Binuclear silver(I) complexes of p-xyllyl/2,6-lutidinyl linked bis-N-heterocyclic carbene ligands: Synthesis, crystal structures and biological evaluation

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N-heterocyclic carbene (NHC) supported transition metal complexes are of wide interest because of their diverse catalytic [1] and biological and clinical applications [2]. Within this frame, late transition metal NHC complexes have attracted considerable interest as antibacterial and or anticancer agents and are also known as the most popular scaffolds in bioorganometallic chemistry [3]. The introduction on the NHC system of a functional arm having additional donor site such as N, S or O may coordinate/showing interaction to/with the metal center providing additional stability through the chelation. This would implement a rigid conformation to the metal–NHC system, which is suitable for sustained release of the metal ions at the required site. Late transition metal complexes bearing N-functionalized NHC ligands appear more general and involve nitrile or amide functions [4] or an N-heterocyclic system [5]. In particular, binuclear complexes of functionalized bis-NHCs having aromatic/aliphatic spacers have evidenced the higher biological activity due to the cooperative effects existing between the metal centers. In fact, several studies have demonstrated the promising biological applications of binuclear complexes of both, functionalized and non-functionalized NHCs, as potent anticancer/anti-microbial agents against a variety of pathogens [6]. The most crucial aspect in designing the biologically active candidate is the control and tuning of the sterically and electronic properties of the NHC system. This can be easily targeted by varying the N-substituents of the 1,3-diazole ring, thus allowing for synthetic control over hydrophilic and lipophilic properties of the resulting complexes.

There has been a recent emphasis especially, on mono and binuclear Ag complexes of functionalized NHC ligands as biologically relevant entities because of their low toxicity profile [7]. Imidazole and benzimidazole-based 1,3-disubstituted salts and their Ag–NHC complexes may offer interesting chemical differences from other Ag complexes of N-donor or related ligands, which may turn into a variety of applications as antimicrobial and or anticancer agents. Binuclear Ag complexes bearing a pyridine-functionalized NHC system were found active as antimicrobial agents [8]. In similar cases, pyridine-nitrogen atom showing interaction with the Ag center, which reduces the ligand dissociation tendency as donor/interacting groups, is composed of different chemical nature. This pseudo-chelation effect brings about stereoelectronic differentiation within the Ag–NHC coordination sphere leading to the formation of an additionally stable system. These additionally stable complexes are expected to show superior activity over other silver compounds because of the slow dissociation of the Ag–NHC complex and thus continual and sustained release of Ag ions at the required site. The first Ag–NHC complexes showing antimicrobial activity against a series of pathogens were composed of a pyridine-functionalized NHC system [9]. In view of these promising facts, we designed a series of new bis-([benz]imidazolium salts bearing different aromatic spacers which can provide additional stability to the corresponding binuclear Ag–NHC complexes that are possible scaffolds as antimicrobial agents. We thus report herein on the synthesis,
characterization and antimicrobial activity of a series of binuclear Ag–NHC complexes.

The reactivity of bis-(benz)imidazole bearing p-xylyl/2,6-lutidinyl spacer toward propargyl bromide in methanol was examined (Scheme 1), which yielded desired bromide salts as highly viscous oils. These bromide salts were readily amenable to salt metathesis reaction with potassium hexafluorophosphate in methanol to yield white solids of hexafluorophosphate salts, 1 and 2. The same method, however, failed to yield almost similar salts 3 and 4. Latter salts were prepared by similar procedure based on the route used by Dias and Jin for the synthesis of a tri-imidazolium analogue of salt 3 or 4 [10]. An alkyl group (pentyl or hexyl) is attached to the imidazole ring followed by the insertion of a spacer unit. The reaction of two equivalents of N-pentyl/hexyl imidazole were treated with p-xylyl bromide in acetonitrile at refluxing temperature for 24 h to afford desired bromide salts, which are then treated with a solution of potassium hexafluorophosphate in methanol to afford hexafluorophosphate salts 3 and 4, respectively which is in agreement with the formation of the desired (benz)imidazolium salts [11]. Apart from these characteristic peaks, the spectra also evidenced the resonance peaks corresponding to aliphatic and aromatic proton and carbon nuclei in the range of 5.60–7.60 and 13.0–180.0 indicating the successful complexation after treating Ag2O with (benz)imidazolium salts 1–4. The spectra also evidenced the resonance peaks corresponding to aliphatic and aromatic proton and carbon nuclei in the range of δ 9.20–9.76 and 6.80–8.12 attributed to the C2 proton and carbon of (benz)imidazole, respectively which is in agreement with the formation of the desired (benz)imidazolium salts 1–4. The spectra also evidenced the resonance peaks corresponding to aliphatic and aromatic proton and carbon nuclei in the range of δ 9.20–9.76 attributed to the C2 proton and carbon of (benz)imidazole, respectively which is in agreement with the formation of the desired (benz)imidazolium salts 1–4.

