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Silver(I) *N*-heterocyclic carbene complexes: Synthesis, characterization and antibacterial activity

Mariagrazia Napoli [a,*], Carmela Saturnino [b,**], Elena Immacolata Cianciulli [b], Mario Varcamonti [c], Anna Zanfardino [c], Giuseppina Tommonaro [d], Pasquale Longo [a,***]

[a] Dipartimento di Chimica e Biologia, Università di Salerno, Via Ponte don Melillo, 84084 Fisciano, SA, Italy
[b] Dipartimento di Scienze Farmaceutiche e Biomediche, Università di Salerno, Via Ponte don Melillo, 84084 Fisciano, SA, Italy
[c] Dipartimento di Biologia Strutturale e Funzionale, Università di Napoli-Federico II, Complesso Universitario di Monte Sant'Angelo Via Cinthia, 84126 Napoli, Italy
[d] Istituto di Chimica Biomolecolare, Consiglio Nazionale delle Ricerche CNR, Via Campi Flegrei, 80078 Pozzuoli, NA, Italy

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ABSTRACT

Five new silver complexes having bidentate *N*-heterocyclic carbene ligands were synthesized and characterized by elemental analysis, IR, NMR and mass spectroscopic methods. Four of the ligands used are neutral, having an alcohol group on alkyl substituent of one of the two nitrogen atoms of the heterocycle [NHC–OH], the fifth, having a ligand alkoxide, is mono-anionic [NHC–O]. A study on the rate of hydrolysis of complexes synthesized, showed that they are stable to hydrolysis even after 24 h. Probably, the pincer effect of both [NHC–OH] and [NHC–O] ligands stabilizes these compounds. All the synthesized complexes do not show cytotoxicity, but they have a significant antibacterial activity. They were tested on *Escherichia coli* and *Bacillus subtilis* and data obtained demonstrate that the synthesized Ag-complexes are very promising candidates to be used as antimicrobial compounds.

1. Introduction

Silver has been since antiquity used as antimicrobial agent, it was earlier employed in the purification of drinking water, wine and vinegar, and also Hippocrates remarked its therapeutic properties [1]. Still today silver salts are used particularly in the treatment of chronic ulcers, extensive burns and to prevent conjunctivitis into newborn's eyes as well as other bacterial infections [2].

The activity of silver against Gram-positive and Gram-negative bacteria, fungi and yeast is due to Ag cations, which can interact with the cell membrane, interfere with the electron transport system of the cell and interact with thiol groups of the vital enzymes of bacteria [3].

The efficacy of a silver-drug as antibacterial agent is, of course, linked to its bioavailability which must be slow and continues for an appropriate time in the affected area [4]. In fact, silver nitrate and silver sulfadiazine, commonly used topical antibacterials, have very quick bactericidal action, but rapidly lose their effectiveness by exposing the wound-site to a possible re-infection.

Thus, the search for a silver-compound that slowly releases silver cations into the wound, maintaining a constant source of antibacterial agents to prevent infection, is a topic of great interest in the medical community.

The slow release of Ag cation at the wound-site is closely related to the choice of ancillary ligands to silver, which can play an important role stabilizing the complexes, thus retaining the antibacterial effect over a longer period of time.

N-heterocyclic carbenes (NHC), due to their excellent σ-donating properties and, as theoretical and structural studies suggest, π-backbonding ability [5,6], are widely used as ancillary ligands to stabilize both main group and transition metals [7–9].

In recent years many *N*-heterocyclic silver compounds have been synthesized, mainly for use in transmetalation of *N*-heterocyclic carbenes from silver to other metals, making it the most important synthetic method to give other metal NHC compounds [10].

Silver-*N*-heterocyclic carbene complexes can slowly release silver ions into the wound, enabling better prevention of infection and promoting healing [11,12].

Pyridine linked NHC-[1-benzyl-3-tert-buthylimidazol-2-ylidene]-, *N,N′*-methylate-NHC, with varying substituents on the 4

* Corresponding author. Tel.: +39 089969571; fax: +39 089969603.
** Corresponding author. Tel.: +39 089969769; fax: +39 089969602.
*** Corresponding author.
E-mail addresses: mnapoli@unisa.it (M. Napoli), saturnino@unisa.it (C. Saturnino), elena.cianciulli@libero.it (E.I. Cianciulli), mario.varcamonti@unina.it (M. Varcamonti), anna.zanfardino@unina.it (A. Zanfardino), giuseppina.tommonaro@icb.cnr.it (G. Tommonaro), plongo@unisa.it (P. Longo).

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and 5 position of the imidazole ring, -silver complexes have been synthesized and tested on clinically important microorganisms [10,13–15].

NHC having a pendant amine-alkoxide, and cyclopentadienyl-group have been recently synthesized. The concomitant presence of a strong electron-donating ylidene carbon with coordinating group generates ligand [NHC–X] able to produce complexes with noticeable stability and having steric rigidity [16,17].

In this paper, we report the preparation and characterization of some iodide silver complexes stabilized by *N*-heterocyclic carbene ligands having an alcohol group on alkyl substituent of one of the two nitrogen atoms of the heterocycle (NHC–OH). Moreover, we also detail on the synthesis of a silver complex having an *N*-heterocyclic carbene ligand bringing an alkoxy group on one of two nitrogen of the heterocycle (NHC–O). The latter ligand is bidentate–monoanionic, while the former are bidentate–neutral, however in all cases they can act as a chelating agent, and therefore significantly stabilize the resulting complex.

In Scheme 1 are reported the ligands utilized, which produced the corresponding complexes **1**, **2**, **3**, **4** and **1′**.

The antibacterial activity was evaluated for all the reported compounds, using a standard assay against a Gram negative (*Escherichia coli*) and a Gram positive (*Bacillus subtilis*) strain.

