In vitro antimicrobial studies of naphthalen-1-ylmethyl substituted silver N-heterocyclic carbene complexes

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Abstract Seven novel naphthalen-1-ylmethyl substituted silver N-heterocyclic carbene (Ag–NHC) complexes (1–7) were synthesized by the interaction of benzimidazolium salts with silver carbonate in dry dichloromethane at room temperature and characterized by means of spectroscopic methods and elemental analysis techniques. The Ag–NHC compounds were tested for their in vitro antibacterial and antifungal activity against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Candida albicans and Candida tropicalis and showed high antimicrobial activities. The synthesized complexes, in particular, demonstrated better results against both fungi and gram-positive bacteria.

Key Words Benzimidazol-2-ylidene; N-Heterocyclic carbene; Silver complex; Antibacterial activity; Antifungal activity

1. Introduction

The first study on N-heterocyclic carbene (NHC) was conducted by Wanzlick and Schikora in 1960 (Wanzlick and Schikora, 1960). Since the report by Ofele in 1968 (Ofele, 1968), N-heterocyclic carbenes (NHCs) have received much attention (Akköç et al., 2014a,b; Cardin et al., 1972; Gök et al., 2014a,b; Inomata et al., 2011; Taige et al., 2007). In 1991, Arduengo isolated the first free carbene (Arduengo et al., 1991), and since then NHCs and the transition metal complexes derived from them have found a wide range of uses in coordination and organometallic chemistry (Akköç and Gök, 2013).
have good σ-donating but weak π-accepting ability and low toxicity (Wu et al., 2011). In addition to this, it is possible to control the steric and electronic effects of substituents on the nitrogen atom, and NHC pre-ligands are more stable against air and moisture compared to phosphate types. Various metal–NHC complexes, such as silver, palladium, gold, ruthenium and rhodium, have been synthesized by using such ligands. The metal–NHC complexes display perfect catalytic activities for very useful organic transformations, particularly the C–N and C–C coupling reactions, C–H bond activation, and metathesis reactions (Danopoulos et al., 2007; Göttker-Schnetmann et al., 2004; Karami et al., 2013; Pozo et al., 2012; Rudolph and Hashmi, 2011; Wiedemann et al., 2006). Among the metal-NHC complexes, silver complexes have been utilized for several purposes. Silver N-heterocyclic carbene (Ag–NHC) complexes are of particular interest, due to their wide use as ligand transfer agents to transition metals (Huang et al., 2014; Kumar and Cisarova, 2013; Saito et al., 2012; Wang et al., 2005; Zhu et al., 2012) and also because of their biological activities as anticancer (Gandin et al., 2012; Rudolph and Hashmi, 2011; Wiedemann et al., 2006).

In this study, seven new naphthalen-1-ylmethyl substituted Ag–NHC complexes containing benzimidazole moiety were prepared and their structures were fully characterized. All compounds were tested for in vitro antibacterial and fungal activities against the following strains: Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Candida albicans and Candida tropicalis. All compounds demonstrated antimicrobial activity against the tested gram-negative and gram-positive bacteria and fungal strains.

2. Experimental

2.1. General

All reactions were made under argon gas using standard Schlenk techniques. Some of the necessary reagents were synthesized in our laboratory while others were purchased commercially. Reagents and solvents such as benzyl chloride, 2-methylbenzyl chloride, 4-methylbenzyl chloride, 3,4,5-trimethoxybenzyl chloride, 2,4,6-trimethylbenzyl chloride, 1-(chloromethyl)naphthalene, 2,3,4,5,6-pentamethylenbenzyl chloride, silver carbonate, o-phenylenediamine, formic acid, ethyl alcohol, dimethylformamidine, hexane, dichloromethane and diethyl ether were purchased from commercial suppliers like Alfa Aesar, Merck, Aldrich and Fluka. The hexane and dichloromethane solvents were distilled before being used over Na and P2O5, respectively. The NMR spectra were recorded by using a Bruker AC300P FT spectrometer. Chemical shifts (δ) were given in ppm relative to tetramethylsilane. The melting points were restrained in open capillary tubes by using an Electrothermal-9200 device. Elemental analyses were performed with CHNS-932 LECO apparatus.

Minimal inhibitory concentrations (MICs) for all silver NHC complexes were examined against two gram-negative and two gram-positive bacteria strains which were taken from the American Type Culture Collection (Rockville, MDi USA): P. aeruginosa (ATCC 27853), E. coli (ATCC 25922), E. faecalis (ATCC 29212) and S. aureus (ATCC 29213). The two fungal strains C. albicans and C. tropicalis were taken from Ege University in Turkey. Fungal strains were subcultured on RPMI 1640 Broth (Sigma–Aldrich Chemie GmbH Taufkirchen, Germany) and bacterial strains were subcultured on Muller Hinton Broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India).

