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N-Heterocyclic carbene–silver complexes: A new class of antibiotics

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Contents

Abstract

N-Heterocyclic carbene (NHC)–silver complexes were synthesized from pyridine linked pincer ligands and methylated caffeine. Pincer NHC–silver complexes were found to have more potent antimicrobial activity than the conventionally used silver antimicrobials. Encapsulation of a gem-diol pincer NHC–silver complex in a polymer mat demonstrated a promising method for the sustained delivery of silver ions in wound care applications. An NHC precursor derived from caffeine was found to have a low toxicity and the resulting silver complex showed encouraging antimicrobial activity against numerous pathogens including resistant organisms isolated from the lungs of patients with cystic fibrosis (CF). Bacteria studied included members of the *Burkholderia cepacia* complex, which cause significant morbidity and mortality in infected CF patients. This review explores this newly growing area, focusing on the synthesis from pincer and xanthine ligands of new silver–NHC complexes and their antimicrobial activities.

Keywords: *N*-Heterocyclic carbene; Silver; Antimicrobial; Encapsulation; Caffeine; Cystic fibrosis

1. Introduction

N-Heterocyclic carbenes (NHCs), are cyclic carbenes (Fig. 1) that are usually derived from the deprotonation of imidazolium salts. NHC chemistry was first investigated by Wanzlick et al. [1] in the early 1960s leading to the synthesis of the first NHC transition metal complexes of chromium and mercury by Öfele [2] and Wanzlick and Schönherr [3] in 1968. The following decades saw limited activity in this area, the most notable by Lappert and co-workers who synthesized several NHC–metal complexes from electron-rich olefins [4]. The isolation of the first stable NHC, 1,3-diadmantylimidazol-2-ylidene, by Arduengo et al. [5] was a breakthrough and lead to significant interest in this field of chemistry. The coordination chemistry of NHC–metal complexes continues to be actively studied, particularly for catalytic applications [6].

NHCs are strong nucleophiles and bind to both main group and transition metals often with greater stability than phosphines [6]. The carbene carbon atom of NHCs is stabilized by the p_π–p_π electron donation of the two adjacent nitrogen atoms accounting

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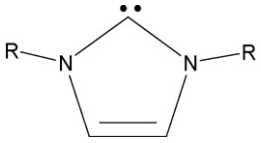


Fig. 1. General representation of an NHC.

for a stabilization energy of approximately 70 kcal/mol. For unsaturated systems aromaticity can contribute an additional 25 kcal/mol of stabilization (Fig. 2) [6]. The nitrogen atoms also stabilize the lone pair electrons of the carbene inductively [6b]. Although NHCs are viewed mainly as sigma-donors, recent theoretical and structural studies [7] suggest the existence of some π-backbonding for certain metal centers [8] (Fig. 3).

The first silver–NHC complex was synthesized using a free carbene (Scheme 1) [10]. However, this method has been applied to the synthesis of only a limited number of silver–NHC complexes [11–13] due to the difficulty of generating most free carbenes, which have sensitivities to air, moisture and heat [9].

The *in situ* deprotonation of imidazolium salts with basic silver precursors is the most commonly used method to synthesize silver–NHC complexes. The most commonly used base is silver(I) oxide and other bases such as silver(I) acetate and silver(I) carbonate are also used. The first example of this method was the reaction of a triazolium salt and silver acetate by Bertrand and co-workers [14]. The use of silver oxide to give silver complexes of 1,3-diethylbenzimidazole-2-ylidine was pioneered by Lin and co-worker (Scheme 2) [15]. More recently, Danopoulos and co-workers reported the use of silver carbonate to deprotonate imidazolium salts to give silver–NHC complexes [16].

The use of silver oxide has made the synthesis of silver–NHC complexes much easier [17]. The reactions can be performed

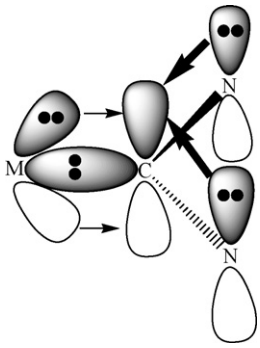


Fig. 3. Bonding of NHCs to metal centers [9b].

at ambient conditions, in a variety of solvents including water with little work up [18–20]. The formation of silver complexes in water suggests that the deprotonation of the imidazolium salt and coordination to metal center is a concerted process because free NHCs are water sensitive [9b].

Silver–NHC complexes show diverse structures in the solid state depending on the ratio of silver reagent to the imidazolium salts used in the synthesis, the nature of the NHC ligand, and the source of the silver. Counter anions, solvent and temperature can also be factors in determining structure [9a,21]. A more detailed discussion on the synthesis, characterization and structural diversity of the silver–NHC complexes can be found in reviews by Lin and Vasam and Garrison and Youngs [9].

The lack of ^{13}C–$^{107/109}Ag$ couplings in the NMR spectra in various silver–NHC complexes suggests that the silver–carbene bond is labile, and has lead to their use as carbene transfer reagents. This method was first used by Lin and co-worker to synthesize palladium and gold complexes [15], and several transition metal complexes have been synthesized utilizing this method [9].

