Article

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Synthesis, characterization and anticancer activity of new benzimidazolium salts with their Ag(I) and Au(I) bis-NHC complexes

Ihab Hamid Mohammed,and Mohammed Zghair Ghdhayeb

Kufa University, Faculty of sciences, Department of Chemistry

*mohammed.alhallaqi@uokufa.edu.iq

Abstract

The research shows how to the synthesis and characterization of new substituted benzimidazolium salts, identify their Ag(I), and Au(I) bis-NHC complexes, and assess their anticancer activity of these compounds. Benzimidazolium salts have been derived from various substitution such as aliphatic chains and phenylacetamide chloride to produce asymmetrically substituted salts. New Ag(I) bis N-heterocyclic carbene complexes were synthesized from the in situ deprotonation process of asymmetrically di substituted benzimidazolium salts with Ag_2O. Afterwards, Ag(I) bis-NHC complexes were used as transfer reagents in the transmetallation process to create Au(I) bis-NHC. The prepared complexes were characterized using (^{1}H-NMR, ^{13}C-NMR, and FT-IR) spectro techniques. The anticancer activity of benzimidazolium salts and Ag(I) and Au(I) complexes showed good activity compared to cisplatin as standard substance.

Keywords: Benzimidazole, Carbene, Ag(I)bis-NHC, Au(I)bis-NHC, Anticancer activity.

1. Introduction

Following the synthesis of the first *N*-heterocyclic carbene by Öfele and Wandslick in 1968 [1,2] and the isolation of the first stable NHC by Arduengo in 199 [3], there has been a significant increase in research interest in the synthesis of new N-heterocyclic carbenes and their complexes. A variety of new metal-NHC complexes involving transition metals and main group elements have been synthesized and used in various fields, making it an appealing field of study [4–7]. The synthesis of benzimidazole derived compounds and their uses have received increased attention in the last ten years because of their pharmacological agents antibacterial, antifungal, antiulcer, and anticancer effects [8–10]. Benzimidazole and its derivatives are now used as powerful antimicrobial medications to treat a variety of bacterial and fungal infections [11]. Many studies are currently being conducted to create safer and more effective medications of these kinds of NHC compounds for their clinical applications, as benzimidazole derivatives have a wide range of pharmacological applications [12,13]. Silver, gold and copper are the most studied transition metals, due to their distinct structural qualities and used in wide range of applications[14–19]. Silver NHC complexes have gained much attention in the field of organometallic chemistry because they are easy to synthesize by reacting benzimidazolium salts with Ag_2O in one step [20,21]. The possibility of using Ag-NHC complexes as a reagent in carbene transfer reactions to form other transition metal complexes, such as (Ni, Pt, Au, Cu, Ir, Rh, and Ru) NHC complexes, is another factor that makes them interesting. This provides a simpler method to create metal carbene complexes [22–24]. It's interesting to note a lot of research that has shown that silver carbene complexes have demonstrated biological activity as antimicrobial and anticancer drugs [25]. Ag-NHC complexes, which are produced when silver is added to bis-benzimidazolium salts, have shown as potent anticancer drugs [26]. The use of gold (I) organometallic complexes as possible anticancer agents has also attracted a lot of attention in recent years [27–31]. Gold complexes are actually thought to be viable substitutes for platinum-based medications (such as cisplatin and its analogues) because of their more selective mode of action [32,33]. By doing this, it appears that some of the primary problems with the cancer chemotherapy used today, such as drug resistance and poor selectivity, may be resolved [34,35].

2. Experimental

2.1. Materials and instrumentation

All chemicals (Ag_2O, $[AuCl(SMe)_2]$, benzimidazole, aliphatic chain bromide and p-aminophenol) quality and used solvents were of the highest analytical quality and were not purified before use. A 400 MHz Bruker spectrometer was utilized to capture room temperature nuclear magnetic resonance (NMR) spectra. The solvent d_6-DMSO signals were compared to the chemical shifts (ppm). FT-IR spectra were obtained in the 4000–600 cm^{-1} wavenumber range using a Bruker FTIR spectrophotometer.

2.2. Synthesis of 4-(2-chloroacetamido)phenyl 2-chloroacetate (1)

4-(2-chloroacetamido)phenyl 2-chloroacetate was synthesized according to a reported procedure [36] (1.09g, 10 mmol) of p-aminophenol dissolved in DMF (20 mL). Then triethylamine (TEA) (1.5 mL) was added, and the mixture stirred for 20 minutes, after which chloroacetyl chloride (1:2) (3 g, 20 mmol) was added dropwise at (0–5 °C), and the mixture was stirred for another 30 minutes at room temperature (i- in scheme 1). After completion of the reaction, the reaction mixture was filtered and washed with distilled water, and then recrystallized with absolute ethanol, to give 3.4 g (85 % yield) as a fine pale brown powder (m.p = 257-260°C). FT-IR cm^{-1}: 3268 (N-H), 3060 ($C\text{-}H_{aromatic}$), 2947 ($C\text{-}H_{aliph}$), 1761 ($C{=}O_{ester}$), 1673 ($C{=}O_{amide}$), 1200 (C-N). ^1H-NMR (400 MHz, DMSO-*d6*)δ, ppm: 10.42 (s, H, N-H), 7.14-7.66 (m, 4H, Ar-H), 4.69 (s, 2H, ester-CH_2), 4.21 (s, 2H, amide-CH_2). ^{13}C-NMR (100 MHz, DMSO-*d6*)δ, ppm: 167.01 (C=O amide), 165.17 (C=O ester), 146.37 (Ar-C-O), 136.95 (Ar-C-N), 122.35, 120.88 (Ar-C), 43.99 (amide-CH_2), 41.79 (ester-CH_2).

2.3. General procedure for the syntheses of *N*-substituted benzimidazole

All the *N*-substituted benzimidazole **(2-4)** were synthesized according to previously reported procedures [37]. (20 mmol) of benzimidazole dissolved in 20 ml of DMSO, then was added (20 mmol) of NaOH powder, the mixture was refluxed for 2 hours at 90 °C. After cooling to 30 °C, alkylbromoide ($C_nH_{2n+1}X$, n =12,14 ,16) (20 mmol) was added dropwise, then the temperature was raised to 40 °C and the mixture was stirred for 1 hour. The mixture was added to 100 ml of crushed ice of distilled water and the solution stirred for 15 min, the mixture was then extracted using DCM (3 x 10 ml). The DCM layer was collected and dried over $MgSO_4$ then the solvent was removed using a rotary evaporator to obtain the product.

2.3.1. 1-dodecyl-1H-benzo[d]imidazole (2)

It was prepared as pure yellow oil, 5.8 g (78 % yield). FT-IR cm^{-1}: 3050 (C-H $_{aromatic}$), 2920 (C-H $_{asy\text{-}aliph}$), 2853 (C-H $_{sy\text{-}aliph}$), 1558 (C=C), 1203 (C-N). ^{1}H-NMR (400 MHz, DMSO-*d6*)δ ppm: 7.83 (s, 1H, NCHN), 7.46-6.68 (m, 4H, Ar-H), 4.17 (t, *J* = 8.0 Hz, 2H, N-CH_2), 1.78 (p, *J* = 7.0 Hz, 2H, N-CH_2-CH_2), 1.27-1.17 (m, 18H, 9 x CH_2 chain), 0.85 (t, *J* = 8.4 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-*d6*)δ ppm: 136.41 (NCHN), 133.89-115.61 (Ar-C), 49.31 (N-CH_2), 31.78-22.58 (10 x CH_2 chain), 14.43 (CH_3).

