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# Synthesis, characterization, and investigation of antiproliferative activity of novel Ag (I)-N-Heterocyclic Carbene (NHC) compounds

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ABSTRACT

The aim of this study was to present the synthesis, characterization, and investigation of the antiproliferative activity of new metal *N*-Heterocyclic Carbene (NHC) salts (**1a-c**) and their Ag(I) complexes (**2a-c**). All synthesized compounds were characterized using elemental analysis, LC-MS, FT-IR, $^{1}$H NMR, and $^{13}$C NMR spectroscopy techniques. Salts and complexes were tested for antiproliferative activities on human breast and prostate cancer cell lines (MCF-7, MDA-MB-23, DU-145) and L-929 normal cells for 24 h, 48 h and 72 h using MTT assays. The Ag(I)-NHC complexes **(2a-c)** showed dose and time-dependent cytotoxic activity against all cell lines. MDA-MB-231 and MCF-7 human breast carcinoma cells were the most sensitive to displaying $IC_{50}$ lower than 1 μM at all time points for **2a** and **2b** complexes respectively. The $IC_{50}$s for Ag(I)-NHC were higher in normal cells especially compared to the breast cancer cells, suggesting that complexes possessed noteworthy selectivity for human breast cancer cells. Complex **2a** showed high selectivity (≥13-fold) for MDA-MB-231 breast cancer cells at all time points. These results also demonstrated that complex **2b** has 4-7-fold selectivity against MCF-7 breast cancer cells.



## 1. Introduction

N-Heterocyclic Carbenes (NHCs) have become a well-known class of organometallic ligands [1–5]. In addition to the catalytic applications of NHCs, numerous studies have been conducted on the biological activities of NHC based organometallic compounds [6–15]. The variability of the biological activities of NHCs greatly depends on the ring size and structure of the substitutions over the ring [16–18]. In 1944, Wooley reported that benzimidazole can act in a similar way to purines [19]. Following that study, it was demonstrated that benzimidazole derivatives have biological activities, such as antihypertensive, anti-inflammatory, antimicrobial, antiviral, antioxidant, antitumor, lipid modulator, and anticoagulant effects [20–27]. Silver salts have historically been used as antimicrobial agents for the prevention of eye infections in newborns and the purification of drinking water as well as in wound healing [28,29]. The lower toxicity of silver salts has attracted the attention of researchers for the investigation of the biological activities of silver-NHC complexes [30–34]. Since Ghosh et al. first reported the cytotoxic properties of silver(I)-NHC complex against MCF-7 breast cancer cells, HCT 116 colon adenocarcinoma cells and HeLa cervical cancer cells, a great number of new silver(I)-NHC complexes synthesized and investigated against different cancer cells [35–41]. The structural similarity of benzimidazole derivatives with naturally occurring nucleotides makes them biologically important structures [42]. Some studies have shown that a number of organic derivatives of benzimidazole have important therapeutic activities including anticancer activity [43–47]. Due to both the lower toxicity of silver and useful biological activity of benzimidazole these two structures were used to synthesize salts and complexes in this study.

Cancer is one of the most formidable diseases and a major cause of death worldwide, with an estimated 9.6 million deaths due to cancer in 2018 [48]. Breast cancer is the most frequently diagnosed form of cancer in women worldwide, with approximately 2.3 million new cases and 627,000 deaths in 2018. For males, prostate cancer is the second most common cancer, with approximately 1.3 million new cases in 2018 [48]. Different kinds of treatment protocols have been developed for cancer treatment; including radiotherapy, chemotherapy and drug therapy. Treatment protocols

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vary depending on the cancer types. *Cis*-platin, discovered by Rosenberg et al., was the first example of metal-based anticancer drugs [49]. Although *cis*-platin has several side effects, such as hair loss, neurotoxicity, vomiting, nephrotoxicity, diarrhea and the development of intrinsic and acquired resistance in some cancer cells, *cis*-platin and its analogs are mostly used for the medical treatment of cancer patients [50–52]. Therefore, it is important to obtain new anti-cancer agents that are more effective and have low cytotoxicity.

The aim of this study was to present the synthesis, characterization, and investigation of antiproliferative activity of new metal *N*-Heterocyclic Carbene (NHC) salts (**1a-c**) (Scheme 1) and their Ag(I) complexes (**2a-c**) (Scheme 2. All synthesized compounds were characterized using elemental analysis, LC-MS, FT-IR, $^1$H NMR, and $^{13}$C NMR spectroscopy techniques. Salts and complexes were tested for antiproliferative activities against human breast cancer cells (MCF-7, MDA-MB-23), human prostate cancer cells (DU-145) and L-929 normal cells for 24 h, 48 h and 72 h using MTT assays.

## 2. Experimental

### 2.1. Materials and methods

All the synthesis processes of benzimidazolium salts and Ag-NHC complexes were prepared under argon in flame-dried glassware using standard Schlenk line techniques. The 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 [53]. All $Ag_2O$ reactions were carried out in the absence of light. Elemental analyses were performed in İnönü University Scientific and Technology Center. Melting points were determined using the Electrothermal 9100 melting point detection apparatus. Fourier transform infrared (FT-IR) spectra were obtained in the range of 400–4000 cm$^{-1}$ on a PerkinElmer Spectrum 100 FT-IR. $^1$H NMR and $^{13}$C NMR spectra were taken using a Bruker As 400 Mercury spectrometer operating at 400 MHz ($^1$H), 100 MHz ($^{13}$C) in $CDCl_3$ with tetramethylsilane as the internal reference. $^1$H peaks were labeled as singlet (s), doublet (d), triplet (t), quintet (quint.) and multiplet (m). Chemical shifts and coupling constants were reported in ppm and in Hz, respectively. LC-MS was performed using LC/MSD SL mass spectrometer (AgilentTechnologies 1100). Samples were introduced on a continuous flow of 1 mL/min with $C_{18}$ column (250 × 4.6 mm) at 25 °C. Nitrogen served both as the nebulizer and dry gas.

