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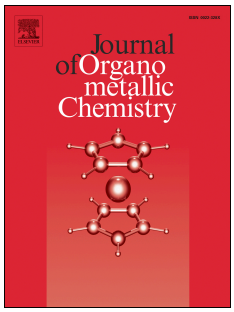

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Novel Silver-NHC Complexes: Synthesis and Anticancer Properties

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Abstract

The aim of this study was to present the synthesis, characterization and anticancer activities of two novel benzimidazole-based NHC salts (**1a-b)** and their silver(I) complexes **(2a-b)** with methylbenzyl and isopropylbenzyl chains. All compounds were prepared using Schlenk techniques in an inert atmosphere. The carbene complexes were prepared with the interaction of carbene precursors and Ag_2O. All new compounds were characterized by elemental analysis, LC-MS, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopic techniques. The anticancer activities of the salts and complexes were determined by cell proliferation analysis using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on human prostate cancer cells (DU-145) and human breast cancer cells (MCF-7, MDA-MB-231). L-929, mouse adipose tissue fibroblasts were used as model normal cells. The cells were plated at a cell density of $1x10^5$ cells in 96-well plates and treated with different concentrations (1-20 µM) of salts and complexes during 24, 48 and 72 hours. The MTT assay results indicated that the salts (**1a-b)** have lower anticancer activity on cancer cells than their silver(I) complexes **(2a-b).** It was also observed that benzimidazole salts (**1a-b)** and their Ag-NHC complexes **(2a-b)** displayed lower anticancer activities on L-929 normal cells than on cancer cells. These results highlight that the anticancer activities of N-heterocyclic carbene-silver complexes vary according to the structure of the silver complex and cell line type.

Keywords: N-Heterocyclic carbene; Benzimidazole-2-ylidene; Silver; Prostate cancer; Breast cancer; Anticancer activity

1. Introduction

Cancer is one of the major causes of death worldwide and deaths due to cancer are increasing. According to global cancer statistics (GLOBOCAN 2018), there will be an estimated 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 [1]. In both

sexes combined, lung cancer is the most commonly diagnosed cancer, followed by breast, prostate, colorectal, stomach and liver cancer. Prostate cancer is the second most commonly occurring cancer in men and there will be approximately 1.3 million new cases in 2018. Breast cancer is the most commonly occurring cancer in women and there will be over 2 million new cases in 2018. Therefore, treatment for cancer is one of the most researched areas.

Cancer treatment varies according to the type of cancer that develops. Different strategies have been developed for treating various types of cancers, including drug treatment, radiotherapy, and surgical treatment. Drug treatment includes hormonal therapy, chemotherapy, and targeted therapy. In chemotherapy, different types of drugs are used to treat cancer patients in combination with surgery and/or radiotherapy [2]. Cis-platin, which was discovered by Rosenberg et al, was the first example of metal-based anticancer drugs [3]. Cis-platin and its analogs are used mostly for the medical treatment of cancer patients [4-6]. However, platinum-based anticancer drug cis-platin has several side effects, such as vomiting, nephrotoxicity, hair loss, neurotoxicity, diarrhea, ototoxicity and the development of intrinsic and acquired resistance in some cancer cells, and these are considered to be serious health problems [7]. These side-effects may result in the patient being reluctant to progress with treatment [8]. Therefore, it is important to design and synthesize transition metal-containing new anti-cancer agents that are chemically different from those currently available, and which have a broad spectrum and low cytotoxicity.

N-Heterocyclic Carbenes (NHCs) have become a well-known class of organometallic ligands. Recently, several metal N-heterocyclic carbene (M-NHC) complexes have been widely investigated in medicinal chemistry due to their noteworthy biological properties [9-18]. In biological systems, the selection of metals is also an important issue. Silver salts have historically been used for the purification of drinking water, as antimicrobial agents, for prevention of eye infections in newborns, and in wound healing [19-20]. The low toxicity of silver salts for humans has also attracted researchers to further investigate their *in vitro* and *in vivo* biological properties, including antimicrobial and anticancer features [21-25]. A number of new silver(I)-NHC complexes were synthesized and screened against several carcinoma cell lines after the first report of cytotoxic activities of silver(I)-NHC complex on breast cancer (MCF-7), cervical cancer (HeLa), and colon adenocarcinoma (HCT 116) [26-32]. Although the imidazole structure is very frequently used in organometallic chemistry, benzimidazole derivatives have been rarely used, especially for biological applications [33-

34]. In organic chemistry, a number of organic derivatives of benzimidazole have shown important therapeutic activities including anti-cancer activity [35-36]. The structural similarity of benzimidazole derivatives with naturally occurring nucleotides makes them biologically important structures [35]. For those reasons, benzimidazole and silver were used to synthesize organometallic compounds in this study.

The aim of this study was to design two novel NHC salts and their Ag(I) complexes (Scheme 1) and investigate their anticancer activities. All salts and complex structures were characterized by elemental analysis, LC-MS, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy. The anticancer activities of the compounds were examined on DU-145 (HTB-81, human prostate carcinoma), MCF-7 (HTB-22, human breast adenocarcinoma), MDA-MB-231 (HTB-26, human breast adenocarcinoma) cell lines with the MTT assay. L-929, mouse adipose tissue fibroblast cells were used as model normal cells.

2. Experimental

2.1. Materials and Methods

The synthesis of benzimidazolium salts and Ag(I)-NHC complexes were carried out 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. 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 (FTIR) spectra were obtained in the range 400–4000 cm^{-1} on Perkin Elmer Spectrum 100 FT-IR. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance III spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. ^{1}H peaks are labeled as singlet (s), doublet (d), multiplet (m) and heptet (hept.). Chemical shifts and coupling constants are reported in ppm and in Hz, respectively.

2.2. General procedure for the preparation of benzimidazolium salts, (1a-b)

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. 2-methyl-2-propenyl chloride (10.1 mmol) was added dropwise to the obtained solution and heated for 24 h at 60 ^{0}C. Then, the solvent was removed under the vacuum. Dichloromethane (50 mL) was added to solid. The mixture was filtered and the obtained clear solution was concentrated under vacuum. The last solution was distilled and 1-(2-methyl-2-propenyl) benzimidazole was obtained. The 1-(2-

methyl-2-propenyl) benzimidazole (1 mmol) and alkyl halide (1 mmol) were stirred in DMF (5 mL) for 24 h at 80 ^{0}C. White product was collapsed. After the solution was filtered, solid was rinsed out with diethylether and dried under vacuum. The crude product was recrystallized from dichloromethane/diethylether [37].

