ORIGINAL ARTICLE

Synthesis, Characterization, and Anticancer Studies of Dimethylene Bridged Bis Tri-n-heterocyclic Carbene Trinuclear Silver(I)

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ABSTRACT

Introduction: Cancer therapy generally commences precisely following some cells in a specified location of the body begin growing uncontrollably and additionally penetrate the whole body; they keep on with their anomalous spread and consume entire nutrients from the non-infected cells, which ultimately causes the demise of cells. The admiration of N-heterocyclic carbenes commenced following the work of Ülfele and Wanzlick when they first synthesized them and identified them as ligands in metal complexes. This research aims to synthesize, characterize, and study the anti-tumour potential of the tris-NHC salts and their corresponding Ag(I) complexes. **Materials and methods:** Tris-benzimidazolium salts (1 and 2) and their respective open-chain Ag1 and Ag2 complexes were synthesized. The salts formed from the reaction between 3-(2-bromoethyl)-alkylbenzimidazolium bromide and benzimidazole in equimolar concentrations. The complexes were obtained by *in situ* deprotonation reaction of the salts with 1.5 equivalent of Ag2O in 1,4-dioxane and methanol. FT-IR, 1H, and 13C NMR, elemental analysis, melting point, and solubility test fully characterized the compounds. The anti-tumour study was carried out with MCF-7 cells. **Results:** All the composites were afirmed by various characterization methods. Salts did not show any activity; the IC_{50} obtained for Ag1 and Ag2 were 6.35±0.6, 6.00±0.2 µM while tamoxifen gave 19.7±0.3 µM IC_{50}, which is lower than the tested compounds. **Conclusion:** Two salts and two Ag(I) complexes were synthesized and characterized, and their anticancer potential was studied.
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Keywords: Benzimidazolium salt, Tri-NHC, Trinuclear Silver(I), Breast cancer, Tamoxifen

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INTRODUCTION

Cancer therapy often starts just after some cells in a specific location of the body start growing uncontrollably as well as infiltrate the entire body; the tumour cells continue their aberrant development and devour all the foods from the normal cells, ultimately causing the demise of cells (1). It is the dominant reason for death in the United States and a substantial global public health matter (1). According to the "American Cancer Society's" projections for 2023, there will be "1,958,310" new cancer cases and "609,820" demise by cancer in the country (1). The Society presented that the, "breast cancer" (BC) has become the number two giant cancer-related reason for demise in women, and its prevalence is rising every year (1) (2).

The World Health Organization reports that cancer was Malaysia's leading cause of death in 2018, accounting for 43,837 cases and 26,395 fatalities, with breast cancer having the highest incidence (17.3%) and mortality rate (11.0%) (3). In 2020, new cases increased to 48639, and the mortality rate to 29530. Breast cancer has the most significant number of new cases (17.%) and the second mortality rate (11.9%) (4). In most cases, women have the highest number of breast cancer cases. In 2020, women's breast cancer cases in Malaysia were 8418 (32.9%) (4). The popularity of the *N*-heterocyclic carbenes began after the work of Öfele (5) and Wanzlick (6), when they first identified them as ligands in metal complexes, accompanied by the champion confinement of the *N*-heterocyclic carbenes freely resulted in their metalation to numerous leading group and transition metal complexes by Arduengo (7).

Furthermore, 20 years back, a thorough assessment of NHC ligands for transition metals disclosed vibrant coordination chemistry with improving applications (8).

Formerly, *N*-heterocyclic carbenes coordinating with transition metals are earning more attention because these complexes have potential in several research fields, such as medicine (9,10), nanomaterials (11), and catalysis (12,13), luminescent. The *N*-heterocyclic carbenes are perfectly recognized, exceptionally relevant, and promising ligands in organometallic chemistry because they form stable composites in "higher" and lower "oxidation" states "transition metals" (14,15). The generation of these metal complexes is ascribed to their bonding to transition metals more strongly caused by their powerful α-donating and delicate π-accepting ability once reacted amidst metals (13,16). The NHCs systems' neighbouring chains that are annexed to the nitrogen atoms are typically changed in order to achieve conformational changes. This reveals a sequence of minor modifications to the ligand's electronic and steric properties that subsequently have an impact on the physical characteristics of the corresponding complexes, including their solubility, stability, and lipophilicity (17).

In a few numbers, open-chain and cyclic tris-benz/imidazolium salts and their corresponding metal composites were described (16–20). In this, we presented the synthesis, characterization, and anti-tumour investigation of novel open-chain tris-benzimidazolium salts (H_3L) emanating from the reaction between benzimidazole and two equimolar 3-(2-bromomethyl)-1-alkyl benzimidazolium bromide. Three Ag(I) ions are located in the middle of the two tri-carbene ligands in the open-chain structures $[Ag_3L_2]$. $3PF_6$ produced by the generated salts with Ag_2O reaction.

