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ARTICLE *in* INORGANICA CHIMICA ACTA · JANUARY 2013

Impact Factor: 2.05 · DOI: 10.1016/j.ica.2012.09.013

Inorganica Chimica Acta 394 (2013) 519–525

Contents lists available at SciVerse ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Non-symmetrically substituted *N*-heterocyclic carbene–Ag(I) complexes of benzimidazol-2-ylidenes: Synthesis, crystal structures, anticancer activity and transmetallation studies

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ARTICLE INFO

Article history:
Received 14 July 2012
Received in revised form 6 September 2012
Accepted 19 September 2012
Available online 29 September 2012

Keywords:
Anticancer agent
Ag(I)–carbene complex
N-Heterocyclic carbene
Transmetallation
X-ray diffraction

ABSTRACT

Non-symmetrically substituted benzimidazolium salts (**1** and **2**) having bromide counterion undergo metallation with Ag_2O at stiochiometric ratio, giving mononuclear bis-carbene Ag(I) complexes (**3** and **4**), active as anticancer agents against Human Colorectal (HCT 116) cell lines. Both the complexes provide good activity (IC_{50} = 13.9 μM for **3** and 14.6 μM for **4**) in the anticancer studies and so distinctly better than the ligand precursors (IC_{50} = 119.3 μM for **1**, and 70.0 μM for **2**). The molecular structure of Ag complexes **3** and **4** is elucidated by X-ray diffraction studies. The Pd(II)– and Au(I)–NHC complexes (**5** and **6**) were synthesized from **4** via the technique of transmetallation. However, attempts to produce transmetallated complexes using **3** were unsuccessful.

1. Introduction

Benzimidazole derivatives are important constituents in most pharmacologically, catalytically, and biologically active compounds, and therefore correspond to significant synthetic targets [1,2]. Transition metal derivatives of *N*-heterocyclic carbene (NHC) ligands encompassing benzimidazole hub demonstrate great utility across a wide variety of metal based drugs [3]. Imidazole and benzimidazole derived NHC precursors are known in the field of organometallic chemistry for more than five decades, and imidazole-based derivatives may have been the first NHC precursors ever used by the organometallic chemists. However, transition metal derivatives of benzimidazole-derived NHCs have a rich history in catalysis and pharmacology, starting with pioneering contributions by Hahn and co-workers at the end of twentieth century [4,5]. Among the wide range of biological applications, *N*-substituted benzimidazolium salts are known to inhibit a vast number of cytochrome P450 enzymes employed in hepatic drug metabolism and steroid biosynthesis. In particular, *N*-substituted benzimidazolium salts have been reported to poses antithrombotic, herbicidal, anthelmintic and catalytic properties since long back [6].

N-Allyl substituted versions of these popular ligand precursors for the preparation of transition metal–carbene complexes are rapidly emerging, however, only a few unique mono- and bis-NHC architectures exist [7]. Interestingly, *N*-allyl-substituted dibenzotetraazafulvalenes react through rearrangement and produce dealkylated products [8]. With the successful applications of symmetric and asymmetric allyl/alkyl substituted ligands in preparation of biologically active compounds, the rational design and such ligands has been an important strategy for the development of silver based drugs, which can significantly reduce the toxicity and other side effects. In the recent years researchers have explored silver, gold and platinum-based NHCs having benzimidazole core as excellent antitumor and anticancer agents in the form of complexes. Their various biological applications with fewer side effects are now attracting the world's attention [9]. Group-XI elements are so medicinal that simple amine derivatives of which are known as "Drugs" in cancer treatment (cisplatin, carboplatin, etc.) [10]. Recently, palladium complexes are also used for the treatment of cancer with fewer side effects [11]. Complexes designed for this particular work are on the basis of active sites of cisplatin and its analogs used for cancer. Following cisplatin administration, one of the chlorides is displaced by water, and forms aqua-coordinated platinum, which is responsible to bind to guanine bases. Either in a similar way or by the slow displacement of one of the NHCs by water to form aqua-Ag-complex, which can

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http://dx.doi.org/10.1016/j.ica.2012.09.013

further target the DNA of an infected cell. Most notable among the similarities of reported complexes with cisplatin are the linear C–Ag–C fragment (N–Pt–Cl in cisplatin) and the basicity of the Ag ion to bind DNA of the infected cell to arrest cell growth. A literature survey revealed that the Ag(I)–NHC complexes of imidazole and benzimidazole show an appreciable level of anticancer activity against different cancer cell lines (Chart 1). Associated activity of the complexes is mainly due to the presence of number of (benz)imidazolium units and the nature of coordinated anions. Herein we report synthesis, crystal structure, anticancer activity and transmetallation studies of two new Ag(I)–NHC complexes, in which the ligand scaffolds have been modified by installation of allyl/butyl substituents.

2. Experimental

2.1. Reagents and instruments

All chemicals and solvents were obtained from commercial sources and used without further purifications. The benzimidazole, Ag_2O, benzyl bromide, allyl bromide, butyl bromide, 5-fluorouracil (standard), and 3-[4,5-yl]-2,5-diphenyltetrazolium bromide for the MTT assay were purchased from Sigma–Aldrich. 1-Benzylimidazole and 1-benzylbenzimidazole were prepared according to the literature method with slight modifications. The carbene ligand precursors, 1-allyl-3-benzylbenzimidazole bromide (**1**) and 1-benzyl-3-butylbenzimdazole bromide (**2**) were prepared according to an established procedure [12,13] with slight modifications. The FTIR spectra of the compounds were recorded in potassium bromide disks using a Perkin Elmer 2000 system spectrometer in the range 4000 to 400 cm^{-1}. The NMR spectra were recorded in d_6-DMSO using Bruker 500 MHz Ascend spectrometer at ambient temperature with TMS as an internal reference. All the compounds were analyzed for C, H, and N using a PerkinElmer 2400 Series II microanalyzer. The X-ray diffraction data were collected using a Bruker SMART APEX2 CCD area-detector diffractometer. Calculations, structure refinement, molecular graphics and the material for publication were performed using the SHELXTL and PLATON (Spek, 2009) software packages.