2.2.1. 1-(2-Methyl-2-propenyl)-3-(4-methylbenzyl)benzimidazolium chloride, 1a

Yield: 82%, m.p. 195-197 °C. FT-IR $\nu_{(CN)}$: 1551 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.72 (s, 3H, $NCH_2C(CH_3)CH_2$), 2.23 (s, 3H, $CH_2C_6H_4(CH_3)$-4), 4.90 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.04 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.21 (s, 2H, $NCH_2C(CH_3)CH_2$), 5.78 (s, 2H, $CH_2C_6H_4(CH_3)$-4), 7.09 (d, 2H, Ar-*H*, *J* = 8 Hz), 7.31 (d, 2H, Ar-*H*, *J* = 8 Hz), 7.45-7.51, 7.53-7.55 and 7.59-7.61 (m, 4H, Ar-*H*), 11.74 (s, 1H, NC*H*N). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 19.7 ($NCH_2C(CH_3)CH_2$), 21.2 ($CH_2C_6H_4(CH_3)$-4), 51.4 ($CH_2C_6H_4(CH_3)$-4), 53.6 ($NCH_2C(CH_3)CH_2$), 113.7 ($NCH_2C(CH_3)CH_2$), 139.2 ($NCH_2C(CH_3)CH_2$), 113.9, 116.1, 127.1, 128.2, 128.3, 129.8, 130.0, 131.2, 131.7 and 137.5 (Ar-*C*), 144.0 (N*C*HN). % Anal. Calcd for $C_{19}H_{21}N_2Cl$: C, 72.95; H, 6.77; N: 8.95. Found: C, 72.83; H, 6.68; N, 8.87.

2.2.2. 1-(2-Methyl-2-propenyl)-3-(4-isopropylbenzyl)benzimidazolium chloride, 1b

Yield: 91%, m.p. 158-160 °C. FT-IR $\nu_{(CN)}$: 1558 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.20 (d, 6H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 1.79 (s, 3H, $NCH_2C(CH_3)CH_2$), 2.87 (hept., 1H, $CH_2C_6H_4(CH(CH_3)_2)$-4, *J* = 8 Hz), 4.98 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.12 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.30 (s, 2H, $NCH_2C(CH_3)CH_2$), 5.87 (s, 2H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 7.22 (d, 2H, Ar-*H*, *J* = 8 Hz), 7.43 (d, 2H, Ar-*H*, *J* = 8 Hz), 7.54-7.57, 7.64-7.70 (m, 4H, Ar-*H*), 11.78 (s, 1H, NC*H*N). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 19.8 ($NCH_2C(CH_3)CH_2$), 23.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 33.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 51.3 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 53.6 ($NCH_2C(CH_3)CH_2$), 113.7 ($NCH_2C(CH_3)CH_2$), 137.5 ($NCH_2C(CH_3)CH_2$), 113.8, 116.1, 127.1, 127.4, 128.3, 128.4, 130.2, 131.3, 131.7 and 150.1 (Ar-*C*), 144.0 (N*C*HN). % Anal. Calcd for $C_{21}H_{25}N_2Cl$: C, 73.99; H, 7.39; N: 8.22. Found: C, 73.86; H, 7.26; N, 8.17.

2.3. General procedure for preparation of Ag(I)-NHC complexes, (2a-b)

A solution of 1 mmol of Ag_2O and 0.5 mmol of benzimidazolium salts **(1a-b)** in dichloromethane (25 mL) were stirred at room temperature for 24 h under the dark condition. After that, the mixture was filtered through celite. The clear filtrate was evaporated under vacuum to afford the crude product. The crude product was recrystallized from dichloromethane/diethylether.

2.3.1.

Chloro[1-(2-Methyl-2-propenyl)-3-(4-methylbenzyl)benzimidazole-2-ylidene]silver(I), 2a

Yield: 83%, m.p. 198-200 °C. FT-IR $\nu_{(CN)}$: 1391 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.67 (s, 3H, $NCH_2C(CH_3)CH_2$), 2.23 (s, 3H, $CH_2C_6H_4(CH_3)$-4), 4.76 (s, 1H, $NCH_2C(CH_3)CH_2$), 4.92 (s, 2H, $NCH_2C(CH_3)CH_2$), 4.96 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.51 (s, 2H, $CH_2C_6H_4(CH_3)$-4), 7.04-7.11, 7.23-7.30 and 7.37-7.39 (m, 8H, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.1 ($NCH_2C(CH_3)CH_2$), 21.2 ($CH_2C_6H_4(CH_3)$-4), 53.4 ($CH_2C_6H_4(CH_3)$-4), 55.6 ($NCH_2C(CH_3)CH_2$), 112.1 ($NCH_2C(CH_3)CH_2$), 139.1 ($NCH_2C(CH_3)CH_2$), 112.2, 114.6, 124.3, 127.1, 127.2, 129.8, 131.8, 133.7, 134.1 and 138.4 (Ar-*C*), 189.1 ($C_{carbene}$-Ag). % Anal. Calcd for $C_{19}H_{20}N_2ClAg$: C, 54.37; H, 4.80; N: 6.67. Found: C, 54.22; H, 4.71; N, 6.54; LC–MS: 659.3 $[AgL_2]^+$.

2.3.2.

Chloro[1-(2-Methyl-2-propenyl)-3-(4-isopropylbenzyl)benzimidazole-2-ylidene]silver(I), 2b

Yield: 90%, m.p. 163-165 °C. FT-IR $\nu_{(CN)}$: 1387 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.22 (d, 6H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 1.75 (s, 3H, $NCH_2C(CH_3)CH_2$), 2.87 (hept., 1H, $CH_2C_6H_4(CH(CH_3)_2)$-4, *J* = 8 Hz), 4.84 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.00 (s, 2H, $NCH_2C(CH_3)CH_2$), 5.04 (s, 1H, $NCH_2C(CH_3)CH_2$), 5.59 (s, 2H, $CH_2C_6H_4(CH(CH_3)_2)$-4), 7.16-7.22 and 7.30-7.40 (m, 7H, Ar-*H*), 7.46 (d, 1H, Ar-*H*, *J* = 8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.1 ($NCH_2C(CH_3)CH_2$), 23.9 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 33.8 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 53.3 ($CH_2C_6H_4(CH(CH_3)_2)$-4), 55.6 ($NCH_2C(CH_3)CH_2$), 112.0 ($NCH_2C(CH_3)CH_2$), 139.1 ($NCH_2C(CH_3)CH_2$), 112.2, 114.6, 116.7, 120.2, 124.3, 127.1, 127.2, 132.1, 133.7, 134.0 and 149.3 (Ar-*C*), no peak ($C_{carbene}$-Ag). % Anal. Calcd for $C_{21}H_{24}N_2ClAg$: C, 56.33; H, 5.40; N: 6.26. Found: C, 56.21; H, 5.32; N, 6.17; LC–MS: 715.4 $[AgL_2]^+$.

2.4. In-vitro anticancer studies

2.4.1. Cell culture;

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), 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. L-929 (normal cells mouse adipose tissue fibroblast) were purchased from ECACC (European Collection of Animal Cell Culture, Salisbury, U.K.). All absorbance values were measured with a microplate reader (BioTek, Epoch, USA) at 570 nm.

