Research article

Molecular docking and *in vitro* anticancer studies of silver(I)-*N*-heterocyclic carbene complexes

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ABSTRACT

A series of symmetric and unsymmetrical benzimidazolium-based N-heterocyclic carbene (NHC) precursors (**1a-i**) and their silver complexes (**2a-i**) have been synthesized. The Ag(I)–NHC complexes were characterized by ^1H, ^{13}C {^1H} NMR, FTIR, LC/MS-QTOF, and elemental analysis. Anticancer and cytotoxic activity of all Ag(I)–NHC complexes were tested against healthy fibroblast cell line (L929), breast cancer cell line (MCF-7), and neuro-blastoma cell line (SH-SY5Y) by MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfo-phenyl)-2H-tetrazolium] assay. The **2b**, **2c**, **2e**, **2g**, **2h**, and **2i** complexes showed higher cytotoxicity than cisplatin against SH-SY5Y and MCF-7 and lower cytotoxic activity against L929 cell lines. Because of their high cytotoxic activity against cancer cells and low cytotoxicity against healthy fibroblast cell lines, the **2b**, **2c**, **2e**, **2g**, **2h**, and **2i** are expected to be new lead compounds. In addition, molecular docking studies were performed to explore the binding interactions of silver complexes with the enzyme to explore new anticancer compounds. Furthermore, ADME properties of all complexes were predicted to explore lead-like characteristics and may be a potential drug candidate for cancer treatment.

1. Introduction

Metallopharmaceutics such as cisplatin and its analogues are used in clinical practices; however, they have unavoidable neurotoxic side effects [1]. Cisplatin is one of the most known drugs to cure many cancers. Although cisplatin is effective in treating a variety of cancer, its adverse effects, such as toxicity, immunization of cancer cells, and low solubility, limit its use. Cisplatin use in cancerous cell treatment is a milestone in using metal-derived compounds in chemotherapy. However, platinum-based drugs treat both types of cancer, though hematological, and gastrointestinal side effects should be avoided [2, 3, 4, 5]. Synthesis of metal-derived compounds that do not show platinum-based drugs' side effects and give better cytotoxicity than cisplatin in chemotherapy remains a challenge [6]. Even though non-platinum metal anticancer drugs have not been approved for use in chemotherapy until now, various transition-metal complexes are now being under-investigated as drug candidates for future development [7, 8, 9, 10, 11]. NAMI-A, KP1019, Budotitane, and gallium-based compounds are some drug candidates [7, 8, 9, 10, 11].

N-heterocyclic carbenes (NHCs) are easily synthesized, chemically modified and have donor properties similar to phosphine ligands. NHC can form a stronger bond with the metal canters, leading to more stable complexes against moisture, heat, and air. NHC complexes are primarily used in catalysis as catalysts [12, 13, 14, 15, 16, 17]. Recently, NHCs have been beginning to be excellent cytotoxic metal carriers due to their ready prerequisites for an efficient drug design and fast optimizations [2, 13, 18, 19, 20, 21, 22]. The lipophilic terminal is essential in drug molecules. It needs to be chemically modified to serve this lipophilic terminal on NHC. Thus, easy chemical modification of NHCs to serve lipophilic terminals in NHC-based drug molecules is very important. Last two decades, an increasing number of publications have focused on the biological application of Ag(I), Au(I), Ru(II), Rh(II), Pt(II), and

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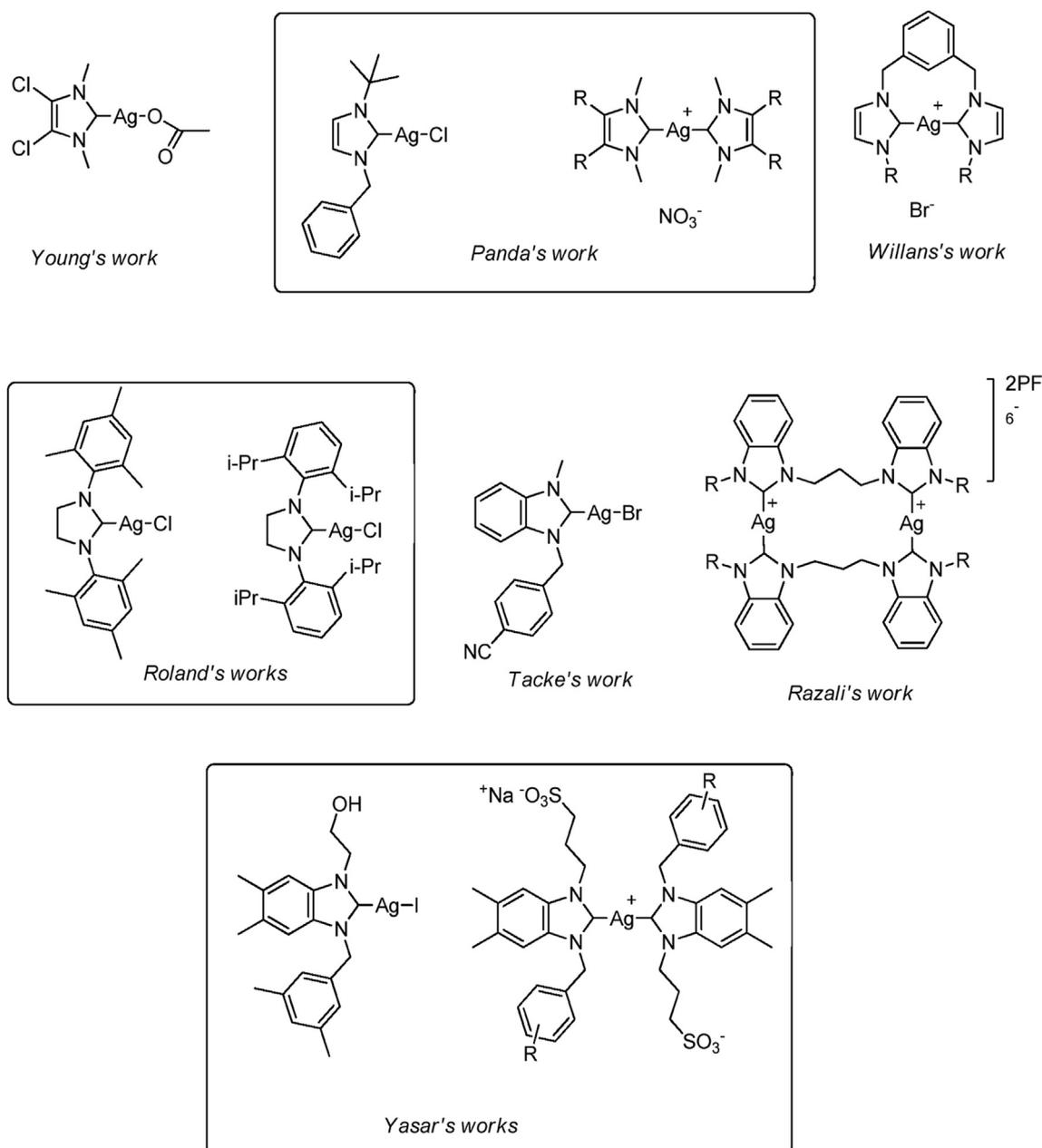
Scheme 1. Structure of cytotoxic properties studied Ag–NHC complexes against different cancer cells.

