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Synthesis, Structural and Anticancer Studies of Asymmetrical Dinuclear Silver(I) Di-*N*-heterocyclic Carbene Complexes

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A series of asymmetric bis-benzimidazolium salts (**1**-**6**) and their respective dinuclear silver(I) di-NHC complexes (**7**-**12**) were successfully synthesized and characterized (where NHC = *N*-heterocyclic carbene). Each asymmetric salt was prepared through a quaternization reaction with the resulting NHC ligand being a bridging ligand to two adjacent metal ions. All synthesized compounds were characterized by elemental analysis, FTIR, and ^{1}H and ^{13}C NMR. All salts and dinuclear silver(I) di-NHC complexes were evaluated against the cervical adenocarcinoma (HeLa) cancer cell line using MTT assays. Preliminary results indicated that all compounds demonstrated dose-dependent cytotoxic activity, while compound **6** demonstrated moderate cytotoxicity against non-cancerous Hs27 cells. The dinuclear silver(I) di-NHC complexes showed significant IC_{50} values in the range of 2.91 – 5.97 µg/mL, compared to the standard drug, etoposide (IC_{50} = 3.15 µg/mL).

Keywords: *N*-heterocyclic carbene; dicarbene; silver(I)-NHC complexes; anticancer studies; human cervical; IC_{50}

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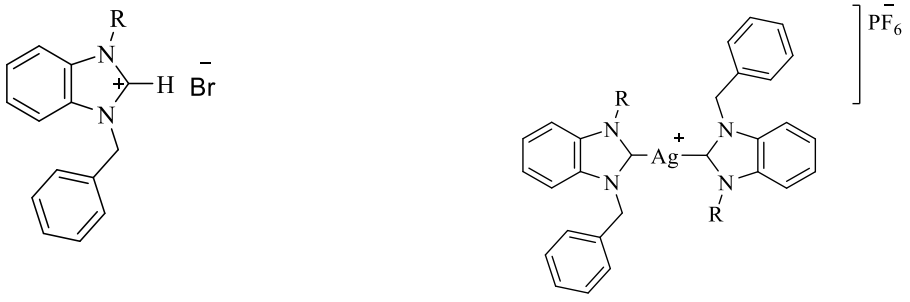
In the past decade, the *N*-heterocyclic carbene (NHC) complexes of late transition metals have emerged as compounds of increasing interest, owing to their significant properties, not only in terms of catalytic activity but also in biological and pharmacological aspects [1]. In 1991, Arduengo and co-workers successfully isolated and characterize5d the NHC compounds and found them to be suitable as ligands, with characteristics such as being excellent σ-donors, so that the ligand can efficiently donate electron density to metal centres which eases the preparation of stable organometallic complexes [2]. Moreover, the carbon atoms in NHC ligands are highly electron-rich, and thus nucleophilic, which allows NHC ligands to participate in many chemical reactions such as nucleophilic substitution, addition, and catalysis [3]. Since then, NHCs have experienced an unprecedented growth in research and applications including magnetic susceptibility and optical properties. Metal-NHC complexes have received considerable attention since the discovery of silver(I)-NHC complexes [4], notably in biomedical applications, because these complexes can be easily handled and are resistant to air and moisture. Nevertheless, studies have shown that the silver(I)-NHC complexes have been found to interact with DNA molecules by binding to their bases, hence interfering with DNA replication and transcription processes. These bonds can lead to DNA damage and subsequently cell death [5].



(a) (b)

Figure 1. The proposed structures of (a) synthesized asymmetrical benzimidazolium salts (*R* = methyl/ethyl/propyl/butyl/pentyl/hexyl) and (b) synthesized asymmetrical silver(I) di-NHC complexes

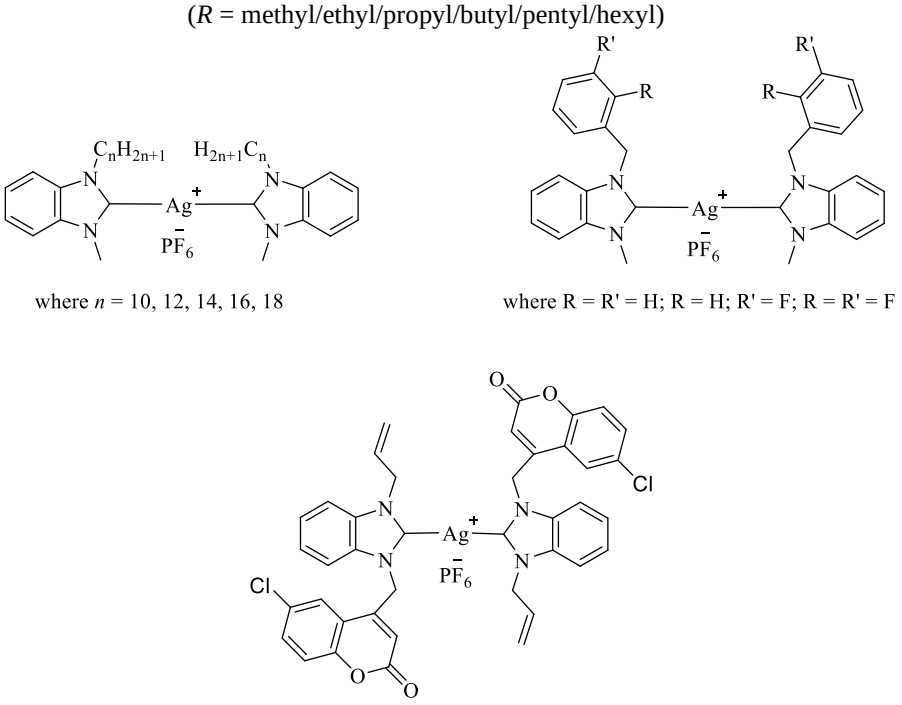


Figure 2. The structure of the studied silver(I)-NHC complexes with anticancer properties.

A broad range of silver(I) complexes with both functionalized and non-functionalized NHC ligands have been reported to date, which utilize benzimidazole moieties as anticancer agents [6] (**Figure 2**). Dinuclear silver(I) complexes with bulky non-functionalized NHC ligands have shown remarkable supramolecular chemistry via attractive interactions between the two silver centres through the favourable d^{10}-d^{10} argentophilic interaction that enhances the stability of the complexes formed [7]. Interestingly, by varying the metal to NHC ratio, the coordination behaviour between the NHC ligands and the silver(I) ion can be regulated in terms of topology [8]. In fact, numerous silver(I)-NHC complexes show spectacular supramolecular structures resulting from the interactions of metal complexes with various counter ions or solvent molecules in the lattice [9].

The identification of a new silver-coordinated metallotherapeutic compound has several advantages due to its lower toxicity [10]. Even though cisplatin has a high cure rate, the use of this drug is relatively limited due to side effects and toxicity concerns [11]. Moreover, over time, novel anticancer and antibacterial medicines with improved pharmacological effects have been made possible by the binding of various NHC ligands to silver atoms [12]. Akkoç and co-workers synthesized a series of silver(I) complexes with mixed NHC ligands [12]. The silver(I)-NHC complexes have shown promising results in anticancer studies, by exhibiting superior or comparable activity compared to cisplatin, a widely used chemotherapeutic agent. Silver(I)-NHC complexes have also exhibited efficacy against a wide range of cancer types, including both solid tumours and haematological malignancies. This versatility is valuable as it suggests the potential for silver-NHC complexes to be effective against different types of cancers, including those that are resistant to cisplatin. Compared to the platinum derivatives of cisplatin, carboplatin, and oxaliplatin, silver(I)-NHC complexes have shown anticancer qualities with higher activity, less side effects, and less development of resistance [12]. One drawback of platinum-based drugs is the development of resistance in cancer cells over time. Cancer cells can acquire mechanisms to neutralize or repair the DNA damage caused by platinum drugs, leading to reduced effectiveness and treatment failure. It is also known that platinum-based drugs can cause significant toxicity to healthy cells and tissues. They can induce various side effects, including nephrotoxicity (kidney damage), ototoxicity (hearing loss), peripheral neuropathy, and gastrointestinal disturbances. This toxicity limits the dose and duration of treatment, thus affecting the overall efficacy of the drug [13,14].

In recent years, we have reported the methodology for synthesizing a series of unprecedented asymmetrical bis-benzimidazolium salts and their respective dinuclear silver(I) di-NHC complexes in order to investigate their antimicrobial activity [15]. As these complexes have shown significant antimicrobial activity, we now expand our work to explore the potential cytotoxic activity of some new dinuclear silver(I) di-NHC complexes. Hence, in this study, a full series of asymmetrical bis-benzimidazolium salts and their dinuclear silver(I) di-NHC complexes was developed and evaluated on the human cervical cancer (HeLa) cell line.