2.4.2. Anticancer properties, (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 37ºC humidified air condition. Anticancer activity of the salts **(1a-b)** and complexes **(2a-b)** was evaluated using the MTT assay [38]. L-929, mouse adipose tissue fibroblast was used as model normal cells to determine the anticancer activity of salts and complexes against normal cells. DU-145, MCF-7, MDA-MB-23, and L-929 cells were seeded in 96-well plate 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 four different concentrations (1, 5, 10 and 20 µM) of salts and complexes, for 24 h, 48 h and 72 h. 0.5% dimethyl sulfoxide in media (DMSO) and nutrient media without cells were used were used as negative control and a blank, respectively. After 24 h, 48 h, and 72 h treatment 10 mL of MTT solution (5 mg/mL in PBS, pH 7.2) was added to each well and samples were further incubated for 2 h at 37 °C in 5% CO_2. The medium was aspirated and added 100 µL/well DMSO to dissolve the formazan crystals. Absorbance values were measured with a microplate reader (Epoch, USA) at 570nm. Cytotoxicity curves and IC_{50} (µM) concentrations (defined as a concentration of drug that decreases the cell viability by 50% compared to non-treated control cells), were fitted by GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Selectivity index was calculated by comparing the IC_{50} values of the test sample in normal and cancer cell lines.

2.5. Statistical analysis

Each experiment was repeated at least three separate experiment and three replicates (n=9). All data expressed as mean ± 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).

3. Result and Discussion

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

Nitrogen-containing heterocyclic salts have attracted attention in the fields of coordination chemistry due to their metal complexes show high reactivity as catalyst and biologically agents. Benzimidazolium salts (**1a-b**) were synthesized by reaction of 2-methyl-2-propenyl substituted benzimidazolium precursor with different alkyl halides as shown Scheme 1.

Scheme 1.

Ag(I)-NHC complexes (**2a-b**) were obtained from reacting Ag_2O with corresponding benzimidazolium salts (**1a-b**) in dichloromethane at room temperature under dark condition. Synthesis of the Ag(I)-NHC complexes (**2a-b**) is summarized in Scheme 2. Benzimidazolium salts (**1a-b**) and Ag(I)-NHC complexes (**2a-b)** were characterized by elemental analysis, LC-MS, FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy (Figure S1-S10). Ag(I)-NHC complexes (**1a-b**) are air and moisture stable in the solid state but unstable to light and soluble in dimethylformamide, dimethylsulfoxide and halogenated solvents such as chloroform, dichloromethane but insoluble in petroleum ether, diethyl ether. NMR spectra of all the compounds were analyzed in d-$CDCl_3$.

Scheme 2.

The benzimidazolium salts (**1a-b**) have an acidic NC*H*N proton which came to 11.74 and 11.78 ppm as a characteristic sharp singlet for **1a** and **1b** respectively, in the ^{1}H NMR spectrum. When benzimidazolium salts form Ag(I)-NHC complexes, they lose an acidic proton. Therefore, in the ^{1}H NMR spectrum Ag(I)-NHC complexes (**1a-b**), the disappearance of an acidic proton is evidence of formation complex. In the ^{13}C NMR spectra of **1a-b,** the characteristic signals of carbon (N*C*HN) were seen as singlets at 144.0 ppm for both benzimidazolium salts. For complex **2a,** a signal of carbon (N*C*N) was seen to have shifted greatly downfield region compared to the corresponding benzimidazolium salt **1a** and was observed at 189.1 ppm as slight. No signal of carbon (N*C*N) of complex **2b** was observed. These values and the lack of the carben peak are in agreement with reported data for similar Ag(I)-NHC complexes [45]. At the same time, formation of the Ag(I)-NHC complexes (**2a-b**) was proven by IR spectra, which showed *CN* bond vibrations at 1391 and 1387, respectively, and these vibrations for benzimidazolium salts (**1a-b**) were 1551 and 1558 cm^{-1}, respectively. However LC–MS spectrometry can be used in order to clarify the structures of silver(I)–NHC complexes in the solution in the absence of crystallographic data. LC–MS spectrometry of the

prepared complexes showed that maximal peak intensities for each complex were attributable to $[Ag(NHC)_2]^+$ as the molecular ion, which is not uncommon for these types of complexes.

3.2. *In vitro* anticancer properties of benzimidazolium salts and Ag(I)-NHC complexes

The anticancer activities of novel benzimidazole-based NHC salts (**1a-b**) and their silver(I) complexes (**2a-b**) in prostate cancer DU-145 cells, breast cancer MCF-7, MDA-MB-231 cells, and normal fibroblasts L-929 cells were investigated after exposing the cells to the compounds for 24, 48 and 72 hours, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) cell viability assay [46]. The percentage inhibition of cell proliferation was determined at different concentrations of the test compounds (**1a-b**) and (**2a-b**) ranging from 1 to 20 µM (Figure 1-4).

Figure 1-4

The anticancer efficacy parameters, in terms of IC_{50} values after 24 h, 48 h and 72 h of incubation time, are demonstrated in Table 1. Tests were made to determine whether the salts by themselves could exert any anticancer activity on cancer cells. The MTT assay results indicated that the salts had an inhibitory effect on cancer cells in a time- and dose-dependent manner except for **1a** on DU-145 cell line. **1a** had no anticancer effects on DU-145 cells at all time points, on MCF-7 cells at 24 h and on MDA-MB-231 cells at 24 and 48 hours, at least at doses equal to 20 µM.

Table 1

The benzimidazole salts (**1a-b)** displayed the following IC_{50} values against the DU-145, MCF-7 and MDA-MB-231 cell lines; >20, 12.9 ± 0.28, >20, 5.83 ± 0.14, 9.91 ± 0.11, 8.56 ± 0.18 µM respectively for 48 h. As **1b** was determined with lower IC_{50} values than **1a** for cancer cells, the anticancer activity of **1b** was higher than that of **1a.** All the complexes showed higher anticancer activity compared to their benzimidazolium salts. Similarly, IC_{50} values demonstrated that the anticancer activity of **2a** was lower than that of **2b.** This feature was attributed to the different chemical properties (such as lipophilicities, steric and electronic) of the ligands around the benzimidazole nucleus. The differences in these ligands enable various inhibition activities in the complexes. Isopropyl substituted complex, **2b**, was more active than methyl substituted complex, **2a.** The isopropyl group is bulkier and could interfere with the uptake mechanism across cell or organelle membranes. The Ag-NHC complexes **(2a-b)** displayed the following IC_{50} values against the DU-145, MCF-7 and MDA-

MB-231 cell lines; 4.04 ± 0.11, 8.25 ± 0.10, 8.06 ± 0.21, 2.08 ± 0.11, 1.20 ± 0.19, 1.10 ± 0.17 µM respectively for 48 h. Increasing doses of the salts and complexes were also tested on normal cells, L-929. The MTT assay results indicated that the salts displayed no dose- and time-dependent anticancer activity, even when tested at various concentrations and times on L-929 cells. The IC_{50} values of the benzimidazole salts (**1a-b)** and their Ag-NHC complexes **(2a-b)** on L-929 normal cells were determined; >20, >20, 14.0 ± 0.30, 3.81 ± 0.22 and >20, >20, 6.10 ± 0.10, 2. 80 ± 0.23µM respectively, at 48 h. As the complexes (**2a-b**) had larger IC_{50} values for normal cell lines, these compounds are more effective toward breast cancer cells and prostate cancer cells. Calculated selectivity index values for salts (**1a-b**) were 1, 1.55, 1, 3.43, 2.08, 2.33, and for Ag-NHC complexes **(2a-b**), they were 3.46, 1.70, 1.73, 1.83, 3.18, 3.46 on DU-145, MCF-7 and MDA-MB-231 cell lines respectively, for 48 h.