2.3.2. 1-tetradecyl-1H-benzo[d]imidazole (3)

It was prepared as pure yellow oil, 5.8 g (78 % yield). FT-IR cm^{-1}: 3035 (C-H $_{aromatic}$), 2919 (C-H $_{asy\text{-}aliph}$), 2851 (C-H $_{sy\text{-}aliph}$), 1554 (C=C), 1200 (C-N).

2.3.3. 1-hexadecyl-1H-benzo[d]imidazole (4)

It was prepared as pure yellow oil, 5.5 g (79 % yield). FT-IR cm^{-1}: 3049 (C-H $_{\text{aromatic}}$), 2917 (C-H $_{\text{asy-aliph}}$), 2849 (C-H $_{\text{sy-aliph}}$), 1557 (C=C), 1206 (C-N).

2.4. General synthesis of 1,3-disubstituted benzimidazolium salt

All the 1,3-disubstituted benzimidazolium salts **(5-7)** were synthesized according to previously reported procedures [38]. (5 mmol) of compound (1) dissolved in 10 ml of 1,4-dioxane, (10 mml) of compounds (2,3 and 4) dissolved in 20 ml of 1,4-dioxane then was added dropwise (1:2) on the compound (1). The solution was refluxed with stirring for 24 hours at 90 °C, reaction progress was monitored by TLC. After the reaction was completed, the solvent was removed using a rotary evaporator to obtain the product.

2.4.1. 1-dodecyl-3-(2-(4-(2-(3-dodecyl-1*H*-benzo[*d*]imidazole-3-ium-1-yl)acetamido)phenoxy)-2-oxoethyl)-1*H* benzo [*d*] imidazol-3-ium) chloride salt (5)

It was prepared as dark brown oil, 3.3 g (yield 79 %). FT-IR cm^{-1}: 3249 (N-H), 3032 (CH $_{\text{aromatic}}$), 2920 (C-H $_{\text{asy-aliph}}$), 2852 (C-H $_{\text{sy-aliph}}$), 1764 (C=O $_{\text{ester}}$), 1689 (C=O $_{\text{amide}}$), 1558 (C=C), 1250 (C-N). ^{1}H-NMR (400 MHz, DMSO-*d6*)δ ppm: 10.51 (s, 1H, N-H), 10.07,10.05 (s, 2H, NCHN), 7.81-7.15 (m, 6H, Ar-H), 4.60 (t, *J* = 8.0 Hz, 2H, N-CH_2), 4.29 (s, 2H, ester-CH_2), 4.24 (s, 2H, amide-CH_2), 1.91 (P, *J* = 7.0 Hz, 2H, N-CH_2-CH_2), 1.32-1.20 (m, 18H, 9 x CH_2 chain), 0.85 (t, *J* = 6.0 Hz, 3H, CH_3). ^{13}C-NMR (100 MHz, DMSO-*d6*)δ ppm: 168.32 (C=O $_{\text{ester}}$), 163.02 (C=O $_{\text{amide}}$), 154.36, 154.27 (NCHN), 143.69 (Ar-C-N), 136.98 (Ar-C-O), 132.16-120.61 (Ar-C), 47.28 (amide-CH_2), 43.99 (ester-CH_2), 40.86 (N-CH_2), 31.77- 22.57 (10 x CH_2 chain), 14.40 (CH_3).

2.4.2. 1-tetradecyl-3-(2-(4-(2-(3-tetradecyl-1*H*-benzo[*d*] imidazole-3-ium-1-yl)acetamido)phenoxy)-2-oxoethyl)-1*H*-benzo[*d*]imidazol-3-ium) chloride salt (6)

It was prepared as dark brown oil, 3.7 g (yield 83 %). FT-IR cm^{-1}: 3202 (N-H), 3038 (CH $_{aromatic}$), 2920 (C-H $_{asy-aliph}$), 2852 (C-H $_{sy-aliph}$), 1745 (C=O $_{ester}$), 1687 (C=O $_{amide}$), 1558 (C=C), 1248 (C-N).

2.4.3. 1-hexadecyl-3-(2-(4-(2-(3-hexadecyl-1*H*-benzo[*d*] imidazol-3-ium-1-yl)acetamido)phenoxy)-2-oxoethyl)-1*H*-benzo[*d*] imidazol-3-ium) chloride salt (7)

It was prepared as dark brown oil, 3.5 g (yield 74 %). FT-IR cm^{-1}: 3204 (N-H), 3052 (CH $_{aromatic}$), 2920 (C-H $_{asy-aliph}$), 2852 (C-H $_{sy-aliph}$), 1764 (C=O $_{ester}$), 1688 (C=O $_{amide}$), 1556 (C=C), 1245 (C-N).

2.5. General syntheses of silver(I) bis-NHC complexes

The silver complexes **(8-10)** were prepared via in the situ deprotonation method according to previously reported procedures [39]. Silver oxide (Ag_2O) (1 mmol l) was added to compounds (5,6 and 7) solution (1 mmol) dissolved in 20 ml DCM (1:1). The mixture stirred for 10 hours at room temperature in glassware covered by aluminum foil to block from light, the reaction progress was monitored by TLC. After the reaction was completed, the mixture black was filtered by celite to remove the excess (Ag_2O) particles, and the solvent was removed using a rotary evaporator to obtain the product.

2.5.1. Complex (8)

It was prepared as dark-brown oil, 1g (78 % yield). FT-IR cm^{-1} : 3201 (N-H), 3057 (CH $_{aromatic}$), 2922 (C-H $_{asy-aliph}$), 2854 (C-H $_{sy-aliph}$), 1746 (C=O $_{ester}$), 1682 (C=O $_{amide}$), 1558 (C=C), 1243 (C-N). ^{1}H-NMR (400 MHz, DMSO-*d6*)δ ppm: 10.15 (s, 1H, N-H), 8.01-7.32 (m, 10H, Ar-H), 4.49 (t, J = 8.1 Hz, 4H, 2 x N-CH_2), 3.90 (s, 2H, ester-CH_2), 3.87 (s, 2H, amide-CH_2), 1.50 (P, J = 8.8 Hz, 4H, 2 x N-CH_2-CH_2), 1.21-1.09 (m, 36H, 18 x CH_2 chain), 0.79 (t, J = 6.6 Hz, 6H, 2 x CH_3). ^{13}C-NMR (100 MHz, DMSO-*d6*)δ ppm: 188.16, 187.69 (NCHN), 170.63 (C=O ester), 163.06 (C=O amide), 143.84 (Ar-C-N), 132.23 (Ar-C-O), 130.50-114.15 (Ar-C), 49.42 (amide-CH_2), 47.26 (ester-CH_2), 40.78 (N-CH_2), 35.12- 22.57 (10 x CH2 chain), 14.41 (CH_3).

