), 4.07 (2H, t, R-CH_2–N, $J = 4$ Hz), 7.17 (1H, s, Ar–H), 7.58 (1H, s, Ar–H), 7.78 (1H,

s, N–CH–N). GC–MS, m/z, (%): ($[C_{12}H_{16}N_2]^+$ 188.2 (100). Anal. Calcd. for $C_{12}H_{16}N_2$: C 76.6, H 8.6, N 14.9. Found C 77.1, H 8.65, N 14.2%.

Preparation of 5,6-dimethyl-1-butyl-1H-benzo[d] imidazole (b)

The synthesis of **b** followed the above procedure, but using 1-bromobutane (2.84 mL, 20 mmol). Compound **b** was obtained as a white solid. Yield 3.64 g (90%); M.P 50–52 °C. FTIR (cm^{-1}): 2961(s), 2933(s), 2863(s) (C_{aliph}–H str), 1558(w), 1493(w), 1468(w), 1328(s) (C=$N_{benzimid}$ str), 881(s), 842(s), 732(S), 62(S) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 0.97 (3H, t, 1×CH_3, J = 7.46 Hz), 1.37 (2H, m, 1×CH_2), 1.86 (2H, m, 1×CH_2), 2.39 (3H, s, 1×CH_3–Ar), 2.42 (3H, s, CH_3–Ar), 4.13 (2H, t, R-CH_2–N, J = 7 Hz), 7.17 (1H, s, Ar–H), 7.58 (1H, s, Ar–H), 7.78 (1H, s, N–CH–N). GC–MS, m/z, (%): ($[C_{13}H_{18}N_2]^+$ 202.3 (100). Anal. Calcd. for $C_{13}H_{18}N_2$: C 77.18, H 8.97, N 13.85. Found C 77.62, H 8.65, N 13.18%.

Preparation of L1

A mixture of compound **a** (2 g, 10.6 mmol) and 1,4-dibromobutane (0.6 mL, 5.3 mmol) was refluxed in 1,4 dioxane (30 mL) at 100 °C until the reaction was complete (as monitored by GC–MS). After 4 h, white precipitates of **L1** appeared; these were filtered off, washed with water (3×5 mL), and dried at ambient temperature. Yield 3.5 g (75%); M.P 170–173 °C. FTIR (cm^{-1}): 2940(w), 2874(w) (C_{aliph}–H str), 1569(w), 1456(w), 1399(w) (C=$N_{benzimid}$ str), 829(s), 741(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 1.02 (6H, t, 2×CH_3, J = 7.43 Hz), 2.07 (8H, m, 4×CH_2), 2.50 (12H, t, 4×Ar–CH_3, J = 10.62 Hz), 4.57 (4H, m, R-CH_2–N), 4.71 (4H, t, J = 5.96 Hz, R-CH_2–N), 7.88 (4H, d, Ar–H, J = 24.58), 7.73 (2H, s, Ar–H), 9.3 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, acetone-d_6, δ ppm) 10.12 (CH_3), 19.5, 22.3, 25.8 (R-CH_2), 46.6, 48.6 (Ar–CH_3, 2×CH_3), 113.1, 113.2 (N–CH_2-R, 2×CH_2), 130.1, 130.2, 137.2, 137.2 (Ar–C), 140.2 (N=C=N). Anal. Calcd. for $C_{28}H_{40}Br_2N_4$: C 56.7, H 6.8, N 9.4. Found C 56.9, H 6.6, N 9.2.