While the majority of organometallic pharmaceutical research has been focused on platinum and gold, NHC-silver complexes have become an important class of anticancer agents in medicinal applications. Tacke et al. synthesized 6 different cyanobenzyl-NHC-silver complexes and determined their antibacterial and anticancer activity [13]. All 6 NHC–silver complexes were tested to determine their cytotoxic activity on human renal-cancer cell line Caki-1 using MTT *in vitro* tests. The IC_{50} values of NHC-silver complexes 4a–f were found to be 6.2 (±1.0), 7.7 (±1.6), 1.2 (±0.6), 10.8 (±1.9), 24.2 (±1.8) and 13.6 (±1.0) µM, respectively. From that study, compound **4c** was determined to be approximately three-times more cytotoxic (1.2 ±0.6 µM) than cisplatin (3.3 µM). The current study results indicated that compound **2a** showed similar activity as **4c** against DU-145 and as compound **2b** showed against MCF-7 and MDA-MB-231 cell lines. It has been previously reported that researchers synthesized seven novel non-symmetrically p-Benzyl-Substituted imidazole/benzimidazole NHC- Silver(I) Acetate Complexes and evaluated their *in vitro* biological activities [14]. Cytotoxic activities of the seven complexes were determined against human renal cancer cell line Caki-1. The IC_{50} values, of complexes were found to be 25 (±1), 15 (±2), 5.4 (±0.8), 16 (±2), 7.1 (±1), 20 (±4), and 14 (±1) µM, respectively. The complex **2c**, gave the smallest IC_{50} value of 5.4 (±0.8) µM indicating the highest anticancer activity. Tacke et al, also tested *in vitro* and *in vivo* cytotoxic activity of the anticancer drug candidate (1-methyl-3-(p-cyanobenzyl)benzimidazole-2-ylidene)silver(I)acetate (SBC1) against UKF-NB-3 and UKF-NB-6 (neuroblastoma) HCT8 (colon), and paclitaxel-resistant cell line PC-3 (prostate) [24]. They found that the compound was effective *in vitro* against platinum-resistant cell lines human neuroblastoma cells, UKF-NB-3, human colon carcinoma cell line HCT8 as well as paclitaxel-resistant cell line human prostate cancer cell line PC-3. From the first *in vivo*

experiments using non-tumor bearing mice, the toxicity of SBC1 was also demonstrated by Tacke et al, [24]. They first determined the MTD value. For this purpose, in three groups of two mice, each was treated with single doses of 25, 50 and 100 mg/kg/d of SBC1. The first two groups of mice showed a body weight loss of 1 or 2%, respectively, while the value extended to 5% in the group receiving 100 mg/kg/d of SBC1. The highest dose led to one toxic death within one day after treatment, while the other mice recovered and the body weight loss was found to be reversible. After these results, SBC1 at 25 and 50 mg/kg/d, in four injections were administered to two cohorts of eight CAKI-1 tumor-bearing NMRI:nu/nu mice, while a further cohort was treated with solvent only. It was observed that these two dosages of SBC1 showed borderline toxicity leading to mortality and body weight loss, while there was no significant tumor growth reduction or influence on blood parameters compared to the solvent-treated control group. It was concluded that SBC1 is a potent cytotoxic and resistance-breaking anticancer agent, which shows very little selectivity *in vivo*. Youngs et al were the first to report the anticancer activity of Ag(I)-NHC compounds with a series of 4,5-dicholorimidazole based Ag(I)-NHC compounds [25]. These were evaluated for their *in vitro* antitumor activity against the cancer cell lines OVCAR-3 (ovarian), MB157 (breast), and HeLa (cervical). The silver compounds were determined to be the most active against MB157 but compounds **6**–**8** were ineffective against the HeLa cell line (>200 μM). They were also tested in an *in vivo* xenograft model developed utilizing OVCAR-3 and **6**. According to the pathological studies, significant tumor cell death was observed while no ill-effects to the major organs of the mice were detected. According to the results of the Tacke and Youngsstudies, the structure of the compound affects the *in vivo* cytotoxic activity of the compound.

4. Conclusions

In this study, two new benzimidazolium salts and their Ag(I)-NHC complexes were synthesized, characterized by elemental analysis, LC-MS, FT-IR, ^{1}H NMR, and ^{13}C NMR spectroscopy and anticancer activities were evaluated. The *in vitro* anticancer activities of novel benzimidazole-based NHC salts (**1a-b**) and their silver(I) complexes (**2a-b**) against DU-145 prostate cancer cells, MCF-7, MDA-MB-231breast cancer cells, and L-929 normal fibroblasts cells were demonstrated after exposing the cells to the compounds for 24, 48 and 72 hours, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) cell viability assay. L-929, mouse adipose tissue fibroblasts were used as model normal cells. The MTT assay results showed that the compounds had an inhibitory effect on cancer cells in a

time- and dose-dependent manner. The results also indicated that the salts (**1a-b)** have lower anticancer activity on cancer cells than their silver(I) complexes **(2a-b).** The benzimidazole salts (**1a-b)** and their Ag-NHC complexes **(2a-b)** were seen to display lower anticancer activities on L-929 normal cells than cancer cells. The isopropyl substituted complex, **2b**, was more active than the methyl substituted complex, **2a.** These results showed that the *in vitro and in vivo* anticancer activities of N-heterocyclic carbene-silver complexes vary according to the structure of the silver complex and cell line type.

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References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018, 68(6):394-424.

[2] Waxman J, Cancer Treatment Handbook, Springer-Verlag, London, 2013; (b) T. Priestman, Cancer Chemotherapy in Clinical Practice, Springer-Verlag, London, 2012.

[3] Rosenberg B, Vancamp L, Krigas T: Inhibition of cell division in escherichia coli by electrolysis products from a platinum electrode. Nature 1965, 205:698-699.

[4] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur. J. Pharmacol. 2014, 740:364-378.