Cu(I)–NHC complexes as metallopharmaceutic [23, 24, 25, 26, 27, 28, 29]. Silver complexes showed considerable anticancer activity, some of them in vitro and in vivo [19, 30, 31, 32]. The mechanism of action of silver complexes is based on the release of the Ag^+ ions that enter the cell membranes and disrupt the cell function. Additionally, silver complexes lose their activity very quickly because of the rapid release of the Ag^+ ions. There is a need to synthesize a stable complex that can control or slow Ag^+ ions release. Slow release of Ag^+ ions is only possible when firmly coordinated between the silver and the ligand. Due to the firm σ-donor properties of NHC, Ag–NHC complexes remain therapeutically active for a longer time than $AgNO_3$, which may be rationalized by a slower release of Ag^+ ions from stable Ag(I)–NHC complex [20]. Ag(I)–NHC complexes gained increased stability via strong σ-donor properties of NHC. They could easily overcome the fast loss of activity problems and sulphonamide resistance of the pathogens against conventional silver-based antibiotics [21]. Different groups reported that Ag–NHC complexes could act as antimicrobial agents since the release of Ag^+ ions from Ag–NHC is behind the usual schedule [13, 22, 33, 34]. After

these encouraging results, Youngs et al. investigated the cytotoxic activity of Ag–NHC complexes against OVCAR-3, MB157 cancer cells (Scheme 1). These Ag–NHC complexes showed cytotoxic activity but did not affect HeLa cancer cells [34]. Panda et al. reported a similar cytotoxic effect of Ag–NHC complex against HeLa cancer cells [35] (Scheme 1). However, Youngs et al. reported that the same complex had good activity against ovarian cancer in mice. After these results, activity of Ag–NHC complex can differ according to the type of cancer cell [34]. The Ag-Bis(NHC) complexes showed similar efficiency against H460 lung cancer cells [36]. The anticancer potential of Ag–NHC complexes and their synthesis have been the attention of various research teams. Ag–NHC complexes have been reported to show good to excellent anticancer potential against many cell lines [23, 36, 37, 38, 39, 40, 41, 42].

Herein, molecular docking and investigation of the cytotoxic activity of benzimidazole-based Ag–NHC complexes were reported. The MCF-7 and SH-SY5Y cell lines were significantly inhibited by these complexes, and the L929 cell lines showed moderate cytotoxic activity.

2. Experimental

2.1. General considerations

All these experiments were performed using normal Schlenk techniques in freshly dried solvents under in air or nitrogen atmosphere.

Synthesis of Ag(I)–NHC complex reactions were performed in the absence of light. Ag_2O and solvents used in this work were purchased from Sigma-Aldrich. The compounds' elements were examined using the ElementarVario EL III Carlo Erba 1108 instrument. The melting points of the compounds were calculated using a Stuart automated melting point apparatus (SMP-40). The ^1H and $^{13}C\{^1H\}$ NMR spectra were obtained using Bruker Avance III HD 300 and 400 MHz NMR spectrometers. The *J* values for coupling constants are displayed in hertz. For the HRMS study, an Agilent 6530 Accurate-Mass spectrometer was used. A PerkinElmer Spectrum 100 GladiATR FT/IR spectrometer was used to record FTIR spectrum.

2.2. In vitro *cytotoxic activity using MTS assay*

The DMEM (Dulbecco's Modified Eagles Medium) containing penicillin-streptomycin (1%) and fetal bovine serum (10%) was used to maintain the MCF-7, SH-SY5Y and L929 cell lines. They were then kept in 5% CO_2 at 37 °C in a CO_2 incubator. The cells' medium was replaced twice a week. When the cells were 85% confluent, the cells were removed from flasks using a trypsin-EDTA solution. Each 96-wells contained 5 × 10^3 healthy cells and was analyzed by MTS assay. The DMEM-containing plates were incubated in an incubator at 37 °C for 24 h. Following 24 h incubation at 37 °C in the CO_2 incubator, the silver compounds were dissolved in dimethyl sulfoxide (DMSO). All cells were serially diluted (1, 10, 25, 50, and 100 μM) for 24 and 48 h. After incubation, the MTS solution (10 μL) was added to it and put in incubation for next 2 h.

Using an ELISA reader (BioTek Instruments, Inc. Winooski, Vermont, USA), the absorbance at 450 nm was measured to determine the viability of the cells. The cell viability percentage of each group was obtained focused on the control cells (cells not treated with silver compounds) as 100%. The MTS analyses were repeated for each concentration at 24 and 48 h (n: 4).

2.3. Molecular docking

Molecular docking was enforced for the **2a-i** complexes to compare their theoretical and experimental binding affinity with MCF-7 and SH-SY5Y Cell Line. The 3D structure of MCF-7 (PDB ID: 1E31) at 2.71 Å resolution and SH-SY5Y cell line (PDB ID: 2H9V) at 3.10 Å resolution were downloaded from PDB (Protein Data Bank). The 2D structures of all nine complexes and cisplatin were drawn using Chem3Draw Ultra 16.0.1.2 software and were converted into a 3D structure using a discovery studio visualizer [43]. These two protein structures were the best in the PDB with their low-resolution values and thus were chosen for docking studies. The PyRx was use to investigate the rmsd (root-mean-square deviation) of the ligands and the amino acid residue surrounded to it. Ten conformations for each ligand were generated; the top 5 docking poses were retained for each compound to analyze the interactions of the docked conformations within the active site.

2.4. ADME prediction

An in-silico ADME study was conducted to predict the pharmacokinetic parameters of all the complexes using SwissADME prediction software [44]. The Structures were drawn by ChemDraw 16.0.1.4 and converted to smile format. These SMILE formats were loaded into the SwissADME database and evaluated their ADME profile.

2.5. Synthesis

2.5.1. Synthesis pathway for benzimidazole-based NHCs (**1a**–**i**)

NHC precursors **1a-i** were prepared based on the relevant literature (Scheme 2) [45]. The spectroscopic data (NMR and FTIR) of **1a-i** are shown in the supporting information (Figures S1–S9 and S19–S24, respectively).

1-(methylpyridine)-3-(2-methoxyethyl)benzimidazolium chloride, **1a**: The **1a** is produced in an excellent yield when 1-(methylpyridine) benzimidazole (10 mmol) and 2-methoxyethyl chloride (12 mmol) are reacted using DMF (5 mL) at 80 °C under Ar atmosphere. Thereafter, the precipitates were subsequently filtered out, repeatedly washed with diethyl ether, and dried in vacuo. Methanol/diethyl ether (1:1) solvent was used to crystallize the resultant brown solid (2.17 g, 75 percent).

1-(methylpyridine)-3-(3,5-dimethylbenzyl)-5,6-dimethylbenzimidazolium chloride, **1b**: **1b** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(methylpyridine)-5,6-dimethylbenzimidazole (10 mmol) and 3,5-dimethylbenzyl chloride (11 mmol) to give **1b** as brown powder (3.09 g, 85%).

1,3-Bis(ethylmorpholine)benzimidazolium chloride, **1c**: **1c** was synthesized using synthetic pathway of **1a** by the reaction of 1-(ethylmorpholine)benzimidazole (10 mmol) and ethylmorpholine chloride (11 mmol) to give **1c** as white powder (2.67 g, 70%).

1,3-bis(4-tert-butylbenzyl)-5,6-dimethylbenzimidazolium bromide, **1d**: **1d** was synthesized following the **1a** synthesis pathway by reacting 1-(4-*tert*-butylbenzyl)-5,6-dimethylbenzimidazole (10 mmol) and 4-*tert*-butylbenzyl chloride (11 mmol) to give **1d** as white powder (4,00 g, 77%).

1-(methylpyridine)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride, **1e**: **1e** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(methylpyridine)benzimidazole (10 mmol) and 2,4,6-trimethylbenzyl chloride (11 mmol) to give **1e** as white powder (2.95 g, 78%).

1-(methylpyridine)-3-(3-methoxybenzyl)benzimidazolium chloride, **1f**: **1f** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(methylpyridine)benzimidazole (10 mmol) and 3-methoxybenzyl chloride (11 mmol) to give **1f** as white powder (4.43 g, 94%).