2. Results and discussion

2.1. Chemistry

Main Imidazolium-*N*-methyl-*N′*-cyclopentan-2-ol-iodide, imidazolium-*N*-methyl-*N′*-cyclohexane-2-ol-iodide, imidazolium-*N*-methyl-*N′*-ethane-2-ol-iodide were prepared following the strategy proposed by Arnold and co-workers [16], and applying the procedures previously reported by us [17].

2.1.1. Synthesis of N-methyl, N′-[(2-hydoxy-2-phenyl)ethyl] imidazolium iodide

The *N*-methyl, *N′*-[(2-hydroxy-2-phenyl)ethyl]-imidazolium iodide was synthesized by reacting phenylethylenoxide with imidazole, followed addition of iodomethane in acetonitrile (see Scheme 2). After distillation of the solvent, the product was purified by crystallization in acetone, obtaining the imidazolium salt as a white solid in high yield.

Elemental analysis (C, H, N) is in agreement with the proposed formulation. ^{1}H COSY and NOESY experiments allowed to assign all proton resonances of the ^{1}H NMR spectrum, whereas resonances of ^{13}C NMR spectrum were attributed using support of DEPT and HMQC experiments (see Fig. 1). All signal attributions are reported in the experimental part. The singlet at 8.23 ppm is assigned to imidazolium proton on carbon 2, whereas the resonances of hydrogen atoms of methines of the backbone fall at 6.84 and 6.82 ppm, respectively. At 6.60–6.40 ppm are the signals of protons of aromatic ring. The doublet at 5.10 was attributed to –OH and the multiplet at 4.11 to –CH–O, while the protons of methylene –CH_2–N, give rise to two signals at 3.57 and 3.36 ppm, because they are not equivalent, probably because the free rotation around the C–C bond is prevented by the coordination of oxygen to the carbocation. The protons of the methyl bonded to nitrogen atom give a singlet at 3.01 ppm.

Signal at 136.9 is assigned to C_2 of imidazolium and that at 123.0 ppm to two carbons of imidazolium backbone. Aromatic carbons resonate at 141.2, 128.3, 127.8, 125.9. The resonances of methine carbons bonded to oxygen and to nitrogen atoms are at 70.6 and 55.6 ppm, respectively, whereas the chemical shift of methyl carbon is at 35.8 ppm.

2.1.2. Synthesis of silver complex bearing [NHC–O] ligand (*1′*)

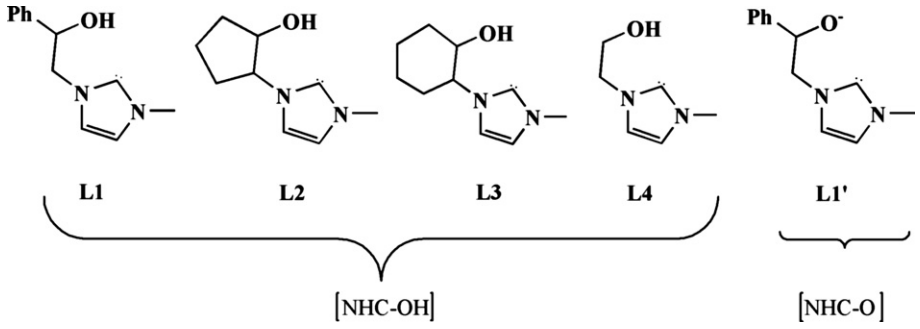
Reaction of *N*-methyl, *N′*-[(2-hydroxy-2-phenyl)ethyl] imidazolium iodide with an excess of strong base (*i.e.* potassium-hexamethyldisilazide), used both for the deprotonation of the alcohol group and for the formation of carbene, produced potassium alkoxide carbene, to which was added silver acetate CH_3COOAg to give, after purification (see Section Experimental part), [NHC–O]Ag ({*N*-methyl,*N′*-[(2-phenyl)-2-ethoxy]imidazole-2-ylidine}-silver(I)) as a white microcrystalline solid. Elemental analysis, mass spectroscopy analysis, NMR and FT-IR measurement are according with formulation reported in Scheme 3.

In fact, MS spectrum of complex showed the presence of the signals at 309 and 311 *m*/*z* assignable to the molecular ion $C_{12}H_{13}AgN_2O$. The presence of the signals M and M + 2 are due to the two isotopes of silver (^{107}Ag and ^{109}Ag) of abundance nearly equal. The elemental analysis data of [NHC–O]Ag found are in good agreement with those calculated (see Section Experimental part) and the IR spectrum does not show absorbance attributable to –OH. ^{1}H and ^{13}C NMR spectra show the signals of phenyl ring, of methine of the backbone, of *N*-methyl group, and of methine and methylene bonded to heteroatoms, O and N, respectively. In the ^{13}C NMR it is possible to observe only one sharp resonance attributable to carbene at 180.9 ppm. This fact, evidently due to the non coupling ^{13}C–^{107,109}Ag, is understandable given the lability of the carbene–silver bond and therefore explainable considering the fluxional behavior of **1′** [18]. In Fig. 2 are reported ^{1}H and ^{13}C NMR and in the experimental part are detailed all the attribution of ^{1}H and ^{13}C NMR signals.

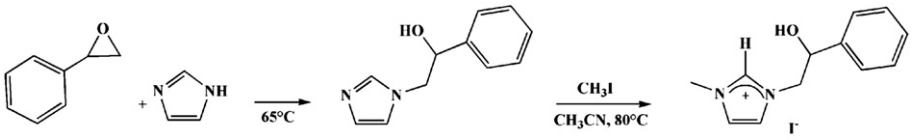
2.1.3. Synthesis of silver complexes bearing [NHC–OH] ligands (*1*–*4*)

The *in situ* deprotonation of imidazolium salt with basic silver precursors (*i.e.* silver oxide) was used to give *N*-methyl-*N′*alkylhydroxy-imidazole-2-ylidine [14,18].

