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Amine-fnctionalized silver and gold *N*-heterocyclic carbene complexes: Synthesis, characterization and antitumor properties

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This work is dedicated to Professor Wolfgang Hermann on the occasion of his 70th birthday.

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ABSTRACT

Di*iso*propilamine-tethered benzimidazolium salts have been prepared as precursors for Ag(I)–NHC and Au(I)–NHC complexes. These NHC ligands were metallated with Ag_2O on moderate conditions to give novel silver-NHC complexes. Gold-NHC complexes have been obtained by transmetalation using the silver-NHC precursor. The structures of all compounds were characterized by ^1H NMR, ^{13}C NMR, IR and elemental analysis techniques. The cytotoxic properties of the silver(I) and gold(I) complexes have been assessed in various human cancer lines, including cisplatin-sensitive and resistant cell. IC_{50} values of these four complexes were determined by the MTS based assay on three human cancer cell lines (SHSY5Y, HTC116 and HEP3B) and human healthy cell line (HF). These silver and gold *N*-heterocyclic carbene complexes have been highlighted metal-based cancer therapeutic agent with unique structures and functions. These strategies provide exciting opportunities for discovering new type metalodrug.

1. Introduction

Cancer is rapidly becoming the top killer in the World. Most of the FDA approved anticancer drugs are organic molecules, while metallodrugs are very scarce. The advent of the first metal based therapeutic agent cisplatin launched a new era in the application of transition metal complexes for therapeutic design. The discovery of the anticancer properties of cisplatin and its successful introduction in the clinic in 1979 as chemotherapeutic agents revolutionized the research in inorganic chemistry [1–3]. Cisplatin and its analogues are frequently used for the treatment for many cancers, including ovarian, cervical, lung, testicular cancer as well as myelomas, melanomas and lymphomas [4]. However, their use has been associated with dose limiting side effects, including neurotoxicity, nephrotoxicity and nausea [5]. To overcome these problems, it is necessary to create new cytotoxic complexes by the metal variation and development of new carrier ligands. These ligands should be easily accessible in a few reaction steps and allow for a wide variation of their reactivity in biological media. *N*-heterocyclic carbene (NHC) ligands fulfill all these requirements and permit the design and synthesis of cytotoxic and antibacterial transition metal complexes [6–10]. NHCs display bonding properties roughly comparable to phosphines when applied as ligands [11–14]. Furthermore, NHC ligands are two electron σ-donors with little π-accepting ability [15,16]. They are considered to behave in several aspects similar to tertiary phosphines, but bind more strongly to the metal center and are additionally excellent electron donors.

Among the non-platinum drugs, silver(I) and gold(I) NHC complexes have attracted the most attention [17,18]. Recent studies have shown that Ag(I)–NHC complexes display *in vitro* anticancer

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activity against ovarian and breast cancer cells [19] whereas Au(I)–NHC complexes inhibit the enzyme thioredoxin reductase, which promotes the proliferation of tumor tissue [20] whose inhibition is related to the triggering of anti-mitochondrial effects. These results have encouraged further medicinal applications of NHC complexes of Ag(I) and Au(I) [21].

In a recent study we have reported the synthesis of azolium salts and their silver(I) complexes, which were tested as potential antimicrobial agents [22–24]. In the present article, we now described the synthesis and characterization of three benzimidazolium salts and three novel silver(I) and three gold(I) complexes of the general formula [MCl(NHC)] (M: Ag and Au) **2a-c** and **3a-c** (Scheme 1, 2) All the synthesized silver and gold complexes (**2a-c** and **3a-c**) were tested against the three human cancer cell lines (SHSY5Y, HTC116 and HEP3B) and healthy cell lines (human fibroblast).

