Inorganic Chemistry

Article

pubs.acs.org/IC

Synthesis and Biological Activity of Ester- and Amide-Functionalized Imidazolium Salts and Related Water-Soluble Coinage Metal N-Heterocyclic Carbene Complexes

Maura Pellei,[†] Valentina Gandin,[‡] Marika Marinelli,[†] Cristina Marzano,[‡] Muhammed Yousufuddin, H. V. Rasika Dias,*[,§] and Carlo Santini*[,†]

[†]School of Science and Technology, Chemistry Division, University of Camerino, via S. Agostino 1, 62032 Camerino, Macerata, Italy

[‡]Department of Pharmaceutical and Pharmacological Sciences, University of Padova, via Marzolo 5, 35131 Padova, Italy

[§]Department of Chemistry and Biochemistry, The University of Texas at Arlington, Box 19065, Arlington, Texas 76019-0065, United States

Supporting Information

ABSTRACT: *N*-Heterocyclic carbene (NHC) ligand precursors, namely, $HIm^{A}Cl$ [1,3-bis(2-ethoxy-2-oxoethyl)-1*H*-imidazol-3-ium chloride] and $HIm^{B}Cl$ {1,3-bis[2-(diethylamino)-2-oxoethyl]-1*H*-imidazol-3-ium chloride}, functionalized with hydrophilic groups on the imidazole rings have been synthesized and were used in the synthesis of corresponding carbene complexes of silver(I) and copper(I), $\{[Im^{A}]AgCl\}$, $\{[Im^{A}]CuCl\}$, and $\{[Im^{B}]_2Ag\}Cl$. Related Au^{I}NHC complexes $\{[Im^{A}]AuCl\}$ and $\{[Im^{B}]AuCl\}$ have been obtained by transmetalation using the silver carbene precursor. These compounds were characterized by several spectroscopic techniques including NMR and mass spectroscopy. $HIm^{B}Cl$ and the gold(I) complexes $\{[Im^{A}]AuCl\}$ and $\{[Im^{B}]AuCl\}$ were also characterized by X-ray crystallography. The cytotoxic properties of the NHC complexes have been assessed in various human cancer cell lines, including cisplatin-sensitive and -resistant cells. The silver(I) complex $\{[Im^{B}]_2Ag\}Cl$ was found to be the most active, with IC_{50} values about 2-fold lower than those achieved with cisplatin in C13*-resistant cells. Growth-inhibitory effects evaluated in human nontransformed cells revealed a preferential cytotoxicity of $\{[Im^{B}]_2Ag\}Cl$ versus neoplastic cells. Gold(I) and silver(I) carbene complexes were also evaluated for their ability to in vitro inhibit the enzyme thioredoxin reductase (TrxR). The results of this investigation showing that TrxR appeared markedly inhibited by both gold(I) and silver(I) derivatives at nanomolar concentrations clearly point out this selenoenzyme as a protein target for silver(I) in addition to gold(I) complexes.

INTRODUCTION

The widespread success of platinum compounds in the clinical treatment of various types of neoplasias has placed the coordination chemistry in the front line in the fight against cancer. Extensive investigations are currently focused on the synthesis of metal complexes with a better chemotherapeutic index in terms of increased bioavailability, more antiproliferative activity, and fewer side effects than the platinum drugs. Complexes of group 11 metals (copper, silver, and gold) are promising in this regard[1,2] with the particular focus on phosphine complexes. A number of copper(I),[1,3−7] silver(I),[8,9] and gold(I)[10−15] complexes comprising aromatic tertiary phosphines and diphosphines[15−21] were investigated for their tumoricidal properties,[16,22−25] making possible the assessment of a structure−activity relationship for a wide range of phosphine ligands and their metal complexes.

