Research Article

Synthesis, Characterization, and Crystal Structures of Bis-Imidazolium Salts and Respective Dinuclear Ag(I) N-Heterocyclic Carbene Complexes: In Vitro Anticancer Studies against “Human Colon Cancer” and “Breast Cancer”

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This paper describes synthesis, characterization (NMR, FT-IR, microanalysis, and X-ray crystallography), in vitro anticancer activity of ortho/para-xylyl linked bis-imidazolium salts (4-5) and respective dinuclear Ag(I) N-heterocyclic carbene (NHC) complexes (6-7). All the compounds were tested for their cytotoxicity against human colorectal cancer (HCT 116) and breast cancer (MCF-7) cell lines. According to cell viability measurements using MTT assay, all the complexes (6-7) showed a dose-dependent cytotoxic activity against both the cell lines, whereas respective salts (4-5) proved to be inactive. The complexes (6-7) demonstrated significant activity with IC_{50} values range 5.6–20.3 μM for HCT 116 and 1.12–6.38 μM for MCF-7. 5-Fluorouracil was used as standard drug (IC_{50} = 5.9 μM) for HCT 116, whereas tamoxifen was used as standard drug for MCF-7 (IC_{50} = 14 μM) cell line.

1. Introduction

New drugs to fight cancer are constantly needed. Every year, more than 10 million new cases of cancer are registered around the globe and more than half of the patients die [1]. Among various types of cancers, breast cancer is the second and human colon cancer is the third most common and leading cause of deaths worldwide [1, 2]. Every year more than 945000 people develop colorectal cancer and 492000 patients die, whereas the number is even higher for breast cancer [2]. Therapeutic strategies like chemotherapy, radiotherapy, and surgery are used to treat cancer. In chemotherapy, metal-based drugs have recently emerged as excellent anticancer medicament [3–10]. Today a number of platinum based drugs (oxaliplatin, carboplatin, nedaplatin, and lopablatin) are serving the cancer patients worldwide [3, 11]. Except platinum based drugs, a number of transition-metal drugs are currently at various stages of development [12–17]; however, none of them could pass all the stages of clinical development until today [5].

Recently, metal N-heterocyclic carbene (M-NHC) complexes appeared as an emerging field of research in medicinal chemistry [18–25] where NHC complexes of coinage metals (Cu, Au, and Ag) proved to be better anticancer agents [4, 10]. It was found that coinage metal complexes have a broader spectrum of anticancer potential with lower toxicity to the normal cells [10]. Among coinage metals, perhaps, silver is more suitable because of its compatibility with the biological system. Silver salts have a safe history to be used in maintaining human health. Many civilizations used silver salts for purification of drinking water and healing of wound infections [8, 26, 27]. The low toxicity of silver salts for humans
has attracted researchers to further explore their biomedical applications, specifically antimicrobial and anticancer [8, 28–30]. On the other hand, azoles (imidazole, benzimidazole, triazole, etc.) are heterocyclic moieties possessing wide spectrum of biological activities, specifically benzimidazole [31–39]. The biological importance of azole derivatives is due to their structural resemblance to the naturally occurring nucleotides, which allows them to interact with the biopolymers of the living system [32]. Coupling of these biologically compatible moieties generates a pharmaceutically enhanced class of compounds known as Ag(I)-NHC complexes. Azolium (imidazolium, benzimidazolium, triazolium, etc.) salts are used as stable precursors to synthesize N-heterocyclic carbene (NHCs) that serve as very reactive ligands to produce metal NHC complexes [40–43]. Anticancer properties of Ag(I)-NHC complexes are dynamically under investigation [8, 18, 19, 25, 44–48]. The current work is an effort to further explore this area of research.

2. Experimental

2.1. Reagents and Instruments. Nuclear magnetic resonance spectra were recorded on Bruker 500 MHz Ultrashield spectrometer at ambient temperature. FT-IR spectra were recorded on Perkin Elmer-2000. Elemental analysis was carried out on a Perkin Elmer series II, 2400 microanalyzer. X-ray diffraction data were taken with Bruker SMART APEX2 CCD area-detector diffractometer. The melting and boiling points were assessed by using a Stuart Scientific SMP-1 (UK) instrument. Chemicals and solvents were used as received without further purifications.