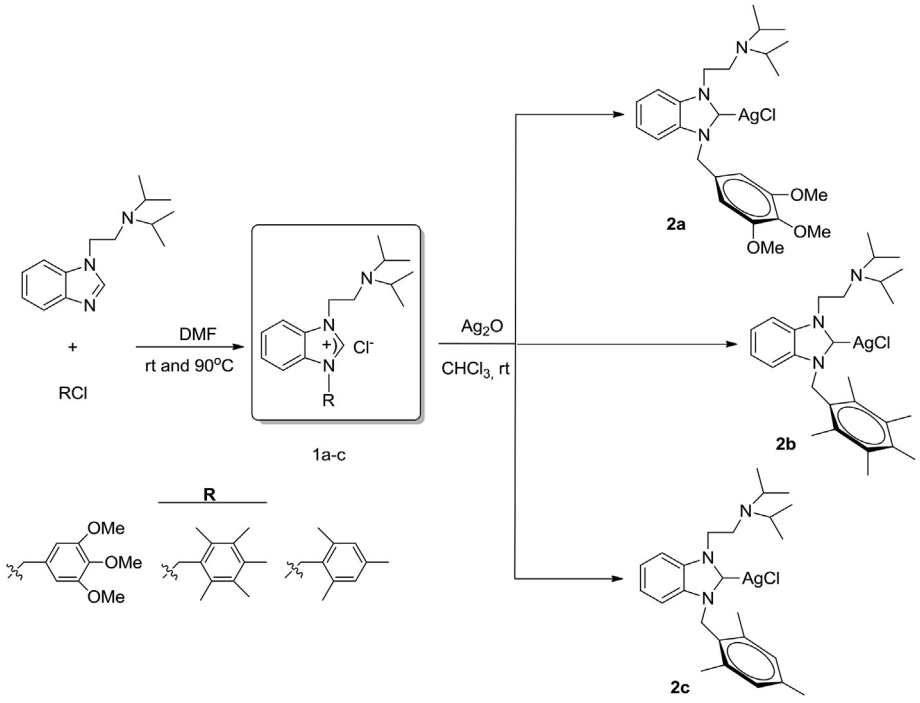
2. Results and discussion

2.1. Chemistry

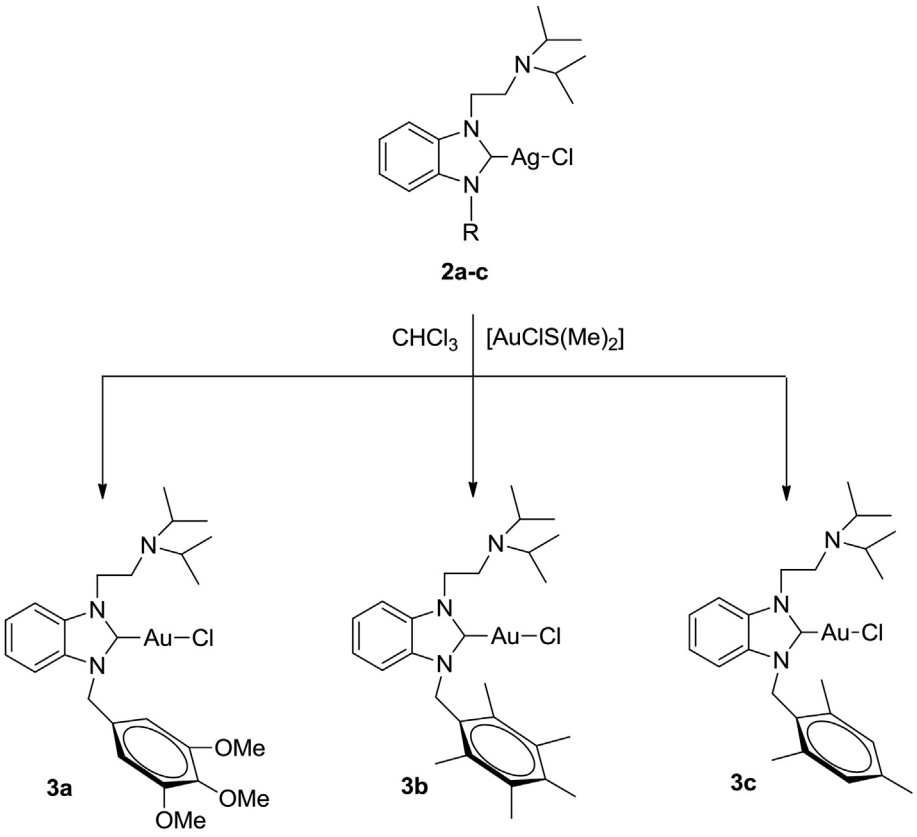
Di*iso*propilamine-tethered benzimidazolium salts **1a-c** {1-[2-(di*iso*propylamino)ethyl]-3-R}benzimidazolium chloride (**1a-c**) were prepared in good to excellent yields as previously described [25] starting from {1-[2-(di*iso*propylamino)ethyl]}benzimidazole and the corresponding alkyl chloride (Scheme 1). The salts **1a** and **1b** were fully characterized by elemental analysis, ^{1}H and $^{13}C\{^{1}H\}$ NMR and infrared spectroscopies (experimental part). The FT-IR spectra of the free ligands showed a broad band at 1595 and 1550 cm^{-1}, which corresponds to the –C=N- bond vibration of **1a** and **1b**, respectively. The signal of the NC*H*N proton appears in the expected range, at $\delta = 11.27$ and 9.92 ppm for **1a** and **1b**, respectively. Their ^{13}C NMR spectra display the characteristic singlet at 144.0 and 142.9 ppm for the NCHN carbon for **1a** and **1b**, respectively [26,27]. The silver complexes **2a-c** were synthesized according to the general method described by Wang and Lin[28] by reacting the corresponding benzimidazolium salts with Ag_2O in chloroform under exclusion of light at room temperature for 24 h (Scheme 1).

The air and moisture-stable silver and gold carbene complexes (**2a-c, 3a-c**) were soluble in solvents such as dichloromethane, chloroform, and insoluble in aqua and nonpolar solvents. The complexes, which are very stable both in the solid state and in solution have been characterized by analytical and spectroscopic techniques. The spectroscopic data are similar to those found for other silver(I)–NHC complexes [26,29,30]. The two silver(I) complexes exhibit a characteristic $\nu_{(NCN)}$ band at 1461, 1462 and 1454 cm^{-1} for **2a-c**, respectively. In their ^{1}H NMR spectra, the NC*H*N protons were disappeared upon complexation with silver. In the **2a-c** complexes, the resonances for Ag–carbene were not observed, which has also been mentioned in the literature and given as a reason for the fluxional behavior of the NHCs complexes [31–33].