2.2. Syntheses

2.2.1. Synthesis of Ag–carbene complex *3*

To a suspension of Ag_2O (0.46 g, 0.2 mmol) and **1** (0.249 g, 0.1 mmol) in methanol (20 mL) was stirred at room temperature for 10 h. The flask was wrapped with aluminum foil to avoid light. The reaction mixture was filtered through a pad of Celite, and the resulted colorless filtrate was concentrated to 5 mL under vacuum. The white solid was afforded by addition of 75 mL diethyl ether. So obtained solid was redissolved in acetonitrile and 150 mL of diethyl ether was added to reprecipitate the solid, isolation by filtration and vacuum dried to yield **3**. Yield: 0.149 g, 71.8%, M.P.: 212°. ^{1}H NMR (500 MHz, d_6-DMSO, 298 K): δ 5.05 (2H, d, N–CH_2, J = 7.5 Hz), 5.16 (1H, m, CH_2–CH=CH_2), 5.59 (2H, s, N–CH_2 benzyl), 5.96 (2H, m, CH=CH_2), and 7.16–7.37 (5H, m, Ar–H). ^{13}C{^{1}H}NMR (125 MHz, d_6-DMSO, 298 K): δ 50.01 (N–CH_2CH), 51.4 (N–CH_2–CH=CH_2), 114.8 (N–CH_2Ar), 121.5 (CH=CH_2), 127.6, 129.8 (ArC), 131.8, 134.3 (benzimidazolium ArC), and 189.07 ($C_{carbene}$–Ag). FTIR (KBr disc) cm^{-1}: 2868, 2923 ν(C–H), 1476, 1192 ν(benzimidazole ring vibration). *Anal.* Calc. for $C_{34}H_{34}N_4AgBr$: C, 59.5; H, 5.0; N, 8.2. Found: C, 59.8; H, 5.2; N, 7.9%.

2.2.2. Synthesis of Ag–carbene complex *4*

This compound was prepared in a manner analogous to that for **3**, only with **2** (0.347 g, 1 mmol) instead of **1**. Yield: 0.16 g, 71.1%, M.P.: 236 °C. ^{1}H NMR (500 MHz, d_6-DMSO, 298 K): δ 0.96 (3H, t, CH_3, J = 7.25 Hz), 1.35 (6H, m, CH_2–CH_2–CH_3), 1.93 (5H, m, CH_2–CH_2–CH_3), 4.53 (2H, t, N–CH_2, J = 7.75 Hz), 5.79 (2H, s, N–CH_2–Ar), 7.38–7.66 (5H, m, Ar–H), and 7.96 (4H, t, benzimidazole ArH, J = 7.5 Hz). ^{13}C{^{1}H}NMR (125 MHz, d_6-DMSO, 298 K): δ 13.3 (CH_3), 19.2 (CH_3–CH_2–CH_2), 30.5 (CH_3–CH_2–CH_2), 46.4 (N–CH_2), 50.2 (N–CH_2, benzyl), 113.3, 126.5.7, 128.6 (Ar–C), 131.2–133.6

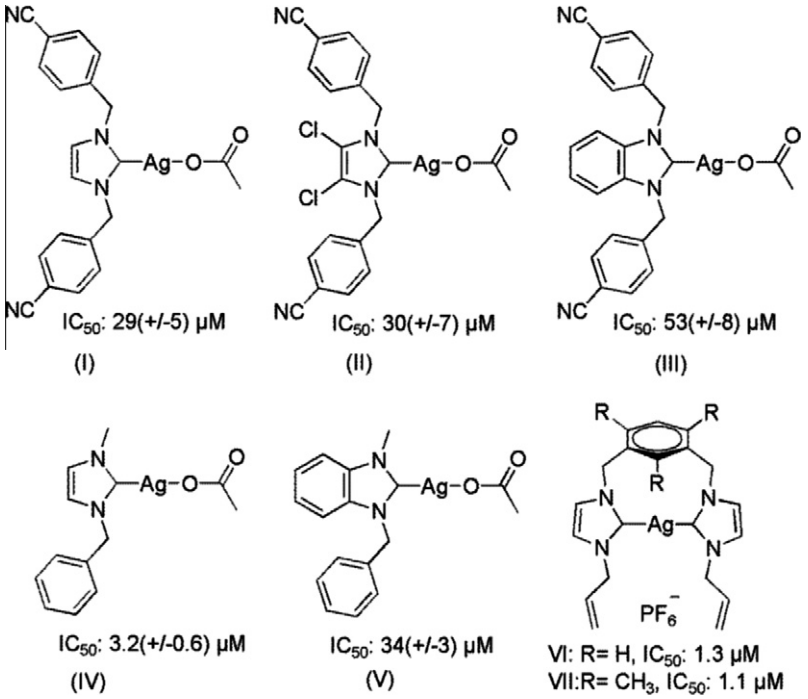


Chart 1. Anticancer potential of (benz)imidazole based silver complexes (I–V against Caki-1, and VI and VII against HCT cells from MTT assays).

