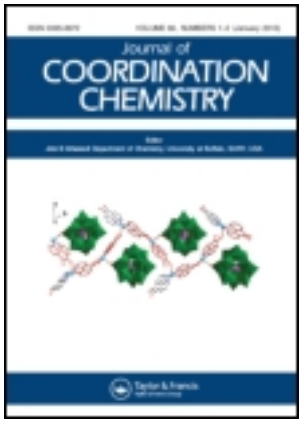

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A new dinuclear Ag(I)–*N*-heterocyclic carbene complex derived from *para*-xylyl linked *bis*-imidazolium salt: synthesis, crystal structure, and *in vitro* anticancer studies

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This manuscript describes synthesis, spectral (FT-IR and NMR), and structural studies of a new *para*-xylyl linked *bis*-imidazolium salt (**1**) and the dinuclear Ag(I)–*N*-heterocyclic carbene complex (**2**). Both **1** and **2** were tested for their potential against human colon cancer (HCT 116) and breast cancer (MCF-7) cell lines. According to cell viability measurements using MTT assay, the test compounds showed dose-dependent cytotoxic activities against both cell lines. The complex displayed significant activity ($IC_{50} = 20.9\,\mu M$ for HCT 116 and $2.4\,\mu M$ for MCF-7) compared to their respective imidazolium salt ($IC_{50} > 200\,\mu M$ for HCT 116 and $= 137\,\mu M$ for MCF-7). The photomicrographs of the cells treated with **2** revealed that the cytotoxic efficacy of **2** is mainly by deposition of silver in the cytoplasm of the affected cells since clear signs of black silver deposits in the cytoplasm of the affected cells were observed.

Keywords: *Bis*-imidazolium salt; Ag(I)–*N*-heterocyclic carbene; Anticancer; HCT 116; MCF-7; Crystal structure

1. Introduction

Since the discovery of cisplatin and its incorporation in medicine (against cancer) [1], a number of its derivatives were synthesized and studied against cancer [2]. However, very few of them (oxaliplatin, carboplatin, nedaplatin, and lobaplatin) [3] showed better results with fewer side effects. These new platinum-based drugs have been approved worldwide and are currently being used for cancer therapy [2(a), 3(a), 4]. These encouraging results induced researchers to study other transition metal complexes against cancer; however, none of them could pass all the pre-clinical and clinical trials. These facts have been described well by Gautier and Cisnetti [5]. Hence, the exploration was further extended to coinage metals (Cu, Au, and Ag) where the complexes of these metals showed much better antitumor results [6]. It was noticed that coinage metal complexes have a broader spectrum of anticancer activity with lower toxicity to non-cancerous cells [6(a)].

Among coinage metals, silver is more suitable because of its compatibility with biological systems. This is also evident from its usage by early civilizations to purify and store drinking water [7]. Till the end of the eighteenth century, silver nitrate was used as

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an antiseptic in wound care [8]. In the nineteenth century, it was discovered that silver compounds kill certain microorganisms [9] and also 1% silver nitrate solution was first used to prevent the eye infections of newborns [8, 10]. In the early twentieth century, colloidal solutions of silver were incorporated to avoid the irritation due to silver nitrate [11] and silver sulfadiazines were used for the treatment of burn wounds [12]. In the current era, silver complexes have established their worth as antimicrobial [13], antibacterial [13(c), 14], and antifungal [15] compounds. Silver complexes (Ag–N or Ag–O) have been studied against various types of cancer [16]; silver complexes with weak Ag–O and Ag–N bonds lose their effect quickly by rapid release of Ag^+ [3(b), 6(a)]. This drawback was compensated by introducing Ag(I)–NHC complexes since *N*-heterocyclic carbenes (NHCs) are strong σ-donating and weak π-accepting ligands and release Ag^+ at relatively slower rate [3(b), 17]. During the last five years, a number of review articles on Ag(I)–NHC complexes and their biological applications have been compiled, which signify their medicinal importance [2(a), 3(b), 4, 6, 10, 18].

Recently, we reported that dinuclear Ag(I)–NHC complexes, derived from *para*-xylyl linked *bis*-benzimidazolium salts, exhibit potential anticancer activity against human colon cancer (HCT 116) and leukemia (HL-60) [19]. We also reported the synthesis and crystal structure of a dinuclear Ag(I)–NHC complex, where the dinuclear silver carbene complex was aggregated in pair with a short Ag–Ag separation [20]. These compounds, i.e. ligand and its silver complex, also showed potential anticancer activity against HCT 116 and HL-60. We have reported that xylyl-linked *bis*-benzimidazolium salts and their respective dinuclear silver–NHC complexes have potential anticancer activities against various types of cancer [21]. Imidazole-based NHC precursors (*bis*-imidazolium salts) are either entirely inactive or negligibly active compared to similar salts derived from benzimidazole; however, their Ag(I)–NHC complexes have comparable anticancer potential [22]. Compounds **1** and **2** were synthesized to further investigate this phenomenon.

