Inorganica Chimica Acta 437 (2015) 143–151

Contents lists available at ScienceDirect

Inorganica Chimica Acta

journal homepage: www.elsevier.com/locate/ica

Novel silver(I)– and gold(I)–N-heterocyclic carbene complexes. Synthesis, characterization and evaluation of biological activity against tumor cells

Oriel Sanchez [a,b], Sorenlis González [b], Mercedes Fernández [c], Angel R. Higuera-Padilla [b], Yokoy Leon [b], David Coll [b], Alba Vidal [b], Peter Taylor [c], Izaskun Urdanibia [c], María Cristina Goite [b,*], William Castro [b,*]

[a] *Universidad de los Andes, Facultad de Ciencias, Departamento de Química, Venezuela*
[b] *Centro de Química, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020-A, Venezuela*
[c] *Medicina Experimental, Instituto Venezolano de Investigaciones Científicas (IVIC), Caracas 1020-A, Venezuela*

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 7 July 2015
Accepted 21 August 2015
Available online 5 September 2015

Keywords:
N-heterocyclic carbene
Metal–NCH complexes
DNA
Lipophilicity
Cytostatic
Cytotoxic

ABSTRACT

Two novel neutral N-heterocyclic carbene (NHC) complexes of silver (**1**) and gold (**2**), derived from 1,1′-(1,2-ethylene)-3,3′-dimethyldiimidazolium dibromide ($\mathbf{L_1}$), were synthesized, characterized (experimentally and computationally) and evaluated for biological activity in terms of binding to calf thymus (CT) DNA and inhibition of tumor cell lines. Spectroscopic absorption and viscosity measurements suggested the interaction with DNA to be noncovalent, probably π–π stacking or electrostatic. Additionally, electrophoresis and melting point studies and competitive studies showed a moderate interaction with CT DNA, principally complex **2**. The compounds exhibited lipophilic properties in the order: $\mathbf{2} > \mathbf{1} > \mathbf{L_1}$. Compounds **1** and **2** exerted growth inhibitory and cytotoxic activity against tumor cell lines in the lower micromolar range.

1. Introduction

Metal–NHC (N-heterocyclic carbene) complexes are widely studied for their intriguing structural properties and numerous applications [1,2]; in particular, an interest in medicinal applications of stable NHC complexes has recently appeared [3–8]. These carbene ligands interact with metal centers primarily through strong σ-donation and to a lesser degree through π-back-donation, producing stable metal–NHCs with strong metal—carbon bonds. Additionally, simple functionalization of the imidazole ring can be used to vary the structure of metal–NHC complexes and to modulate their therapeutic activity [9].

Due to their easy preparation under mild conditions via the Ag_2O route, and their usefulness as transmetalating agents [10], Ag(I)–NHCs have become the most synthesized and studied among coinage metal–NHCs. In the last decade, Ag(I)–NHCs complexes have emerged as improved antibacterial agents due to their low toxicity in humans and slower release of silver leading to prolonged biological activity. Youngs and co-workers first demonstrated the exceptional antimicrobial efficacy of Ag(I)–NHCs against a broad spectrum of both Gram-positive and Gram-negative bacteria as well as fungi. Although the mechanism of antimicrobial activity was not established, it was found that N-atom substitution affected the antimicrobial activity [11,12]. Some complexes have shown cytotoxicity against cancer cell lines; Haque and coworkers reported a series of Ag(I)–NHC complexes of N-allyl substituted imidazol-2-ylidenes and their activity against human colorectal (HCT 116) cancer cell lines [13]. Ag(I) insertion in a NHC precursor increased inhibitory activity although this depended on the nuclearity of the complex and the presence of appropriate substitutions on the ligand. More recently, Iqbal reported Ag(I)–NHCs with *para*-xylyl linked to *bis*-benzimidazolium, that exhibited dose-dependent inhibition against human colon cancer cells, with some compounds showing IC_{50} values significantly lower than 5-fluorouracil [14].

Another metal center of great interest is gold, playing an important role in the search for new Au(I)–NHC drugs with biological activity against different molecular targets and diseases [3,15–18]. Au(I)–NHC complexes have been obtained principally by carbene-transfer from the corresponding Ag(I)–NHC [15,19]. The similarities between NHCs and phosphines led to the

---

* Corresponding authors. Tel.: +58 212 5041740; fax: +58 212 5041350 (M.C. Goite). Tel./fax: +58 212 5041642 (W. Castro).

