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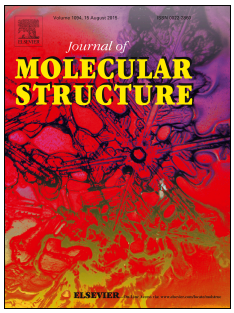

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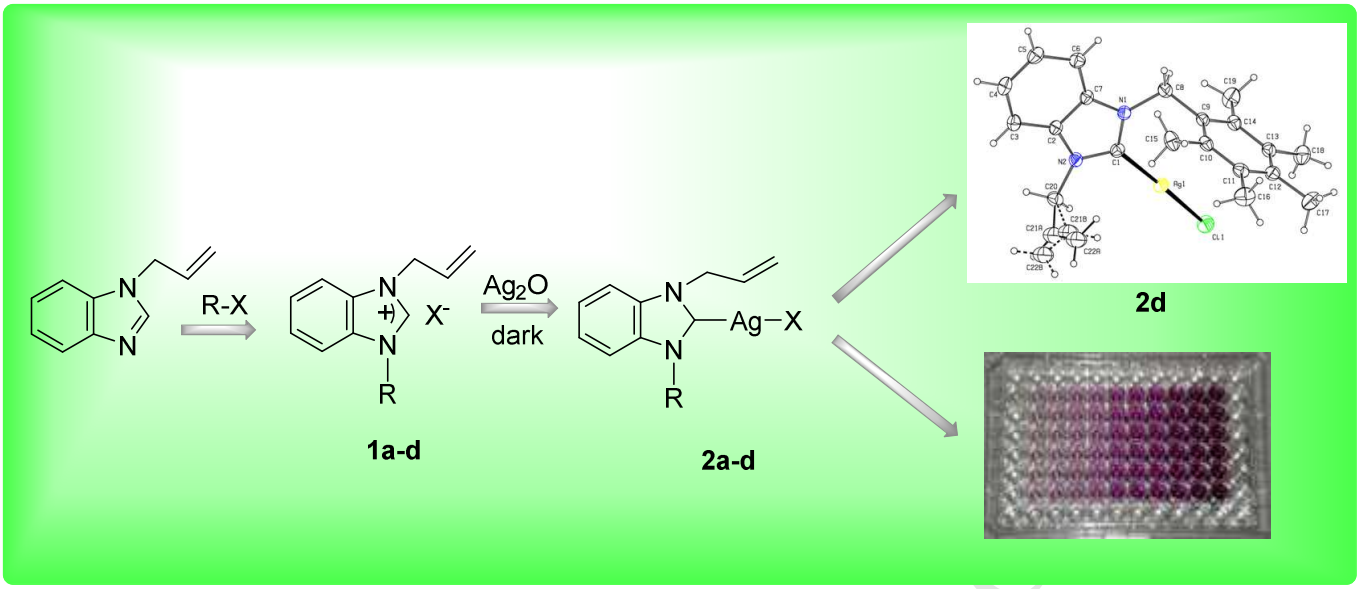

Synthesis, Characterization and Anticancer Activity of Allyl Substituted N-Heterocyclic Carbene Silver(I) Complexes

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Abstract

Metal N-heterocyclic carbene (NHC) complexes have attracted considerable attention in biological fields for their potential applications in cancer and antimicrobial therapies. In this study, four new benzimidazole-based N-heterocyclic carbene salts (**1a-d**) and their silver (I) complexes (**2a-d**) were synthesized. All new compounds were characterized by elemental analysis, FT-IR, ^{1}H NMR and, ^{13}C NMR spectroscopy. Additionally, single crystal structural studies for complex **2d** show that the benzene rings (C9-C14) and the central benzimidazole ring system make dihedral angles of 83.58(13)°. The Ag-C*l* and Ag-C single bond lengths are 2.3267(8) and 2.087(3) Å, respectively. The C-Ag-C*l* bond angle is 175.20(7)°. The prop-1-ene moiety attached at the second N-atom of benzimidazole is disordered at two set of sites with an occupancy ratio of 0.592(6): 0.408(6). There is one intramolecular hydrogen bond interaction between C22A-H22A...N2. The salts and Ag-complexes were further evaluated for their *in vitro* anticancer activities against DU-145 human prostate cancer cells, MCF-7, MDA-MB-231 human breast cancer cells and L-929 non-cancer cell for 24 h, 48 h and 72 h using the MTT assay. The Ag(I)-NHC complexes **(2a-d)** showed a dose and time-dependent cytotoxic activity against all cell lines. The IC_{50}s for all Ag(I)-NHC complexes lower than 1 μM for 72 h time points on cancer cells. The results showed that complex **2d** exhibited the highest activity against all cancer cell lines studied. Further, the complexes had relatively higher cytotoxicity to cancer cells than to non-cancer cell lines.

Keywords: N-Heterocyclic carbene; silver complex; benzimidazole-2-ylidene; anticancer activity.

1. Introduction

The heterocyclic substituent, including ligands, have constituted the basis of organometallic and coordination chemistry. N-Heterocyclic Carbenes (NHCs) are a well-known class of organometallic ligands that are strong σ-donating and weak π-accepting when connected with metal ions. Since the first NHC was isolated and characterized by Arduengo in 1991, NHCs have attracted interest due to their all-purpose features as ligands for diverse metallic complexes [1].

Metal-NHC complexes have been popularly investigated due to their noteworthy applications as catalysis [2-5] and potential biological properties [6-8]. These compounds are prepared by the different process such as via free carbene, in situ and using transmetallation methods, but the more commonly used process is deprotonation of azolium precursors with a simple metal salt or strong bases, such as sodium hydride or potassium *tert*-butoxide [9,10].

For years metallic silver and silver compounds have been applied to a variety of applications like water purification, wound management, eye-drops, anti-infective coatings in medical devices and in burn treatment as they have potent antimicrobial properties [11-16]. One of the most commonly used compounds of silver is silver(I) sulfazine, which is used to treat bacterial infections caused by severe burns [17]. Silver has low toxicity as compared to other transition metals.

In the family of metal-NHCs, Ag-NHCs have received significant attention because of their medicinal applications such as antibacterial activity [18] or anticancer activity [19]. Due to the high stability of Ag-NHC complexes and slowly release of silver ions to the diseased area from Ag-NHC complexes, the Ag-NHC complexes exhibit high efficacy influence against various types of cancer cell lines. Ag-NHC complexes are synthesized by the reaction of free NHC and silver salt or silver base, mostly Ag_2O [20]. A number of new Ag-NHC complexes were also synthesized after initial reports of cytotoxic properties of Ag-NHC complexes against cervical cancer (HeLa), breast cancer (MCF-7), and colon adenocarcinoma (HCT 116) cells [21].