EXPERIMENTAL

Materials and Instruments

All chemicals and solvents were purchased from commercial sources and used as received. Melting points were tested using a Stuart Scientific SMP-1 (UK) instrument. Elemental analyses were carried out on a PerkinElmer Series II, 2400 microanalyzer. The FTIR spectra were obtained using a PerkinElmer Spotlight 200 FTIR Microscope in the range of 4000 cm^{-1} to 600 cm^{-1}. Nuclear magnetic resonance (NMR) spectra were recorded in DMSO-d_6 using a Bruker 500-MHz Ascend NMR spectrometer at ambient temperature with TMS as the internal standard.

Cell line and Culture Conditions

Cervical adenocarcinoma cells (HeLa cell line) and Fibroblast (Skin; Foreskin) human normal (Hs27) cells were obtained from the American Type Culture Collection (ATCC), USA. The cells were cultured in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 µ/mL of penicillin and 100 µ/mL of streptomycin. The cell line was maintained as an adherent culture in a humidified atmosphere of 5 % CO_2 at 37 °C.

Synthesis

N-methylbenzimidazole is commercially available and was used as received. *N*-alkyl-benzylbenzimidazoles (alkyl = methyl, ethyl, propyl, butyl, pentyl, hexyl) and *N*-(2-bromoethyl)-N'-benzylbenzimidazolium bromide were synthesized according to the reported procedure with minor modifications [16-18]. In order to complete the series of compounds with methyl to hexyl substituents in the benzimidazolium moiety, the reported bis-benzimidazolium salt **4** and respective dinuclear silver(I) di-NHC complex **10** that contained a butyl substituent were prepared in this study using a method reported previously [15]. The formation of salt **4** and complex **10** in this current work was confirmed by spectroscopic analyses.

Synthesis of Bis-benzimidazolium salts (1-3, 5, 6)

Synthesis of N, N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-methylbenzimidazolium dibromide (**1**)

A mixture of *N*-(2-bromoethyl)-*N*'-benzyl benzimidazolium bromide (1.00 g, 2.52 mmol) and *n*-methylbenzimidazole (0.33 g, 2.50 mmol) was stirred and refluxed at 80 – 100 °C for 24 hours in acetonitrile (20 mL). A white precipitate formed in the reaction medium was filtered, washed with acetonitrile (2 × 5 mL) and diethyl ether (5 mL), and then air-dried to obtain **1**. Yield: 1.02 g (77 %), MP: 254 - 257 °C. **FTIR** (ATR, cm^{-1}): 3026 (Csp^3-H_{arom} stretching); 2989 (Csp^3-$H_{aliphatic}$ stretching); 1558 (C=N stretching); 1456 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 4.07 (3H, s, N-C**H**$_3$), 5.17 (4H, s, N-C**H**$_2$C**H**$_2$-N), 5.70 (2H, s, N-C**H**$_2$-Ar), 7.37 – 7.42 (5H, m, arene-**H**), 7.61 – 8.00 (8H, m, benzimi-**H**), 9.82 (1H, s, CH_3-N-C**H**-N-CH_2), 9.93 (1H, s, Ar-N-C**H**-N-CH_2). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 33.99 (N-**C**H_3), 45.82, 46.58 (N-**C**H_2**C**H_2-N), 50.54 (N-**C**H_2-Ar), 112.81, 113.75, 114.15, 114.50, 127.13, 127.24, 127.47, 127.52, 128.77, 128.83, 129.31, 129.50, 131.15, 131.24, 131.54, 132.01, 133.84 (arene-**C**/benzimi-**C**), 143.33 (CH_3-N-**C**H-N-CH_2), 143.81 (Ar-N-**C**H-N-CH_2). Anal. Calc. for $C_{24}H_{24}N_4Br_2$: C, 54.57; H, 4.55; N, 10.61 %. Found: C, 54.94; H, 4.59; N, 10.22 %.

Synthesis of N, N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-ethylbenzimidazolium dibromide (**2**)

The preparation of **2** was similar to that of **1**, but used *n*-ethylbenzimidazole (0.37 g, 2.53 mmol) instead of *n*-methylbenzimidazole. Yield: 1.03 g (75 %), MP: 272 – 275 °C. **FTIR** (ATR, cm^{-1}): 3123 (Csp^3-H_{arom} stretching); 3042, 2978 (Csp^3-$H_{aliphatic}$ stretching); 1555 (C=N stretching); 1455 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 1.44 (3H, t, C**H**$_3$CH$_2$-N, J = 7.5 Hz), 4.45 (2H, q, CH_3C**H**$_2$-N , J = 7.5 Hz), 5.18 (4H, s, N-C**H**$_2$C**H**$_2$-N), 5.71 (2H, s, N-C**H**$_2$-Ar), 7.39 – 7.63 (5H, m, arene-**H**), 7.64 – 8.02 (8H, m, benzimi-**H**), 9.78 (1H, s, CH_3CH_2-N-C**H**-N-CH_2), 9.85 (1H, s, Ar-N-C**H**-N-CH_2). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 14.38 (N-CH_2**C**H_3), 33.92 (N-**C**H_2CH_3), 42.78, 46.43 (N-**C**H_2**C**H_2-N), 50.65 (N-**C**H_2-Ar), 113.19, 114.40, 127.40, 127.51, 128.72, 128.76, 129.35, 129.39, 129.50, 129.53, 129.56, 131.04, 131.61, 133.76 (arene-**C**/benzimi-**C**), 143.20 (CH_3CH_2-N-**C**H-N-CH_2), 143.37 (Ar-N-**C**H-N-CH2). Anal. Calc. for $C_{25}H_{26}N_4Br_2$: C, 55.4; H, 4.80; N, 10.33 %. Found: C, 55.5; H, 4.58; N, 10.68 %.

Synthesis of N, N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-propylbenzimidazolium dibromide (**3**)

The preparation of **3** was similar to that of **1**, but used *n*-propylbenzimidazole (0.40 g, 2.50 mmol) instead of n-methylbenzimidazole. Yield: 1.03 g (74 %), MP: 279 - 281 °C. **FTIR** (ATR, cm-1): 3123 (Csp^3-H_{arom} stretching); 3039, 2975 (Csp^3-$H_{aliphatic}$ stretching); 1555 (C=N stretching); 1456 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 0.81 (3H, t, C**H**$_3$CH$_2$CH$_2$-N, J = 7.0 Hz), 1.80 (2H, q, CH_3C**H**$_2$CH$_2$-N, J = 7.0 Hz), 4.37 (2H, t, CH_3CH_2C**H**$_2$-N, J = 7.0 Hz), 5.14 (4H, s, N-C**H**$_2$C**H**$_2$-N), 5.69 (2H, s, N-C**H**$_2$-Ar), 7.39 – 7.70 (5H, m, arene-**H**), 7.71 – 8.03 (8H, m, benzimi-**H**), 9.74 (1H, s, $CH_3CH_2CH_2$-N-C**H**-N-CH_2), 9.83 (1H, s, Ar-N-C**H**-N-CH_2). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 8.97 (N-CH_2CH_2**C**H_3), 20.36 (N-CH_2**C**H_2CH_3), 44.12 (N-**C**$H_2CH_2CH_3$), 44.30, 46.76 (N-**C**H_2**C**H_2-N), 48.63 (N-**C**H_2-Ar), 110.97, 111.18, 112.21, 112.35, 112.38, 116.59, 125.31, 125.40, 125.48, 125.52, 126.71, 127.37, 127.51, 129.30, 129.35, 129.56, 131.65 (arene-**C**/benzimi-**C**), 140.93

($CH_3CH_2CH_2$-N-**C**H-N-CH_2), 141.22 (Ar-N-**C**H-N-CH_2). Anal. Calc. for $C_{26}H_{28}N_4Br_2$: C, 56.12; H, 5.04; N, 10.01 %. Found: C, 56.52; H, 5.14; N, 10.15 %.