MATERIALS AND METHODS

Chemicals and types of equipment used

All of the reagents and solvents were obtained from Sigma-Aldrich and utilized directly. FT-IR spectra in the 600–4000 cm^{-1} range were established employing the Perkin Elmer FT-IR Microscope Spotlight 200. The ^1H NMR and ^{13}C NMR were generated from a Bruker 500 MHz spectrometer at room temperature in deuterated dimethylsulfoxide with tetramethylsilane internal standard in the scan range δ 0-16 and 0-200 ppm for ^1H and ^{13}C NMR., respectively. The NMR peaks were labelled as singlet (s), doublet (d), triplet (t), and multiplet (m), and chemical shifts were referenced to the solvent signals peaks as standard internal references; d_6-DMSO (^1H NMR δ 2.50, ^{13}C NMR δ 39.51. Using a PerkinElmer series II 2400 micro-analyzer, elemental analysis was performed. A Stuart Scientific SMP-1 (UK) device was used to measure melting points. Human breast cell lines (MCF-7) were used in the anti-tumour study, along with full medium (DMEM combined with 10% FBS and 1% Pen Strep), dimethylsulfide as a solvent, and 96 well plates. Using a Microplate Reader-Multiskan Spectrum, the optical density (OD) was measured at 540 nm (Thermo Scientific).

Salts Synthesis

3-(2-bromoethyl)-1-pentylbenzimidazolium bromide is synthesis (i)

Pentylbenzimidazole (1.00 g, 5.31 mmol) was refluxed for 12 hours at 80 °C in a neat mixture with extra 1,2-dibromoethane (4 ml, 21.30 mmol). Dichloromethane was used to filter out and clean the white precipitate once it had formed. Dichloromethane and 3-(2-bromoethyl)-1-pentylbenzimidazolium bromide are present in the clear filtrate. To allow the dichloromethane to evaporate, the mixture was placed in the fume hood for two days, and the precursor (i) was found as a clear crystal. Yield: 1.78 g (90%) %). M.P: 59-63 °C. Anal. Calcd. for $C_{14}H_{20}Br_2N_2$: C, 44.73; H, 5.36; N, 7.45%; found: C, 44.51; H, 5.05; N, 7.87%; IR (ATR, cm^{-1}): 3022 (C-H_{arom} stretching); 2929-2856 (C-H_{aliph} stretching), 1559 (C=N stretching), 1425-1375 (C-$N_{arom.}$ stretching); 1197-1010 (C-$N_{aliph.}$ stretching), 765 (C-H bending) 638 (C-Br). ^1H NMR (500 MHz, d_6-DMSO) in ppm: 0.85 - 0.88 (3H, t, pentyl CH_3×1, J = 6.8 Hz); 1.30 -1.36 (4H, m, pentyl CH_2CH_2×1, J = 6.4 Hz), 1.92 - 1.98 (2H, m, pentyl CH_2×1, J = 6.0 Hz); 4.10 - 4.12 (2H, t, ethyl Br-CH_2×1, J = 6.0 Hz); 4.58 – 4.61 (2H, t, pentyl NCH_2×1, J = 6.0 Hz); 5.03 – 5.05 (2H, t, ethyl NCH_2×1, J = 7.1 Hz); 7.72 – 8.22 (4H, m, benzimi H×4); 10.10 (1H, s, NCHN, H×1); ^{13}C NMR (125 MHz, d_6-DMSO) in ppm: 14.2, (pentyl CH_3), 22.0 (pentyl CH2); 28.2 (pentyl CH_2); 28.6 (pentyl CH_2); 31.6 (ethyl BrCH_2); 47.3 (pentyl NCH_2); 48.5 (ethyl NCH_2) 114.3, 114.4, 127.2, 127.3, 131.3, 131.4 (benzimi C'); 143.1 (NCHN)

Synthesis of propyl-substituted trisbenzimidazolium tribromide bridged by ethyl group (1)

