Synthesis and Antimicrobial Activities of Gold(I) N-heterocyclic Carbene Complexes

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Abstract

Water-soluble gold(I) complexes [1-mesityl-3-(3-sodiumsulfonatopropyl)imidazol-2-ylidene] gold(I) Chloro (**3**) and [1,3-bis(2,6-diisopropyl-4-sodiumsulfonatophenyl)imidazol-2-ylidene] gold(I) Chloro (**4**) were prepared, in high yield, by carbene transmetallation from the appropriate NHC silver complex with chloro tetrahydrothiophene gold ([AuCl(tht)]). The antimicrobial properties of both gold(I) compounds and the respective ligands were assessed by agar diffusion assay and the broth macrodilution method against both Gram-positive and negative bacteria. MICs values (> 128 ug/ml) were superior to those displayed by the reference antibiotics penicillin G and streptomycin.

Keywords: Water-soluble Au(I), N-heterocyclic carbene, Antimicrobial agent

Introduction

Since 1991, when Arduengo isolated the first free carbene N-heterocyclic (NHC) [1], a wide variety of NHC complexes have been synthesized involving both transition and main group metals [2]. Its neutral monodentate ligands, whose properties may be modified by the introduction of different substituents allowing the incorporation of water-soluble functional groups. These ligands have been compared with phosphanes for the way they bond to metals. However, some evidence has suggested that NHCs form more stable bonds with metals [3]. Although many water-soluble phosphane ligands have been synthesized [4], there is little bibliography related to water-soluble NHC complexes. These reports are

mainly based on the effectiveness of these catalysts in aqueous medium [5]. There is even less information available about their chemical behavior or the stability of the metal-carbene bonds [6]. On the other hand, the use of gold complexes as catalysts has increased substantially in the last two decades [7], as well as the interest on the antimicrobial and antitumoral properties of these compounds [8], expanding the application of transition metals.

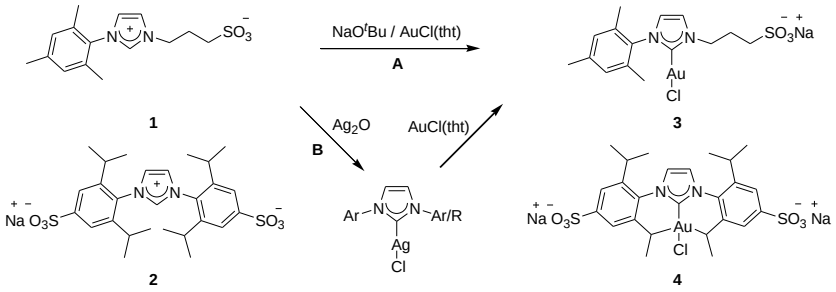
The biological properties of Au-complexes such as Au(I) thiocyanate, Au(III) and Au(I) phosphane complexes have been previously documented [9]. Among Au-complexes, NHCs are easily manipulated and can be readily functionalized. As mentioned before, they also form a stronger bond to metals than phosphanes do and, therefore, more stable metal complexes. The Silver, Gold, Rhodium, Ruthenium and Palladium NHC complexes are known to display antimicrobial and antitumoral activities. Ghosh et al. conclude that Pd-NHC complexes exhibit potent anticancer activities while Ag and Au-NHC complexes show significant antimicrobial activities [10]. One factor that could influence the activity and the interaction with living tissues is the lipophilicity of these complexes. Studies on antitumoral activities of Au complexes show that a moderate lipophilicity improves their biological behavior. On the contrary, a higher degree of lipophilicity enhances the activity of antitumoral drugs targeted to mitochondria.

Because water is the biologically most relevant solvent, the synthesis of metal-based drugs with enough solubility and stability in water or physiological media is a matter of increasing interest. Herein, we report the synthesis and antimicrobial efficacy of two water-soluble NHC gold(I) complexes.