1,3-bis-(3,4,5-trimethoxybenzyl)benzimidazolium chloride **1g**: **1g** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(3,4,5-trimethoxybenzyl)benzimidazole (10 mmol) and 3,4,5-trimethoxybenzyl chloride (11 mmol) to give **1g** as white powder (4.22 g, 82%).
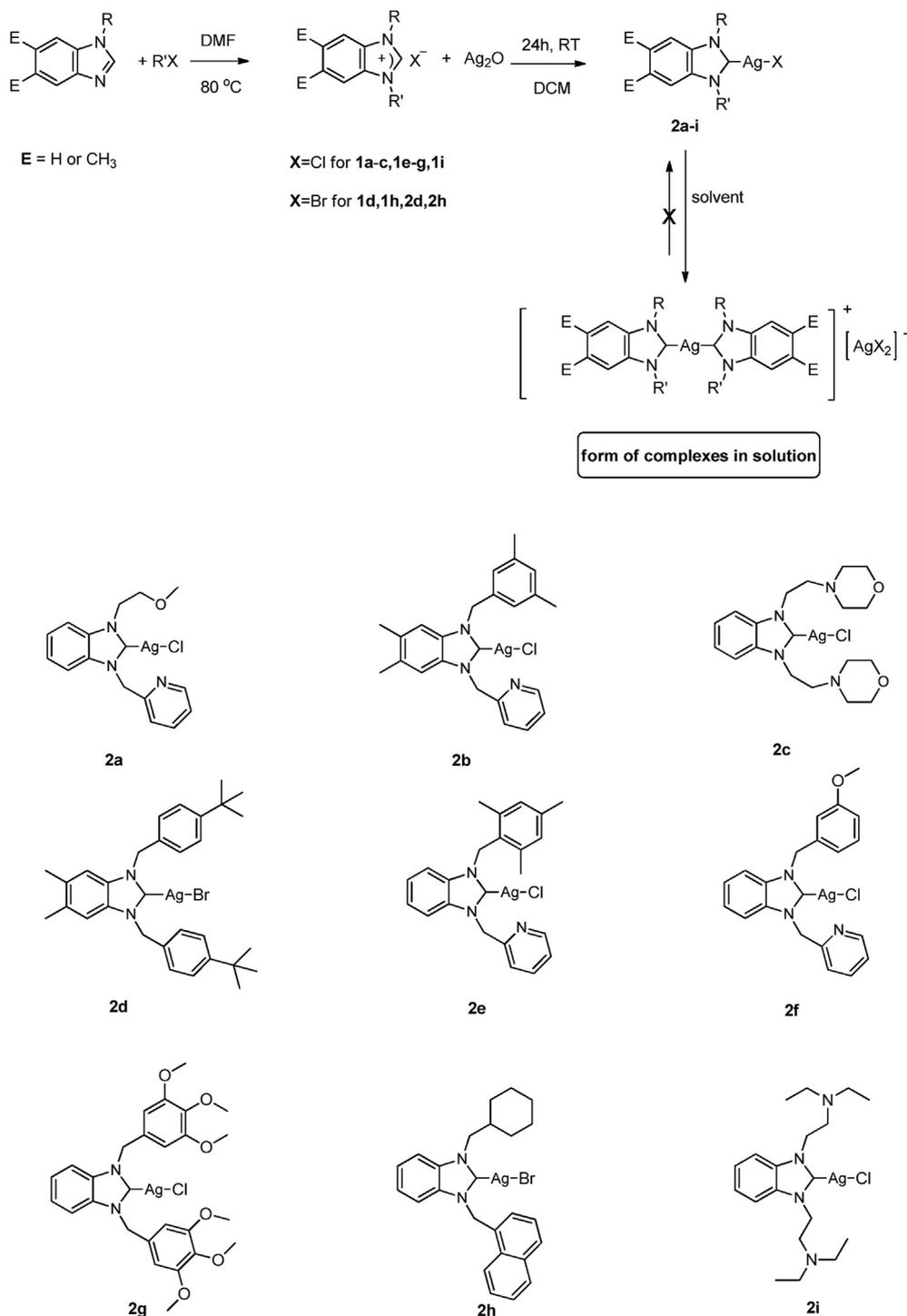
1-(methylcyclohexyl)-3-(2-methylnaphthalene)-benzimidazolium Bromide **1h**: **1h** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(methylcyclohexyl)benzimidazole (10 mmol) and 2-methylnaphthalene bromide (11 mmol) to give **1h** as white powder (2.96 g, 68%).

1,3-(bis-(2-diethylamino)ethyl)benzimidazolium chloride **1i**: **1i** was synthesized with the synthesis pathway of **1a** by the reaction of 1-(2-diethylaminoethyl)benzimidazole (10 mmol) and 2-diethylaminoethyl chloride (11 mmol) to give **1i** as white powder (2.51 g, 71%).

2.5.2. Synthesis pathway for Ag–NHC complexes (**2a-i**)

The synthesis of **2a-i** complexes were done according to the literature [24]. The spectroscopic data (NMR and FTIR) of Ag(I)-NHC complexes are shown in the supporting information (Figures S10–S18 and S25–S33, respectively).

Chloro[1-(methylpyridine)-3-(2-methoxyethyl)benzimidazol-2-yliden]silver(I), **2a**: **2a** was synthesized in goodyield by the reaction of **1a** (1 mmol) and Ag_2O (1 mmol) in 10 ml dichloromethane (DCM) at rt for overnight in the absence of light leading to give Ag–NHC complex. After the reaction, reaction solution was filtered by passing over a small layer of Celite and the amount of filtrate was reduced by half and diethylether (15 mL) was added to the solution to obtain a brown solid. This brown solid was then washed several times with diethylether, dried under vacuum and stored in the dark (0.34 g, 82%).

E = H or CH$_3$

X=Cl for **1a-c,1e-g,1i**

X=Br for **1d,1h,2d,2h**

form of complexes in solution

2a

2b

2c

2d

2e

2f

2g

2h

2i

Scheme 2. Synthesis of NHC salts (**1a-i**) and their Ag–NHC complexes (**2a-i**).

Chloro[1-(methylpyridine)-3-(3,5-dimethylbenzyl)-5,6-dimethylbenzimida-zol-2-yliden]silver(I), **2b**: **2b** was synthesized using the synthesis method of complex **2a** by the reaction of compound **1b** (1 mmol) and Ag$_2$O (1 mmol) in 10 ml DCM at rt for overnight to give **2b** as brown powder (0.38 g, 77%).

Chloro[1,3-Bis(ethylmorpholine)benzimidazol-2-yliden]silver(I), **2c**, *(C$_{19}$H$_{28}$N$_4$O$_2$ClAg)*: **2c** was synthesized using the synthesis method of complex **2a** by the reaction of compound by the reaction of **1c** (1 mmol) and Ag$_2$O (1 mmol) to give **2c** as white powder (0.39 g, 75%).

Bromo[1,3-bis(4-tert-butylbenzyl)-5,6-dimethylbenzimidazol-2-yliden] silver(I), **2d**: **2d** was synthesized using the synthesis method of complex

2a by the reaction of compound **1d** (0.34 g, 1 mmol) and Ag$_2$O (1 mmol) to give **2d** as white powder (0.43 g, 70%).

Chloro [1-(methylpyridine)-3-(2,4,6-trimethylbenzyl)benzimidazol-2-yli-den] silver(I), **2e**: **2e** was synthesized using the synthesis method of complex **2a** by the reaction of **1e** (1 mmol) and Ag$_2$O (1 mmol) to give **2e** as white powder (0.39 g, 78%).

Chloro [1-(methylpyridine)-3-(3-methoxybenzyl)benzimidazol-2-yliden] silver(I), **2f**: **2f** was synthesized using the synthesis method of complex **2a** by the reaction of **1f** (1 mmol) and Ag$_2$O (1 mmol) to give **2f** as white powder (0.35 g, 76%).