2. Experimental

2.1. *Reagents and instruments*

Nuclear magnetic resonance spectra were recorded on a Bruker 500 MHz Ultrashield^{TM} spectrometer at ambient temperature with samples prepared in DMSO-d_6. FT-IR spectra were recorded on Perkin Elmer-2000 using KBr pellets. Elemental analyses were carried out on a Perkin Elmer series II, 2400 microanalyzer. X-ray diffraction data were taken with a Bruker SMART APEX-2 CCD area-detector diffractometer. The melting and boiling points were assessed by using a Stuart Scientific SMP-1 (UK) instrument. Chemicals and solvents were purchased from Sigma-Aldrich, Germany, and were used as received.

RPMI 1640 and DMEM growth media were purchased from ScienCell, USA. Trypsin and heat-inactivated fetal bovine serum (HIFBS) were obtained from GIBCO, UK. Phosphate buffered saline (PBS), penicillin/streptomycin (PS) solution, MTT reagent and the reference standards 5-fluorouracil (5-FU), and tamoxifen were purchased from Sigma–Aldrich, Germany. All other chemicals used in this study were of analytical grade or better.

2.2. *Cell lines and culture conditions*

Human colorectal tumor (HCT 116) and breast cancer (MCF-7) cell lines were purchased from American type culture collection (Rockville, MD, USA). HCT 116 cell line is

derived from colonic epithelial carcinoma and MCF-7 cell line is derived from pleural metastasis of a ductal human breast carcinoma. The HCT 116 cells were maintained in RPMI 1640 culture medium, whereas the MCF-7 cells were grown in DMEM medium. Both growth media were supplemented with 10% HIFBS and 1% PS. Cells were cultured in 5% CO_2-humidifed atmosphere at 37 °C.

2.3. *Synthesis*

To a solution of 1,4-*bis*((1*H*-imidazol-1-yl)methyl)benzene (1.0 g, 0.0042 M) in 30 mL of acetonitrile, 1-bromopropane (1.0 g, 0.0084 M) was added. The mixture was refluxed at 100 °C for 24 h. **1** appeared as white precipitate, filtered, and washed with fresh acetonitrile (2 × 5 mL). Crystals suitable for X-ray diffraction were obtained by slow diffusion of the salt solution using diethyl ether and methanol at ambient temperature to collect the compound as colorless cubes. The general reaction involved in the preparation of *N*-propyl-substituted *bis*-imidazolium salt is shown in scheme 1.

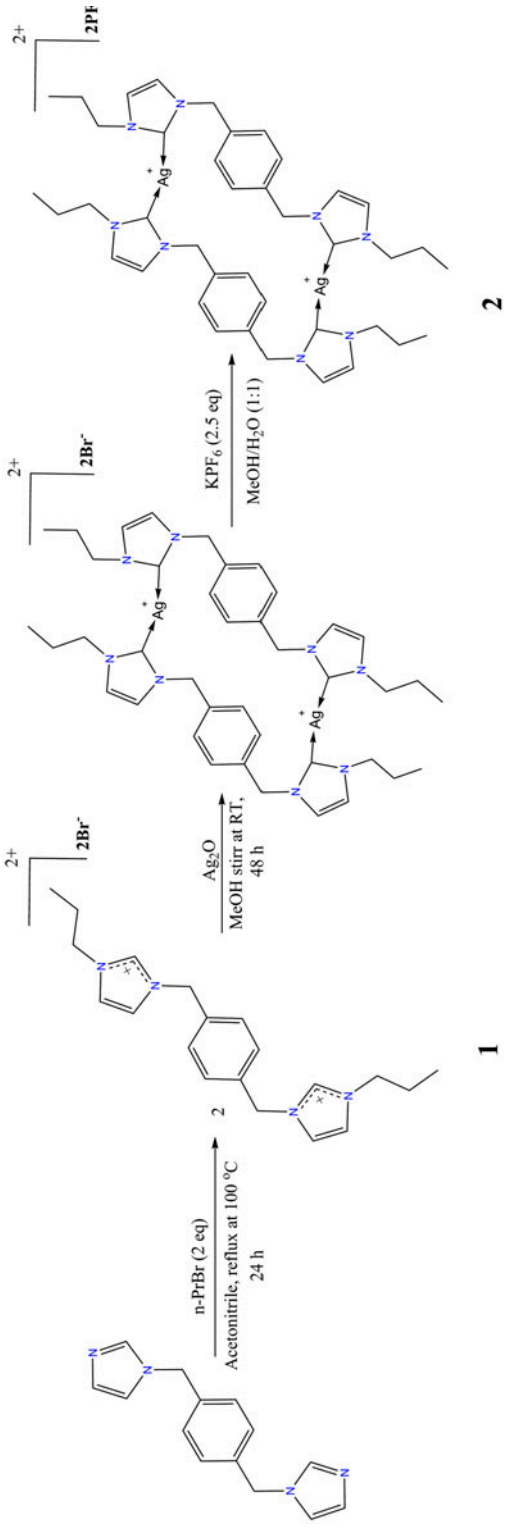
Colorless cubes. Yield: 1.3 g (93%), m.p.: 248–250 °C. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 0.85 (6H, t, 2 × CH_3, J = 7.0 Hz), 1.82 (4H, sext., 2 × CH_2), 4.13 (4H, t, 2 × N–CH_2–R, J = 8.0 Hz), 5.41 (4H, s, 2 × N–CH_2–Ar), 7.46 (4H, s, Ar–CH), 7.73 (4H, s, Ar–CH), 9.22 (2H, s, 2 × NCHN); ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6, δ ppm): 10.2 (2 × CH_3), 22.6 (2 × CH_2), 50.5 (N–CH_2–R), 51.5 (Ar–CH_2–N), 122.7, 123.1 (Ar–C), 128.8, 135.1 (Ar–C) and 135.9 (NCHN). FT–IR (KBr pellets): υ (cm^{-1}); 34.21 (C_{aliph}–$N_{benzimi}$); 3165, 3116 (C–H_{arom}); 2979, 2943, 2910, 2875 (C–H_{aliph}); 1613, 1564 (C_{arom}–C_{arom}); 1471, 1455, 1442, 1340, 1320 (C_{arom}–$N_{benzimi}$). Anal. Calcd for $C_{20}H_{28}F_{12}N_4P_2$: C, 39.10; H, 4.59; N, 9.12%. Found: C, 38.90; H, 4.68; N, 8.91%.