Benzimidazole (0.25 g, 2.12 mmol) and 3-(2-bromoethyl)-1-n-propyl benzimidazolium bromide (1.46 g, 4.23 mmol) were dissolved in a mixture of 1,4-dioxane (20 mL) and methanol (2 mL). The reaction mixture was left to reflux for 24 hours. The tris-benzimidazolium tribromide was a white precipitate that was filtered, followed by successive rinsing with original 1,4-dioxane and dichloromethane. A white precipitate of salt 1 was achieved; it was recrystallized in methanol and allowed to air dry. Yield:1.20 g (77.4 %). MP: 172 - 176 °C. Anal. Calcd. for $C_{31}H_{37}Br_3N_6$: C, 50.77; H, 5.10; N, 11.46 %; found: C, 51.25; H, 4.87; N, 1.87%; IR (ATR, cm^{-1}): 3159 - 3102 (C-$H_{arom.}$ stretching); 2940 (C-$H_{aliph.}$ stretching), 1568 (C=N stretching), 1490 - 1464 (C-$Na_{rom.}$ stretching); 1194 (C-$N_{aliph.}$ stretching); ^1H NMR (500 MHz, d_6-DMSO) in δ ppm: 0.82 – 0.85 (6H, t, propyl CH_3×2, J = 7.3 Hz), 1.75 – 1.82 (4H, m, propyl CH_2×2, J = 7.0); 4.37 - 4.40 (4H, t, propyl NCH_2×2, J = 7.0 Hz), 5.03 (4H, s, ethyl bridge NCH_2×2); 5.09 (4H, s, ethyl bridge NCH_2×2); 7.54 – 8.11 (12H. m, benzimi 4H×3); 9.58 (2H, s, NC*H*N H×2); 9.70 (1H, s, NC*H*N H×1); ^{13}C NMR (125 MHz, d_6-DMSO) in ppm: 11.1 (propyl CH_3); 22.5 (propyl CH_2); 46.2 (propyl NCH_2); 48.6 (ethyl bridge NCH_2); 113.4, 113.6, 114.3, 114.4, 127.3, 127.4, 127.5, 127.6, 131.3, 131.4, 131.4, 131.5

(benzimi C′); 143., 144.1 (NCHN).

Synthesis of Pentylsubstituted trisbenzimidazolium tribromide bridged by ethyl group (2)

Salt 2 was obtained using the same method as salt 1; besides that, (i) was exchanged with 3-(2-bromoethyl)-1-n-pentyl benzimidazolium bromide (1.58 g, 4.23 mmol). Salt 2 was produced as a white precipitate, purified by recrystallization in methanol, and allowed to air dry. Yield: 1.28 g (77%). MP: 181 - 186 °C. Anal. Calcd. for $C_{35}H_{45}Br_3N_6$: C, 53.23; H, 5.75; N, 10.64 %; found: C, 54.45; H, 5.24; N, 10.15 %; IR (ATR., cm^{-1}): 3158 - 3102 (C-$H_{arom.}$ stretch); 2963 - 2875 (C-$H_{aliph.}$ stretch), 1567 (C=N stretching), 1489 - 1378 (C-$N_{arom.}$ stretching); 1190 (C-Naliph. stretching); 1H NMR (500 MHz, d_6-DMSO) in δ ppm: 0.84 – 0.86 (6H, t, pentyl CH_3×2, J = 7.2 Hz), 1.20 – 1.25 (4H, m, pentyl CH_2×2, J = 7.2), 1.28 – 1.34 (4H, m, pentyl CH_2×2, J = 7.4); 1.72- 1.78 (4H, m, pentyl CH_2×2, J = 7.4); 4.40 - 4.42 (4H, t, pentyl NCH_2×2, J = 7.25 Hz), 5.03 (4H, s, ethyl bridge NCH_2×2); 5.10 (4H, s, ethyl bridge NCH_2 ×2); 7.52 – 8.10 (12H. m, benzimi 4H×3); 9.59 (2H, s, NCHN H×2); 9.72 (1H, s, NCHN H×1); 13C NMR (125 MHz, d_6-DMSO) in ppm: 14.2 (pentyl CH_3); 22.0 (pentyl CH_2); 28.2 (pentyl CH_2); 28.7 (pentyl CH_2); 46.2 (pentyl NCH_2); 47.21 (ethyl bridge NCH_2); 113.3, 113.6, 114.3, 127.4, 127.4, 127.5, 127.5, 131.3, 131.5 (benzimi C′); 143.1, 144.1 (N*C*HN).

Synthesis of Ag1 and Ag2 complexes

Synthesis of propyl-substituted tris-benzimidazol-2-ylidene trinuclear silver(I) tris-hexafluorophosphate bridged by ethyl group (Ag1)