2.1.1. Chloro-[1,3-bis(naphthalen-1-ylmethyl)benzimidazol-2-ylidene]silver(1), 1

Ag–NHC complex 1 was synthesized from 1,3-bis(naphthalen-1-ylmethyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.16 g, 1 mmol) in dry dichloromethane (25 mL) at 25 °C for one day in dark conditions. Yield: 65%, m.p.: 171–172 °C. 1H NMR (300 MHz, DMSO-d6, 25 °C), δ: 6.06 (s, 4 H, CH2C6H4); 6.86–8.02 (m, 18 H, Ar–H). 13C NMR (75 MHz, DMSO-d6, 25 °C), δ: 55.4 (CH2C6H4); 112.9, 123.5, 124.9, 125.5, 125.9, 126.8, 127.2, 129.0, 129.3, 130.7, 132.2, 133.8 and 134.2 (Ar–C). IR ν(C=N) = 1612.6 cm⁻¹. Anal. calcd. for C29H22N2AgCl (541.82 g/mol): C: 64.29, H: 4.09, N: 5.17. Found: C: 64.44, H: 4.21, N: 5.11%.

2.1.2. Chloro-[1-(naphthalen-1-ylmethyl)-3-benzylbenzimidazol-2-ylidene]silver(1), 2

Ag–NHC complex 2 was synthesized from 1-(naphthalen-1-ylmethyl)-3-benzylbenzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.18 g, 1 mmol) in dichloromethane (25 mL). Yield: 62%, m.p.: 93–94 °C. 1H NMR (300 MHz, DMSO-d6, 25 °C), δ: 5.71 (s, 2 H, CH2C6H4); 6.22 (s, 2 H, CH2C6H4); 6.99–8.16 (m, 16 H, Ar–H). 13C NMR (75 MHz, DMSO-d6, 25 °C), δ: 50.3 (CH2C6H4); 112.9, 113.0, 123.6, 124.8, 125.2, 125.9, 126.7, 127.2, 127.7, 127.8, 128.5, 128.9, 129.2, 129.5, 130.1, 131.2, 133.7, 133.8, 134.4 and 136.7 (Ar–C). IR ν(C=N) = 1607.5 cm⁻¹. Anal. calcd. for C32H22N2AgCl (491.76 g/mol): C: 61.06, H: 4.10, N: 5.70. Found: C: 61.25, H: 4.27, N: 5.67%.

2.1.3. Chloro-[1-(naphthalen-1-ylmethyl)-3-(4-methylbenzyl)benzimidazol-2-ylidene]silver(1), 3

Ag–NHC complex 3 was synthesized from 1-(naphthalen-1-ylmethyl)-3-(4-methylbenzyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.17 g, 1 mmol) in dry dichloromethane (25 mL). Yield: 55%, m.p.: 175–176 °C. 1H NMR (300 MHz, DMSO-d6, 25 °C), δ: 2.23 [3, 3 H, CH2C6H4(CH3)-4]; 5.65 [s, 2 H, CH2C6H4(CH3)-4]; 6.21 (s, 2 H, CH2C6H4); 7.08–8.13 (m, 15 H, Ar–H). 13C NMR (75 MHz, DMSO-d6, 25 °C), δ: 21.1 [CH2C6H4(CH3)-4]; 50.3 [CH2C6H4(CH3)-4]; 52.4 (CH2C6H4); 112.8, 113.0, 123.6, 124.7, 125.2, 125.9, 126.8, 127.2, 127.8, 128.9, 129.2, 129.8, 129.9, 130.7, 132.3, 133.6, 133.8, 134.4 and 137.8 (Ar–C). IR ν(C=N) = 1607.3 cm⁻¹. Anal. calcd. for C34H24N2AgCl (505.79 g/mol): C: 61.74, H: 4.45, N: 5.58. Found: C: 61.61, H: 4.45, N: 5.58%.