Ligand **1** (1.23 g, 0.002 M) was dissolved in methanol (50 mL) along with Ag_2O (0.7 g, 0.003 M) with the exclusion of light by enveloping the flask with aluminum foil. The reaction mixture was stirred for 2 days at room temperature and filtered by Celites 545 to collect a clear solution. The solution was evaporated under reduced pressure and converted directly to its hexafluorophosphate counterpart by metathesis using KPF_6 (1.90 g, 0.007 M) in 40 mL of methanol/water. The white precipitates were collected and washed with fresh methanol (2 × 3 mL) to obtain the product as a white powder that was further recrystallized by acetonitrile/water. Single crystals suitable for X-ray diffraction were obtained by slow evaporation in an acetonitrile/water mixture (3 : 1).

Colorless cubes. Yield 0.91 g (79.13%), m.p.: 268–270 °C. ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 0.86 (12H, t, 4 × CH_3, J = 7.5 Hz), 1.82 (8H, sext., 4 × CH_2), 4.12 (8H, t, 4 × N–CH_2–R, J = 6.5 Hz), 5.30 (8H, s, 2 × N–CH_2–Ar), 7.09 (8H, s, Ar–H), 7.54 (8H, d, 4 × imidazolium H, J = 6.5 Hz); ^{13}C{^{1}H} NMR (125 MHz, DMSO-d_6, δ ppm): 10.7 (4 × CH_3), 24.4 (4 × CH_2), 52.6 (N–CH_2–R), 53.6 (Ar–CH_2–N), 122.7, 123.1 (Ar–C), 127.4, 132.0 (Ar–C) and 180.1 [d, $^1J_{(C-109Ag)}$ = 210 Hz] & [d, $^1J_{(C-107Ag)}$ = 180 Hz]. FT-IR (KBr pellets): υ (cm^{-1}); 3407 (C_{aliph}–$N_{benzimi}$); 3172, 3145, 3108 (C–H_{arom}); 2964, 2932, 2875 (C–H_{aliph}); 1616, 1566, 1581 (C_{arom}–C_{arom}); 1466, 1445, 1420, 1382, 1351 (C_{arom}–$N_{benzimi}$). Anal. Calcd for $C_{40}H_{52}Ag_2F_{12}N_8P_2$: C, 41.76; H, 4.56; N, 9.74%. Found: C, 41.50; H, 4.59; N, 9.47%.

2.4. *Preparation of cell culture*

Initially, HCT 116 and MCF-7 cells were allowed to grow under optimal incubator conditions. Cells that had reached a confluence of 70–80% were chosen for cell plating



Scheme 1. Synthesis of *bis*-imidazolium salt (**1**) and corresponding silver complex (**2**).

purposes. The old medium was aspirated out of the plate. Next, the cells were washed using sterile PBS (pH 7.4), 2–3 times. PBS was completely discarded after washing. Subsequently, trypsin was added and distributed evenly onto the cell surfaces. Cells were incubated at 37 °C in 5% CO_2 for 1 min. Then, the flasks containing the cells were gently tapped to aid cell segregation and observed under an inverted microscope (if cell segregation is not satisfactory, the cells were incubated for another minute). Trypsin activity was inhibited by adding 5 mL of fresh complete media (10% FBS). Cells were counted and diluted to get a final concentration of 2.5×10^5 cells/mL and were inoculated into wells (100 µL cells/well). Finally, plates containing the cells were incubated at 37 °C in an atmosphere of 5% CO_2.