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-flourouracil 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 ducal 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% CO₂–humidified atmosphere at 37°C.

2.3. Syntheses

2.3.1. Synthesis of N-Substituted Bis-Imidazolium Salts (4-5)

Synthesis of 3,3’-[(1,2-Phenylenbis(methylene)]bis(1-propylimidazolium) Bis(hexafluorophosphate) (4). To a solution of 1, 2-bis[(1H-imidazol-1-yl)methyl]benzene (2.00 g, 0.008 mol) in 30 mL of acetonitrile, 1-bromopropane (2.06 g, 0.016 mol) was added. The mixture was refluxed at 90°C for 24 h. The resultant sticky brownish liquid was decanted, washed with fresh acetonitrile (2 x 5 mL), and converted directly to its hexafluorophosphate counterpart by metathesis reaction using KPbF₆ (3.09 g, 0.016 mol) in 40 mL of methanol/water. The white precipitates were collected and washed with fresh methanol (2 x 3 mL) to give the product as a white solid. Crystals were obtained by slow diffusion method. According to this method, the salt solution in acetonitrile was exposed to diethyl ether vapours at ambient temperature. General reaction involved in the preparation of bis-imidazolium salts is shown in Scheme 1.

White Powder. Yield: 3.16 g, (84%), mp: 120–122°C. 1H NMR (500 MHz, d₆-DMSO): 0.87 (6H, t, 2 × CH₃, J = 7.5 Hz), 1.81 (4H, sext., 2 × CH₂), 4.14 (4H, t, 2 × N–CH₂–R, J = 7.0 Hz), 5.56 (4H, s, 2 × N–CH₂–Ar), 7.69 (2H, d, Ar 2 × CH, J = 1.5 Hz), 7.31 (2H, sext., Ar–H), 7.51 (2H, sext., Ar–H), 7.69 (2H, t, 2 × imidazolium H), 7.82 (2H, t, 2 × imidazolium H), 9.15 (2H, s, 2 × NCHN); 13C¹H NMR (125 MHz, d₆-DMSO): 10.3 (2 × CH₃), 22.3 (2 × CH₂), 49.0 (N–CH₂–N), 50.5 (Ar–CH₂–N), 122.8 (Ar–C, d, J = 18.7 Hz), 129.7 (Ar–C, d, J = 15.0 Hz), 133.7 (Ar–C) and 136.3 (NCHN). FT-IR (KBr): ν (cm⁻¹): 3423 (C aliph-NH₂-Nenzim); 1317, 3130 (C–H arom); 2978, 2944, 2885 (C–H aliph₂); 1666, 1607, 1569 (C arom–C arom); 1473, 1456, 1383, 1364, 1347 (C arom–N Benzim). Anal. Cal. For: C₄₀H₃₂N₄F₁₂P₂: C, 39.10; H, 4.59; N, 9.12%. Found: C, 39.01; H, 4.82; N, 9.31%.

Synthesis of 3,3’-[(1,3-Phenylenbis(methylene)]bis(1-propylimidazolium) Bis(hexafluorophosphate) (5). Compound 5 was prepared according to the same procedure for 4, 1,3-bis[(1H-imidazol-1-yl)methyl]benzene (2.20 g, 0.009 mol) and 1-bromopropane (2.25 g, 0.018 mol). The product appeared as a white solid. Crystals suitable for X-ray diffraction studies were obtained by vapor diffusion method of the salt solution by using diethyl ether and acetonitrile at ambient temperature.