Following the encouraging approach of diminishing the lipophilic character of phosphine complexes, mixed compounds were prepared in our group by combining hydrophilic N_2-scorpionate ligands ($Na[HC(CO_2)(tz)_2]$, $Na[HC(CO_2)(pz^{Me2})_2]$, and $K[H_2B(tz^{NO_2})_2]$) with lipophilic "CuP_2" unsaturated moieties (P_2 = bidentate diphenylphosphine or two monodentate phosphines) in an attempt to enhance the hydrophilic character of the overall phosphine−scorpionate copper assembly.[6,7,26,27] Recently, we synthesized and tested hydrophilic, monocationic $[M(L)_4]PF_6$ complexes (M = Cu^{I}, Ag^{I}, or Au^{I}; L = tertiary phosphine) as cytotoxic agents against a panel of several human tumor cell lines: copper derivatives were found to be the most efficient drugs, exceeding its potency of more than 30 times that of the reference drug cisplatin.[27−29] These studies showed that reduction of the lipophilicity of the overall molecule is a successful strategy to increase the cytotoxic activity for $[Cu(L)_4]^+$-type compounds, but this approach is limited by the more difficult synthesis of appropriate phosphine ligands.

In this context, N-heterocyclic carbenes (NHCs)[30] are an interesting class of ligands with donor properties similar to those of phosphines. Their chemical versatility implies not only a wide variety of structural diversity and coordination modes

Received: June 21, 2012
Published: September 4, 2012

dx.doi.org/10.1021/ic3013188 | Inorg. Chem. 2012, 51, 9873−9882

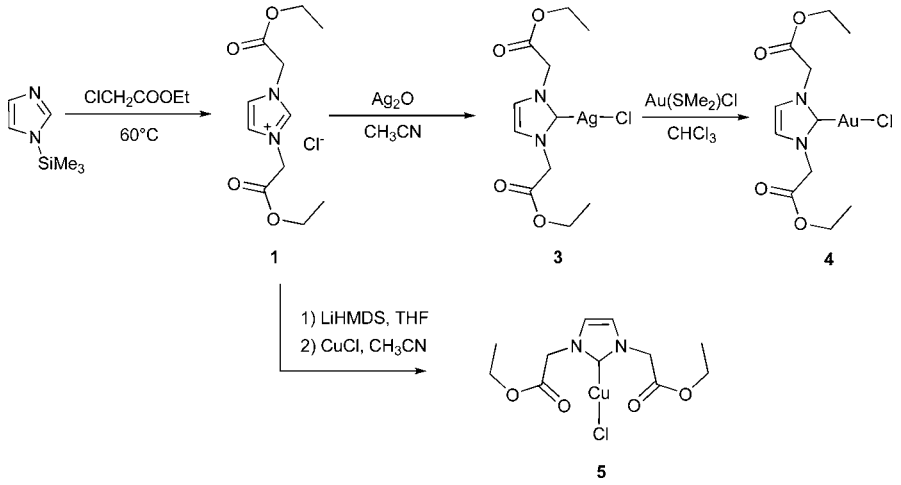

Figure 1. Reaction scheme of compounds **1** and **3**−**5**.

but also a capability to form stable complexes with a large number of transition metals with different oxidation states.[31−33] An attractive feature of NHC chemistry is the ease with which a series of structurally similar complexes with varying lipophilicity can be synthesized simply by changing the substituents on the imidazolium salt precursor.[34,35] As potential anticancer drugs, metal NHCs constitute a recent and very rapidly growing field of research.[36,37] Berners-Price and co-workers reported the synthesis of a family of fascinating dinuclear Au^{I}NHC complexes with the formula $[Au_2L_2]^{2+}$ (L = bidentate cyclophane NHC ligand) with significant antimitochondrial activity.[38,39] Nolan et al.[40] also reported the synthesis of a carbenic gold(I) saccharin complex, as a well-behaved class of compounds analogous to gold drugs such as Auranofin[41] and Solganol,[42] by using for the first time a cationic monoligated NHC gold(I) as the reagent.[43] Metzler-Nolte and co-workers[44] synthesized amino acid and dipeptide conjugate NHC gold(I/III) complexes and tested them as antitumor agents. A series of cationic Au^{I}NHC complexes has been prepared as analogues of Auranofin,[45] and it was shown that mononuclear, cationic, linear Au^{I}NHC species induced dose-dependent mitochondrial swelling in isolated rat liver mitochondria, which increased with an increase in the lipophilicity of the alkyl residue at the carbene nitrogen atom.[46,47] The selectivity toward tumorigenic cell lines and apoptosis induction via the mitochondrial pathway was further confirmed by studies in breast cancer cells. By fine-tuning the ligand-exchange reactions at the Au^{I} center, it was demonstrated that lipophilic and cationic Au^{I}NHC complexes selectively induce apoptosis in cancer cells but not normal cells and allow selective targeting of mitochondrial selenoproteins, such as thioredoxin reductase (TrxR).[48,49] The inhibition of protein tyrosine phosphatase activity was determined for gold(I) complexes containing NHC ligands.[50] This is of special interest because protein tyrosine phosphatases contain a cysteine residue in the active site, which supposedly is the target structure of these agents and might also be of high relevance for other bioactive gold species. In addition, Raubenheimer and co-workers[51] reported a heterobimetallic Au^{I}NHC complex containing a conjugatively attached ferrocenyl moiety. This phosphine-free complex is selective for cancer cells and active against the human colon adenocarcinoma line, the Jurkat leukemia cell line, and the MCF-7 breast cancer cell line.

