Mert Olgun Karataş*, Selami Günal, Ahmet Mansur, Bülent Alıcı and Engin Çetinkaya

Synthesis and antimicrobial properties of cycloheptyl substituted benzimidazolium salts and their silver(I) carbene complexes

DOI 10.1515/hc-2016-0105
Received June 28, 2016; accepted August 27, 2016; previously published online November 22, 2016

Abstract: Due to increasing infections caused by microbes, there is an urgent need for the development of new effective antimicrobial agents. Silver-*N*-heterocyclic carbene (silver-NHC) complexes are a new class of antimicrobial agents. In this study, we aimed to synthesize highly lipophilic silver-NHC complexes. Four new complexes were synthesized by the reaction of the corresponding benzimidazolium salts and Ag_2O in dichloromethane at room temperature. The synthesized compounds were characterized by ^1H NMR, ^{13}C NMR, IR and elemental analysis. The antimicrobial performances of benzimidazolium salts and silver complexes were tested against the standard bacterial strains *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and the fungi *Candida albicans* and *Candida tropicalis*. Minimum inhibitory concentrations (MICs) of all compounds were determined. The obtained data demonstrate that all benzimidazolium salts and silver complexes inhibit the growth of bacteria and fungi. Silver complexes are more active than the corresponding benzimidazolium salts (MIC: 6.25 μg/mL for Gram-positive bacteria and fungi).

Keywords: antimicrobial; benzimidazole; cycloheptyl; *N*-heterocyclic carbine; silver.

Introduction

Infections caused by bacteria and fungi are a major threat to world health. In recent decades, antibiotic-resistant microbes have emerged due to overuse of antibiotics [1, 2]. Therefore, discovery of more effective antimicrobial compounds is an important challenge. For this purpose, many classes of compounds are being investigated for potential use as antimicrobial agents. It is known that silver salts and silver-based compounds show significant antibacterial effects. For example, silver nitrate was known as an antimicrobial agent before the 1800s and it had been used in wound care for more than 200 years [3]. Another silver-based compound, silver sulfadiazine, was also used as an antimicrobial agent in the past [4]. Although silver-based compounds are well known as antimicrobial compounds, their mechanisms of action are not clear. It is assumed that a slow release of silver cation at the wound sites is responsible for antimicrobial activity [5]. Silver nitrate and silver sulfadiazine lose their effects quickly, which causes the wound site to be re-infected [6, 7]. Therefore, the usage of these silver-based compounds as antimicrobial agents has limitations. Due to these problems, researchers began searching for new and more effective silver-based antimicrobial agents. In 2004, Young and co-workers reported silver *N*-heterocyclic carbenes (silver-NHCs) as a new class of antibiotics [8].

After the first isolation of a stable NHC by Arduengo [9], metal-NHC complexes attracted much attention and today NHC ligands have an important place in organometallic chemistry [10–12]. These carbenes act as excellent σ-donor ligands and they can form stable complexes with metal cations [12, 13]. The NHC complexes release silver ion more slowly than traditional silver-based antimicrobial compounds. Thus, the effect of the silver NHC complexes is retained over a longer period of time [10]. Metal-NHC complexes are generally used as catalysts in organic reactions but after the first report of their antimicrobial properties, studies of their biological properties increased rapidly [14–17].

It is known that lipophilicity of benzimidazolium salts and their metal complexes is important in contributing to their antimicrobial effects [18]. We have previously reported the antimicrobial properties of coumarin functionalized silver-NHC complexes and observed that lipophilicity of complexes is a primary factor for antimicrobial activity [19]. Therefore, in this study, we aimed to prepare highly lipophilic new benzimidazole-based silver-NHC

***Corresponding author: Mert Olgun Karataş,** Inonu University, Faculty of Science, Department of Chemistry, 44280 Malatya, Turkey, e-mail: mert.karatas@inonu.edu.tr
Selami Günal and Ahmet Mansur: Inonu University, Faculty of Pharmacy, Department of Microbiology, 44280 Malatya, Turkey
Bülent Alıcı: Inonu University, Faculty of Science, Department of Chemistry, 44280 Malatya, Turkey
Engin Çetinkaya: Ege University Faculty of Science, Department of Chemistry, 35100, Izmir, Turkey

complexes and evaluate their antimicrobial properties. We used cycloheptyl moiety as the lipophilic substituent on the benzimidazole scaffold. For this purpose, *N*-cycloheptyl substituted benzimidazolium salts were synthesized first. The antimicrobial activities of salts and silver complexes were tested against Gram-positive, Gram-negative bacteria and fungi *Candida albicans* and *Candida tropicalis*.