Synthesis of N, N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-pentylbenzimidazolium dibromide (**5**)

The preparation of **5** was similar to that of **1**, but used *n*-pentylbenzimidazole (0.48 g, 2.55 mmol) instead of *n*-methylbenzimidazole. Yield: 1.04 g (71 %), MP: 269 – 271 °C. **FTIR** (ATR, cm^{-1}): 3123 (Csp^3-H_{arom} stretching); 3026, 2958 (Csp^3-$H_{aliphatic}$ stretching); 1556 (C=N stretching); 1457 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 0.86 (3H, s, C**H**$_3$CH$_2$ CH$_2$CH$_2$CH$_2$-N), 1.26 (2H, t, CH$_3$C**H**$_2$CH$_2$CH$_2$CH$_2$-N, *J* = 10 Hz), 1.30 (2H, t, CH$_3$CH$_2$C**H**$_2$CH$_2$CH$_2$-N, *J* = 10 Hz), 1.79 (2H, t, CH$_3$CH$_2$CH$_2$C**H**$_2$CH$_2$-N, *J* = 10 Hz), 4.44 (2H, t, CH$_3$CH$_2$CH$_2$CH$_2$C**H**$_2$-N, *J* = 10 Hz), 5.21 (4H, s, N-C**H**$_2$C**H**$_2$-N), 5.77 (2H, s, N-C**H**$_2$-Ar), 7.40 – 7.55 (5H, m, arene-**H**), 7.65 – 8.10 (8H, m, benzimi-**H**), 10.02 (1H, s, CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$-N-C**H**-N-CH$_2$), 10.09 (1H, s, Ar-N-C**H**-N-CH$_2$). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 14.20 (N-CH$_2$CH$_2$CH$_2$ CH$_2$**C**H$_3$), 22.05 (N-CH$_2$CH$_2$CH$_2$**C**H$_2$CH$_3$), 28.20 (N-CH$_2$CH$_2$**C**H$_2$CH$_2$CH$_3$), 28.66 (N-CH$_2$**C**H$_2$CH$_2$CH$_2$CH$_3$), 46.19 (N-**C**H$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 46.33, 47.26 (N-**C**H$_2$**C**H$_2$-N), 50.54 (N-**C**H$_2$-Ar), 113.45, 113.55, 114.30, 114.47, 127.16, 127.26, 127.37, 128.85, 129.25, 129.44, 131.15, 131.42, 131.44, 131.71, 134.05 (arene-**C**/ benzimi-**C**), 143.35 (CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$-N-**C**H-N-CH$_2$), 143.70 (Ar-N-**C**H-N-CH$_2$). Anal. Calc. for $C_{28}H_{32}N_4Br_2$: C, 57.53; H, 5.49; N, 9.59 %. Found: C, 57.18; H, 5.62; N, 9.22 %.

Synthesis of N, N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-hexylbenzimidazolium dibromide (**6**)

The preparation of **6** was similar to that of **1**, but used *n*-hexylbenzimidazole (0.51 g, 2.52 mmol) instead of *n*-methylbenzimidazole. Yield: 1.07 g (71 %), MP: 265 - 269 °C. **FTIR** (ATR, cm^{-1}): 3121 (Csp^3-H_{arom} stretching); 3025, 2934 (Csp^3-$H_{aliphatic}$ stretching); 1556 (C=N stretching); 1458 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 0.87 (3H, t, C**H**$_3$CH$_2$ CH$_2$CH$_2$CH$_2$CH$_2$-N, *J* = 5 Hz), 1.27 (6H, s, CH$_3$C**H**$_2$ C**H**$_2$C**H**$_2$CH$_2$CH$_2$-N), 1.79 (2H, t, CH$_3$CH$_2$CH$_2$CH$_2$ C**H**$_2$CH$_2$-N, *J* = 5 Hz), 4.45 (2H, t, CH$_3$CH$_2$CH$_2$ CH$_2$CH$_2$C**H**$_2$-N, *J* = 5 Hz), 5.24 (4H, s, N-C**H**$_2$C**H**$_2$-N), 5.78 (2H, s, N-C**H**$_2$-Ar), 7.40 – 7.55 (5H, m, arene-**H**), 7.65 – 8.10 (8H, m, benzimi-**H**), 10.03 (1H, s, CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-N-C**H**-N-CH$_2$), 10.10 (1H, s, Ar-N-C**H**-N-CH$_2$). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 14.34 (N-CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$**C**H$_3$), 22.33 (N-CH$_2$CH$_2$CH$_2$CH$_2$**C**H$_2$CH$_3$), 25.82 (N-CH$_2$CH$_2$CH$_2$ **C**H$_2$CH$_2$CH$_3$), 28.96 (N-CH$_2$CH$_2$**C**H$_2$CH$_2$CH$_2$CH$_3$), 31.10 (N- CH$_2$**C**H$_2$CH$_2$CH$_2$**C**H$_2$CH$_3$), 46.18 (N-**C**H$_2$ CH$_2$CH$_2$CH$_2$CH$_2$CH$_3$), 46.34, 47.28 (N-**C**H$_2$**C**H$_2$-N), 50.55 (N-**C**H$_2$-Ar), 113.43, 113.57, 114.30, 114.47, 127.15, 127.25, 127.36, 128.85, 128.88, 129.24, 129.43, 129.45, 131.15, 131.43, 131.70, 134.05 (arene-**C**/ benzimi-**C**), 143.35 (CH$_3$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$-N-**C**H-N-CH$_2$), 143.70 (Ar-N-**C**H-N-CH$_2$). Anal. Calc. for $C_{29}H_{34}N_4Br_2$: C, 58.19; H, 5.69; N, 9.36 %. Found: C, 58.36; H, 5.74; N, 9.81 %.

Synthesis of Silver(I) di-NHC Complexes (7-9, 11, 12)

Synthesis of [Bis(N,N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-methylbenzimidazolium)disilver(I)] dihexafluorophosphate (**7**)

Compound **1** (0.30 g, 0.57 mmol) was dissolved and stirred in methanol (15 mL) with Ag_2O (0.26 g, 1.14 mmol) in the absence of light. The mixture was stirred for 48 hours at room temperature before being filtered through a pad of Celite to remove any unreacted Ag_2O. The clear solution obtained was subjected to a metathesis reaction by stirring with KPF_6 (0.20 g, 1.09 mmol) for 3 hours at room temperature before being left to stand overnight. The grey precipitate that appeared was collected and washed with distilled water (2 × 3 mL) and air-dried before being recrystallized using acetonitrile (20 mL) and diethyl ether (100 mL) to afford the grey precipitate of complex **7**. Yield: 0.25 g (36 %), MP: 259 – 261 °C. **FTIR** (ATR, cm^{-1}): 2924, 2853 (Csp^3-$H_{aliphatic}$ stretching); 1441, 1398 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 5.13 (6H, s, N-C**H**$_3$), 5.38 (8H, s, N-C**H**$_2$C**H**$_2$-N), 5.49 (4H, s, N-C**H**$_2$-Ar), 6.45 – 7.19 (10H, m, arene-**H**), 7.25 – 7.71 (16H, m, benzimi-**H**). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 36.25 (N-**C**H$_3$), 45.38, 50.30 (N-**C**H$_2$**C**H$_2$-N), 51.23 (N-**C**H$_2$-Ar), 112.51, 112.65, 124.63, 125.09, 126.56, 128.20, 129.29, 132.90, 133.47, 134.17, 136.95 (arene-**C**/benzimi-**C**), 185.29, 191.28 ($C_{carbene}$-Ag). Anal. Calc. for $C_{48}H_{48}Ag_2N_8P_2F_{12}$: C, 46.37; H, 3.86; N, 9.02 %. Found: C, 46.71; H, 4.11; N, 9.26 %.

Synthesis of *[Bis(N,N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-ethylbenzimidazolium)disilver(I)] dihexafluorophosphate* (**8**)

The preparation of **8** was similar to that of **7**, but used **2** (0.30 g, 0.55 mmol) instead of **1**, Ag_2O (0.26 g, 1.11 mmol) and KPF_6 (0.20 g, 1.07 mmol) to obtain **8** as a white precipitate. Yield: 0.23 g (33 %), MP: 273 – 275 °C. **FTIR** (ATR, cm^{-1}): 2925, 2864 (Csp^3-$H_{aliphatic}$ stretching); 1445, 1401 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 1.13 – 1.36 (6H, m, N-CH$_2$C**H**$_3$), 4.14 – 4.15 (4H, m, N-C**H**$_2$CH$_3$), 5.38 (8H, s, N-C**H**$_2$C**H**$_2$-N), 5.52 (4H, s, N-C**H**$_2$-Ar), 6.63 – 7.22 (10H, m, arene-**H**), 7.28 – 7.63 (16H, m, benzimi-**H**). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 15.12 (N-CH$_2$**C**H$_3$), 43.32 (N-**C**H$_2$CH$_3$), 46.26, 47.54 (N-**C**H$_2$**C**H$_2$-N), 51.25 (N-**C**H$_2$-Ar), 110.73, 111.05, 111.15, 111.73, 118.82, 121.19, 121.99, 123.60, 123.98, 125.68 126.82, 127.24, 128.04, 128.10, 132.39, 132.86, 135.06, 135.15, 136.23 (arene-**C**/benzimi-**C**), 188.23, 189.43 ($C_{carbene}$-Ag). Anal. Calc. for $C_{49}H_{50}Ag_2N_8P_2F_{12}$: C, 46.82; H, 3.98; N, 8.92 %. Found: C, 46.83; H, 4.14; N, 8.63 %.