Colorless Cubes. Yield: 4.02 g, (73%), mp: 145–147°C. 1H NMR (500 MHz, d₆-DMSO): 0.81 (6H, t, 2 × CH₃, J = 7.5 Hz), 1.78 (4H, sext., 2 × CH₂), 4.15 (4H, t, 2 × N–CH₂–R, J = 7.0 Hz), 5.46 (4H, s, 2 × N–CH₂–Ar), 7.46 (1H, d, Ar 1 × CH, J = 4.0 Hz), 7.63 (1H, s, Ar 1 × CH), 7.74 (2H, t, imidazolium H), 7.77 (2H, t, imidazolium H), 9.39 (2H, s, 2 × NCHN); 13C¹H NMR (125 MHz, d₆-DMSO): 10.2 (2 × CH₂), 22.6 (2 × CH₂), 50.5 (N–CH₂–R), 51.6 (Ar–CH₂–N), 122.7 (Ar–C, d, J = 51.2 Hz), 128.8 (Ar–C, d, J = 8.7 Hz), 135.33 (Ar–C) and 135.82 (NCHN). FT-IR (KBr): ν (cm⁻¹): 3149 (C aliph-NH₂-Nenzim); 3172, 3199 (C–H arom); 2981, 2945, 2933, 2885 (C–H aliph₂); 1617, 1565 (C arom–C arom); 1474, 1458, 1447, 1345, 1327 (C arom–N Benzim). Anal. Cal. For: C₄₀H₃₂F₁₁N₄P₂: C, 39.10; H, 4.59; N, 9.12%. Found: C, 38.21; H, 4.56; N, 8.80%.

2.3.2. Synthesis of Ag(I)-NHC Complexes (6-7)

Synthesis of 3,3’-[(1,2-phenylenbis(methylene)]bis(1-propylimidazolium) Disilver (I) Bis(hexafluorophosphate) (6). The ligand 4,2Br (1.70 g, 0.006 mol) was dissolved in methanol (50 mL) along with Ag₂O (2.84 g, 0.012 mol) with exclusion of light by enveloping flask with aluminum foil. The reaction
mixture was stirred for a period of 2 days at room temperature. The reaction mixture was filtered by Celite 545 to collect a crystal clear solution. The solution was evaporated using rotary evaporator and the solid obtained was converted directly to its hexafluorophosphate counterpart by metathesis reaction using KPF$_6$ (1.90 g, 0.007 mol) in 40 mL of methanol/water. The white precipitates were collected and washed with fresh methanol (2 × 3 mL) to give the product as a white powder that was further recrystallized by acetonitrile/water system. Single crystals suitable for X-ray diffraction study were obtained by slow evaporation of title compound in acetonitrile/water mixture (3:1).

Colorless Cubes. Yield: 0.83 g (72.15%), mp: 220–222°C. $^1$H NMR (500 MHz, $d_6$-DMSO): 0.73 (12H, t, 2 × CH$_3$, $J$ = 7.5 Hz), 1.66 (8H, sext., 2 × CH$_2$), 3.83 (8H, t, 2 × N–CH$_2$–R, $J$ = 6.5 Hz), 5.36 (8H, s, 2 × N–CH$_2$–Ar), 7.14 (2H, t, Ar 2 × CH, $J$ = 4.5 Hz), 7.13 (4H, sext., Ar–H), 7.29 (4H, s, 2 × imidazolium H), 7.39 (4H, sext., Ar–H), 7.49 (4H, s, 2 × imidazolium H). $^{13}$C$^{1}$HNMR (125 MHz, $d_6$-DMSO): 10.6 (4 × CH$_3$), 24.2 (4 × CH$_2$), 51.6 (N–CH$_2$–R), 52.5 (Ar–CH$_2$–N), 122.6 (Ar–C, d, $J$ = 61.25 Hz), 129.0 (Ar–C, d, $J$ = 21.2 Hz), 134.4 (Ar–C) and 180 (br C–Ag). FT-IR (KBr): $\nu$ (cm$^{-1}$); 3393 (Caliph–Nbenzimi); 3171, 3041 (C–H$_{arom}$); 2967, 2937, 2877 (C–H$_{aliph}$); 1606, 1567 (C$_{arom}$–C$_{arom}$); 1493, 1456, 1422, 1386, 1358 (C$_{arom}$–Nbenzimi). Anal. Cal. For: C$_{49}$H$_{22}$Ag$_2$F$_{12}$N$_{6}$P$_2$: C, 41.76; H, 4.56; N, 9.74%. Found: C, 41.59; H, 4.81; N, 9.85%.