(benzimidazolium ArC), and 188.4, 189.0 ($C_{carbene}$–Ag). FTIR (KBr disc) cm^{-1}: ~2860, 2925 ν(C–H), 1488, 1187 ν(benzimidazole ring vibration). *Anal.* Calc. for $C_{39}H_{48}N_4AgBrO$: C, 60.3; H, 6.2; N, 7.2. Found: C, 60.6; H, 6.0; N, 7.8%.

2.2.3. Synthesis of Pd–carbene complex ***5***

A mixture of Ag–carbene complex **4** (0.194 g, 0.25 mmol) and $Pd(COD)Cl_2$ (0.071 g, 0.25 mmol) in dichloromethane (25 mL) was stirred for 10 h at room temperature, and a pale yellow solution was formed. The resulting yellow solution was filtered through a pad of Cilite, and concentrated to 5 mL, and petroleum ether (50 mL) was added to precipitate the pale yellow colored powder. So obtained solid was redissolved in acetonitrile (10 mL) and 150 mL of diethyl ether was added to reprecipitate the solid, isolation by filtration and vacuum dried to yield **5**. Yield: 0.114 g, 64.9%. M.P.: 244 °C. ^{1}H NMR (500 MHz, d_3-CD_3CN, 298 K): δ 0.82 (3H, t, C*H*$_3$, J = 6.75 Hz), 1.14 (3H, t, C*H*$_3$, J = 6.5 Hz), 1.28 (6H, m, C*H*$_2$–CH_2–CH_3), 1.65 (6H, m, CH_2–C*H*$_2$–CH_3), 2.15 (5H, m, CH_2–C*H*$_2$–CH_3), 2.31 (5H, m, CH_2–C*H*$_2$–CH_3), 4.77 (2H, t, N–C*H*$_2$, J = 7.0 Hz), 4.95 (2H, t, N–C*H*$_2$, J = 6.5 Hz), 6.10 (2H, s, N–C*H*$_2$–Ar), 6.24 (2H, s, N–C*H*$_2$–Ar), 7.13–7.68 (5H, m, Ar–*H*), and 7.90–8.19 (4H, t, benzimidazole Ar*H*, J = 6.25 Hz). ^{13}C{^{1}H}NMR (125 MHz, d_6-DMSO, 298 K): δ 13.5 (*C*H$_3$), 20.7 (CH_3–*C*H$_2$–CH_2), 32.6 (CH_3–CH_2–*C*H$_2$), 48.5 (N–*C*H$_2$), 52.9 (N–*C*H$_2$, benzyl), 110.6, 123.2, 127.9 (Ar–*C*), 134.2, 135.1 (benzimidazolium Ar*C*), and 182.1 ($C_{carbene}$–Pd). FTIR (KBr disc) cm^{-1}: ~2860, ~2920 ν(C–H), 1496, 1182 ν(benzimidazole ring vibration). *Anal.* Calc. for $C_{36}H_{42}N_4PdCl_2$: C, 61.1; H, 6.0; N, 7.9. Found: C, 61.6; H, 6.3; N, 8.1%.

2.2.4. Synthesis of Au–carbene complex ***6***

This compound was prepared in a manner analogous to that for **5**, only with $[AuCl(PPh_3)_2]$ (0.189 g, 0.25 mmol) instead of $Pd(COD)Cl_2$. Yield: 0.151 g, 79.0%, M.P.: 210 °C. ^{1}H NMR (500 MHz, d_6-DMSO, 298 K): δ 0.91 (3H, t, C*H*$_3$, J = 6.25 Hz), 1.35 (6H, m, C*H*$_2$–CH_2–CH_3), 1.96 (5H, m, CH_2–C*H*$_2$–CH_3), 4.58 (2H, t, N–C*H*$_2$, J = 7.0 Hz), 5.76 (2H, s, N–C*H*$_2$–Ar), 7.28–7.65 (5H, m, Ar–*H*), and 7. 89 (4H, t, benzimidazole Ar*H*, J = 6.25 Hz). ^{13}C{^{1}H}NMR (125 MHz, d_6-DMSO, 298 K): δ 13.0 (*C*H$_3$), 19.5 (CH_3–*C*H$_2$–CH_2), 31.8 (CH_3–CH_2–*C*H$_2$), 48.4 (N–*C*H$_2$), 51.5 (N–*C*H$_2$, benzyl), 112.0, 125.2, 127.0 (Ar–*C*), 133.2, 135.6 (benzimidazolium Ar*C*), and 190.3 ($C_{carbene}$–Au). FTIR (KBr disc) cm^{-1}: 2862, 2932 ν(C–H), 1493, 1181 ν(benzimidazole ring vibration). *Anal.* Calc. for $C_{18}H_{21}N_2AuCl$: C, 43.4; H, 4.3; N, 5.6. Found: C, 43.9; H, 5.9; N, 5.8%.