Synthesis of *[Bis(N,N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-propylbenzimidazolium)disilver(I)] dihexafluorophosphate* (**9**)

The preparation of **9** was similar to that of **7**, but used **3** (0.30 g, 0.54 mmol) instead of **1**, Ag_2O (0.25 g, 1.08 mmol) and KPF_6 (0.20 g, 1.09 mmol) to obtain **9** as a grey precipitate. Yield: 0.28 g (40 %), MP: 277 – 280 °C. **FTIR** (ATR, cm^{-1}): 2964, 2967 (Csp^3-$H_{aliphatic}$ stretching); 1446, 1401 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 1.20 – 1.38 (6H, m, N-$CH_2CH_2CH_3$), 1.60 – 1.80 (4H, m, N-$CH_2CH_2CH_3$), 4.12 – 4.31 (4H, m, N-$CH_2CH_2CH_3$), 5.44 (8H, s, N-CH_2CH_2-N), 5.70 (4H, s, N-CH_2-Ar), 6.70 – 7.25 (10H, m, arene-**H**), 7.31 – 7.78 (16H, m, benzimi-**H**). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 10.14 (N-$CH_2CH_2CH_3$), 22.70 (N-$CH_2CH_2CH_3$), 30.08 (N-$CH_2CH_2CH_3$), 47.29, 49.63 (N-CH_2CH_2-N), 51.25 (N-CH_2-Ar), 110.49, 110.84, 110.96, 111.78, 111.89, 118.67, 121.69, 122.47, 123.54, 123.95, 125.40, 125.66, 127.32, 128.05, 132.30, 132.77, 135.04, 135.95 (arene-**C**/benzimi-**C**), 187.73, 189.45 ($C_{carbene}$-Ag). Anal. Calc. for $C_{50}H_{52}Ag_2N_8P_2F_{12}$: C, 47.24; H, 4.09; N, 8.82%. Found: C, 47.20; H, 4.36; N, 8.45 %.

Synthesis of [Bis(N,N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-pentylbenzimidazolium)disilver(I)] dihexafluorophosphate (**11**)

The preparation of **11** was similar to that of **7**, but used **5** (0.30 g, 0.51 mmol) instead of **1**, Ag_2O (0.24 g, 1.04 mmol) and KPF_6 (0.20 g, 1.09 mmol) to obtain **11** as a white precipitate. Yield: 0.27 g (40 %), MP: 267 – 269 °C. FTIR (ATR, cm^{-1}): 2964, 2928 (Csp^3-$H_{aliphatic}$ stretching); 1448, 1402 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 0.84 (6H, s, N-$CH_2CH_2CH_2CH_2CH_3$), 0.85 – 0.90 (4H, m, N-$CH_2CH_2CH_2CH_3$), 1.03 – 1.17 (4H, m, N-$CH_2CH_2CH_2CH_3$), 1.18 – 1.24 (4H, m, N-$CH_2CH_2CH_2CH_3$), 4.18 – 4.33 (4H, m, N-$CH_2CH_2CH_2CH_3$), 5.36 (8H, s, N-CH_2CH_2-N), 5.67 (4H, s, N-CH_2-Ar), 6.70 – 7.24 (10H, m, arene-**H**), 7.34 – 7.83 (16H, m, benzimi-**H**). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 12.86 (N-$CH_2CH_2CH_2CH_2CH_3$), 21.05 (N-$CH_2CH_2CH_2CH_2CH_3$), 27.24 (N-$CH_2CH_2CH_2CH_2CH_3$), 29.34 (N-$CH_2CH_2CH_2CH_2CH_3$), 47.78 (N-$CH_2CH_2CH_2CH_2CH_3$), 48.35, 48.40 (N-CH_2CH_2-N), 51.35 (N-CH_2-Ar), 110.99, 111.59, 111.74, 111.91, 123.54, 123.82, 123.94, 124.02, 125.65, 127.26, 128.12, 132.23, 132.80, 132.95, 135.11 (arene-**C**/benzimi-**C**), 187.95, 189.48 ($C_{carbene}$-Ag). Anal. Calc. for $C_{52}H_{56}Ag_2N_8P_2F_{12}$: C, 48.52; H, 4.35; N, 8.71%. Found: C, 48.86; H, 4.53; N, 8.54 %.

Synthesis of [Bis(N,N'-(ethane-1,2-diyl)-N-benzylbenzimidazolium-N'-hexylbenzimidazolium)disilver(I)] dihexafluorophosphate (**12**)

The preparation of **12** was similar to that of **7**, but used **6** (0.30 g, 0.50 mmol) instead of **1**, Ag_2O (0.23 g, 0.99 mmol) and KPF_6 (0.20 g, 1.09 mmol) to obtain **12** as a white precipitate. Yield: 0.27 g (40 %), MP: 270 – 272 °C. **FTIR** (ATR, cm^{-1}): 2960, 2928 (Csp^3-$H_{aliphatic}$ stretching); 1450, 1403 (C-N stretching). **^{1}H NMR** (500 MHz, d_6-DMSO) in δ ppm: 0.66 (6H, t, N-$CH_2CH_2CH_2CH_2CH_2CH_3$, J = 3 Hz), 0.80 – 1.15 (12H, m, N-$CH_2CH_2CH_2CH_2CH_2CH_3$), 1.21 (4H, t, N-$CH_2CH_2CH_2CH_2CH_2CH_3$, J = 4 Hz), 4.22 (4H, t, N-$CH_2CH_2CH_2$ $CH_2CH_2CH_3$, J = 9 Hz), 5.28 (8H, s, N-CH_2CH_2-N), 5.66 (4H, s, N-CH_2-Ar), 6.65 – 7.25 (10H, m, arene-**H**), 7.29 – 7.83 (16H, m, benzimi-**H**). **^{13}C NMR** (125 MHz, d_6-DMSO) in δ ppm: 13.13 (N-$CH_2CH_2CH_2CH_2CH_2CH_3$), 21.30 (N-$CH_2CH_2CH_2$ $CH_2CH_2CH_3$), 24.91 (N-$CH_2CH_2CH_2CH_2CH_2CH_2CH_3$), 29.69 (N-$CH_2CH_2CH_2CH_2CH_2CH_3$), 30.10 (N-$CH_2CH_2CH_2CH_2CH_2CH_3$), 45.24 (N-$CH_2CH_2CH_2CH_2$ CH_2CH_3), 47.90, 48.48 (N-CH_2CH_2-N), 51.22 (N-CH_2-Ar), 110.97, 111.02, 111.69, 123.61, 123.84, 124.05, 125.66, 127.31, 128.31, 132.25, 134.87, 135.10 (arene-**C**/benzimi-**C**), 188.35, 189.74 ($C_{carbene}$-Ag). Anal. Calc. for $C_{53}H_{58}Ag_2N_8P_2F_{12}$: C, 48.92; H, 4.46; N, 8.62 %. Found: C, 49.21; H, 4.75; N, 8.99 %.

Preparation of Cell Cultures

The human cervical (HeLa) cells and normal human skin fibroblast (Hs27) cells were allowed to grow under optimal incubator conditions. Subculturing of cells was performed after the cells achieved 70 – 80% confluence. The old medium was first aspirated out of the plate. The cells were then washed twice with sterile phosphate-buffered saline (PBS) (pH = 7.4), and then completely discarded. A solution of 2 mL trypsine-EDTA was then added and distributed evenly onto the surface of the cells in a 75 cm^2 cell culture flask. The cells were then placed in an incubator at 37 °C with 5% CO_2 for 5 min. To aid cell segregation, the flask containing the cells was gently tapped. Trypsin activity was inhibited by adding 2 mL of fresh complete media (10 % FBS). The cells were then transferred to a sterile centrifuge tube and upon centrifugation, the cell pellet was resuspended in the media and counted with the aid of a haemocytometer. Cells were then plated into a new tissue culture flask containing fresh media and incubated at 37 °C with an internal atmosphere of 5 % CO_2.

MTT Assays

The cancer cells (HeLa) were harvested and approximately 3000 cells in 100 µL media were seeded in each well of a sterile 96-well plate and incubated overnight in a CO_2 incubator. Different concentrations of bis-benzimidazolium salts (**1** – **6**) and their respective silver(I) di-NHC complexes (**7** – **12**) were prepared from the stock solutions by serial dilution in media, ranging from 50.00 µg/mL to 0.39 µg/mL/well. These were then used to treat the HeLa cells for 24 hrs. The media was then carefully aspirated out and the formazan crystals formed in each well were dissolved using 100 µL of DMSO. Finally, the absorbance was measured at 540 nm using a

Multiskan Spectrum microplate reader (Thermo Scientific). Etoposide was used as the positive control and the negative control was represented by the untreated medium containing DMSO. DMSO at 0.32 % (v/v) was used as a polar solvent to dissolve the complexes. All experiments were performed in triplicate. Following this MTT assay, further analysis was conducted to evaluate the toxicity effect of 0.32% (v/v) DMSO against the HeLa cell cancer line.