Synthesis of 3,3′-[1,3-Phenylenebis(methylene)]bis(1-propyl-imidazolium) Disilver(I) Bis(hexafluorophosphate) (7). Compound 7 was synthesized by following the same procedure for 6. Compound 5 (1.23 g, 0.002 mol) and Ag$_2$O (0.7 g, 0.003 mol) were reacted and recrystallized in the same way as 6.

White Crystalline Powder. Yield: 0.71 g (61.73%), mp: 182–184°C. $^1$H NMR (500 MHz, $d_6$-DMSO): 0.79 (12H, t, 4 × CH$_3$, $J$ = 7.0 Hz), 1.74 (8H, sext., 4 × CH$_2$), 3.95 (8H, t, 4 × N–CH$_2$–R, $J$ = 6.5 Hz), 5.14 (8H, s, 4 × N–CH$_2$–Ar), 6.94 (2H, s, Ar–H), 7.07 (4H, d, Ar–H, $J$ = 7.0 Hz), 7.28 (2H, t, Ar–H), 7.43 (4H, s, 2 × imidazolium H). 7.49 (4H, s, 2 × imidazolium H); $^{13}$C$^{1}$HNMR (125 MHz, $d_6$-DMSO): 10.8 (4 × CH$_3$), 24.4 (4 × CH$_2$), 52.5 (N–CH$_2$–R), 54.1 (Ar–CH$_2$–N), 122.4 (Ar–C, d, $J$ = 53.7 Hz), 125.1 (Ar–C), 126.5 (Ar–C, d, $J$ = 65.0 Hz), 128.4, 129.3, 137.7 (Ar–C) and 178.6 & 180.2 [two br, C–Ag signals, $^1$J$_{C-Ag}$ = 192.5 Hz]. FT-IR (KBr): $\nu$ (cm$^{-1}$); 3415 (C$_{aliph}$–Nbenzimi); 3175, 3144, 3112 (C–H$_{arom}$);
2962, 2933, 2875 (C–Haliph); 1613, 1567 (Carom–Carom); 1462, 1442, 1421, 1383, 1350 (C arom). Anal. Cal. For: C_{40}H_{52}Ag_{12}N_{6}P_{2}: C, 41.76; H, 4.56; N, 9.74%. Found: C, 41.69; H, 4.93; N, 9.06%.

2.4. In Vitro Anticancer Activity

2.4.1. 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 purposes. Old medium was aspirated out of the plate. Next, cells were washed using sterile phosphate buffered saline (PBS) (pH 7.4), 2–3 times. PBS was completely discarded after washing. Following this, trypsin was added and distributed evenly onto cell surfaces. Cells were incubated at 37°C in 5% CO₂ for 1 min. Then, the flasks containing the cells were gently tapped to aid cells segregation and observed under inverted microscope (if cells segregation is not satisfying, the cells will be 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 × 10⁵ cells/mL and inoculated into wells (100 μL cells/well). Finally, plates containing the cells were incubated at 37°C with an internal atmosphere of 5% CO₂.

