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Remarkable *in vitro* anti-HIV activity of new silver(I)– and gold(I)–N-heterocyclic carbene complexes. Synthesis, DNA binding and biological evaluation

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A novel metallomacrocyclic silver complex $[Ag(C_{13}H_{13}N_5)]_2Br_2$ **1** and a bimetallic bridged gold complex $[Au_2(C_{13}H_{13}N_5)Cl_2]$ **2** derived from 2,6-bis(3-methylimidazolin-2-yliden-1-yl)pyridine dibromide **L1** have been synthesized and fully characterized by UV–Vis, elemental analysis, FT-IR, NMR techniques and DFT calculations. The DNA interactions with the compounds were investigated by UV-spectrophotometric studies, viscosity measurements and DNA electrophoresis. Additionally, the lipophilicity values were determined from the water/*n*-octanol partition coefficient. Experimental data indicated that all the compounds interacted with DNA, through a non-covalent binding mode. **L1** and **2** were found to be hydrophilic character while **1** was somewhat lipophilic. The biological activities of **L1**, **1** and **2** were tested against a panel of cancer cell lines (MCF-7, PC-3, A459, HeLa, HT-29 and the 4T1 murine tumor cell line). *In vitro* antiviral studies against HIV-1 were performed in infected MT4 leukemia cells. All the compounds exhibited a low activity against the cell lines, associated possibly with low lipophilicity values. However, complexes **1** and **2** showed remarkable viral inhibition at low concentrations. The complexes may inhibit virus infectivity through interaction with the HIV-1 envelope proteins.

1. Introduction

Following the isolation of the first stable N-heterocyclic carbene (NHC) 1,3-bis(adamantyl)imidazol-2-ylidene by Arduengo et al. [1], there has been intense research on such as compounds due to their versatility as ligands for metallic complexes. Their bonding properties have been described by spectroscopic [2–4] and computational studies [5,6], showing that NHCs have strong $\sigma \rightarrow d$ donation, and the filled and empty orbitals π, π^* may participate in NHC–metal bond. Modifications of the N-substituents and architectures of the NHC would produce drastic changes in the donor electronic properties of the carbene moieties. N-substituents have been used to control the steric pressure both on the carbene and the coordinated metal center, and are a powerful tool in the design of NHC–metal complexes.

The introduction of N-substituents with additional donor atoms such as N, O or S on NHC moieties offers the possibility of coordination or interaction with the metal center through chelation. This kind of coordination provides additional stability and rigidity to the NHC complex, improving their ability for slow metal release [7]. These features make them promising candidates in the field of organometallic chemistry and more recently for bioorganometallic chemistry. NHC with late transition metal complexes (Ag, Au) have generated great interest due to effectiveness as antimicrobial and anticancer agents [8,9]. Among their principal advantages is found the stabilization that the NHC offers to the metal center, allowing slow cation release and providing a constant

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concentration of the therapeutic agent [8]. Furthermore, easy functionalization of NHC through N-substituents permits the modulation of the lipophilic/hydrophilic properties of such complexes, an important feature for their use as therapeutics agents.

Thus, chelated carbenes containing rigid aromatic linkers between imidazole rings have been used to obtain bimetallic silver and gold complexes. Youngs et al. synthetized the first water-soluble Ag(I)–NHC complexes based in 2,6-bis(ethanolimidazolemethyl)pyridine hydroxide and 2,6-bis(propanolimidazolemethyl)pyridine hydroxide, which showed a improved antimicrobial activities against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* over silver nitrate [10]. In the same year, Berners-Price et al. reported a series of dinuclear Au(I) complexes of imidazolium-linked cyclophanes and studied their biological behavior in isolated mitochondria [11,12]. The mitochondrion plays a fundamental role in programmed cellular death or apoptosis and is considered as a target in the development of new cancer chemotherapeutic agents [12]. These Au(I) complexes could induce Ca^{2+} sensitive Mitochondrial Permeability Transition (MPT) as evidenced by mitochondrial swelling, leading to cellular death [12,13]. More recently, a new family of benzimidazole and imidazole-based Ag(I)–NHC complexes, with N-allyl [7,14] and N-alkyl [15] substituents using p-xylyl or 2,6-lutidinyl as linker have been reported. Anticancer studies using HCT-116 cells showed that these complexes are active with IC_{50} values (50% inhibitory concentration) almost five times lower than the control drug 5-fluorouracil. In fact, Ag(I)–NHC and Au(I)–NHC complexes have presented excellent antitumor, antiproliferative and antimitochondrial activities, suggesting them to be good candidates as anticancer agents [16,17] as well as stimulating the study of their potential activity against other diseases.

Metals drugs containing Au(I) have been studied as anti-HIV agents from the late 80's showing minimal toxicity compared to most Au(III) complexes, due to the thermodynamic stability of Au(I) in biological media [18]. In the two principal mechanisms of action, inhibition of reverse transcriptase (RT) or inhibition of viral entry (infectivity), the key role of Au(I) is the formation of reactive gold-thiols intermediaries through ligand exchange with cysteinyl residues on the surface of enzymes or proteins of the viral envelope [18]. Most of the complexes tested possess ligands of biological interest, such as thioglucose [19], thiomalate [19,20] and phosphines [21]. However, as far as we know, anti-HIV activity of Ag(I) or Au(I)–NHC complexes has not been reported and the relative simple manipulation of these NHC ligands remains unexploited.