Salt 1 (0.50 g, 0.68 mmol) dissolved in 50 ml methanol, and Ag2O (0.24 g, 1.02 mmol) was incorporated. Ambient temperature stirring was conducted for 48 hours in a flask covered with aluminium foil to prevent light interference. Following the reaction period, the compound was filtered via a celite column to separate it from the insoluble. Employing KPF_6 (0.19 g, 1.02 mmol), the resultant Ag1 complex in bromide was exposed to metathesis. The resultant compound was agitated for 2 hrs; the precipitate was filtered, cleaned with deionized water, and allowed to dry openly. The Ag1 complex was generated as a white precipitate undergoing recrystallization in methanol to obtain it in a pure form. Yield 0.42 g (71%). MP.: 251 - 257°C. Anal. Calcd. for the $C_{62}H_{68}Ag_3F_{18}N_{12}P_3$: C, 42.80, H, 3.94, N, 9.66 %; found: C, 43.24; H, 3.56; N, 9.64%. IR (ATR, cm^{-1}): 2953 (C-H_{aliph} stretching); 1448 - 1396 (C-N_{arom}. Stretching), 1207 (C-$N_{aliph.}$ stretching); 1H NMR (500 MHz, d_6-DMSO) in ppm: 0.46 – 0.49 (12H, t, propyl CH_3×4, J = 7.2 Hz); 1.16 - 1.11 (8H, m, propyl CH_2×4, J = 7.7 Hz); 4.15 - 4.17 (8H, m, propyl NCH_2×4, J = 5.5 Hz); 5.03 (8H, s, ethyl bridge NCH_2CH_2N×2); 5.30 (8H, s, ethyl bridge NCH_2CH_2N×2); 6.19 - 7.21 (32H, m, benzimi 4H×8); 13C NMR (125 MHz, d_6-DMSO) in ppm: 11.3 (propyl CH_3); 23.9 (propyl CH_2); 43.4 (propyl NCH_2); 50.0, 50.1 (ethyl bridge NCH_2); 109.5, 112.6, 112.5, 118.8, 123.8, 124.4, 124.8, 124.9, 127.2, 127.2, 133.1, 133.7, 134.1, 139.9, 146.2 (benzimi C′); 185.9, 186.0, 187.9, 188.1 (C-Ag, d, $J_{C\text{-}Ag107}$ = 242.3 Hz, $J_{C\text{-}Ag109}$ = 271.0 Hz).

Synthesis of pentyl-substituted tris-benzimidazol-2-ylidene trinuclear silver (I) tris-hexafluorophosphate bridged by ethyl group (Ag2)

The Ag2 was formed using the same method that resulting Ag1. Besides that, salt 1, silver oxide, and potassium hexafluorophosphate were substituted with salt 2 (0.5 g, 0.63 mmol), silver oxide (0.22 g, 0.95 mmol) and KPF_6 (0.18 g, 0.95 mmol). It was obtained as a white precipitate and recrystallized with methanol. Yield 0.43g (73%). MP.: 261 - 267 °C. Anal. Cal. for the $CH_{70}H_{84}Ag_3F_{18}N_{12}P_3$: C, 45.40, H, 4.57, N, 9.08 %; found: C, 44.89; H, 4.78; N, 9.45%. IR (ATR., cm^{-1}): 2945 – 2863 (C-H_{aliph} stretching); 1454 - 1402 (C-$N_{arom.}$ stretching), 1229 (C-$N_{aliph.}$ stretching); 1H NMR (500 MHz, d_6-DMSO) in ppm: 0.82 – 0.85 (12H, t, pentyl CH_3×4, J = 7.00 Hz); 1.15 – 1.22 (8H, m, pentyl CH_2×4, J = 5.6 Hz); 1.28 – 1.34 (8H, m, pentyl CH_2×4, J = 7.30 Hz); 1.78 – 1.84 (8H, m, pentyl CH_2×4, J = 7.60 Hz); 4.27 – 4.30 (8H, m, pentyl NCH_2×4, J = 7.2 Hz); 5.01 (8H, s, ethyl bridge NCH_2CH_2N×2); 5.30 (8H, s, ethyl bridge NCH_2CH_2N×2); 6.55 – 8.22 (24H, m, benzimi 4H×6); 13C NMR (125 MHz, d_6-DMSO) in ppm: 14.2 (pentyl CH_3); 22.1 (pentyl CH_2); 28.6 (pentyl CH_2); 30.2 (pentyl CH_2); 44.8 (pentyl NCH_2); 56.3, 56.9 (ethyl bridge NCH_2); 111.2, 112.6, 119.6, 123.3, 124., 133.8, 141.6, 142.9, 144.9, (benzimi C′); 187.2, 189.2 (C-Ag).

Methodology for Cytotoxicity Study

The cytotoxic effect of 4 samples on the MCF-7 cell line was determined using an MTT assay. The cells were treated with samples at different concentrations. The 10 mg/mL of stock solution of each sample was prepared by dissolving 10 mg of the sample in 100 µL of dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA), then 900 µL of complete media (DMEM incorporated with 10% FBS, 1% Pen Strep) was added. 5000 cells/well were seeded in 96 well plates in complete media for 24 hours. Breast cancer cells were then treated with increasing concentrations of the different samples ranging from 50, 25, 12.5, 6.25, 3.125, and 1.56 µg/mL and incubated for 24 hours. Tamoxifen was used as a positive control at concentrations of 50, 25, 12.5, 6.25, and 3.125, 1.5625 µg/mL. Upon completion of the incubation period, an MTT assay was performed. The optical density (OD) was recorded at 540 nm using a microplate reader- Multiskan Spectrum (Thermo Scientific). The final concentration of DMSO was 0.2% in the highest concentration of the treatment (200 µg/mL) and the vehicle control. The concentration for each sample varies. All experiments were performed in triplicates. The inhibition of cells and the finding of IC_{50} values have been statistically analyzed using Two-way ANOVA (Multiple Comparisons) with Graphpad Prism (8.4.2).