2.4.2. MTT Assay. Cancer cells (100 μL cells/well, 1.5 × 10⁵ cells/mL) were inoculated in wells of microtitre plate. Then the plate was incubated overnight in CO₂ incubator in order to allow the cell for attachment. Various concentrations of 100 μL of test substance were added into each well containing the cells. Test substance was diluted with media into the desired concentrations from the stock. The plates were incubated at 37°C with an internal atmosphere of 5% CO₂. After 72 hours treatment period, 20 μL of MTT reagent was added into each well and incubated again for 4 hours. 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 CO₂ incubator. Finally, plates were read at 570 and 620 nm wavelengths using a high-end Tecan M200Pro multimode microplate reader. Data were recorded and analyzed for the assessment of the effects of test substance on cell viability and growth inhibition. The percentage of growth inhibition was calculated from the optical density (OD) obtained from 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 either with 1,2-bis((1H-imidazol-1-yl)(methyl)benzene or 1,3-bis((1H-imidazol-1-yl)(methyl)benzene in acetonitrile at 100°C for 24 h afforded the bis-imidazolium salts (4-5) in good yield. The use of acetonitrile or 1,4-dioxane as a reaction medium for the synthesis of xyllyl (ortho/meta/para) linked bis-azolium salts is highly recommended because by using either of the solvents, bis-azolium salts can be collected either directly as a solid from reaction medium using common filtration method or by decantation of reaction medium when the product settles as a thick yellowish fluid at the bottom of the flask (Scheme 1) [49]. Synthesis of Ag(I)-NHC complexes (6-7) using bis-imidazolium salts with bromide as counter anions was found to be more convenient compared to the same compounds as hexafluorophosphate (PF₆⁻) as counter anion. Preliminary confirmation for the successful synthesis of complexes was done by difference in melting points of ligands and respective silver complexes. Also, the difference in FT-IR spectra of both the compounds indicated the formation of complexes. Scheme 1 shows three simple steps for the synthesis of ligands and respective silver complex.

For ligands conversion of counter anion from halide to hexafluorophosphate is usually done for the ease of handling whereas for complexes this method is the way to purity if and only if halide salt of ligand is used as starting material in methanol as described above. For details see our previous article [25].

The compounds 4-7 are stable to the air and moisture. These are soluble in acetonitrile, DMSO, and DMF but not soluble in methanol, ethanol, and water.

3.2. FT-IR Spectra of the Compounds. Imidazolium salts and respective silver NHC complexes do not have many functional groups to be characterized by IR spectroscopic technique. However, it is possible to study some spectral features of these salts in comparison to the respective metal complexes. Some specific patterns can be observed, which may be used as primary indicators of a successful synthesis [25, 47].

For ligands (4-5), strong and sharp stretching vibrations (3419–3423 cm⁻¹) appeared for tertiary nitrogens of azolium ring (C₈H₇N₉). The pure modes of the C–H stretching vibrations appeared at around 2900 to 3000 cm⁻¹ (–C₉H₇N₉). This variation in the range is due to the presence of C–H (sp³–s) stretching of alkyl chains and methylene (N–CH₂–Ar) group. The vibrations due to C–H (sp³–s) due to aromatic hydrogens appeared at around 3191–3172 cm⁻¹. A strong and sharp intense band in the range 1350 to 1500 cm⁻¹ ascribed to the stretching modes of vibrations of imidazole ring due to the presence of –HC=N– module [50]. This region is interesting that provided the preliminary information about formation of silver NHC complexes [25, 47]. We observed that bonding of NHC carbon with silver metal ion strengthens vibrations in the range 1350–1500 cm⁻¹ and a characteristic “four fingers (f·fs)” pattern appears for all the dinuclear Ag-NHC complexes. This region is specific for –C=N (C₈H₇N₈) ring stretchings. The observed “f·fs” pattern is entirely different than all the respective vibrations in azolium salts and is easily distinguishable [25].

3.3. FT-NMR Spectra of the Compounds. FT-NMR spectra of all the compounds were analyzed in d₆-DMSO over the scan range 0 to 12 δ ppm for ¹H NMR and 0 to 200 δ ppm for ¹³C NMR studies. In the ¹H NMR spectra of imidazolium salts (4-5), a characteristic sharp singlet in the range δ 9.15–9.40 for acidic proton (NCHN) indicated the successful formation of
target ligands that is in accordance with our previous reports [40, 51]. The characteristic peaks for benzylic protons (N–CH2–Ar) and methylene protons (N–CH3–R) appeared at δ 5.46–5.56 and 4.15 ppm, respectively [51].