### 2.2. Synthesis

Benzimidazolium salts **(1a-c)** and the respective Ag(I)-NHC complexes **(2a-c)** were synthesized according to the previous study by the current authors [54]. **1c** and bromo salt of **1a** have been synthesized previously in literature [55,56]. In this study, chloro salt of 1-(allyl)-3-benzylbenzimidazolium (**1a**) was synthesized.

### 2.3. General procedure for the preparation of benzimidazolium salts, (1a-c)

Benzimidazole (10 mmol) was added to a solution of NaH (10 mmol) in dry THF (30 mL), and the mixture was stirred for 1 h at room temperature. Allyl bromide (10.1 mmol) was added dropwise to obtain a solution that was heated for 24 h at 60 °C. Then, the THF was removed under vacuum. Dichloromethane (50 mL) was added to the 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 °C and the salts (**1a-c**) were precipitated. Following the completion of the process the solution was filtered, the solids were rinsed with diethylether and dried under vacuum. Crude products were recrystallized from dichloromethane/diethylether.

#### 2.3.1. 1-Allyl-3-benzylbenzimidazolium chloride, (1a)

Yield: 83%; m.p. 166-168 °C. FT-IR $\nu_{(CN)}$: 1551 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 5.26 (d, 2H, NC*H*$_2$CHCH$_2$, $J$ = 8 Hz), 5.38–5.44 (m, 2H, NCH$_2$CHC*H*$_2$), 5.84 (s, 2H, C*H*$_2$C$_6$H$_5$), 6.04 (quint, 1H, NCH$_2$C*H*CH$_2$, $J$ = 4 Hz), 7.25–7.31 (m, 3H, Ar-*H*), 7.45–7.57 (m, 5H, Ar-*H*), 7.65 (d, 1H, Ar-*H*, $J$ = 8 Hz), 11.90 (s, 1H, NC*H*N).$^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm): 50.2 (*C*H$_2$C$_6$H$_5$), 51.5 (NCH$_2$CHCH$_2$), 121.9 (NCH$_2$CHCH$_2$), 131.3 (NCH$_2$CHCH$_2$), 113.7, 113.9, 127.1, 127.2, 128.4, 129.2, 129.3, 129.6, 131.5, and 132.8 (Ar-*C*), 143.8 (N*C*HN). % Anal. Calcd for $C_{17}H_{17}N_2Cl$: C, 71.70; H, 6.02; N: 9.84; Found: C, 71.62; H, 5.93; N, 9.77.

#### 2.3.2. 1-Allyl-3-(naphthylmethyl)benzimidazolium chloride, (1b)

Yield: 88%; m.p. 173-175 °C. FT-IR $\nu_{(CN)}$: 1555 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 5.33 (d, 2H, NC*H*$_2$CHCH$_2$, $J$ = 4 Hz), 5.42–5.47 (m, 2H, NCH$_2$CHC*H*$_2$), 6.11 (quint, 1H, NCH$_2$C*H*CH$_2$, $J$ = 4 Hz), 6.37 (s, 2H, C*H*$_2$C$_{10}$H$_7$), 7.40–7.55 (m, 6H, Ar-*H*), 7.62 (t, 1H, Ar-*H*, $J$ = 8 Hz), 7.69 (d, 1H, Ar-*H*, $J$ = 8 Hz), 7.86 (t, 2H, Ar-*H*, $J$ = 8 Hz), 8.21 (d, 1H, Ar-*H*, $J$ = 8 Hz), 11.85 (s, 1H, NC*H*N).$^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm): 49.6 (*C*H$_2$C$_{10}$H$_7$), 50.2 (NCH$_2$CHCH$_2$), 121.7 (NCH$_2$CHCH$_2$), 131.5 (NCH$_2$CHCH$_2$), 113.7, 114.0, 122.5, 125.3, 126.5, 127.0, 127.2, 127.3, 127.7, 128.0, 129.2, 129.7, 130.1, 130.7, 131.5, 131.6 and 133.8 (Ar-*C*), 144.2 (N*C*HN). % Anal. Calcd for $C_{21}H_{19}N_2Cl$: C, 75.33; H, 5.72; N: 8.37; Found: C, 75.21; H, 5.63; N, 8.32.

### 2.4. General procedure for preparation of Ag-NHC complexes, (2a-c)

A solution of 0.5 mmol of $Ag_2O$ and 1 mmol of the corresponding benzimidazolium salt (**1a-c**) 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.

#### 2.4.1. Chloro[1-allyl-3-benzylbenzimidazole-2-ylidene]silver(I), (2a)

Yield: 81%; m.p. 192-194 °C. FT-IR $\nu_{(CN)}$: 1395 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 5.09 (d, 2H, NC*H*$_2$CHCH$_2$, $J$ = 4 Hz), 5.24 (d 1H, NCH$_2$CHC*H*$_2$, $J$ = 16 Hz), 5.33 (d 1H, NCH$_2$CHC*H*$_2$, $J$ = 12 Hz), 5.64 (s, 2H, C*H*$_2$C$_6$H$_5$), 6.02 (quint, 1H, NCH$_2$C*H*CH$_2$, $J$ = 4 Hz), 7.27–7.39 (m, 8H, Ar-*H*), 7.46–7.49 (m, 1H, Ar-*H*). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm): 52.1 (*C*H$_2$C$_6$H$_5$), 53.5 (NCH$_2$CHCH$_2$), 119.3 (NCH$_2$CHCH$_2$), 131.8 (NCH$_2$CHCH$_2$), 111.9, 112.2, 124.3, 124.4, 127.1, 128.5, 129.1, 133.7, 134.0, 134.9 (Ar-*C*), 189.1 (slightly, $C_{carbene}$-Ag). % Anal. Calcd for $C_{17}H_{16}N_2AgCl$: C, 52.14; H, 4.12; N: 7.15; Found: C, 52.06; H, 4.03; N, 7.07. LC-MS: 603.2 $[AgL_2]^+$