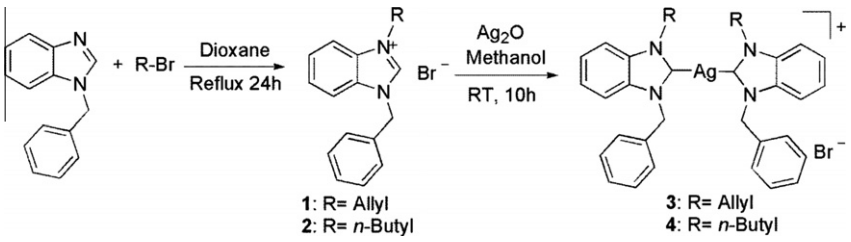
2.3. Anticancer activity

2.3.1. Preparation of cell culture

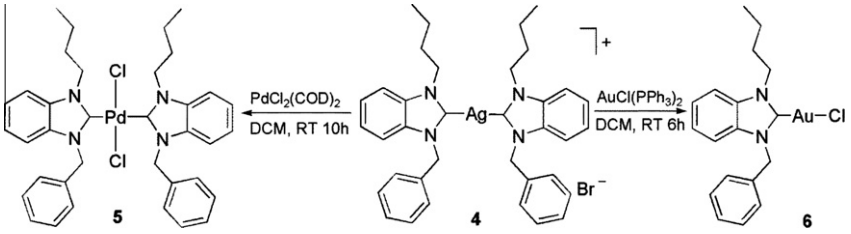
Initially, cells (HCT 116) were allowed to grow under optimal incubator conditions. Cells that have reached a confluency of 70–80% were chosen for cell plating purposes. Old medium was aspirated out of the plate. Next, cells were washed using sterile phosphate buffered saline (PBS) (pH 7.4), 2–3 times. PBS was completely discarded after washing. Following this, trypsin was added and distributed evenly onto cell surfaces. Cells were incubated at 37 °C in 5% CO_2 for 1 min. Then, the flasks containing the cells were gently tapped to aid cells segregation and observed under inverted microscope (if cells segregation is not satisfying, the cells will be incubated for another minute). Trypsin activity was inhibited by adding 5 ml of fresh complete media (10% FBS). Cells were counted and diluted to get a final concentration of 2.5×10^5 cells/mL, and inoculated into wells (100 μL cells/well). Finally, plates containing the cells were incubated at 37 °C with an internal atmosphere of 5% CO_2.

2.3.2. MTT assay

Cancer cells (100 μL cells/well, 1.5×10^5 cells/mL) were inoculated in wells of microtitre plate. Then the plate is incubated in CO_2 incubator for overnight in order to allow the cell for attachment. One hundred microliters of test substance were added into each well containing the cells. Test substance was diluted with media into the desired concentrations from the stock. The plates were incubated at 37 °C with an internal atmosphere of 5% CO_2 for 72 h. After this treatment period, 20 μL of MTT reagent was added into each well and incubated again for 4 h. After this incubation period, 50 μL of MTT lysis solution (DMSO) was added into the wells. The plates were further incubated for 5 min in CO_2 incubator. Finally, plates were read at 570 and 620 nm wavelengths using a standard ELISA microplate reader. Data were recorded and analyzed for the assessment of the effects of test substance on cell viability and growth inhibition. The percentage of growth inhibition was calculated from the optical density (OD) that was obtained



Scheme 1. Synthesis of allyl/alkyl substituted benzimidazol-2-ylidenes and their Ag–carbene complexes.



Scheme 2. Synthetic pathway to Pd– and Au–carbene complexes via transmetallation reactions.

Table 1
Crystal data and structure refinement details for Ag–carbene complex **4**.

	3	**4**
Formula	$C_{37}H_{34}AgBrN_5O$	$C_{39}H_{48}N_4AgBrO$
Formula weight	697.19	701.66
Crystal system	triclinic	triclinic
Space group	$P\bar{1}$	$P\bar{1}$
Unit cell dimensions		
a (Å)	10.5279(2)	12.3083(4)
b (Å)	10.7966(3)	13.0893(5)
c (Å)	14.6437(2)	13.5459(4)
α (°)	108.4830(10)	115.974(2)
β (°)	91.926(2)	102.260(2)
γ (°)	100.9250(10)	103.788(2)
V (Å^3)	1542.15(6)	1775.58(10)
Z	2	2
D_{calc} (g/cm^3)	1.501	1.312
Absorption coefficient (mm^{-1})	0.698	0.604
$F(000)$	717	732
Crystal size (mm)	0.39 × 0.25 × 0.13	0.56 × 0.5 × 0.17
T (K)	293	293
Radiation (Å)	Mo Kα 0.71073	Mo Kα 0.71073
θ Minimum, maximum (°)	1.98, 30.12	1.79, 34.18
Dataset	−14:14, −15:15, −20:20	−19:18, −20:20, −21:21
Total; unique data	32999	51931
R_{int}	0.0253	0.0679
N_{ref}, N_{par}	8981, 725	14382, 772
R, wR_2, S	0.0894, 0.2182, 1.318	0.0679, 0.1771, 1.085

from MTT assay, i.e., hundredth multiple of subtracted OD value of control and survived cells over OD of control cells.

3. Results and discussion

3.1. Syntheses and structural characterization

Ag–carbene complexes are known to adopt different architectures, depending on the reaction conditions employed, but above all they are the most popular compounds used in bioorganometallic chemistry and carbene transfer reactions which can be used to get access to a number of other late transition/inner transition metal–carbene complexes [3]. In the present study, we describe the preparations of carbene complexes and also investigated anticancer activity of benzimidazolium salts and their Ag complexes. Reaction of the precursor **1**/**2** with Ag_2O in a 2:1 molar ratio in methanol gave the complex **3**/**4** in good yield, after recrystallization from methanol–diethyl ether. Synthetic routes to the complexes **3** and **4** are shown in Scheme 1.

Further, Pd(II)– and Au(I)-carbene complexes (**5** and **6**) were synthesized in quantitative yields at a milder condition by transmetallation using Ag(I) complex **4** as a carbene transfer reagent (Scheme 2). Similar transmetallation reactions of Ag(I)–carbene complexes having cyclic and acyclic carbene ligands with [AuCl(SMe$_2$)] in acetonitrile have been reported [14–16]. However, all our attempts to get transmetallated products using Ag–carbene complex **3** were unsuccessful, even variable amounts of starting materials were used. Diffusion of diethyl ether into an acetonitrile solution of complex **4** afforded colorless block crystals suitable for single crystal X-ray diffraction studies. The benzimidazolium salts, Ag(I)–, Pd(II)–, and Au(I)–carbene complexes are thermally stable up to their melting points and are nonhygroscopic in nature. The complexes are insoluble in water, but are readily soluble in DMSO, DMF, (m)ethanol and acetonitrile. Both the ligands and their Ag–carbene complexes were evaluated for their *in vitro* cytotoxicity towards Human Colorectal (HCT 116) cell lines. Cytotoxic activity was evaluated by colorimetric MTT assay to calculate cell viability and growth inhibition.