For normal cells, a total of 15000 Hs27 cells/well were seeded in complete media (DMEM incorporated with 10 % FBS, 1 % Pen Strep) in a 96-well plate for 24 h. The Hs27 cells were treated with increasing concentrations of different samples of bis-benzimidazolium salts (**4** – **6**) (3 samples were selected to study their cytotoxicity against non-cancerous Hs27 cells) ranging from 50, 25, 12.5, 6.25, 3.125 and 1.56 µg/mL and incubated for 24 h. Further analysis was conducted following the MTT assay.

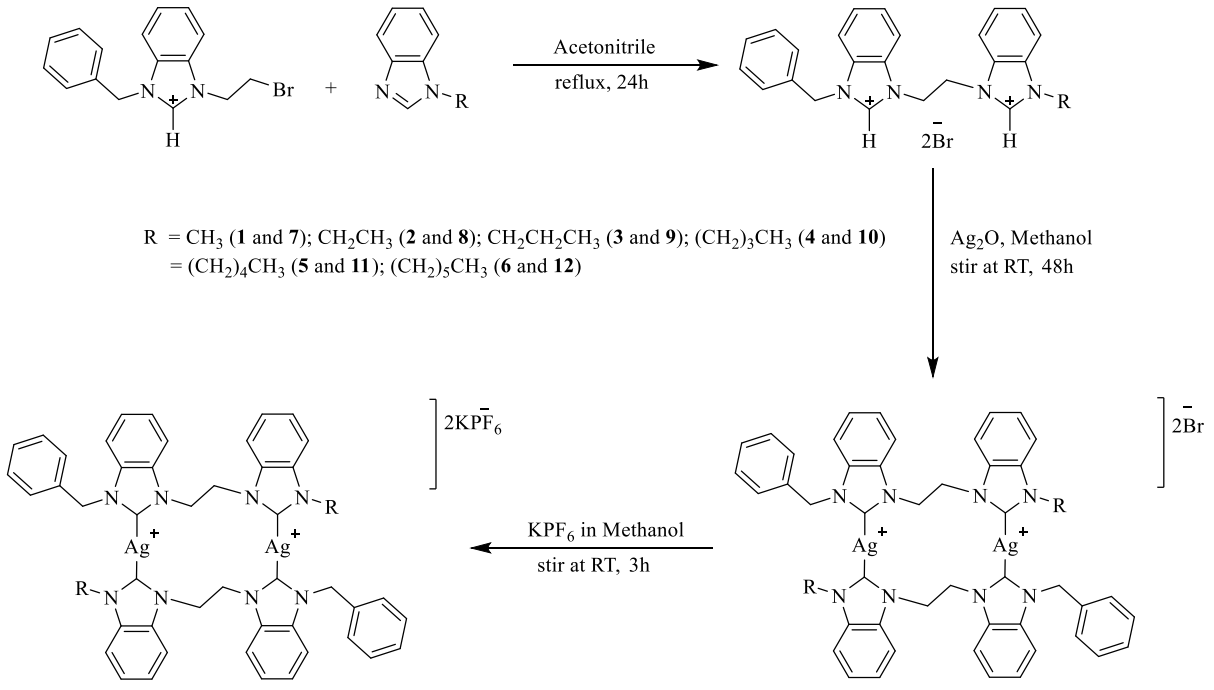
RESULTS AND DISCUSSION

Syntheses

Benzimidazole derivatives bearing *N*-alkyl/arylated substituents provide access, through the quaternization reaction with alkyl/aryl halide, to the wide variety of *N*,*N'*-disubstituted benzimidazolium salts. As mild bases were used, these benzimidazolium salts can be transformed to NHCs that are readily available to attach to metal ions [9]. While the formation of symmetrical bis-benzimidazolium salts is common and widely reported, the formation of asymmetrical bis-benzimidazolium salts is relatively scarce. In fact, the first examples of asymmetrical dibenzimidazolium salts were developed and reported by our group [15].

Herein, the synthesis of asymmetrical bis-ben zimidazolium salts was performed in accordance with the reported methodology. *N*-(2-bromoethyl)-*N'*-benzylbenzimidazolium bromide was left to react with various *n*-alkylbenzimidazoles in acetonitrile to yield *N*,*N'*-(ethane-1,2-diyl)-*N*-benzylbenzimidazolium-*N'*-alkylbenzimidazolium dibromide (alkyl = methyl, ethyl, propyl, butyl, pentyl and hexyl). The formation and purity of *N*-(2-bromoethyl)-*N'*-benzylbenzimidazolium bromide is crucial to achieve asymmetrical benzimidazolium salts, as small changes in molar ratio or the presence of solvent in the preparation of this compound may result in a different product.

The corresponding dinuclear silver(I) di-NHC complexes **7** – **12** were prepared through *in-situ* deprotonation of the bis-benzimidazolium salts **1** – **6** with a molar ratio of 1:2 (salts: Ag_2O) in methanol at room temperature for 2 days. This was followed by a metathesis reaction with KPF_6 to facilitate the formation of dinuclear silver(I) di-NHC complexes with two hexafluorophosphate being counter anions. All silver(I) complexes comprised white or grey solids that were soluble in polar organic solvents such acetonitrile, pyridine, DMSO and DMF, but insoluble in diethyl ether, hexane and benzene. A detailed synthetic route for the asymmetrical substituted bis-benzimidazolium salts **1** – **6** and their respective dinuclear silver (I) di-NHC complexes **7** – **12** is provided in **Scheme 1**.



Scheme 1. The synthesis of asymmetrical bis-benzimidazolium salts **1** – **6** and their respective dinuclear silver(I) di-NHC complexes **7** – **12**.

Spectral Studies

The bis-benzimidazolium salts **1** – **6** and their respective dinuclear silver(I) di-NHC complexes **7** – **12** were defined by FTIR, ^{1}H and ^{13}C NMR spectroscopy. Even though FTIR spectroscopy was not fully utilized in this work due to the lack of functional groups, specific patterns were identified in the spectra of these salts and their corresponding metal complexes, which could be used to confirm a successful synthesis. The FTIR spectra of all the bis-benzimidazolium salts showed a sharp medium intensity band at around 1456 – 1458 cm^{-1}, indicating the stretching of C-N in the benzimidazole ring as well as the *N*-substituent. In addition, the stretching suggests a large shift in the spectra of the complexes, revealing that the C2 carbon was coordinated directly with the silver(I) ion. Nevertheless, the spectra exhibited equivalent stretching bands for aliphatic and aromatic C-H at around 2500 cm^{-1} [19].

NMR spectra were obtained in DMSO-d_6 over a scan range of δ 0 – 11 pm and δ 0 – 200 ppm for ^{1}H and ^{13}C, respectively. In the ^{1}H NMR spectra of **1** - **6**, the resonances for the asymmetrical bis-benzimidazolium salts differed among the alkyl groups as methyl/ethyl/propyl/butyl/pentyl/hexyl were attached to the benzimidazole moiety. The most significant observation in these spectra was the presence of two singlet peaks in the range of δ 9.74 – 10.10 ppm that corresponded to the two benzimidazolium C2 protons (Hb and He, **Figure 3**). This characteristic is important for asymmetrical bis-benzimidazolium salts, as these peaks indicate the presence of two benzimidazolium groups that experienced different chemical environments (benzyl *vs*. alkyl chain). It must be noted that for a symmetrical bis-benzimidazolium salt, only one sharp peak would be observed in this region.

In the ^{13}C NMR spectra, two pre-carbenic carbon C2 peaks were observed in the range of δ 140.93 – 143.81 ppm. The benzylic CH_2- showed a singlet peak in the range of δ 48.63 – 50.65 ppm, while the 1,2-ethylene bridge showed a doublet peak at δ 46.06 – 47.28 ppm. These indicate the successful attachment of a bridge with two different benzimidazolium moieties. Other peaks, such as arene carbon peaks and alkyl carbon peaks, were observed at common resonances as reported previously [16,17].

The successful formation of the dinuclear silver(I) di-NHC complexes was confirmed by the disappearance of the characteristic acidic proton of the benzimidazolium moiety in the ^{1}H NMR spectra of the complexes. In the ^{1}H NMR spectra of complexes **7** – **12**, the presence of the peaks were analogous to the spectra of their respective pre-carbenic salts, except in the downfield region, where the peak that corresponded to the acidic proton was not observed. This evidence illustrates the formation of dinuclear silver(I) di-NHC complexes. The ^{13}C NMR spectra of all complexes showed the absence of the pre-carbenic C2 peaks and the presence of additional peaks around δ 185.79 – 191.15 ppm, which relate to the carbene carbons coordinated to the silver(I) ion [19,20]. Besides this major change, the ^{13}C NMR spectra of the complexes displayed the resonances of aromatic, benzylic, 1,2-ethanediyl bridge and aliphatic carbon nuclei in the range of δ 110.49 – 136.95, 51.20 – 51.35, 45.38 – 50.30 and 5.13 – 45.24, respectively.