#### 2.4.2. Chloro[1-allyl-3-naphthylmethylbenzimidazole-2-ylidene] silver(I), (2b)

Yield: 84%, m.p. 203-204 °C. FT-IR $\nu_{(CN)}$: 1399 cm$^{-1}$. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 5.10 (d, 2H, NC*H*$_2$CHCH$_2$, $J$ = 4 Hz), 5.23 (d 1H, NCH$_2$CHC*H*$_2$, $J$ = 20 Hz), 5.33 (d 1H, NCH$_2$CHC*H*$_2$, $J$ = 8 Hz), 6.02 (quint, 1H, NCH$_2$C*H*CH$_2$, $J$ = 4 Hz), 6.08 (s, 2H, C*H*$_2$C$_{10}$H$_7$), 6.97 (d, 1H, Ar-*H*, $J$ = 4 Hz), 7.25–7.26, 733-7.39 and 7.49–7.60 (m, 7H, Ar-*H*), 7.83 (d, 1H, Ar-*H*, $J$ = 8 Hz), 7.90–7.92 (m, 1H, Ar-*H*), 8.00–8.03 (m, 1H, Ar-*H*). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ (ppm): 51.2 (*C*H$_2$C$_{10}$H$_7$), 52.2 (NCH$_2$CHCH$_2$), 122.3 (NCH$_2$CHCH$_2$), 131.8 (NCH$_2$CHCH$_2$), 112.0, 112.2, 119.3, 124.4, 124.9, 125.3, 126.3, 127.1, 129.2, 130.1, 130.5, 133.8, 133.9 and 134.1 (Ar-*C*), 189.7 (slightly, $C_{carbene}$-Ag). % Anal. Calcd for $C_{21}H_{18}N_2AgCl$: C, 57.10; H, 4.11; N:

6.34; Found: C, 57.04; H, 4.06; N, 6.26. LC-MS: 703.2 $[AgL_2]^+$

*2.4.3. Chloro[1-allyl-3-(anthracen-9-yl-methyl)benzimidazole-2-ylidene]silver(I), (2c)*

Yield: 87%; m.p. 205-206 °C. FT-IR $\nu_{(CN)}$: 1395 $cm^{-1}$. $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ (ppm): 4.96 (d, 2H, $NCH_2CHCH_2$, $J = 8$ Hz), 5.11 (d, 1H, $NCH_2CHCH_2$, $J = 16$ Hz), 5.24 (d, 1H, $NCH_2CHCH_2$, $J = 8$ Hz), 5.91 (quint, 1H, $NCH_2CHCH_2$, $J = 4$ Hz), 6.40 (s, 2H, $CH_2C_{14}H_9$), 7.07–7.15 and 7.28–7.30 (m, 3H, Ar-*H*), 7.39 (d, 1H, Ar-*H*, $J = 8$ Hz), 7.47–7.56 (m, 4H, Ar-*H*), 8.08 (d, 2H, Ar-*H*, $J = 8$ Hz), 0.8.19 (d, 2H, Ar-*H*, $J = 8$ Hz), 8.59 (s, 1H, Ar-H). $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ (ppm): 46.5 ($CH_2C_{14}H_9$), 52.4 ($NCH_2CHCH_2$), 122.9 ($NCH_2CHCH_2$), 130.0 ($NCH_2CHCH_2$), 111.9, 112.1, 119.0, 123.1, 124.2, 125.3, 127.7, 130.4, 131.1, 131.4, 131.7, 133.9, 134.2 (Ar-*C*), 189.3 (slightly, $C_{carbene}$-Ag). % Anal. Calcd for $C_{25}H_{20}N_2AgCl$: C, 61.06; H, 4.10; N: 5.70; Found: C, 60.99; H, 4.01; N, 5.62. LC-MS: 804.2 $[AgL_2]^+$

### 2.5. Antiproliferative activity

The antiproliferative activity of the newly-synthesized salts and complexes was determined against MCF-7 (HTB-22, human breast adenocarcinoma), DU-145 (HTB-81, human prostate carcinoma), MDA-MB-231 (HTB-26, human breast adenocarcinoma) cancer cells and L-929 normal cells using the MTT assay [57]. MCF-7 and MDA-MB-231 cells were grown in DMEM (Sigma, D6429) medium. DU-145 cancer cells and L-929 normal cells (ECACC, European Collection of Animal Cell Culture, Salisbury, U.K.) were grown in EMEM (ATTC, 30-2003) and RPMI-1640 media (ATTC, 30-2001), respectively. All media contained 10% (v/v) FBS (ATTC, FBS, 30-2020), and 100 Units/ml penicillin and 100 μg/ml streptomycin (ATTC, 30-2300). Exponentially growing DU-145, MCF-7, MDA-MB-231, and L-929 cells, in a 37 °C humidified incubator with 5% $CO_2$, were seeded into 96-well plates at a density of 1 x $10^5$ cells/well and allowed to attach for 24 h. A stock solution of the tested compounds was prepared in DMSO and diluted in the complete culture medium. DMSO concentration did not exceed 1% (v/v). The cells were treated with 1–20 μM of compounds in 5% $CO_2$, at 37 °C for 24 h, 48 h, and 72 h. The control wells contained cells and 1% DMSO. At the end of the exposure period, 10 μL MTT solution was added to each well (5 mg/mL, in PBS) and the cells were incubated for 2 h at 37 °C in 5% $CO_2$. After removal of the medium, the purple-blue precipitated crystals were dissolved in 100 μL of DMSO (Sigma, St. Louis, MO, USA). The absorbance was read at 570 nm with a Biotek plate reader (Biotek, Epoch, USA). Data were based on the means from at least three independent experiments, each comprising three replicates per concentration. The $IC_{50}$ values of compounds were calculated using GraphPad Prism 7 software (GraphPad Software, San Diego, CA, USA).