Within this manuscript, we present the synthesis and characterization of four new allyl substituted NHC salts and their silver(I) complexes. All the new synthesized compounds (**1a-d**, **2a-d**) were tested as anticancer agents against three human cancer cell lines (DU-145, MCF-7, MDA-MB-231) and non-cancer cells (L-929) for 24 h, 48 h and 72 h by MTT assays.

2. Experimental

2.1. Materials and Methods

All synthesis of benzimidazolium salts and Ag-NHC complexes were prepared under argon in flame-dried glassware using standard Schlenk line techniques. Chemicals and solvents were purchased from Sigma Aldrich Co. (Dorset, UK). The solvents used were purified by distillation over the drying agents indicated and were transferred under Argon [22]. All Ag_2O reactions were carried out in the absence of light. Elemental analyses were performed by İnönü University Scientific and Technology Center. Melting points were determined using Electrothermal 9100 melting point detection apparatus. Fourier transform infrared (FT-IR) spectra were obtained in the range 400–4000 cm^{-1} on a Perkin Elmer Spectrum 100 FT-IR. ^1H NMR and ^{13}C NMR spectra were recorded using a Bruker As 400 Mercury spectrometer operating at 400 MHz (^1H), 100 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane as the internal reference. ^1H peaks were labeled as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.) and multiplet (m). Chemical shifts and coupling constants were reported in ppm and in Hz, respectively. The intensity data were collected using a Bruker Kappa ApexII CCD diffractometer [23], using Mo-K_α radiation (λ= 0.71073 Å). The structure was solved by direct methods using the solution program SHELXS97 [24] and then refined with full-matrix least-square methods based on F^2 *SHELXL2014*/6 [25]. All non-hydrogen atoms were first refined isotropically and then with anisotropic atomic displacement parameters by the full matrix least square methods. All the measurements were taken at room temperature for freshly prepared solutions.

The cells lines DU-145 (HTB-81, human prostate carcinoma), MCF-7 (HTB-22, human breast adenocarcinoma), MDA-MB-231 (HTB-26, human breast adenocarcinoma), F-12K Medium (30-2004), Eagles Minimum Essential Medium (EMEM, 30-2003), RPMI-1640 (30-2001), fetal bovine serum (FBS, 30-2020) and penicillin and streptomycin (30-2300) were purchased from American Type Culture Collection (ATTC, Manassas, VA). Dulbecco's Modified Eagle's Medium (DMEM, D6429) and Trypsin-EDTA solution (T-3924) were purchased from Sigma Aldrich.(Sigma-Aldrich Chemie GmbH , Steinheim, Deutschland). L-929 (non-cancer cells from the adipose tissue of mice) were purchased from ECACC (European Collection of Animal Cell Culture, Salisbury, U.K.). All absorbance values were measured with a microplate reader (Bio-Tek, Epocch, USA) at 570nm.

2.2. Statistical analysis

All experiments were carried out in triplicates and results are expressed as means ± SEM. Data were analyzed using one-way analysis of variance and differences were considered significant at $p < 0.0001$. The IC_{50} were determined by statistical software, GraphPad Prism7 (GraphPad Software, San Diego, CA, USA).

The benzimidazolium salts **(1a-d)** and Ag-NHC **(2a-d)** compounds were prepared under argon gas atmosphere. The unsymmetrically N1, N3-substituted benzimidazolium salts **(1a-d)** and respective Ag(I)-NHC complexes **(2a-d)** were synthesized as described in our previous study [26].

2.3. General procedure for the preparation of salts, 1a-d

Benzimidazole (10 mmol) was added to a solution of NaH (10 mmol) in dry THF (30 mL), the mixture was stirred for 1 h at room temperature. Allyl bromide (10.1 mmol) was added dropwise to obtained a solution that was heated for 24 h at 60 ^{0}C. Then, the THF was removed under the vacuum. Dichloromethane (50 mL) was added upon to solid. The mixture was filtered and the obtained clear solution was concentrated under vacuum. Then the solution was distilled to 1-allyl benzimidazole. The 1-allyl benzimidazole (1 mmol) and alkyl halide (1 mmol) were stirred in DMF (5 mL) for 24 h at 80 ^{0}C. White product was precipitated. Following the completion of the process the solution was filtered, the solid was rinsed with diethylether and dried under vacuum. Crude product was recrystallized from dichloromethane/diethylether.

1-(Allyl)-3-(4-isopropylbenzyl)benzimidazolium chloride, 1a

Yield: 88%; m.p. 153-155 $^{\circ}$C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.19 (m, 6H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 2.62-2.63 (m, 1H, $CH_2C_6H_4(CH(CH_3)_2)$-4),5.35 (d, 2H, NCH_2CHCH_2, J = 8 Hz), 5.45-5.50 (m, 2H, NCH_2CHCH_2), 5.84 (s, 2H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 6.08-6.16 (m, 1H, NCH_2CHCH_2), 7.21-7.23, 7.44-7.46, 7.56-7.58, 7.65-7.67 and 7.70-7.72 (m, 8H, NC_6H_4Nand $CH_2C_6H_4(CH(CH_3)_2)$-4), 11.85 (s, 1H, NCHN).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm**):**23.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 33.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4),50.2 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 51.3 (NCH_2CHCH_2),113.7 (NCH_2CHCH_2),121.8 (NCH_2CHCH_2),113.9, 127.0, 127.1,127.4,129.7,128.4,130.0,131.3, 131.5 and 150.1 ($CH_2C_6H_4(CH(CH_3)_2)$-4 and NC_6H_4N), 143.6 (NCHN); FT-IR $\nu_{(CN)}$: 1547 cm^{-1}; % Anal. Calcd for $C_{20}H_{23}N_2Cl$: C, 73.49; H, 7.09; N: 8.57; Found: C, 73.47; H, 7.08; N, 8.54.

1-(Allyl)-3-(4-*ter*-butylbenzyl)benzimidazoliumbromide, 1b

Yield: 86%; m.p.196-198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):1.26 (s, 9H, $CH_2C_6H_4(C(CH_3)_3$-4), 5.34 (d, 2H, NCH_2CHCH_2, *J* = 4 Hz), 5.45-5.53 (m, 2H, NCH_2CHCH_2),5.85 (s, 2H, $CH_2C_6H_4(C(CH_3)_3)$-4),6.11-6.23 (m, 1H, NCH_2CHCH_2),7.36-7.38, 7.48-7.50, 7.57-7.58 and 7.69-7.75 (m, 8H, NC_6H_4Nand $CH_2C_6H_4(C(CH_3)_3)$-4,11.55 (s, 1H, NCHN).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 31.2 ($CH_2C_6H_4(C(CH_3)_3$-4), 34.6 ($CH_2C_6H_4(C(CH_3)_3$-4), 50.2 ($CH_2C_6H_4(C(CH_3)_3$-4), 51.2 (NCH_2CHCH_2), 113.7 (NCH_2CHCH_2), 122.0 (NCH_2CHCH_2),113.9, 126.3, 127.1, 127.2, 128.2, 129.6, 129.7, 131.3, 131.4 and 152.4 ($CH_2C_6H_4(C(CH_3)_3$-4 and NC_6H_4N), 142.7 (NCHN); $\nu_{(CN)}$: 1551 cm^{-1}; % Anal. Calcd for $C_{21}H_{25}N_2Br$: C, 65.46; H, 6.54; N: 7.27; Found: C, 65.49; H, 6.57; N, 7.30.