Chloro [1,3-bis(3,4,5-trimethoxybenzyl)benzimidazol-2-yliden]silver(I), 2g: **2g** was synthesized using the synthesis method of complex **2a** by the reaction of **1g** (1 mmol) and Ag₂O (1 mmol) to give **2g** as white powder (0.32 g, 65%).

Bromo [1-(methylcyclohexyl)-3-(2-methylnaphthalene)benzimidazol-2-yliden]silver(I), 2h: **2h** was synthesized using the synthesis method of complex **2a** by the reaction of **1h** (1 mmol) and Ag₂O (1 mmol) to give **2h** as white powder (0.30 g, 69%).

Chloro[1,3-bis-(2-(diethylamino)ethyl)benzimidazol-2-yliden]silver(I), 2i: **2i** was synthesized using the synthesis method of complex **2a** by the reaction of **1h** (1 mmol) and Ag₂O (1 mmol) to give **2i** as white powder (0.3 g, 70%).

3. Results and discussion

3.1. Synthesis and characterization

The NHC salts and Ag(I)–NHC complexes, which are given in Scheme 2, were synthesized following reported procedures [24]. Both air-stable **1a-i** and **2a-i** (82%, 77%, 75%, 70%, 78%, 76%, 65%, 69%, and %70, respectively) were prepared with good yields using the methods in which wide range Ag(I)–NHC complexes prepared from the corresponding NHC salts and Ag₂O in DCM under the absence of light at room temperature. All the synthesized compounds were fully characterized by spectroscopic techniques (^{1}H NMR, ^{13}C{^{1}H} NMR, HRMS, FTIR, and Elemental analysis) and melting point determination.

The resonance of C_2 protons and C_2 carbons of **1a-i** in the ^{1}H NMR and ^{13}C{^{1}H} NMR appears at 11.23, 11.64, 11.21, 11.84, 9.44, 11.71, 12.21, 10.03, 11.08, 152.5, 152.8, 144.3, 162.6, 153.1, 152.5, 153.9, 144.1, and 144.1 ppm in CDCl₃, respectively. The loss of the C2 proton of **1a-i** in ^{1}H NMR and the downfield shift of the C2 carbon to a new area in ^{13}C{^{1}H} NMR spectra of **2a-i** complexes indicate the formation of **2a-i** complexes. The C2 carbon of **1a** and **1f** downfield shifted to 189.2 and 189.4 ppm, respectively, indicating the formation of **2a** and **2f** complexes. However, the C2 carbon of **2b**, **2c**, **2d**, **2e**, **2g**, **2h**, and **2i** have not been observed in ^{13}C{^{1}H} NMR spectra. According to the literature [46, 47, 48, 49, 50, 51, 52], the fluxional behaviour of the Ag–NHC complexes may cause not to be observed the resonance of the C2 carbene carbon in the ^{13}C{^{1}H} NMR spectrum. However, based on these data, C2 carbon of 2a and 2f complexes was observed in ^{13}C{1H} NMR spectra; it can be said that fluxional movements are effective in **2b**, **2c**, **2d**, **2e**, **2g**, **2h**, and **2i** complexes.

The HRMS analyses were performed to elucidate the **2a-i** complexes structures, and obtained spectra are shown in the supporting information as Figures S34–S42 showed that the proposed structures for the **2a-i** complexes are in bis-carbene ([Ag(NHC)₂]$^+$ [AgX₂]$^−$) form in solution. According to LC/MS-Q-TOF spectra, it was understood that **2a-i** complexes were in the form of bis-carbene in the solution and did not

transform into mono-carbene form. Although we do not have clear information about the fluxional behaviour of **2a-i** complexes in solid form, it has been observed that **2a-i** complexes in solution have a [Ag(NHC)₂]$^+$ [AgX₂]$^-$ structure. We made every attempt to obtain a suitable crystal by single crystal X-ray diffraction analysis to ascertain the structure of silver-NHC complexes in solid form, but we were unable to do so. According to the literature [53], the Ag–NHC complexes' structure was characterized by X-ray analysis found in the form of mono-carbon. Due to the structural similarity of Ag(I)–NHC complexes with literature results [53], we think that **2a-i** complexes have the same structure in solid form.

3.2. Biological evaluation

3.2.1. Antitumor activity

The inhibitory effect of **2a-i** complexes and cisplatin on the growth of L929, MCF-7 and SH-SY5Y cell lines were measured using the MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfo-phenyl)-2H-tetrazolium] assay (Table 1). Antiproliferative effects of the Ag(I)–NHC complexes were assessed for 24 and 48 h at various concentrations (1, 10, 25, 50, and 100 μM). The concentration corresponding to 50% growth inhibition (IC₅₀) of **2a-i** on MCF-7, SH-SY5Y, and L929 is shown in Figures 1, 2, and 3 and Table 1. The results show that all Ag(I)–NHC complexes had more significant inhibitory effects than cisplatin on MCF-7 and SH-SY5Y cells at all concentrations.

3.2.1.1. The antitumor activity comparison of the complexes on the SH-SY5Y cell line. **2a**, **2b**, **2c** and **2d** complexes showed moderate inhibitory effects on SH-SY5Y cell growth at a 1 μg/mL concentration
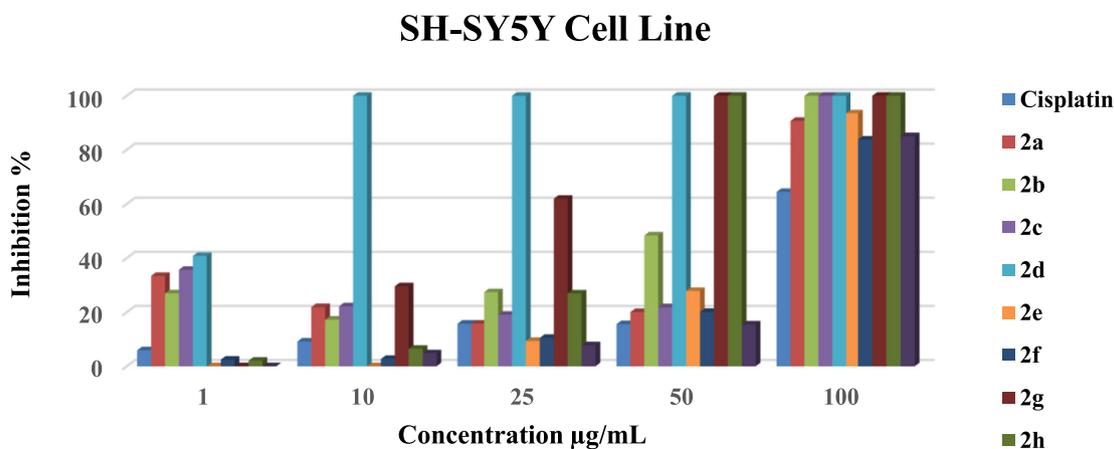
Table 1. The IC₅₀ (μM) ± S.E.[a] for Ag–NHC complexes **2a-i** on L929, MCF-7, and SH-SY5Y cell lines and relative activity of Ag(I)–NHC complexes to cisplatin.

Compound	L929 Cell Line	MCF-7 Cell Line	SH-SY5Y Cell Line
Cisplatin	11.6 ± 0.5	7.2 ± 0.4	10.2 ± 0.4
2a	63.48 ± 0.4	98.29 ± 1	23.60 ± 0.7
2b	37.11 ± 0.9	20.67 ± 0.6	2.55 ± 0.7
2c	53.53 ± 0.9	76.02 ± 0.4	8.86 ± 0.4
2d	1.18 ± 0.3	2.66 ± 0.6	0.25 ± 0.8
2e	155.9 ± 0.5	21.32 ± 1	71.17 ± 1
2f	404.3 ± 0.7	35.63 ± 0.5	82.46 ± 0.6
2g	18.71 ± 0.5	6.56 ± 0.6	13.74 ± 0.5
2h	45.41 ± 1	16.33 ± 0.8	24.73 ± 0.9
2i	140.7 ± 0.5	53.64 ± 0.6	88.89 ± 0.5

*Values are mean value ±standard error of three different replicates.