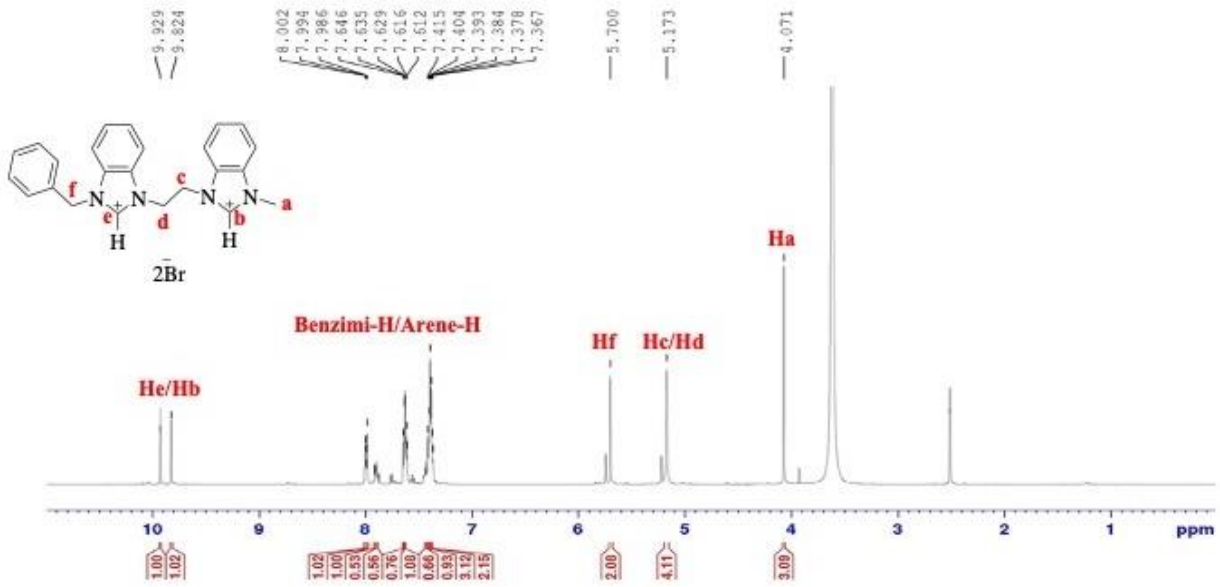


Figure 3. A representative ^{1}H NMR spectrum (d_6-DMSO, 500 MHz) of **1** indicating the chemical shifts of different protons types and highlighting the appearance of the resonance signals for the two C2-protons (Hb & He) which confirm the successful formation of bis-benzimidazolium salts.

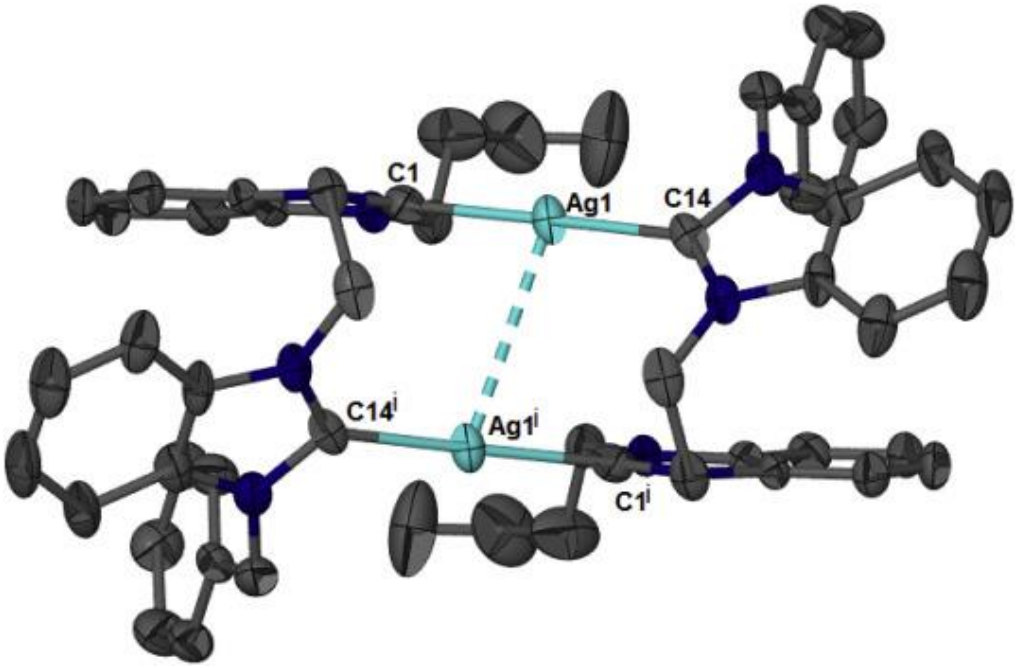


Figure 4. Crystal structure of complex **10** according to Haziz and co-workers[1].

Our attempts to crystallize all the new salts and dinuclear complexes were unsuccessful even under multiple crystallization means. However, through elemental analysis, FTIR and NMR (^{1}H and ^{13}C) data, we proposed that the molecular structures of the complexes **7 - 9**, **11** and **12** were similar to the reported complex **10**, with the only difference being the substituent group used in each NHC moiety (**Figure 4**). The re-synthesis of complex **10** in this current work allowed us to provide a complete series for cytotoxic activity studies. It should be noted that compound **10** showed medium inhibition of antibacterial activities against *E. coli* and *S. aureus* compared to the standard drug ampicillin, and should thus be explored further.

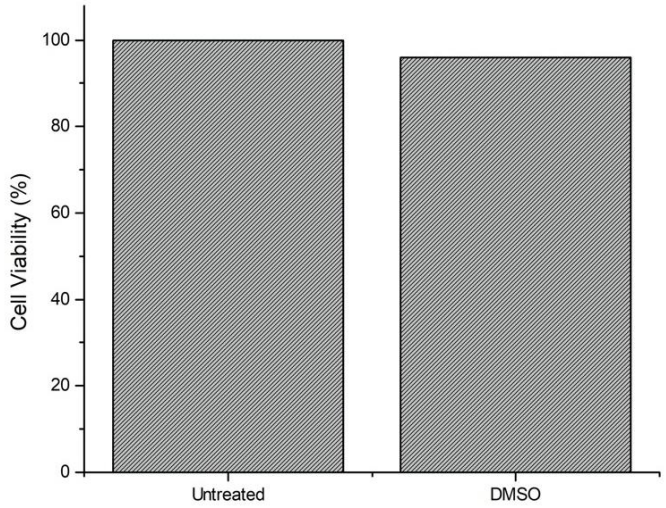


Figure 5. The cell viability of a HeLa cancer cell line treated with 0.32% (v/v) DMSO by MTT assay.

[1]Reprinted with permission from Journal of Organometallic Chemistry, Volume 899, Haziz, U. F., Haque, R. A., Amirul, A. A., Aidda, O. N., and Razali, M. R., New-class of non-symmetrical homo-dibenzimidazolium salts and their dinuclear Silver(I) di-NHC complexes, page 4, Copyright (2019), with permission from Elsevier.

Anticancer Activity

The cytotoxic effects of all bis-benzimidazolium salts and their respective dinuclear silver(I) di-NHC complexes were tested against a HeLa cervical cancer cell line and evaluated by MTT assays at a range of 0.39 – 50.00 µg/mL after a 24 h treatment period, as it was based on the live cells' ability to transform tetrazolium salt into purple formazan. The cell viability was quantitatively assessed by the colour intensity of the purple formazan dye. As the drug concentration declined, the intensity of the purple formazan dye intensified [21]. The cytotoxic effect of 0.32 % (v/v) DMSO was tested against the HeLa cancer cell line using an MTT assay, and the result is depicted in **Figure 5**. The 0.32 % (v/v) DMSO-treated HeLa cells recorded a 96 + 16.13% cell viability compared with the untreated HeLa cells. The results proved that 0.32 % (v/v) DMSO did not exhibit cytotoxicity against the HeLa cancer cell line.

All the bis-benzimidazolium salts **1** – **6** were investigated for cytotoxicity against the HeLa cancer cell line compared with a negative control and the standard drug, etoposide. The cytotoxicity of all salts and dinuclear silver(I) di-NHC was described as the half maximal inhibitory concentration (IC_{50}). The percentage cell viability graph was plotted using the absorbance values received from the microplate reader for each concentration of the bis-benzimidazolium salts and their dinuclear silver(I) di-NHC complexes. The results (**Figure 6**) proved that a majority of the bis-benzimidazolium salts exhibited moderate cytotoxicity against the HeLa cancer cell line as the cell growth was inhibited by a lower percentage, very similar to the negative cell control. The IC_{50} values (the test substance concentration required to suppress cell growth by 50 %) were found to be in the range of 31.00 – 58.14 µg/mL.