Results and Discussion

***Synthesis and Characterization*.** The water-soluble gold(I)-carbene complexes **3** and **4** were prepared in high yield in DMSO from [AuCl(tht)] (tht= tetrahydrothiophene) complex [11] and the corresponding imidazolium compounds **1** [6] and **2** [5d] using sodium *tert*-butoxide as the deprotonating agent (Scheme 1, method A). Purification of these products was straightforward due to their insolubility in acetone. They are very stable at air and can be stored for prolonged periods, protected from light. Complexes **3** and **4** were alternatively obtained by carbene transmetallation [12] from the appropriate NHC silver complex, prepared according to reported procedures [6], to precursor complex (Scheme 1, method B). The silver

oxide route turned out to be the most effective (95% and 90% yield, respectively), avoiding the formation of the respective bis-carbene complexes [$(NHC)_2Au(0)$] observed by the direct route [5e].



Scheme 1. Synthesis of water-soluble NHC-Au(I) complexes **3** and **4**

These complexes were fully characterized by ^1H and ^{13}C NMR spectroscopy, elemental analysis and mass spectroscopy. Electrospray ionization-mass spectra (ESI-TOF) obtained in methanol showed the molecular anion corresponding to the lost of a sodium(1+) ion for both complexes. The partitioning constant of the new gold complexes in mixtures of water and common organic solvents could be determined by Vis-UV spectra of these complexes. Qualitative information obtained by ^1H NMR spectroscopy showed that both complexes are completely dissolved into the aqueous phase in mixtures of this solvent with diethyl ether or toluene.

***Antimicrobial activity*.** Antimicrobial activities of compounds **3** and **4** were first assayed by the agar diffusion method (Table 1, Figure 1). Both complexes displayed growth inhibition at 10 mg/ml, while ligands remained inactive at this concentration.

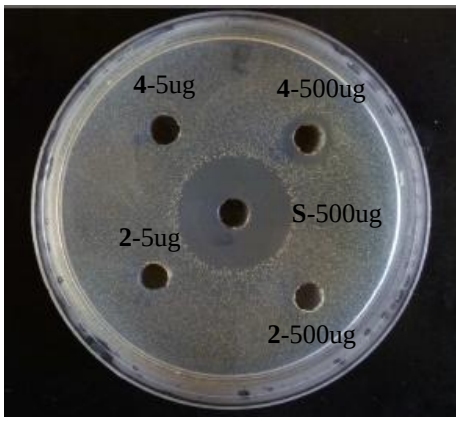
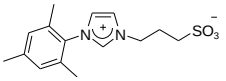
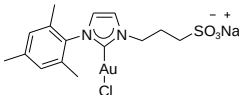
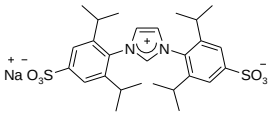
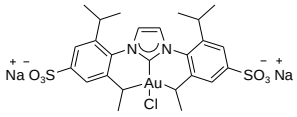


Figure 1. Antimicrobial activity of **2**, **4** and streptomycin (**S**) against *Y. ruckeri*. Growth inhibition of test compounds was evaluated at 10 and 0.1 mg/ml (50 μl each)

Table 1. Antimicrobial activity of Au complexes determined by the agar diffusion assay. Diameter of the inhibition zone is measured in mm.

Compound	Concentration mg/ml	*Lactococcus garvieae*	*Yersinia ruckeri*	*Pseudomonas aeruginosa*
 **1**	10	-	-	-
 **3**	0.1	-	-	-
	10	10	10	11
 **2**	10	-	-	-
 **4**	0.1	-	-	-
	10	-	12	10
Streptomycin	0.1	-	11	-
	10	17	29	33
Penicillin G	0.1	29	24	-
	10	35	36	-

In order to determine the Minimal Inhibitory Concentration, complexes and reference substances were tested by the broth macrodilution method at a dilution range of 0.25 to 128 μg/ml. MICs of Penicillin G were 0.5 μg/ml against *L. garvieae* and 1 ug/ml against *Y. ruckeri*. MICs of Streptomycin were 8, 64 and 64 μg/ml against *L. garvieae, Y. ruckeri* and *P. aeruginosa*, respectively.