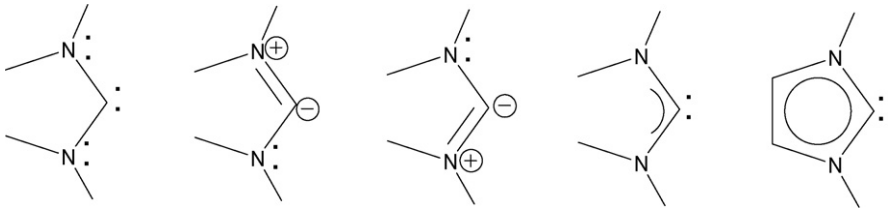
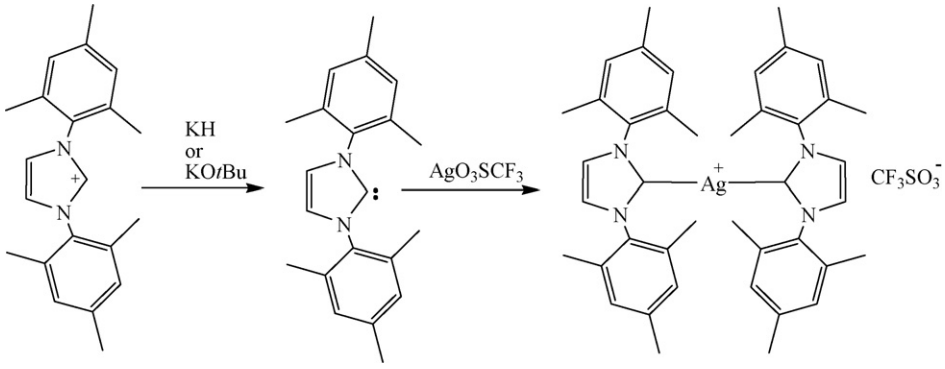
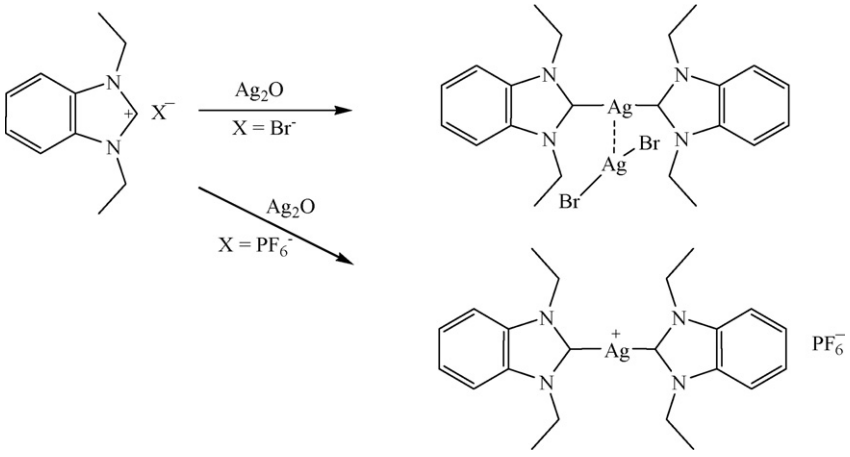


Fig. 2. The resonance structures depicted on the diamino carbene part of NHC and the aromaticity [6b].



Scheme 1. Synthesis of the first Ag(I) NHC complex.



Scheme 2. The first Ag(I) NHC complexes by Ag_2O deprotonation.

2. The use of silver compounds as antimicrobials

2.1. Background on silver antimicrobials

The use of silver as an antimicrobial can be traced to ancient times. Early civilizations used silver metal to purify and store drinking water [22]. The antimicrobial properties of silver nitrate were well known long before the 1800s, and it was recognized as an antiseptic in wound care for more than 200 years [23]. In the late 19th century, it was reported that at very low concentrations silver compounds killed certain microorganisms and the term oligodynamic, active with few ions, was used for the first time to explain this property [22,24]. In 1881, Créde began the use of 1% silver nitrate solution to prevent eye infections in newborns, a method which is still used today [23]. Colloidal silver solutions were introduced in the early 20th century to avoid the irritation associated with silver nitrate and remained popular until the 1940s [25,26]. Silver compounds then lost favor following the discovery of penicillin and other new antibiotics [23]. The use of 0.5% silver nitrate solution for the treatment of burn wounds by Moyer [27] rekindled an interest in the area. However, the true revival of the use of silver antibiotics came with the discovery of silver sulfadiazine by Fox [28]. Silver sulfadiazine (Fig. 4) is used for the treatment of burn wounds, and was designed to combine the antibiotic sulfonamide, sulfadizine, with silver in order to obtain a wide spectrum antibiotic. It is a water insoluble complex and is polymeric in the solid state [29]. Silver sulfadiazine has been shown to be effective against a number of gram-positive and gram-negative bacteria, and is marketed as a water soluble cream, Silvadene® Cream 1%. It remains one of the most widely used antimicrobials for infections associated with burns.

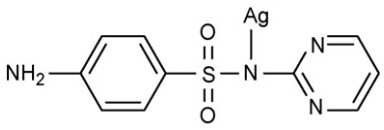


Fig. 4. Simplified drawing of the structure of silver sulfadiazine.

Silver has also been introduced into wound dressings in the form of organic and inorganic silver compounds and as silver metal, usually in the nanocrystalline form [30]. The aim of the silver containing dressings is not only the sustained release of silver to the wound site creating a barrier for infection, but also ease of use, management of wound exudates and provision of moisture required for optimal wound healing [23,30a]. Silver has been impregnated in several different kinds of dressing materials including nylon fabrics, meshes, biodegradable collogens, low adherent materials, carbon fibers, and hydrofiber alginates [23,30a]. Such silver containing dressings have been used in the treatment of acute and chronic wounds, leg ulcers and several degrees of burn wounds [30].

2.2. Mechanism of action and toxicity of silver

Silver is effective against a broad range of gram-negative and gram-positive bacteria, fungi and yeast. The pure metal is inactive; however, in the presence of moisture, silver readily ionizes to give silver cations, which show antimicrobial activity [31,23]. As early as the 1800s, Von Nägeli reported that 10^{-5} to 10^{-8} M of silver cations derived from metallic silver were effective to stop the growth of *A. niger* spores [24].

The activity of silver cations depends on their bioavailability [32]. Delivery methods, solubility and ionization of the silver sources and the presence of biological ligands such as proteins, chloride, and sulfides all affect the bioavailability [21,32]. For example, recent studies have shown that in the presence of high concentrations of chloride anions, silver becomes more bioavailable forming soluble anionic $AgCl_2^-$ compounds rather than precipitating as AgCl. Both sensitive and resistant bacteria show increased sensitivity to silver in the presence of chloride anions which is likely due to increased access of the Ag^+ ion to the cell membrane [33].