Results and discussion

Chemistry

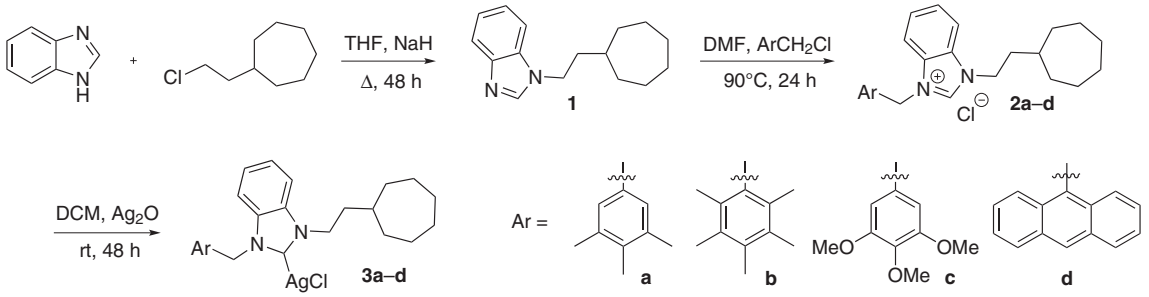
The ligand precursors, cycloheptyl substituted benzimidazolium chlorides **2**, were synthesized by quaternization of *N*-(2-cycloheptylethyl)benzimidazole (**1**) with various substituted benzyl chlorides. Compound **1** was synthesized by treatment of benzimidazole with 2-cycloheptylethyl chloride in tetrahydrofuran (THF) in the presence of sodium hydride (NaH). The reaction conditions for the synthesis of compound **1** and benzimidazolium chlorides **2a–d** are given in Scheme 1. The structures of benzimidazolium salts were confirmed by the ^{1}H NMR, ^{13}C NMR, IR spectroscopic methods and elemental analysis [20].

Various procedures have been reported for the synthesis of silver-NHC complexes. Among them, reaction of an azolium salt with an alkaline silver compound is the most commonly used method [21, 22]. In this study, Ag_2O was used as the source of alkaline silver (I). Silver-NHC complexes **3a-d** were synthesized in 16%–34% yields by reaction of the benzimidazolium chlorides **2a–d** with 0.5 equivalent Ag_2O in dichloromethane. All reactions were carried out in the dark and complexes were also stored in the dark. The synthetic route to the silver-NHC complexes **3a–d** is given in Scheme 1. The structures of complexes were established by spectroscopic data and elemental analysis. In the ^{1}H NMR spectra of **3a–d**, the disappearance of a downfield signal of acidic benzimidazolium proton (NC*H*N) is consistent with the formation of silver-NHC complexes. In the ^{13}C NMR spectra of **3a–d**, the lack of the signal for the imino carbon (N*C*HN) also supports the proposed structures. Unfortunately, the signal of the carbene carbon could not be detected because of the fluxional behavior of silver-NHC complexes. This feature has been discussed in the literature [22]. Fluxional behavior between (NHC)AgX and $[(NHC)_2Ag]^+[AgX_2]^-$ species in solution has been shown for many complexes [22]. However, it has been demonstrated that monomeric (NHC)AgX species are the most favorable form in dichloromethane [23]. All attempts to obtain a single crystal of **3** suitable for X-ray crystallographic analysis failed.

Antimicrobial evaluation

Minimal inhibitory concentrations (MICs) were determined against *Staphylococcus aureus*, *Enterococcus faecalis* (Gram-positive), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) bacterial strains and fungal strains *C. albicans* and *C. tropicalis*. The MIC values of synthesized compounds are summarized in Table 1. Ampicillin, ciprofloxacin and fluconazole were used as standard drugs for comparison. As shown in Table 1, MICs against bacteria and fungi are in the range of 800–6.25 μg/mL. All compounds inhibit the growth of the selected bacteria and fungi strains. The silver-NHC complexes are more active than benzimidazolium chlorides.