2.5.2. Complex (9)

It was prepared as dark-brown oil, 1 g (80 % yield). FT-IR cm^{-1} : 3218 (N-H), 3058 (CH $_{aromatic}$), 2921 (C-H $_{asy\text{-}aliph}$), 2852 (C-H $_{sy\text{-}aliph}$), 1744 (C=O $_{ester}$), 1685 (C=O $_{amide}$), 1558 (C=C), 1248 (C-N).

2.5.3. Complex (10)

It was prepared as dark-brown oil, 0.9 g (73 % yield). FT-IR cm^{-1}: 3256 (N-H), 3060 (CH $_{aromatic}$), 2920 (C-H $_{asy\text{-}aliph}$), 2852 (C-H $_{sy\text{-}aliph}$), 1751 (C=O $_{ester}$), 1683 (C=O $_{amide}$), 1556 (C=C), 1225 (C-N).

2.6. General syntheses of gold(I) bis-NHC complexes

The Gold complexes **(11-13)** were prepared via transmetallation method by used [AuCl$(SMe)_2$] with Ag(I) bis-NHC complexes according to previously reported procedures [40]. A mixture of complexes (8,9 and 10) (0.34 mmol) and gold complex [AuCl$(SMe)_2$] (0.1 g, 0.34 mmol), was dissolved in DCM 20 ml and the mixture stirred for 24 hours at room temperature in glassware, the reaction progress was monitored by TLC. After the reaction was completed, the mixture was filtered by celite to remove (AgCl) particles, and the solvent was removed using a rotary evaporator to obtain the product.

2.6.1. Complex (11)

It was prepared as brown oil, 0.48 g (69 % yield), FT-IR cm^{-1}: 3264 (N-H), 3096 (CH $_{aromatic}$), 2917 (C-H $_{asy\text{-}aliph}$), 2853 (C-H $_{sy\text{-}aliph}$), 1738 (C=O $_{ester}$), 1665 (C=O $_{amide}$), 1552 (C=C), 1269 (C-N). ^1H-NMR (400 MHz, DMSO-d_6)δ ppm: 10.71 (s, 1H, N-H), 8.07-7.32 (m, 10H, Ar-H), 4.52 (t, J = 8.0 Hz, 4H, 2 x N-CH_2), 4.15 (s, 2H, ester-CH_2), 3.90 (s, 2H, amide-CH_2), 1.85 (P, *J* = 6.0 Hz, 4H, 2 x N-CH_2-**CH_2**), 1.25-1.13 (m, 36H, 18 x CH_2 chain), 0.79 (t, *J* = 6.5 Hz, 6H, 2 x CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6)δ ppm: 188.90, 188.16 (NCHN), 170.63 (C=O $_{ester}$), 163.06 (C=O $_{amide}$), 149.34 (Ar-C-N), 143.84 (Ar-C-O), 132.22-114.17 (Ar-C), 49.42 (amide-CH_2), 47.26 (ester-CH_2), 43.97 (N-CH_2), 35.12- 19.10 (10 x CH_2 chain), 14.41 (CH_3).

2.6.2. Complex (12)

It was prepared as brown oil, 0.5 g (70 % yield), FT-IR cm^{-1}: 3207 (N-H), 3053 (CH $_{aromatic}$), 2922 (C-H $_{asy-aliph}$), 2855 (C-H $_{sy-aliph}$),1719 (C=O $_{ester}$), 1628 (C=O $_{amide}$), 1557 (C=C), 1261 (C-N).

2.6.3. Complex (13)

It was prepared as brown oil, 0.53 g (69 % yield), FT-IR cm^{-1}: 3241 (N-H), 3048 (CH $_{aromatic}$), 2921 (C-H $_{asy-aliph}$), 2854 (C-H $_{sy-aliph}$),1752 (C=O $_{ester}$), 1688 (C=O $_{amide}$), 1554 (C=C), 1244 (C-N).

2.7 . Anticancer activity

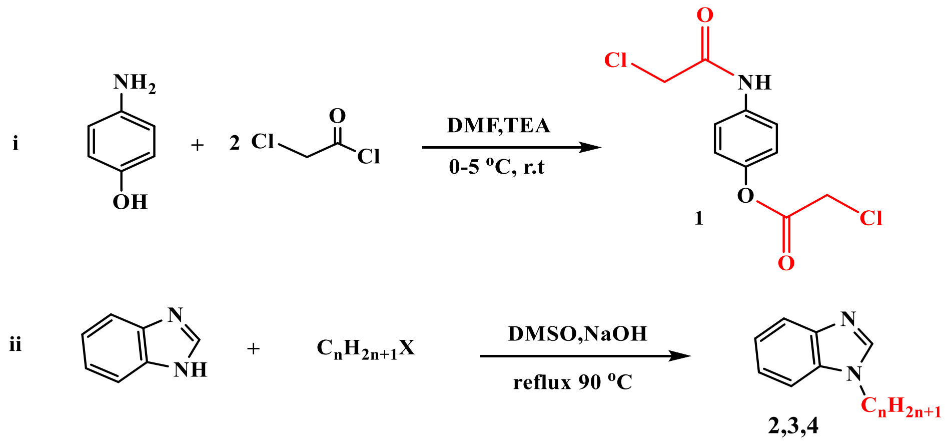
2.7.1. MTT assay

Cell growth and cell viability were quantified using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide] (Sigma-Aldrich) assay. In brief, for monolayer culture, cells were digested with trypsin, harvested, adjusted to a density of 1.4 × 104 cells/well and seeded to 96-well plates filled with 200 µl fresh medium per well for 24 h. When cells formed a monolayer, they were treated with 600-7.4 µg/ml of the compounds for 24 h at 37 °C in 5% CO_2. At the end of the treatment (24 h), while the monolayer culture was left untouched in the original plate, the supernatant was removed and 200 µl/well of MTT solution (0.5 mg/ml in phosphate-buffered saline [PBS]) was added and the plate was incubated at 37 °C for an additional 4 h. MTT solution (the supernatant of cells was removed and dimethyl sulfoxide was added (100 µl per well). Cells were incubated on a shaker at 37 °C until crystals were completely dissolved. Cell viability were quantified by measuring absorbance at 570 nm using an ELISA reader (Model wave xs2, BioTek, USA). The concentration of the compounds that resulted in 50% of cell death (IC_{50}) was determined from respective dose-response curves.