2.5. *MTT assay*

Cancer cells (100 µL cells/well, 1.5×10^5 cells/mL) were inoculated in the wells of a microtitre plate. Then, the plate was incubated overnight in a CO_2 incubator to allow the cell for attachment. Various concentrations of 100 µL of the test substance were added into each well containing the cells. The test substance was diluted with media into the desired concentrations from the stock. The plates were incubated at 37 °C in an atmosphere of 5% CO_2. After 72 h treatment, 20 µL of MTT reagent was added into each well and incubated again for 4 h. After this incubation period, 50 µL of MTT lysis solution (DMSO) was added into each well. The plates were further incubated for 5 min in a CO_2 incubator. Finally, the plates were read at 570 and 620 nm using a high-end Tecan M200Pro multimode microplate reader. Data were recorded and analyzed for assessment of effects of the test substance on the cell viability and growth inhibition. The percentage of growth inhibition was calculated from the optical density (OD) obtained from the MTT assay. 5-FU and tamoxifen were used as the standard reference drugs for HCT 116 and MCF-7 cells, respectively.

3. Results and discussion

3.1. *Synthesis*

The reaction of two equivalents of *n*-propyl bromide with 1,4-*bis*((1*H*-imidazol-1-yl) methyl)benzene in acetonitrile at 100 °C for 24 h afforded the *para*-xylyl linked *bis*-imidazolium salt (**1**) in good yield. The use of acetonitrile or 1,4-dioxane as a reaction medium for the synthesis of xylyl (*ortho*/*meta*/*para*)-linked *bis*-azolium salts is highly recommended, because by using either of these solvents as the reaction medium, *bis*-azolium salts can be collected either directly as a solid from the reaction medium using common filtration methods or by decantation of the reaction medium when the product settles as a thick yellowish fluid at the bottom of the flask [23]. Synthesis of Ag(I)–NHC complex (**2**) using an azolium halide salt as the starting material was more convenient than the same compound having hexafluorophosphate (PF_6) as the counter anion. Preliminary confirmation for synthesis of **2** was done by the difference in melting points of **1** (248–250 °C) and **2** (268–270 °C). Also, the difference in FT-IR spectra of both the compounds indicated the formation of a new compound. Scheme 1 shows three simple steps for the synthesis of ligand and silver complex.

3.2. *FT-IR spectra of the compounds*

Dinuclear Ag(I)–NHC complexes show some characteristic peaks compared to ligands and can be used as a preliminary confirmation for synthesis [19(a)]. For **1**, strong and sharp stretching vibrations (3421 cm^{-1}) indicate tertiary nitrogens of imidazolium ring (C_{aliph}–$N_{benzimi}$). The pure modes of the C_{sp3}–H stretch in *bis*-imidazolium salt appeared at 2875–2979 cm^{-1} (C–H_{aliph}). This range is due to the presence of C–H (sp^3-s) stretch of propyl chain and methylene (N–CH_2–Ar) group. This can be further classified as vibrational bands at 2875 cm^{-1} that appeared for the CH_3 stretch, 2910–2943 cm^{-1} for the CH_2 antisymmetric stretch, and 2975 cm^{-1} for the CH_2 symmetric stretch. Intense bands at 1200–1500 cm^{-1} from stretch of the imidazole ring are due to the presence of –HC=N– [19(a), 21(a)].

The *N*-heterocyclic carbene carbon on bonding with silver changes the vibrational bands in the range of 1200–1500 cm^{-1} and a characteristic "four fingers (f.fs)" pattern appeared for **2**. Previously, for a number of benzimidazole-based dinuclear silver–NHC complexes, such "(f.fs)" pattern has been reported by our group [19(a)]. This region is specific for –C=N (C_{arom}–$N_{benzimi}$) and CH_2 bending vibrations. The observed pattern is strong and entirely different than all the respective vibrations in the *bis*-imidazolium salt (**1**) and is easily distinguishable. This information has been helpful in predicting the synthesis of dinuclear Ag(I)–NHC complexes.

3.3. *FT-NMR spectra of the compounds*

FT-NMR characteristics of **1** and **2** were analyzed in DMSO-d_6 over the scan range of 0–12 δ ppm for ^{1}H NMR and 0–200 δ ppm for ^{13}C NMR. ^{1}H NMR spectrum of **1** evidenced a sharp singlet at 9.22 ppm ascribed to the imidazolium ring (NCHN) proton. These signals are in accord with previous reports [13(b), 19(a), 21(a) and (b), 24]. Synthesis of 2 was primarily confirmed by the disappearance of this acidic proton peak and observable changes in benzylic signals [24, 25]. The benzylic protons are a sharp singlet at 5.41 ppm in **1,** whereas the same signal shifted 5.30 ppm for **2**. Finally, the resonance of *N*-substituted propyl chain protons appeared at 0.85–4.15 ppm for both **1** and **2**.

Structural features of the salt were further confirmed by ^{13}C NMR data. The spectra of **1** displayed a peak in the most downfield region at 135.9 ppm ascribed to the imidazole ring carbon (NCN). This signal for benzimidazole-based ligands appears at 142–144 ppm [3(b), 19(a), 20]. The signals for benzylic carbon are at 51.52 ppm and for alkyl chain at 10.0–50.54 ppm. Upon complexation with Ag, two doublets are at *ca.* δ 180 for imidazole-based Ag–NHC complexes with Ag–C coupling constants *ca.* 204–220 and 180–189 Hz. These doublets appear in dimeric complexes $[L_2Ag_2]^{2+}$ due to carbene carbon bonding to C–Ag^{107} and C–Ag^{109}, respectively [25(b)]; however, single Ag–$C_{carbene}$ peak has also been observed at 175–185 ppm [26]. For benzimidazole-based Ag–NHC complexes such doublets at *ca.* δ 189 with coupling constant of 180 Hz for Ag^{107} and 204 Hz for Ag^{109} are commonly observed [24(c)]. In ^{13}C NMR of complexes, resonances of aromatic carbons were found at δ 122–133. The benzylic carbon (N–C–Ar) and alkyl chain carbon resonances were at 53.60 δ ppm and δ 10.0–53.0, respectively, 2–3 ppm downfield compared to corresponding ligands.