Structures of both, ligand precursors and carbene complexes, in solution were deduced from the ^{1}H, ^{13}C and HMQC NMR spectra. The benzimidazolium C(2)–H showed a typical absorption in the ^{1}H NMR spectra of **1** and **2** at δ 10.05, which is consistent with those of other reported benzimidazolium salts [17]. Signals due to allyl and butyl protons in the spectra of **1** and **2** resonated at δ 5.42, 5.44, 5.82 and δ 0.96, 1.35, 1.93, 4.53, respectively. Singlets at δ 5.82 and 5.79 and multiplates in the range δ 7.38–7.72 are attributed to the benzylic methylene and aromatic protons, respectively. As a simple indicator for the success of the formation of carbene

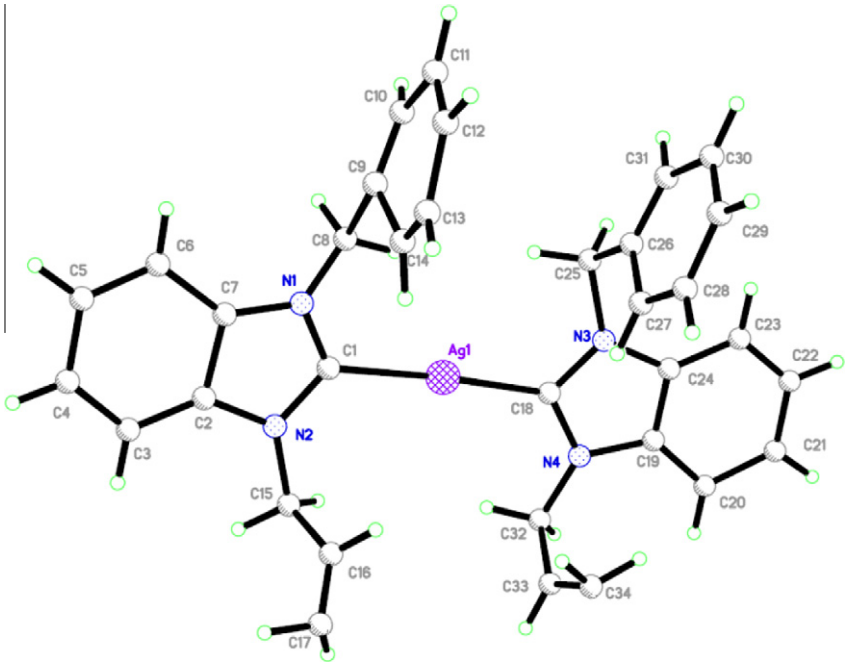


Fig. 1. A perspective view of the complex **3** with the displacement ellipsoids drawn at 50% probability. Bromide and solvent molecules were omitted due to clarity. Selected bond lengths (Å): C1–Ag1 1.73(3); C18–Ag1 2.051(16); C1–N1 1.27(2); C1–N2 1.366(17); N3–C18 1.37(2); N4–C18 1.381(15); N1–C8 1.34(5); N2–C15 1.47(3); N3–C25 1.57(2); N4–C32 1.41(2). Selected bond angles (°): C1–Ag1–C18 172.2(14); N1–C1–N2 124(2); N3–C18–N4 108.7(14); N1–C8–C9 107.3(18); N2–C15–C16 113.4(17); N3–C25–C26 113(3); N4–C32–C33 114.5(13).