Figure 1. The antiproliferative effects of **2a-i** complexes on SH-SY5Y cells were analyzed by MTS assay.
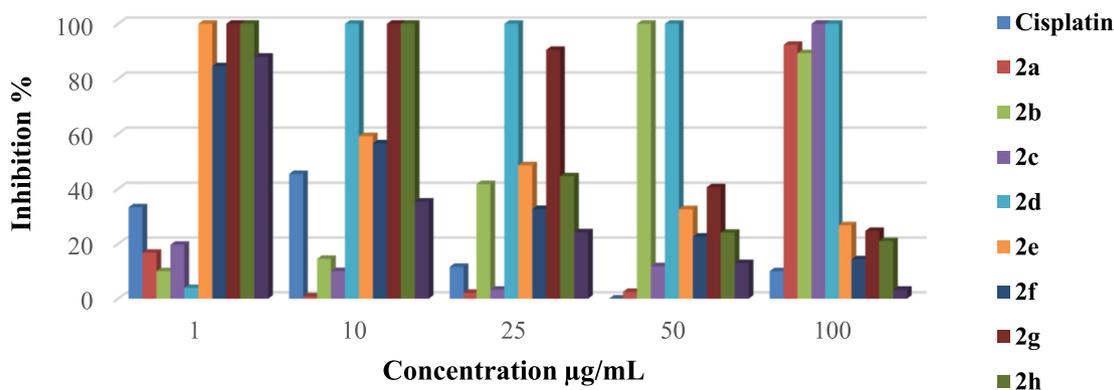
MCF-7 Cell Line

Figure 2. The antiproliferative effects of **2a-i** complexes on MCF-7 cells were analyzed by MTS assay.
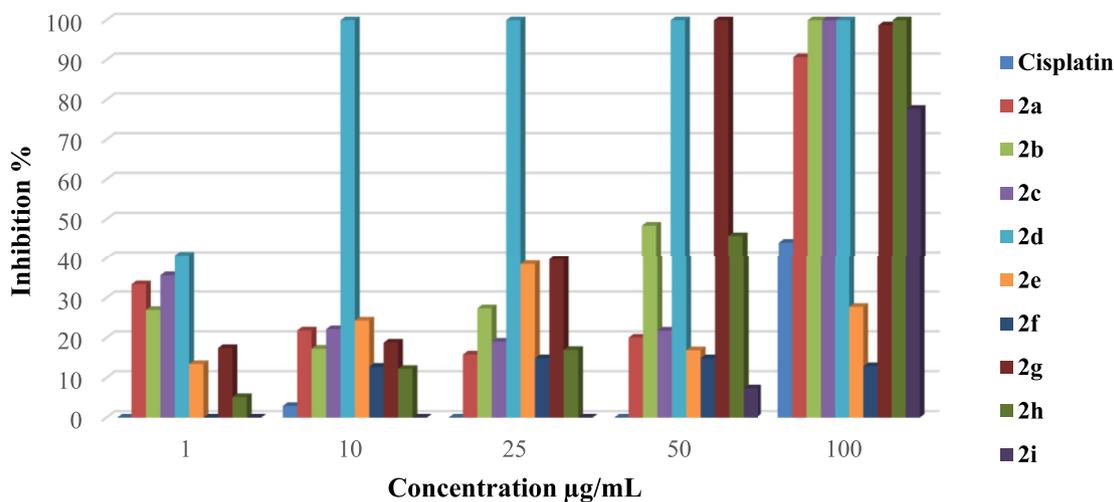
L929 Cell Line

Figure 3. The antiproliferative effects of **2a-i** complexes on L929 cells were evaluated using MTS assay.

(Figure 1). However, complex **2d**, **2g** and **2h** showed excellent inhibitory effects on SH-SY5Y cell growth at concentrations of 10 μg/mL, 50 μg/mL, and 50 μg/mL, respectively (Figure 1). The growth inhibition effects were more significant than 99%, and the cell viability of the SH-SY5Y cells was <0.5%. The rest of the complexes had excellent inhibitory effects on SH-SY5Y cell growth at a 100 μg/mL concentration. The cell viability of complex **2c** against SH-SY5Y cells decreased dose-dependently.

3.2.1.2. The antitumor activity comparison of the complexes on the MCF-7 cell line. **2d**, **2e**, **2g**, and **2h** complexes showed excellent inhibitory effects on MCF-7 cell growth at a concentration of 1 μg/mL (Figure 2). However, complex **2f** and **2i** showed inhibitory effects on MCF-7 cell growth at the same concentration with very low cell viability. The growth inhibition effects were greater than 99%, and the cell viability of the MCF-7 cells was 85% and 88%, respectively. The rest of the complexes reached fewer inhibitory effects than the cisplatin on MCF-7 cell growth at a concentration of 1 μg/mL. When Ag(I)–NHC complex concentration increased to 10 μg/mL, inhibition of the complex **2d** reached 100% inhibition. Some of Ag(I)–NHC complexes (**2e**, **2f**, **2h**, **2i**) exhibit increase proliferative activity instead of antiproliferative activity in the 10, 20, 25, 50, and 100 μg/mL concentrations (Figure 2).

3.2.1.3. The proliferative activity comparison of the complexes on the L929 cell line. Against the L929 cell line, complex **2f** and **2i** complexes showed no cytotoxicity at a concentration of 1 μg/mL, which is very important in terms of the less harmful effect on healthy cells. However, all other complexes exhibited low cytotoxicity at the same concentration (Figure 3). Except for **2d** and **2i** complexes, all complexes showed low and similar cytotoxicity effects on L929 cell growth at the 10, 25, and 50 μg/mL concentrations with low cell inhibitions (Figure 3).

As a result, all **2a-i** complexes except **2d** have a low cytotoxic effect against L929 cells at 1–25 μg/mL. Complexes **2a**, **2b**, **2c**, **2d**, **2g**, and **2h** can be anticancer drug candidates at 1–50 μg/mL concentrations on the SH-SY5Y cell lines. However, Complexes **2e-i** can be considered an anticancer drug candidate at 1–25 μg/mL concentrations and **2d** complex 10–25 μg/mL on the MCF-7 cell lines.