1-(Allyl)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride, 1c

Yield: 89%; m.p. 223-225 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.25 and 2.32 (s, 9H, $CH_2C_6H_2(CH_3)_3$-2,4,6),5.32-5.37 (m, 4H, NCH_2CHCH_2), 5.79 (s, 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 5.99-6.06 (m, 1H, NCH_2CHCH_2), 6.86 (s, 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 7.19 (d, 1H, NC_6H_4N, *J*= 8 Hz), 7.40 (t, 1H, NC_6H_4N, *J*= 8 Hz), 7.50 (t, 1H, NC_6H_4N, *J*= 6 Hz), 7.65 (d, 1H, NC_6H_4N, *J*= 8 Hz), 11.36 (s, 1H, NCHN). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):20.2 and 21.0 ($CH_2C_6H_2(CH_3)_3$-2,4,6), 47.2 ($CH_2C_6H_2(CH_3)_3$-2,4,6),50.1 (NCH_2CHCH_2),113.6 (NCH_2CHCH_2),121.2 (NCH_2CHCH_2),113.7, 125.0, 127.0, 127.2, 129.9, 130.1, 131.4, 131.5, 137.9 and 139.7 ($CH_2C_6H_2(CH_3)_3$-2,4,6 and NC_6H_4N),143.6 (NCHN); $\nu_{(CN)}$: 1554 cm^{-1}; % Anal. Calcd for $C_{20}H_{23}N_2Cl$: C, 73.49; H, 7.09; N: 8.57; Found: C, 73.46; H, 7.08; N, 8.55.

1-(Allyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride, 1d

Yield: 88%; m.p. 205-206 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.18, 2.21 and 2.23 (s, 15H, ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 5.29-5.38 (m, 4H, NCH_2CHCH_2), 5.77 (s, 2H, ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 5.96-6.04 (m, 1H, NCH_2CHCH_2), 7.31 (d, 1H, NC_6H_4N, *J*= 8 Hz),7.42-7.53 (m, 2H, NC_6H_4N), 7.64 (d, 1H, NC_6H_4N, *J*= 8 Hz), 10.91 (s, 1H, NCHN). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 17.0,17.2 and 17.3, ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 48.9 ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 50.2 (NCH_2CHCH_2), 113.6 (NCH_2CHCH_2), 121.0 (NCH_2CHCH_2),113.7, 124.8, 127.0, 127.2, 130.2, 131.5, 131.7, 133.6, 134.0 and 137.4 (NC_6H_4N and $CH_2C_6(CH_3)_5$-2,3,4,5,6), 143.3 (NCHN); $\nu_{(CN)}$: 1558 cm^{-1}; % Anal. Calcd for $C_{22}H_{27}N_2Cl$: C, 74.45; H, 7.67; N: 7.89. Found: C, 74.44; H, 7.65; N, 7.86.

2.4. General procedure for preparation of Ag-NHC complexes, 2a-d

A solution of 1 mmol of Ag_2O and 0.5 mmol of the corresponding benzimidazolium salt (**1a-d**) in dichloromethane (25 mL) were stirred at room temperature for 24 h under dark conditions. The reaction mixture was then filtered through celite. The clear filtrate was evaporated under vacuum to a crude product, which was recrystallized from dichloromethane/diethyl ether.

Chloro[1-allyl-3-(4-isopropylbenzyl)benzimidazole-2-ylidene]silver(I), 2a

Yield: 83%; m.p. 159-160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.19-1.21 (m, 6H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 2.83-2.90 (m, 1H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 5.08 (d, 2H, NCH_2CHCH_2, J = 4 Hz), 5.22-5.35 (m, 2H, NCH_2CHCH_2), 5.59 (s, 2H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 5.97-6.07 (m, 1H, NCH_2CHCH_2), 7.16-7.22, 7.28-7.40 and 7.45-7.49 (m, 8H, NC_6H_4N and $CH_2C_6H_4(CH(CH_3)_2)$-4). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm**):** 23.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 33.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 52.0 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 53.3 (NCH_2CHCH_2), 112.2 (NCH_2CHCH_2), 119.3 (NCH_2CHCH_2), 112.0, 124.3, 127.1, 127.2, 128.4, 131.8, 133.8, 134.0 and 149.3 ($CH_2C_6H_4(CH(CH_3)_2)$-4 and NC_6H_4N), 189.0 ($C_{carbene}$-Ag); $\nu_{(CN)}$: 1395 cm^{-1}; % Anal. Calcd for $C_{20}H_{22}N_2AgCl$: C, 55.38; H, 5.11; N: 6.46; Found: C, 55.37; H, 5.09; N, 6.44; LC-MS: 687.2 $[AgL_2]^+$

Bromo[1-allyl-3-(4-*ter*-butylbenzyl)benzimidazole-2-ylidene]silver(I), 2b

Yield: 80%, m.p. 199-200 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm):1.27 (s, 9H, $CH_2C_6H_4(C(CH_3)_3$-4), 5.11-5.13 (m, 2H, NCH_2CHCH_2), 5.22-5.32 (m, 2H, NCH_2CHCH_2), 5.63 (s, 2H, $CH_2C_6H_4(C(CH_3)_3$-4), 5.98-6.07 (m, 1H, NCH_2CHCH_2), 7.24-7.27, 7.31-7.33, 7.36-7.41 and 7.44-7.49 (m, 8H, NC_6H_4N and $CH_2C_6H_4(C(CH_3)_3$-4. ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm**):**31.2 ($CH_2C_6H_4(C(CH_3)_3$-4), 34.7 ($CH_2C_6H_4(C(CH_3)_3$-4), 51.2 ($CH_2C_6H_4(C(CH_3)_3$-4), 53.0 (NCH_2CHCH_2), 111.8 (NCH_2CHCH_2), 119.1 (NCH_2CHCH_2), 112.1, 121.8, 124.0, 125.9, 127.1, 128.2, 132.1, 133.9, 134.1and 151.4 ($CH_2C_6H_4(C(CH_3)_3$-4 and NC_6H_4N), 191.2 ($C_{carbene}$-Ag); $\nu_{(CN)}$: 1391 cm^{-1}; % Anal. Calcd for $C_{21}H_{24}N_2AgBr$: C, 51.24; H, 4.91; N: 5.69; Found: C, 51.22; H, 4.89; N, 5.67; LC-MS: 716.3 $[AgL_2]^+$.