Preparation of L2

This synthesis followed the same procedure as for **L1**, but using 1,5-dibromopentane (0.75 mL, 5.3 mmol). **L2** was obtained as a white solid. Yield 3.6 g (75%); M.P 100–101 °C. FTIR (cm^{-1}): 3483(s) (C_{aliph}–$N_{benzimid}$ str), 2934(s) (C_{aliph}–H str), 1561(s), 1485(s), 1359(s) (C=$N_{benzimid}$ str), 1261(m), 1212(m) (C_{arom}=C_{arom} str), 898(s), 640(m) (C_{arom}–H oop ben).^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 0.89 (6H, t, 2×CH_3, J = 7.25 Hz), 1.38 (2H, m, CH_2), 1.93 (8H, m, 1×CH_2), 2.42 (12H, d, 2×CH_3–Ar, J = 4.42), 4.41 (8H, m, R-CH_2–N), 7.83–7.91 (4H, s, Ar–H), 9.72 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 11.1 (CH_3), 20.3, 20.6, 22.7, 22.8, 28.1, (R-CH_2) 46.7, 48.9 (Ar–CH_3, 2×CH_3), 112.5, 113.3 (N–CH_2-R, 2×CH_2), 129.7, 129.8, 137.2, 137.4 (Ar–C), 141.0 (N=C=N). Anal. Calcd. for $C_{29}H_{42}Br_2N_4$: C 57.4, H 6.9, N 9.2. Found C 57.9, H 6.6, N 8.9%.

Preparation of L3

This synthesis followed the same procedure for as for **L1**, but using 1,3-dichloro metaxylene (0.927 g, 5.3 mmol). **L3** was obtained as a white solid. Yield 3.5 g (76%). M.P = 145–150 °C. FTIR (cm^{-1}) 2935(w), 2873(w) (C_{aliph}–H str), 1564(m), 1456(w), 1400(m), 1359(w) (C=$N_{benzimid}$ str), 862(s), 743(s), 622(w) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 0.92 (6H, t, 2×CH_3, J = 7.45 Hz), 1.92 (4H, m, 2×CH_2), 2.29 (6H, s, R-CH_3), 2.40 (6H, s, R-CH_3), 4.41 (4H, t, N–CH_2, J = 7.7 Hz), 5.61 (4H, s, Ar–CH_2–N), 7.42 (3H, t, Ar–H, J = 3.5 Hz), 7.55 (1H, s, Ar–H), 7.58 (2H, s, Ar–H), 7.90 (2H, s, Ar–H), 9.74 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 11.1 (CH_3), 20.3 (2×Ar–CH_3), 22.4 (R-CH_2-R), 48.6 (CH_2–N), 49.9 (N–CH_2–Ar), 113.4, 113.7, 128.6, 129.8, 130.2, 130.2, 135.4, 136.8, 136.9 (Ar–C), 141.6 (N=C=N). ^{19}F (470.4 MHz, $CDCl_3$, δ ppm) − 71.7 (d, 6F). ^{31}P (202.4 MHz, $CDCl_3$, δ ppm) − 144.6 (h, 1P). Anal. Calcd. for $C_{32}H_{40}Cl_2N_4$: C 69.7, H 7.3, N 10.2. Found C 69.1, H 7.1, N 10.3%.

Preparation of L4

A mixture of compound **b** (1.5 mL, 4.9 mmol) and 1,4-dibromobutane (5 mL) was refluxed without solvent at 100 °C, and the progress of the reaction was monitored by GC–MS. After 24 h, a white solid of **L4** was obtained. This was filtered off, washed with copious amounts of ether, and dried at room temperature. Yield 2.75 g (75%); M.P 120 °C. FTIR (cm^{-1}): 3433(s), 3015(s) (C_{aliph}–$N_{benzimid}$), 2956(s), 2872(s) (C_{aliph}–H str), 1563(s), 1455(s) (C=$N_{benzimid}$ str), 1223(m), 1145(m) (C_{arom}–$N_{benzimid}$), 852(m), 775(m), 636(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 0.97 (6H, t, 2×CH_3, J = 7.57 Hz), 1.41 (4H, m, 2×CH_2), 2.07 (8H, m, 4×CH_2), 2.15 (12H, d, 2×Ar–CH_3), 4.26 (4H, t, 2×R-CH_2–N, J = 7.57 Hz), 4.71 (4H, t, 2×R-CH_2–N, J = 11.36 Hz), 7.82 (2H, s, Ar–H), 7.92 (2H, s, Ar–H), 9.31 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 13.4 (CH_3), 19.8, 20.7, 26.6, 2, 29.1, 31.2, 44.2 (R-CH_2) 46.5, 47.3 (Ar–CH_3, 2×CH_3), 112.7 (N–CH_2-R, 2×CH_2), 129.7, 129.8, 137.3, 137.4 (Ar–C), 142.4 (N=C=N). Anal. Calcd. for $C_{30}H_{44}Br_2N_4$: C 58.1, H 7.2, N 9.1. Found C 58.1, H 7.1, N 9.3%.