The mechanism of action of silver cations is not yet completely understood. Silver cations bind to bacteria cell surfaces, and interact with enzymes and proteins important for cell wall synthesis. Silver can also affect cell respiration, transport and metabolism, as well as DNA, RNA, and subcellular organelle

structure [21,30]. Evidence for the action of the silver cations on the cell wall of the yeast *C. albicans* has been reported [34]. It was found that silver cations bind to the cysteine residues of the essential enzyme phosphomannose isomerase used in the synthesis of the yeast cell wall resulting in loss of cell wall integrity. In addition to its antimicrobial activity, silver helps the healing process by blocking matrix metalloproteinases, which delay healing of chronic wounds [35,36].

Silver antimicrobials have been known to cause a rarely seen cosmetic side effect known as argyria. Argyria is a gray to blue discoloration of the skin due to the irreversible deposition of silver sulfide in the dermis or eyes by the occupational or therapeutic exposure of the body to silver for long periods time [37]. Silver taken into systematic circulation is excreted in the urine. The levels of silver in urine of burn patients treated with topical silver antimicrobials has been shown to increase [38,39]. Some cytotoxicity studies have shown that silver salts affect the growth of keratin producing epidermal cells [40], bone marrow [41], connective tissue cells [42], hepatocytes [43] and lymphocytes [44] by inhibiting cellular respiration with the loss of ATP [42]. However, other studies have reported no observed cytotoxicity of silver [45–47]. Therefore, the present literature is inconclusive regarding the potential toxicities of silver antimicrobials.

Bacterial resistance to silver has been rarely reported. Silver sulfadiazine resistant strains of *P. aeruginosa* have arisen in burn units; the mechanism of resistance is currently unknown [48–50]. In contrast, the mechanism of resistance of a *Salmonella* strain that resulted in numerous patient deaths and the closing of a burn ward [51] has been well studied. This strain carries a plasmid, pMG101 that encodes a peri-plasmic silver binding protein (SilE) and two parallel efflux pumps (SilCBA and SilP) [32,52]. Subsequently, plasmid-mediated resistance to silver has been identified in several other strains of bacteria [53–55].

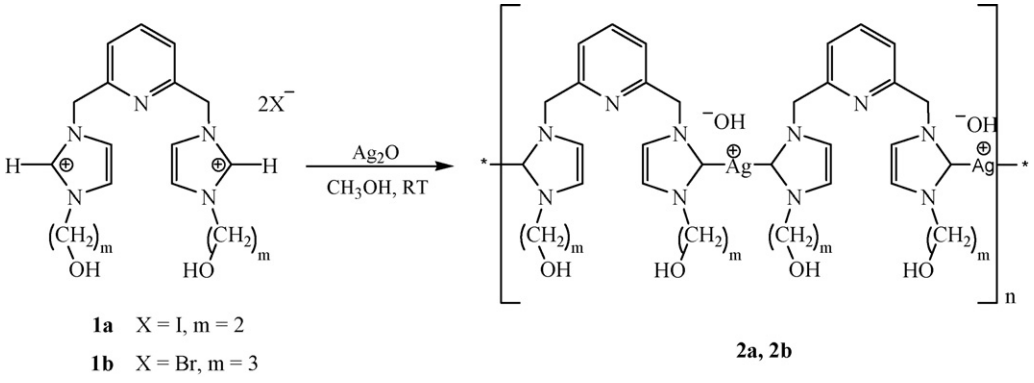
3. Synthesis and the antimicrobial properties of silver–NHC complexes

3.1. Pyridine linked pincer NHC–silver complexes

Widely used topical antimicrobials such as silver sulfadiazine and silver nitrate have been observed to kill bacteria quickly, but loose their effect in a short time causing the wound site to be reinfected. Moreover, discoloration of the skin and development of resistance of some organisms to sulfonamides limit the use of conventional silver antibiotics [48–50]. The slow release of silver at the wound sites is essential for faster healing and the prevention of infections [36,56]. The strong binding ability of NHCs to silver can result in more stable complexes that can slowly release silver ions, thus retaining the antimicrobial effect over a longer period of time. We have synthesized NHC–silver complexes **2a** and **2b** from pincer ligands **1a** and **1b** substituted by hydroxyethyl and hydroxypropyl groups, which increase the water solubility of the complexes (Scheme 3). The antimicrobial properties of these silver–NHCs against clinically important microorganisms was investigated [57].

The pyridine linked pincer ligands were prepared by the reaction of 2,6-bis(imidazolemethyl)pyridine [58] with iodoethanol and 3-bromopropanol, respectively. The reaction of **1a** and **1b** with silver oxide in aqueous methanol gave the corresponding silver–NHC complexes, **2a** and **2b**, in high yields. Complex **2a** was observed to be a one dimensional polymer in the solid state (Fig. 5) with the carbene–silver–carbene bond angle between the repeating units of 174.7(4)° and the silver–carbene bond lengths of 2.108(11) Å and 2.060(13) Å. However, mass spectrometry suggested that **2a** exists as a monomer in the gas phase.

Complexes **2a** and **2b** were tested against the clinically relevant bacteria, *E. coli*, *S. aureus* and *P. aeruginosa* to determine the minimum inhibitory concentration (MIC). MIC denotes the lowest concentration that inhibits the visible growth of the microorganism after overnight incubation [59] and is accomplished through a standard serial dilution method. Upon dissolving the complexes in the culture medium (Luria–Bertani broth), both NHC–silver complexes and silver nitrate precipitate a small amount of silver chloride. The precipitate was filtered and a dilution series of the complexes was prepared. Freshly grown organisms at a constant volume were added to the dilution series on a daily basis. As shown in Table 1, **2a** and **2b** showed better bacteriostatic effect (lower MIC value) than silver nitrate at about 2.7 times lower initial silver ion concentration. This result showed that although the theoretical amounts of the silver cations released from **2a** and **2b** were lower than silver nitrate, more silver cations were in the solution from **2a** and **2b**. Most of the silver cations from silver nitrate were precipitated as silver



Scheme 3. Synthesis of **2a** and **2b**.