Reaction of *N*-methyl, *N′*-[(2-hydroxy-2-phenyl)ethyl] imidazolium iodide (1.0 mmol) with the stoichiometric amount of silver



Scheme 1. Structures of substituted carbene ligands: *N*-methyl-*N′*-alkylhydroxy [NHC–OH] and *N*-methyl-*N′*-(2-alkoxyde-2-phenyl-ethyl) [NHC–O].



Scheme 2. Synthesis of proligands.

oxide (0.5 mmol) in CH_3CN produced, after usual work up, a white solid whose MS spectrum showed a maximal peak at 513 *m*/*z*. FT-IR analysis revealed −OH absorbance at 3400 cm^{-1}, and ^1H and ^{13}C NMR spectra gave the signals expected (see Fig. 3 and Section Experimental part), with one sharp carbene resonance at 185.3 ppm, evidently for the same reason hypothesized for complex **1′**. Elemental analysis of **1** found for $C_{12}H_{14}AgIN_2O$ is in agreement with that calculated (see Section Experimental part).

Mass spectrometry can provide fundamental data on the structure of compounds in the gas phase. In fact, the maximal peak intensity at 513 *m*/*z* (associate, of course, with M + 2) is attributable to $[(L1)_2Ag]^+$, on the other hand the elemental analysis gives a molar ratio among silver, ligand and iodide of 1:1:1. These data suggest that complex may consist of $[(L1)_2Ag]^+$ cation and of $[AgI_2]^-$ anion.

From literature it is known that anion $[AgX_2]^-$ subsist both in solution and in the solid state, counter-balanced by cation $[Ag(NHC)_2]^+$ [18–21], *e.g.* Wang and Lin reported structure of $[Ag(Et_2\text{-Bimy})_2]^+[AgBr_2]^-$ (Et_2-bimy = diethylbenzilimidazol-2-ylidene) and pointed out the existence of these ions in solution [18], so it is likely that our complex has a structure such as that shown in Scheme 4: {$[Ag(L1)_2]^+[AgI_2]^-$} (bis{*N*-methyl,*N′*-[(2-hydroxy-2-phenyl)ethyl]imidazole-2-ylidine}-silver(I))(di-iodo-argentate(I)).

The complexes **2**–**4** were prepared in the similar manner. In all three cases in the FT-IR spectra is observed the absorbance of −OH group around 3400 cm^{-1}, ^1H and ^{13}C NMR spectra show the expected signals (see attribution in the Section Experimental part) with one sharp resonance for carbene of each complex at 180.3, 179.7, 181.0 ppm for **2**, **3** and **4**, respectively. The elemental analysis for all three complexes (reported in Section Experimental part) gives a ratio among ligand, silver and iodide of 1:1:1.

MS spectra show the peak leading, associated each one with the respective M + 2, to 439, 469 and 275 *m*/*z* for the complexes **2** {(bis{*N*-methyl,*N′*-[1-cyclopentenyl-2-hydroxy]imidazole-2-ylidine}-silver(-I))(di-iodo-argentate(I))}, **3** {(bis{*N*-methyl,*N′*-[1-cyclohexenyl-2-hydroxy-]imidazole-2-ylidine}-silver(I))(di-iodo-argentate(I))}. and **4** {(bis{*N*-methyl,*N′*-[2-hydroxy-ethyl]imidazole-2-ylidine}-silver(-I))(di-iodo-argentate(I))}, respectively. These peaks are attributable to $[Ag(L2)_2]^+$, $[Ag(L3)_2]^+$ and $[Ag(L4)_2]^+$, respectively. So it is likely also believe that the structure of these complexes is similar to that of (**1**) ($[Ag(L1)_2]^+[AgI_2]^-$).

2.1.4. Hydrolysis tests

Starting from the reflection that the slow release of Ag cations in the wound site is certainly related to the stability of the complexes, the stability to hydrolysis is a first indication on the possible antibacterial effect over a long period of time by the compounds synthesized. Thus, we have determined the stabilities of all synthesized compounds in D_2O-10% of DMSO-d_6 and in 100% DMSO-d_6. All investigated complexes are stable in aqueous solution for 24 h. In fact, the ^1H NMR spectra are not changed at this time. As an example in Fig. 4 is reported, ^1H NMR spectra of complex **1′** after 15 min and after 24 h of dissolution in aqueous solution-10% of DMSO. Obviously the situation under physiological conditions may be different, but these preliminary results are certainly worthy of note.