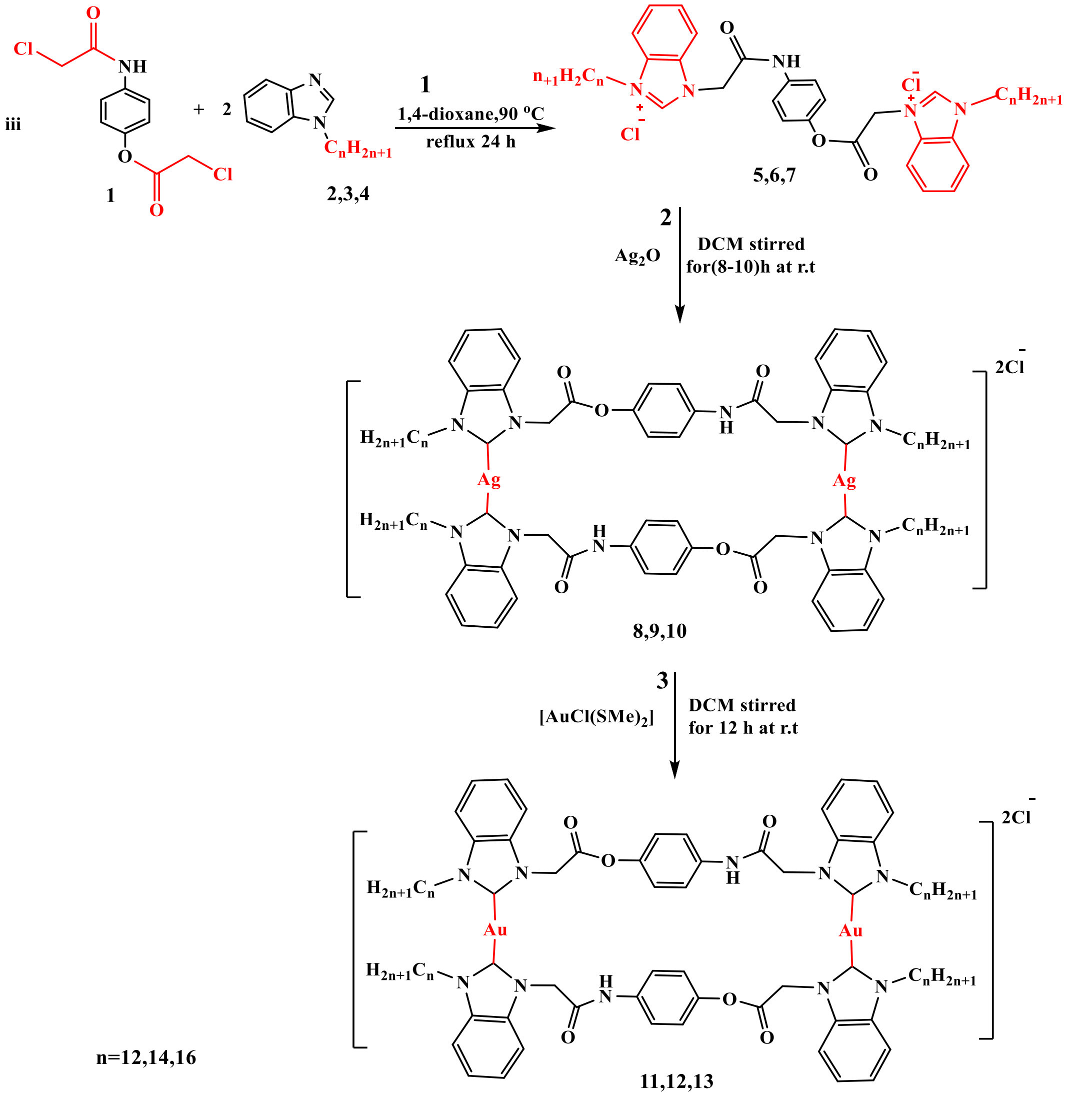
3. Results and discussion

3.1. NHCs precursors and complexes

The compound 4-(2-chloroacetamido)phenyl-2-chloroacetate (1) was prepared as shown in (i- Scheme 1), and wes used to prepare compounds (7, 6, 5) in 4-dioxane as shown in (iii-1 in Scheme 2), after reacting with the substituted benzimidazoles (2, 3, 4) as in (ii- Scheme 1). Ag(I) bis-NHC (8, 9, and 10) were prepared as shown in (iii-2 Scheme 2), by in situ reaction of Ag_2O with the corresponding benzimidazolium salts (7, 6, and 5), respectively. Reacting Ag_2O with an benzimidazolium salt in dichloromethane for 10 hours at room temperature produces complexes with good yields. All reactions produced a dark suspension that was filtered using a celite pad to remove excess Ag_2O.The solvent is then removed at low pressure, to give the complexes a dark brown oil. Gold complexes (11, 12 and 13) were synthesized using the transmetallation method as shown in (iii-3 Scheme 2), where $[AuCl(SMe)_2]$ reacted with Ag(I) bis-NHC complexes by stirring them in dichloromethane for 6 hours at room temperature. This resulted in the production of new Au(I) bis-NHC complexes with good yields. All reactions produced a dark suspension that was filtered using a celite pad to remove remove precipitated AgCl. The solvent is then removed under reduced pressure, to give the complexes a pale brown oil. The synthesis of compounds (1-13) is shown in (Schemes 1 and 2).



Scheme 1. (i) Synthesis of phenyl acetamide chloride (1), (ii) N-substituted benzimidazole (2, 3, 4).



Scheme 2. (iii-1) 1,3-disubstituted benzimidazolium salts (5, 6, 7) (NHC) precursors, (iii-2) their corresponding Ag(I) bis-NHC (8, 9, 10) and (iii-3) Au(I) bis-NHC (11, 12, 13) complexes.

3.2. FT-IR spectroscopy

When comparing ligands (NHCs) and their appropriate metal complexes (M-NHCs), the use of FT-IR spectra can provide some useful information. All benzimidazolium salts, as well as their Ag(I) and Au(I) complexes, had FT-IR spectra recorded in (Figures 1, 2, and 3). The bands appearing in the benzimidazolium salt (5) 3249 cm^{-1} are due to N-H stretching. The stretching bands of (C=O $_{\text{ester}}$) appear at 1764 cm^{-1}, and (C=O $_{\text{amide}}$) at 1689 cm^{-1}. The C-N stretching appears in the 1250 cm^{-1}. Most of the above bands are displaced upwards or downwards in the Ag(I) bis-NHC and Au(I) bis-NHC complexes, and this can be considered as an initial sign of effective multimerization with Ag and Au [41] (Table 1).