RESULTS

Synthesis

The synthesis section is the most important factor to be considered to confirm the formation of the proposed salts and their corresponding silver(I) complexes. 3-(2-bromoethyl)-1-n-alkylbenzimidazolium bromide (i – ii) were the precursor ligands employed for synthesizing the open-chain trisbenzimidazolium bromide salts (where alkyl = propyl (i) and pentyl (ii). They were synthesized using the reported procedure (16,21). The reaction involves two stages, beginning with synthesizing n-alkyl benzimidazole (alkyl = propyl and pentyl), as reported previously (22). Further reaction of the prepared n-alkyl benzimidazole with an excess amount of 1,2-dibromoethane in a neat resulted i – ii in a good yield (Scheme 1). Worthy of mention, salt i was reported before for open-chained tetrakis benzimidazolium salts synthesis (Figure 1) (23,24). The open-chained tris-benzimidazolium bromide salts (1 and 2) and their corresponding trinuclear silver(I) (Ag1 and Ag2) were obtained by a "multistep designed scheme" reported earlier by our group (18). Reacting benzimidazole with 3-(2-bromoethyl)-1-n-alkyl benzimidazolium bromide in a 1:2 molar ratio in their 1,4-dioxane and methanol solution yielded the salts (Scheme 2). Moreover, the corresponding Ag1 and Ag2 complexes were generated via *in situ* deprotonations using methanol and an Ag_2O:H_3L ratio of 3:2. (Scheme 3). Both the salts and the complexes were obtained in a good yield as indicated in the experimental section.

Figure 1: Reported 3-(2-bromoethyl)-1-propylbenzimidazolium bromide (i) by our group (23-24).

Solubility test

Based on the obtained results, all the ligands and their corresponding Ag1 and Ag2 complexes were soluble in solvents, like dichloromethane, DMSO, acetonitrile, DMF, methanol, and ethanol (25,26). Similarly, all the compounds are stable in air and moisture in solid and solution forms (25,27). The result indicated that the significant variation between these novel composites to those presented earlier by our group is the alkyl chain connected to the terminal nitrogen atoms (16) (Scheme 1).

FTIR Spectroscopic Study

The formation of the salts and their corresponding silver(I) complexes was confirmed based on the results obtained from various characterization methods, including FTIR by showing the presence of the important functional groups present in the structures and the data obtained were presented in the experimental section.

^{1}H and ^{13}C NMR Analyses

All the synthesized salts and their corresponding silver(I) complexes were confirmed to be formed by 1H and 13C NMR spectroscopy and the data were presented in the experimental and discussion sections. This is in addition to a good correlation between the calculated and obtained results for elemental analysis, as indicated in the experimental section (28).

Anticancer study

All the salts and their corresponding silver(I) complexes were tested against the breast cancer cell line using MTT assay method. The Salts did not show any activity based on the results obtained; the IC50 obtained for Ag1 and Ag2 were 6.35±0.6, 6.00±0.2 µM while tamoxifen gave 19.7±0.3 µM IC50, which is lower than the tested compounds (Table 1).

DISCUSSION

The tris-benzimidazolium salts and their corresponding Ag1 and Ag2 complexes were generated, and the successful synthesis was confirmed by FTIR spectral analysis (29). In the benzimidazolium system, sharp peaks were obtained from the FTIR spectra of Salt1 and salt2 at 1568 and 1567 cm^{-1}, individually attributed to the C=N bond stretching vibration (30). Likewise, C-N stretching vibration can be noticed ranging from 1490-1464 and 1489-1378 cm-1 for the salts (Fig.2 (a) and (b) (bottom)) (30). The C=N stretching vibration bands vanished after the successful metal insertion in the salts, as found in the FTIR spectral data of the Ag1 and Ag2 complexes. The influential peaks in the spectra of the synthesized Ag1 and Ag2 complexes are for C-N stretching vibrations, which were observed in the range of 1448-1396 and 1454-1402 cm-1, respectively (Figure 2 (a) and (b) (Top)) (31,32).