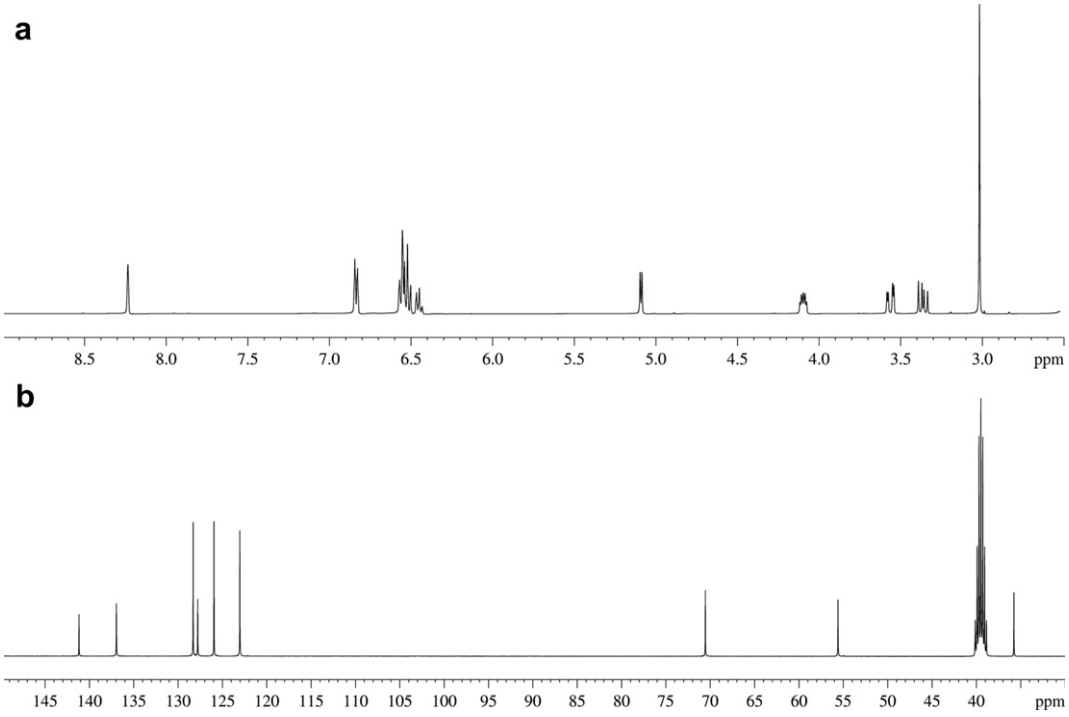
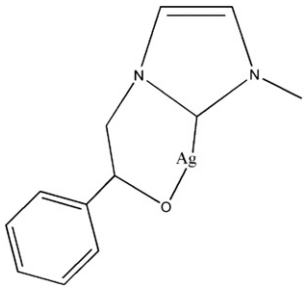


Fig. 1. ^1H (a) and ^{13}C (b) NMR spectra of *N*-methyl, *N′*-[(2-hydroxy-2-phenyl)ethyl] imidazolium iodide.



Scheme 3. Sketch of complex **1′**.

2.2. Pharmacology

Before going to evaluate the bactericidal activity of the complexes synthesized we have preferred to be sure that they were not cytotoxic. For this reason we carried out the Brine shrimp assay which is a general bioassay, indicative of cytotoxicity [22–24]. The brine shrimp is a marine crustacean. It lives in most of the world, including environments with high salinity, feeding mainly of phytoplankton. This crustacean, at stage of larvae (48 h after hatching), was used to test the cytotoxicity of samples and to calculate the LD_{50}, which is the minimum lethal dose for which we observe a 50% mortality of larvae. Results obtained by using this test showed that all samples were not toxic on brine shrimp larvae; in fact after 24 h of exposure to samples at concentration of 100, 10 and 1 μg/ml, all larvae were live and motile.

2.2.1. Determination of the minimum inhibitory concentration (MIC) for the synthesized silver compounds

Our data indicate that many of the tested silver compounds have a significant antibacterial activity. As shown in Table 1, complex **1′** inhibits *E. coli* and *B. subtilis* growth at a concentration of 5 μg/ml; same concentration is required for complex **1** to inhibit the growth of both tested strains. The complexes **2** and **3** kill *E. coli* cells at a concentration of 5 μg/ml, but a higher concentration was necessary to inhibit the *B. subtilis* growth (50 μg/ml). The complex **4** was tested at many concentrations, at 50 μg/ml the bacteria still showed residual growth. The conclusion of these experiments is that the synthesized silver complexes represent very promising candidates to be used as antibacterial compounds.

3. Conclusions

Bidentate *N*-heterocyclic carbene ligands, bringing on a nitrogen a methyl group and on the other one an alcohol or an alkoxide group, were reacted with suitable compounds of Ag(I) to give five new silver-complexes. The pincer effect of the ligands used produced stable compounds to hydrolysis even after 24 h.

Bioassay, indicative of cytotoxicity, carried out using brine shrimp, showed that all samples were not cytotoxic, whereas antibacterial activity, tested on *E. coli* and *B. subtilis*, gave very interesting results, *e.g.* complexes **1** and **1′** have a MIC of 5 μg/ml.

Thus, the remarkable antibacterial activity of [NHC–O]- and [NHC–OH]-silver complexes, their stability to hydrolysis, which is the first indication on the possible slow-release of Ag cations into the wound-site, it suggests that they can be very promising candidates for use as antibacterial compounds.

4. Experimental section

4.1. Spectroscopic measurements

The elemental analyses for C, H, N were recorded on a Thermo-Finnigan Flash EA 1112 and were performed according to standard microanalytical procedures. The elemental analyses for I, Ag were carried out by atomic absorption spectrophotometer AAnalyst model 100 (Perkin Elmer) equipped hollow-cathode lamp Lumina Ag (Perkin Elmer) using the software AAwinLabAnalyst. Silver was determined with a burner (FIAS-100) air-acetylene flame. Solution of Ag at known concentration prepared from a stock solution of 1 g/