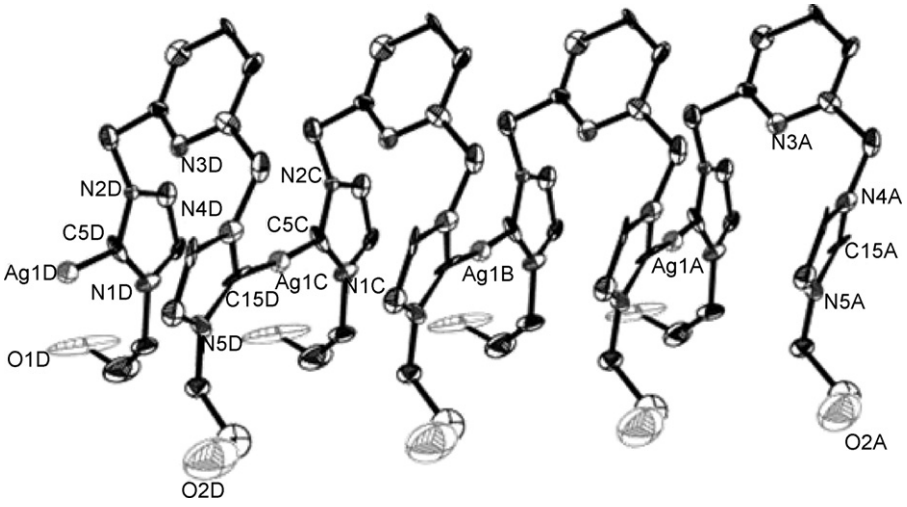


Fig. 5. Molecular structure of **2a**.

chloride and lost activity. The amount of 0.1% chloride anions in the culture medium is close to physiological concentrations of chloride (0.15 M sodium chloride). Under these conditions, **2a** and **2b** were more stable than silver nitrate. Moreover, when the solutions demonstrating the lowest MIC value were inoculated onto agar plates, the growth of organisms treated with **2a** and **2b** was delayed for a longer time than was the growth of organisms treated with silver nitrate. The observed results can be explained by the slow decomposition of the silver–NHC complexes in the aqueous culture medium to imidazolim cation and biologically active silver species. **2a** and **2b** were observed to decompose over a period of weeks in deionized water.

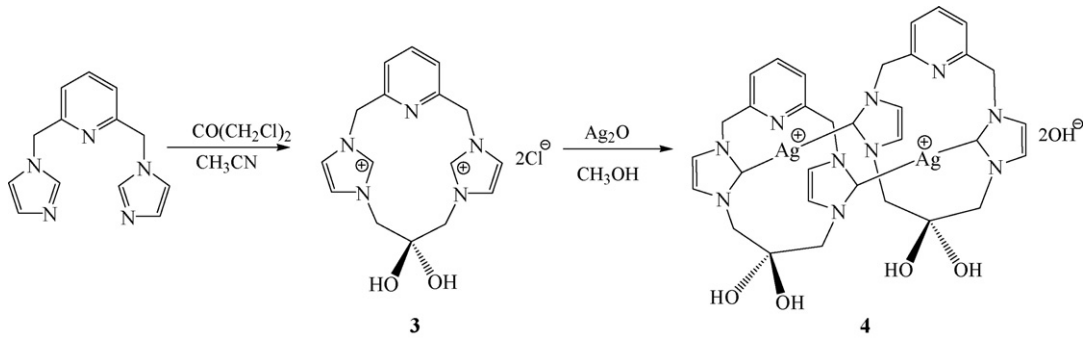
Another pyridine linked pincer NHC precursor was obtained by the reaction of 2,6-bis(imidazolemethyl)pyridine with 1,3-dichloroacetone. The expected imidazolium salt obtained as a gem-diol **3** instead of carbonyl linked cyclophane, which can be explained by an acid catalyzed process. The reaction of **3** with silver oxide in aqueous methanol gave complex **4** in high yields (Scheme 4) [60]. The single crystals of **4** have silver–carbene bond lengths ranging between 2.072(0) Å and 2.085(5) Å. There is a weak interaction between the silver centers at a distance of 3.375(10) Å, which is within the van der Waals distance (3.44 Å) for two silver atoms (Fig. 6).

Complex **4** was encapsulated into an electrospun polymer mat and the antimicrobial activity of this mat was investigated against the bacteria *E. coli*, *P. aeruginosa*, *S. aeureus* and the fungi *C. albicans*, *A. niger* and *S. cerevisiae*. Electrospun polymer mats have found several applications in drug delivery, wound dressing, filtration, protective clothing, reinforced composites and structural elements of artificial organs. The polymer mats are obtained by generating an electrically charged jet of polymer solution or polymer melt, which creates fibers upon elongation

Table 1
MIC results of the silver compounds (silver chloride removed)

Test compounds	Ag (mg/mL)	*E. coli*		*P. aeruginosa*		*S. aureus*	
		Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
2a	1186	−	−	−	−	−	−
1DF		−	+	−	−	−	+
2DF		−	+	−	+	+	
3DF		+		+		+	
4DF		+		+		+	
2b	1125	−	−	−	−	−	−
1DF		−	+	−	+	−	+
2DF		−	+	−	+	−	
3DF		+		+		+	
4DF		+		+		+	
$AgNO_3$	3176	−	+	−	+	+	
1DF		+		+		+	
2DF		+		+		+	
3DF		+		+		+	
4DF		+		+		+	

DF: dilution factor (1 mL); growth (+); no growth (−).



Scheme 4. Synthesis of **3** and **4**.

and solidification. The diameter of the fibers range from a few nanometers to several microns [21,61]. Complex **4** is slightly soluble in water, but very soluble in ethanol and stable for more than 24 h. Complex **4** was encapsulated into a medical grade polymer Tecophilic® (polyurethane), which can be electrospun from ethanol. Tecophilic® can absorb water up to 150% of its dry weight. Absorption of water by the polymer mat provides the required hydration for the slow release of the silver active species from the encapsulated complex and also provides essential moisture for wound healing [62]. The fiber mat encapsulated with **4** was characterized with transmission electron microscopy (TEM). In dry conditions, a uniform mixing of **4** and the polymer fibers was observed. However, as the fiber mat was exposed to humidity, slow decomposition of **4** resulted in the deposition of nano-sized silver particles in the fiber mats (Fig. 7).