Concerning the silver derivatives, most of the biomedical studies on Ag^{I}NHC complexes have been conducted on their antimicrobial properties, mainly by Youngs' research groups.[52−55] In particular, Youngs and colleagues have shown that the stability of Ag^{I}NHC complexes was greatly enhanced by the addition of electron-withdrawing groups on the 4 and 5 positions of the imidazole ring.[56] Regarding the anticancer activity of silver complexes, Youngs and co-workers[57] recently reported that Ag^{I}NHC complexes, based on a 4,5-dichloroimidazolydene core, are active against the ovarian and breast human cancer cell lines, but no significant activity was observed on HeLa cells (cervical cancer). In accordance with these results, Ghosh and co-workers reported poor antiproliferative effects of the Ag^{I}NHC complex of 1-benzyl-3-*tert*-butylimidazol-2-ylidene on HeLa cells.[58] The anticancer properties of Ag^{I}NHC complexes based on fully aromatic substituted carbenes of high lipophilicity was determined by Gautier and co-workers.[59] They also studied the influence of the ligand structure on the cytotoxicity: interestingly, all of the complexes exhibited higher cytotoxicities than cisplatin, and the cytotoxicity could be increased by about a factor of 10 by using bulkier ligands.[59]

Besides the above-mentioned gold and silver derivatives, NHC complexes with copper have also been recently reported to exhibit antiproliferative properties. In particular, the CuNHC complex [CuCl(SIMes)] [SIMes = 1,3-bis(2,4,6-trimethylphenyl)imidazolin-2-ylidene] was found to be more cytotoxic than cisplatin, induced apoptosis, and, unlike cisplatin, arrested the cell cycle progression in the G1 phase.[36,59,60]

In this line of research, we are interested in exploring the possible use of NHC ligands as alternatives to phosphines in the synthesis of new biologically active group 11 metal complexes.[61−64] To the best of our knowledge, hydrophilic and water-soluble Au^{I}NHC complexes have not been accomplished so far. The first example of using remarkably water-soluble imidazolium inner salts with covalently bonded sulfonate or carboxylate anions as ligand precursors for the synthesis of gold(I) and palladium(II) complexes was described by Shaughnessy and co-workers.[65] We synthesized sulfonate- or carboxylate-functionalized N-heterocyclic bis-carbene ligands and related water-soluble silver complexes.[63] Recently, Joo and co-workers reported a series of water-soluble gold(I) complexes using sulfonated N-heterocyclic imidazolylidene carbenes as

dx.doi.org/10.1021/ic3013188 | *Inorg. Chem.* 2012, 51, 9873−9882

ligands, prepared by transmetalation from the corresponding bis-carbene gold(I) complexes.[66]

In this work, we report the synthesis of N-heterocyclic precarbene ligands functionalized with hydrophilic groups (such as esters or amides) and their use in coinage metal chemistry. In particular, NHC ligand precursors $HIm^{A}Cl$ [1,3-bis(2-ethoxy-2-oxoethyl)-1*H*-imidazol-3-ium chloride, **1**] and $HIm^{B}Cl$ {1,3-bis[2-(diethylamino)-2-oxoethyl]-1*H*-imidazol-3-ium chloride, **2**} have been synthesized and used in the preparation of copper(I), silver(I), and gold(I) adducts. The cytotoxic properties of the newly synthesized NHC complexes were investigated in various human cancer cell lines, including cisplatin-sensitive and -resistant cells. Growth-inhibitory effects were compared with those induced in human nontransformed cells. Moreover, gold(I) and silver(I) carbene complexes were evaluated for their ability to in vitro inhibit the selenoenzyme TrxR, which is recognized as the most relevant molecular target for gold(I,III) species and was recently proposed as a potential protein target also for silver derivatives.[28]