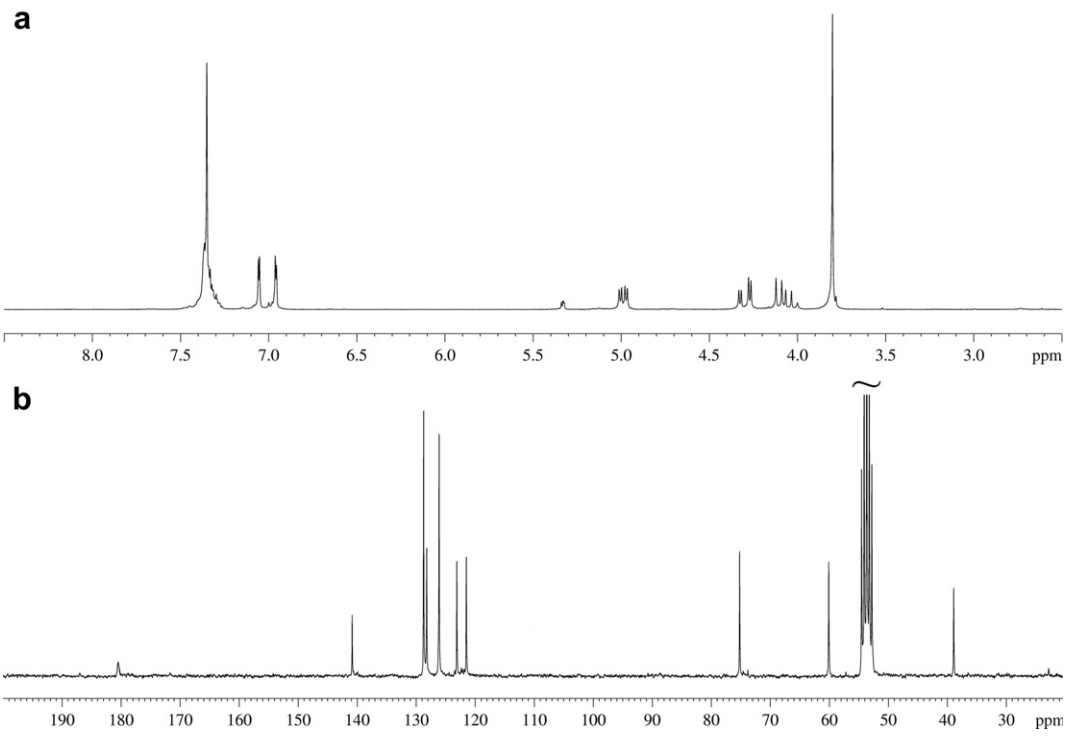


Fig. 2. ^{1}H (a) and ^{13}C (b) NMR spectra of complex **1′**. TMS scale.

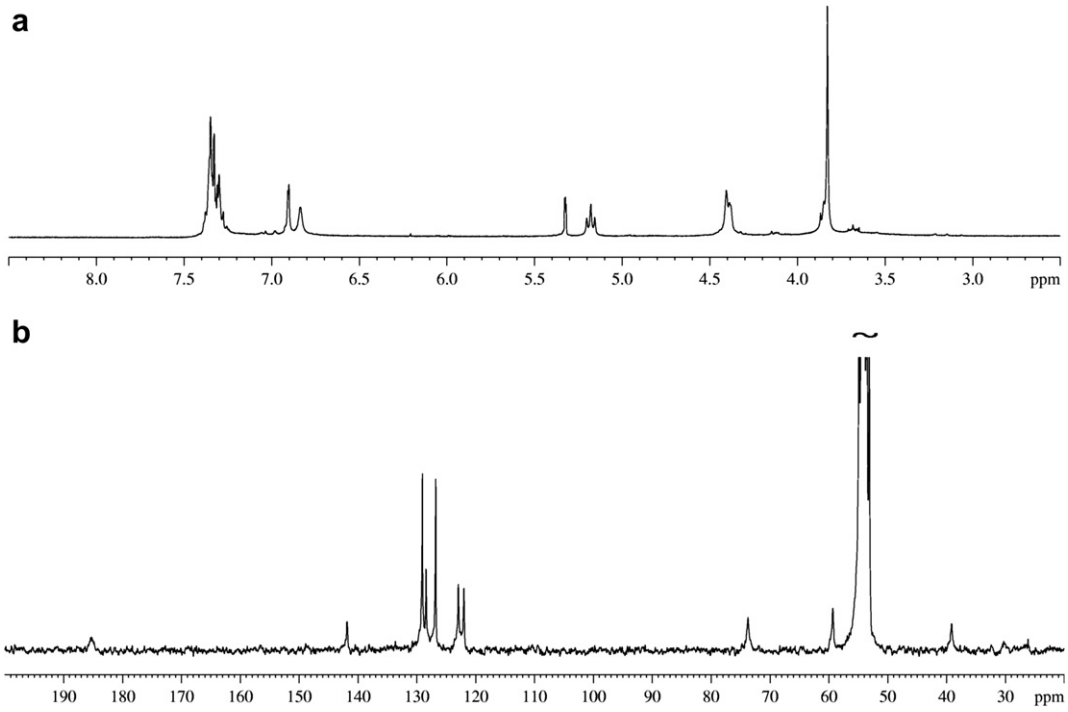


Fig. 3. ^{1}H (a) and ^{13}C (b) NMR spectra of complex **1**. TMS scale.

l (Carlo Erba) was used as standards. The instrument was set at zero using a 1% solution of HNO_3. Sample scripts were analyzed along with their white. Iodide was determined indirectly by reaction of $AgNO_3$ with I^-, precipitation of AgI which was dissolved in $Na_2S_2O_3$. Silver content in the solution was determined by FAAS and the iodide content was calculated using the content of silver.

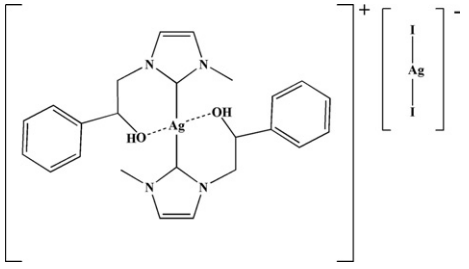
^{1}H NMR, homodecoupled ^{1}H NMR, ^{1}H COSY, ^{1}H NOESY, HSQC and ^{13}C {^{1}H} NMR spectra were recorded at 298 K on a Bruker Avance 300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C) and referred to internal tetramethylsilane.