Chloro[1-allyl-3-(2,4,6-trimethylbenzyl)benzimidazole-2-ylidene]silver(I), 2c

Yield: 81%; m.p. 205-206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.24and 2.35 (s, 9H, $CH_2C_6H_2(CH_3)_3$-2,4,6),5.03-5.01 (m, 2H, NCH_2CHCH_2), 5.16 (d, 1H, NCH_2CHCH_2, J= 16 Hz), 5.29 (d, 1H, NCH_2CHCH_2,J = 12 Hz), 5.49 (s, 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 5.99-5.90 (m,

1H, NCH_2CHCH_2), 6.99 (s, 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 7.27-7.40 and 7.45-7.47 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.4 and 21.2 ($CH_2C_6H_2(CH_3)_3$-2,4,6), 47.8 ($CH_2C_6H_2(CH_3)_3$-2,4,6), 52.6 (NCH_2CHCH_2), 111.7 (NCH_2CHCH_2), 119.0 (NCH_2CHCH_2), 111.9, 124.2, 124.3, 126.5, 130.3, 131.8, 133.8, 134.3, 137.5 and 139.6 ($CH_2C_6H_2(CH_3)_3$-2,4,6 and (NC_6H_4N), $C_{carbene}$-Ag: not observed; $\nu_{(CN)}$: 1388 cm^{-1}; % Anal. Calcd for $C_{20}H_{22}N_2AgCl$: C, 55.38; H, 5.11; N: 6.46; Found: C, 55.36; H, 5.10; N, 6.44; LC-MS: 687.2 $[AgL_2]^+$.

Chloro[1-allyl-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazole-2-ylidene]silver(I), 2d

Yield: 76%; m.p. 218-219 °C, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 2.35, 2.22 and 2.31 (s, 15H, ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 5.02 (s, 2H, NCH_2CHCH_2), 5.16 (d, 1H, NCH_2CHCH_2, J = 17.2 Hz), 5.28 (d, 1H, NCH_2CHCH_2, J = 10 Hz), 5.50 (s, 2H, $CH_2C_6(CH_3)_5$-2,3,4,5,6), 5.95 (quint, 1H, NCH_2CHCH_2, J = 6.54 Hz), 7.44-7.52 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):17.2 and 17.4 ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 47.7 ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 52.9 (NCH_2CHCH_2), 111.4, 112.0, 118.8, 124.1, 124.4, 126.6, 132.0,133.0, 133.8, 134.3, 134.5 and 137.4 (NC_6H_4N, NCH_2CHCH_2 and $CH_2C_6(CH_3)_5$-2,3,4,5,6); $\nu_{(CN)}$: 1387 cm^{-1}; % Anal. Calcd for $C_{22}H_{26}N_2AgCl$: C, 57.22; H, 5.68; N: 6.07; Found: C, 57.20; H, 5.66; N, 6.05; LC-MS: 743.4 $[AgL_2]^+$.

2.5. Cell viability (MTT) assay

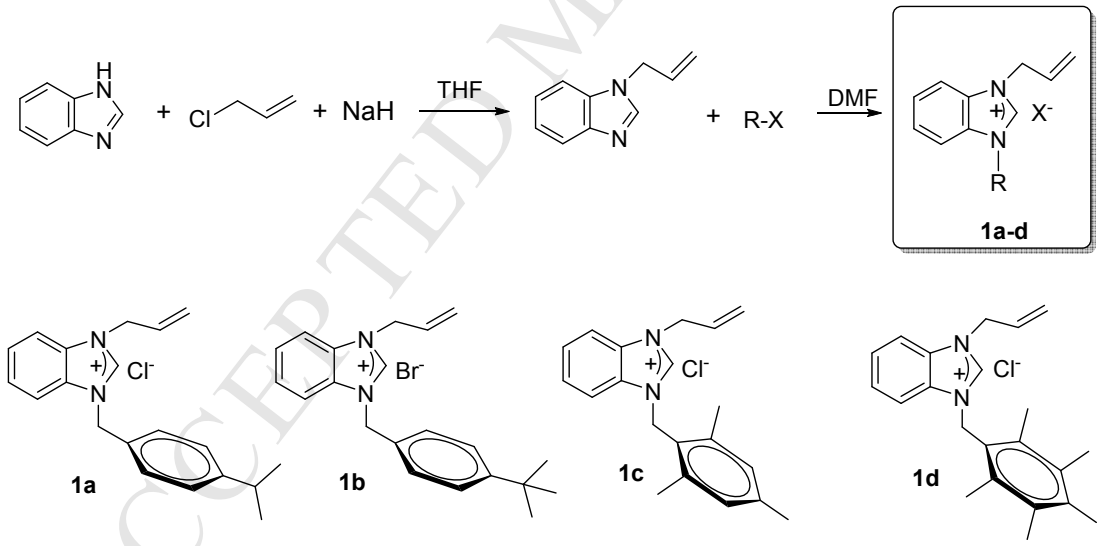
DU-145 prostate cancer cell lines grown in EMEM medium, MCF-7 and MDA-MB-231 breast cancer cell lines were grown in DMEM medium and L-929 cell lines grown in RPMI-1640 medium with L-glutamine supplemented with 10% FBS and 1% penicillin/streptomycin solution under 5% CO_2 at 37 ºC humidified air condition. Cells were passaged at 70–80% confluence and the media were changed every two/three days. The anticancer activity of the salts and complexes were evaluated using the MTT assay [27]. Cells including DU-145, MCF-7, MDA-MB-231, and L-929 were seeded in 96-well plates at a volume of 100 µL per well ($1x10^5$ cell/well) and incubated for 24 h at 37 °C in 5% CO_2. Then the cells were further incubated with various concentrations of the salts and Ag-complexes (1, 5, 10 and 20 µM) for 24 h, 48 h, and 72 h. Media without cells were used as a blank and the control wells contained cells with media and 0.5% DMSO. After the incubation period, 10 mL of MTT solution (5 mg/mL in PBS, pH 7.2) was added to each well. Samples were further incubated for 2 h at 37 °C in 5% CO_2. The medium was replaced with DMSO (100 µL/well) to dissolve the formazan crystals. Cytotoxicity curves and IC_{50} (µM) concentrations (defined as the concentration of drug that decreases MTT staining by 50% as compared to non-treated control cells), were

fitted using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Each experiment was repeated at least three times and all data expressed as mean ± SEM.