Synthesis of Ag-NHC complexes was confirmed by the disappearance of acidic proton peak (NCHN). The signals caused by benzylic (N–CH2–Ar) group, which connects xylxyl unit with azolium units, displayed sharp singlets in the range δ 5.10–5.40 for silver(I)-NHC complexes. These resonance values are comparable with corresponding ligands.

Similarly, the structural features of the salts were further confirmed by the 13C NMR data. In 13C NMR spectra, the chemical shift values of C–2 carbon (NCN) were observed within the range δ 135–136, which is also in agreement with reported data for similar azolium salts [40, 42]. Upon complexation with Ag, two doublets appear at ca. δ 189 for benzimidazole based Ag-NHC complexes with Ag–C coupling constants ca. 208 Hz and 180 Hz [25, 47]. These doublets appear in dimeric complexes of structure [L2Ag]+ due to carbene carbon bonding to C–Ag107 and C–Ag109, respectively [52]. In case of imidazole based Ag-NHC complexes such doublets appear at ca. δ 180 with splitting patterns 180–189 Hz for Ag107 and 204–220 Hz for Ag109 [53]. In 13C NMR of complexes, resonance of aromatic carbons was disturbed and was found in the comparable region around δ 122–137 ppm in Ag-NHC complexes. Also, the benzylic carbon (N–C–Ar) and alkyl chain carbon resonances were observed in the chemical shift regions δ 52.5–53.6 and δ 10.6–10.8 ppm, respectively. These resonance values are δ 2–3 ppm downfield as compared to corresponding ligands.

3.4. Crystallography. The molecular structure of the Ag-NHC complex 6 was determined by single crystal X-ray diffraction studies. Crystal refinement data, selected bond lengths, and angles of complex 6 are tabulated in Tables 1 and 2. Crystal structures of bis-imidazolium salt 5 have been published elsewhere [54, 55].

Ag(I)-carbene complex 6 crystallizes in the orthorhombic space group Pbca, with one cation of the complex and two hexafluorophosphate anions. A perspective view of the complex 6 is illustrated in Figure 1(a). Both the central ortho-xylxyl spacers are parallel to each other, having imidazole units on either side. Ag(I) ions exist in an almost linear coordination geometry, 176.22(10)° for Cl–Ag1–Cl21 and 177.76(10)° for Cl4–Ag2–C34 formed by two carbene carbon atoms of the benzimidazole rings. The internal ring angles of benzimidazole (N–C–N) at the carbene center are 104.03(2)° for N1–Cl1–N2 and 104.44(2)° for N5–C21–N6. These values decreased by 4 ± 0.5° compared to the same angles in similar bis-imidazolium salts [54, 55]. For example, the bond angles at N1–Cl1–N2 and N3–Cl4–N4 in 5 (Figure 3) are 108.30° and 108.95°, respectively. These bond angles are 4 ± 0.5° greater compared to the same angles in 6. This shows that after coordination, the charge density drifts from NHC to silver ion that deviates the imidazole ring shape. A general presentation has been shown in Figure 1(b). This phenomenon has been recently described [48]. This observation has also been reported in benzimidazole derived Ag(I)-carbene complexes [27, 56]. Two xylxyl units are on the same direction, parallel to each other making angles in the range 112.95–113.95° with all the four imidazolium units. In the extended structure, one hexafluorophosphate counter ion resides right above the gap between Cl1–Ag1–Cl21 and Cl4–Ag2–C34 bonds and connects benzylic hydrogens through C–H⋯F to neighboring complex cations. Second hexafluorophosphate counter ion resides beside cations of the complexes and connects benzylic and imidazolium H4/H5 hydrogens through C–H⋯F hydrogen bonding, forming 3-dimensional network. A perspective view crystal packing is shown in Figure 2.