Preparation of L5

A mixture of compound **b** (1.51 g, 7.5 mmol) and 1,5-dibromopentane (0.5 mL, 3.7 mmol) in 1,4 dioxane (30 mL) was refluxed at 100 °C, and the progress of the reaction was monitored by GC–MS. After 24 h, the sticky material was allowed to settle, decanted, washed with fresh 1,4-dioxane and dried until a shiny crystalline material **L5** was obtained. Yield 3.5 g (75%); M.P 75 °C. FTIR (cm^{-1}): 3386(s) (C_{aliph}–$N_{benzimid}$), 2934(s), 2871(s) (C_{aliph}–H str), 1559(s), 1486(s), 1454(s), 1362(m) (C=$N_{benzimid}$ str), 1205(s) (C_{arom}–$N_{benzimid}$), 853(m), 684(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 1.02 (6H, t, 2 × CH_3, J = 8.07 Hz), 1.48 (4H, m, 2 × CH_2), 1.76 (2H, m, R-CH_2-R), 2.05 (4H, m, 2 × CH_2), 2.24 (4H, q, 2 × CH_2), 2.48 (12H, d, 4 × CH_3–Ar, J = 12.10 Hz), 4.51 (4H, t, J = 7.5 Hz, R-CH_2–N), 4.70 (4H, t, J = 7.5 Hz, R-CH_2–N), 7.42 (2H, s, Ar–H), 7.71 (2H, s, Ar–H), 11.11 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 13.5 (CH_3), 19.8, 20.5, 20.7, 28.1, 31.3, (R-CH_2) 46.7, 47.5 (Ar–CH_3, 2 × CH_3), 112.5, 113.3 (N–CH_2-R, 2 × CH_2), 129.8, 129.9, 137.2, 137.4 (Ar–C), 141.1 (N=C=N). Anal. Calcd. for $C_{30}H_{44}Br_2N_4$: C 58.1, H 7.2, N 9.1. Found C 58.3, H 7.3, N 9.2%.

Preparation of L6

The synthesis of **L6** followed the same procedure as for **L5**, but using 1,3-dichloro metaxylene (0.65 g, 3.7 mmol). The product was obtained as a white solid. Yield 3.7 g (78%); M.P = 170 °C. FTIR (cm^{-1}): 3381(s) (C_{aliph}–$N_{benzimid}$), 2957(s), 2873(s) (C_{aliph}–H str), 1557(s), 1485(s), 1425(m), 1388(m) (C=$N_{benzimid}$ str), 1143(m) (C_{arom}–$N_{benzimid}$), 952(w), 750(s), 665(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 1.03 (6H, t, 2 × CH_3, J = 7.40 Hz), 1.5 (4H, m, 2 × CH_2), 2.11 (4H, m, 2 × CH_2), 2.50 (12H, d, 2 × R-CH_3, J = 13.7 Hz), 4.60 (4H, t, N–CH_2, J = 7.4 Hz), 5.81 (4H, s, Ar–CH_2–N), 7.31 (1H, s, Ar–H), 7.42 (3H, t, Ar–H, J = 8.46 Hz), 7.55 (2H, d–d, Ar–H, J = 7.47 Hz), 7.74 (2H, s, Ar–H), 11.61 (2H, s, N–CH–N). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 13.5 (CH_3), 19.8, 20.7 (Ar–CH_3), 20.8, 31.2 (R-CH_2-R), 47.4 (CH_2–N), 50.4 (N–CH_2–Ar), 112.5, 113.6, 129.2, 129.6, 130, 134.4, 137.3, 137.8 (Ar–C), 141.9 (N=C=N). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 71.8 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 146.3 (h, 1P). Anal. Calcd. for $C_{34}H_{44}Cl_2N_4$: C 70.4, H 7.6, N 9.6. Found C 70.1, H 7.2, N 9.9%.