Benzimidazolium salts and complexes are more active against Gram-positive bacteria compared to Gram-negative bacteria strains. The activities of the newly synthesized silver complexes are comparable with the activities of standard drugs ampicillin and fluconazole for *S. aureus* and fungi strains. As already mentioned, the lipophilicity of complexes is the primary factor for enhanced antimicrobial activities. The lipophilicity enhances the passage of the drug through a lipid that surrounds the microorganism's cell wall [18, 24]. We previously reported the



Scheme 1 Synthesis of benzimidazolium salts and silver(I)-NHC complexes.

Table 1 Minimal inhibitory concentrations (μg/mL) of benzimidazolium salts **2a–d** and silver NHC complexes **3a–d** against tested bacteria and fungi.

Compound	Bacteria				Fungi	
	Gram-positive		Gram-negative			
	Staphylococcus aureus	*Enterococcus faecalis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Candida tropicalis*
2a	25	25	100	200	25	25
2b	25	25	100	200	25	25
2c	25	25	800	800	25	25
2d	25	25	100	200	25	25
3a	6.25	6.25	25	25	6.25	6.25
3b	6.25	6.25	25	25	6.25	6.25
3c	6.25	6.25	25	25	6.25	6.25
3d	6.25	6.25	50	50	6.25	6.25
Ampicillin	3.12	1.56	3.12	–	–	–
Ciprofloxacin	0.39	0.78	1.56	3.12	–	–
Fluconazole	–	–	–	–	3.12	3.12

synthesis and antimicrobial properties of coumarin functionalized silver-NHC complexes [19] and when antimicrobial activities of complexes **3a–d** are compared with those of the complexes of the coumarin derivatives, it is obvious that complexes **3a–d** have better antimicrobial properties.

Conclusion

The synthesis, characterization and antimicrobial properties of cycloheptyl substituted benzimidazolium salts and their silver(I)-NHC complexes are described. The complexes are candidates for practical antimicrobial agents.

Experimental

All reactions for the preparation of benzimidazolium salts and their silver derivatives were carried out in standard Schlenk-type flasks under an atmosphere of dry argon. Chemicals and solvents were purchased from Sigma-Aldrich (Istanbul, Turkey). THF was dried and distilled over Na/K alloy. Dichloromethane was dried over P_2O_5. Melting points were determined in open capillary tubes on an Electrothermal-9200 melting point apparatus. Fourier transform infrared (FT-IR) spectra were recorded on a Perkin-Elmer 100 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker FT spectrometers operating at 300 MHz (^{1}H) and 75 MHz (^{13}C). Elemental analyses were performed using an LECO CHNS-932 elemental analyzer.

Synthesis of 1-(2-cycloheptylethyl)benzimidazole (1)

A mixture of benzimidazole (3 g, 25 mmol) (50 mL) and NaH (600 mg, 25 mmol) in anhydrous THF (50 mL) was stirred for 1 h at ambient temperature, then treated with 1-chloro-2-cycloheptylethane (4 g, 25 mmol) and heated at 70°C for 48 h with continuous stirring. After cooling, the mixture was filtered through celite and the filtrate was concentrated. Compound **1** was obtained in 68% yield as a yellow oil; ^{1}H NMR ($CDCl_3$): δ 8.14 (s, 1H, -NC*H*N-), 7.07–7.59 (m, 4H, Ar*H*), 4.13 (t, 2H, J = 7 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 0.85–1.70 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR ($CDCl_3$): δ 143.9, 143.4, 133.7, 122.1, 121.3, 119.4, 110.3, 44.0, 34.9, 32.1, 29.5, 25.2, 24.6.

General procedure for preparation of benzimidazolium salts 2a–d

A solution of **1** (3 mmol, 0.73 g) in dried dimethylformamide (DMF) (5 mL) was treated with the corresponding benzyl chloride (3 mmol) and the mixture was heated for 24 h at 80°C, after which it was cooled and concentrated under reduced pressure. The residue was crystallized from ethanol/ether (1/1).