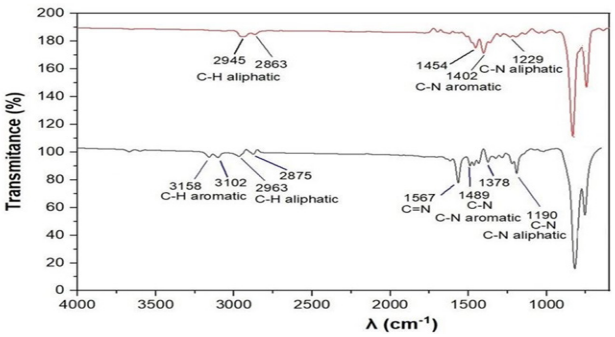

Figure 2 (a): Compared FTIR Spectra of salt1 (bottom) and Ag1 (top). The spectra indicate the functional groups available in the salt and the complex with some variation after metalation.

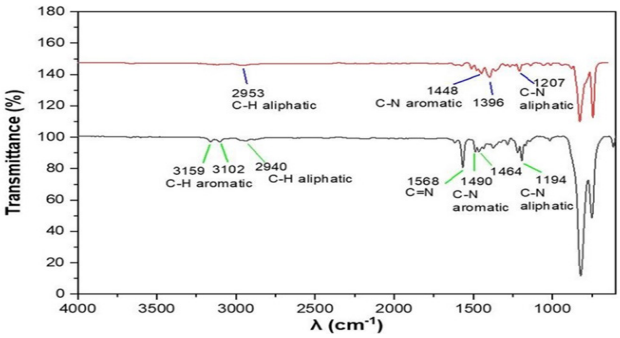

Figure 2 (b): Compared FTIR Spectra of 2 (bottom) and Ag2 (top). The spectra indicate the functional groups available in the salt and the complex with some variation after metalation.

The synthesis of the precursor salts ii, the main salts 1 and 2, and their corresponding complexes (Ag1 and Ag2) was confirmed as well with ^{1}H and ^{13}C NMR. The essential characteristic resonance that confirms the synthesis of the precursor salts (ii) in the 1H NMR spectra are the additional triplet peaks for CH_2Br ranges between δ 4.06 - 4.12 ppm (23). While the two other triplet peaks present are those of the terminal nitrogen atoms in the benzimidazolium ring (NCH_2), as indicated in Figure 3 (a). The ^{13}C NMR spectra of ii served as another evidence for their synthesis, N*C*HN, which appeared at δ 143.1 ppm, as shown in Figure 3 (b). These values are also similar to the reported literature (23).

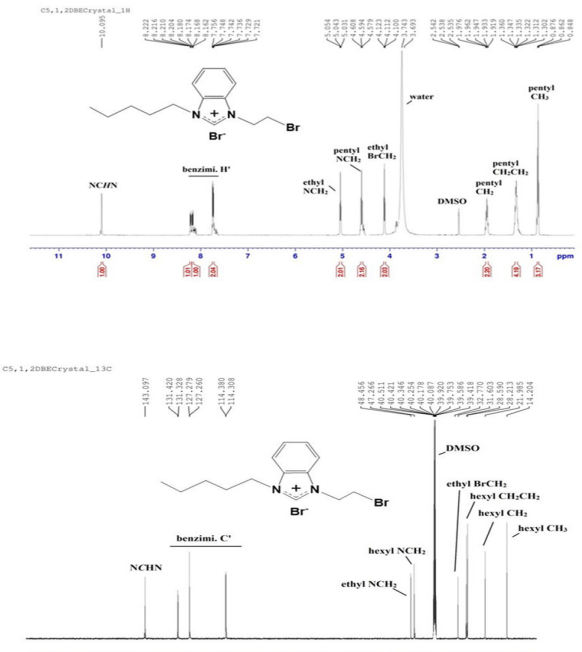

Figure 3: (a) ^{1}H NMR of ii (b) ^{13}C NMR of ii.
The (a) Spectra indicated the presence of NCHN at the most downfield region, and the $BrCH_2$, NCH_2 (b) Spectra indicated the presence of NCHN at the most downfield region, and the $BrCH_2$, NCH_2.

The synthesis of the salts 1 and 2 and their corresponding Ag1 and Ag2 complexes was similarly probed with ^{1}H and ^{13}C NMR. The ^{1}H NMR spectral data of the 1 and 2 indicate the occurrence of the two singlet peaks at the most downfield region of the spectra correspond to three carbene centres within the range of δ 9.58 - 9.71 ppm, confirming the successful synthesis of the salts as reported earlier (26,33). The two singlet peaks for the three protons indicate two different chemical environments; thus, two protons are the same, given only one peak (18). All the dimethylene protons occurred as a singlet peak ranging from δ 5.03 - 5.10 ppm for all the salts, which denotes the flexibility of the conformation (34). The proton resonances of each salt's terminal substituents also occur in their expected areas in the spectra, in addition to the core benzimidazolium moieties, indicating the successful synthesis of the desired salts (Figure 4 (a)) and Fig. S1 (a) in *Supplementary Materials*) (35,36). The ^{13}C NMR spectral data of the salts displayed the two resonances by the N*C*HN in the downfield area at δ 143.1-144.1 ppm in each of the salts, similar to the earlier reports (Figure 4 (b)) and Figure S2 (b) in *Supplementary Materials*) (26,37).