Preparation of C1

Solid **L1** (1.5 g, 2.5 mmol) was added to a suspension of silver oxide (1.2 g, 5 mmol) in methanol (50 mL) in a flask covered with aluminium foil, and the mixture was stirred for 48 h at room temperature. A silver mirror formed on the wall of flask. The black suspension was filtered through Celite, and a solution of KPF_6 (0.85 g, 5 mmol) was added to the filtrate at room temperature under continuous stirring. The mixture was stirred for 4 h, and the precipitates formed were filtered off and dried at room temperature. Complex **C1** was obtained as a beige powder. All efforts to obtain single crystals were unsuccessful. Yield 1.74 g (57%); M.P 260–262 °C. FTIR (cm^{-1}): 2934(w), 2874(w) (C_{aliph}–H str), 1559(s), 1496(m), 1401(m) (C=$N_{benzimid}$ str), 876(m), 824(s), 749(m) (C_{arom}–H ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.83 (12H, t, 4 × CH_3, J = 7.53 Hz), 1.49 (8H, m, 4 × CH_2), 1.81 (8H, m, 4 × CH_2), 2.41 (24H, d, 4 × Ar–CH_3, J = 7.35 Hz), 4.54 (8H, t, 4 × N–CH_2, J = 7.65 Hz), 4.45 (8H, t, 4 × N–CH_2, J = 7.01 Hz) 7.56 (8H, s, Ar–H). ^{13}C NMR (125.72 MHz, DMSO-d_6, δ ppm) 11.5 (CH_3), 20.3, (R-CH_2), 23.4, 24.5 (Ar–CH_3, 2 × CH_3), 30.5, 48.9, 50.3, (R-CH_2) 112.4, 112.9 (N–CH_2-R, 2 × CH_2), 132.1, 132.2, 132.2, 132.9, 133.1, 133.9 (Ar–C), 184.3, 185.7 (d, N=C=N, J = 15.9 Hz), 186.7, 187.0 (d, N=C=N, J = 15.9 Hz). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 71.01 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 145.2 (h, 1P). Anal. Calcd. for $C_{56}H_{76}Ag_2F_{12}N_8P_2$: C 49.2, H 5.6, N 8.2. Found C 48.9, H 5.2, N 7.9%.

Preparation of C2

This synthesis followed the same procedure as for **C1**, but using **L2** (1.6 g, 2.5 mmol). Complex **C2** was obtained as a beige powder. All efforts to get single crystals failed. Yield 1.74 g (52%); M.P 250 °C. ESI$^+$, m/z, %): $[C_{58}H_{80}Ag_2N_8PF_6]^+$ 1247.43, 56). FTIR (cm^{-1}): 2937(w), 2875(w) (C_{aliph}–H str), 1566(w), 1456(m), 1401(m) (C=$N_{benzimid}$ str), 1030(m) (C_{arom}–$N_{benzimid}$), 830(s), 743(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.78 (12H, t, 4 × CH_3, J = 7.39 Hz), 1.44 (4H, m, 2 × CH_2), 1.75 (8H, m, 4 × CH_2), 1.96 (8H, m, CH_2), 2.37 (24H, d, 4 × Ar–CH_3, J = 7.53 Hz), 4.31 (8H, t, 4 × N–CH_2, J = 7.53 Hz), 4.45 (8H, t, 4 × N–CH_2, J = 7.01 Hz) 7.51 (8H, s, Ar–H). ^{13}C NMR (125.72 MHz, DMSO-d_6, δ ppm) 11.5 (CH_3), 20.3, (R-CH_2), 23.9, 24.4 (Ar–CH_3, 2 × CH_3), 30.5, 48.9, 50.3, (R-CH_2) 112.4, 112.5 (N–CH_2-R, 2 × CH_2), 132.1, 132.9, 132.2, 132.3, 133.7, 133.7 (Ar–C), 184.5, 185.9, 186.1, 187.9 (d, N=C=N, J = 13.93 Hz). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 71.01 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 145.2 (h, 1P). Anal. Calcd. for $C_{58}H_{80}Ag_2F_{12}N_8P_2$: C 49.9, H 5.7, N 8.1. Found C 49.4, H 5.6, N 7.9%.

Preparation of C3

This synthesis followed the same procedure as for **C1**, but using **L3** (1 g, 1.8 mmol) and silver oxide (0.89 g, 3.6 mmol). Complex **C3** was obtained as a beige powder.