RESULTS AND DISCUSSION

Synthesis. The NHC ligand precursor **1** has been synthesized by a solventless route using ethyl chloroacetate and 1-(trimethylsilyl)imidazole (Figure 1). Compound **1** is a pale-yellow solid, soluble in water, acetonitrile, chlorinated solvents, methanol, and dimethyl sulfoxide (DMSO) and insoluble in diethyl ether and *n*-hexane.

Deprotonation by the use of a silver base has been the most widely used method in the syntheses of NHC complexes of silver. This procedure can be accomplished using a variety of silver bases such as Ag_2O, AgOAc, and Ag_2CO_3. Silver oxide is the most commonly used of the metal bases; reactions can be easily monitored by the uptake of the insoluble silver oxide. A wide variety of solvents have been used with Ag_2O in the synthesis of AgNHCs, such as CH_2Cl_2, 1,2-dichloroethane, DMSO, acetone, methanol, acetonitrile, *N*,*N*-dimethylacetamide, and water.[67] The silver(I) complex $\{[Im^{A}]AgCl\}$ (**3**) was prepared in a degassed acetonitrile solution via deprotonation of the imidazolium species **1** with Ag_2O (Figure 1). The resulting adduct **3** is soluble in chlorinated solvents, but it is unstable in water. Although transition-metal complexes can be resistant to moisture and some reactions can even be performed in aqueous media, free carbenes, if generated in solution, are usually considered to be particularly prone to hydrolysis.[68]

Compound **3** was dissolved in water (pH = 7) and was stirred at room temperature for 24 h. A purple coloration of the solution was observed within 3 h. Spectroscopic analysis of the resulting precipitate points to C−Ag bond cleavage and the presence of several different species including the starting ligand $[HIm^{A}]^{+}$, the totally hydrolyzed compound $[HIm^{A} - 2Et]^{-}$, and the partially hydrolyzed species $[HIm^{A} - Et + H]^{+}$. This particular behavior was studied at different pH values: the same situation was observed at pH = 4, while only $[HIm^{A} - 2Et]^{-}$ was obtained at pH = 8. Facile formation of an imidazolium cation is in accordance with C−Ag bond cleavage, leading to the generation of extremely basic carbenes.

Ag^{I}NHC complexes have proven themselves to be very suitable at transferring to a variety of other metals: gold(I), copper(I), nickel(II), palladium(II), platinum(II), rhodium(I,III), iridium(I,III), and ruthenium(II,III,IV). In most cases, these transmetalation reactions can be carried out under aerobic conditions and in the presence of water.[63] The gold(I) complex $\{[Im^{A}]AuCl\}$ (**4**) was prepared by dissolving compound **3** in a degassed chloroform solution and then adding $(CH_3)_2SAuCl$ (Figure 1). Compound **4** has been obtained as a microcrystalline solid by recrystallization from $CHCl_3$/diethyl ether; it is soluble in chlorinated solvents and shows the same behavior in the water and methanol solution previously described for the silver(I) complex **3**. A single crystal of **4** suitable for X-ray diffraction analysis was obtained from a $CHCl_3$/*n*-hexane solution (Figure 2).

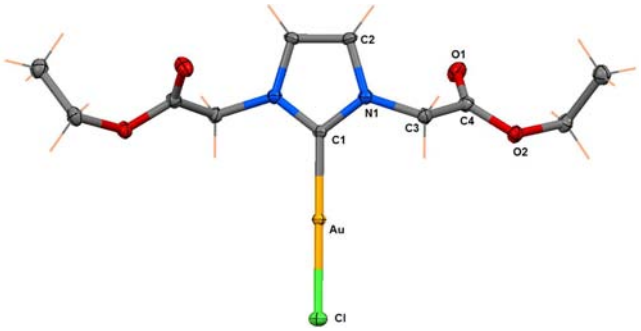

Figure 2. Molecular structure of **4** and selected bond lengths (Å) and angles (deg): Au−Cl 2.2886(11), Au−C1 1.992(4), N1−C1 1.347(3), N1−C2 1.392(3); C1−Au−Cl 180.0, N1−C1−Au 127.21(17), N1−C1−N1a 105.6(3), C1−N1−C3 123.3(2).