The gold(I)–NHC carbene complexes (**3a-c**) were prepared by carbene transfer from Ag(I)–NHC complexes (**2a-c**) respectively to $[AuCl(CH_3)_2]$ in a degassed chloroform solution (Scheme 2) [26]. After filtration and evaporation to dryness, **3a**-c were obtained in 78%, 81% and 80% yields, respectively. The structures of the gold(I)–NHCs were determined by their characteristic spectroscopic data and elemental analyses techniques. Their ^{13}C NMR spectra compare well to those of analogous complexes described in the literature. The characteristic peak of the Au(I)–carbene resonance for compound **3a-c** displays as a singlet at 178.7, 179.2, and 179.5 ppm respectively in the ^{13}C NMR spectra. The chemical shift is well consistent with those of the known gold(I)–NHC complexes in the range of 173–195 ppm [28,34]. Gold(I)–NHC complexes (**3a-c**) exhibit characteristic $\nu_{(CN)}$ band typically at 1403, 1404 and 1405 cm^{-1} respectively. Additional elemental analyses of these complexes are in agreement with the molecular formula proposed (Table 1).



Scheme 1. Synthesis of benzimidazolium salts and silver complexes.



Scheme 2. Synthesis of gold complexes.

Table 1
Physical and spectroscopic properties of compounds.

Comp	Formula	Isolated yield (%)	m.p. (°C)	ν(CN) (cm^{-1})	H(2) ^{1}H NMR (ppm)	C(2) ^{13}C NMR (ppm)
1a	$C_{25}H_{36}N_3O_3Cl$	75	167.6	1595	11.27	144.0
1b	$C_{27}H_{40}N_3Cl$	85	216.8	1550	9.92	142.9
1c	$C_{25}H_{36}N_3Cl$	83	274.3	1550	10.62	143.6[25]
2a	$C_{25}H_{35}N_3O_3AgCl$	70	172.0	1461	–	not observed
2b	$C_{27}H_{39}N_3AgCl$	73	180.5	1462	–	not observed
2c	$C_{25}H_{35}N_3AgCl$	75	167.1	1454	–	not observed
3a	$C_{25}H_{35}N_3O_3AuCl$	81	177.4	1403	–	178.7
3b	$C_{27}H_{39}N_3AuCl$	78	143.5	1404	–	179.2
3c	$C_{25}H_{35}N_3AuCl$	80	175.5	1405	–	179.5

Table 2
IC_{50} values for NHC complexes (μM).

Complex	IC_{50}			
	Hep3B	SH-SH5Y	HTC116	HF
2a	106.8 ± 10.1	94.99 ± 9.23	51.34 ± 6.76	100.8 ± 10.4
2b	6.19 ± 1.09	5.23 ± 0.95	8.44 ± 1.07	60.6 ± 9.54
2c	7.89 ± 1.41	15.4 ± 2.43	3.39 ± 0.59	46.57 ± 8.43
3a	10.69 ± 1.86	18.83 ± 2.11	10.9 ± 0.98	20.26 ± 6.23
3b	11.49 ± 1.38	4.74 ± 0.91	7.14 ± 1.14	54.16 ± 6.85
3c	6.2 ± 1.12	8.15 ± 1.24	30.98 ± 3.65	64.84 ± 7.12

2.2. Antitumor activity

The IC_{50} values of all complexes against three human cancer cell lines (SHSY5Y, HTC116, HEP3B) and healthy cell line (HF) were measured and listed in Table 2. It was determined that Complexes **2a** showed the lowest cytotoxic activity against all cell lines. The cytotoxic effects of the other compounds were close to each other and have no significant differences between them. On the other hand, the **2b** (6.19 ± 1.09) and **3c** (6.2 ± 1.12) complexes were relatively more cytotoxic against Hep3B cell line than the other compounds. Similarly, **2b** (5.23 ± 0.95) and **3b** (4.74 ± 0.91) were more effective against SHSY5Y and **2c** (3.39 ± 0.59) was more cytotoxic against HTC116. For the all tested compounds, the IC_{50} values was determined higher in HF cells than the rest of tumor cell lines and we showed that, except 2a all the other compounds (**2b-c**, **3a-c**) were safer for human fibroblasts (healthy cell) compared to the other tumor cell lines.

According to these results we claimed that the **2b** complex has the most cytotoxic effects against the three cancer cell lines as having the lowest IC_{50} values compared to other compounds.

The cell viability of complexes against cancer cell lines and healthy cell line were given in Figs. 1–4. For Ag(I)–NHC (**2a-c**), it was determined that **2a** was ineffective against HF and moderately effective towards the three cancer cell lines at >50 μM in 3rd day

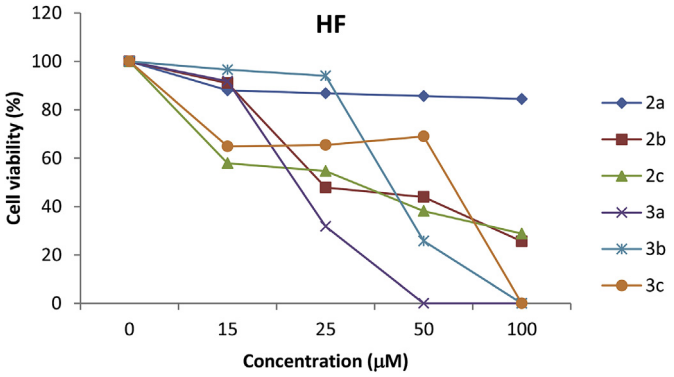


Fig. 1. The dose-dependence of cell viability for HF cell line at the end of 72 h.