All efforts to get single crystals failed. Yield 1.58 g (60%); M.P 260–262 °C. ESI$^+$, m/z, %): ($[C_{64}H_{76}Ag_2N_8PF_6]^+$ 1315.39, 50). FTIR (cm^{-1}): 2938(w), 2874(w) (C_{aliph}–H str), 1568(m), 1456(m), 1400(m), 1359(m) ($C{=}N_{benzimid}$ str), 830(s), 741(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.90 (12H, t, 4 × CH_3, J = 7.41 Hz), 1.87 (8H, m, 4 × CH_2), 2.20 (12H, s, 4 × R-CH_3), 2.41 (12H, s, 4 × R-CH_3), 4.42 (8H, t, 4 × N–CH_2, J = 7.1 Hz), 5.50 (8H, s, 4 × Ar–CH_2–N), 6.81 (2H, s, Ar–H), 7.07 (4H, d, Ar–H, J = 7.57 Hz), 7.17 (2H, d, Ar–H, J = 7.32 Hz), 7.32 (4H, s, Ar–H), 7.63 (4H, s, Ar–H). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 11.6 (CH_3), 20.2 (2 × Ar–CH_3), 20.3 (R-CH_2-R), 50.5 (CH_2–N), 51.3 (N–CH_2–Ar), 112.3, 112.5, 124.5, 126.3, 129.6, 132.2, 132.3, 132.4, 133.7, 133.8, 137.7 (Ar–C), 187.0, 189.0 (d, Ag–C–Ag, J = 195.7 Hz) (N=C=N). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 70.5 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 143.8 (h, 1P). Anal. Calcd. for $C_{64}H_{76}Ag_2F_{12}N_8P_2$: C 52.5, H 5.2, N 7.6; Found: C 51.8, H 5.3, N 7.5%.

Preparation of C4

This synthesis followed the same procedure as for **C1**, but using **L4** (1.5 g, 2.5 mmol) and silver oxide (1.2 g, 5 mmol). Complex **C4** was obtained as a beige powder. All efforts to get single crystals failed. Yield 1.91 g (57%); M.P 175–178 °C. FTIR (cm^{-1}): 2928(w), 2871(w) (C_{aliph}–H str), 1455(w), 1403(m), 1360(m) ($C{=}N_{benzimid}$ str), 873(m), 830(s), 736(s) (C_{arom}–H ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.85 (12H, t, 4 × CH_3, J = 7.53), 1.27 (8H, m, 4 × CH_2), 1.78 (8H, t, 4 × CH_2, J = 7.75 Hz), 1.83 (8H, m, 4 × CH_2), 2.45 (24H, d, 2 × Ar–CH_3, J = 8.47 Hz), 4.41 (8H, t, 4 × N–CH_2, J = 7.15 Hz), 4.43 (8H, t, 4 × N–CH_2, J = 7.53 Hz) 7.50 (8H, s, Ar–H). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 11.4 (CH_3), 20.3, 23.9 (R-CH_2), 24.4 (Ar–CH_3, 2 × CH_3), 30.5, 38.5 (R-CH_2), 48.9, 50.3 (N–CH_2-R, 2 × CH_2), 112.4, 112.5, 132.1, 132.2, 132.3, 133.7, 133.8 (Ar–C), 183.1, 184.4 (s, N=C=N, J = 190.18 Hz). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 71.1 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 143.1 (h, 1P). Anal. Calcd. for $C_{60}H_{84}Ag_2F_{12}N_8P_2$: C 50.6, H 5.9, N 7.8; Found: C 51.0; H 6.1; N 7.5%.