### 2.6. Statistical analysis

All experiments were carried out in triplicate and the data represented the average values of three independent measurements with standard error means (±SEM). Data were analyzed using one-way analysis of variance and differences were considered significant at $^*p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0005$, $^{****}p < 0.0001$. The $IC_{50}$ were determined using statistics software, GraphPad Prism7 (GraphPad Software, San Diego, CA, USA).

## 3. Results and discussions

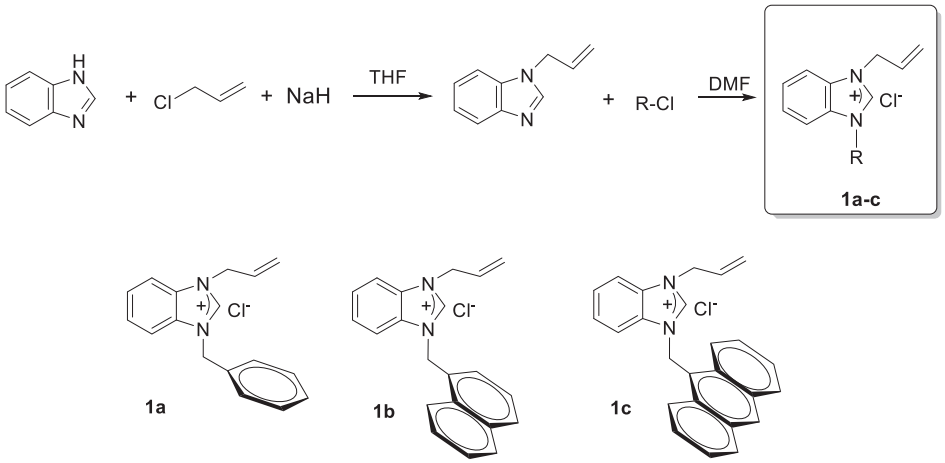
### 3.1. Synthesis and characterization of benzimidazolium salts and corresponding Ag(I)-NHC complexes

The synthetic route for the synthesis of benzimidazolium salts (**1a-c**) is shown in Scheme 1. The salts were synthesized with a reaction of *N*-allyl benzimidazole with different alkyl halides in DMF at 80 °C. Collapsed products were crystallized in dichloromethane/diethyl ether for purification.

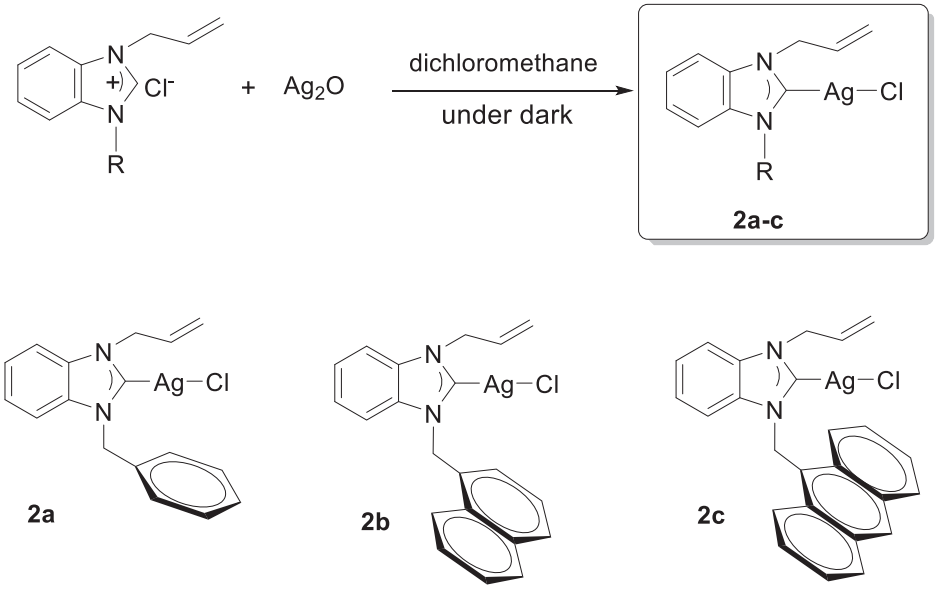
As shown in Scheme 2, synthesized benzimidazolium salts (**1a-c**) were reacted with $Ag_2O$ in dichloromethane at room temperature under dark conditions and their Ag(I)-NHC complexes (**2a-c**) were obtained. Crude products were purified by crystallization in dichloromethane/diethyl ether. The structures of all new compounds were characterized by elemental analysis, LC-MS, FT-IR, $^1H$ NMR and, $^{13}C$ NMR spectroscopy.

In the elemental analyses, the accepted deviation is ±0.4%. For all compounds (**1a-c** and **2a-c**), deviation of experimental elemental analysis was < ±0.4%. Thus, the experimental elemental analysis values of all the compounds were compatible with the theoretical values.

The experimental FT-IR spectra of benzimidazolium salts and Ag(I)-NHC complexes are given in the Supporting Information (Figs. S2, S4, S6, S9, S12). The FT-IR spectra of all compounds contained some characteristic bands of the stretching vibrations of the C=N, C-N, C-H and C=C groups. The aliphatic and aromatic C-H stretching vibrational bands for all compounds reached a peak at around 2700–3200 $cm^{-1}$. Benzimidazole ring CN vibrations of




**Scheme 1.** Synthesis of benzimidazolium salts (**1a-c**).




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

benzimidazolium salts (**1a-c**) were assigned at around 1551-1555 $cm^{-1}$. These vibrations show a shift in the Ag(I)-NHC complexes (**2a-c**) at 1395, 1399 and 1595 $cm^{-1}$, respectively. This negative shift is because of the electropositive metal center which pulls electron density towards itself and as a result, CN vibrations shift to the lesser energy region in the complex.