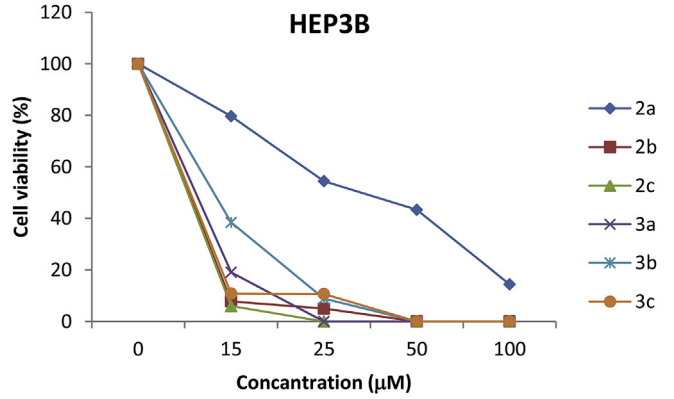


Fig. 2. The dose-dependence of cell viability for HEP3B cell line at the end of 72 h.

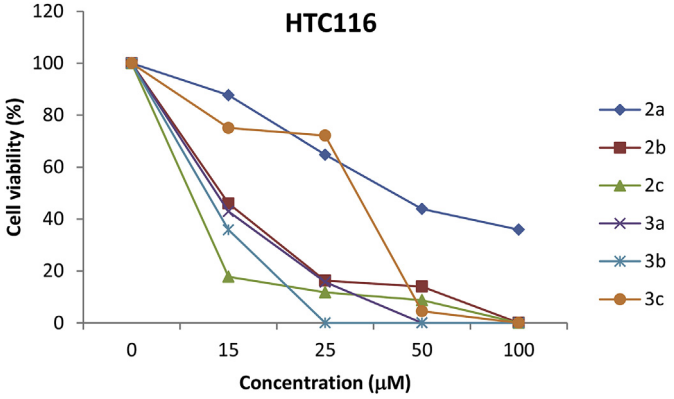


Fig. 3. The dose-dependence of cell viability for HTC116 cell line at the end of 72 h.

(Fig. 1). On the other hand, **2b** and **2c** were highly effective against cancer cell lines at <30 μM in 1st, 2nd and 3rd day (Figs. 2–4). For this reason, we believe that, according to the anticancer activity of Ag (I) –NHC the derivate compounds could be classified as **2b** > **2c** > **2a** (Figs. 1–4).

For gold(I)–NHC complexes (**3a**-**c**), all compounds exhibited anticancer and cytotoxic activity towards the three tested cancer cell lines. The most effective compounds were; 3b against SHSY5Y and HTC116 cell lines and **3c** toward Hep3Bones. Also, it is worth noting that the effectiveness of the **3a**-**3c** obvious at the 1st day of treatment and lasted at the 3rd day similarly to **2a**-**2c** (see supporting information). The gold(I)–NHC complexes affected the

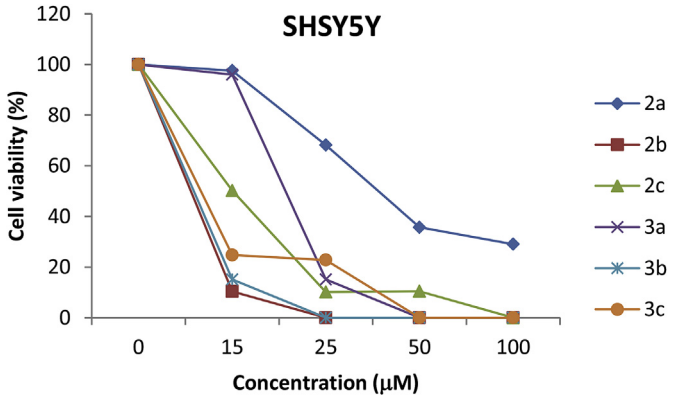


Fig. 4. The dose-dependence of cell viability for SHSY5Y cell line at the end of 72 h.

cancer cells at <30 μM and human fibroblasts at >50 μM. Therefore, we claimed that these compounds could have selective cytotoxic activity towards cancer cells.

The metal based complexes such as silver and gold are important for selective and effective cancer therapy in recent years. Zeng et al. claimed that metal complexes move inside living cells, they overcome the cell membrane and then penetrate within the nucleus [35]. Generally, it was known that metal complexes such as gold and silver bind the DNA and could affect the DNA replication activity of cells which could partially explain the cytotoxic activity with metal drugs [36].

Similarly, we determined that The silver(I)–NHC complexes (**2a**-**c**) and the gold(I)–NHC complexes (**3a**-**c**) have cytotoxic activity against the three tested cancer cell lines (SHSY5Y, HTC116, HEP3B). In this context, it was shown that gold complexes are most often cytotoxically active than silver complexes. Compared to our results, Sanchez et al. have reported that similar biological activity of silver and gold complexes in terms of DNA binding and inhibition of tumor cell lines [37]. This study confirmed our findings for these two metal compounds. Besides, Li et al. determined that the Au(I)–NHC and Ag(I)–NHC complexes have antitumor activity and they claimed that the Au(I) complexes display higher anticancer activity than Ag(I) [38]. These results corroborate with our findings. In addition, it was reported in previous studies that these complexes could selectively target mitochondria organelles and could influence their integrity, inducing cancer cell death through different mechanisms [33–37]. Finally, we believe that more investigations should be undertaken in order to highlight the exact molecular mechanism responsible for this selective cytotoxic effect.