Fourier transform infrared (FTIR) spectra were obtained at a resolution of 2.0 cm^{-1} with a Bruker-Vector 22 FTIR spectrometer equipped with a deuterated triglycine sulfate (DTGS) detector and Ge/KBr beam splitter. The frequency scale was internally calibrated to 0.01 cm^{-1} using a He–Ne reference laser. Thirty-two scans were signal-averaged to reduce spectral noise.

ESI-MS spectra were performed on a Quattro Micro triple quadrupole mass spectrometer equipped with an electrospray ion source.

4.2. Chemistry

All manipulations were carried out under oxygen- and moisture-free atmosphere in a MBraun MB 200 glove-box. All the solvents were thoroughly deoxygenated and dehydrated under argon by refluxing over suitable drying agents, while NMR deuterated solvents (Euriso-Top products) were kept in the dark over molecular sieves.



Scheme 4. Sketches of probable structure of complex **1**.

The organic compounds imidazole, styrene oxide and ethylene oxide, iodomethane, potassium hexamethyldisilazide (Strem, Aldrich) were used as received. The silver compounds Ag_2O and CH_3COOAg were purchased from Aldrich. The imidazolium salts (Imidazolium-*N*-methyl-*N*′-cyclopentan-2-ol-iodide, imidazolium-*N*-methyl-*N*′-cyclohexane-2-ol-iodide, imidazolium-*N*-methyl-*N*′-ethane-2-ol-iodide) were prepared by following reported procedure [17].

4.3. Synthesis of imidazolium salt (imidazolium-N-methyl-N′-benzyl-2-hydroxy-iodide) ($C_{12}H_{15}IN_2O$ FW = 330.05.) (**L1**)

The synthesis of imidazolium salt was carried out in the same way as reported in the ref. [17] for imidazolium-*N*-methyl-*N*′-cyclopentenyl-2-hydroxy-iodide, by the reaction between 0.1 mol of styrene oxide (11.4 ml, FW = 120.15, d = 1.05 g/l) and a stoichiometric amount of imidazole. The reaction was carried out at 50 °C overnight and then 0.1 mol of iodomethane and 30 ml of acetonitrile were added. The solution was warmed up 80 °C for 2 h, then the solvent was removed to under pressure, the product was precipitated as white solid by added cool acetone and isolated in good yield.

Yield = 70%.

Elemental analysis of **L1**: found (%): C 43.6, H 4.4, N 8.6, O 4.9, I 38.5. Calc. for $C_{12}H_{15}IN_2O$ (%): C 43.7, H 4.5, N 8.5, O 4.8, I 38.4.

^{1}H NMR (DMSO-d_6, 298 K): 8.23 (s, 1H, NC***H***N); 6.84 (d, 1H, NC***H***CHN); 6.82 (d, 1H, NCHC***H***N); 6.60–6.40 (m, 5H, **Ph ring**); 5.10 (d, 1H, O*H*); 4.11 (dd, 1H, OC***H***); 3.60–3.10 (m, 2H, NC$\boldsymbol{H_2}$); 3.01 (s, 3H, NC$\boldsymbol{H_3}$).

^{13}C {^{1}H} NMR (DMSO-d_6, 298 K): 136.9 (N***C***HN), 123.0 (N***C***HCHN), 123.0 (NCH***C***HN), 128.3, 141.2, 128.3, 127.8, 125.9 (**Carbons** of **Ph ring**); 70.6 (O***C***H), 55.6(N***C***H_2), 35.8 (N***C***H_3).

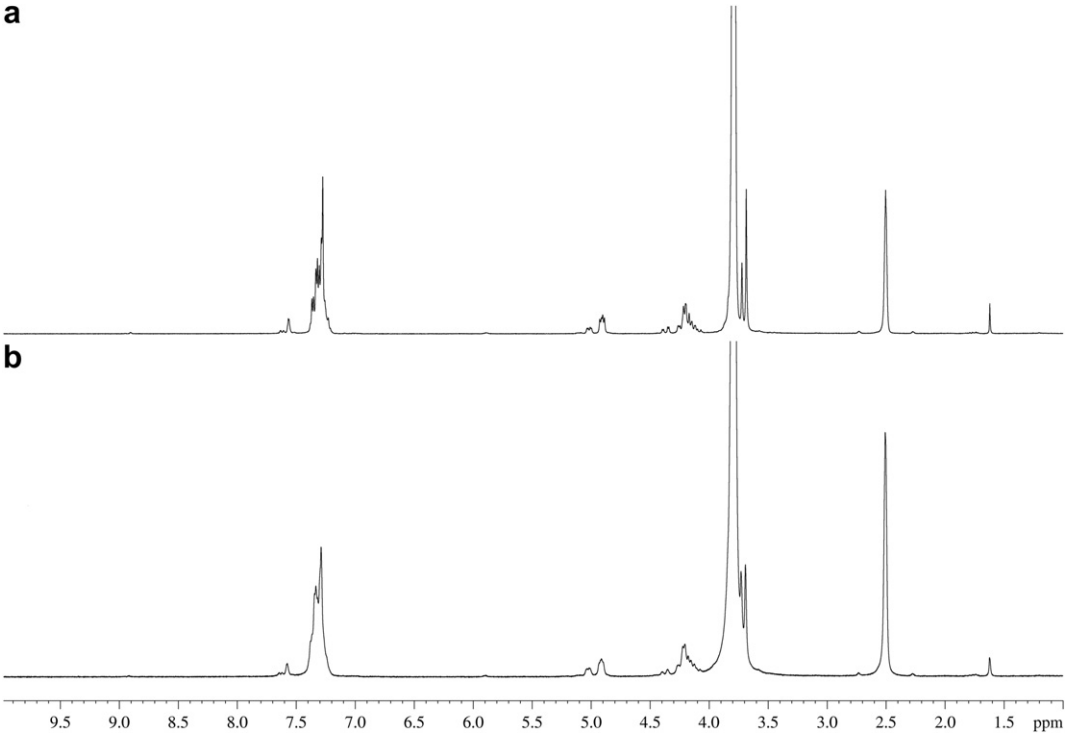


Fig. 4. ^{1}H NMR spectra of complex **1′** after 15 min (a) and after 24 h (b) from dissolution in D_2O-10% of DMSO-d_6.