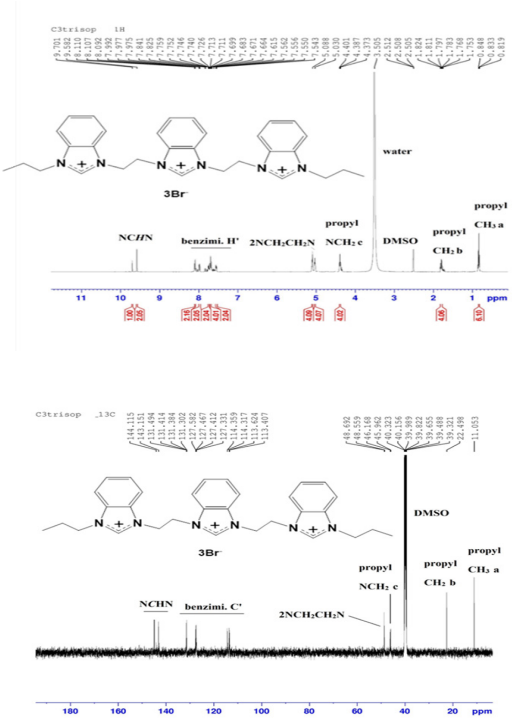

Figure 4: The (a) ^{1}H and (b) ^{13}C NMR spectra of salt1 as a representative for the other members in the series.
The (a) Spectra showed the appearance of NC*H*N at the most downfield region, and the NCH_2 for both the dimethylene ^{1}H and NCH_2 ^{1}H in the pentyl chain and other CH_2 in the pentyl group (b) Spectra indicated the presence of N*C*HN carbon at the most downfield region, the dimethylene carbons NCH_2, the NCH_2 in the pentyl chain and other CH_2 in the pentyl group.

The ^{1}H NMR spectra of Ag1 and Ag2 complexes showed the disappearance of the resonance for the azolium group protons, which confirmed the success of the complexes synthesis (38,39). As previously observed, the resonances of the remaining protons are broadened and overlapped, indicating that the Ag1 and Ag2 complexes

possess flexible structures (Figure 5 (a)) and in Fig. S3 (a) in *Supplementary Materials* (16).

The ^{13}C NMR spectra provide proof that the $C_{carbene}$-Ag(I) bond has formed because the complexes exhibit two doublets in complex Ag6 (δ 185.8, 186.0, 187.9, and 188.0 ppm) with coupling constant of 239.9 for C- ^{107}Ag, 271.0 Hz C-^{109}Ag (Figure 5 (b)) (9,40–42). However, δ 185.9 and 189.2 ppm were obtained for Ag1 (C-Ag); the coupling for C- $^{107/109}Ag$ was not detected; this might be because of the high broadening of the peaks that made the resonances invisible Figs. S3 (b) in *Supplementary Materials*) (18). All attempts to generate single crystals failed; hence, X-ray crystallography did not hold up the conformation of any of the Ag1 and Ag2 complexes, as reported earlier by our group (18,24,32). Despite our inability to obtain the crystal structure of the compounds after several attempts, we could still, based on the 1H, 13C NMR, FTIR, and elemental analysis, put forward that the Ag1 and Ag2 compounds in this research exist as tri-nuclear assemblies containing 2 tri-NHC units with linear conformation. The three carbene centres coordinated to the 3 Ag(I) ions in a tri-metal bi-ligand conformation that holds up the stoichiometry of the complexes to be $[Ag_3L_2]\cdot 3PF_6$ as earlier presented (42) (*vide supra*). Prior research has revealed more compounds with similar coordination types, such as bis-benzimidazol-2-ylidene dinuclear silver (I) $[Ag_2L_2]\cdot 2PF_6$, which has a di-metal bi-ligand arrangement and complex stoichiometry (43–46).