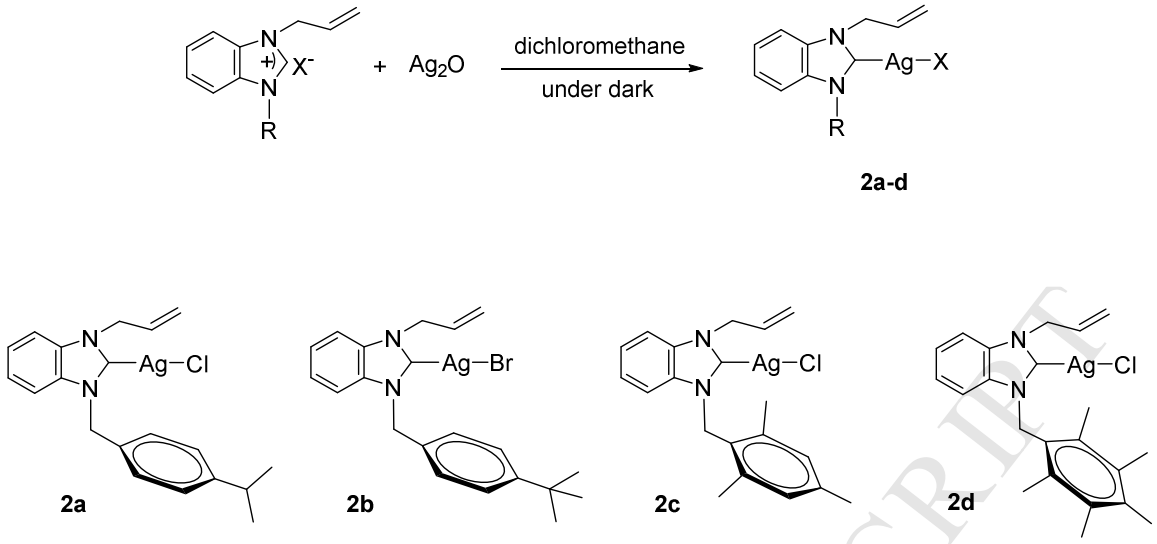
3. Result and discussion

3.1. Chemistry

N-heterocyclic carbene salts and Ag-NHC complexes were synthesized as shown and their chemical structures are summarized in Scheme 1 and Scheme 2. N-heterocyclic carbene salts were synthesized by reaction of allyl substituted benzimidazolium precursor with different alkyl halides in DMF at 80 ^{0}C. Collapsed products were crystallized in dichloromethane/diethyl ether for purification. Synthesized NHC salts were reacted with Ag_2O in dichloromethane at room temperature under the dark condition and their Ag(I)-NHC complexes were obtained. Crude products were purified by crystallization in dichloromethane/diethyl ether. The structure of all new compounds characterized by elemental analysis, FT-IR, ^{1}H NMR and, ^{13}C NMR spectroscopy. And for compound **2d** was made X-ray crystallography studies.



Scheme 1. Synthesis of 1-allyl substituted N-heterocyclic carbene salts (**1a-d**)



Scheme 2. Synthesis of Ag-NHC complexes (**2a-d**)

The FT-IR spectra for all NHC salts (**1a-d**) showed the aliphatic and aromatic C-H stretching vibrational bands at around 2867–2997 cm^{-1} and 3015-3185 cm^{-1}, respectively. C=C stretching vibration modes of all compounds were seen at around 1609-1680 cm^{-1}. Benzimidazole ring C=N vibrations of benzimidazolium salts (**1a-d**) were assigned at around 1545-1556 cm^{-1}. These vibrations have shown a shift in the Ag-NHC complex (**2a-d**) at around 1387-1394 cm^{-1}. This negative shift is because of the electropositive metal center which pulls electron density towards itself and as a result C=N vibrations shifts to the lesser energy region in the complex.

NMR spectra of all the compounds were analyzed in d-$CDCl_3$. In the ^1H NMR spectra, acidic protons (NCHN) for benzimidazolium salts (**1a-d**) were seen at 11.85, 11.55, 11.36 and 10.91 ppm, respectively, as a characteristic sharp singlet. Formation of Ag-NHC complexes (2a-d) were evidenced by the disappearance of the acidic proton peak. For salts (**1a-d**), aromatic protons appeared in the range 7.19-7.75 ppm. Allylic N-CH_2 protons at **1a** and **1b** salts came to at 5.35 and 5.34 ppm as the doublets. For **1a** and **1**, protons at the terminal -CH_2 and -C*H*- protons on allyl group were observed at 5.54-5.50 and 6.08-6.16 ppm as multiplet for **1a** and at 5.45-5.53 and 6.11-6.23 ppm as the multiplets for **1b**. N-CH_2 protons and terminal -CH_2 protons of allyl group belonging to **1c** and **1d** salts came to together as multiplet at 5.32-5.37 and 5.29-5.38 ppm, respectively. The –C*H* protons of allyl groups at **1c** and **1d** salts were detected at 5.99-6.09 and 5.96-6.04 ppm as a multiplet. Benzylic -CH_2 protons at benzimidazolium salts (**1a-d**) were observed at 5.84, 5.85, 5.79 and 5.77 ppm as a sharp singlet, respectively. Methyl protons of benzimidazolium salts (**1a-d)** appeared at 1.19 ppm as a multiplet, at 1.26 ppm as a singlet, at 2.25 and 2.32 ppm as two singlets and at 2.18,

2.21 and 2.23 ppm as three singlets, respectively. In the ^{13}C NMR spectra, aromatic carbons on benzimidazolium salts (**1a-d**) were detected in the range at 113.7-152.4 ppm. N*C*HN carben carbon on salts (**1a-d**) signaled at 143.6, 142.7, 143.6 and 143.3 ppm, respectively. Terminal -*C*H$_2$ carbons of allyl group on **1a** and **1b** gave a peak at 113. 7 ppm and the same carbons on **1c** and **1d** gave a peak at 113.6 ppm. For **1a-d** salts, -N*C*H$_2$ and –*C*H- carbons on allyl groups appeared in the range at 50.1-51.3 ppm and 121.0-122.0 ppm, respectively. Benzylic carbons of benzyl groups were observed at 50.2, 50.2, 47.2 and 48.9 ppm for **1a-d**, respectively. Observed peaks in the range at 17.0-31.2 ppm were attributed to methyl carbons on the salts. ^{1}H NMR spectra and ^{13}C NMR spectra of benzimidazolium salts (**1a-d**) are compatible with each other. After complexation, ^{1}H NMR and ^{13}C NMR spectra of Ag-NHC complexes were observed to almost close of spectra corresponding benzimidazolium salts, except for two characteristic peaks. The first of them was the disappearance of an acidic proton peak at spectra of Ag-NHC complexes which confirmed that the Ag-NHC complexes (**2a-d**) were synthesized. The second one was N*C*N carben resonance on the Ag-NHC complexes (**2a-d**) which shifted much downfield region compared to the corresponding the benzimidazolium salts (**1a-d**). Carben peaks of Ag-NHC complexes **2a** and **2b** were observed at 189.0 and 191.2 ppm slightly and was not observed for **2c** and **2d**. These values, and the lack of the carben peak are in agreement with reported data for similar Ag-NHC complexes.[26]

3.2. X-ray crystallography for 2d

Crystal data and collection details of chloro[1-allyl-3-(2,3,4,5,6-pentamethylbenzyl) benzimidazole-2-ylidene]silver(I), **2d**, (Figure 1) are given in Table 1. The relevant crystallographic for **2d** has been deposited at Cambridge Crystallographic Data Center (CCDC) with No. 1588742. See the Supplementary Materials for more details on complete NMR, FT-IR, LC-MS data and X-ray crystallography studies.