1-(2-Cycloheptylethyl)-3-(2,4,6-trimethylbenzyl)benzimidazolium chloride (2a) The salt was obtained in 84% yield as a white solid; mp 203–205°C; IR: 1554 cm^{-1} (N=C); ^{1}H NMR (DMSO-d_6): δ 9.29 (s, 1H, -NC*H*N-), 7.12–8.15 (m, 4H, Ar*H*), 7.02 (s, 2H, Ar*H*), 5.67 (s, 2H, -NCH_2Ph), 4.48 (t, 2H, J = 7.1 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 2.29 (s, 3H, ArCH_3), 2.25 (s, 6H, ArCH_3), 0.94–1.84 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR (DMSO-d_6): δ 141.1, 138.7, 138.3, 131.6, 131.2, 129.5, 126.7, 126.6, 125.8, 113.9, 113.7, 46.6, 45.0, 34.7, 32.0, 24.8, 24.6, 20.6, 19.3. Anal. Calcd for $C_{26}H_{35}ClN_2$: C, 75.98; H, 8.58; N, 6.82. Found: C, 75.88; H, 8.51; N, 6.84.

1-(2-Cycloheptylethyl)-3-(2,3,4,5,6-pentamethylbenzyl)benzimidazolium chloride (2b) This salt was obtained in 89% yield as a white solid; mp 212–214°C; IR: 1561 cm^{-1} (N=C); ^{1}H NMR (DMSO-d_6): δ 9.18 (s, 1H, -NC*H*N-), 7.72–8.23 (m, 4H, Ar*H*), 5.72 (s, 2H, -NCH_2Ph), 4.48 (t, 2H, J = 7.1 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 2.26 (s, 3H, ArCH_3), 2.22 (s, 6H, ArCH_3), 2.19 (s, 6H, ArCH_3), 0.96–1.82 (m,15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR (DMSO-d_6): δ 140.9, 136.2, 133.8, 132.9, 131.5, 131.2, 126.8, 126.6, 125.7, 113.9, 113.8, 46.5, 46.3, 34.7, 32.0, 28.9, 24.8, 24.6, 17.0, 16.7, 16.4.

Anal. Calcd for $C_{28}H_{39}ClN_2$: C, 76.59; H, 8.95; N, 6.38. Found: C, 76.61; H, 8.71; N, 6.49.

1-(2-Cycloheptylethyl)-3-(3,4,5-trimethoxybenzyl)benzimidazolium chloride (2c) This salt was obtained in 92% yield as a white solid; mp 219–220°C; IR: 1572 cm^{-1} (N=C); ^1H NMR (DMSO-d_6): δ 10.28 (s, 1H, -NC*H*N-), 7.67–8.18 (m, 4H, Ar*H*), 7.02(s, 2H, Ar*H*), 5.67 (s, 2H, -NCH_2Ph), 4.53 (t, 2H, J = 7.1 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 3.78 (s, 6H, ArOCH_3), 3.63 (s, 3H, ArOCH_3), 1.00–1.93 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR (DMSO-d_6): δ 153.1, 142.4, 137.6, 131.2, 130.8, 129.3, 126.6, 126.5, 114.0, 113.8, 106.5, 59.9, 56.1, 50.1, 46.7, 34.9, 32.1, 28.6, 24.9, 24.6. Anal. Calcd for $C_{26}H_{35}N_2ClO_3$: C, 68.03; H, 7.69; N, 6.10. Found: C, 68.08; H, 7.61; N, 6.05.

1-(2-Cycloheptylethyl)-3-(anthracen-9-ylmethyl)benzimidazolium chloride (2d) This salt was obtained in 66% yield as a yellow solid; mp 216–217°C; IR: 1544 cm^{-1} (N=C); ^1H NMR (DMSO-d_6): δ 9.15 (s, 1H, -NC*H*N-), 8.92 (s, 1H, Ar*H*), 7.59–8.41 (m, 12H, Ar*H*), 6.76 (s, 2H, -NCH_2Ant.), 4.36 (t, 2H, J = 7 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 0.83–1.71 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR (DMSO-d_6): δ 141.2, 131.7, 131.1, 131.0, 130.4, 129.4, 127.8, 126.8, 126.7, 125.6, 123.4, 122.0, 114.3, 113.9, 46.4, 43.5, 34.6, 32.0, 28.7, 24.6. Anal. Calcd for $C_{31}H_{33}ClN_2$: C, 79.38; H, 7.09; N, 5.97. Found: C, 79.42; H, 7.11; N, 5.84.