A susceptibility test to determine the bactericidal activity of encapsulated complex **4** was performed by using a modified Kirby Bauer method. Pure Tecophilic® fiber mat was used as the control agent. Fiber mats with a composition of 25% **4** (25 wt.%) and 75% Tecophilic® (75 wt.%) and 75 wt.% **4** and 25 wt.% Tecophilic® were put on to LB agar plates containing a lawn of organisms. The plates were incubated at 35 °C overnight for the bacteria and at 25 °C for 48 h for fungi. Zones of inhibition around the fiber mats containing different silver complexes were observed (Fig. 8). However, this test was not a quantitative tool to evaluate the antimicrobial activity, since the diameters of the zones of inhibition were found to be in non-linear relationship to the amount of the silver complex encapsulated (25 wt.% **4**–75 wt.% Tecophilic®, 2.00 mm versus 75 wt.% **4**–25 wt.% Tecophilic®, 4.00 mm).

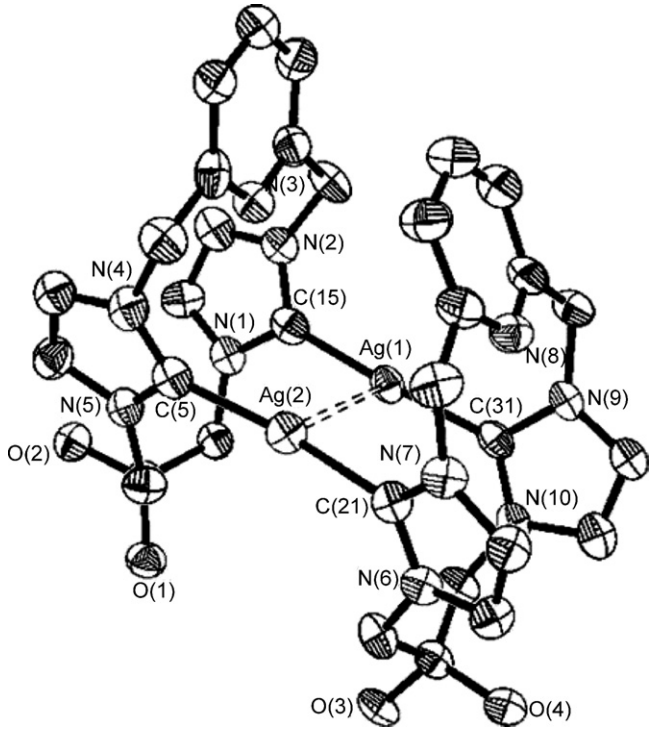


Fig. 6. Molecular structure of **4**.

Complex **4** is sparingly soluble and decomposes quickly in water. Silver nitrate showed better antimicrobial activity (MIC of 433 μg/mL) than the unencapsulated complex (838 μg/mL) after a 2 day-incubation period. However when **4** was encapsulated, the polymer mat containing 25 wt.% **4**–75 wt.% Tecophilic® having the lowest initial silver concentration of 140 μg/mL ($[Ag^+] = 140$ μg/mL) showed a complete killing of bacteria for days with the addition of a constant volume of freshly grown organisms on a daily basis. Moreover, when the killing rate of the encapsulated complex was compared with the conventionally used 0.5% silver nitrate and 1% silver sulfadiazine cream (SSD) (in the culture environment on a certain amount of bacteria with respect to time) both polymer mats 75 wt.% **4**–25 wt.% Tecophilic® ($[Ag^+] = 424$ μg/mL) and 25 wt.% **4**–75 wt.% Tecophilic® ($[Ag^+] = 140$ μg/mL) showed a better killing rate than SSD ($[Ag^+] = 3020$ μg/mL). The fiber mat having about eight-fold lower silver concentration ($[Ag^+] = 424$ μg/mL) than the silver nitrate ($[Ag^+] = 3176$ μg/mL) showed almost the same kill rate. The fiber mat with 75 wt.% **4**–25 wt.% Tecophilic® showed longer bacteriocidal activity (over 2 weeks) than the 25 wt.% **4**–75 wt.% Tecophilic® on *P. aeruginosa* by the addition of higher amounts of freshly grown organisms (200 μg/mL day) on a daily basis than used before (25 μg/mL day). Encapsulation of the silver–NHC complex increased the antimicrobial activity by enabling a slower release of active silver species. The fibers of the polymeric mat also provided a greater surface area for the active silver species to be released and increased the efficiency of the silver. The deposition of the active silver species in the fiber mat helps the culture medium to retain its original color

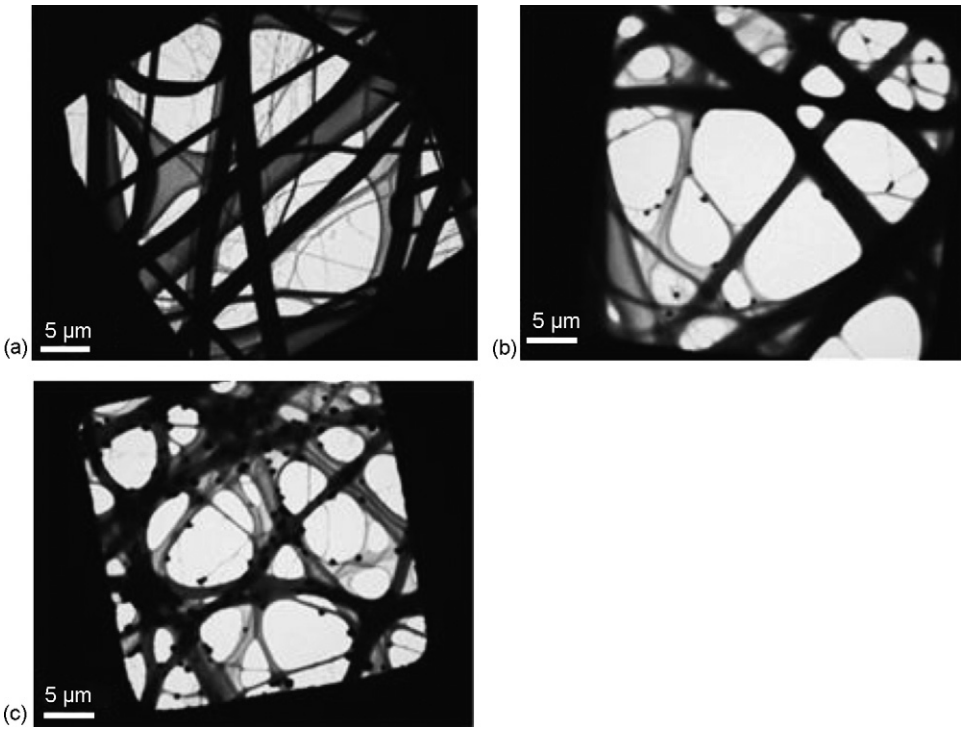


Fig. 7. TEM images of 50 wt.% **4**–50 wt.% Tecophilic® embedded into electrospun polymer mat (a) before exposure to a high moisture environment (b) after exposure to a high moisture environment for 30 min (c) after exposure to a high moisture environment for 65 h.