NMR spectra of all the compounds were analyzed in d-$CDCl_3$. In the $^1$H NMR spectra, acidic protons (NCHN) for benzimidazolium salts (**1a-c**) were seen at 11.90, 11.85 and 9.14 ppm [50], respectively, as a characteristic sharp singlet. The formation of Ag(I)-NHC complexes was evidenced by the disappearance of the acidic proton peak as seen in $^{13}$C NMR spectra (Figs. S1, S3, S5, S8, S11). For all compounds, aromatic protons appeared in the range 6.97−8.59 ppm. Single protons at $CH_2CHCH_2$ reached peaks in the range 5.91−6.22 ppm as quint for all compounds. In $^{13}$C NMR spectra, while the sharp characteristic signals of the imino carbon (NCHN) on the **1a-c** salts were seen around 140.0 ppm, after complexation, NCN carben resonance on the **2a-c** shifted highly to the downfield region at around 190.0 ppm as slight peaks compared to the corresponding benzimidazolium salts. In the complexes, the resonances for carbene carbon was not detected exactly, which has also been mentioned in the literature and given as a reason for the fluxional behavior of NHCs complexes [58−61]. These values are in agreement with reported data for similar compounds [62,63]. In the LC-MS spectrum of Ag(I)-NHC complexes **2a-c**, it was seen that, complexes exist as $[Ag(NHC)_2]^+$ in solution (Figs. S7, S10, S13).

### 3.2. Antiproliferative activity

The antiproliferative activity of synthesized benzimidazolium salts **(1a-c)** and their Ag(I)-NHC complexes **(2a-c)** were determined using MTT assay against MCF-7, DU-145, and MDA-MB-231 cancer cells, as well as L-929 non-cancer cells for 24, 48, and 72 h of exposure. Fig. 1 shows the time and dose-dependent antiproliferative activities of salts **(1a-c)** and complexes **(2a-c)** against MCF-7 breast cancer cells.

According to Fig. 1 the antiproliferative activity of the compounds was found to be concentration- and time-dependent against MCF-7 breast cancer cells. Fig. 1 (Figs. S14, S15, S16) also show that Ag-complexes have more antiproliferative activity than their own salts. The $IC_{50}$ (concentration of the test compound to achieve 50% inhibition) values of salts **(1a-c)** and complexes **(2a-c)** for 24, 48, and 72 h are listed in Table 1.

The $IC_{50}$ values of **1a** against MDA-MB-231, MCF-7 breast cancer cells, and L-929 normal cells ranged from 7.07 to >20 μM indicating lower antiproliferative activity. Similarly, **1b** did not display any activity toward MDA-MB-231 breast cancer cells and L-929 normal cells even at a concentration of 20 μM. **1a** and **1b** were determined to have time-dependent $IC_{50}$ values towards DU-145 prostate cancer cells and MCF-7 breast cancer cells, respectively. These $IC_{50}$ values indicated high antiproliferative activity. Notably, the $IC_{50}$ values of **1c** were lower than those of **1a,** and **1b,** suggesting higher antiproliferative activity than **1a,** and **1b.** The silver complexes (**2a-c**) decreased MTT staining in all cells, compared to the controls. **2a** showed better antiproliferative activity against cancer cells with $IC_{50}$ ranging from <1 to 16.3 μM. MDA-MB-231 human breast carcinoma cells were the most sensitive to **2a** displaying $IC_{50}$ lower than 1 μM at all time points. These results showed that the $IC_{50}$ values of **2b** differ from <1 to 6.94 μM against cancer cells. Considering **2b**, MCF-7 human breast carcinoma cells were the most sensitive to displaying $IC_{50}$ lower than 1 μM at all time points. The $IC_{50}$ values of complex **2c, 2b,** and **2a** on DU-145 human prostate cancer cells were found to be range 1.74−3.9 μM, 2.11−6.94 μM and 2.74−16.3 μM at 24, 48 and 72 h respectively. These results indicated that antiproliferative activity followed the order of **2c**>**2b**>**2a** and the increased ring numbers bound on the benzimidazole ring may be attributed to the increased activities.

The pharmaceutical research on organometallic compounds has generally been focused on platinum and gold complexes. However recent reports of lower toxicity and biological activities of silver have increased interest in Ag-NHC complexes. In a previous study three series of silver(I) complexes bearing sterically−modulated coumarin−functionalized benzimidazole−based NHC ligands were synthesized and characterized (Scheme 3, Scheme 4) [64].

An anticancer activities against human lung cancer cells (H1975 and A549) were also evaluated over a period of 72 h. The results of that study showed that, all the azolium salts were found to be inactive, while complexes demonstrated significant activities. The $IC_{50}$ values of NHC-silver complexes **13**−**24** were found to range