3. Conclusions

In summary, in this study, a series of di*iso*propilamine-tethered benzimidazolium salts and silver(I)–NHC complexes (**2a-c**) of them were synthesized from the benzimidazolium salts (**1a-c**) with Ag_2O. Di*iso*propilamine-tethered gold(I)–NHC complexes (**3a-c**) have been obtained by carbene-transfer from the corresponding Ag(I)–NHC (**2a-c**). All these compounds were characterized by spectroscopic and analytic techniques. A preliminary anticancer study of these compounds were evaluated against the three human cell lines brain (SHSY5Y), colon (HTC116), and liver (HEP3B). Both silver and gold complexes were found to be active against the tested cell lines showing comparable activity with examples in the literature. An important effort is currently being done in our laboratories in order to develop the potential of these complexes for biological or catalytic applications.

4. Experimental

4.1. Chemistry

All reactions for the preparation benzimidazolium salts and their complexes were carried out under argon in flame-dried glassware using standard Schlenk techniques. Melting points were measured in open capillary tubes with Stuart SMP 40 melting point apparatus and are uncorrected. IR spectra were recorded using an ATR unit in the range 400–4000 cm^{-1} with a PerkinElmer Spectrum 100 spectrophotometer.^{1}H NMR and ^{13}C NMR spectra were recorded using a Varian As 400 Merkur spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in $CDCl_3$ with tetramethylsilane as an internal reference. Coupling constants (*J* values) are given in hertz. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, m = multiplet signal.

4.1.1. General method for the preparation of benzimidazolium salts

To a solution of 1-(2-(di*iso*propylamino)ethylbenzimidazole (1 mol) in DMF (5 mL) was slowly added benzylic halide (1 mmol) and the resulting mixture was stirred at room temperature for 5 h and then heated for 5h at 90 °C. Diethyl ether (10 mL) was added to obtain a white crystalline solid, which was filtered off. The solid was washed with diethyl ether (3 × 10 mL) dried under vacuum and the crude product was recrystallized from ethanol/diethyl ether.

4.1.2. Synthesis of 1-[2-(diisopropylamino)ethyl]-3-(3,4,5-trimethoxybenzyl)benzimidazolium chloride, **1a**

Yield: 3.47 g, 75%; mp: 167.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (d, 12H, *J* = 8 Hz, NCH(CH_3)$_2$), 2.92 (t, 2H, *J* = 4 Hz, CH_2CH_2N(Pr*i*)$_2$), 2.99 (h, 2H, *J* = 8 Hz, NC*H*(CH_3)$_2$), 3.80 (s, 3H, $CH_2C_6H_2$(OCH_3)$_3$-4), 3.86 (s, 6H, $CH_2C_6H_2$(OCH_3)$_3$-3,5), 4.58 (t, 2H, *J* = 4 Hz CH_2CH_2N(Pr*i*)$_2$], 5.79 (s 2H, $CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 6.93 (s 2H, $CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 7.57–7.79 (m, 4H, NC_6H_4N), 11.27 (s, 1H, NC*H*N). ^{13}C NMR (δ, 100 MHz, $CDCl_3$): δ = 20.6 (NCH(CH_3)$_2$), 44.0 (CH_2CH_2N(Pr*i*)$_2$), 47.7 (NCH(CH_3)$_2$), 47.3 (CH_2CH_2N(Pr*i*)$_2$), 51.3 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 56.6 ($CH_2C_6H_2$(OCH$_3$)$_3$-4), 60.8 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,5), 106.5, 1321.5, 138.7 and 153.8 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 113.2, 113.6, 126.9 and 128.7(NC_6H_4N), 144.0 (NC*H*N). IR(ATR): CN{*ν*} = 1595 cm^{-1}; Anal. calcd for $C_{25}H_{36}N_3O_3Cl$: C 64.99, H 7.85, N 9.09. Found: C 65.03, H 7.88, N 9.05.