[5] Mehmood RK. Review of Cisplatin and oxaliplatin in current immunogenic and monoclonal antibody treatments. Onco Rev. 2014, 8(2):256.

[6] Ho GY, Woodward N, Coward JIG. Cisplatin versus carboplatin: comparative review of therapeutic management in solid malignancies. Crit. Rev. Oncol.-Hematol. 2016, 102:37-46.

[7] McWhinney SR, Goldberg RM, McLeod HL. Platinum neurotoxicity pharmacogenetics. Mol Cancer Ther. 2009, 8(1):10-16.

[8] Wheate NJ, Walker S, Craig GE, Oun R. The status of platinum anticancer drugs in the clinic and in clinical trials. Dalton Trans. 2010, 39(35):8113-8127.

[9] Boselli L, Carraz M, Mazeres S, Paloque L, Gonzalez G, Benoit-Vical F, Valentin A, Hemmert C, Gornitzka H. Synthesis, structures, and biological studies of heterobimetallic Au (I)-Ru (II) complexes involving n-heterocyclic carbene-based Multidentate ligands. Organometallics, 2015, 34(6):1046-1055.

[10] Haque RA, Choo SY, Budagumpi S, Abdullah AAA, Ahamed MBK, Majid AMA. Synthesis, crystal structures, characterization and biological studies of nitrile-functionalized silver (I) N-heterocyclic carbene complexes. Inorganica Chim. Acta 2015, 433:35-44.

[11] Garner ME, Niu W, Chen X, Ghiviriga I, Abboud KA, Tan W, Veigne, AS. N-heterocyclic carbene gold (I) and silver (I) complexes bearing functional groups for bio-conjugation. Dalton Trans. 2015, 44(4):1914-1923.

[12] Dahm G, Bailly C, Karmazin L, Bellemin-Laponnaz S. Synthesis, structural characterization and in vitro anti-cancer activity of functionalized N-heterocyclic carbene platinum and palladium complexes. J. Organomet. Chem. 2015, 794:115-124.

[13] Patil S, Deally A, Gleeson B, Müller-Bunz H, Paradisi F, Tacke M. Novel Benzyl-Substituted N-Heterocyclic Carbene-Silver Acetate Complexes: Synthesis, Cytotoxicity and Antibacterial Studies. Metallomics, 2011, 3:74-88.

[14] Hackenberg F, Deally A, Lally G, Malenke S, Müller-Bunz H, Paradisi F, Patil S, Quaglia D, Tacke M. Novel non-symmetrically p-benzyl-substituted (benz)imidazole N-heterocyclic carbene-silver(I) acetate complexes-synthesis and biological evaluation Int. J. Inorg. Chem, 2012, Article ID 121540, 13 pages. http://dx.doi.org/10.1155/2012/121540

[15] Napoli M, Saturnino C, Sirignano E, Popolo A, Pinto A, Longo P. Synthesis, characterization and cytotoxicity studies of methoxy alkyl substituted metallocenes. Eur. J. Med. Chem. 2011, 46(1):122-128

[16] Grisi F, Costabile C, Grimaldi A, Viscardi C, Saturnino C, Longo P. Synthesis of unsaturated macrocycles by Rucatalyzed ring-closing metathesis: a comparative study. Eur. J. Org. Chem. 2012, 30:5928-5934.

[17] Chimento A, Saturnino C, Iacopetta D et al. Inhibition of human topoisomerase I and II and anti-proliferative effects on MCF-7 cells by new titanocene complexes. Bioorg. Med. Chem. 2015, 23(22):7302-7312.

[18] Teyssot M-L, Jarrousse A-S, Manin M, Chevry A, Roche S, Norre F, Beaudoin C, Morel L, Boyer D, Mahiou R, Gautier A. Metal-NHC complexes: a survey of anti-cancer properties. Dalton Trans 2009, 35:6894-6902.

[19] Graham C: The role of silver in wound healing. Br J Nutr 2005, 14(19):22-26.

[20] Garrison JC, Youngs WJ. Ag(I) N-heterocyclic carbene complexes: synthesis, structure, and application. Chem Rev 2005, 105(11):3978-4008.

[21] Gautier A, Cisnetti F. Advances in metal–carbene complexes as potent anti-cancer agents. Metallomics 2012, 4(1):23-32.

[22] Melaiye A, Simons RS, Milsted A, Pingitore F, Wesdemiotis C, Tessier CA, Youngs WJ. Formation of water-soluble pincer silver(I)−carbene complexes: a novel antimicrobial agent. J Med Chem 2004, 47(4):973-977.

[23] Austin LA, Kang B, Yen CW, El-Sayed MA. Plasmonic imaging of human oral cancer cell communities during programmed cell death by nucleartargeting silver nanoparticles. J Am Chem Soc 2011, 133(44):17594-17597.

[24] Fichtner I, Behrens D, Cinatl J, Michaelis M, Sanders LC, Hilger R, Kennedy BN, Reynolds A L, Hackenberg F, Lally G, Quinn SJ, McRae I, Tacke M. In Vitro and In Vivo Investigations into the Carbene Silver Acetate Anticancer Drug Candidate SBC1 Letters in Drug Design & Discovery, 2012, 9:815 - 822.

[25] Medvetz DA, Hindi K.M, Panzner MJ, Ditto AJ, Yun YH, Youngs, WJ. Anticancer Activity of Ag(I) N-Heterocyclic Carbene Complexes Derived from 4,5-Dichloro-1H-Imidazole. Met. Based Drugs 2008, 2008:1–7.

[26] Patil SA, Patil SA, Patil R, Keri RS, Budagumpi S, Balakrishna GR, Tacke M. N-heterocyclic carbene metal complexes as bio-organometallic antimicrobial and anticancer drugs. Future Med. Chem. 2015, 7(10):1305-1333.

[27] Hu XL, Castro-Rodriguez I, Olsen K, Meyer K. Group 11 metal complexes of N-heterocyclic carbene ligands: natüre of the metal-carbene bond. Organometallics 2004, 23(4):755-764.

[28] Parisi OI, Morelli C, Puoci F et al. Magnetic molecularly imprinted polymers (MMIPs) for carbazole derivative release in targeted cancer therapy. J. Mater. Chem. B 2014, 2(38):6619-6625.

[29] Bruno G, Nicolo F, Loschiavo S, Sinicropi MS, Tresoldi G. Synthesis and spectroscopic properties of Di-2-Pyridyl Sulfide (Dps) compounds - crystal-structure of [Ru(Dps)2cl2]. J. Chem. Soc. Dalton Trans. Rans. 1995, 1:17-24.

[30] Klasen HJ. A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns 2000, 26(2):131-138.

[31] Hindi KM, Siciliano TJ, Durmus S et al. Synthesis, stability, and antimicrobial studies of electronically tuned silver acetate N-heterocyclic carbenes. J. Med. Chem. 2008, 51(6):1577-1583.