In order to have access to binuclear Ag–NHC complexes of 1–4, we used the in situ deprotonation methodology to attempt the synthesis of [NHC-Ag–NHC]2·2PF6 type complexes. Imidazolium salts 1, 3 and 4 were stirred with Ag2O in acetonitrile at room temperature for 24 h and afforded the corresponding binuclear bis-NHC Ag complexes 5, 7 and 8, respectively in good yield while the reaction of salt 2 bearing 2,6-lutidinyl spacer with Ag2O in acetonitrile at 50 °C afforded the desired binuclear complex 6 as a white precipitate. All these targeted compounds were recrystallized from methanol–diethyl ether mixture to afford pure Ag complexes, which were isolated in an overall yield of 63–68%. Reactions involved in the preparation of binuclear Ag complexes are outlined in Scheme 2. Both azolium salts and their corresponding Ag complexes are readily soluble in highly polar solvents such as acetonitrile, DMF, and DMSO, while insoluble in benzene, toluene and diethyl ether at room temperature. The new (benz)imidazolium salts and their respective binuclear Ag–NHC complexes are consistent with their elemental analysis data.

1H and 13C NMR spectroscopies supported the presence of a resonance peak at the downfield region in the range of δ 9.20–9.76 and 13.7–142.6 attributed to the C2 proton and carbon of (benz)imidazole, respectively which is in agreement with the formation of the desired (benz)imidazolium salts 1–4. Apart from these characteristic peaks, the spectra also evidenced the resonance peaks corresponding to aliphatic and aromatic proton and carbon nuclei in the range of δ 9.20–9.76 attributed to the C2 proton and carbon of (benz)imidazole, respectively which is in agreement with the formation of the desired (benz)imidazolium salts 1–4.

The complex spectra display two apparent doublets for the imidazole backbone (additionally, two triplets for benzimidazole) protons in the range of δ 7.1–7.8. In the case of 13C NMR spectra of complexes 5–8, illustrating successful complexation after treating Ag2O with (benz)imidazolium salts 1–4. Thus the complex spectra display two apparent doublets for the imidazole backbone (additionally, two triplets for benzimidazole) protons in the range of δ 7.1–7.8. In the case of 13C NMR spectra of complexes, the carbene carbon resonance signals appear at ca. δ 180.0 indicating the successful complex formation; these values are in the range of those reported for other binuclear bis-NHC Ag complexes [12]. The spectra also evidenced the aromatic and related aliphatic carbon resonance signals of the

Scheme 1. Synthesis of p-xylyl/2,6-lutidinyl linked bis-(benz)imidazolium salts.

NHC groups are almost unaffected in comparison to the corresponding (benz)imidazolium salts.

The FTIR spectra of (benz)imidazolium salts 1-4 evidenced the presence of two sharp medium intensity bands at ca. 1600 and 1050 cm⁻¹, which are assigned to the stretching vibrations of (benz)imidazole ring C≡N and C=N modules, respectively. In the case of benzimidazolium salt havinglutidinyl spacer, a new band is observed at 1650 cm⁻¹, ascribable to pyridine ring vibrations. In the complex spectra, both the former vibrations were shifted to the lesser energy region by ca. 10–15 cm⁻¹, suggesting the formation of Ag complexes [13]. In the case of Ag complex having lutidinyl spacer, pyridine ring vibrations were unaltered compared to the corresponding benzoimidazolium salt which ruled out the possible involvement of pyridine ring nitrogen in the coordination. All the spectra displayed two medium intensity sharp bands at ca. 2860 and 2930 cm⁻¹, assignable to the stretching vibrations of aliphatic and aromatic C–H modules, respectively. These values are in accordance with the range reported for similar binuclear bis-NHC Ag complexes [14].

The structure of the benzoimidazolium salt having lutidinyl spacer 2 and a binuclear Ag–NHC complex 7 was established by X-ray diffraction on single crystals obtained by slow evaporation of the salt solution in acetonitrile and slow diffusion of diethyl ether into a solution of 7 in acetonitrile, respectively at ambient temperature. It is worth mentioning that there is no solvent or water molecule in the unit cell of 2 or 7 and no disorders were observed. Salt 2 crystallizes in the monoclinic system with the C2/c space group. Molecular structure of the salt along with the pertinent bond angles and distances is shown in Fig. 1. An asymmetric unit of salt 2 is composed of one half of the benzoimidazolium cation and one hexafluorophosphate anion. The planes of the benzoimidazole rings are making a dihedral angle of 110.54(9)° (for C3–C4–N2) with the plane of the central pyridine ring, while the propargyl modules are making a dihedral angle of 111.85(10)° (for N3–C12–C13) with the benzoimidazole ring systems. The internal bond angles at carbene carbon center (N2–C11–N3) and at sp-hybridized carbon (C12–C13–C14) are found as 110.03(11) and 177.63(15)°, respectively. These values are in well agreement with the similar compounds available in the literature [15]. Furthermore, a weak face-to-face π–π stacking interaction was observed between benzoimidazole rings of one molecule with the same adjacent molecule with an interaction distance of ca. 3.835 Å. In the extended structure, benzoimidazolium cations are connected with the hexafluorophosphate anions via intermolecular hydrogen bonding interactions (C–H–F) ranging from 2.824 to 2.664 Å.