unlike silver nitrate which changes the LB broth to dark brown (Fig. 9). The active species released from the spun mat in the culture environment may include silver cations, nano-sized silver particles, clusters of silver cations, anionic silver chloride complexes and silver chloride.

3.2. Silver–NHC complexes derived from xanthines

The presence of imidazole moieties in the structures of some biologically relevant molecules, such as xanthines, provides the opportunity for the synthesis of new NHC–silver complexes. The medicinal use of xanthines as diuretics, central nervous system stimulants, and inhibitors of cyclic adenosine monophosphate (cAMP) phosphodiesterase is well established [63]. Caffeine is the most popular xanthine due to its commercial availability, low cost, and low toxicity.

It is possible to couple the nucleophilic nitrogen (N9) with different substituents to obtain NHC precursors. The formation of the imidazolium cation methylated caffeine, 1,3,7,9-tetramethylxanthinium, has been reported using a variety of methylating agents such as methyl iodide [64], dimethyl sulfate [63a,65], methyl tosylate [65] and recently trimethyloxonium tetraflouroborate [66]. Several transition metal complexes of methyl xanthines, including caffeine and silver, have displayed the coordination of the xanthine ligand to metal centers via the nitrogen atoms of the imidazole ring [8,67]. NHC transition metal complexes of protonated and methylated caffeine have been reported as early as the 1970s, however there are only a handful of examples [8,19,68–71].

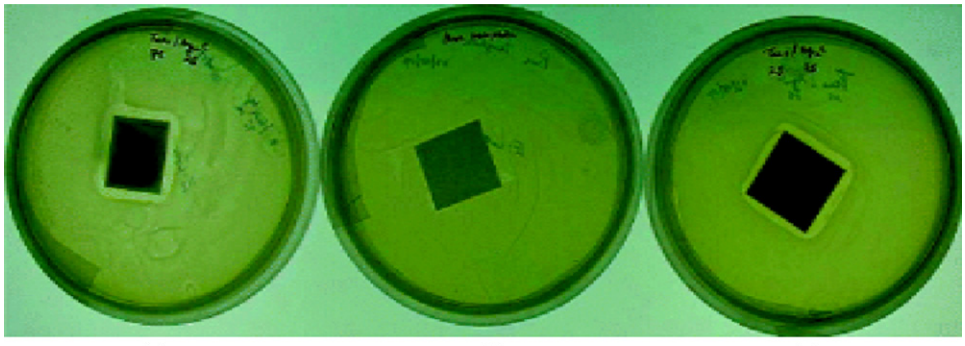
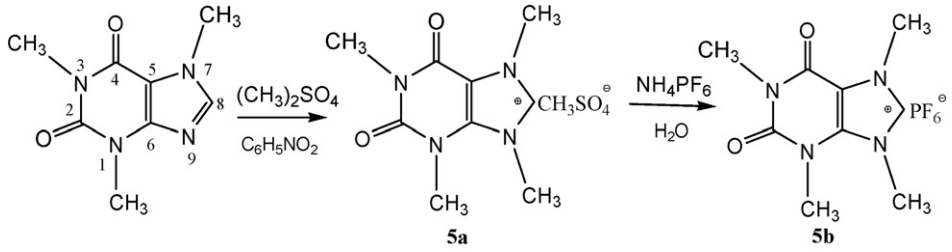


Fig. 8. Kirby Bauer susceptibility test showing the bacteriocidal activity of encapsulated complex in different ratio vs. Tecophilic®. (a) 25 wt.% **4**–75 wt.% Tecophilic®; (b) pure Tecophilic®; (c) 75 wt.% **4**–25 wt.% Tecophilic®.

Fig. 9. LB broth solutions after 1 week of bactericidal testing. From left to right: (a) 0.25% $AgNO_3$; (b) 0.50% $AgNO_3$; (c) 25 wt.% **4**–75 wt.% Tecophilic®; (d) 75 wt.% **4**–25 wt.% Tecophilic®; (e) pure Tecophilic® (100 wt.%); (f) LB broth solution with organisms only.



Scheme 5. Synthesis of **5a** and **5b** from caffeine.

We have synthesized methylated caffeine **5a** by the reaction of caffeine with dimethyl sulfate. Anion exchange with ammonium hexafluorophosphate gave **5b** which was crystallographically characterized (Scheme 5 and Fig. 10).

The reaction of **5a** with Ag_2O in water resulted in the formation of the water soluble silver–NHC complex **6a**. This was the first reported silver–NHC complex from caffeine (Scheme 6)