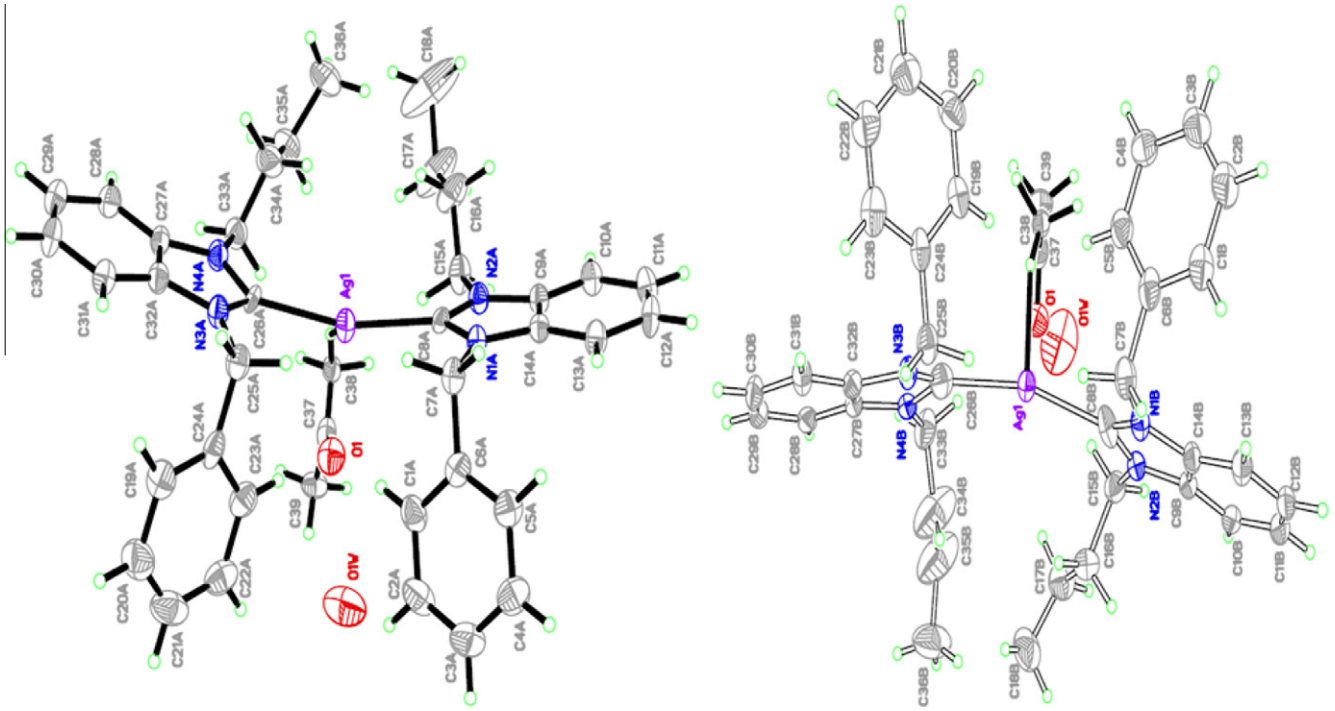


Fig. 2. Perspective views of two crystallographically independent structural units of **4** in a single crystal, displacement ellipsoids drawn at 50% probability. Selected bond lengths (Å): Ag(1)–C(8A) 2.096(18); Ag(1)–C(26A) 2.111(11); C(8A)–N(1A) 1.325(14); C(8A)–N(2A) 1.38(2); C(26A)–N(3A) 1.267(15); C(26A)–N(4A) 1.415(14); Ag(1)–O 2.608; O–Br 2.657. Selected bond angles (°): C(26A)–Ag(1)–C(8A) 157.1(6); C(8A)–Ag(1)–O 101.33; C(26A)–Ag(1)–O 99.69; N(1A)–C(8A)–Ag(1) 127.1(14); N(2A)–C(8A)–Ag(1) 126.3(9); N(3A)–C(26A)–Ag(1) 130.9(9); N(4A)–C(26A)–Ag(1) 121.9(9). Almost similar bond lengths and angles are found for other crystallographically different unit.

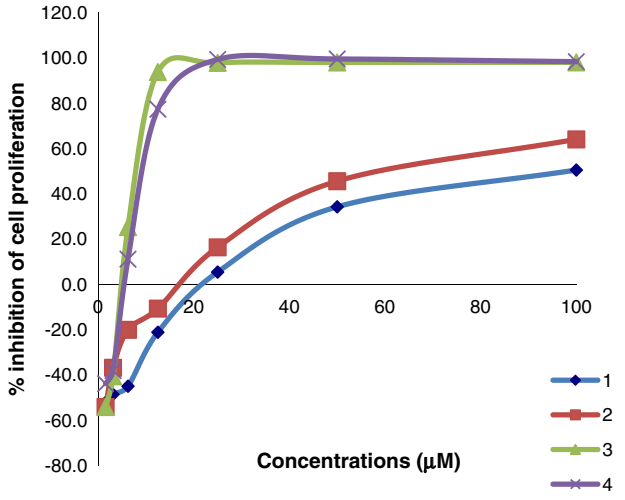


Fig. 3. MTT assay results of NHC precursors **1** and **2**, and Ag–carbene complexes **3** and **4** vs. the HCT 116 cell lines.

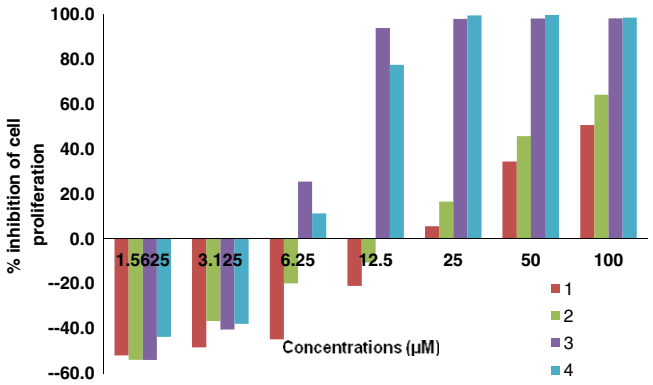


Fig. 4. Effects of increasing amounts of **1**–**4** on the percentage inhibition of cell proliferation.

complexes **3**–**6**, the disappearance of benzimidazoliumC(2)–H in the corresponding ^{1}H NMR spectrum could be used, proving that during the complex formation the acidic proton of the benzimidazole was deprotonated followed by subsequent coordination to the Ag(I), Au(I), and Pd(II) [18–20]. In the ^{13}C NMR spectra, for the same solutions, a signal in the range δ 182–190.4 is attributed to the carbene carbon bound to transition metal ion. Most likely due to the unsymmetrical arrangement and steric bulk of the NHCs, complex **5** exist in solution as a mixture of two rotamers.