Table 1. The crystal data and collection details of **2d**

Crystal data of chloro[1-allyl-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazole-2-ylidene]silver(I)	
Chemical formula	$C_{22}H_{26}AgClN_2$
M_r	461.77
Crystal system, space group	Monoclinic, $P2_1/c$
Temperature (K)	296
a, *b*, *c* (Å)	10.9722 (15), 14.5472 (19), 13.1839 (19)
β (°)	99.309 (4)
V (Å^3)	2076.6 (5)

Z	4
Radiation type	Mo $K\alpha$
μ (mm^{-1})	1.11
Crystal size (mm)	0.44 × 0.40 × 0.37
T_{min}, T_{max}	0.570, 0.746
No. of measured, independent and observed [$I > 2\sigma(I)$] reflections	12120, 4011, 3350
R_{int}	0.034
$(\sin\theta/\lambda)_{max}$ ($Å^{-1}$)	0.617
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.031, 0.087, 1.04
No. of reflections	4011
No. of parameters	241
No. of restraints	8
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e $Å^{-3}$)	0.34, -0.36

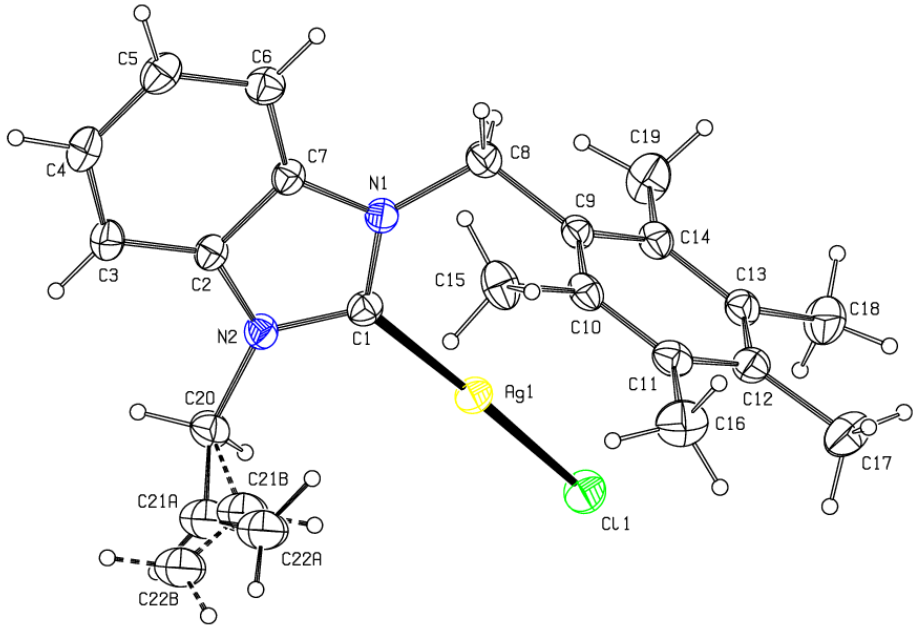


Figure 1: PLATON drawing of the **2d** compound with the atomic numbering scheme. Displacement ellipsoids are drawn at 50 % probability level. The H-atoms are shown as small circles of arbitrary radii.

3.3. In vitro anticancer effects of NHC salts and Ag complexes

The cytotoxicity of the salts **(1a-d)** and Ag-complexes **(2a-d)** in DU-145 human prostate cancer cells, MCF-7, MDA-MB-231 breast cancer cells, and L-929 normal adipose cells were done by assessing their ability to alter 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining over a period of 24 h, 48 h and 72 h.[27] The cytotoxicity of the complexes was found to be concentration- and time-dependent (Figure 2-5**).**

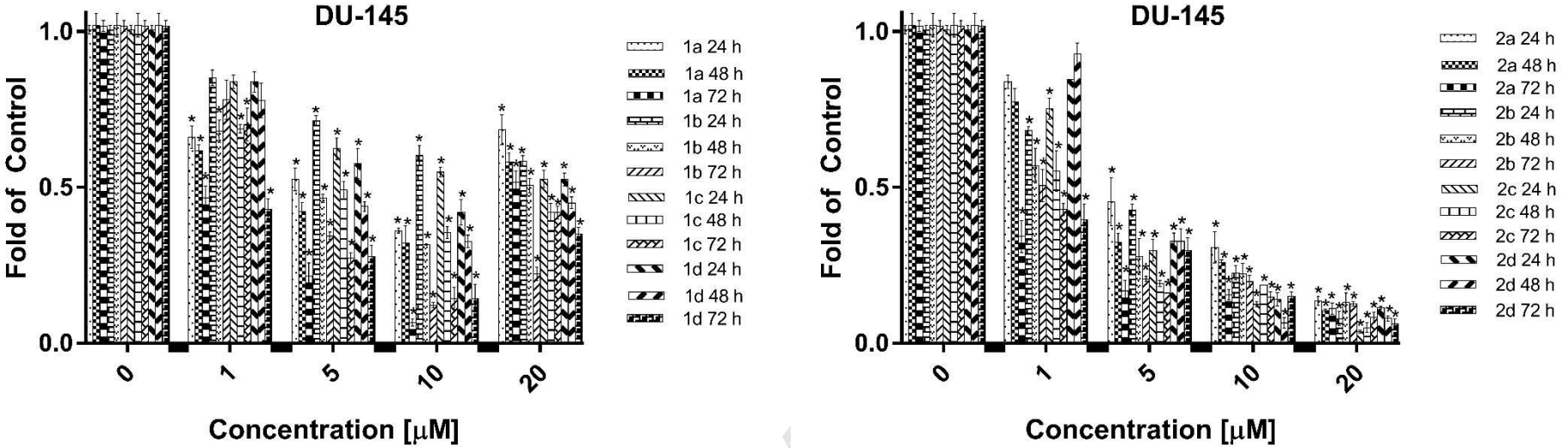


Figure 2. Cell proliferation was determined by MTT assay. DU-145 cells treated with 1, 5, 10 and 20 μM of salts **(1a-d)** and Ag-NHC complexes **(2a-d)** for 24, 48 and 72 h. DMSO treated cells were used as vehicle control. Data are representative of the mean of three separate experiments done in triplicate (± SEM). (*$p<0.0001$ vs control).