E-mail addresses: hamarkeys@gmail.com (M.C. Goite), wcastro10@gmail.com (W. Castro).

http://dx.doi.org/10.1016/j.ica.2015.08.017
0020-1693/

investigation of Au–NHC complexes as potential new antitumour agents [20–23]. Berners-Price and co-workers reported the antitumor activity and the underlying cytotoxic mechanisms of Au(I)–NHC complexes [24], one of which, a bidentate NHC induced significant mitochondrial swelling at 10 μM. Baker and co-workers also reported the synthesis of linear Au(I)–NHC complexes as the first NHC analog for the Au(I) phosphine drug auranofin. The advantage of these compounds is the ease with which NHCs may be chemically manipulated to synthesize a range of structurally similar complexes with varying degrees of lipophilicity for biological evaluation [25]. Dinda et al. found the activity of a gold(I) complex with 1-methyl-2-pyridin-2-yl-2H-imidazo[1,5-a] pyridin-4-ylium, to be more active against four cell lines than cisplatin [18]. Some Au(I)–NHC complexes inhibit bacterial proliferation. Silbestri, reported the properties of water-soluble gold(I)–NHC complexes against six different bacterial strains and compared them with both reference antibiotics and silver nitrate as an antiseptic [26,27]. The bactericidal activity of these complexes was comparable to $AgNO_3$.

Based on these observations, we have chosen 1,1′-(1,2-ethylene)-3,3′-dimethyldiimidazolium dibromide **L₁** as an NHC precursor to undertake the synthesis and characterization of two new complexes: $[Ag_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Br_2]$ **1** and $[Au_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Cl_2]$ **2**. We studied the interaction of L_1 and both complexes with CT-DNA through diverse physical and spectroscopy techniques. Due to the interesting biological activities presented for this family of complexes, especially gold and silver as anticancer agents, we have evaluated the activity of the compounds synthesized in this work against six tumor cell lines.

2. Experimental

2.1. Materials and methods

All syntheses were carried out under inert atmosphere and in the dark. The solvents were previously dried and distilled following standard methods prior to use [28,29]. 1,1′-(1,2-Ethylene)-3,3′-dimethyldiimidazolium dibromide (**L₁**) and the gold precursor salt Au(Cl)(tetrahydrothiophene) were prepared according to reported methods [30]. ^1H and ^{13}C NMR spectra were obtained on a Bruker Advance 300 spectrometer. IR spectra were recorded on a Perkin Elmer 100/100N spectrometer using KBr disks. Mass analyses were performed by electrospray ionization [ESI] with a Thermo Funnagam TSQ Quantum Ultra AMm spectrometer. UV–Vis spectra were recorded in an HP-8450A spectrophotometer in cells with a 1 cm optical pathway at 298 K, using the corresponding solvent. Conductivity measurements were performed with a LaMotte CDS 5000 conductimeter. Elemental analyses were performed with an EA 1108 FISONS instrument. Fluorescence measurements were carried out using a Perkin Elmer LS45 with pulse xenon lamp.

2.2. Synthesis of silver(I)- and gold(I)–N-heterocyclic carbene complexes

2.2.1. Synthesis of 1,1′-(1,2-ethylene)-3,3′-dimethyldiimidazolium dibromide (*L₁*)

1,2-Dibromoethane (2.60 mL; 30.0 mmol) and N-methylimidazole (6.00 mL, 75.6 mmol) were placed in a Schlenk tube, and 10 mL of ethanol were added. The reaction mixture was heated to 80 °C for 2 h. Subsequently, the mixture was cooled and the product was washed with dichloromethane (×4) to give **L₁** (8.67 g; yield 83%) mp 239 °C. Elemental analysis Calc. for $C_{10}H_{16}N_4Br_2$: C, 34.11; N, 15.91; H, 4.58; Found: C, 34.13; N, 15.79; H, 4.58%. ^1H NMR (300 MHz, DMSO-d_6, δ ppm) 9.15 (s, 2H, imidazolium-H2), 7.73 (t, 2H, imidazolium-H4), 7.67 (t, 2H, imidazolium-H5), 4.72 (s, 4H, N-ethylene H), 3.85 (s, 6H, 2× N-CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 137.11 (imidazolium-C2); 123.81 (imidazolium-C4); 122.30 (imidazolium-C5); 48.30 (N-ethylene C); 35.95 (N-CH_3C). IR-TF (υ_{max} cm^{-1}): 3084 (m, C-H_{arom}, C-H), 1561 (s, C=N, C=C), 1450 (m, C-H), 1342 (w, C-H), 1170 (s, C-N). UV–Vis DMSO/MeOH (λ, nm) (ε, cm^{-1} M^{-1}): 235 (8032.20). MS (ESI): *m*/*z*: 191.14 $[M-H]^+$.