The copper(I) complex $\{[Im^{A}]CuCl\}$ (**5**) was prepared in a dry tetrahydrofuran (THF) solution via deprotonation of the imidazolium species **1** with bis(trimethylsilyl)amide and the addition of an acetonitrile solution of CuCl (Figure 1). The resulting adduct **5** has been obtained as a chloride complex; it is soluble in chlorinated solvents.

The NHC ligand precursor **2** has been synthesized by a solventless process using 2-chloro-*N*,*N*-diethylacetamide and 1-(trimethylsilyl)imidazole (Figure 3). Compound **2** is a white solid soluble in water, acetonitrile, methanol, DMSO, and chlorinated solvents and insoluble in diethyl ether and *n*-hexane. Spectroscopic data (IR and ^{1}H and ^{13}C NMR) suggest that in compound **2** the rotation is hindered around the N−CO bond, as is evident from two different signals attributable to the CH_2CH_3 groups in the ^{1}H and ^{13}C NMR spectra.[69,70] Crystals suitable for X-ray diffraction analysis were obtained from a CH_2Cl_2/THF solution (Figure 4).

The silver(I) complex $\{[Im^{B}]_2Ag\}Cl$ (**6**) was prepared in water by a reaction between the imidazolium species **2** with Ag_2O at about a 4:1 molar ratio (Figure 3). The resulting adduct **6** has been obtained as a chloride salt with a 2:1 ligand-to-metal ratio. The unusual coordination geometry is confirmed by spectroscopic and analytical results, and it is in accordance with the literature data.[67,71−75] Unfortunately, we have not been successful so far in obtaining suitable crystals of **6** for X-ray crystallography. We note that the reaction between **2** and Ag_2O at a 2:1 molar ratio also gave **6** as the product. The gold(I) complex $\{[Im^{B}]AuCl\}$ (**7**) was prepared by dissolving $(CH_3)_2SAuCl$ in a degassed dichloromethane solution and then adding compound **6** (Figure 3). Compound **7** has been obtained as a microcrystalline solid; it is soluble in water, chlorinated solvents, acetonitrile, and DMSO. The crystal structure of **7** is illustrated in Figure 5.

The ligand precursors **1** and **2** and the metal derivatives **3**−**7** have been characterized by several analytical methods. The Fourier tranform infrared (IR) spectra showed weak

dx.doi.org/10.1021/ic3013188 | *Inorg. Chem.* 2012, 51, 9873−9882

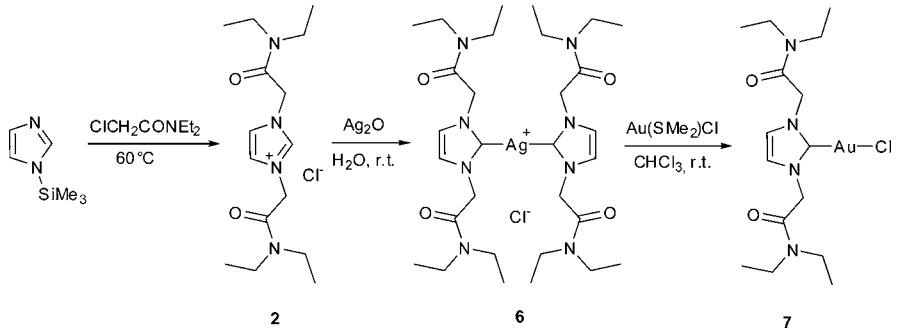

Figure 3. Reaction scheme of compounds **2**, **6**, and **7**.