3.4. *Crystallography*

Single crystals suitable for X-ray diffraction study were grown by exposing a saturated solution of **2** in acetonitrile (0.5 mL) to diethyl ether at room temperature (vapor diffusion method). Single crystals appeared as colorless blocks. Crystal refinement data and selected bond lengths and angles are tabulated in tables 1 and 2. Complex **2** crystallizes in the triclinic space group with P-1 symmetry, whereas **1** grew in monoclinic space group with P-2 symmetry [27]. A perspective view of **1** and **2** is shown in figure 1. Figure 1(a) shows that the ligand is comprised of a *bis*-imidazolium cation and two bromides, whereas the Ag(I)–NHC complex (figure 1(b)) is comprised of two silver cations sandwiched by two units of **1** through NHC carbon and two hexafluorophosphates. The imidazolyl units in **1** are on either side of the central *para*-xylyl core with the dihedral angle 110.61(8)°. Internal ring angles of imidazole (N–C–N) at the carbene center are 108.29(16) for N1–C7–N2. These internal ring angles are 3–6° smaller in Ag(I)–NHC complex, whereas angles at each nitrogen increase 2–3°. This is in accord with the phenomenon that the NHC carbon after bonding with Ag(I), shifts charge density to the metal cation due to which the shape of the imidazole ring deviates. The bond lengths between Ag ion and the bonded carbene carbons are Ag1–C1/C12 = 2.101(9)/2.077(9) Å. These values are in accord with those of other Ag–NHC complexes [19(a), 24(c), 28]. The two silver cations are connected to the carbene centers through C1–Ag1–C12 and C1A–Ag1A–C12A linear (174.9°) bridges. Comparison of bond distances and angles between ligand and dinuclear complex is shown in figure 2. The figure clearly shows that after bonding with Ag, the bond angle at carbene carbon (NCN) shrinks from 108.29° to 105.2° and 102.9° whereas the bond angles at both nitrogens expands 2–4°, i.e. from 108.42°–108.74° to 110.20°–112.10°. This shows that the charge density drifted from imidazolium ring (ligand) to metal ion which causes deviation in the ring shape. This phenomenon is further supported by significant changes in the

Table 1. Crystal data and structure refinement details of **2**.

Formula	$C_{40}H_{52}Ag_2F_{12}N_8P_2$
Formula weight	1150.58
Crystal system	Triclinic
Space group	*P*1
Unit cell dimensions	
a (Å)	11.2288(6)
b (Å)	11.2410(5)
c (Å)	11.5714(6)
α (°)	65.003(4)
β (°)	61.859(3)
γ (°)	89.477(4)
V (Å^3)	1133.75(12)
Z	1
Density (Calcd) (gm/cm^3)	1.685
Abs. coeff. (mm^{-1})	1.024
F (000)	580
Crystal size (mm)	0.07 × 0.08 × 0.52
Temperature (K)	100
Radiation (Å)	MoKα 0.71073
θ min, max (°)	2.1, 25.0
Dataset	−13: 12; −13: 13; −13: 13
Total; unique data	14,789
R (int)	0.082
N ref, N par	3895, 279
R, wR_2, S	0.0620, 0.1918, 1.26

Table 2. Selected bond lengths (Å) and angles (°) of **2**.

Ag1–C1	2.101(9)	C1–N1	1.354(11)	N1–C2	1.373(13)
Ag1–C12	2.077(9)	C2–C3	1.349(14)	N2–C3	1.385(12)
C13–C14	1.340(15)	N2–C4	1.462(12)	N1–C7	1.448(13)
C12–N3	1.361(13)	C15–N4	1.470(14)	P1–F1	1.592(6)
N4–C12	1.367(11)	N4–C14	1.374(13)		
C1–N2	1.368(13)	N3–C13	1.372(11)		
C1–Ag1–C12	174.9(4)		C12–N3–C13	112.1(8)	
N1–C1–N2	105.2(8)		N3–C18–C19	111.9(8)	
N3–C12–N4	102.9(8)		N1–C7–C8	111.6(8)	
C1–N2–C3	110.2(8)		F1–P1–F2	89.8(3)	
C1–N1–C2	110.4(8)		F1–P1–F5	179.5(4)	
C12–N4–C14	111.4(8)				