From this, it seems silver complexes with different structures are quite effective in destroying different cancerous cells. When examining the complexes structures, it was seen that structural differences cause antiproliferative activity differences in different cancer cells. O- or N- heteroatom containing NHCs led to a difference in the antiproliferative activity of Ag–NHC complexes against both cancer cell lines and have low cytotoxicity against L929. However, complex **2d** showed superior cytotoxic activity for all types of cancer cell lines at all concentrations. Thus,
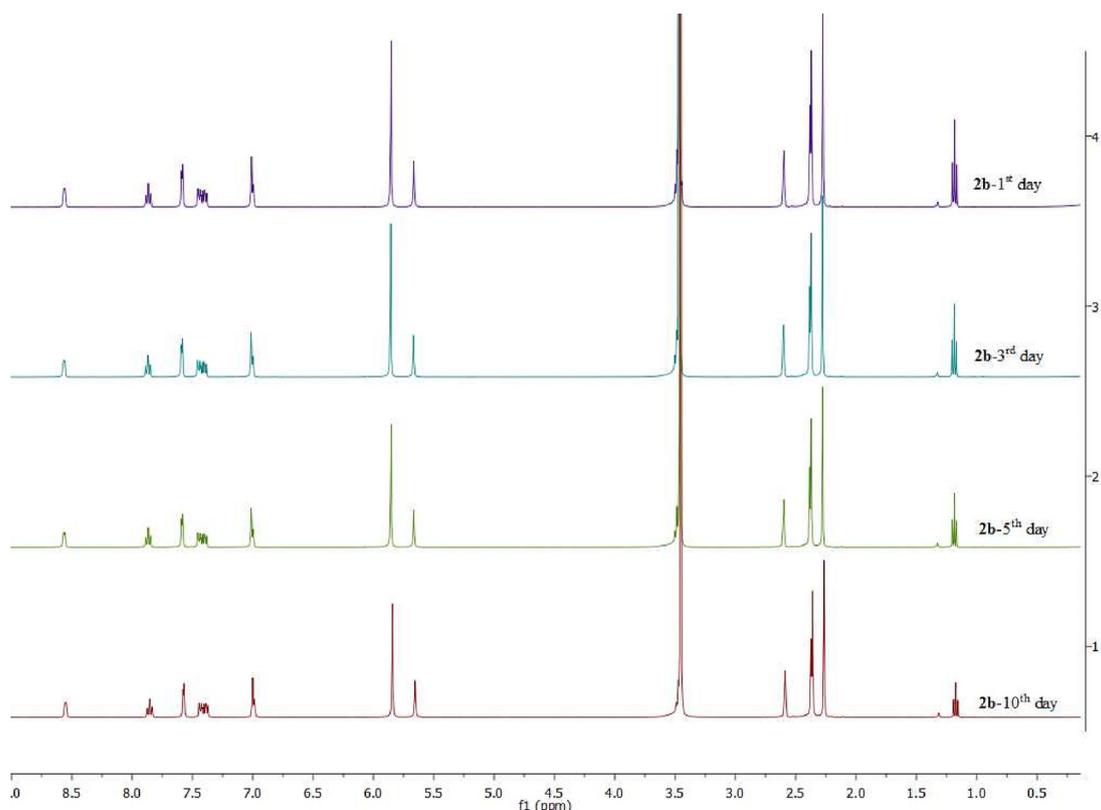
Figure 4. Stability test of the **2b** complex by NMR spectroscopy.

2d has not been considered a drug candidate due to its high mortality in L929 cell lines. We think that the antiproliferative activity of the **2d** complex is also affected by the 4-*tert*-butyl group or Br anion. These differences may lead to a sensitive balance on the release of Ag$^+$ ions from the stable Ag–NHC complex. This result may indicate the importance of the anions or NHCs on silver(I)–NHC complexes in anticancer activity.

All the **2a-i** complexes were soluble in DMSO and remained stable in the biological medium over the testing period (Figure 4 and please see supporting info Figures S43-S45). The degradation of the **2a-d** complexes

was examined by NMR spectroscopy dissolved in DMSO-d$_6$ for ten days. According to the measurements, **2a-d** complexes can remain stable in DMSO-d$_6$ in the open air without decomposition. This good stability is essential for the slow release of Ag$^+$ ions as required.

3.2.2. Molecular docking studies

A molecular docking study is an alternate tool for analyzing the biologically active drug candidate. In this study, the complexes exhibiting the significant anticancer potential towards MCF-7 and SH-SY5Y cell lines docked with the protein PDBs: 1E31 & 2H9V. The results obtained

Table 2. Report of predicated interactions of MCF-7 cell line docking confirmations and docking scores.

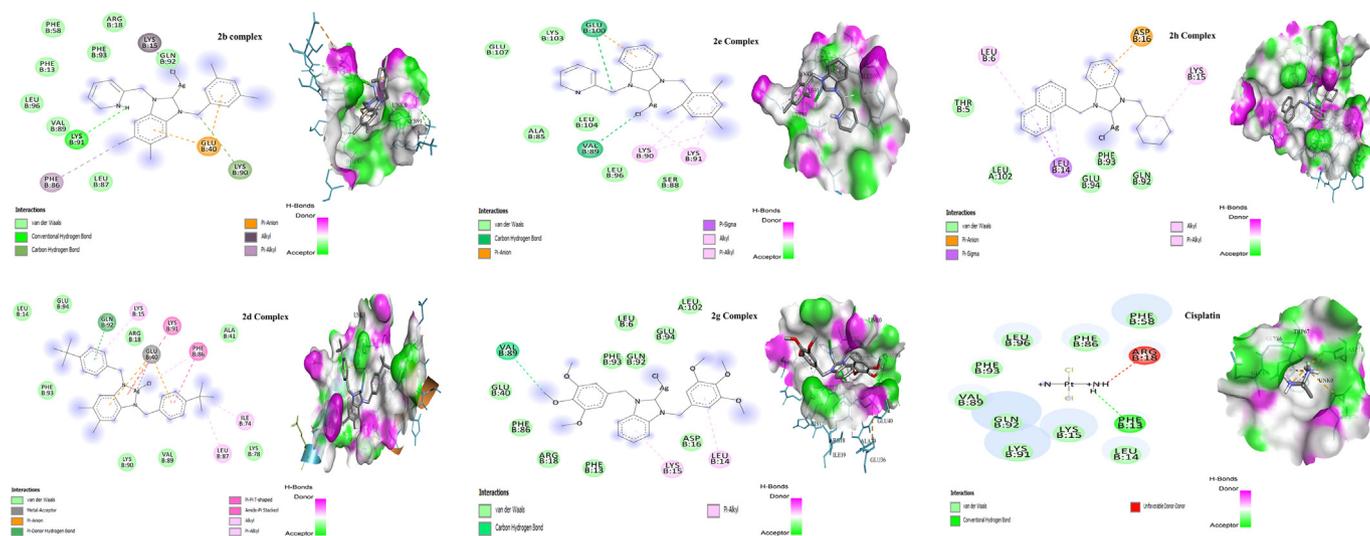| S. No	Bonding interaction	Interacting amino acid residue	Bond type with	Bond distance (Å)	Binding affinity (Kcal/mol)
2b	Hydrogen bonding	LYS:91	NH	2.40	-7.5
	C–H bonding	LYS:90	CH$_2$N	3.48	
	Pi-Anion	GLU:40	C$_6$H$_6$	3.90, 4.23	
	Alkyl pi-alkyl	LYS:15	Cl	3.81	
		PHE:86	CH$_3$	4.26	
2d	Pi-Anion pi-sigma,	GLU:94	C$_6$H$_6$	3.69	-7.1
	Alkyl pi-alkyl	LEU:102, LEU:14	C$_6$H$_6$	3.77, 3.83	
		ARG:106, LEU:102, LEU:14	CH$_3$, C(CH$_3$)$_3$	3.54, 4.69, 4.84	
		TRP: 10	CH$_3$	5.29	
2e	C–H bonding	GLU:100, VAL:89	CH$_2$N, Cl	3.64, 3.14	-6.3
	Pi-Anion	GLU:100	C$_6$H$_6$	3.81	
	Pi-sigma	GLU:100	C$_6$H$_6$	3.86	
	Alkyl pi-alkyl	VAL:89, LYS:90, LYS:91	C$_6$H$_6$, CH$_3$, Cl	3.42, 4.02, 3.99,	
		LYS:90	C$_6$H$_3$	5.39	
2g	C–H bond pi-alkyl	VAL:89	OCH$_3$	3.48	-6.4
		LEU:14, LYS:15	C$_6$H$_6$	5.08, 3.92	
2h	Pi-Anion pi-sigma,	ASP:16	C$_6$H$_6$	3.70	-6.8
	Alkyl pi-alkyl	LEU:14	C$_6$H$_6$	3.79	
		LYS:15	C$_6$H$_{12}$	4.66	
		LEU:6, LEU:14	C$_6$H$_6$	5.27, 5.25	
Cisplatin	C–H bonding	PHE:13	NH	1.98	-3.1

Figure 5. 2D and 3D diagram of the binding interactions of **2b, 2d, 2e, 2g, 2h** silver complexes and Cisplatin with the active site residues of the MCF-7 cell line receptor.

via docking was compared *in vitro* results. In this study, the highest binding energy of silver complexes with MCF-7 cell line was calculated for **2b** is -7.50 kcal/mol, while −6.30 kcal/mol is the lowest binding energy calculated for the complex **2e**. Hydrogen bonding with LYS91, carbon-hydrogen bonding with LYS90, pi-anion with GLU40, alkyl with LYS15, pi-alkyl with PHE86 and van der Waals interaction have been identified. The detailed binding interaction of the most active complexes within the protein pockets is shown in Table 2 and Figure 5. The complexes docking results were compared standard cisplatin (−4.93 kcal/mol) as shown in Table 2 and Figure 5. The less binding energy indicates the formation of stable ligand-complex. According to the molecular modelling and *in vitro* results, all complexes are more effective than cisplatin.