MICs for compounds **3** and **4** were not detected at concentrations equal or lower than 128 μg/ml. According to these results, the complexes show a low inhibitory activity against the bacterial strains tested. Neither ligand **1** nor **2** inhibited the growth of the strains tested even at high concentrations in both macrodilution and agar diffusion tests. It suggests that the activity is due to the complex itself. Structurally similar Au(I)-NHCs reported in the literature inhibited growth of Gram-positive and negative bacteria at

12.5 μg/ml [13], but the activities displayed by the respective ligands were superior to that of the Au-complexes. Another gold(I)-NHC complex reported by Ghosh and co-workers exhibited antimicrobial activity (6 μg/ml) against the Gram-positive *Bacillus subtilis*, although it was not active against the Gram-negative *Escherichia coli* [10]. The main advantage of complexes **3** and **4** in comparison with those previously reported is the lower hydrophobicity which allows the preparation of aqueous solutions without addition of solvents such as DMSO.

Conclusions

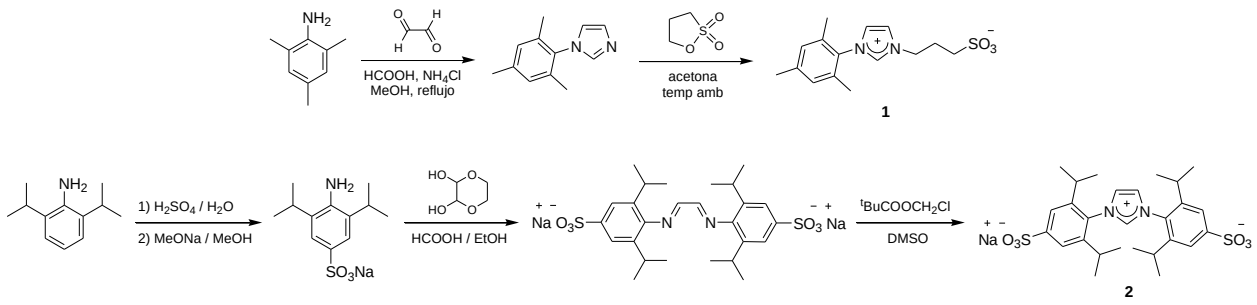
The synthesis of complex **3** and **4** by the NHC-Ag intermediate resulted to be the most effective method (80% and 75% respectively), avoiding the formation of the respective bis-carbene complexes $[(NHC)_2Au(0)]$ observed by the direct route. Although compounds **3** and **4** displayed low activity against the strains tested, further research is needed to assess the antimicrobial potency against a wider bacterial spectrum, including strains such as *B. subtilis* and *E. coli* and also fungal strains. One of the advantages of complex **3** and **4** is the hydrophilic nature conferred by the sulfonic groups, which improves the physicochemical properties of these compounds for their use in living tissues and pharmaceutical formulations. Moreover, the activities observed were not due to the ligands. It was suggested that intermediate lipophilicity of Au(I)-NHCs is desired for a better biological effect while the functionalization of the nitrogen atoms of the NHC ligands and the complexation with Au(I) at the C2 site influence the antimicrobial activity [9]. This supports further studies on the synthesis of novel sulfonated Au(I)-NHCs in order to find ligands which improve the antimicrobial activity exerted by the complex.

Experimental Section

All operations were performed under a nitrogen atmosphere by using Schlenk techniques. Organic solvents were dried and distilled under nitrogen and degassed prior to use. Unless otherwise stated, reagents were obtained from commercial sources and used as received. [AuCl(tht)] was prepared according to reported procedures [11]. ^{1}H and ^{13}C spectra were recorded with a Bruker 300 spectrometer. The Analytical Services of the Universidad de Alcalá performed the C, H, S and N analyses in a Heraeus

CHN-O-Rapid microanalyzer, and the ESI mass spectra in an Automass Multi, ThermoQuest spectrometer.