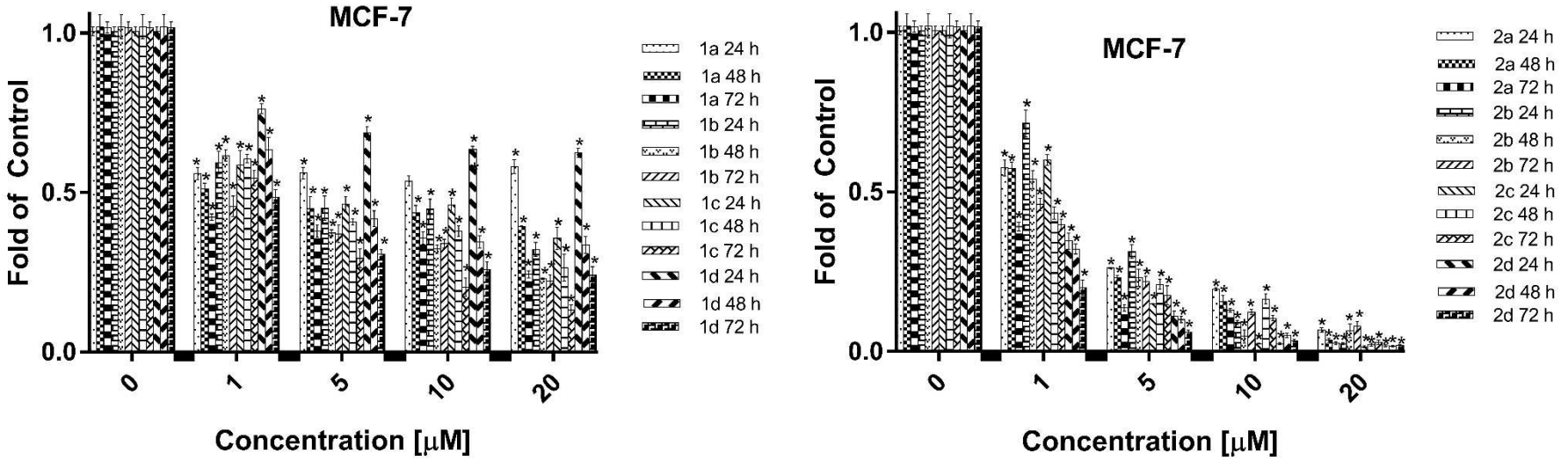


Figure 3. Cell proliferation was determined by MTT staining. MCF-7 cells treated with 1, 5, 10 and 20 μM of salts (**1a-d**) and Ag-complexes (**2a-d**) for 24, 48 and 72 h. DMSO treated cells were used as vehicle control. Data are representative of the mean of three separate experiments done in triplicate (± SEM). (*$p<0.0001$ vs control).

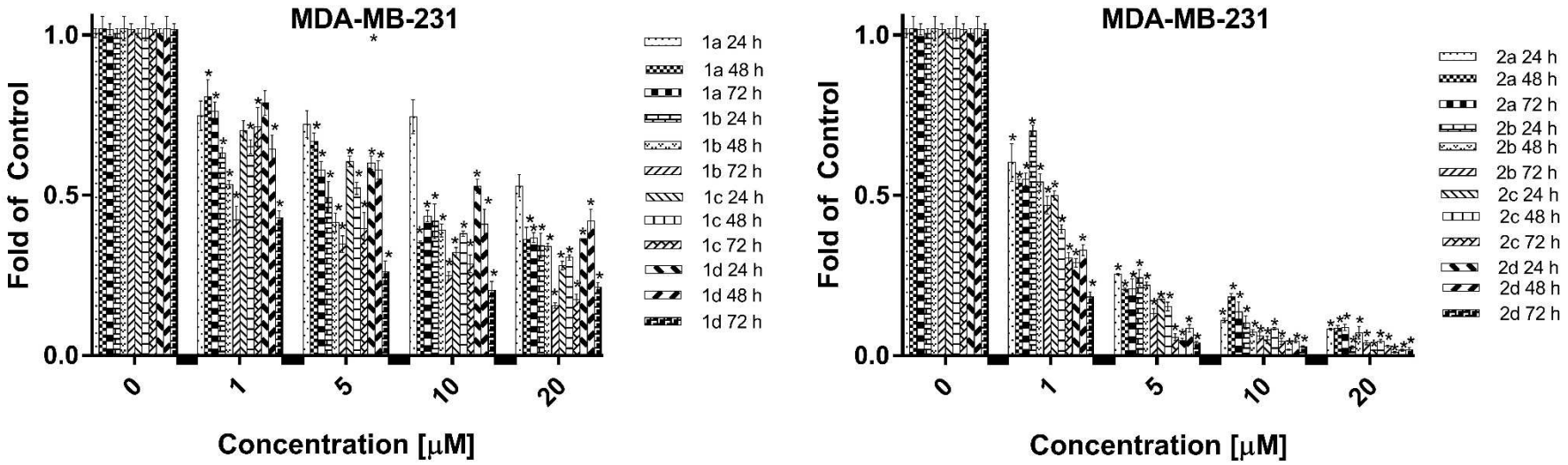


Figure 4. Cell proliferation was determined by MTT staining. MDA-MB-231 cells treated with 1, 5, 10 and 20 μM of salts (**1a-d**) and Ag-complexes (**2a-d**) for 24, 48 and 72 h. DMSO treated cells were used as vehicle control. Data are representative of the mean of three separate experiments done in triplicate (± SEM). (*$p<0.0001$ vs control).