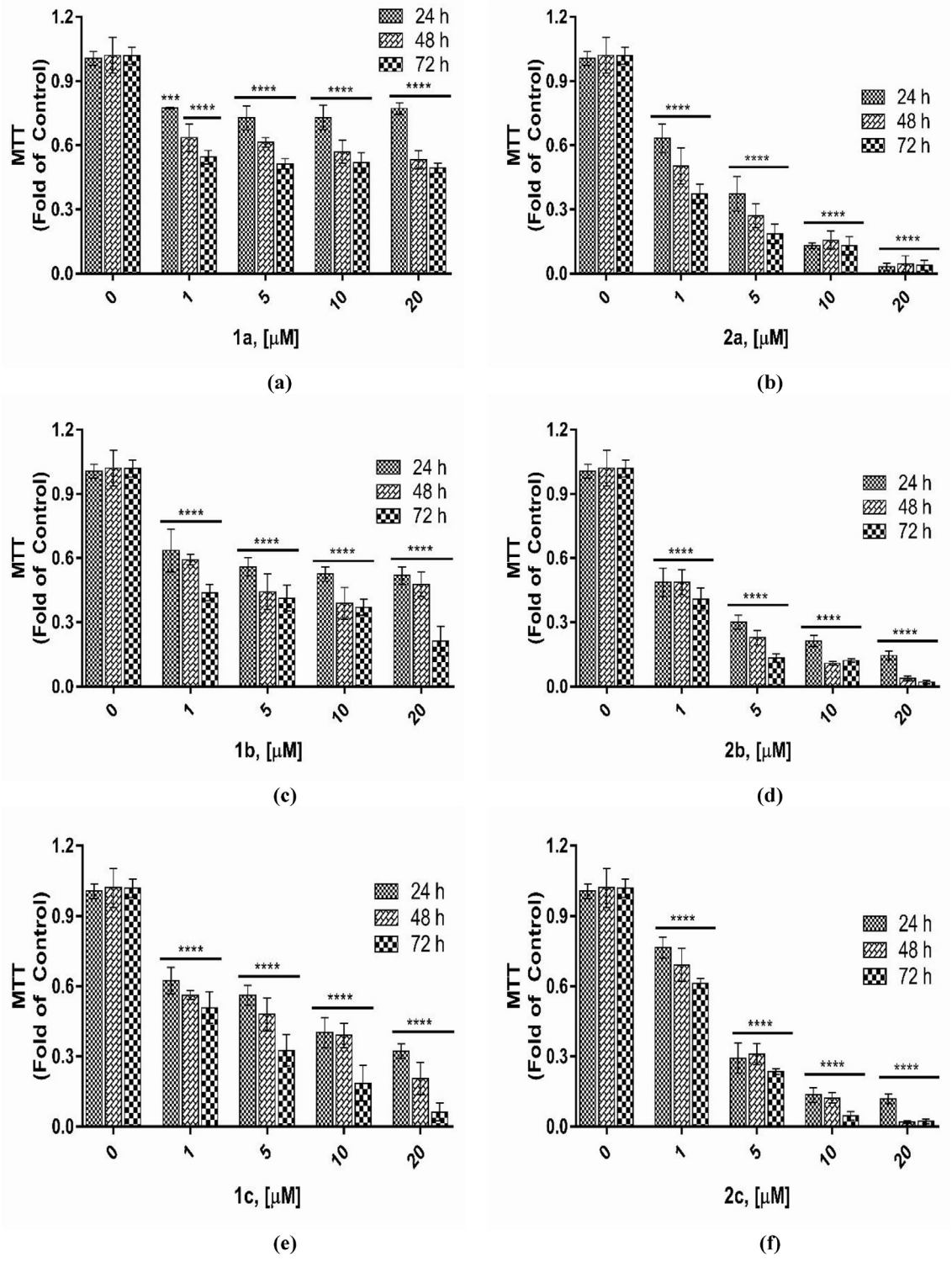


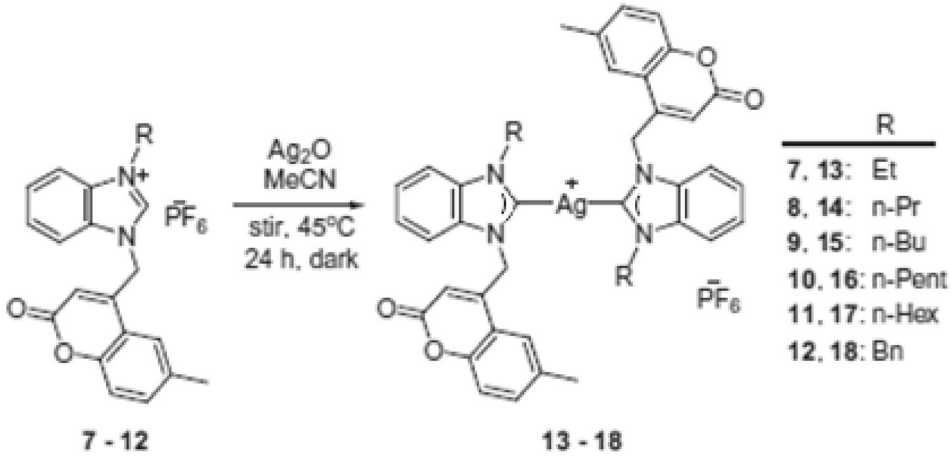

**Fig. 1.** Time-dependent antiproliferative activities of salts **(1a-c)** and complexes **(2a-c)** on MCF-7 breast cancer cells. Data are representative of the mean of three separate experiments and are reported at the ±SEM. (***p < 0.0005 vs control, ****p < 0.0001 vs control).

from 9.8 to 19.4 μM, 8.3 to 17.7 and 7.7−18.3 μM against H1975 and A549 lung cancer cells and Hs68 normal cell line, respectively. Complexes **14, 15, 16** and **18** were found to be cytotoxic against the human lung cancer cell lines A549 and H1975 with the $IC_{50}$ value under 10 μM, while mono−NHC complex **20** had the $IC_{50}$ of 13.7 ± 2.70 and 14.5 ± 1.20 μM against the cancer cell lines H1975 and A549, respectively. The current study results indicated that all complexes have lower $IC_{50}$ values and showed higher antiproliferatife activity than these complexes against MCF-7, MDA-MB-231 and DU-145 cancer cell line at 72 h. Fatima et al. synthesized and evaluated the cytotoxicity of a group of benzimidazole compounds with varied alkyl chains of the nitrogen positions of the ring using the MTT assay on HCT 116 cells [65]. The results showed that increasing the alkyl chain length to 6 carbons or more marked