General procedure for preparation of silver complexes 3a–d

A mixture of Ag_2O (120 mg, 0.5 mmol), a benzimidazolium salt **2a–d** (1 mmol) and activated 4 Å molecular sieves in dichloromethane (25 mL) was stirred at room temperature for 48 h. Then the mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The residue was crystallized from dichloromethane/*n*-hexane at ambient temperature and washed with *n*-hexane (3×5 mL). All manipulations for the synthesis of silver-NHC complexes were carried out in the dark.

[1-(2-Cycloheptylethyl)-3-(2,4,6-trimethylbenzyl)-2-benzimidazolylidene]silver(I) chloride (3a) This complex was obtained in 23% yield as a white solid; mp 113–117°C; IR: 1598 cm^{-1} (N-C); ^1H NMR ($CDCl_3$): δ 7.17–7.41 (m, 4H, Ar*H*), 6.91 (s, 2H, Ar*H*), 5.41 (s, 2H, -NCH_2Ph), 4.30 (t, 2H, J = 7.3 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 2.28 (s, 3H, ArCH_3), 2.16 (s, 6H, ArCH_3), 0.92–1.82 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR ($CDCl_3$): δ 138.5, 136.4, 133.2, 132.7, 129.3, 125.6, 123.1, 123.0, 110.7, 110.4, 49.1, 46.8, 38.9, 34.6, 31.6, 29.5, 24.9, 24.1, 20.2, 19.4. Anal. Calcd for $C_{26}H_{34}N_2AgCl$: C, 60.30; H, 6.62; N, 5.41. Found: C, 60.14; H, 6.54; N, 5.28.

[1-(2-Cycloheptylethyl)-3-(2,3,4,5,6-pentamethylbenzyl)-2-benzimidazolylidene]silver(I) chloride (3b) This complex was obtained in 31% yield as a white solid; mp 180–183°C; IR: 1612 cm^{-1} (N-C); ^1H NMR ($CDCl_3$): δ 7.31–7.38 (m, 4H, Ar*H*), 5.39 (s, 2H, -NCH_2Ph), 4.27 (t, 2H, J = 7.3, Hz -NCH_2CH$_2$C$_7$H$_{13}$), 2.22 (s, 3H, ArCH_3), 2.21 (s, 6H, ArCH_3), 2.11 (s, 6H, ArCH_3), 0.92-1.79 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR ($CDCl_3$): δ 137.3, 134.2, 132.9, 126.6, 124.2, 123.9, 111.5, 111.4, 50.4, 47.7, 40.0, 35.6, 32.6, 30.5, 25.9, 25.1, 17.4, 17.2, 17.1. Anal. Calcd for $C_{28}H_{38}N_2AgCl$: C, 61.60; H, 7.02; N, 5.13. Found: C, 61.44; H, 7.14; N, 5.22.

[1-(2-Cycloheptylethyl)-3-(3,4,5,-trimethoxylbenzyl)-2-benzimidazolylidene]silver(I) chloride (3c) This complex was obtained in 34% yield as a white solid; mp 143–144°C; IR: 1608 cm^{-1} (N-C); ^1H NMR ($CDCl_3$): δ 7.20–7.44 (m, 4H, Ar*H*), 6.47 (s, 2H, Ar*H*), 5.43 (s, 2H, -NCH_2Ph), 4.35 (t, 2H, J = 7 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 3.74 (s, 9H, ArOCH_3), 0.97–1.86 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR ($CDCl_3$): δ 152.7, 137.2, 132.8, 132.7, 129.6, 123.3, 123.2, 111.0, 110.6, 103.7, 59.9, 55.3, 52.5, 48.8, 38.9, 34.6, 31.6, 29.6, 25.0, 24.1. Anal. Calcd for $C_{28}H_{38}N_2O_3AgCl$: C, 55.18; H, 6.06; N, 4.95. Found: C, 55.27; H, 6.13; N, 5.09.