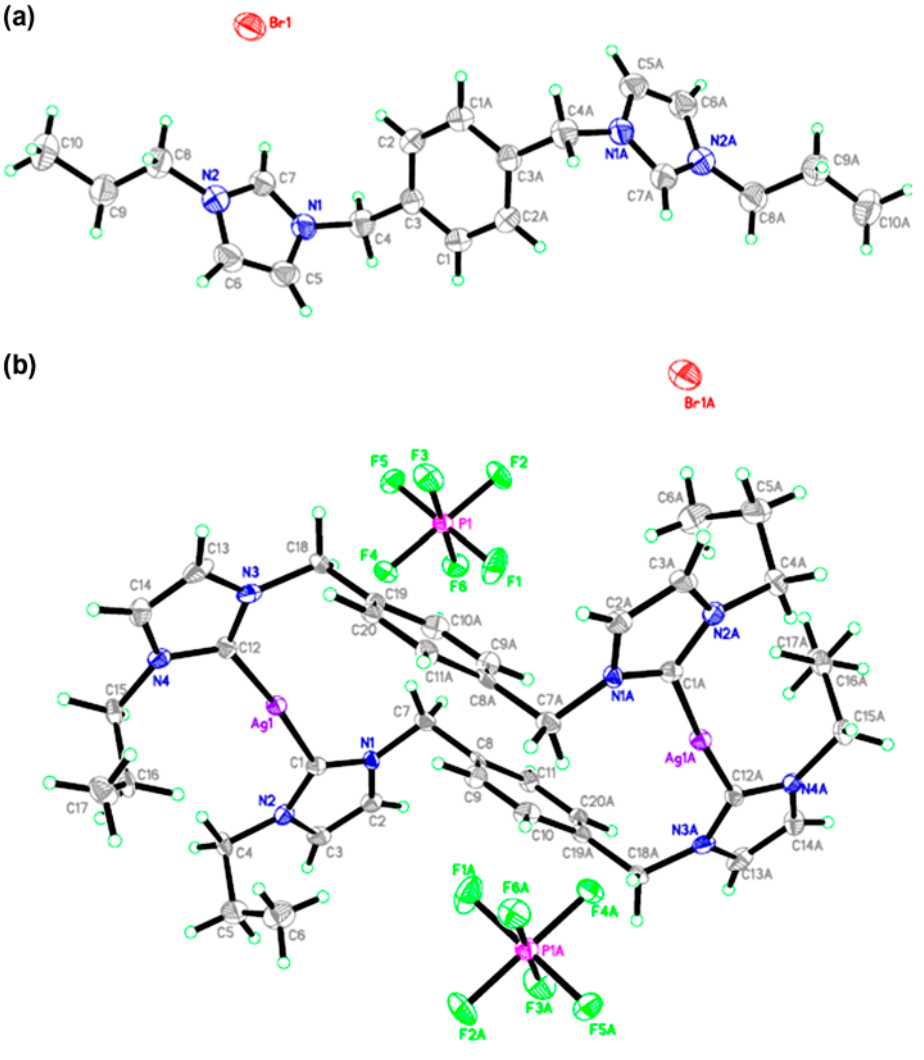


Figure 1. ORTEP of *bis*-benzimidazolium salt **1** [27] and respective dinuclear silver complex **2** with displacement ellipsoids drawn at 50% probability.

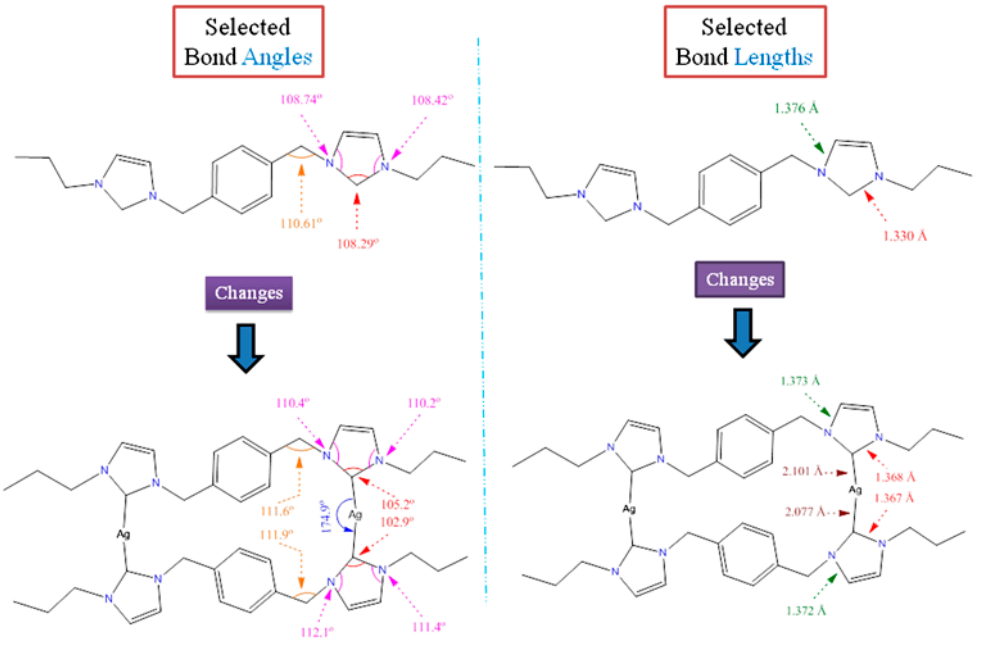


Figure 2. Comparison of selected bond angles and bond lengths between **1** and **2**. All the labeled values were selected from X-ray crystallographic data.