In ^{13}C NMR spectrum of **4**, the chelating carbene carbon is observed as two doublets centered at δ 188.3 with a C–Ag coupling constant ($^{1}J_{C-Ag}$ = 197.12 Hz), which is consistent with the literature [21]. These doublets are attributed to carbene carbons bound to ^{107}Ag and ^{109}Ag centers. Apart from this major change all other peaks are appeared in the complex spectra with negligible change in peak position. HMQC spectra of the complexes showed correlation peaks corresponding to the proposed structures. FTIR spectra of the ligands showed a band of medium intensity in the range 1560–1590 cm^{-1}, which is assigned to benzimidazole ring ν(C=N) vibrations. In the complex spectra this band showed a shift of ~120 cm^{-1}, indicating the formation of free C–N module in the benzimidazole ring. Finally, the IR spectra of all compounds contain prominent bands at around 2900, 1220 and 1190 cm^{-1}, and are assigned to ν(C–H) and benzimidazole ring vibrations.

Crystal data and structure refinement details for carbene complexes **3** and **4** are tabulated in Table 1. Single crystals of **3**/**4** suitable for X-ray diffraction analysis were obtained from slow diffusion of diethyl ether into a solution of **3**/**4** in acetonitrile at ambient temperature. A perspective view of complexes **3** and **4** are shown in Figs. 1 and 2, respectively. Complex **3** crystallizes in the triclinic space group $P\bar{1}$ containing one bis-carbene complex

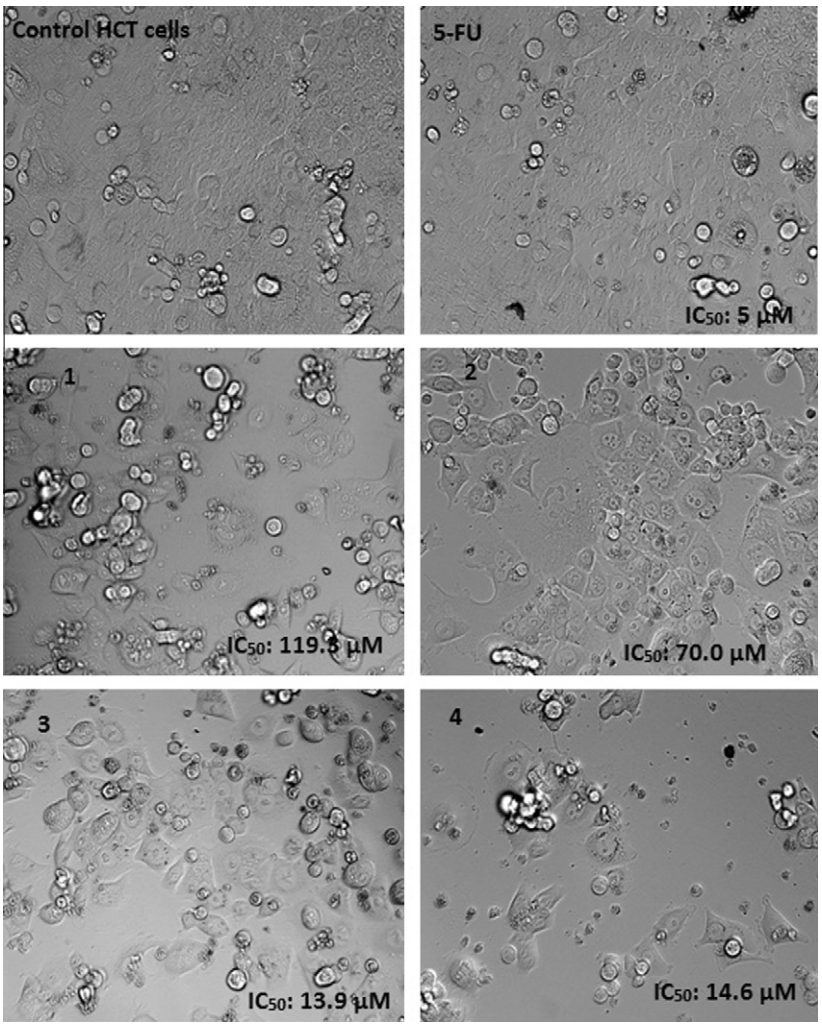


Fig. 5. Images of the control HCT116 cells, cells treated with 5-FU and compounds **1**–**4** after 72 h of incubation.

cation, one bromide anion and one acetonitrile molecule in an asymmetric unit. Both, the carbon and hydrogen atoms, of the general-position benzyl and allyl substitutions are disordered over two sets of sites. In the complex cation, the central Ag(I) is coordinated by two carbene carbon atoms, making an almost linear C1–Ag1–C18 bond with the bond angle 172.2(14)°. The carbene ligands adopt a *syn*-arrangement around the metal center, probably due to the sterically demanding nature of the benzyl substituents. Both the benzimidazole rings are twisted out of the C–Ag–C axis, forming a dihedral angle of 7.8(6)°. The internal ring angle at the carbene center (N–C–N) are 108(2) and 108.7(14) for N1–C1–N2 and N3–C18–N4, respectively, which agree well with the reported complexes [22,23]. In the extended crystal structure, the bromide anions link the Ag-complex cations into three-dimensional networks by C–H···Br hydrogen bonds with bond distances ranging from 2.86 to 3.170 Å, and are further consolidated by π–π stacking interactions with the bond distances 3.619 Å.