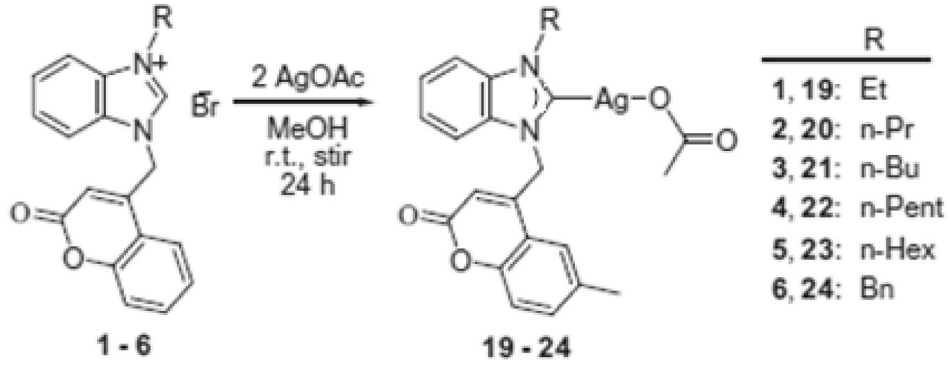
**Table 1**
Antiproliferative activities of salts **(1a-c)** and complexes **(2a-c)** against Prostate Cancer Cells, Breast Cancer Cells, and Normal Cells.

| Cell lines | Time | Salts ($IC_{50}$, μM)[a] | | | Complexes ($IC_{50}$, μM)[a] | | |
|---|---|---|---|---|---|---|---|
| | | 1a | 1b | 1c | 2a | 2b | 2c |
| MCF-7 | 24 h | >20 | >20 | 8.21 ± 0.15 | 5.82 ± 0.15 | <1 | 2.05 ± 0.72 |
| | 48 h | >20 | 4.70 ± 0.42 | 3.67 ± 0.07 | 1.12 ± 0.08 | <1 | 1.21 ± 0.03 |
| | 72 h | 15.8 ± 0.03 | <1 | 1.84 ± 0.08 | <1 | <1 | 1.02 ± 0.05 |
| DU-145 | 24 h | >20 | >20 | 13.5 ± 0.17 | 16.3 ± 0.23 | 6.94 ± 0.15 | 3.90 ± 0.09 |
| | 48 h | 8.72 ± 0.60 | >20 | 9.33 ± 0.20 | 10.4 ± 0.06 | 6.10 ± 0.07 | 2.95 ± 0.19 |
| | 72h | 7.07 ± 0.31 | 5.38 ± 0.25 | 4.43 ± 0.16 | 2.74 ± 0.32 | 2.11 ± 0.05 | 1.74 ± 0.21 |
| MDA-MB-231 | 24 h | >20 | >20 | 2.37 ± 0.11 | <1 | 2.19 ± 0.15 | 1.83 ± 0.03 |
| | 48 h | >20 | >20 | 1.76 ± 0.12 | <1 | 1.60 ± 0.04 | 1.21 ± 0.22 |
| | 72 h | 18.8 ± 0.17 | >20 | 1.31 ± 0.50 | <1 | 1.26 ± 0.02 | <1 |
| L-929[b] | 24 h | >20 | >20 | >20 | 13.0 ± 0.41 | 6.87 ± 0.03 | 2.89 ± 0.04 |
| | 48 h | >20 | >20 | 18.7 ± 0.08 | 11.3 ± 0.21 | 6.70 ± 0.04 | 2.50 ± 0.03 |
| | 72 h | >20 | >20 | 18.3 ± 0.20 | 5.75 ± 0.07 | 4.14 ± 0.05 | 2.01 ± 0.04 |

[a] The $IC_{50}$ value was determined as the concentration causing 50% decrease in cell growth after 24 h, 48 h and 72 h of incubation. The $IC_{50}$ values were calculated from an MTT assay. Values are means ± SEM and represent one of three representative experiments.
[b] Normal Cells.




**Scheme 3.** Synthesis of cationic, bis−NHC coordinated silver(I) hexafluorophosphate complexes, 13−18 [64].




**Scheme 4.** Synthesis of neutral, mono−NHC coordinated silver(I) acetate complexes 19−24 [64].

a significant decrease in $IC_{50}$ value for both the ligand and the Ag(I)-NHC complex. While the $IC_{50}$ values of silver complexes with less than 6 carbons in the alkyl chain were found to be in the range of 13.2 (±1.50) to 26.8 (±2.30), with 6 or more carbons in the alkyl chain, the range was were found to be range of 0.02 (±0.02) to 3.9 (±0.62). It has been previously reported that researchers synthesized, characterized and evaluated the *in vitro* anticancer activity of *para*-xylyl linked bis-benzimidazolium salts and respective dinuclear Ag−NHC complexes against human colorectal cancer (HCT 116) and promyelocytic leukemia (HL-60) cells using the MTT assay [66]. It was observed that all tested compounds showed dose-dependent cytotoxic activity against both cell lines. The $IC_{50}$ values of complexes were found to be in the range of 0.01−18.7 μM for HCT 116 and 0.7−55.7 μM for HL-60 cells. The current study results indicated that complexes have higher cytotoxic activity than these complexes against HL-60 cells but similar activity against HCT 116 cell lines. Lukevics et al. synthesized bromo-salt of 1-(allyl)-3-benzylbenzimidazolium (current study **1a**) and investigated its

*in vitro* cytotoxic activity against HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and 3T3 normal mouse fibroblast cells [55]. The $IC_{50}$ values of bromo salt of 1-(allyl)-3-benzylbenzimidazolium were found to be 21, 45 and 29 μg/mL against HT-1080, MG-22A and B16 cells, respectively. The salt was also found not to have a cytotoxic effect on Neuro 2A cancer cells and 3T3 cells. In the current study, the chloro-salt of 1-(allyl)-3-benzylbenzimidazolium (**1a**) was synthesized and the $IC_{50}$ values of **1a** were found to range from 2.76 to 7.82 μg/mL on MCF-7, DU-145, and MDA-MB-231 cancer cells. These results showed that **1a** did not display any activity toward MDA-MB-231 breast cancer cells and L-929 normal cells even at a concentration of 7.82 μg/mL, clearly indicating that the cytotoxic activity of the salt depends on the type of substituted ion over the benzimidazole ring. It was found that chloro salt was more cytotoxic than bromo-salt against cancer cells. It has been previously reported that researchers synthesized 1c (4b in the paper) and evaluated its tyrosinase inhibitory activity with catechol as substrate [56]. The IC50 value of 4b was found to be 0.52 μM.