3.5. In Vitro Anticancer Activity. In this study, antiproliferative potential of bis-imidazolium salts (4-5) and respective dinuclear Ag(I)-NHC complexes (6-7) was evaluated using MTT assay on human colorectal tumor (HCT 116) and breast cancer (MCF-7) cell lines. The results were reported as mean percentage inhibition of cell proliferation (±SD). Both the imidazolium salts (4-5) were found to be almost inactive (IC50 > 200 μM) against HCT 116 whereas they showed a mild cytotoxicity (IC50 = 158 to >200 μM) for MCF-7 cell line.
Table 2: Selected bond lengths (Å) and angles (°) of silver complex 6.

<table>
<thead>
<tr>
<th>Bond Length</th>
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<tr>
<td>C17–C16</td>
<td>1.514(5)</td>
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<tr>
<td>C4–C5</td>
<td>1.516(4)</td>
</tr>
<tr>
<td>C14–N4</td>
<td>1.347(3)</td>
</tr>
<tr>
<td>C15–N1</td>
<td>1.456(4)</td>
</tr>
<tr>
<td>C5–C10</td>
<td>1.402(4)</td>
</tr>
<tr>
<td>N4–C18</td>
<td>1.466(4)</td>
</tr>
<tr>
<td>C1–N1</td>
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<tr>
<td>C10–C11</td>
<td>1.517(4)</td>
</tr>
<tr>
<td>C19–C20</td>
<td>1.512(6)</td>
</tr>
<tr>
<td>Ag1–C1</td>
<td>2.088(3)</td>
</tr>
<tr>
<td>Ag2–C14</td>
<td>2.089(3)</td>
</tr>
<tr>
<td>C17–C16–C15</td>
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<td>C1–Ag1–C21</td>
<td>176.22(10)</td>
</tr>
<tr>
<td>C14–Ag2–C34</td>
<td>177.76(10)</td>
</tr>
</tbody>
</table>

After complexation

Before complexation

The silver complexes (6-7) of respective salts showed relatively better antiproliferation activity with IC$_{50}$ (concentration of the test compound to achieve 50% inhibition) 5.6–20.3 μM for HCT 116 and 1.12–6.38 μM for complexes.

The complex 7, having meta-xylyl bridge, showed better cytotoxicity (IC$_{50}$ = 5.6 μM for HCT 116 and 1.12 μM for MCF-7) compared to complex 6, having ortho-xylyl bridge (IC$_{50}$ = 20.3 μM for HCT 116 and 6.38 μM for MCF-7).
The metals with zero oxidation state are considered biologically inactive; however, the activity of metal cations further depends on their bioavailability. Consequently, delivery methods, solubility, and ionization of the metal sources are significant parameters to deal with metals in biological systems [57]. Possibly this is the reason that the bonding of silver cations to biologically compatible ligands enhances the bioavailability and ultimately the activity of silver cations. This is very obvious with our current and previously reported results [25, 44, 47, 58], where ligands were found to be relatively less active compared to respective silver complexes. The mechanism of action of silver cations is still not clear; however, it has been proved that silver cations bind to the cell surfaces and interact with the enzymes and proteins that are important for the cell wall synthesis [25, 26, 57]. The variation in N-alkyl chain length might further affect the potency of these compounds as the substituent produces variation in the lipophilicity of the drugs [25]. Thus, the antiproliferative effect of test complexes is likely due to the lipophilicity of the complexes that alleviates the transport of silver cations into the cell and subsequently into the organelles where silver may possibly contribute to toxicity by inhibiting cellular respiration and metabolism of biomolecules. Figures 4 and 5 show dose dependent antiproliferative effect of synthetic complexes (6-7), whereas the ligands (4-5) were found totally inactive for HCT 116 cell line, whereas for MCF-7 salt 5 showed a mild cytotoxicity; hence dose dependent charts for these compounds were included.

Figures 6 and 7 show the cell images taken under an inverted phase-contrast microscope at ×200 magnifications with digital camera.