Preparation of C5

This synthesis followed the same procedure as for **C1**, but using **L5** (1.5 g, 2.4 mmol) and silver oxide (1.14 g, 4.8 mmol). Complex **C5** was obtained as a beige powder. Single crystals were grown by slow evaporation of saturated solution in acetonitrile at room temperature. Yield 1.81 g (52%); M.P 170 °C. FTIR (cm^{-1}): 2934(m), 2872(m) (C_{aliph}–H str), 1561(m), 1465(m), 1398(m) ($C{=}N_{benzimid}$ str) 1163(m) (C_{arom}–$N_{benzimid}$), 830(s), 750(s) (C_{arom}–H ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.80 (12H, t, 4 × CH_3, J = 7.66), 1.24 (8H, m, 4 × CH_2), 1.43 (4H, m, 2 × CH_2), 1.75 (8H, t, 4 × CH_2, J = 7.15 Hz), 1.96 (8H, m, 4 × CH_2), 2.37 (24H, d, 2 × Ar–CH_3, J = 8.17 Hz), 4.34 (8H, t, 4 × N–CH_2, J = 7.15), 4.45 (8H, t, 4 × N–CH_2, J = 7.15) 7.50 (8H, s, Ar–H). ^{13}C NMR (125.72 MHz, $CDCl_3$, δ ppm) 13.9 (CH_3), 20.2, 20.3 (R-CH_2), 24.5 (Ar–CH_3, 2 × CH_3), 30.4, 32.7 (R-CH_2), 48.7, 48.9 (N–CH_2-R, 2 × CH_2), 112.5, 132.2, 133.7 (Ar–C), 186.1, 188.1 (s, N=C=N, J = 191.18 Hz). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 71.1 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 143.1 (h, 1P). Anal. Calcd. for $C_{62}H_{88}Ag_2F_{12}N_8P_2$: C 51.3, H 6.1, N 7.7; Found: C 51.2; H 6.2; N 7.8%.

Crystal data: $C_{62}H_{88}Ag_2F_{12}N_8P_2$; Mol. wt. 1451.08; colourless block, space group: P-1 (2); Crystal size (mm): 0.2 × 0.18 × 0.16; crystal system: triclinic; Z = 0.5; $\boldsymbol{a}$ = 8.5272 (2), $\boldsymbol{b}$ = 12.7654 (3), c = 14.8616 (3) Å; α = 85.717 (1)°, β = 74.708(1)°, γ = 86.197(1)°; V = 1554.27 (6) Å^3; μ (Mo Kα) = 6.251 mm^{-1}; $d_{calcd.}$ = 1.550 g/cm^{-3}); F(000) = 748; Nref = 6147; Npar = 394; R(reflections) = 0.0439(5792); wR2(reflections) = 0.1635(5910); S = 1.082; T = 100 K.

Preparation of C6

This synthesis followed the same procedure as for synthesis of **C1**, but using **L6** (1.2 g, 5.1 mmol) and silver oxide (1.2 g, 5.1 mmol). The complex **C6** was obtained as a beige powder. All efforts to get single crystals failed. Yield 2.24 g (59%); M.P 260 °C. ESI$^+$, m/z, %: ($[C_{64}H_{76}Ag_2N_8PF_6]^+$ 1371.45, 50). FTIR (cm^{-1}): 2959(m), 2875(w) (C_{aliph}–H str), 1559(w), 1445(m), 1402(s), 1358(m) ($C{=}N_{benzimid}$ str), 832(s) (C_{arom}–H oop ben). ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm) 0.90 (12H, t, 4 × CH_3, J = 7.58 Hz), 1.32 (8H, m, 4 × CH_2), 1.84 (8H, m, 4 × CH_2), 2.21 (12H, s, 4 × R-CH_3), 2.41 (12H, s, 4 × R-CH_3), 4.40 (8H, t, 4 × N–CH_2, J = 7.2 Hz), 5.51 (8H, s, 4 × Ar–CH_2–N), 7.01 (4H, t, Ar–H, J = 10.37 Hz), 7.12 (1H, t, Ar–H, J = 7.07 Hz), 7.31 (3H, s, Ar–H), 7.60 (4H, s, Ar–H), 8.31 (4H, s, Ar–H). ^{13}C NMR (125.72 MHz, DMSO-d_6, δ ppm) 14.0 (CH_3), 20.1, 20.3 (CH_2), 30.9, 32.6 (2 × Ar–CH_3), 46.9, 48.9 (CH_2–N), 49.9, 51.3 (N–CH_2–Ar), 112.4, 113.7, 125.0, 126.4, 129.6, 130.37, 132.4, 133.6, 136.9, 137.9, 141.7 (Ar–C), 187.0, 188.9 (s, Ag–C–Ag). ^{19}F (470.4 MHz, DMSO-d_6, δ ppm) − 70.6 (d, 6F). ^{31}P (202.4 MHz, DMSO-d_6, δ ppm) − 141.9 (h, 1P). Anal. Calcd. for $C_{68}H_{84}Ag_2F_{12}N_8P_2$: C 44.6, H 4.7, N 5.8; Found: C 44.7; H 4.8; N 5.9%.