[1-(2-Cycloheptylethyl)-3-(anthracen-9-ylmethyl)-2-benzimidazolylidene]silver(I) chloride (3d) This complex was obtained in 16% yield as a yellow solid; mp 223–225°C; IR: 1582 cm^{-1} (N-C); ^1H NMR ($CDCl_3$): δ 8.56 (s, 1H, Ar*H*), 8.00–8.15 (m, 4H, Ar*H*), 7.00–7.50 (m, 8H, Ar*H*), 6.36 (s, 2H, -NCH_2Ant.), 4.26 (t, 2H, J = 7.4 Hz, -NCH_2CH$_2$C$_7$H$_{13}$), 0.90–1.78 (m, 15H, -NCH$_2$CH_2C$_7$H$_{13}$); ^{13}C NMR ($CDCl_3$): δ 134.2, 133.8, 131.5, 131.2, 130.4, 130.1, 127.7, 125.3, 124.1, 123.1, 122.9, 112.1, 111.4, 50.1, 46.5, 39.9, 35.6, 32.6, 30.5, 25.9, 25.1. Anal. Calcd for $C_{31}H_{32}N_2AgCl$: C, 64.65; H, 5.60; N, 4.86. Found: C, 64.54; H, 5.43; N, 4.96.

Antimicrobial activities of benzimidazolium salts and silver complexes

Antimicrobial performances of the benzimidazolium salts **2a–d** and silver-NHC complexes **3a–d** were determined by using the agar dilution procedure recommended by the Clinical and Laboratory Standards Institute [25, 26]. MICs were obtained against standard bacterial strains *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 and the fungal strains *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 13803 purchased from American Type Culture Collection (Rockville, MD). Bacterial strains were subcultured on a Muller Hinton broth (HiMedia Laboratories Pvt. Ltd. Mumbai, India). The fungal strains were subcultured on an RPMI 1640 broth (Sigma-Aldrich Chemie GmbH Taufkirchen, Germany). Their turbidities matched that of a McFarland no. 0.5 turbidity standard [27]. The stock solutions were prepared in dimethyl sulfoxide (DMSO). All dilutions were done with distilled water. The concentrations of the tested compounds were 800, 400, 200, 100, 50, 25, 12.5 and 6.25 µg/mL. Ampicillin and ciprofloxacin were used as antibacterial standard drugs, while fluconazole was used as an antifungal standard drug. A loopful (0.01 mL) of the standardized inoculum of the bacteria and yeasts (10^6 CFUs/mL) was spread over the surface of agar plates. All inoculated plates were incubated at 35°C and the results were evaluated after 16–20 h of incubation for bacteria and 48 h for yeasts. The lowest concentration of the compounds that prevented visible growth was considered to be the MIC.

References

[1] Walsh, C. T.; Wright, G. Antibiotic resistance. *Chem. Rev.* **2005**, *105*, 391–393.
[2] Fisher, J. F.; Meroueh, S. O.; Mobashery, S. Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. *Chem. Rev.* **2005**, *105*, 295–424.
[3] Klasen, H. J. Historical review of the use of silver in the treatment of burns. *J. Burns* **2000**, *26*, 117–130.
[4] Fox, C. L. Silver sulfadiazine a new topical therapy for pseudomonas in burns. *Arch. Surg.* **1968**, *96*, 184–188.