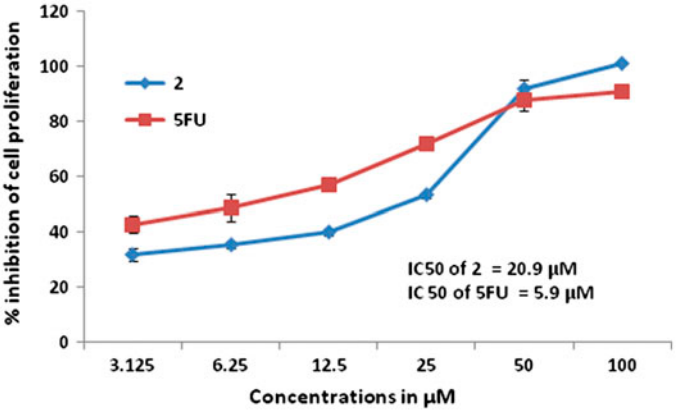


Figure 3. Dose-dependent antiproliferative effect of **2** and 5-FU on human colorectal tumor cells (HCT 116).

C–N bond lengths as shown in figure 2. The crystal packing (Supplementary material) shows that cationic and anionic components are connected *via* C–H···F hydrogen bonding in a 3-dimensional network.

3.5. In vitro *anticancer activity*

3.5.1. Effect of 1 and 2 on proliferation of HCT 116 and MCF-7 cell lines. A wide variety of heterocyclic compounds based on imidazole and benzimidazole are being studied

as anticancer agents against various cancer cell lines [29]. Furthermore, imidazole and benzimidazole-based compounds are promising candidates for anticancer treatment. As reported in recent reviews, there has been a growing interest to examine the anticancer activities of different heterocyclic compounds with specific functional groups [30]. Thus, antiproliferative potencies of **1** and **2** were evaluated using MTT assay on cancer cell lines, HCT 116 and MCF-7.

The results of the antiproliferation test showed a dose-dependent effect of **2** on both cell lines, whereas **1** proved to be inactive against HCT 116 but showed mild cytotoxicity against MCF-7. Figures 1 and 4 illustrate the antiproliferative effect of the tested compounds. The graph clearly shows that **2** inhibited the proliferation of HCT 116 and MCF-7 cells in a dose-dependent manner. In previous reports, having benzimidazole as a heterocyclic moiety, both ligands and dinuclear complexes showed potential anticancer activity [19(a)–21, 28(b)]. This is perhaps due to the relatively higher potential of benzimidazole compared to imidazole against cancer [29(d) and (e)]. However, metal complexes of both of these moieties show significant anticancer activity [24(a) and (c), 28, 31].

Cell images of tested compounds and 5-FU against human colon cancer are shown in Supplementary material. Cells from the control group have fully confluent growth with compactly proliferating HCT 116 cells. **1** has a trivial effect ($IC_{50} > 200\,\mu M$) on cancer cells as the cell growth did not get affected and cellular morphology was similar to that of negative control. HCT 116 cells treated with **2** exhibited marked cytotoxicity ($IC_{50} = 20.9\,\mu M$). The photomicrograph depict that **2** showed significant inhibitory effect on the cellular growth as compared to untreated cells; characteristic features of apoptosis that can be noticed by observing membrane blebbing, chromatin condensation and formation of apoptotic bodies. These observations are in accord with previous report, where Ag (I)–NHC complexes induced caspase-independent apoptotic cellular death [28(a)]. However, both the newly synthesized compounds proved to be less potent than the standard drug ($IC_{50} = 5.9\,\mu M$, 5-FU).

Cell images of **1** and **2** and Tamoxifen against breast cancer (MCF-7) are provided in Supplementary material. Photomicrograph of MCF-7 cells from the control group formed a compact confluent layer, where all the cells displayed aggressive multiplication and intact cellular membrane, similar to HCT 116 cells. Treatment with **1** showed moderate cytotoxic-

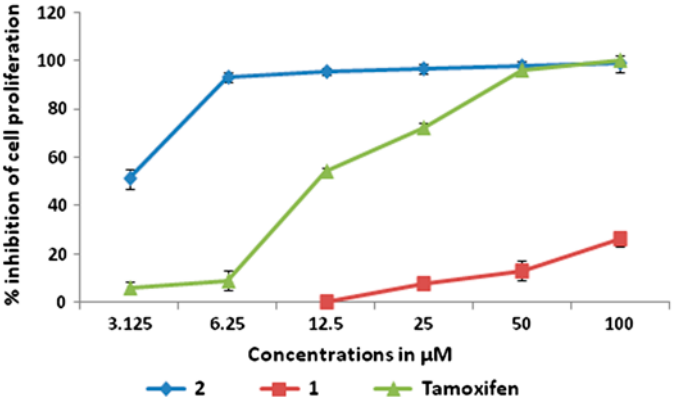


Figure 4. Dose-dependent antiproliferative effect of **1**, **2** and tamoxifen on breast cancer (MCF-7).

ity, with IC_{50} value of 137 μM, whereas **2** was found to be strongly cytotoxic ($IC_{50} = 2.4$ μM). The antiproliferative effect of **2** was 6 times more pronounced than the standard reference drug (tamoxifen). The viability of the cells was severely affected, as the photomicrograph showed that all the treated cells lost their viable characteristics. Treatment with tamoxifen (standard) showed marked inhibition in cell proliferation with $IC_{50} = 14$ μM.