The series of dinuclear silver(I)-NHC complexes **7** – **12** were evaluated for their anticancer activity against the HeLa cancer cell line using MTT assays. The results (**Figure 7**) showed better activity than their respective bis-benzimidazolium salts **1** – **6**. In this series, complexes **8** - **12** containing ethyl/propyl/butyl/pentyl/hexyl with benzyl moieties were found to be relatively more active, with IC_{50} values of 2.91 – 5.97 µg/mL (**Table 1**). It is clear that the IC_{50} values decreased with longer carbon side chain lengths, as the cytotoxic potential for dinuclear silver(I) di-NHC complexes increased [22]. Thus, adjusting the *N*-alkyl chain length of the dinuclear complexes as well as the number of silver(I) ions coordinated with the NHC-ligands might affect their potential, especially in biological activities [23].

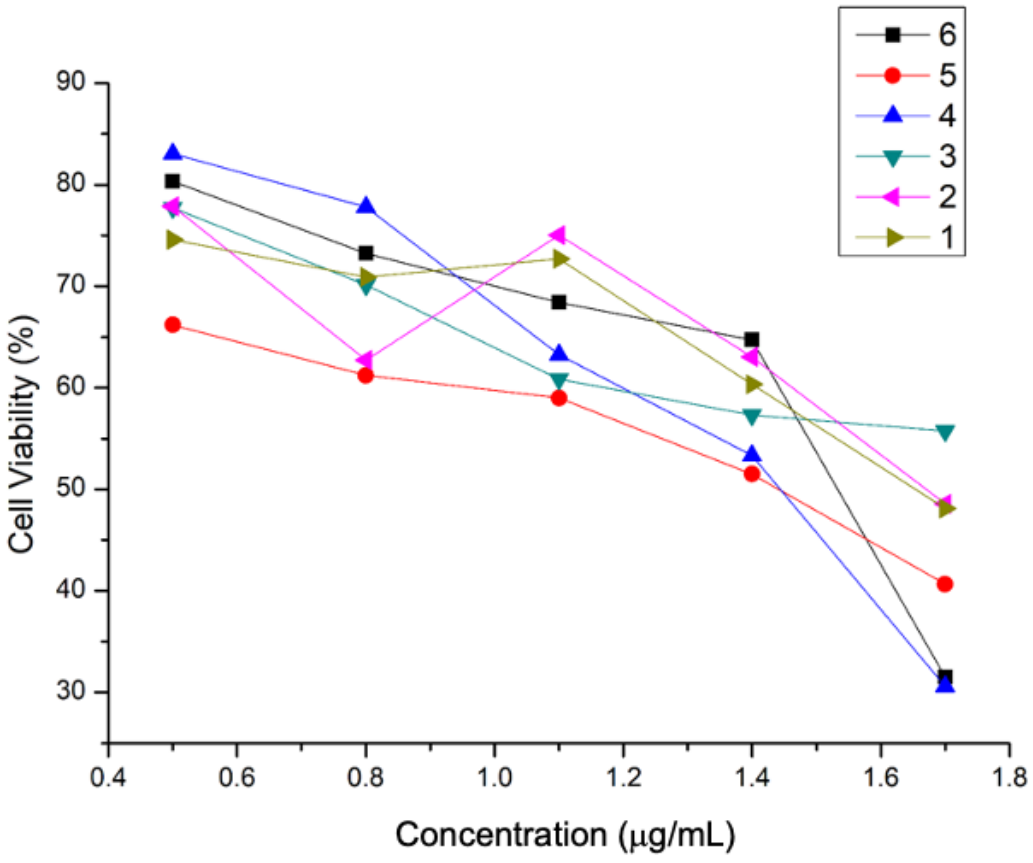


Figure 6. The cell viability of a HeLa cancer cell line treated with bis-benzimidazolium salts **1 – 6**.

Previous research on silver(I)-NHC complexes that focused on anticancer activity has shown the correlation between the complexes' stability and antiproliferation activity [18]. It has been theorized in earlier studies that the stability of silver(I) complexes is critical for ensuring maximum cytotoxic action. Furthermore, all silver(I) complexes were hypothesized to have the same mechanism of action, which includes the release of Ag^+ ions into the cell membrane, interrupting their activity [24]. So, it is vital for the silver(I) ions to coordinate strongly with the ligands to prevent their quick release into the cell membrane [25-27]. Increasing the carbon alkyl chain length from a methyl to a hexyl group proved that increasing stability (forming relatively strong Ag-C bonds) could prevent the quick release of Ag^+ ions into the cell membrane [14]. Based on these results, the IC_{50} values fell as the carbon alkyl chain length increased, implying that the cytotoxicity increased as well. [28]. **Figure 8** and **Figure 9** show the dose dependence of cell proliferation effects on the bis-benzimidazolium salts **1** – **6** and Ag(I) complexes **7** – **12** on HeLa cells, respectively. All the compounds displayed dose dependent cytotoxic activity against the HeLa cancer cell lines. Furthermore, the MTT assay proved that the 0.32 % (v/v) DMSO used as the solvent in this study was not toxic towards HeLa cells, and was suitable for cell culture work.

In a nutshell, the dinuclear silver(I) di-NHC complexes exhibited promising potential in terms of metallopharmaceutical chemistry, mainly as anti-cancer agents against the HeLa cancer cell line. Notably, the silver(I)-NHC complexes' anticancer properties highlight the importance of the sterically and electrically regulated NHC system [29,30].

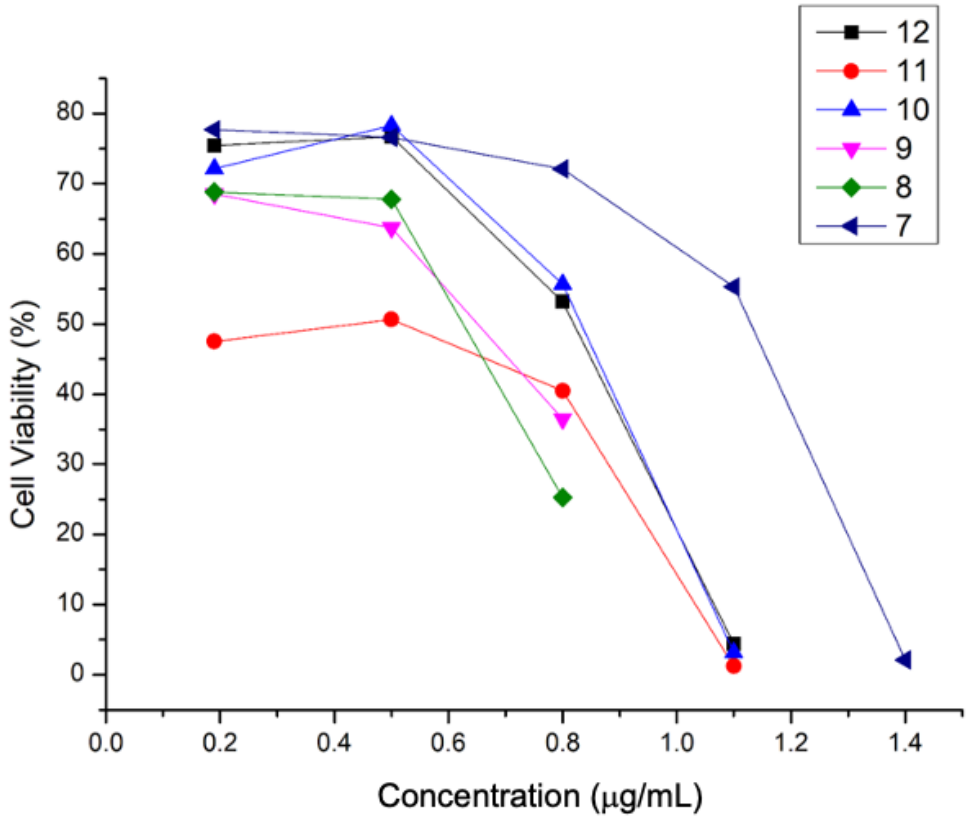


Figure 7. The cell viability of a HeLa cancer cell line treated with dinuclear silver(I) di-NHC complexes **7 – 12**.