2.1.4. Chloro-[1-(naphthalen-1-ylmethyl)-3-(2-methylbenzyl)benzimidazol-2-ylidene]silver(1), 4

Ag–NHC complex 4 was synthesized from 1-(naphthalen-1-ylmethyl)-3-(2-methylbenzyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.17 g, 1 mmol) in dichloromethane (25 mL). Yield: 60%, m.p.: 118–119 °C. 1H NMR
In vitro antimicrobial studies of naphthalen-1-ylmethyl substituted silver N-heterocyclic carbene complexes

2.1.5. Chloro-[1-(naphthalen-1-ylmethyl)-3-(2,4,6-trimethoxybenzyl)benzimidazol-2-ylidene]silver(I), 5

Ag–NHC complex 5 was synthesized from 1-(naphthalen-1-ylmethyl)-3-(2,4,6-trimethoxybenzyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.16 g, 1 mmol) in dichloromethane (25 mL). Yield: 65%, m.p.: 160.7 °C. $\text{^{13}C}$ NMR (75 MHz, DMSO-$d_6$): 160.7 cm$^{-1}$. Anal. calcd. for C$_{28}$H$_{26}$N$_2$AgCl (505.79 g/mol): C: 64.13, H: 4.99%. Found: C: 64.6, H: 4.49, N: 5.50%.

2.1.6. Chloro-[1-(naphthalen-1-ylmethyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazol-2-ylidene]silver(I), 6

Ag–NHC complex 6 was synthesized from 1-(naphthalen-1-ylmethyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.16 g, 1 mmol) in dichloromethane (25 mL). Yield: 59%, m.p.: 260–261 °C. $\text{^{13}C}$ NMR (300 MHz, DMSO-$d_6$): 160.0 cm$^{-1}$. Anal. calcd. for C$_{26}$H$_{26}$N$_2$AgCl (533.84 g/mol): C: 63.00, H: 4.91, N: 5.25%. Found: C: 63.19, H: 4.84, N: 5.21%.

2.1.7. Chloro-[1-(naphthalen-1-ylmethyl)-3-(3,4,5-trimethoxybenzyl)benzimidazol-2-ylidene]silver(I), 7

Ag–NHC complex 7 was synthesized from 1-(naphthalen-1-ylmethyl)-3-(3,4,5-trimethoxybenzyl)benzimidazolium chloride (0.5 g, 2 mmol) and silver carbonate (0.15 g, 1 mmol) in dichloromethane (25 mL). Yield: 63%, m.p.: 180–181 °C. $\text{^{13}C}$ NMR (300 MHz, DMSO-$d_6$): 160.0 cm$^{-1}$. Anal. calcd. for C$_{26}$H$_{26}$N$_2$AgCl (581.84 g/mol): C: 57.80, H: 4.50, N: 4.81. Found: C: 57.63, H: 4.59, N: 4.85%.

2.2. General preparation of benzimidazolium salts

1-(Chloromethyl)naphthalene (1 mmol) and potassium hydroxide were slowly added to a solution of benzimidazole (1 mmol) in ethyl alcohol (10 ml) and refluxed for 12 h. Aryl halide was then added to the solution of 1-(naphthalen-1-ylmethyl)benzimidazole in N,N-dimethylformamide (DMF) and stirred at 80 °C for 24 h. The benzimidazolium salt was crystallized from C$_2$H$_5$OH–Et$_2$O at room temperature.

2.3. General process for the synthesis of Ag–NHC complexes

1-(Naphthalen-1-ylmethyl)-3-alkylbenzimidazolium chloride (2 mmol), silver carbonate (1 mmol) and molecular sieves 4 Å were stirred in dry CH$_2$Cl$_2$ (20 mL) at 25 °C for one day. To prevent light exposure, the Schlenk reaction was covered with aluminum foil. After the reaction was completed, it was filtered through Celite and silica gel. The solvent in the reaction medium was removed by applying pressure. The product obtained was crystallized in a dichloromethane/n-hexane (2/1) mixture at 25 °C.

2.4. Antibacterial and antifungal activities of Ag–NHC complexes

Using the agar dilution method proposed by the Clinical and Laboratory Standards Institute, the antibacterial and antifungal activities of the Ag–NHC complexes were determined (National Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2002, 2003). MICs for silver complexes were tested against standard bacterial and fungal strains. The stock solutions of all complexes were prepared in dimethyl sulfoxide and all of the dilutions were carried out with distilled water. The concentrations of the compounds were prepared at between 6.25 μg/mL and 800 μg/mL (800, 400, 200, 100, 50, 25, 12.5 and 6.25). Over the surface of the agar plates, a loopful (0.01 mL) of the standardized inocula of the fungi and bacteria ($10^6$ CFUs/mL) was spread. All the plates were inoculated after 16–20 h and 48 h of incubation for bacteria and fungi, respectively.