Synthesis of the Imidazolium Salts. Compounds **1** [5d] and **2** [13, 14] were prepared according to the reported procedures. The yield in the preparation of **2** was raised from the reported 70% to 98% due to the replacement of the final chromatographic purification by the precipitation of the product from the DMSO solution with acetone. Water solubility at 25 °C: 72.5 (**1**) and 160 (**2**) g/L.



Scheme 2. Synthesis of the Imidazolium Salts **1** and **2**

General procedure for preparation of NHC-gold(I) complexes

Method A. The corresponding imidazolium salt (1.00 mmol), [AuCl(tht)] (0.288 g, 1.00 mmol), sodium *tert*-butoxide (0.192 g, 2.00 mmol), and DMSO (10 mL) were introduced into a 25 mL Schlenk tube, and the mixture was stirred over night at room temperature. Once the reaction was completed, the mixture was filtered using a plug of celite, and the DMSO partially removed under vacuum up to a remaining volume of 2-3 mL. Finally, acetone (15 mL) was added to cause the precipitation of the gold product. Filtration of the solid, followed by washings with acetone (3 × 5 mL), and drying under vacuum for 2 h at 50 ºC, yielded the corresponding complex as a pure solid.

Method B. The imidazolium salt (0.60 mmol), silver oxide (0.083 g, 0.36 mmol) and sodium chloride (0.035 g, 0.60 mmol) were combined in a 25 mL Schlenk tube together with water (3 mL), and the mixture was stirred over night at room temperature. Upon completion of the reaction, the mixture was filtered through a plug of celite. AuCl(tht) (0.192 g, 0.60 mmol) and NaCl (0.035 g, 0.60 mmol) were

added and the mixture was stirred for 3 hours. Once the reaction was completed, the mixture was filtered using a plug of celite. The solvent was completely removed under vacuum at 50 ºC, affording the corresponding complex as a pure solid.

[1-mesityl-3-(3-sodiumsulfonatopropyl)imidazol-2-ylidene]gold(I) Chloro (3). Complex **3** was obtained as a white solid (0.269 g, 80%) employing method B. Water solubility at 25 °C: 109 g/L.

[1,3-bis(2,6-diisopropyl-4-sodiumsulfonatophenyl)imidazol-2-ylidene]gold(I) Chloro (4). Complex **4** was obtained as an off-white solid (0.361 g, 73%) employing method B. Water solubility at 25 °C: 111 g/L.

Antimicrobial activity assays. Antimicrobial activities of compounds **3**, **4** and the respective ligands were tested by agar dilution procedures according to BSAC guidelines [15] against the Gram-negative bacterial strains *Yersinia ruckeri* ATCC 29473, *Pseudomonas aeruginosa* and the Gram-positive *Lactococcus garvieae*. Complexes, ligands, and antibiotics standards penicillin G and streptomycin were tested at a dilution range of 0.25 to 128 μg/mL. Stock solutions of all compounds were dissolved in distilled water. Agar diffusion tests were performed according to Sequeiros et al [16]. Briefly, molten agar (45°C) was seeded (1% v/v) with a standardized suspension of the indicator strain. The inoculated medium was rapidly dispensed in sterile Petri dishes. After solidification, wells of uniform diameter (6 mm) were bored in the agar. 50 μl aliquots of tested compounds were dispensed into each well. Plates were allowed to diffuse for 2 h at 4°C. Diameters of inhibition zones were measured after 24 h incubation at 30°C. *Y. ruckeri* ATCC 29473 and *P. aeruginosa* were subcultured on Tripticase Soy Agar and *L. garvieae* on de Man-Rogosa-Sharpe Agar.

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