3.6. *Mechanism of action*

During the last decade, a number of Ag(I)–NHC complexes were studied for their antimicrobial potential [6(b), 32]. These complexes release Ag^+ that bind with bacterial cell surfaces and interact with the proteins involved in the synthesis of cell wall and disrupt the cell functions [33]. The anticancer mechanism of Ag(I)–NHC complexes is also monitored by release of silver ions and its binding with the proteins and DNA [34]. A number of studies have proved that released Ag^+ deposits in the cytosol of the cell that hinders the cellular functions by interacting with the enzymes and proteins that are crucial for essential biochemical pathways [10, 22(a), 35]. The current study also reveals clear signs of black spots in the cytoplasm of the affected cells, which confirms that the cytotoxic efficacy of the complexes is primarily contributed by deposition of silver.

3.6.1. Comparison of IC_{50} values of 1 and 2 with the reported azolium salts and M–NHC complexes (M = Ag and Au) tested against HCT 116 and MCF-7 cell lines.

The literature shows that very similar *bis*-benzimidazolium salt **3** ($IC_{50} = 1.4$ μM) and respective dinuclear complex **4** ($IC_{50} = 0.4$ μM) are many fold more active than **1** and **2** (chart 1) against HCT 116 cell line [19(a)]. This might be due to association of benzene with the cellular system [36]. However, some Au(I)–NHC complexes (**6**–**9**), having entirely different structures than **1** and **2**, also showed mild toxicity against HCT 116 and MCF-7 [37]. Substitutions at 1,3 and 4,5 positions of the imidazole ring and metal center play a significant role in chemical and biological properties of benzimidazole. This has been described well in recent reviews [2(a), 18(a)]. In the field of NHCs, **3** and **4** show significant results against human colon cancer (HCT 116) cell line [19(a), 20, 38]. Compound **2** showed much better results than a number of benzimidazole derived Au(I)–NHC complexes (**5**–**8**), whereas some Cu(I)–NHC and Ag(I)–NHC complexes [39] have shown extraordinary cytotoxicity against MCF-7 cell line (table 3).

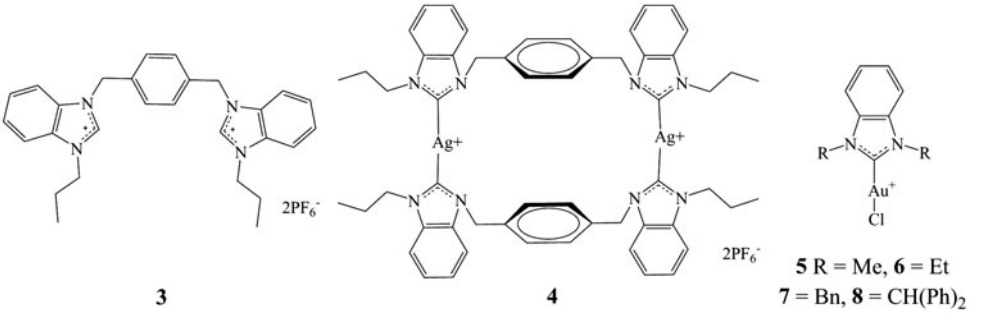


Chart 1. Structures of M–NHC complexes (M = Ag, Au) applied on either HCT 116 or MCF-7 cell lines.

Table 3. IC_{50} values of M(I)–NHC (M = Au, Ag) complexes **2**–**8** tested against HCT 116 or MCF-7 cell lines.

	IC_{50} (μM)	
Complex	HCT 116	MCF-7
1	>200	137
2	20.9	2.4
3	1.4	–
4	0.4	–
5	6.7	7.5
6	8.4	4.6
7	10.0	10.2
8	24.6	10.3

4. Conclusions

Benzimidazole-based NHC precursors (*bis*-benzimidazolium salts) and respective dinuclear Ag(I)–NHC complexes are many fold more active than imidazole-based compounds having similar structures. Also, Ag(I)–NHC complexes were always active against cancer cells compared with respective NHC precursors, **1** to **2** and **3** to **4**, respectively (table 3). This indicates that Ag^+ ions play a vital role in the death of cancer cells. The photomicrographs (Supplementary material) of the cells treated with **2** revealed clear signs of black silver deposits in the cytoplasm of the affected cells, which confirms that the cytotoxic efficacy of **2** is primarily by deposition of silver.

Supplementary data

CCDC 917,387 contains the supplementary crystallographic data for **2**. This data can be obtained free of charge from http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: 44-1223-336-033; or E-mail: deposit@ccdc.cam.ac.uk.

Authors' contribution

The compounds were synthesized, characterized, and tested against cancer by SFN and MAI under the supervision of RAH. The article was prepared and communicated by MAI.

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