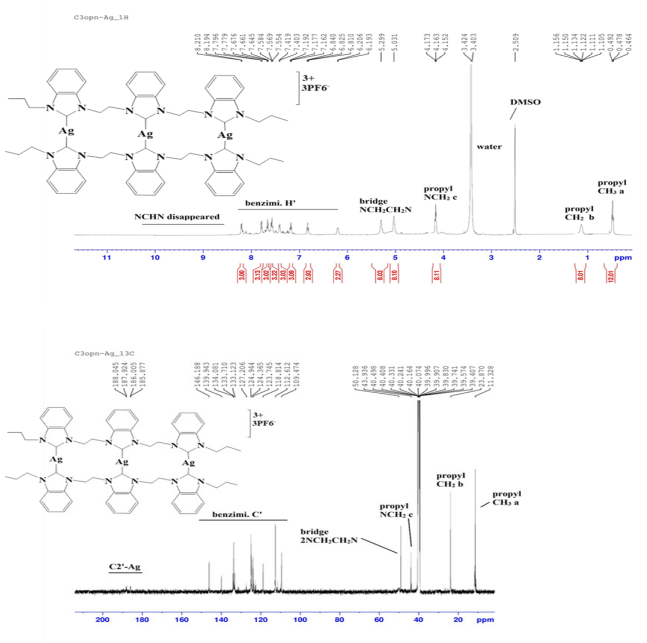

Figure 5: (a) ^{1}H NMR of Ag1 (b) ^{13}C NMR of Ag1 as representative. The (a) Spectra indicated the disappearance of NC*H*N at the most downfield region. The presence of NCH_2 for both the dimethylene ^{1}H and the methylene 1H in the pentyl chain and other pentyl group ^{1}H (b) Spectra indicated the presence of C-Ag peaks at the most downfield region, the dimethylene carbons NCH_2 and the NCH_2 in the pentyl chain other pentyl group carbons.

It was reported earlier that silver(I) mononuclear ben/imidazolium complexes indicated title cytotoxicity potentiality on several tumour cell lines (24). By increasing the N-heterocyclic carbene numbers and the quantity of Ag(I) ions connected, the latter can be improved, creating more reactive complexes against a variety of tumour cells, notably breast cancer cell line (MCF-7) (24). In the meantime, trisbenzimidazolium salts and their corresponding trinuclear silver(I) were tested on colon, breast, and HeLa cell lines, and improved outcomes were achieved, especially for the silver(I) complexes (42).

As such, all the salts (1 and 2) and their corresponding Ag(I)-NHC complexes (Ag1 and Ag2) synthesized were assessed by MTT assay on breast tumour cell lines to determine the IC_{50} value of each sample (Table I). None of the salts displayed any effect at the same concentration as the complexes. Similarly, dose dependence cytotoxicity of the complexes was tested and compared with the tamoxifen as the positive control Figure 6 (42,47).

Most importantly, the complexes were relatively active against the tested cell lines with the IC_{50} values of 6.35±0.6 and 6.0±0.2 µM for Ag1 and Ag2, respectively, comparable to the earlier reports (42). The IC_{50} values indicate that Ag2 delivered more effect on the cancer cell than Ag1 despite the difference is not insignificant (Table I). The complexes showed better activity than the positive control tamoxifen (19.7±0.3 µM) (42) (47). The dose-dependent cytotoxicity study showed that the two complexes and the tamoxifen are related to one another Figure 6 (42).

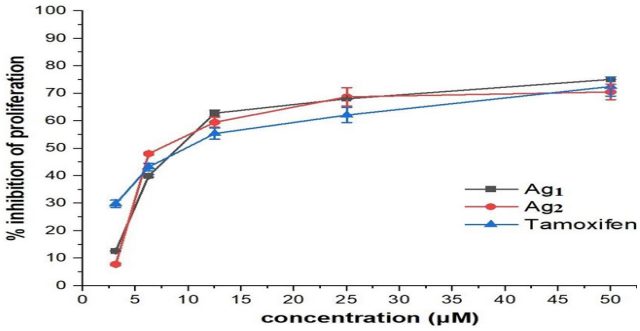

Figure 6: Dose-dependence of % inhibition of breast cancer cell proliferation compared to tamoxifen as standard.
The dose-dependence of inhibition of breast cancer cell proliferation of Ag1, Ag2, and tamoxifen as standard at concentrations range of 3.125-50 µM

Table I: IC50 Values for Ag1 and Ag2 (µM

Compound	IC50 (µM)
Salt1	NA
Salt2	NA
Ag1	6.35±0.6
Ag2	6.00±0.2

*Tamoxifen 19.7±0.3 µM NA = No Activity

The results show that silver(I) ions are essential in anticancer activity by making the silver(I)-NHC bond, which plays a significant role in antiproliferative activity

because of their extreme stability, which enables the release of silver(I) ions slowly (48,49). The impact of the slow release of silver(I) inhibits cellular respiration and metabolism of biomolecules by binding to the surface area. It gives rise to interaction with important cell organelles (41).

CONCLUSION

The synthesized salts 1 and 2 and their corresponding silver(I)-NHC, Ag1 and Ag2 complexes were successfully synthesized and characterized using various characterization methods. The anti-tumour potential of all the compounds was studied. It was confirmed that only the Ag(I) complexes were active against the tested cancer cells. The future study will focus on the synthesis, characterization, crystal structure analysis, and cytotoxicity study of different cancer cell lines on new aryl and longer alkyl chain substituted salts and their silver(I) complexes.

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