[32] Youngs WJ, Knapp AR, Wagers PO, Tessier CA. Nanoparticle encapsulated silver carbene complexes and their antimicrobial and anticancer properties: a perspective. Dalton Trans.2012, 41(2):327-336.

[33] Schrock RR, Fellmann JD. Multiple metal-carbon bonds. 8. Preparation, characterization, and mechanism of formation of the tantalum and niobium neopentylidene complexes, M(CH2CMe3)3(CHCMe3). J. Am. Chem. Soc. 1978, 100(11):3359-3370.

[34] Arduengo AJ Ill, Harlow RL, Kline M. A stable crystalline carbene. J. Am. Chem. Soc. 1991, 113:361-363.

[35] Cavallo L, Correa A, Costabile C, Jacobsen H. Steric and electronic effects in the bonding of N-heterocyclic ligands to transition metals. J. Organomet. Chem. 2005, 690(24):5407-5413.

[36] Hu XL, Castro-Rodriguez I, Olsen K, Meyer K. Group 11 metal complexes of N-heterocyclic carbene ligands. natüre of the metal-carbene bond. Organometallics 2004, 23(4):755-764.

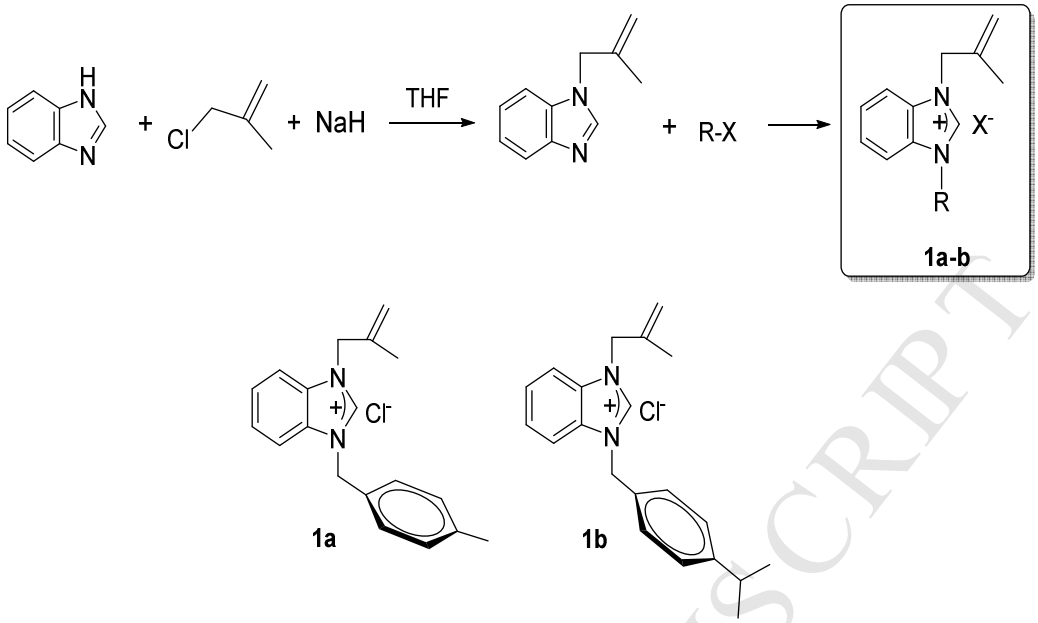
[37] Özdemir İ, Demir S, Günal S, Özdemir İ, Arıcı C, Ülkü D. Synthesis, characterization and antimicrobial activity of new silver complexes with N-heterocyclic carbene salts. Inorganica Chimica Acta, 2010, 363(14):3803-3808**.**

[38] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst. 1990, 82(13):1107-1112.

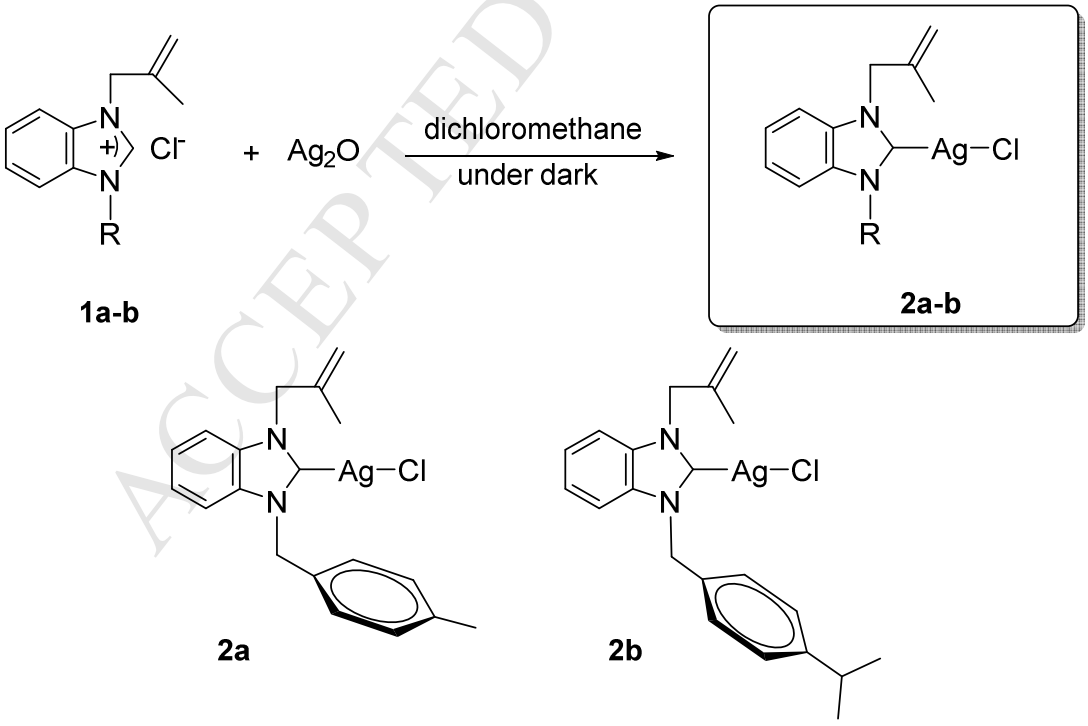
Table 1. IC_{50} (μM) values of salts **(1a-d)** and complexes (**2a-d**) against the selected human cancer cells and normal cells[a] *in vitro* after 24 h, 48 h and 72 h of incubation

Cell lines	Time	Ligands (IC_{50}, μM)[b]		Complexes (IC_{50}, μM)[b]	
		1a	**1b**	**2a**	**2b**
DU-145	24h	>20	19.2 ± 0.2	20.0 ± 0.8	3.0 ±0.1
	48 h	>20	5.8 ± 0.1	4.0 ± 0.1	2.1 ± 0.1
	72h	>20	3.2 ± 0.2	1.2 ± 0.4	< 1
MCF-7	24h	>20	13.9 ± 0.2	9.6 ± 0.1	1.8 ± 0.2
	48 h	12.9 ± 0.2	9.9 ± 0.1	8.2 ± 0.1	1.2 ± 0.2
	72h	4.1 ± 0.2	< 1	< 1	< 1
MDA-MB-231	24h	>20	12.9 ± 0.2	10.4 ± 0.2	1.8 ± 0.7
	48 h	>20	8.5 ± 0.2	8.1 ± 0.2	1.1 ± 0.2
	72h	7.5 ± 0.2	3.8 ± 0.2	2.2 ± 0.2	< 1
L-929[a]	24h	>20	>20	17.9 ± 0.5	5.9 ± 0.1
	48 h	>20	>20	14.0 ± 0.3	3.8 ± 0.2
	72h	>20	>20	6.1 ± 0.1	2.8 ± 0.2

[a] Normal cells,.[b]Cell viability after treatment for 24 h, 48 h and 72 h were determined by MTT staining as described in the Experimental section. Each IC_{50} value represents the mean ± SEM of three independent experiments.