4.1.3. Synthesis of 1-[2-(diisopropylamino)ethyl]-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride, **1b**

Yield: 3.77 g, 85%; mp: 216.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.67 (d, 12H, *J* = 8 Hz, NCH(CH_3)$_2$), 2.25 (s, 6H, CH_2C_6(CH_3)$_5$-2,6), 2.29 (s, 6H, CH_2C_6(CH_3)$_5$-3,5), 2.31 (s, 3H, CH_2C_6(CH_3)$_5$-4), 2.87 (t, 2H, *J* = 4 Hz, CH_2CH_2N(Pr*i*)$_2$), 2.97 (h, 2H, *J* = 8 Hz, NC*H*(CH_3)$_2$), 4.77 (t, 2H, *J* = 4 Hz CH_2CH_2N(Pr*i*)$_2$], 5.74 (s 2H, CH_2C_6(CH$_3$)$_5$-2,3,4,5,6), 7.55–7.85 (m, 4H, NC_6H_4N), 9.92 (s, 1H, NC*H*N). ^{13}C NMR (δ, 100 MHz, $CDCl_3$): δ = 16.9 (CH_2C_6(CH_3)$_5$-2,6), 17.2 (CH_2C_6(CH_3)$_5$-3,5), 17.3 (CH_2C_6(CH_3)$_5$-4), 20.4 (NCH(CH_3)$_2$), 43.4 (CH_2CH_2N(Pr*i*)$_2$), 46.7 (CH_2C_6(CH$_3$)$_5$-2,3,4,5,6), 47.1 (NCH(CH_3)$_2$), 47.3 (CH_2CH_2N(Pr*i*)$_2$), 113.1, 113.5, 124.9, 126.9, 131.3, 133.7, 134.4 and 137.4 (CH_2C_6(CH$_3$)$_5$-2,3,4,5,6 and NC_6H_4N), 142.9 (NC*H*N). IR (ATR): CN{*ν*} = 1550 cm^{-1}; Anal. calcd for $C_{27}H_{40}N_3Cl$: C 73.36, H 9.12, N 9.51. Found: C 73.32, H 9.17, N 9.45.

4.1.4. Synthesis of 1-[2-(diisopropylamino)ethyl]-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride, 1c

This NHC precursor was synthesized according to published procedure.[25]

4.1.5. General procedure for the preparation of the silver(NHC) complexes (**2a-c**)

A schelenk was charged with the alkyl(2-(di*iso*propylamino) ethyl)benzimidazolium (1.0 mmol), Ag_2O (1.2 mmol) and chloroform (6 mL). The solution was stirred at room temperature in for 24 h. The reaction mixture was filtered through celite to remove under reduced pressure. Solid was washed with Et_2O and dried under vacuum. The crude product was recrystallized from dichloromethane–diethyl ether (1:2 v/v) (Scheme 1).

4.1.6. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(3,4,5-trimethoxybenzyl))benzimidazole-2-ylidene}silver(I), **2a**

Yield: 0.40 g, 70%; mp: 172.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.83 (d, 12H, *J* = 8 Hz, NCH(CH_3)$_2$), 2.83 (t, 2H, *J* = 8 Hz, CH_2CH_2N(Pr*i*)$_2$), 2.94 (h, 2H, *J* = 8 Hz, NC*H*(CH_3)$_2$), 3.74 (s, 6H, $CH_2C_6H_2$(OCH_3)$_3$-3,5), 3.75 (s, 3H, $CH_2C_6H_2$(OCH_3)$_3$-4), 4.30 (t, 2H, *J* = 8 Hz CH_2CH_2N(Pr*i*)$_2$], 5.43 (s 2H, $CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 6.49 (s 2H, $CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 7.27–7.46 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.9 (NCH(CH_3)$_2$), 45.4 (CH_2CH_2N(Pr*i*)$_2$), 48.9 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5), 50.7 (NCH(CH_3)$_2$), 53.4 (CH_2CH_2N(Pr*i*)$_2$), 56.4 ($CH_2C_6H_2$(OCH$_3$)$_3$-4), 60.9 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,5), 104.8, 11.8, 111.9, 124.1, 124.2, 130.7, 133.7, 134.2, 138.2 and 153.7 ($CH_2C_6H_2$(OCH$_3$)$_3$-3,4,5 and NC_6H_4N), Ag-$C_{carbene}$ not observed. IR (ATR): CN{*ν*} = 1461 cm^{-1} Anal. calcd for $C_{25}H_{35}N_3O_3AgCl$: C 52.78, H 6.20, N 7.39. Found: C 52.73, H 6.24, N 7.37.

4.1.7. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazole-2-ylidene}silver(I), **2b**

Yield: 0.39 g, 73%; mp: 180.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.81 (d, 12H, *J* = 8 Hz, NCH(CH_3)$_2$), 2.12 (s, 6H, CH_2C_6(CH_3)$_5$-2,6), 2.21 (s, 6H, CH_2C_6(CH_3)$_5$-3,5), 2.26 (s, 3H, CH_2C_6(CH_3)$_5$-4), 2.76 (t, 2H, *J* = 8 Hz, CH_2CH_2N(Pr*i*)$_2$), 2.90 (h, 2H, *J* = 8 Hz, NC*H*(CH_3)$_2$), 4.22 (t, 2H, *J* = 4 Hz CH_2CH_2N(Pr*i*)$_2$), 5.38 (s 2H, CH_2C_6(CH$_3$)$_5$-2,3,4,5,6), 7.28–7.43 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 17.2 (CH_2C_6(CH$_3$)$_5$-2,3,5,6), 17.4 (CH_2C_6(CH_3)$_5$-4), 20.9 (NCH(CH_3)$_2$), 45.5 (CH_2CH_2N(Pr*i*)$_2$), 47.7 (NCH(CH_3)$_2$), 48.7 (CH_2C_6(CH$_3$)$_5$-2,3,4,5,6), 51.1 (CH_2CH_2N(Pr*i*)$_2$), 111.2, 111.7, 123.8124.1, 126.6, 133.0, 134.2 and 137.3 (CH_2C_6(CH$_3$)$_5$-2,3,4,5,6 and NC_6H_4N), Ag-$C_{carbene}$ not observed. IR (ATR): CN{*ν*} = 1462 cm^{-1}; Anal. calcd for $C_{27}H_{39}N_3AgCl$: C 59.08, H 7.16, N 7.65. Found: C 59.05, H 7.18, N 7.61.