Table 1. FT-IR bands of compounds

Com.	N-H	C-H aromatic	C-H asy-aliph	C-H sy-aliph	C=O ester	C=O amide	C=C	C-N
5	3249	3125	2920	2852	1764	1689	1558	1250
8	3201	3057	2922	2854	1746	1682	1558	1243
11	3264	3096	2917	2853	1738	1665	1552	1269

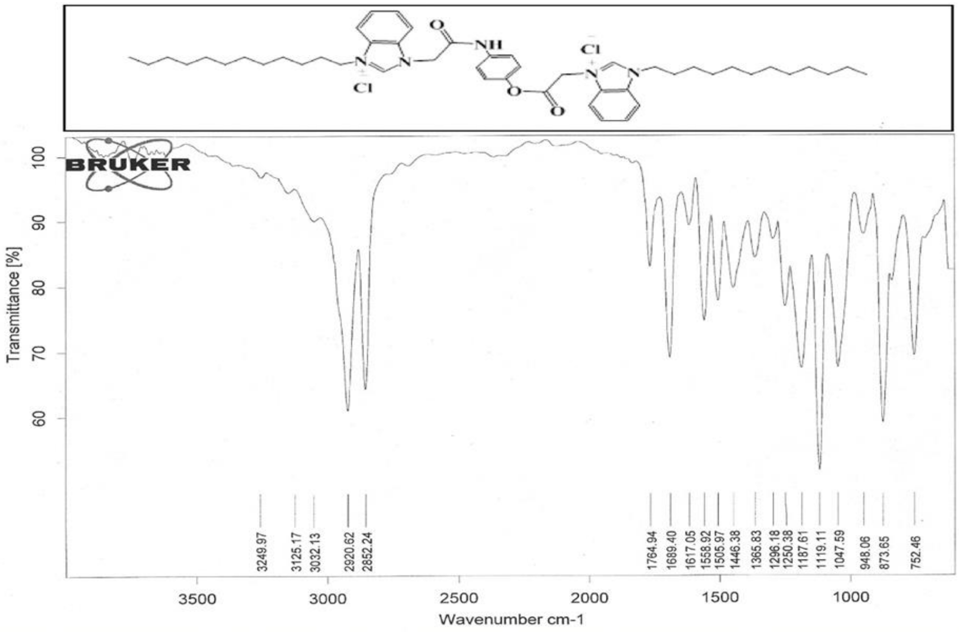


Figure (1) FT-IR spectrum of compound 5

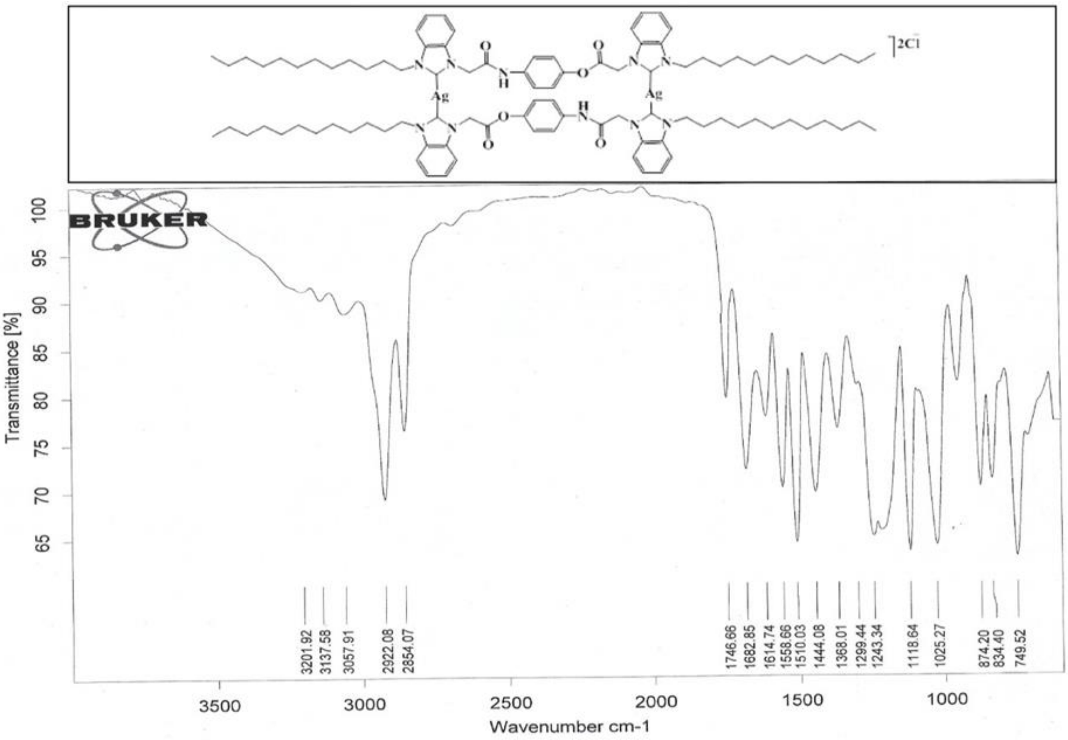


Figure (2) FT-IR spectrum of complex 8

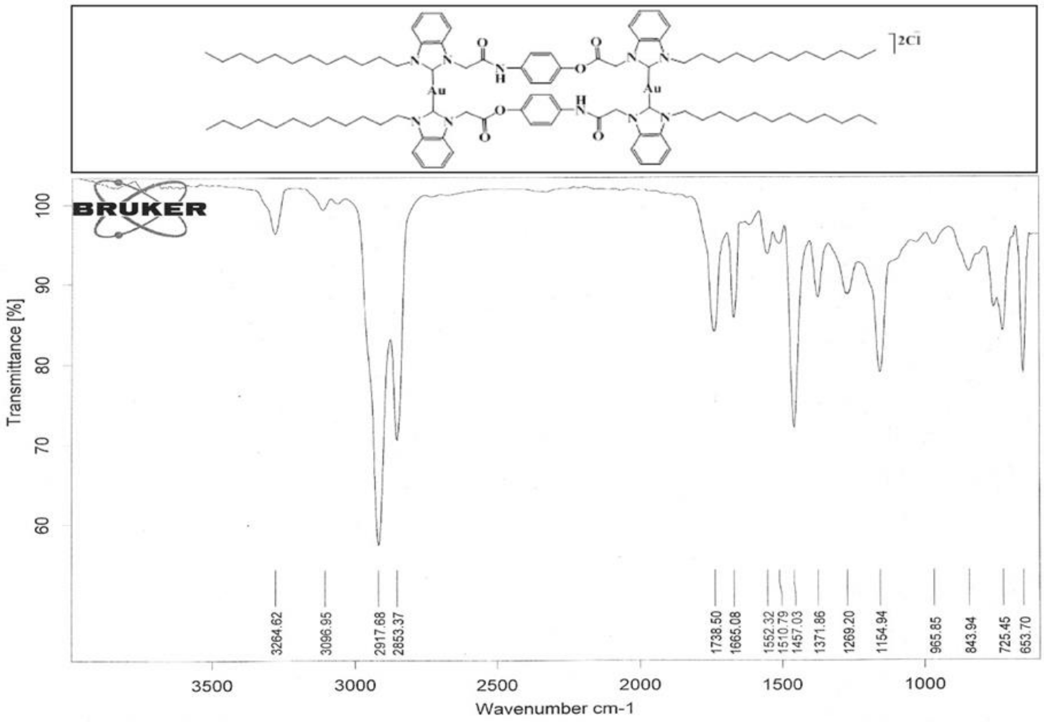


Figure (3) FT-IR spectrum of complex 11

3.3. NMR spectroscopy

All nuclear magnetic resonance spectra of the benzeimidazolium salts and corresponding complexes, Ag(I) bis-NHC and Au(I) bis-NHC in *d6*-DMSO, as shown in (Figures 4, 5, 6, 7, 8, and 9).The ^{1}H-NMR spectra display the expected signals for this group of benzeimidazolium salts and complexes. A specific proton (N-H) signal in (10.25 ppm) was observed in ^{1}H-NMR for benzimidazolium salt (5). In the range of (9.24 - 9.22 ppm), significant signals were seen for benzimidazolium salt H2'. Multiple peaks of aromatic protons (Ar-H) were observed in the range of (7.83-7.37 ppm). In both Ag(I) bis-NHC and Au(I) bis-NHC complexes (8,11), the ^{1}H-NMR spectra showed no H2' signals, which is a specific pattern of imidazolium salts. According to the literature, which is attributed to successful coordination with the metals used, the aromatic protons (Ar-H) range between (7.83-7.37 ppm) it appeared as multiple peaks, (Table 2) [39,42]. In the ^{13}C-NMR spectrum, the most important signals are those given by benzimidazolium salt (5) C2' which can be seen at 153.80 ppm (Figure 7), and the characteristic signals of carbene carbon-Ag and carbene carbon-Au that located at (188.64 ppm) for Ag(I) bis-NHCs and (183.98 ppm) for Au(I) bis-NHC complexes, respectively as shown in (Figures 8, 9) and (Table 3).

Table 2. ^{1}H-NMR Chemical shift range of compounds

Com.	N-H	NCHN	Ar-H	NCH_2	-ester CH_2	-amide CH_2	NCH_2-$\underline{CH_2}$	CH_2 alip	$-CH_3$
5	10.51	10.07, 10.05	7.81-7.15	4.62-4.58	4.29	4.24	1.95-1.88	1.32-1.20	0.87-0.83
8	10.15	-	8.01-7.32	4.52-4.47	3.90	3.87	1.54-1.45	1.21-1.09	0.81-0.77
11	10.71	-	8.07-7.32	4.54-4.50	4.15	3.90	1.88-1.82	1.25-1.13	0.81-0.77