[5] Fox, C. L.; Modak, S. M. Mechanism of silver sulfadiazine action on burn wound infections. *Antimicrob. Agents Chemother*. **1974**, *5*, 582–588.
[6] Modak, S. M.; Fox, C. L. Sulfadiazine silver resistant Pseudomonas in burns: new topical agents. *Arch. Surg*. **1981**, *116*, 854–857.
[7] Modak, S. M.; Fox, C. L. Synergistic action of silver sulfadiazine and sodium piperacillin on resistant Pseudomonas aeruginosa *in vitro* and experimental burn wound infections. *J. Trauma* **1985**, *25*, 27–31.
[8] Melaiye, A.; Simons, R. S.; Milsted, A.; Pignitore, F.; Westemiotis, C.; Tessier, C. A.; Young, W. J. Formation of water soluble pincer silver(I)-carbene complexes: novel antimicrobial agents. *J. Med. Chem*. **2004**, *47*, 973–977.
[9] Arduengo, A. J.; Harlow, R. L.; Kline, M. A stable crystalline carbene. *J. Am. Chem. Soc*. **1991**, *113*, 361–363.
[10] Velazquez, H. D.; Verpoort, F. *N*-heterocyclic carbene transiton metal complexes for catalysis in aqueous media. *Chem. Soc. Rev*. **2012**, *41*, 7032–7060.
[11] Liu, W.; Gust, R. Metal *N*-heterocyclic carbine complexes as potential antitumor metallodrugs. *Chem. Soc. Rev*. **2013**, *47*, 755–773.
[12] Kaufhold, S.; Petermann, C.; Staelher, R.; Rau, S. Transition metal complexes with N-heterocyclic carbine ligands: From organometallic hydrogenation reactions toward water splitting. *Coord. Chem. Rev*. **2015**, *305*, 73–87.
[13] Gusev, D. G. Electronic and steric parameters of 76 N-heterocyclic carbenesin Ni(CO)3(NHC). *Organometallics* **2009**, *28*, 6458–6461.
[14] Dehninger, L.; Rubbiani, L.; Ott, I. *N*-heterocyclic carbine metal complexes in medicinal chemistry. *Dalton Trans*. **2013**, *42*, 3269–3284.
[15] Fernandez, G. A.; Gurovic, M. S. V.; Olivera, N. L.; Chopan, A. B.; Silbestri, G. B. Antibacterial properties of water-soluble gold(I) *N*-heterocyclic carbine complexes. *J. Inorg. Biochem*. **2014**, *135*, 54–57.
[16] Li, J. Y.; Lee, J. Y.; Chang, Y. Y.; Hu, C. H.; Wang, N. M.; Lee, H. M. Palladium complexes with tridentate N-heterocyclic carbine ligands: Selective 'Normal' and 'Abnormal' bindings and their anticancer activities. *Organometallics* **2015**, *34*, 4359–4368.
[17] Gallardo, J. F.; Elie, B. T.; Sanov, M.; Conte,l M. Versatile synthesis of cationic N-heterocyclic carbine-gold(I) complexes containing a second ancillary ligand. Design of heterobimetallic ruthenium-gold anticancer agents. *Chem. Comm*. **2016**, *52*, 3155–3158.
[18] Hindi, K. M.; Panzner, M. J.; Tessier, C. A.; Cannon, C. L.; Youngs, W. J. The medical applications of imidazolium carbine metal complexes. *Chem. Rev*. **2009**, *109*, 3859–3884.
[19] Karatas, M. O.; Olgundeniz, B.; Gunal, S.; Ozdemir I.; Alici B.; Cetinkaya E. Synthesis, characterization and antimicrobial activities of novel silver(I) complexes with coumarin substituted N-heterocyclic carbine ligands. *Bioorg. Med. Chem*. **2016**, *24*, 643–650.
[20] Allegue, A.; Soriano, M. A.; Pastor, I. M. A comperative study of hydroxyl- and carboxyl functionalized imidazolium and benzimidazolium salts as precursors for *N*-heterocyclic carbine ligands. *Appl. Organomet. Chem*. **2015**, *29*, 624–632.
[21] Wang, H. M. J.; Lin, I. J. B. Facile synthesis of silver(I)-carbene complexes: Useful carbine transfer agents. *Organometallics* **1998**, *17*, 972–975.
[22] Lin, I. J. B.; Vasam, C. S. Silver(I)-*N*-heterocyclic carbenes. *Comment Inorg. Chem*. **2004**, *25*, 75–129.
[23] Hayes, J. M.; Viciano, M.; Peris, E.; Ujaque, G.; Lledos, A. Mechanism of formation of silver *N*-heterocyclic carbenes using silver oxide: a theoretical study. *Organometallics* **2007**, *26*, 6170–6183.
[24] Karvembu, R.; Jayabalakrishan, C.; Natajan, N. Thio(β diketonato)-bridged binuclear ruthenium(III) complexes containing triphenylphosphine or triphenylarsine. Synthetic, spectral, catalytic and antimicrobial studies. *Transition Met. Chem*. **2002**, *27*, 574–479.
[25] Clinical and Laboratory Standards Institute: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Seventh Edition, CLSI Document M7-A7, Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2003.
[26] Clinical and Laboratory Standards Institute: Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard-Second Edition. NCCLS document M27-A2 (ISBN 1-56238-469-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA, 19087-1898 USA, 2002.
[27] Hindler, J.; Hochstein, L.; Howell, A. Preparation of Routine Media And Reagents Used In Antimicrobial Susceptibility Testing. Part 1. McFarland standards. In *Clinical microbiology procedures handbook*. Isenberg H. D., Ed. American Society for Microbiology: Washington, DC, 1992; Vol. 1, pp. 5.19.1–5.19.6.