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



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

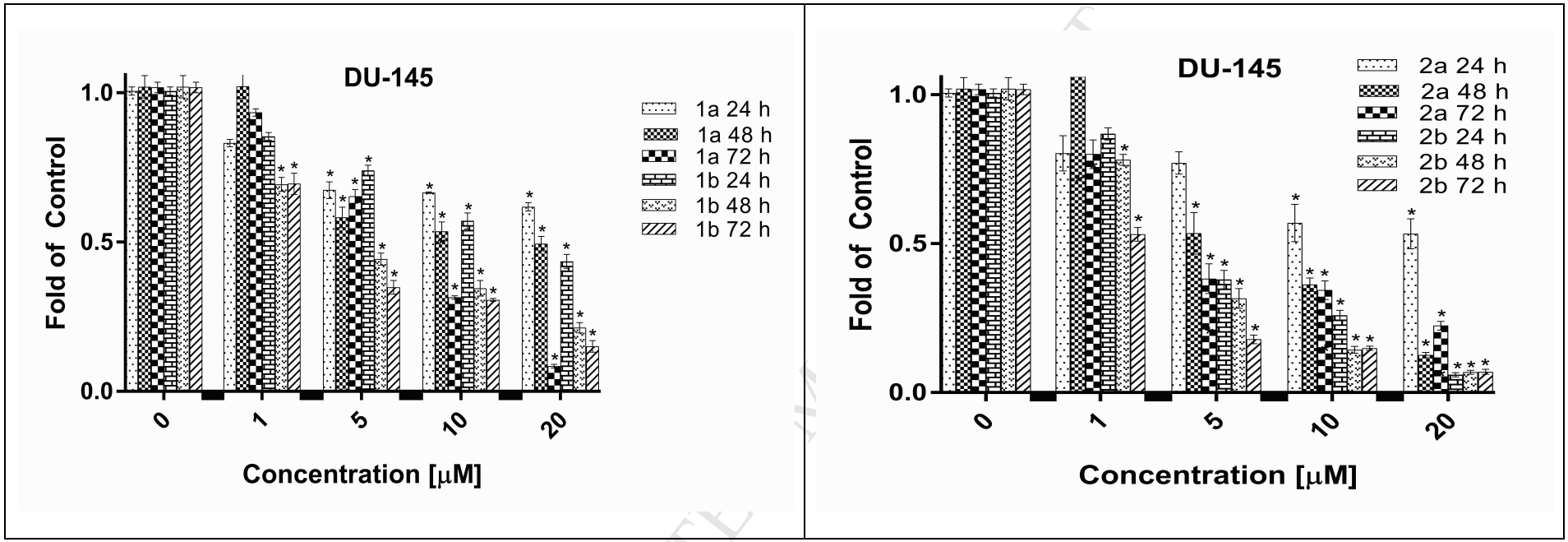


Figure 1. The dose and time-dependence of anticancer activities of ligands (**1a-b**) and Ag-NHC complexes (**2a-b**) for DU-145 cells. Control cells were treated with DMSO. Data are representative of the mean of three separate experiments done in triplicate and are reported at the SEM (* $p<0.0001$ vs control).

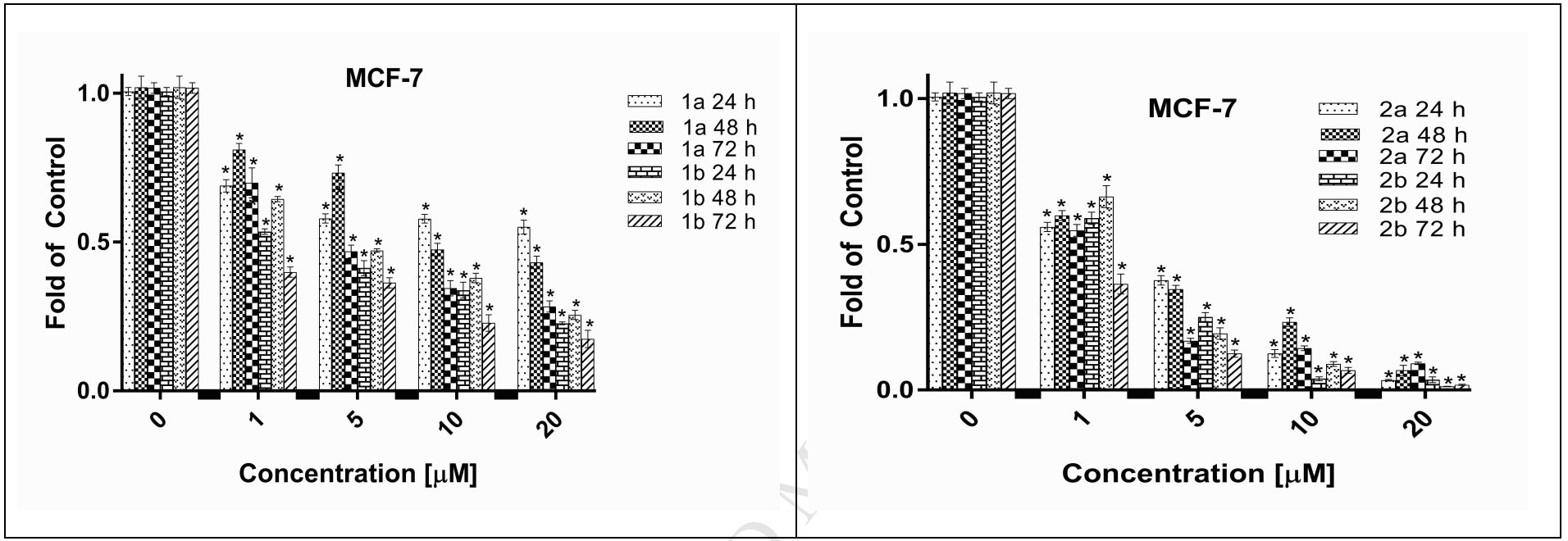


Figure 2. The dose and time-dependence of anticancer activities of ligands (**1a-b**) and Ag-NHC complexes (**2a-b**) for MCF-7 cells. Control cells were treated with DMSO. Data are representative of the mean of three separate experiments done in triplicate and are reported at the ±SEM (* $p<0.0001$ vs control).