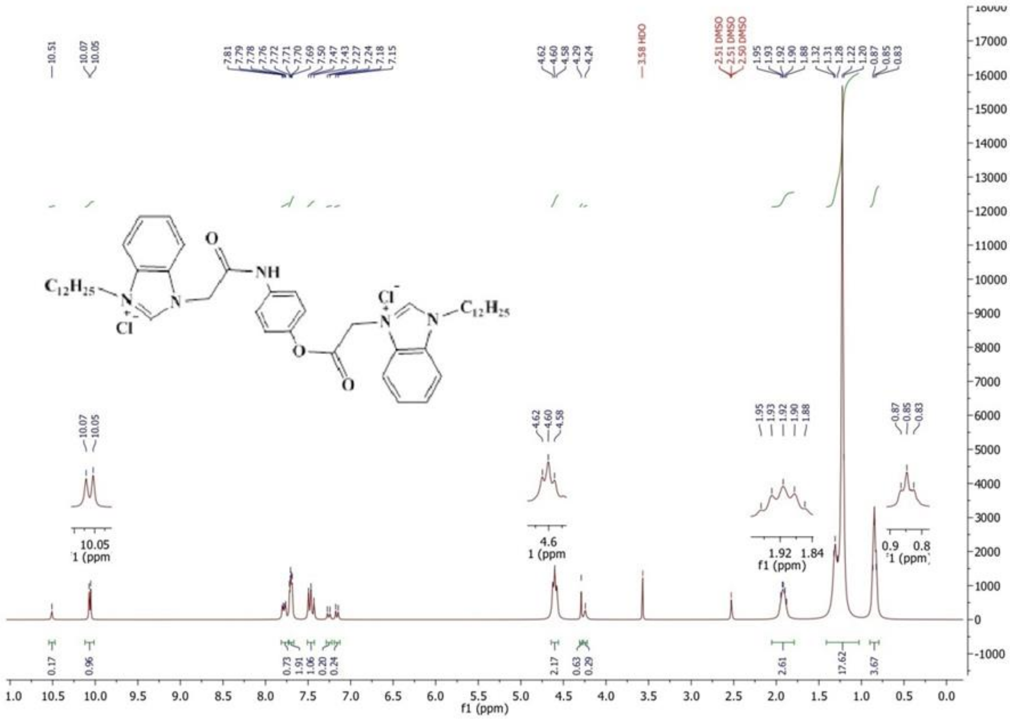


Figure (4) ^{1}H-NMR spectrum of compound 5

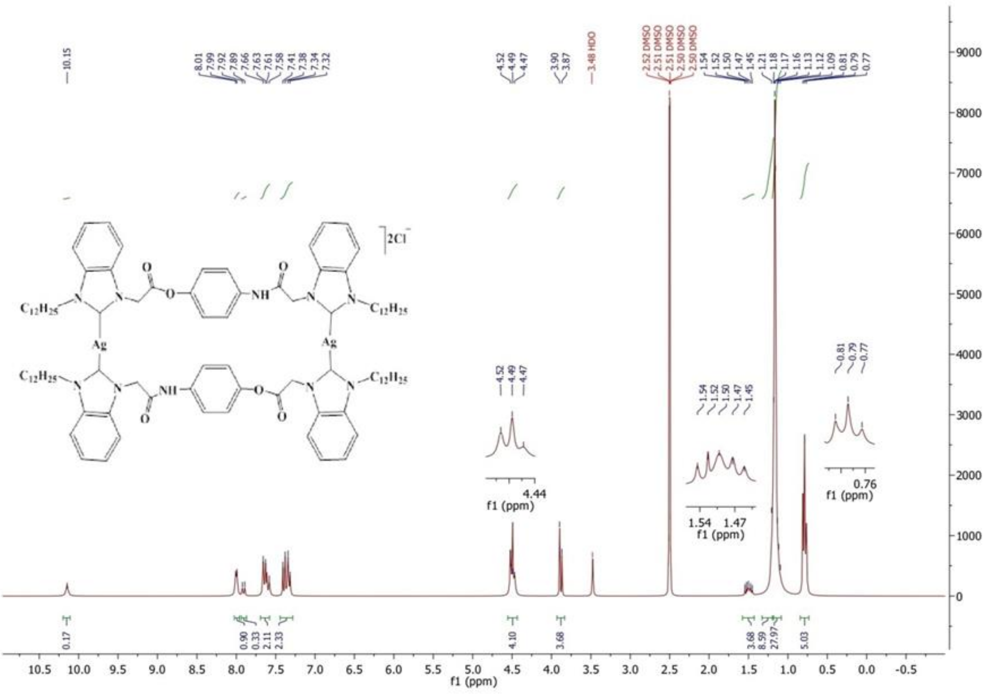


Figure (5) ^{1}H-NMR spectrum of complex 8

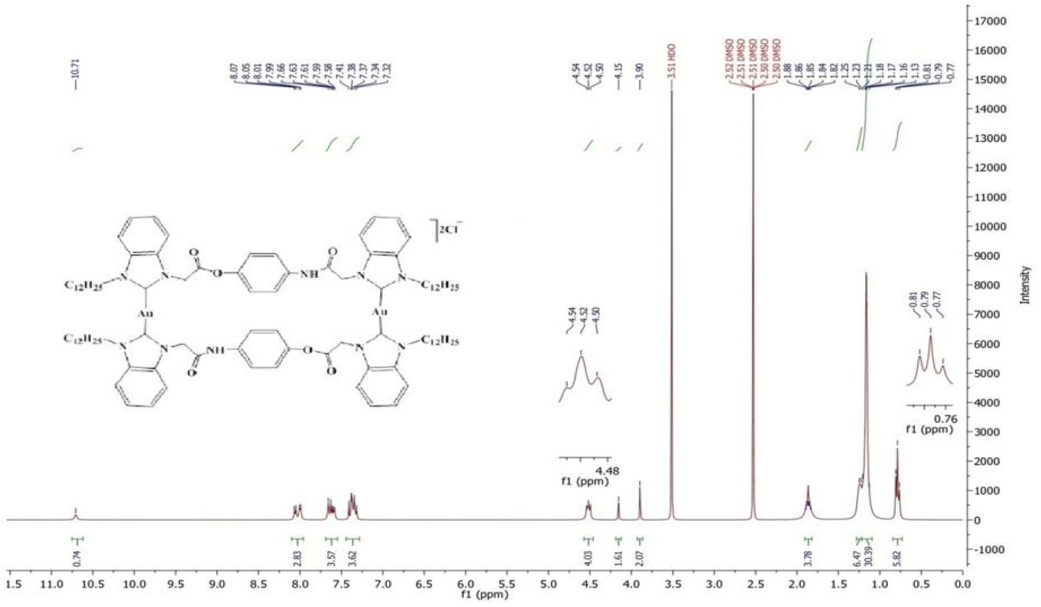


Figure (6) ^{1}H-NMR spectrum of complex 11

Table 3. ^{13}C-NMR Chemical shift range of compounds

Com.	C=O ester	C=O amid	NCN	Ar-C-N	Ar-C-O	Ar-C	ester-CH_2	amide-CH_2	N-CH_2	CH_2	CH_3
5	168.32	163.02	154.36, 154.27	143.69	136.98	132.16- 120.61	47.28	43.99	40.86	31.77- 22.57	14.40
8	170.63	163.06	188.16, 187.69	143.84	132.23	130.50- 114.15	49.42	47.26	40.78	35.12- 22.57	14.41
11	170.63	163.06	188.90, 188.16	149.34	143.84	132.22- 114.17	49.42	47.26	43.97	35.12- 19.10	14.41