4. Conclusions

In conclusion, xylyl (ortho-meta) linked bis-imidazolium salts (4-5) and their Ag-NHC complexes (6-7) were synthesized and characterized. The ligand 5 (salt) and complex 6 were structurally elucidated using single crystal X-ray diffraction technique. All the compounds were tested for their possible cytotoxicity on human colorectal tumor/cancer (HCT 116) and breast cancer (MCF-7) cell lines. The ligands showed mild to inactive cytotoxicity, whereas all the silver complexes showed a dose dependent cytotoxicity. In general, Ag(I)-NHC complexes were found relatively active compared to the respective ligands. The relatively higher cytotoxic effect of Ag-NHC complexes might be due transport of silver cations into the cell and possibly causing the toxicity by interfering with cellular respiration and metabolism of biomolecules. Silver cations have anticancer potential.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors’ Contribution

Rosenani A. Haque supervised the project. Siti Fatimah Nasri designed, synthesized, and characterized compounds. A. M. S. Abdul Majid and Mohamed B. Khadeer Ahamed supervised the anticancer activity whereas the activity was conducted by Muhammad Adnan Iqbal, Siti Fatimah Nasri, Seyedeh Fatemeh Jafari, and Sawsan S. Al-Rawi. The paper was prepared and communicated by Muhammad Adnan Iqbal. All authors read and approved the final paper.

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Figure 6: HCT 116 cell images were taken under an inverted phase-contrast microscope at ×200 magnification with a digital camera at 48 hours after treatment with the samples. (a) Cells from the control group showed fully confluent growth with compactly proliferating HCT 116 cells. (b) Cells treated with the standard drug 5-fluorouracil (IC_{50} = 5.9 μM) showed decreased viability. The cells show apoptotic characteristic features as the cell membrane blebbing, and nuclear condensation can be seen in the treated cells. (c) Treatment with 4 showed negligible cytotoxicity (IC_{50} > 200 μM), as the cell growth did not get affected, and cellular morphology was almost similar to that of negative control. (d) HCT 116 cells treated with the compound 6 exhibited significant antiproliferative activity (IC_{50} = 20.3 μM). As the photomicrograph depicts the treatment of HCT 116 cells with the 6 reduced the doubling time of cells drastically, the population of cells decreased significantly when compared to the negative control. (e) Treatment with 5 showed insignificant inhibitory effect on proliferation of cells (IC_{50} > 200 μM), as the growth and morphology of the cells were unaltered with respect to those of negative control. (f) Photomicrograph of the cells treated with 7 shows the strong cytotoxic effect on HCT 116 cells (IC_{50} = 5.6 μM). The effect can be compared with the standard reference, 5-fluorouracil. The treatment affected the morphology of almost all cells as the cells lost their pseudopodial like cellular extensions.
Figure 7: Human hormone estrogen dependent ductal breast carcinoma cell line MCF 7 cell images were taken under an inverted phase-contrast microscope at ×200 magnification with a digital camera at 48 hours after treatment with the samples. (a) Photomicrograph depicts the MCF 7 cells from the control group formed a compact confluent layer, where all the cells displayed aggressive multiplication and intact cellular membrane. In treatment groups, generally, the complexes demonstrated more significant activity than their respective ligands. (b) Treatment with tamoxifen showed marked inhibition in cell proliferation with IC_{50} = 14 μM. The picture revealed that the population of the cells is reduced drastically. (c) The treatment with 4 showed moderate to least cytotoxicity, with higher IC_{50} value is found to be >200 μM. (d) Treatment of MCF 7 cells with 6 showed strong cytotoxic effect (IC_{50} = 6.38 μM) as the antiproliferative effect was similar to that of the standard reference tamoxifen. The viability of the cells was severely affected, as the photomicrograph showed all the treated cells lost their viable characteristic features. (e) Treatment with 5 showed mild antiproliferative activity on MCF-7 cells. The IC_{50} was determined to be 158 μM. (f) Photomicrograph of MCF-7 cell treated with 7 showed potent inhibition in cell proliferation with IC_{50} = 1.12 μM. The picture revealed the strong cytotoxic activity of the complex as the cells undergo apoptotic changes with the clear evidence of membrane blebbing and nuclear condensation.
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References