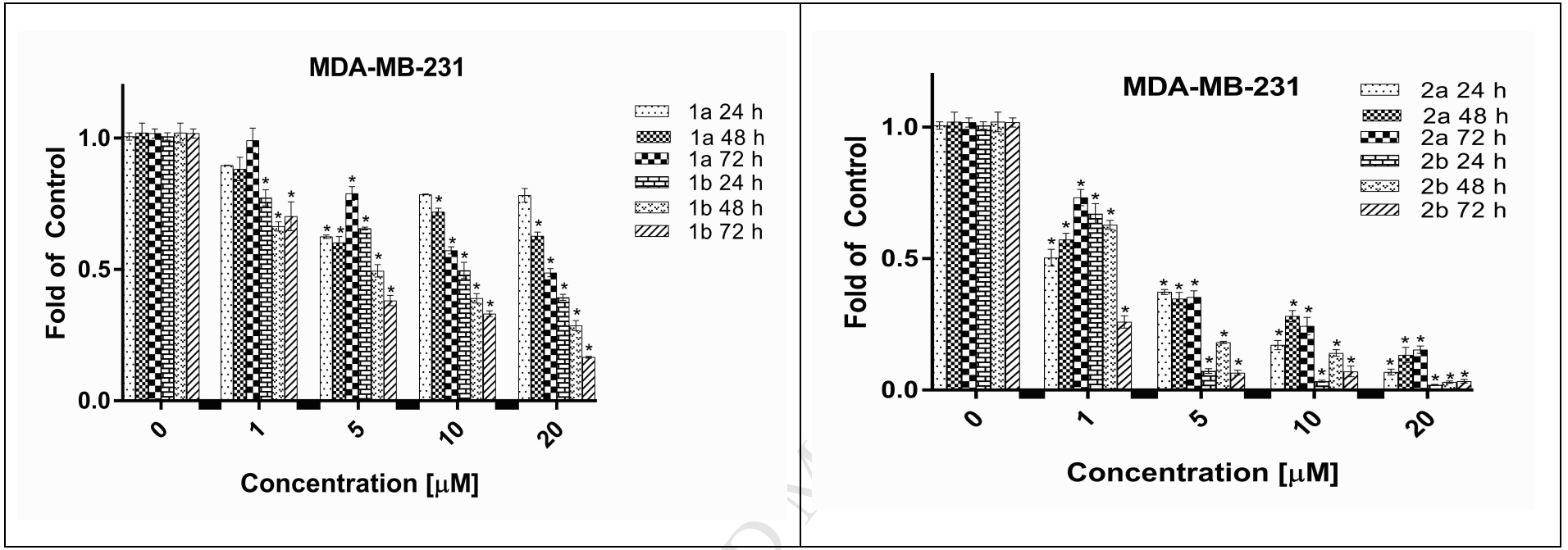


Figure 3. The dose and time-dependence of anticancer activities of ligands (**1a-b**) and Ag-NHC complexes (**2a-b**) for MDA-MB-231 cells. Control cells were treated with DMSO. Data are representative of the mean of three separate experiments done in triplicate and are reported at the ±SEM (* $p<0.0001$ vs control).

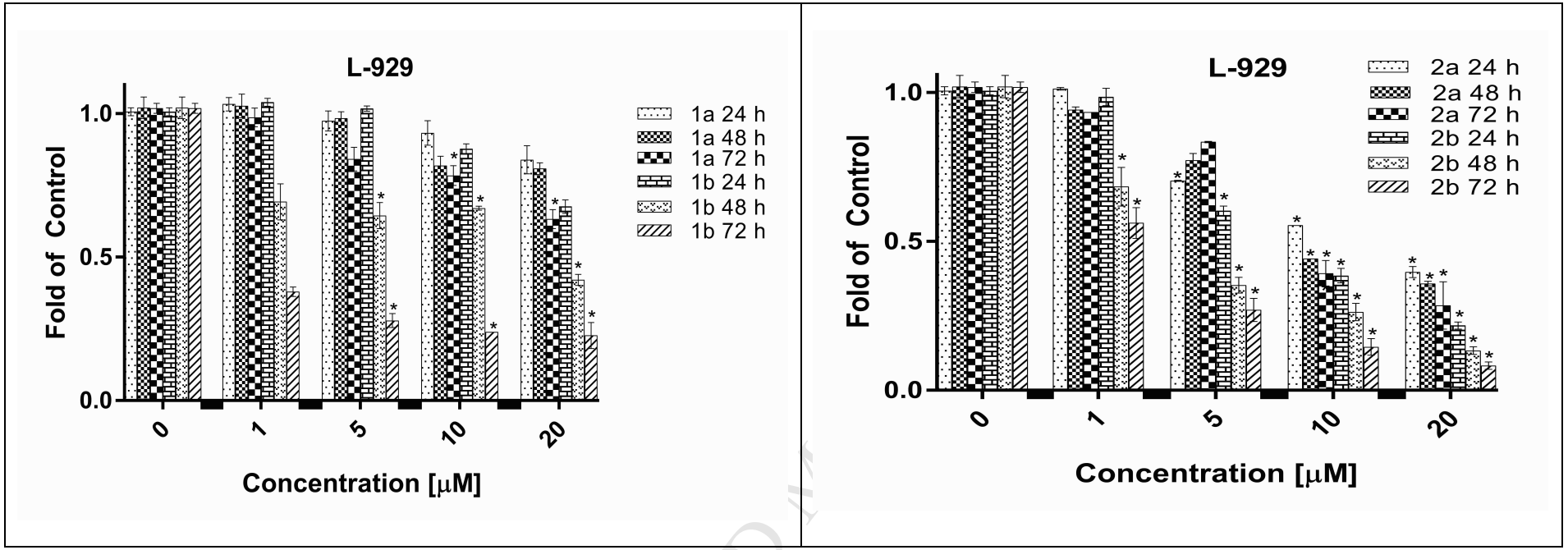


Figure 4. The dose and time-dependence of anticancer activities of ligands (**1a-b**) and Ag-NHC complexes (**2a-b**) for L-929 cells. Control cells were treated with DMSO. Data are representative of the mean of three separate experiments done in triplicate and are reported at the ±SEM (* $p<0.0001$ vs control).

Highlights

- Novel Silver-NHC Complexes
- Elemental analysis, LC-MS, FT-IR, ^{1}H NMR, and ^{13}C NMR techniques.
- Anticancer activities on DU-145 prostate cancer cells and MCF-7, MDA-MB-231 breast cancer cells and L-929 normal cells.
- Comparison of activity according to the structure of complexes and cell line types