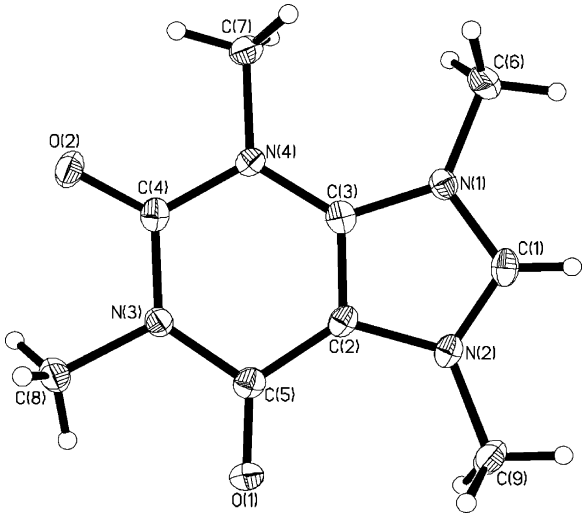
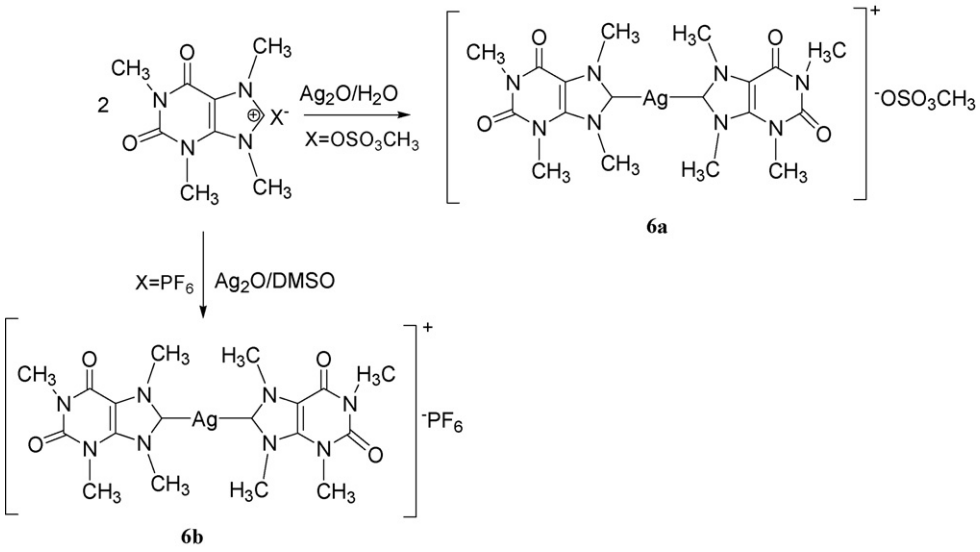


Fig. 10. Molecular structure of **5b**.

[19]. A very similar reaction of methylated caffeine with silver oxide was reported in 1926, but the authors reported it as the hydroxide salt of methylated caffeine [72]. Complex **6a** is an air and light stable solid and is stable for up to 5 days in water in the absence of light. Similarly, the water insoluble salt **5b** reacted with silver oxide in DMSO producing **6b**. Complex **6b** was found to be soluble only in DMSO and is stable in wet DMSO for months. The crystal structure of **6b** revealed a planar complex with a silver–carbene bond length of 2.068(4) Å and carbene–silver–carbene bond angle of 171.4(3)° (Fig. 11).

Recently, we synthesized the iodide salt of methylated caffeine [73] which is more convenient for medicinal applications than the hexafluorophosphate or methyl sulfate salts we have reported earlier. Caffeine was reacted with methyl iodide in DMF using a modified literature procedure [64]. The water soluble iodide salt, **7** was obtained and crystallographically characterized.

Preliminary toxicity studies on rats showed that **7** has a very low toxicity (1.068 g/kg). The low toxicity of **7** was very encouraging and further synthesis to obtain its silver complex was pursued. Compound **7** was reacted with silver oxide in methanol, and attempts to crystallize the silver complex in common alcohols and ethyl acetate mixtures resulted in the formation of a mixed silver–NHC carboxylate complex, **8** [73]. Complex **8** was crystallographically characterized and has a silver–carbene bond length of 2.067(3) Å and a bond angle of 168.2(9)° (Fig. 12). The



Scheme 6. Formation of **6a** and **6b**.

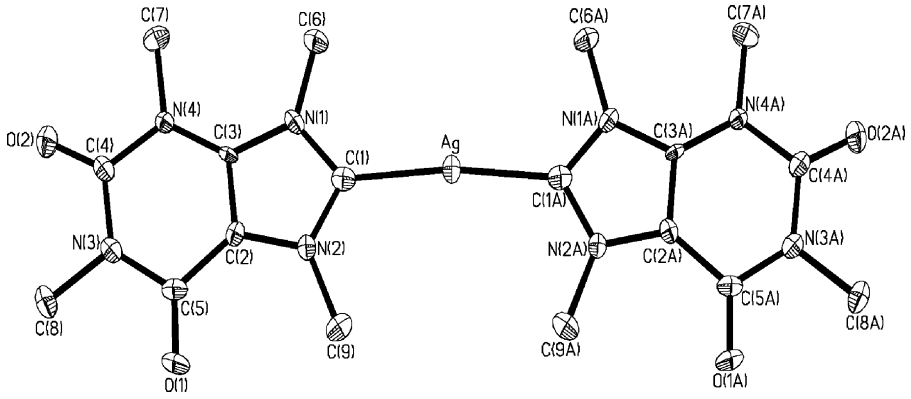


Fig. 11. Molecular structure of **6b**.

complex is water soluble, neutral, and composed of biologically relevant ligands. These features make **8** a good candidate for the antimicrobial applications [73]. Complex **8** has also been synthesized directly from the reaction of **7** with silver acetate in 1:2 molar ratio in methanol (Scheme 7).

Complex **8** is a very effective antimicrobial particularly against highly resistant opportunistic respiratory pathogens including a series of isolates from the lungs of patients with CF. CF is a life threatening genetic disorder caused by mutations in

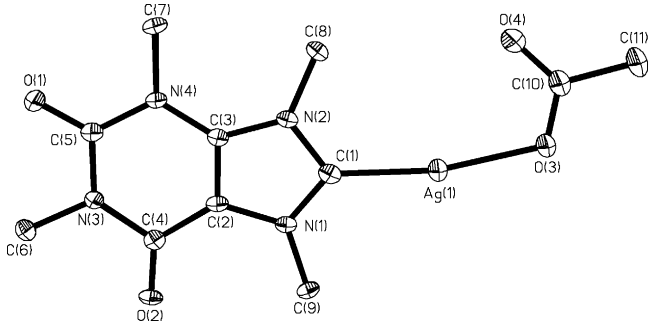
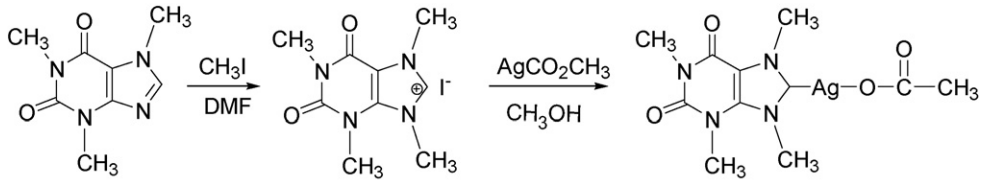