Based on these reports, we decided to use an imidazole-based NHC with a pyridine rigid linker as ligand for Ag(I) and Au(I) complexes. 2,6-Bis(3-methylimidazolin-2-yliden-1-yl)pyridine dibromide (**L1**) is a known pincer carbene widely used in several transition metal reactions [22–26]. Its monometallic complexes exhibit high thermal stability [27] and in the case of metallocycles, the interaction between pyridinic nitrogen and metal center reduces the ligand dissociation tendency through formation of an additional stable system [15]. Thus, **L1** is a good candidate as ligand and as far as we know, the biological activities of its silver and gold complexes have not yet been explored. Here, we report the synthesis and characterization of two new complexes $[Ag(C_{13}H_{13}N_5)]_2Br_2$ **1** and $[Au_2(C_{13}H_{13}N_5)Cl_2]$ **2** with 2,6-bis(3-methylimidazolin-2-yliden-1-yl)pyridine dibromide **L1** as an NHC precursor, as well as studies of their interactions with CT-DNA (Calf thymus DNA) using differents physical and spectroscopic techniques. Moreover, cytotoxicity assays of the compounds were carried out on human tumors cell lines while the *in vitro* antiviral activity was evaluated in MT4 cells infected with HIV-1.

2. Materials and methods

2.1. Reagents, starting materials and instrumentation

All syntheses were carried out under inert atmosphere and in the absence of light. The solvents were previously dried and distilled before use following standard methods [28]. 2,6-Bis(3-methylimidazolin-2-yliden-1-yl)pyridine dibromide **L1** and the gold precursor salt AuCl(THT) were prepared according to reported methods [29,30]. The spectroscopic data for **L1** is shown in Table 1 and in the Supporting information. ^1H and ^{13}C NMR spectra were obtained on a Bruker Advance spectrometer at 300 and 500 MHz.

Table 1
Spectroscopic characterization of compounds **L1**, **1** and **2**.

Compounds	$\nu(CO)^a$ (cm^{-1})	^1H NMR (δ, ppm)[b]	^{13}C NMR (δ, ppm)[c]	UV–Vis λ nm (ε cm^{-1} M^{-1})[d]
Ligand 2,6-bis(3-methylimidazolin-2-yliden-1-yl)pyridine dibromide **L1**	3028m 1610m 1535m 1469s 1362w 1233s	10.63 (s, 2H) Imidazolium-*H2* 8.81 (s, 2H) Imidazolium-*H5* 8.60 (t, *J* = 8.1 Hz) Pyridine-*H8* 8.25 (d, 2H, *J* = 8.1 Hz) Pyridine-*H7* 8.07 (s, 2H) Imidazolium-*H4* 4.04 (s, 6H) N-CH_3	145.22 Pyridine-*C* 144.88 Pyridine-*C6* ×2 136.32 Imidazolium-*C2* ×2 124.98 Imidazolium-*C4* ×2 119.08 Imidazolium-*C5* ×2 114.02 Pyridine-*C7* ×2 36.48N–CH_3 ×2	214 (14438) 281 (7034.5)
Complex $[Ag(C_{13}H_{13}N_5)]_2Br_2$ **1**	3103w 2904sh 1608w 1603m 1461m 1399vw 1233w	8.46 (t, 1H, *J* = 8.0 Hz) Pyridine-*H8* 8.31 (s, 2H) Imidazolium-*H5* 8.02 (d, 2H, *J* = 8.0 Hz) Pyridine-*H7* 7.64 (s, 2H9 Imidazolium-*H4* 3.15 (s, 6H) 2 x N-CH_3	161.46 Imidazolium *C2-Ag* ×2 148.39 Pyridine-*C6* ×2 143.99 Pyridine-*C8* 124.37 Imidazolium–*C4* ×2 119.76 Imidazolium–*C5* ×2 113.88 Pyridine–*C7* ×2 38.65N–CH_3 ×2 (in CDCl3)	249 (11895) 283 (7548.3)
Complex $[Au_2(C_{13}H_{13}N_5)Cl_2]$ **2**	3134w 3104w 2930w 1604m 1446s 1391w 1238m	8.33-8.43 (m, 3H) Pyridine-*H* 8.19 (d, 2H, *J* = 2.0 Hz) Imidazolium-*H5* 7.75 (d, 2H, *J* = 2.0 Hz) Imidazolium-*H4* 3.90 (s, 6H) 2× N–CH_3	168.88 Imidazolium *C2-Ag* ×2 149.09 Pyridine-*C6* ×2 142.67 Pyridine-*C8* 124.05 Imidazolium-*C4* ×2 120.83 Imidazolium-*C5* ×2 117.56 Pyridine-*C7* ×2 38.74N–CH_3 ×2	231 (14700) 284 (6509.8)

[a] C_6H_{12}.
[b] DMSO-d_6, 300 MHz.
[c] DMSO-d_6, 100 MHz.
[d] Solvents indicate in experimental section.