4.1.8. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(2,4,6-trimethylbenzyl)benzimidazole-2-ylidene}silver(I), **2c**

Yield: 0.39 g, 75%; mp: 167.1 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.85 (d, 12H, *J* = 8 Hz, NCH(CH_3)$_2$), 2.17 (s, 6H, $CH_2C_6H_2$(CH_3)$_3$-2,6), 2.29 (s, 3H, $CH_2C_6H_2$(CH_3)$_3$-4), 2.79 (t, 2H, *J* = 8 Hz, CH_2CH_2N(Pr*i*)$_2$), 2.93 (h, 2H, *J* = 8 Hz, NC*H*(CH_3)$_2$), 4.25 (t, 2H, *J* = 8 Hz CH_2CH_2N(Pr*i*)$_2$), 5.39 (s 2H, $CH_2C_6H_2$(CH$_3$)$_3$-2,4,6), 6.92 (s 2H, $CH_2C_6H_2$(CH$_3$)$_3$-2,4,6), 7.17–7.44 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.4 (NCH(CH_3)$_2$), 20.9 ($CH_2C_6H_2$(CH_3)$_5$-2,6), 21.2 ($CH_2C_6H_2$(CH_3)$_5$-4), 45.5 (CH_2CH_2N(Pr*i*)$_2$), 47.7 (NCH(CH_3)$_2$), 48.8 ($CH_2C_6H_2$(CH$_3$)$_5$-2,4,6), 51.1 (CH_2CH_2N(Pr*i*)$_2$), 111.6, 123.9, 124.1, 126.6, 130.3, 134.1, 134.3, 137.6 and 139.6 ($CH_2C_6H_2$(CH$_3$)$_5$-2,4,6 and NC_6H_4N), Ag-$C_{carbene}$ not observed. IR (ATR): CN{*ν*} = 1454 cm^{-1}; Anal. calcd for $C_{25}H_{35}N_3AgCl$: C 57.65, H 6.77, N 8.07. Found: C 57.70, H 6.80, N 8.01.

4.1.9. General procedure for the preparation of the gold(NHC) complexes (**3a-c**)

The gold-NHC complexes were prepared by means of Ag-carbene-transfer method developed by Wang and Lin.[28] To a solution of silver (I) complex (1.0 mmol) in $CHCl_3$ (10 mL) was added [AuClS(CH_3)$_2$] (1.0 mmol). The reaction mixture was then stirred for 24 h at room temperature, and then was filtered through celite. The filtrate was evaporated under vacuum, the solid residue was

washed with Et_2O (3 × 5 mL), dried under vacuum and recrystallized from $CHCl_3/Et_2O$ (1:2, *v/v*).

4.1.10. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(3,4,5-trimethoxybenzyl))benzimidazole-2-ylidene}gold(I), ***3a***

Yield: 0.27 g, 81%; mp: 177.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.93 (d, 12H, *J* = 6.4 Hz, NCH(CH_3)$_2$), 2.97 (t, 2H, *J* = 6.4 Hz, $CH_2CH_2N(Pri)_2$), 3.04 (h, 2H, *J* = 6.4 Hz, NC*H*(CH_3)$_2$), 3.81 (s, 6H, $CH_2C_6H_2(OCH_3)_3$-3,5), 3.82 (s, 3H, $CH_2C_6H_2(OCH_3)_3$-4), 4.43 (t, 2H, *J* = 6.4 Hz $CH_2CH_2N(Pri)_2$], 5.60 (s 2H, $CH_2C_6H_2(OCH_3)_3$-3,4,5), 6.70 (s 2H, $CH_2C_6H_2(OCH_3)_3$-3,4,5), 7.35–7.51 (m, 4H, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.9 (NCH(CH_3)$_2$), 45.4 ($CH_2CH_2N(Pri)_2$), 48.8 ($CH_2C_6H_2(OCH_3)_3$-3,4,5), 49.8 (NCH(CH_3)$_2$), 53.0 ($CH_2CH_2N(Pri)_2$), 56.4 ($CH_2C_6H_2(OCH_3)_3$-4), 60.9 ($CH_2C_6H_2(OCH_3)_3$-3,5), 105.8, 111.8, 124.4, 130.3, 133.9, 138.3 and 153.8 ($CH_2C_6H_2(OCH_3)_3$-3,4,5 and NC_6H_4N), 178.7 (Au-$C_{carbene}$). IR (ATR): CN{*ν*} = 1403 cm^{-1}; Anal. Calcd. for $C_{25}H_{35}N_3O_3AuCl$: C, 45.63, H, 5.36, N, 6.39. Found: C, 45.61, H,5.32, N, 6.42.