Fig. 12. Molecular structure of **8**.

the cystic fibrosis transmembrane regulator (CFTR) gene, and affects approximately 60,000 people world-wide. Malfunction of the CFTR in the airways of CF patients results in production of copious, desicated mucus, which traps bacteria leading to chronic pulmonary infections. Infections with *P. aeruginosa*, *S. aureus* and *Burkholderia cepacia* complex organisms account for most of the morbidity and mortality among CF patients [74]. The thick, abundant CF mucus with denser mucin tends to obstruct diffusion of conventional antibiotics [75]. Complex **8** is a relatively small compound compared with many conventional antibiotics and may diffuse more readily into the mucus to reach the trapped bacteria. Because the complex is water soluble, it may be useful as nebulized therapy in CF patients colonized with resistant organisms.

Complex **8** was found to be effective against a panel of resistant organisms, including *B. dolosa* strains, *Bcc* organisms for which there is no effective therapy, at MIC values between 1–10 μg/mL. Complex **8** was also tested against an *E. coli* J53 strain harboring the silver resistance plasmid, pMG101. The plasmid contains open reading frames for *silP*, *silA*, *silB*, *silC*, *silR*, *silS* and *silE* silver resistance genes originally cloned from a silver nitrate resistant burn ward *Salmonella* isolate [32,52].



7 8

Scheme 7. Synthesis of **7** and **8**.

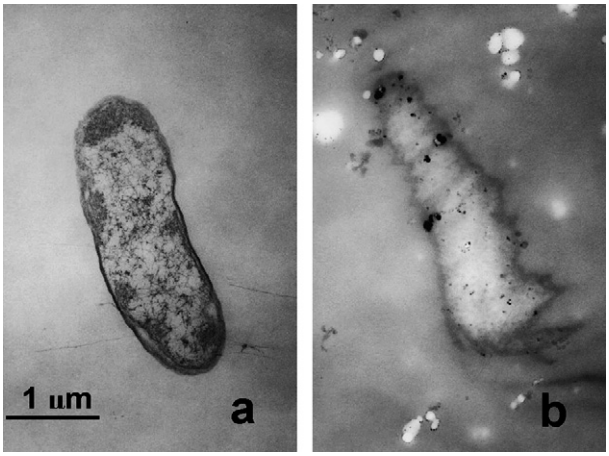


Fig. 13. TEM of B. *dolosa* strain AU4459 (a) before the application of complex **8**; (b) after application of 5 μg/mL complex **8** in Luria broth for 1 h at 37 °C.

The MIC of complex **8** for the J53 strain without the plasmid was 1 μg/mL, however the MIC for the pMG101 containing J53 was greater than 5 mg/mL indicating that the antibacterial activity of complex **8** is mainly due to the presence of silver moiety. Complex **8** also showed some fungicidal activity against *A. niger* and *S. cervisiae* with MIC values of 13 and 4 μg/mL, respectively, and fungistatic activity with an MIC of 4 μg/mL against *C. albicans*.

The mechanism of action of complex **8** as an antimicrobial is poorly understood, but it likely mirrors the mechanism of action of silver cations. *B. dolosa* treated with complex **8** was characterized with transmission electron microscopy (TEM) (Fig. 13) and several cell "ghosts" completely lacking cytoplasm were observed indicating disruption of bacterial cell morphology. The cytoplasm lacking ghost cell membranes were spotted with several electron dense clusters likely representing deposition of silver salts, as observed previously for the *E. coli* after being treated with silver nano-particles [76]. In order to test the potential effects of the silver component of complex **8** as a nebulized antimicrobial, preliminary *in vitro* toxicity studies were performed on primary cultures of murine trachel epithelial cells (MTECs). Treatment with **8** did not cause any transcriptional change at any concentration of the complex tested. Because of the limited solubility of **8** in water (11.6 mg/mL), an LD_{50} could not be reached in the *in vivo* studies on rats. However, no adverse effects were observed for the maximum possible amount of complex injected.

4. Conclusions

The medicinal use of topical silver compounds for wound and burn care is well established. One of the challenges of such treatments is the sustained release of the silver cations to the wound site over a long period of time. Several silver compounds and delivery methods have been reported in order to attain this goal. Slow decomposition of the water soluble pyridine linked NHC–silver complexes **2a** and **2b** to silver cations and active silver species in bacterial culture mediums has been demonstrated. The easy derivatization of NHCs enabled the synthesis of ethanol soluble silver gem-diol complex **4**. Although complex **4** decomposed in water quickly and showed a lower antimicrobial activity than silver nitrate, encapsulation to a medical grade polymer has been observed to increase the bioavailabilty of active silver species. Slow decomposition of **4** in the polymer mat has also provided longer bactericidal activity. Encapsulation of silver–NHCs into a suitable polymer mat is a promising method to achieve the sustained delivery of silver ions for wound treatment.

Only one rare cosmetic side effect of silver in medicinal applications has been reported. Toxicity of the ligands or the anions associated with silver can often determine the safe use of the silver antimicrobials. Therefore, the NHC precursors **5b** and **7** were synthesized by the methylation of biologically relevant molecule caffeine and the formation of silver complexes has been explored. The iodide salt of methylated caffeine **7** has been observed to have a very low toxicity. The silver acetate complex **8** obtained from this salt has been shown to be a very effective antimicrobial activity against numerous resistant respiratory pathogens including members of the *B. cepacia* complex. The potential use of this complex as a useful nebulized therapy for CF oriented lung infections is under exploration.

In short, the potential use of silver–NHCs as effective antimicrobials has been introduced as a new area of research. In addition to their application as traditional topical antimicrobials, silver–NHCs may also be useful as nebulized antimicrobials to treat pulmonary infections.

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