4.4. Synthesis of (*1′*)

To a suspension of ligand **L1** (0.330 g, 1.0 mmol) in CH_2Cl_2 (30 ml), an amount of potassium hexamethyldisilazide (0.690 g, 3.5 mmol) was added at room temperature to obtain the potassium alkoxide-carbenes intermediate. After 5 min was added silver acetate CH_3COOAg (0.600 g, 3.5 mmol) and the brown solution was left stirred overnight at room temperature. The solvent was evaporated at reduced pressure and the reaction mixture was washed with *n*-hexane (3 × 10 ml). The solid was extracted with dichloromethane (3 × 5 ml); the product was obtained as white solid by precipitation at −20 °C and dried *in vacuo*.

Yield = 30%.

Elemental analysis of (**1′**): found (%): C 46.4, H 4.1, N 9.2, O 5.3, Ag 35.0. Calc. for $C_{12}H_{13}AgN_2O$ (%): C 46.6, H 4.2, N 9.1, O 5.2, Ag 34.9.

^{1}H NMR (CD_2Cl_2, 298 K): 7.25–7.40 (m, 5H, **Ph ring**); 7.05 (d, 1H, NC***H***CHN); 6.96 (d, 1H, NCHC***H***N); 4.99 (dd, 1H, OC***H***); 4.25–4.35 (dd, 1H, NC***H*****$_2$**); 3.95–4.15 (dd, 1H, NC***H*****$_2$**); 3.80 (s, 3H, NC***H*****$_3$**).

^{13}C {^{1}H} NMR (CD_2Cl_2, 298 K): 180.9 (N***C***N) 141.1, 129.0, 128.5, 126.4 (**Ph ring**); 123.4 (N***C***HCHN), 121.8 (NCH***C***HN), 75.5 (O***C***H$_2$), 60.4 (N***C***H$_2$), 39.2 (N***C***H$_3$).

Mass spectrum: 309 [Ag(**L1**)]$^+$.

Table 1
MIC results of the silver complexes.

Complexes	M.I.C.[a]	
	Strain *E. coli*[b]	Strain *B. subtilis*[c]
1′	5 μg/ml	5 μg/ml
1	5 μg/ml	5 μg/ml
2	5 μg/ml	50 μg/ml
3	5 μg/ml	50 μg/ml
4	>50 μg/ml	>50 μg/ml

[a] Minimum Inhibitory Concentration.
[b] Escherichia coli.
[c] Bacillus subtilis.

4.5. Synthesis of (*1*)

To a suspension of ligand **L1** (0.330 g, 1.0 mmol) in CH_3CN (60 ml) was added at room temperature silver oxide (0.116 g, 0.5 mmol) and stirred overnight. Afterward the solution was warmed up to 60 °C and left stirred 12 h and then the solvent was concentrated at reduced pressure and the product was obtained as white solid by precipitation at −20 °C and dried *in vacuo*.

Yield = 10%.

Elemental analysis of (**1**): found (%): C 32.8, H 3.1, N 6.5, O 3.6, Ag 24.8, I 29.2. Calc. for $C_{12}H_{14}AgIN_2O$ (%): C 33.0, H 3.2, N 6.4, O 3.7, Ag 24.7; I 29.0.

^{1}H NMR (CD_2Cl_2, 298 K): 7.25–7.40 (m, 5H, **Ph ring**); 6.90 (d, 1H, NC***H***CHN); 6.84 (d, 1H, NCHC***H***N); 5.19 (t, 1H, C***H***OH); 4.39 (d, 2H, NC***H*****$_2$**); 3.86 (s, 3H, NC***H*****$_3$**).

^{13}C {^{1}H} NMR (CD_2Cl_2, 298 K): 185.3 (N***C***N); 141.8, 129.2, 128.6, 126.6 (**Ph ring**); 122.9 (N***C***HCHN), 122.0 (NCH***C***HN), 73.7 (O***C***H$_2$), 59.3 (N***C***H$_2$), 39.2 (N***C***H$_3$).

Mass spectrum: 513 [Ag(**L1**)$_2$]$^+$.

4.6. Synthesis of (*2*)

The complex was prepared as described for analog **1** starting from **L2** ligand (0.294 g, 1.0 mmol) and silver oxide (0.5 mmol, 0.116 g).

Yield = 21%.

Elemental analysis of (**2**): found (%): C 26.8, H 3.4, N 7.1, O 4.1, Ag 26.8; I 31.7. Calc. for $C_9H_{14}N_2OAgI$ (%): C 27.0, H 3.5, N 7.0, O 4.0, Ag 26.9; I 31.6.

^{1}H NMR (DMSO-d_6, 298 K): 7.57 (d, 1H, NC***H***CHN); 7.47 (d, 1H, NCHC***H***N); 4.65 (m, 1H, C***H***OH); 4.20 (m, 1H, NC***H***); 3.82 (s, 3H, NC***H*****$_3$**); 2.30–1.50 (m, 6H, NCHC***H*****$_2$**C***H*****$_2$**C***H*****$_2$**CHOH of cyclopentyl ring).