Cyclic voltammetry experiments

Electrochemical measurements of the Ag(I)–NHC complexes were taken using a BAS Epsilon potentiostat having a three-electrode standard system. Tetrabutyl ammonium hexafluorophosphate [nBu$_4$N][PF_6] (1 mM in CH_3CN) was

used as a supporting electrolyte. Ag/AgCl was used as reference, platinum disc as working and platinum wire as counter electrodes. The solutions were bubbled with nitrogen before each experiment. The electrochemical measurements were taken in acetonitrile with potentials referred to the Ag/AgCl (0.1 mol dm^{-3}) reference electrode ($E_{1/2}$ ($FeCp_2^+$/$FeCp_2$) = + 0.43 V) at a scan rate of 50 and 100 mV/s under N_2 at room temperature.

Anticancer studies

Preparation of cell cultures

Human breast cancer (MDA-MB-231), colon cancer (HCT-116) and normal endothelial (EA.hy926) cells were permitted to grow under optimal conditions in an incubator. After reaching 70–80% confluence rate, the cells were prepared for plating by aspirating the old medium from the plate and washing the cells thrice with slightly basic (pH 7.4) sterile PBS, which was then discarded. Trypsin was then distributed evenly on the cell surfaces and the cells were incubated in 5% CO_2 at 37 °C for 1 min, gently tapping the flasks containing the cells to assist their segregation. Cells were then observed with an inverted microscope. The activity of trypsin was suppressed by adding 10% fresh HIFBS medium (5 mL). A final concentration (2.5×10^5cells/mL) of cells was achieved by diluting with medium and the cultures were inoculated into the wells (100 µL/well). Finally, the plates containing the cells were incubated in 5% CO_2 at 37 °C.

MTT assays

After seeding the cells (100 µL, 1.5×10^5 cells/mL), the 96-well microtiter plate was incubated in a CO_2 environment for 24 h to allow for cellular attachment. The test substance (100 µL) was introduced into each well after making serial dilutions from stock solution with medium, and the plates were further incubated in 5% CO_2 atmosphere at 37 °C for 72 h. MTT reagent was then introduced into each well, and incubation was continued for 4 h. Then 50 µL DMSO was introduced in each well, and plates were incubated in CO_2 for 5 min and then assayed at two different wavelengths (570 and 620 nm) with a multimode monochromator-based microplate reader (Tecan Infinite® M200 PRO). Optical density (OD) obtained during MTT assays was used to calculate the percentage of growth inhibition, which was further employed to calculate IC_{50} values.

Statistical analysis

Statistical difference between the treatments and the control was evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Differences were considered significant at $p < 0.05$, and $p < 0.01$.

Conclusion

In summary, a series of bidendate benzimidazolium-based NHC ligands and their silver complexes were synthesized and characterized by various analytical techniques. Electrochemical studies of the complexes revealed that during the reduction half cycle, an irreversible one-electron reduction process (Ag(I)/Ag(0) produces unstable species which decomposes before the Ag(0)/Ag(I) oxidation event. Both the free azolium salts and their silver complexes were less cytotoxic to normal cells compared to cancer cells, and the silver complexes were more potent than the corresponding NHC precursors (salts). The IC_{50} values of the NHC salts decreased with increasing terminal chain length, but increased for the respective complexes. Complex **C6** exhibited the highest selectivity towards cancer cell lines. Overall, increased chain length, presence of methyl substituents on benzimidazole ring and aryl linker and two silver centres enhance the biopotency of Ag(I)–NHC complexes. Further studies of these complexes as potential chemotherapeutic agents are in progress.

Additional information

CCDC 1890988 contains the supplementary crystallographic data for complex **C5** included in this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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