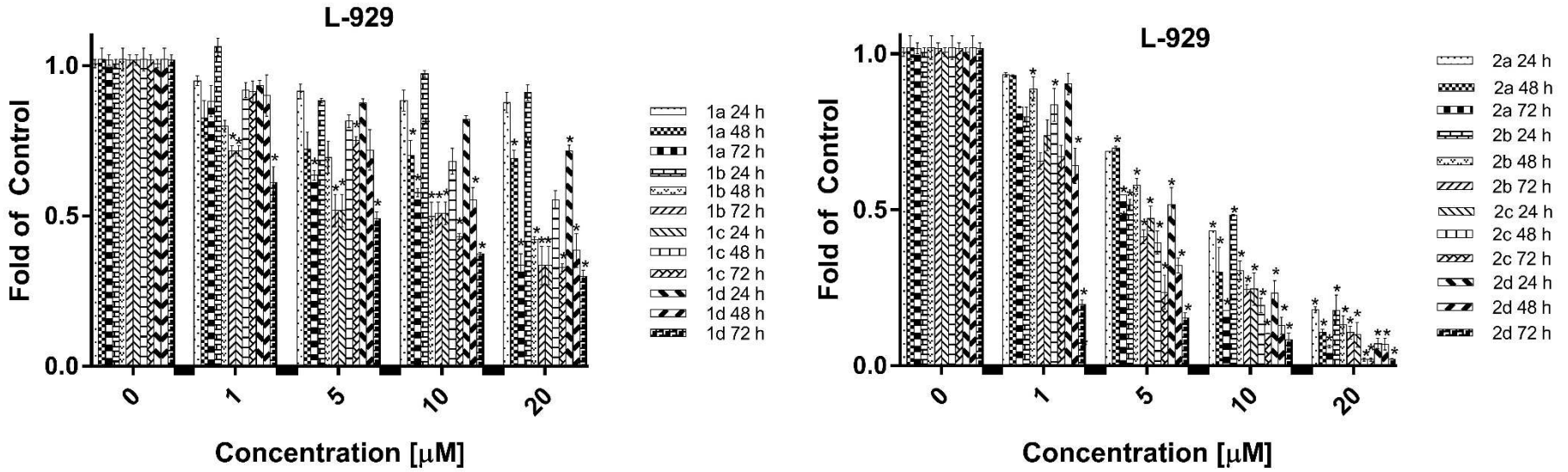


Figure 5. Cell proliferation was determined by MTT staining. L-929 cells treated with 1, 5, 10 and 20μM of salts (**1a-d**) and Ag-complexes (**2a-d**) for 24, 48 and 72 h. DMSO treated cells were used as vehicle control. Data are representative of the mean of three separate experiments done in triplicate (± SEM). (*$p<0.0001$ vs control).

The data were used to calculate IC_{50} values. These values ranged up 1 to 20 µM, suggesting that Ag(I)-NHC complexes exhibited anticancer activity against all cell lines to a different degree (Table 2). As shown in Table 2, the IC_{50} values varied among different complexes and appeared to be cell-line dependent. Complexes **2a-d** show marked potencies compared with the respective the salts **1a-d**. The results indicated that these complexes have potent activity against the three cancer cell lines studied, and the efficacy of complex **2d** is much higher than that of complexes **2a, 2b** and **2c.** In general, on the IC_{50} followed the order of **2d> 2c>2a>2b>.**

Table 2. Inhibition of cell viability (IC_{50} ± SEM, µM) of the free salts **(1a-d)** and Ag-complexes **(2a-d)** on DU-145 (prostate carcinoma), MCF-7, MDA-MB-231(breast carcinoma) and L-929 (non-cancer cell lines)[a] after 48 h of incubation

Compounds	DU-145	MCF-7	MDA-MB-231	L-929[a]
		48 h (IC_{50}, µM[b])		
1a	4.96 ± 0.35	6.17 ± 0.84	10.4 ± 0.33	>20
1b	3.62 ± 0.18	2.61 ± 0.18	3.85 ± 0.31	15.62 ± 0.33
1c	4.91 ± 0.20	5.10 ± 0.29	10.8 ± 0.17	>20
1d	2.99 ± 0.16	1.15 ± 0.48	6.18 ± 0.58	14.7 ± 0.40
2a	1.69 ± 0.21	2.14 ± 0.13	1.10 ± 0.19	14.2 ± 0.22
2b	2.09 ± 0.43	2.19 ± 0.22	1.57 ± 0.19	8.93 ± 0.12
2c	1.41 ± 0.55	<1	<1	4.67 ± 0.11
2d	1.21 ± 0.23	<1	<1	3.56 ± 0.20

[a]Non-cancer cells, [b] Cytotixicity as IC_{50} for each cell lines is in average of three independent experiments after treatment for 48 h (± SEM).

Compared with healthy cells, it was found that **2a** is 8.4 fold, 6.63-fold and more than 12.9-fold more toxic, **2b** is 1.88-fold, 4-fold and more than 5.68 fold more cytotoxic to DU-145, MCF-7, and MDA-MB-231 cancer cells, respectively. **2c** is more than 4.67-fold more cytotoxic to MCF-7 and MDA-MB-231 cancer cells and more than 3.31-fold to DU-145 cells. **2d** is more than 3.56-fold more cytotoxic to MCF-7 and MDA-MB-231 cancer cells more than 2.94-fold on DU-145 cells, for 48 h.

Complexes **2c** and **2d**, with a $-CH_3$ substituent group on the benzene ring, were more cytotoxic than the other complexes. The efficacy of **2d** was higher than **2c**, which may be attributed to the increased $-CH_3$ substituent group. Moreover, the additional $-CH_3$ group in the benzene ring can also contribute to the lipophilicity of the complexes. The higher efficacy of complex **2c** and **2d** may also be related to their faster rate of hydrolysis. The cytotoxicities of **2a** were higher than **2b,** which may be attributed to the isopropyl benzyl substituent on the

benzene ring more than the *tert*-butyl benzyl substituent on the benzene ring. All Ag-complexes exhibited increased efficacy against MCF-7 cells than cisplatin (IC_{50} values; 3.19 μM) after 48 h [28]. This correlates to the reports that the silver in these complexes play important role in the antitumor activity of these complexes. In addition, the IC_{50} value of salts and Ag-complexes for L-929 mouse adipose fibroblasts (non-cancer cells) were all above the IC_{50} value of the cancer cell lines tested, which suggests that the salts and Ag-complexes act very specifically on cancer cells. Interestingly, all of the complexes exhibit a certain degree of selectivity towards cancer cells.

4. Conclusion

In the present work, four benzimidazole-based N-heterocyclic carbene and their Ag(I)-NHC complexes have been designed, synthesized and characterized. The assessment of efficacy using *in vitro* studies indicate that these complexes inhibit cancer cell growth in at least three different cells (DU-145, MCF-7 and MDA-MB-231) especially **2c** and **2d**. Notably, these complexes showed lower efficacy against non-cancer derived from mouse adipose (L-929 cells). The findings of the present work conclude that the isopropyl benzyl substituents on the benzene ring increased the efficacy of these complexes to a greater degree than *tert*-butyl benzyl substituents on the benzene ring. In addition, the additional CH_3 group in the benzene ring can also contribute to the anticancer activity of these complexes. These results will direct the focus of our future research program to modifications of these derivatives, which may be necessary to further improve efficacy.

Appendix A. Supplementary data

CCDC 1588742 contain the supplementary crystallographic data for the compounds reported in this article. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* https://www.ccdc.cam.ac.uk/structures.

Acknowledgments

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Highlights

-N-heterocyclic carbene ligands (NHC) and their Ag(I)-NHC complexes have been synthesized.

-All the ligands and complexes were characterized by spectroscopic methods.

-All compounds have been applied for the anticancer activity on MCF-7, MDA-MB-231 breast cancer and DU-145 prostate cancer cells.

-The Ag(I)-NHC complexes showed a dose and time-dependent cytotoxic activity against all cell lines.