To select the most sensitive cancer cell line, further calculations were made of the selectivity index (SI) values for complexes (**2a-c**), and these are shown in Table 2. Studies have shown that compounds with SI values of 5 and above represent more toxicity towards cancer cells compared to normal cells [34,67]. The results of the current study showed that **2a** was the most selectively toxic towards MDA-MB-231 breast cancer cells with SI values > 13 at all-time points. **2a** was also determined to be toxic towards MCF-7 breast cancer cells with SI values of 10.1 and > 5.75 at 48 h and 72 h, respectively. **2b** showed the most selective toxicity towards MCF-7 breast cancer cells with SI values > 6 at 24 h and 48 h. As the SI values of **2c** were <5 at all time points, **2c** did not show selective toxicity on any cell lines.

When the relationship between structure and cytotoxic activity is examined, the structure which differentiates **2a-c** complexes is the R groups, which are attached to the benzimidazole ring. The R groups of **2a-c** complexes are benzyl, naphthyl, and anthracene, respectively. Since the **2c** complex had lower $IC_{50}$ values than the **2a** and **2b** complex on the DU-145 human prostate cancer cell line for 24, 48 and 72 h, the anthracene group in the **2c** complex was found to play an important role in increased antiproliferative activity of the compound on the DU-145 human prostate cancer cell line. However **2c** showed lower antiproliferative activity than **2a** and **2b** on MCF-7 and MDA-MB-231 human breast cancer cells. This suggests that the benzyl and naphthyl groups of **2a** and **2b** are responsible for the antiproliferative activities of these compounds on human breast cancer cells. More importantly, the benzyl-bound **2a** compound exhibits the highest antiproliferative activity against MDA-MB-231 human breast cancer cells, whereas the naphthyl-linked **2b** compound has the highest antiproliferative activity on MCF-7 human breast cancer cells. These results show that the groups that bind to the benzimidazole ring have a very important role both in the activity and selectivity of the compounds. The compounds showed selective behaviour even in different types of subtypes of the same cancer type. While the MCF-7 human breast cancer cell line is positive for estrogen receptor (ER), progesterone receptor (PR), and negative for human epidermal growth factor receptor (HER2), the MDA-MB-231 7 human breast cancer cell line is triple negative for the estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2). All these results indicated that the antiproliferative activities of the compounds depend on both the chemical structure of the compounds and the cell line types.

**Table 2**
Selectivity Index (SI) of complexes **(2a-c)**.

| Cell lines | Time | Complexes ($IC_{50}$, μM)[a] | | |
|---|---|---|---|---|
| | | 2a | 2b | 2c |
| MCF-7 | 24 h | 2.23 | >6.87 | 1.41 |
| | 48 h | 10.1 | >6.70 | 2.06 |
| | 72 h | >5.75 | >4.14 | 1.97 |
| DU-145 | 24 h | <1 | <1 | <1 |
| | 48 h | 1.08 | 1.09 | <1 |
| | 72 h | 2.09 | 1.96 | 1.15 |
| MDA-MB-231 | 24 h | >13 | 3.13 | 1.66 |
| | 48 h | >13 | 4.18 | 1.36 |
| | 72 h | >13 | 3.28 | >2 |

The selectivity index was calculated by dividing the $IC_{50}$ values of complexes in normal cells by the $IC_{50}$ value of the same complex in cancer cells for 24 h, 48 h, and 72 h of incubation. SI values > 5 show good activity.

## 4. Conclusions

In conclusion, new metal *N*-Heterocyclic Carbene (NHC) salts (**1a-c**) and their Ag(I) complexes (**2a-c**) were synthesized. All synthesized compounds were characterized using elemental analysis, FT-IR, $^{1}H$ NMR, and $^{13}C$ NMR spectroscopy techniques. The antiproliferative activity of synthesized benzimidazolium salts **(1a-c)** and their Ag(I)-NHC complexes **(2a-c)** were determined using the MTT assay against MCF-7, DU-145, and MDA-MB-231 cancer cells, as well as L-929 non-cancer cells for 24, 48, and 72 h of exposure. According to the results the antiproliferative activity of the compounds was found to be concentration- and time-dependent except **1a** against L-929 normal cells. These results also indicated that Ag-complexes have more antiproliferative activity than ligands. The most important findings of this study were that MDA-MB-231 and MCF-7 human breast carcinoma cells were the most sensitive, displaying $IC_{50}$ lower than 1 μM at all time points for **2a** and **2b** respectively. Furthermore, the $IC_{50}$s for Ag(I)-NHC were higher in normal cells especially compared to the breast cancer cells, suggesting that complexes possessed noteworthy selectivity for human breast cancer cells. **2a** showed high selectivity (>13-fold) for MDA-MB-231 breast cancer cells. The results of this study also demonstrated that **2b** has 4-7-fold selectivity against MCF-7 breast cancer cells. These results clearly indicate that the $IC_{50}$ concentrations of **2a** and **2b** against MDA-MB-231 and MCF-7 were lower than those of *cis*-platin which is one of the currently available chemotherapeutic agents. Therefore, **2a** and **2b** could be powerful anticancer drug candidates for MDA-MB-231 and MCF-7 breast cancer cells, respectively. The detailed mechanism of the cell apoptosis relationship with anti-apoptotic and pro-apoptotic proteins is currently under further investigation.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2019.126987.

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