3. Results and discussion

3.1. Synthesis and characterization of Ag–NHC complexes, 1–7

Ag–NHC complexes were prepared from treatment of the 1-(naphthalen-1-ylmethyl)-3-alkylbenzimidazolium salts with Ag$_2$CO$_3$ in dry CH$_2$Cl$_2$ at room temperature (Scheme 1). The compounds 1–7 were obtained as white solids in 59–65% yields. These complexes (1–7) were soluble in organic solvents such as dichloromethane, chloroform and dimethylsulfoxide. Their structures (1–7) were determined by spectroscopic methods and elemental analysis techniques. In the $\text{^{13}C}$ NMR spectra, loss of the benzimidazolium proton (NC) signal suggests the formation of the silver complexes. The characteristic peak of the carbenic carbon resonance for
compound 1 displays as a singlet at 190.8 ppm in the $^{13}$C NMR spectra. In the 2–7 complexes, the resonances for carbene carbon were not identified. While the IR $\nu$(CN) bands of the benzimidazolium salts were shown between 1561.5 and 1607.3 cm$^{-1}$, the characteristic IR $\nu$(CN) bands of the Ag–NHC complexes were obtained at 1612.6, 1607.5, 1607.3, 1607.6, 1615.3, 1653.8 and 1600.0 cm$^{-1}$ for 1–7, respectively. The results of the elemental analyses are proof verification of the synthesized compounds. Unfortunately, we were not able to obtain a suitable single crystal from these novel Ag–NHC carbene compounds for X-ray diffraction.

3.2. Antibacterial and antifungal properties of Ag–NHC complexes

The in vitro antibacterial and antifungal activities of naphthalen-1-ylmethyl substituted Ag–NHC compounds were tested against two gram-negative bacteria, two gram-positive bacteria and two fungal strains. Under sterile conditions, both the antibacterial and antifungal activities of the compounds were determined in various concentrations of the synthesized compounds using the agar dilution method. The MICs of the novel silver complexes (1–7) against gram-negative (P. aeruginosa and E. coli), gram-positive (S. aureus and E. faecalis) bacteria and fungus (C. tropicalis and C. albicans) are shown in the Table 1.

As demonstrated in the Table 1, the silver complexes were found to be effective against gram-negative and gram-positive bacteria with MIC values between 6.25 and 200 $\mu$g mL$^{-1}$. The Ag–NHC complexes 2–6 exhibited high activities (MIC values between 6.25 $\mu$g mL$^{-1}$ and 25 $\mu$g mL$^{-1}$) against gram-positive bacteria. However compound 2, which contains the benzyl group among the synthesized compounds, had the most effective activity against S. aureus bacteria. All compounds demonstrated moderate activity against gram-negative bacteria (MIC values between 100 $\mu$g mL$^{-1}$ and 200 $\mu$g mL$^{-1}$). The silver compounds 4, which contains the 2-methylbenzyl group, 5

Scheme 1 Synthesis of naphthalen-1-ylmethyl substituted silver N-heterocyclic carbene complexes.
which contains the 2,4,6-trimethylbenzyl group (MIC values: 100 μg mL$^{-1}$), and the other Ag–NHC complexes (1–3 and 7) (MIC values: 200 μg mL$^{-1}$) showed the same antimicrobial activities against E. coli and P. aeruginosa bacteria. On the other hand, compound 7 which contains the 3,4,5-trimethoxy-benzyl group, exhibited the least activity against all bacteria and fungus.

The silver complexes were also found to be effective in inhibiting the growth of fungi, with MIC values between 6.25 μg mL$^{-1}$ and 100 μg mL$^{-1}$. Complexes 1, 3, 5 and 6 demonstrated the same level of activity against both fungi (MIC value 12.5 μg mL$^{-1}$), but the highest activity was obtained with compound 4 (MIC value: 6.25 μg mL$^{-1}$). Compound 7 exhibited the least activity against the fungi (MIC value 100 μg mL$^{-1}$). In general, the synthesized compounds were found to be more effective against fungi than bacteria.

We demonstrated that the synthesized naphthalen-1-ylmethyl substituted Ag–NHC complexes were more effective against two gram-positive bacteria and fungi than other complexes studied in the literature (Akkoç et al., 2014b; Gök et al., 2014b; Günl et al., 2012; Özdemir et al., 2010a,b; Yiğit et al., 2012).

4. Conclusions

In this study, seven novel naphthalen-1-ylmethyl substituted Ag–NHC complexes were synthesized and characterized by spectroscopic methods and elemental analysis. The in vitro activities of these novel compounds toward four bacteria (Gram) and two fungi were studied by using the microdilution technique. The silver complexes (1–7) demonstrated high activity against all bacteria and fungi. The results of this study show that complex 2 is the most effective compound against gram-positive bacteria, and complex 4 displayed the highest activity against the fungal strains. The compounds 2–6, which have potential as antimicrobial agents, exhibited significant activities against gram-positive bacteria and fungi.

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References