Complex **4** has been defined as a well ordered bromide salt. One unit of the complex formula comprises the asymmetric unit, i.e., one complex cation, one bromide anion and an acetone molecule, the former disposed about a crystallographic center of symmetry. While, the carbene ligands adopt a *syn*-arrangement, probably due to the sterically demanding nature of the benzyl substituents. An interesting result was obtained in complex **4**, viz, oxygen atom of acetone has a strong interaction with the Ag center. Therefore, the metal is not in an essentially linear fashion. The Ag atom is in a three-coordinate distorted trigonal-planar environment, with two carbene carbon coordination (Ag–C(8A) = 2.096(18) Å, Ag–C(26A) = 2.111(11) Å) as well as an acetone oxygen interaction. The benzimidazole rings are twisted out of the plane of benzyl benzene ring, and showing a dihedral angle of 22.8(4)°, which is quite large to the reported complexes. The C(8A)–Ag–C(26A) angle in **4** is reduced to 157.1(6)° compared to the somewhat larger angle in the previously reported Ag complexes having similar ligand architectures [22,23]. The interaction bond length between Ag atom and oxygen is being 2.608 Å, which is consistent with the reported similar isoelectronic complexes [24,25]. However, the interaction distance is adequately shorter than the sum of van der Waals radii (3.24 Å) of the respective elements for an Ag–O interaction. Probably, this insight may be useful in determining the mode of action of **4** as anticancer agent, as it may form strong interaction with DNA of an infected cell. In the extended structure, the **4** units are connected through C–H(benzimidazole)···Br and O(acetone)···Br hydrogen bonds with bond distances ranging from 2.405 to 2.657 Å.

3.2. Anticancer activity

Ag–carbene complexes have been sought for antimicrobial and anticancer treatments [26]. Numerous Ag complexes have displayed interesting anticancer potentials; however, their medicinal values have always been hampered by their poor solubility and higher cost of preparation. To increase the anticancer potential and solubility of Ag complexes, we looked for NHC ligand systems that could form the target complex in ionic form. Also, strong

σ-donor effect of NHCs can stabilize the carbene-complexes, which, in turn helps to avoid demetallation. Almost linear C–Ag–C module assists to target DNA directly according to the cisplatin paradigm, which ultimately trigger cell death.

The ability of the NHC precursors **1** and **2** and their respective Ag–carbene complexes **3** and **4** to kill human derived cancer cell was investigated using HCT 116 cell line and a standard bioassay, MTT method. HCT116 cells were treated with 5 μM of 5-fluorouracil (5FU) alone for 72 h, however, this concentration represents IC_{50} (72 h) value for 5FU in this cell line. Similarly, HCT 116 cell lines were continuously exposed to test compounds **1**–**4** for 72 h, and their effects on cellular viability were calculated. The profiles of percentage inhibition of cell proliferation against the concentration of the test compound were established to calculate IC_{50} values of each derivative. Percentage inhibition of cell proliferation was determined at different concentrations of the test compounds ranging from 1.5625 to 100 μM. Figs. 3 and 4 depict the effect of concentration of test compounds on HCT 116 cell lines after 72 h incubation time. The results, expressed as concentrations of the benzimidazolium salts **1** and **2**, required to inhibit the infected cell growth by 50% (IC_{50}) are 119.3 and 70.0 μM, respectively. On the other hand, the IC_{50} values of Ag–carbene complexes **3** and **4** are being 13.9 and 14.6 μM, these values are much lesser than their NHC precursors. In general, HCT 116 cell lines showed higher sensitivities to the antiproliferative effect of Ag–carbene complexes, while with respect to NHC precursors, were found to be bit resistant. This increase in antiproliferative effect of complexes is ascribed to the cellular apoptosis and cell cycle arrest [27,28]. However, the IC_{50} values of the comparable mono-carbene Ag(I) complexes (I–V) with imidazole and benzimidazole cores is 2- to 3-folds higher than the reported complexes [29]. Conversely, bis-imidazole complexes VI and VII with a xylyl spacer showed significantly higher anticancer activity [28].

HCT116 cell images from the control group showed fully confluent growth. Both the treated complexes affected the normal morphology of all most all the cells of the group which rendered the cells to lose viability. However, in the case of benzimidazolium salts, the moderate cytotoxic activity as the population of the HCT 116 cells is decreased drastically. Images of the control HCT116 cells, cells treated with 5-FU and compounds **1**–**4** after 72 h of incubation are depicted in Fig. 5.

4. Conclusion

In conclusion, *N*-benzyl-*N*′allyl/butyl substituted benzimidazole bromides **1** and **2**, and their respective Ag–carbene complexes (**3** and **4**) were synthesized. All compounds were characterized by FTIR, ^{1}H, ^{13}C and HMQC NMR spectroscopy and elemental analysis. A Pd– and a Au–carbene complexes **5** and **6** were prepared from the technique of transmetallation using **4** as carbene transfer agent. Molecular structure of Ag carbene complexes 3 and 4 is established by single crystal X-ray diffraction technique. The data of elemental analysis, FTIR, and different NMR techniques confirmed the bonding of **1** and **2** to metal ions through carbene carbons in 1:2 ratio (except Au-complex). Compounds **1**–**4** were tested for *in vitro* anticancer activities against HCT 116 cell lines. Ag–carbene complexes **3** and **4** possessed greater activity and selectivity than their ligand precursors **1** and **2**, and these values are in comparable range with the IC_{50} value of 5FU. As analogous complexes do exert similar activity, it is speculated from the available data that the increased antiproliferative effect of complexes **3** and **4** is associated with cellular apoptosis and cell cycle arrest. Catalytic and bioorganometallic activities of Pd– and Au–carbene complexes are underway.

Acknowledgments

R.A.H. thanks Universiti Sains Malaysia (USM) for the Short term Grant (203/PKIMIA/6311123) and the Research University (RU). Grant 1001/PKIMIA/811217. M.Z.G. thanks USM for the RU grant 1001/PKIMIA/844137 and Kufa University, Najaf, Iraq for a post-graduate research scholarship. S.B. thanks USM for a post-doctoral research fellowship.

Appendix A. Supplementary material

CCDC 881402 and 853548 contain the supplementary crystallographic data for complexes **3** and **4**, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.09.013.

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