2.2.2. Synthesis of $[Ag_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Br_2]$ **1**

A stock solution of **L₁** (363.00 mg; 1.03 mmol) and Ag_2O (477.10; 2.06 mmol) in 40 mL of acetonitrile were stirred at reflux temperature for 24 h under an argon atmosphere. The yellow solution was filtered and concentrated. Subsequently, diethyl ether was added and a brownish solid was precipitated, which was washed with diethyl ether (×3) and then dried under vacuum to give complex **1** $[Ag_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Br_2]$ (151 mg; Yield 26%; Decomp. >168.3 °C; Elemental analysis Calc. for $C_{10}H_{16}N_4Ag_2Br_2$-×CH_3CN: C, 23.67; N, 11.50; H, 3.15; Found: C, 23.65; N, 11.41; H, 3.19%. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 7.37 (s, 2H, imidazolyl-H4), 7.30 (s, 2H, imidazolyl-H5), 4.58 (s, 4H, N-ethylene H), 3.73 (s, 6H, 2× N-CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 179.45 (imidazolyl-C2); 123.16 (imidazolyl-C4); 122.16 (imidazolyl-C5); 51.27 (N-ethylene C); 38.27 (N-CH_3C). IR-TF (υ_{max} cm^{-1}): 3100 (m, C-H_{arom}), 2949 (sh, C-H), 1575–1562 (C=N, C=C), 1449–1408 (C-H), 1223 (C-N). UV–Vis DMSO/MeOH: λ (nm) (ε, cm^{-1} M^{-1}) 235 nm (9862.20). MS (ESI): *m*/*z* 298.99 $[M-AgBr+H]^+$. Molar conductivity (in DMF), $\Lambda_M = 18 \pm 3$ Ohm^{-1} cm^2 mol^{-1}.

2.2.3. Synthesis of $[Au_2(\mu\text{-}\kappa C,C'\text{-}C_{10}H_{14}N_4)Cl_2]$ **2**

A solution of **L₁** (127.50 mg; 0.36 mmol) and Ag_2O (162.30; 0.70 mmol) in CH_3CN (20 mL) were refluxed under argon for 17 h, filtered, and added dropwise to a solution of Au(THT)(Cl) (95.97 mg; 0.30 mmol) in the same solvent. The mixture was refluxed under argon for 18 h, the volume was reduced to 50% under a nitrogen stream and then maintained at −5 °C overnight. Precipitated AgBr was first removed by filtration then diethyl ether was added until the solution became turbid; a yellowish solid $[Au_2(\mu\text{-}\kappa^2C,C'\text{-}C_{10}H_{14}N_4)Cl_2]$ **2** deposited which was filtered off, washed with diethyl ether, and dried under vacuum. (22.83 mg; Yield 23%; m_p. 287 °C); Elemental analysis Calc. for $C_{10}H_{16}N_4Au_2$-Cl_2×CH_3CN: C, 20.64; N, 10.03; H, 2.74; Found: C, 20.83; N, 10.24; H, 2.74%. ^1H NMR (300 MHz, DMSO-d_6, δ ppm): 7.39 (d, 2H, *J* = 1,9 Hz, imidazolyl-H4), 7.27 (d, 2H, *J* = 1,9 Hz, imidazolyl-H5), 4.47 (s, 4H, N-ethylene H), 3.67 (s, 6H, 2× N-CH_3). ^{13}C NMR (300 MHz, DMSO-d_6, δ ppm): 169.09 (imidazolyl-C2); 123.25 (imidazolyl-C4); 121.51 (imidazolyl-C5); 51.02 (N-ethylene C); 37.69 (N-CH_3C). IR-TF (υ_{max} cm^{-1}): 3123 (w, C-H_{arom}), 2941 (w, C-H), 1566 (m, C=N, C=C), 1465–1371 (C-H), 1236 (m, C-N). UV–Vis (DMSO/MeOH) λ (nm) (ε, cm^{-1} M^{-1}): 229 (8779.8), MS (ESI): *m*/*z* 618.95 $[M-Cl]^+$. Molar conductivity (in DMF), $\Lambda_M = 23 \pm 4$ Ohm^{-1} cm^2 mol^{-1}.

2.3. Computational methods

All structures were optimized using DMol³ [31–33]. This DFT based program permits determination of the relative stability of all studied species based on their electronic structure. The calculations were performed using the Kohn–Sham Hamiltonian with the Perdew–Wang 1991 gradient correction [34] and the double-zeta plus (DNP) numerical basic set [31,33], which provides good accuracy at a relatively low computational cost. The All Electron core treatment was used for all the atoms. Frequency calculations of the structures showed that all frequencies were positive indicating that all structures are real minima.