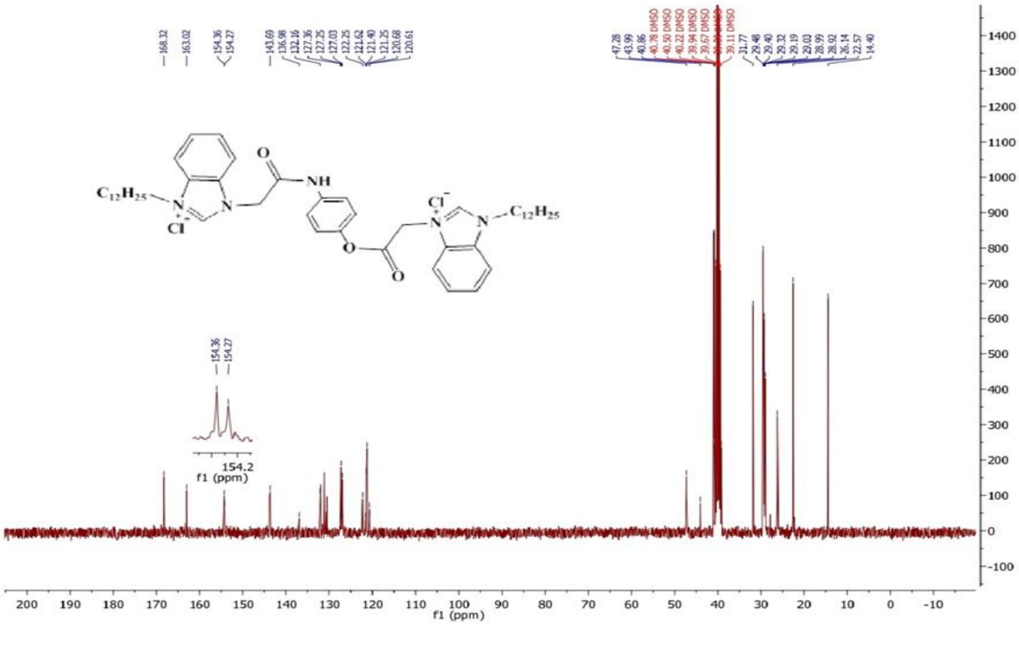


Figure (7) ^{13}C-NMR spectrum of compound 5

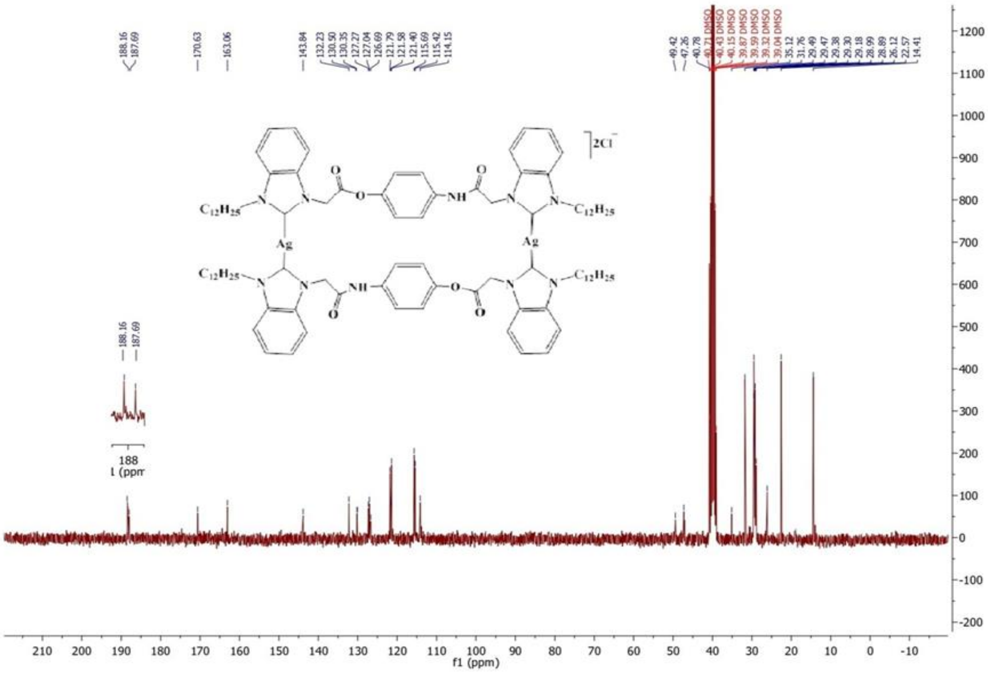


Figure (8) ^{13}C-NMR spectrum of complex 8

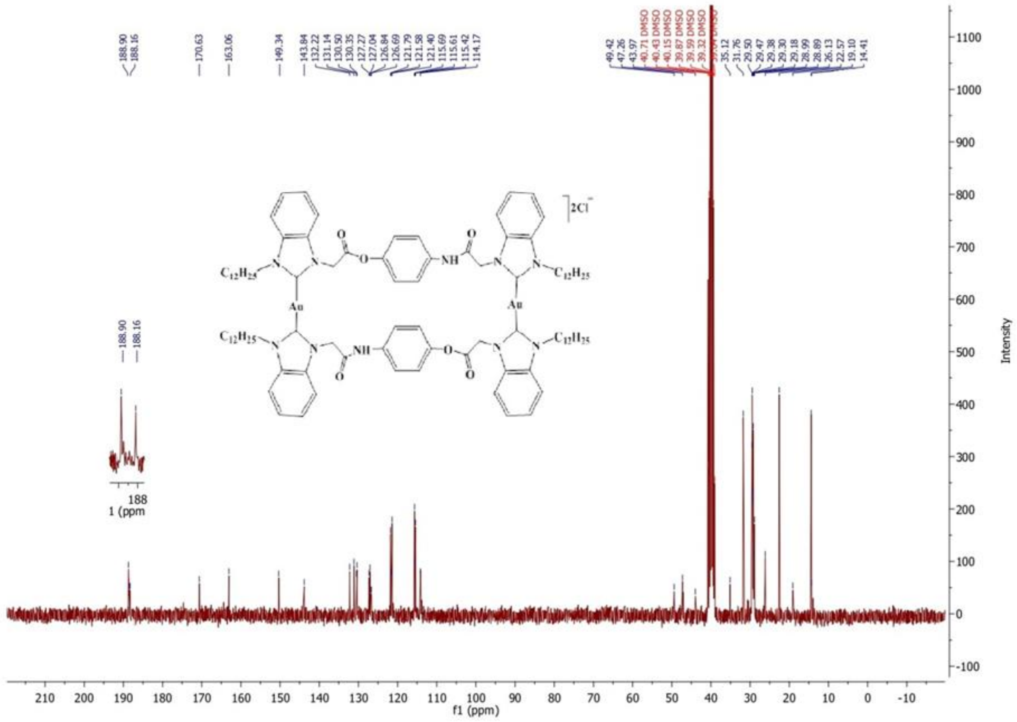


Figure (9) ^{13}C-NMR spectrum of complex 11

3.4. Anticancer activity studies

All compounds showed less cytotoxicity at lower concentrations, and the documented IC_{50} values (21.66 µg/mL, 12.73 µg/mL and 18.53 µg/mL) for (5, 8 and 11) respectively, were close to the IC_{50} value for cisplatin (15.09 µg/ml). Which is evidence that the prepared compounds are highly toxic on the a human breast cancer cell line (MCF7). 5, 8 and 11 compounds showed excellent inhibitory effects on MCF7 cells growth as the percentage was less than (60%) in concentrations (7.4 and 22.22 µg/ml). While the compounds (5, 8 and 1) showed a good ratio between (60% - 90%) at concentrations (66.66, 200 and 600 µg/ml). Interestingly, all the prepared compounds had higher inhibitory effects than cisplatin on MCF7 cells growth at all concentrations, as demonstrated in (Figure 16).