Complex 7 is a well ordered binuclear Ag compound and crystallizes in the triclinic system with the P-1 space group having occupied one half of the complex cation and one hexafluorophosphate anion in an asymmetric unit. The molecular structure of complex 7 along with the pertinent bond angles and distances is shown in Fig. 2. In the molecular structure of 7, two Ag ions are interposed between two units of 3 forming a symmetry equivalent system. The Ag centers display linear coordination geometry (179.37(4)° for C1–Ag1–C14), being surrounded by ligand 3 acting as a carbene carbon donor with bond distances of 2.0874(10) and 2.0925(10) Å for C14–Ag1 and C1–Ag1, respectively. The Ag–C bonds and bond angles at Ag centers are in well agreement with the other reported binuclear Ag complexes [16]. As expected, internal bond angles at the carbene carbon center, N1–C1–N2 and N3–C14–N4, are found to be 104.43(9) and 104.36(8)°, respectively which are in agreement with the literature values for comparable angles [17]. Interestingly, an intermolecular Ag–Ag short separation is observed between the two adjacent Ag atoms of two adjacent binuclear molecules with the bond distance of 3.660(2) Å perpendicular to the ab plane, which is just over the sum of their van der Waals radii (3.44 Å). Besides this, the coordinated imidazole rings are also showing face-to-face π–π stacking interactions with an interaction distance of ca. 3.651 Å. In the crystal packing structure, C–H–F (2.445 Å) hydrogen bonding interactions were observed, which along with the stacking interactions provided the 3-dimensional architecture to the binuclear Ag complex.

Organometallic conjugates based on a metal–NHC system are recently explored to understand their potential as cytotoxic agents, which can interact with both DNA and the disulfide reductase enzyme (thioredoxin reductase, TrxR) [18]. The discovery of the antimicrobial properties of a caffeine derived Ag–NHC complex as an effective antibacterial agent and trademarked as Silvamist® was followed by one of the most inspiring drug success stories in this particular field [19]. Although no other metal-based NHC complexes were effectively used as antimicrobial/anticancer agent, a wide range of transition metal–NHC complexes is currently under consideration for future developments. One promising strategy is the use of bis-NHCS having different aromatic spacers which can hold two metal centers at a specific distance. This can provide stability to the system and promotes slow and sustained release of metal ions at the required site. In the present communication, the antimicrobial potential of the (benz)imidazolium salts and their corresponding binuclear Ag–NHC complexes have been compared. The triggering of antimicrobial effects of all the compounds, 1–8, was investigated against Escherichia coli and Staphylococcus aureus bacteria using the disc diffusion method. Minimum inhibitory concentration potentials of these compounds against both the aforementioned bacteria were
determined based on the lowest concentration that inhibited the bacterial growth.

The Ag free (benz)imidazolium salts displayed almost no activity against both the tested bacteria even at higher concentration levels over a long period of exposure. Transformation of salts 1–4 into Ag–NHC complexes 5–8 led to a strong increase in antibacterial activity against both the bacteria. Antibacterial results revealed that the complexes are more active against S. aureus than E. coli at both the tested concentrations, 50 and 100 μg/mL (Table 1). In the case of complexes having free alkyl-pendent arms, 7 and 8, a strong increase in antibacterial activity was observed with values of 25.6 ± 0.5, 27.0 ± 0.7 and 26.0 ± 0.8, 27.5 ± 2 mm diameter of the bacterial zone of inhibition against E. coli and S. aureus, respectively at 100 μg/mL concentration. The potential of Ag complexes, 5 and 6, having propargyl chain, however, is lesser than the activity of long alkyl chain complexes with 

<table>
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<tr>
<th>Complexes</th>
<th>Concentration in μg/mL</th>
<th>Inhibition zone (mm)</th>
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<tr>
<td></td>
<td></td>
<td>E. coli</td>
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<tr>
<td>5</td>
<td>100</td>
<td>12.4 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>14.0 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>25.6 ± 0.5</td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>27.0 ± 0.7</td>
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<tr>
<td>5</td>
<td>50</td>
<td>9.0 ± 1.5</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>20.2 ± 1</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>21.3 ± 0.6</td>
</tr>
<tr>
<td>AgNO₃</td>
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<td>23.0 ± 3</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>100</td>
<td>31.4 ± 0.5</td>
</tr>
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a Compounds 1–4 showed no activity.

b Test compound volume = 5 μL.

Appendix A. Supplementary material

CCDC 1002219 and 1002220 contain the supplementary crystallographic data for 2 and 7, respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033, or E-mail: deposit@ccdc.cam.ac.uk. Experimental procedure for the synthesis of (benz)imidazolium salts and binuclear Ag–NHC complexes, spectral and analytical characterization data, the method followed to examine their antibacterial potential, and crystal refinement information and packing diagrams for compounds 2 and 7 and 1H and 13C NMR spectra of compounds can also be found as supplementary material. Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.inoche.2014.07.017.

References


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