^{13}C {^{1}H} NMR (DMSO-d_6, 298 K): 180.4 (N***C***N); 123.5 (N***C***HCHN), 120.0 (NCH***C***HN), 76.7 (O***C***H), 69.2 (N***C***H), 38.1 (N***C***H$_3$); 32.1

(NCHCH$_2$***C***H$_2$CH$_2$CHOH of cyclopentyl ring), 30.1 (NCH***C***H$_2$CH$_2$CH$_2$CHOH of cyclopentyl ring), 19.7 (NCHCH$_2$CH$_2$***C***H$_2$CHOH of cyclopentyl ring).

Mass spectrum: 439 [Ag(**L2**)$_2$]$^+$.

4.7. Synthesis of (**3**)

The complex was synthesized following the procedure described for the complex **1**, *i.e.* by reacting, in CH_3CN (60 ml), 1.0 mmol of the ligand **L3** (0.308 g) with 0.5 mmol of silver oxide (0.116 g) at room temperature. The reaction mixture was then refluxed for 12 h and then the solvent was concentrated at reduced pressure and the product was obtained as white solid by precipitation at −20 °C and dried *in vacuo*.

Yield = 18%.

Elemental analysis of (**3**): found (%): C 29.7, H 3.7, N 6.8, O 3.8, Ag 26.1, I 30.7. Calc. for $C_{10}H_{16}AgIN_2O$ (%): C 29.9, H 3.8, N 6.7, O 3.8, Ag 26.0; I 30.6.

^{1}H NMR (DMSO-d_6, 298 K): 7.53 (d, 1H, NC***H***CHN); 7.42 (d, 1H, NCHC***H***N); 4.03 (m, 1H, C***H***OH); 3.86 (m, 1H, NC***H***); 3.83 (s, 3H, NC***H$_3$***); 2.30−1.50 (m, 8H, NCHC***H$_2$***C***H$_2$***C***H$_2$***C***H$_2$***CHOH of cyclohexyl ring).

^{13}C {^{1}H} NMR (DMSO-d_6, 298 K): 178.9 (N***C***N); 122.5 (N***C***HCHN), 120.0 (NCH***C***HN), 71.3 (O***C***H$_2$), 67.0 (N***C***H$_2$), 39.5 (N***C***H$_3$); 35.0 (NCH***C***H$_2$CH$_2$CH$_2$CH$_2$CHOH of cyclohexyl ring), 33.0 (NCHCH$_2$***C***H$_2$CH$_2$CH$_2$CHOH of cyclohexyl ring), 24,8 (NCHCH$_2$***C***H$_2$CH$_2$CH$_2$CHOH of cyclohexyl ring), 23,9 (NCHCH$_2$CH$_2$CH$_2$***C***H$_2$CHOH of cyclohexyl ring).

Mass spectrum: 469 [Ag(**L3**)$_2$]$^+$.

4.8. Synthesis of (**4**)

The complex was prepared as described for analog **1** starting from **L4** ligand (0.254 g, 1.0 mmol) and silver oxide (0.5 mmol, 0.116 g).

Yield = 10%.

Elemental analysis of (**4**): found (%): C 19.8, H 2.7, N 7.9, O 4.3, Ag 30.0, I 35.3. Calc. for $C_6H_{10}AgIN_2O$ (%): C 20.0, H 2.8, N 7.8, O 4.4, Ag 29.9; I 35.2.

^{1}H NMR (DMSO-d_6, 298 K): 7.44 (d, 1H, NC***H***CHN); 7.42 (d, 1H, NCHC***H***N); 4.16 (t, 2H, C***H$_2$***OH); 3.80 (s, 3H, NC***H$_3$***); 3.73 (t, 2H, NC***H$_2$***).

^{13}C {^{1}H} NMR (DMSO-d_6, 298 K): 181.0 (N***C***N); 122.4 (N***C***HCHN), 122.2 (NCH***C***HN), 60.9 (O***C***H$_2$), 53.4 (N***C***H$_2$), 37.9 (N***C***H$_3$).

Mass spectrum: 360 [Ag(**L4**)$_2$]$^+$.

4.9. Hydrolysis tests

NMR samples were prepared by transferring 5 mg of appropriate compound under an N_2 flush into a 10-mm NMR tube that had been charged with 1.00 ml of DMSO-d_6 (or solution DMSO-d_6/D_2O 1/9) previously saturated with N_2 for several minutes. ^{1}H NMR spectra were then recorded at various time intervals with the sample maintained at 37 °C.

4.10. Pharmacology

4.10.1. Determination of the minimum inhibitory concentration (MIC)

Bacterial strains of *E. coli* (DH5α) and *B. subtilis* (PY79) were used in the antibacterial tests to measure the MIC value. Bacteria were grown overnight in LB medium (LBacto-Tryptone10 g, Bacto-yeast extract 5 g, NaCl 10 g per liter), diluted at a density of 4000 colony forming units (CFUs) per ml and incubated with increasing concentrations of the silver compounds. After 18 h (overnight) at 37 °C, bacterial growth was checked by spectrophotometric reading at a wavelength of 600 nm, a sample of bacterial cells grown in LB medium without the addition of any compound was used as control.

For each experiment, carried out in duplicate, triplicate assays were performed.

4.11. Cytotoxicity test by using brine shrimp assay

The Brine shrimps (*Artemia salina*) assay was performed in triplicate with appropriate amounts of samples dissolved in DMSO (1% final volume) to reach final concentrations of 100, 10 and 1 μg/ml, using 10 freshly hatched larvae suspended in 5 ml of artificial sea water [22]. Briefly, for each dose tested, surviving shrimps were left at RT and counted after 24 h, and the data statistically analyzed by the Finney program [25], which affords LD_{50} values with 95% confidence intervals.

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