4.1.11. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazole-2-ylidene}gold(I), ***3b***

Yield: 0.39 g, 78%; mp: 143.5 °C; ^{1}H NMR (400 MHz, CD_3Cl_3): δ = 0.97 (d, 12H, *J* = 6.4 Hz, NCH(CH_3)$_2$), 2.24 (s, 12H, $CH_2C_6(CH_3)_5$-2,3,5,6), 2.29 (s, 3H, $CH_2C_6(CH_3)_5$-4), 2.95 (t, 2H, *J* = 6.8 Hz, $CH_2CH_2N(Pri)_2$), 3.05 (h, 2H, *J* = 6.4 Hz, NC*H*(CH_3)$_2$), 4.41 (t, 2H, *J* = 6.8 Hz $CH_2CH_2N(Pri)_2$), 5.78 (s 2H, $CH_2C_6(CH_3)_5$-2,3,4,5,6), 6.80 and 7.46 (d, H, *J* = 8 Hz, NC_6H_4N), 7.17 and 7.31 (t, 2H, *J* = 8 Hz, NC_6H_4N). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.9 and 17.4 ($CH_2C_6(CH_3)_5$-2,3,5,6), 17.3 ($CH_2C_6(CH_3)_5$-4), 20.9 (NCH(CH_3)$_2$), 46.3 ($CH_2CH_2N(Pri)_2$), 48.9 (NCH(CH_3)$_2$), 50.0 ($CH_2C_6(CH_3)_5$-2,3,4,5,6), 50.5 ($CH_2CH_2N(Pri)_2$), 111.4, 112.3, 124.0, 124.1, 127.3, 133.3, 133.4, 133.6 and 133.8 ($CH_2C_6(CH_3)_5$-2,3,4,5,6 and NC_6H_4N), 179.2 (Au-$C_{carbene}$). IR (ATR): CN{*ν*} = 1404 cm^{-1}; Anal. calcd for $C_{27}H_{39}N_3AuCl$: C 50.83, H 6.16, N 6.59. Found: C 50.81, H 6.19 N, 6.61.

4.1.12. Synthesis of chloro-{1-[2-(diisopropylamino)ethyl]-3-(2,4,6-trimethylbenzyl)benzimidazole-2-ylidene}gold(I), ***3c***

Yield: 0.24 g, 80%; mp: 175.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.98 (d, 12H, *J* = 6.4 Hz, NCH(CH_3)$_2$), 2.26 (s, 3H, $CH_2C_6H_2(CH_3)_3$-4), 2.31 (s, 6H, $CH_2C_6H_2(CH_3)_3$-2,6), 2.96 (t, 2H, *J* = 6.8 Hz, $CH_2CH_2N(Pri)_2$), 3.06 (h, 2H, *J* = 6.4 Hz, NC*H*(CH_3)$_2$), 4.43 (t, 2H, *J* = 6.8 Hz $CH_2CH_2N(Pri)_2$), 5.77 (s 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 6.90 (s 2H, $CH_2C_6H_2(CH_3)_3$-2,4,6), 6.80 and 7.45 (d, 2H, *J* = 8 Hz, NC_6H_4N), 7.15 and 7.32 (t, 2H, *J* = 8 Hz, NC_6H_4N) . ^{13}C NMR (δ, 100 MHz, $CDCl_3$): 20.1 (NCH(CH_3)$_2$), 20.2 ($CH_2C_6H_2(CH_3)_5$-2,6), 21.0 ($CH_2C_6H_2(CH_3)_5$-4), 45.2 ($CH_2CH_2N(Pri)_2$), 48.9 (NCH(CH_3)$_2$), 49.9 ($CH_2C_6H_2(CH_3)_5$-2,4,6), 53.4 ($CH_2CH_2N(Pri)_2$), 111.5, 112.3124.1, 124.3, 127.1, 129.9, 133.1, 133.8137.9 and 138.7 ($CH_2C_6H_2(CH_3)_5$-2,4,6 and NC_6H_4N), 179.5 (Au-$C_{carbene}$). IR (ATR): CN{*ν*} = 1405 cm^{-1}; Anal. calcd for $C_{25}H_{35}N_3AuCl$: C 49.23, H 5.78, N 6.89. Found: C 49.20, H 5.81, N 6.85.

4.2. Antitumor activities of silver and gold-NHC complexes

4.2.1. Cell culture and incubation

SHSY5Y (Neuroblastoma), HEP3B (adenocarcinoma), HTC116 (colorectal carcinoma) and HF (human fibroblast) cells were obtained from the American Type culture collection (ATCC) and maintained at 37 °C in a humidified incubator under 5% CO_2 conditions [39]. Cells were cultured in DMEM containing %10 fetal bovine serum and % 1 antibiotics (100 μg/mL streptomycin and 10000 U/mL penicillin). Media were changed twice a week until cells reach 70–80% confluence.

4.2.2. Cell viability assay

The MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4sulfophenyl)-2H-tetrazolium] assay was performed to identify viable cell ratio according to the manufacturer's instructions (Promega). Briefly cells were seeded in 96 well flat-bottomed tissue culture plates at a concentration of 5 × 103 cells/well. After 24 h, the cells were treated with DMEM medium containing different concentrations (15, 25, 50, and 100 μM) of the test compounds for different incubation periods (24, 48 and 72h). At the end of each time point, fresh complete medium containing 10 μL of MTS solution was added and further incubated 2h in incubator. Cell proliferation was assessed by measuring the absorbance with an ELISA microplate reader (Weida). Each experiment was performed quadruplicate and results are expressed as the percentage growth inhibition with respect to the untreated cells. Statistical deviations for the viability were calculated automatically by Excel 2007 software program (SE ≤ 5%) and for the IC50-by the "Origin Pro 7.5" and "Origin 6.1" (for 7Crf) PC-program.

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Appendix A. Supplementary data

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