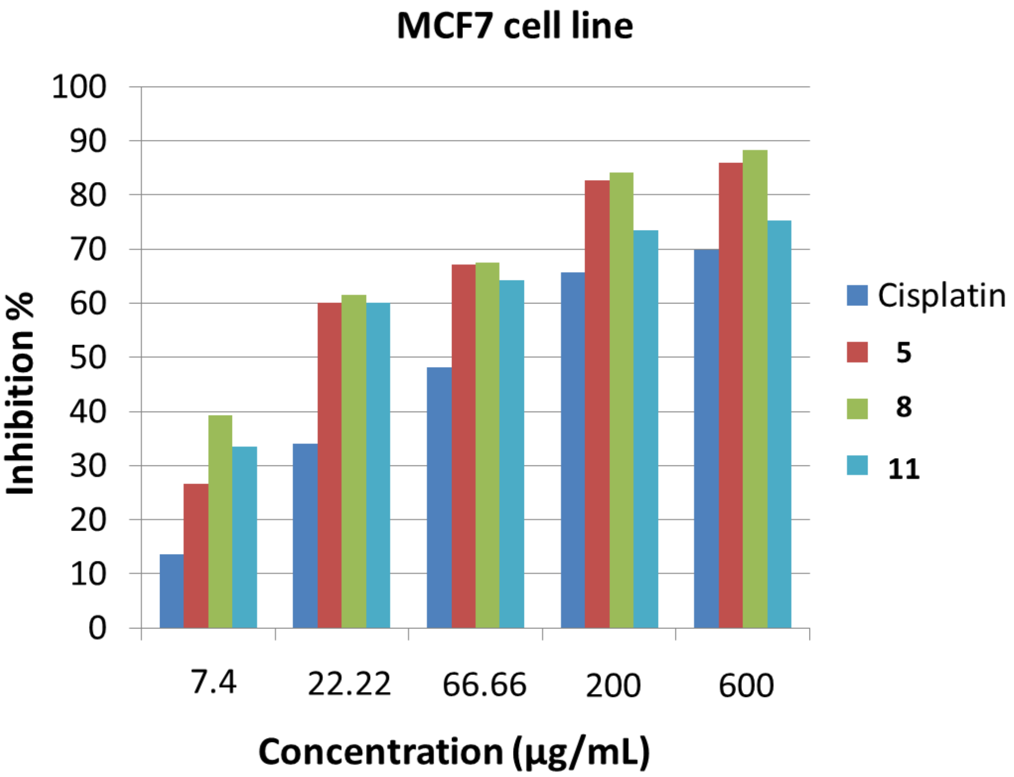


Figure (16) Evaluation of the anticancer inhibition of cisplatin, 5, 8 and 11 complexes on the MCF7 cell lines using the MTS assay

Conclusion

New Ag(I) bis-NHC and Au(I) bis-NHC complexes (8–13) were synthesized from asymmetric benzimidazolium salts (5–7) as NHCs ligands. Ag(I) bis-NHC complexes were synthesized by reacting Ag_2O with ligands via in situ reaction. A transmatellation method by reacting Ag(l) bis-NHC with $[AuCl(SMe)_2]$ was used to synthesized of Au(I) bis-NHC complexes. The results for isotopes of benzimidazolium salts and their complexes are in good agreement with observations shown by (FT-IR, ^{1}H-NMR, and ^{13}C-NMR) spectroscopy. Compounds (5, 8 and 11) were tested for in vitro anticancer activities against MCF7 cell lines. All compounds showed less cytotoxicity at lower concentrations, and the documented IC_{50} values were close to the IC_{50} value for cisplatin. Which indicates that the prepared compounds are highly toxic to human breast cancer cell lines (MCF7).

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