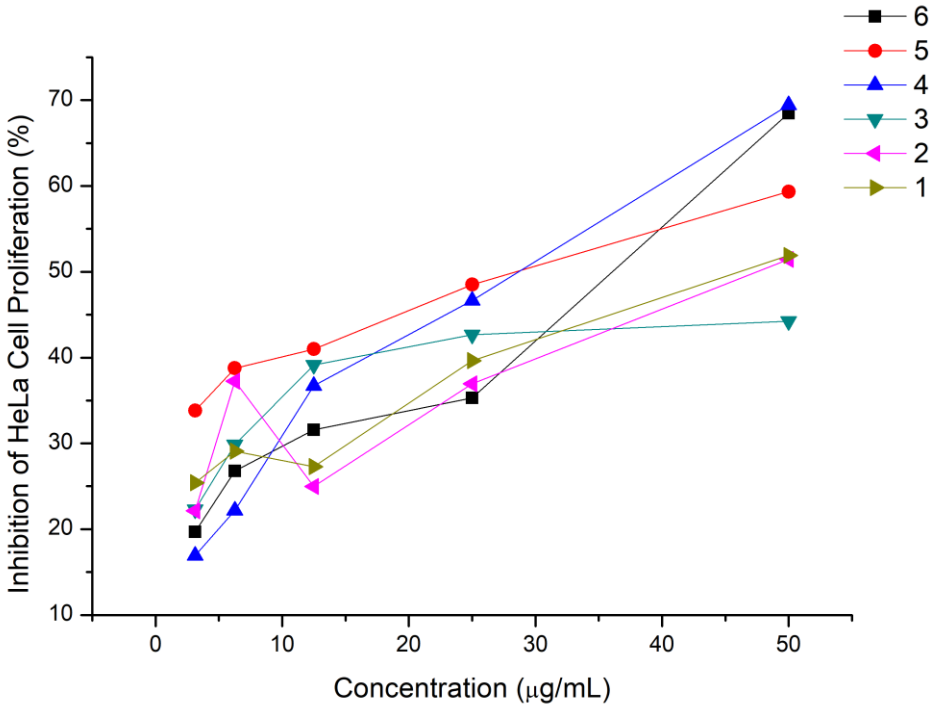


Figure 8. Dose-dependence of cell proliferation effects of the bis-benzimidazolium salts **1 – 6** on a HeLa cancer cell line.

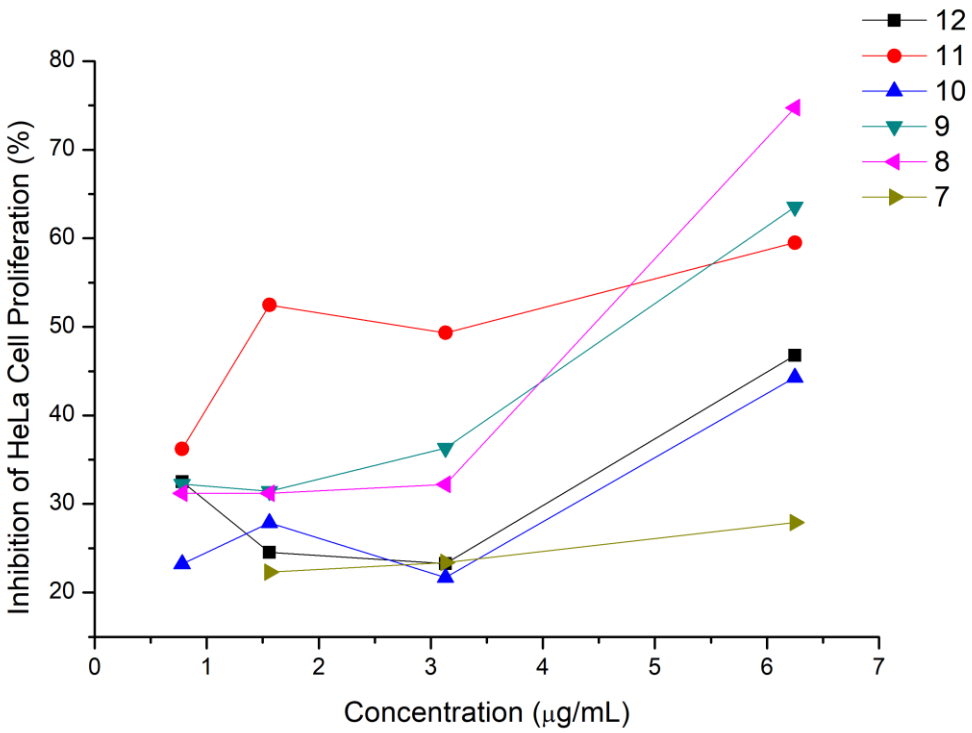


Figure 9. Dose-dependence of cell proliferation effects of the dinuclear silver(I) di-NHC complexes **7 – 12** on a HeLa cancer cell line.

Table 1. The IC_{50} values of bis-benzimidazolium salts (**1 – 6**) and their respective dinuclear silver(I) di-NHC complexes (**7 – 12**) against a HeLa cancer cell line.

Bis-benzimidazolium salts	IC_{50} (µg/mL)	Silver(I) di-NHC complexes	IC_{50} (µg/mL)
1	45.00 ± 16.91	**7**	11.74 ± 8.52
2	48.80 ± 17.09	**8**	3.85 ± 2.09
3	58.14 ± 16.91	**9**	4.50 ± 2.09
4	32.54 ± 15.84	**10**	5.97 ± 4.26
5	31.00 ± 16.91	**11**	2.91 ± 4.26
6	33.45 ± 19.51	**12**	5.75 ± 4.21

**Etoposide, as a positive control, gave an IC_{50} value of 3.15 µg/mL.*
***IC_{50} (µg/mL): 1 – 10 (very strong); 11 – 20 (strong); 21 – 50 (moderate); 51 – 100 (weak); above 100 (non-cytotoxic)*

Selected bis-benzimidazolium salts (**4** – **6**) were also investigated for cytotoxicity against the non-cancerous Hs27 cell line. The cytotoxicity of the tested compounds was described as the half maximal inhibitory concentration (IC_{50}). The percentage cell viability graph was plotted using the absorbance values received from the microplate reader for different drug concentrations in the tested salts, while their IC_{50} values are summarized in **Table 2**. The results showed that bis-benzimidazolium salt **6** showed moderate cytotoxicity against the Hs27 cell line compared to the bis-benzimidazolium salts **4** and **5**. Further detailed studies should be conducted in an animal preclinical model to obtain detailed toxicity profiles of the tested compounds for human applications.

Mechanism of Action

Over the last decade, numerous silver(I)-NHC complexes have been investigated for their anti-bacterial properties [31-33]. These complexes produce Ag^+ ions, which bind to the surfaces of bacterial cells and interact with proteins involved in the cell wall, thus disrupting cell processes. [34]. The anticancer mechanisms of silver(I)-NHC complexes may also be monitored by measuring the release of Ag^+ ions and their interactions with proteins and DNA [35]. The weak π-acceptor and strong σ-donor character of the silver(I)-NHC complexes may explain the gradual release of Ag^+ ions [15, 36]. According to several studies, the released Ag^+ ions accumulate in the cytoplasm of the cell, inhibiting cellular activity by interfering with enzymes and proteins involved in metabolic processes. A previous study by Iqbal and co-workers has proved that the presence of black spots in the cytoplasm of the afflicted cells was due to Ag^+ ions, confirming that the lethal efficacy of the silver(I)-NHC complexes was predominantly due to silver ion deposition [37]. Thus, the bio-potency of silver(I)-NHC complexes was enhanced by the inclusion of longer side chains, an aryl linker, and a greater number of silver centres [38].

CONCLUSION

A series of bis-benzimidazolium salts **1** - **6** were synthesized as precursors for their respective dinuclear silver(I) di-NHC complexes **7** – **12**. All the compounds were characterized by spectroscopic techniques including FTIR, ^1H and ^{13}C NMR, and elemental analysis. All these compounds were evaluated for their anticancer activity and showed dose-dependent cytotoxicity towards the cervical adenocarcinoma (HeLa) cancer cell line. The IC_{50} values of all dinuclear silver(I) di-NHC complexes were in the range of 2.91 – 5.97 µg/mL for the HeLa cancer cell line, which can be considered as active and comparable to the positive control used in this work. From the results of the cytotoxicity study of selected bis-benzimidazolium salts, the bis-benzimidazolium salt **6** showed a moderate cytotoxicity against the non-cancerous Hs27 cell line. Based on these results, it can be concluded that these dinuclear silver(I) di-NHC complexes have potential for use as chemotherapeutic drugs, but a detailed toxicity study in animals is required. The toxicity evaluation and cytotoxicity studies of selected silver complexes against non-cancer cells are suggested as avenues for further study.

Table 2. The IC_{50} values of bis-benzimidazolium salts **4 – 6** against the Hs27 cell line.

Bis-benzimidazolium salts	IC_{50} (µg/mL)
4	11.54 ± 8.48
5	12.25 ± 8.53
6	23.73 ± 16.64

**IC_{50} (µg/mL): 1 – 10 (very strong); 11 – 20 (strong); 21 – 50 (moderate); 51 – 100 (weak); above 100 (non-cytotoxic)*

ACKNOWLEDGEMENTS

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Conflict of Interest

The authors declare that they have no conflict of interest.

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