The mechanism of action of silver complexes against cancer has been studies and revealed that the NHC ligand increased the lipophilicity of silver cations through the cell membrane, where it can easily penetrate the cell membrane resulting in the inhibition of the metabolic mechanism of the cancer cells [39]. Furthermore, the cancer cell has a higher level of reactive oxygen species (ROS) that involve DNA damage. It can induce or inhibit transcription, replication, signal transduction pathways, genomic instability and activation of oncogenes [54]. The silver complexes also contain amine groups with electron-donating properties, thereby donating the lone pair of the electron to neutralize the ROS. The electron density increases on the ring due to inductive effect produced by the presence of alkyl substituents to the benzene ring donates electrons. So, it is proposed that the electron-donating group of the complexes also play a role in breast cancer treatment.

A docking study was also carried out for **2b, 2c, 2d, 2g** and **2h** complexes to determine the its binding interaction with protein pockets. The calculated binding energies of **2b, 2c, 2d, 2g** and **2h** were −10.0, −7.9, −8.00, −7.1 and −9.3 kcal/mol, respectively. Additionally, H-bonds and C–H bonds were observed between ASP218 and **2b**; MET172 and **2c**; GLU170 and **2g**; ASN and **2h**. H-bond with ASP218, C–H bond with ASP176, π-σ interaction with PHE103, LEU221, π-π stacked with PHE103, alkyl interaction with MET:172, MET:169, LYS:121, TYR:171, and pi-alkyl interaction with PHE:384, VAL:106, ALA:231, PHE:103, TYR:171 were noticed for **2b**. In **2c, 2d, 2g** and **2h**, the Hydrogen bonding, C–H bonding, Pi-Anion, Pi-Pi Stacked, Alkyl and Pi-alkyl with amino acid residues are remarkable. The complexes docking results were compared to standard drug (cisplatin). The interaction of cisplatin and the amino acid residue was evaluated, and their binding affinity was calculated as −4.1 kcal/mol, as shown in the table. According to the result of molecular docking, all the complexes showed more activity than

the cisplatin. Significant van der Waals interactions of complexes with several amino acids exist. Among the silver complexes, complex **2b** is noteworthy. The detailed of silver complexes interaction with amino acid residue are shown in Table 3, Figure 6. The bond types with NH, CH_2N, C_6H_6, CH_3, etc., were observed in the docking studies of SH-SY5Y cell lines. The complexes have electron-donating groups that may inhibit oxidative damage, mitochondria dysfunction and cell apoptosis. The Binding of amino acid residue with complexes can stabilize the active pocket and cause lowering bind energy [55]. It is interesting that the silver complexes showed excellent anticancer activity and more negative binding energies than the positive control (cisplatin) in molecular docking. Thus, the docking result showed a resemblance to *in vitro* activities.

Although the exact MOA of silver complexes is not clear, according to the literature [56]. The metabolism, transport and cell respiration can also be effected by the interaction of silver with cell surface. The proposed mechanism of action of silver complexes demonstrated in the literature that silver cations bind to surfaces of cell and interact with proteins and, enzymes which are essential for the cell wall synthesis, may cause apoptosis by internal/external stress interaction [57, 58, 59]. Silver cations are more bioavailable and active when they are bound to target cell [57]. Furthermore, it also described that silver cations inhibit the functionalities of cancer cells by getting deposited in the cytosol and inhibit enzymes and proteins. The NHCs cytotoxic effect could probably be due to high lipophilicity, transporting Ag–NHC complexes into the cell. As a result, Ag–NHCs may be hazardous by hindering biomolecule metabolism and cellular respiration [57]. In line with this information, we think that the antiproliferative effect of Ag(I)–NHCs might be the lipophilic nature of the complexes that transport NHCs to the cells and organelles where silver can causes toxicity by preventing metabolism and respiration of cells.

When the antitumor activities of Ag(I)–NHC complexes presented here were compared with the antitumor activities of similar complexes available in the literature [39, 40, 41, 42, 58, 60, 61, 62, 63, 64], it was seen that the complexes presented here had similar activity to the Ag(I)–NHC complexes presented in the literature. However, **2a-i** complexes have been observed to exhibit better anticancer activity than both cisplatin and many other complexes reported in the literature, even at low concentrations in the MCF-7 and SH-SY5Y cell lines.

These observations point out that (a) the modification or fine-tune of the steric and electronic properties of NHCs through the *N*-substituents (contains hetero atoms) is crucial and that (b) the anions type and electron-donating methyl groups on benzimidazole ring have a

Table 3. Report of predicated interactions of SH-SY5Y cell line docking confirmations and docking scores.

S. No	Bonding interaction	Interacting amino acid residue	Bond type with	Bond distance (Å)	Binding affinity (Kcal/mol)
2b	Hydrogen bonding	ASP:218	NH	2.44	−10.0
	C–H bonding	ASP:176	CH_2N	3.47	
	Pi-Sigma	PHE:103, LEU:221	C_6H_6, CH_3	3.61, 3.91	
	Pi-Pi Stacked	PHE: 103	C_6H_6	4.34	
	Alkyl	MET:172, MET:169, LYS:121, TYR:171,	C_6H_6, CH_3	5.03, 4.77, 5.01, 4.37	
	pi-alkyl	PHE:384, VAL:106, ALA:231, PHE:103, TYR:171	CH_3	4.60, 4.35, 4.14, 3.61, 4.43, 5.49,	
2c	Hydrogen bonding	MET:172	CH_2O	1.97	−7.9
	C–H bonding	MET:172, GLU:170	CH_2O	3.61, 3.25	
	Pi-Anion	ASP:232	C_6H_6	4.13	
	Pi-Pi Stacked	PHE: 103	C_6H_6	4.13	
	Alkyl	VAL:106, ALA:119, LEU:221, MET:172,	$C4H8NO$	5.16, 4.03, 54.67, 5,29	
	pi-alkyl	PHE:384,	Cl	4.73	
2d	Pi-Cation	LYS:116, LYS:224	C6H6	3.01, 3.19	−8.0
	Pi-Pi Stacked	PHE: 156	C6H6 (CH3)3	4.56	
	Alkyl pi-alkyl	PRO:173	(CH3)3, C6H6	4.51	
		PRO:173, LYS:224, PHE:156		5.15, 4.73, 4.59	
2g	C–H bonding	GLU:170	OCH3	3.63	−7.1
	Pi-Sigma	VAL:106, LEU:221	C6H6	3.99, 3.90	
	Pi-Pi T shaped	PHE:103	C6H6	4.71	
	Pi-Alkyl	VAL:89, LYS:90, LYS:91	C6H6, Cl	3.17, 5.35	
2h	C–H bonding	ASN:219	CH2N	3.46	−9.3
	Pi-Sigma	LEU:221	C6H6	3.80, 3.88, 3.91	
	Alkyl	LYS:121	Cl	5.01,	
	Pi-Alkyl	VAL:106, ALA:231, MET:169, PHE:103	C6H6, Cl	4.56, 5.39, 5.26, 4.50, 4.67	
Cisplatin	C–H bonding	TYR:256, ARG:35, ASN:42	NH	2.03, 2.22, 2.53	−4.1
	Salt bridge	GLU:44	NH^+	2.60, 2.99, 5.31	

significant influence on the antiproliferative activity of Ag(I)–NHC complexes and that (c) having properties that facilitate the cellular up-take of complexes into cells. This study clearly showed the metallodrugs potential of this structurally different Ag (I)–NHC complexes. Thus, the outcome of our results is according to the previously reported work in cancer cell lines which is a proof that silver ions deposition in the cell contributes to the cytotoxic activity of the tested compounds.

3.2.2.1. ADME and pharmacokinetic profile. ADME (Absorption, Distribution, Metabolism and Excretion) prediction was calculated to evaluate the druglikeness behaviour of all complexes. ADME is a vital process in discovery and development of drug. An ideal drug candidate, after administration, rapidly absorbs into the systemic circulation and eliminates without affecting the biological activity. The *in silico* studies for lead optimization plays the leading role in determining physicochemical

properties. Because of these reasons, all complexes were screened for its ADME study. A drug consider to be an ideal that must be non-toxic, easily absorbed in the systemic circulation, and show specific biological responses without affecting other organs. SwissADME database was used for the prediction of drug-like characters for all complexes. Except for **2d, 2g** and **2h,** all the complexes showed drug-like characteristics according to the Lipinski rule, as shown in Table 4. According to Lipinski rule, also known as Pfizer's rule of five, should follow the following rule, i.e. five (MW < 500, LogP <5, Hydrogen bond donor ≤5, Hydrogen bond acceptor ≤10) [65]. As shown in Table 4, some of the complexes violate the Lipinski rule but generally, it is acceptable because the drug shows that not more than one violation is considered to be a good orally absorbed [66]. Some drugs, such as sorafenib, atorvastatin and several natural products [67], violate the rule of five but are given orally.
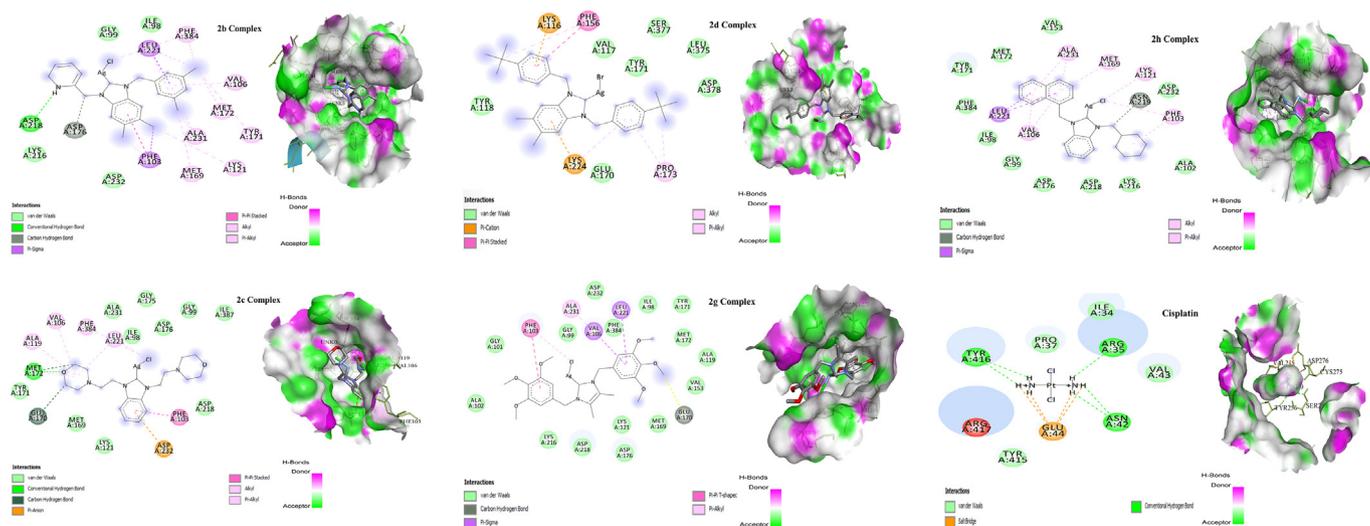

Figure 6. 2D and 3D diagram of the binding interactions of **2b, 2c, 2d, 2g, 2h** silver complexes and Cisplatin with the active site residues of the SH-SY5Y cell line receptor.

Table 4. Lipinski's rule of five (RO5) for the drug-likeness of all NHC complexes.

Compound	M.Wt	HBA	HBD	LogP	TPSA	GI absorption	BBB permeant	P-gp substrate	Lipinski violation
2a	410.64 g/mol	2	0	1.77	28.60 Å^2	High	Yes	Yes	No
2b	498.79 g/mol	1	0	4.04	19.37 Å^2	High	Yes	Yes	No
2c	487.77 g/mol	4	0	0.83	31.42 Å^2	High	No	Yes	No
2d	626.41 g/mol	0	0	6.29	6.48 Å^2	Low	No	Yes	No; 2 violations: MW > 500, LogP>5
2e	484.77 g/mol	1	0	3.75	19.37 Å^2	High	Yes	Yes	No
2f	472.71 g/mol	2	0	2.84	28.60 Å^2	High	Yes	Yes	No
2g	621.85 g/mol	6	0	3.21	61.86 Å^2	High	Yes	Yes	Yes; 1 violation: MW > 500
2h	542.25 g/mol	0	0	4.83	6.48 Å^2	Low	No	Yes	Yes; 1 violation: MW > 500
2i	459.80 g/mol	2	0	2.36	12.96 Å^2	High	Yes	No	No

M.Wt = Molecular weight, HBA = Hydrogen bond acceptors, HBD = Hydrogen bond donors, LogP = octanol–water partition co-efficient, TPSA = Topological Polar Surface Area, GI = Gastrointestinal, BBB = Blood Brain Barrier, P-gb = P-glycoprotein.

SwissADME also provides several parameters to predict whether the compound will be absorbed, distributed, metabolized and excreted. All the complexes except **2d, 2g** and **2h** showed low GI absorption, and these complexes also showed Lipinski rule violation. All the complexes that accept **2i** showed to be the substrate for p-glycoprotein. So that it can be easily entered into the systemic circulation and the brain, it is also essential that the compound be quickly metabolized and excreted from the body-this help in minimizing the possible side effect [68].

Almost all the complexes showed well *in silico* ADME profiles that can be further investigated by *in vivo* and molecular studies.

4. Conclusions

Herein, a series of Ag–NHC complexes were reported, and anticancer activity was investigated against MCF-7, SH-SY5Y, and L929 cell lines. The cytotoxic activities of complex **2a-d** against SH-SY5Y and complex **2d-i** against MCF-7 cell lines showed excellent and better than the positive control cisplatin. Special attention needs for **2b, 2c, 2e, 2g, 2h** and **2i** complexes because these showed excellent anticancer activity and low toxicity against SH-SY5Y and MCF-7 cell lines. This finding indicates that the type of *N*-substituents, which contains heteroatoms such as O- and N- on NHC, may improve the high cytotoxic activity against SH-SY5Y, MCF-7, and low cytotoxic activity against L929 cells. The docking results also revealed that all complexes showed similar potential for *in vitro* anticancer activities. Except for **2d, 2g** and **2h**, all the complexes showed drug-like characteristics and low GI absorption. However, all the complexes that accept **2i** showed to be the substrate for p-glycoprotein.

Declarations

Author contribution statement

Mitat Akkoç: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Siraj Khan: Analyzed and interpreted the data; Wrote the paper.

Hande Yüce; Neşe Başak Türkmen: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Şeyma Yaşar: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sedat Yaşar: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

İsmail Özdemir: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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