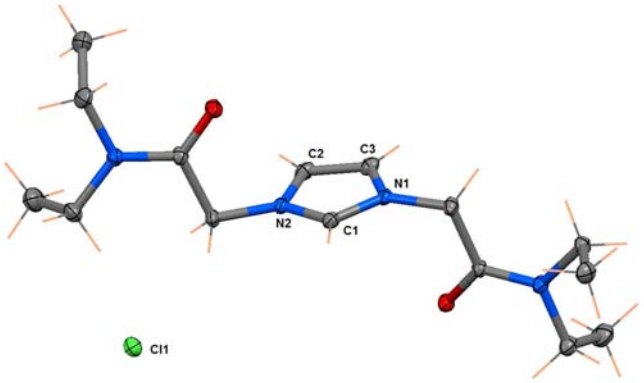

Figure 4. Molecular structure of **2** and selected bond lengths (Å) and angles (deg) (there are two $HIm^{B}Cl$ molecules in the asymmetric unit but only one is shown here): N1−C1 1.325(3), N1−C3 1.378(3), N2−C1 1.327(3), N2−C2 1.381(3), C2−C3 1.353(3); C1−N1−C3 109.21(19), C1−N2−C2 109.1(2), N1−C1−N2 108.2(2).

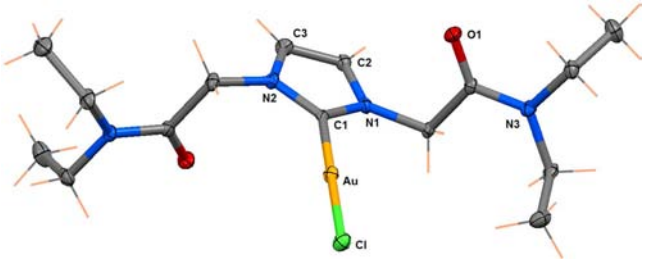

Figure 5. Molecular structure of **7** and selected bond lengths (Å) and angles (deg): Au−Cl 2.2871(11), Au−C1 1.989(4), N1−C1 1.349(6), N1−C2 1.383(6), N2−C1 1.351(5), N2−C3 1.381(6), C2−C3 1.362(6); N1−C1−N2 105.5(4); C1−Au−Cl 179.42(12), C1−N1−C2 110.5(4), C1−N2−C3 110.9(4).

absorptions in the range 2841−3170 cm^{-1} assignable to the azolyl ring C−H stretching bands. In the IR spectra of compounds **1** and **3**−**5**, the presence of the COOEt moiety is detected by intense, broad absorptions at 1730−1749 cm^{-1} due to the COO asymmetric stretching frequencies and by medium absorptions at 1528−1577 cm^{-1} due to the COO symmetric stretching bands. In the IR spectra of compounds **2**, **6**, and **7**, the presence of the amide moiety is detected by intense, broad absorptions at 1640−1643 cm^{-1} due to the $CONEt_2$ asymmetric stretching frequencies and by medium absorptions at 1569−1571 cm^{-1} due to the related symmetric stretching bands.

The ^{1}H and ^{13}C NMR data suggested significant changes between ligands **1** and **2** and the corresponding carbene complexes. In particular, the ^{1}H NMR spectra of **3**−**7** in a $CDCl_3$ solution showed the disappearance of the diagnostic 2-CH_{Im} imidazolium resonance visible respectively at 10.48 and 10.41 ppm in the starting materials **1** and **2**. A ^{1}H NMR study at high temperature has been performed for **6** in a DMSO solution to investigate the different behavior caused by increasing temperature. At room temperature, two different triplets attributable to the CH_2CH_3 groups and a complex multiplet due to the CH_2CH_3 groups are present in the ^{1}H NMR spectrum of **6**, pointing to restricted rotation about Et_2N−CO bonds. At 323 K, the multiplet due to the CH_2CH_3 groups is resolved into two quartets centered at 3.30 and 3.38 ppm. Moreover, at 363 K, a single broad signal attributable to the CH_2CH_3 groups at 1.14 ppm and a well-resolved quartet due to the CH_2CH_3 groups centered at 3.36 ppm are present, showing the possibility of free rotation around Et_2N−CO bonds at this temperature.[76]

Compound **6** is soluble in chlorinated solvents and DMSO, but it is unstable in water. For example, in the NMR spectrum of **6**, recorded 6 h after dissolution of the sample in the D_2O, we observed the appearance of an additional singlet at 7.08 ppm and additional triplets at 1.03 and 0.93 ppm due to a dissociation process of the complex in water. It is interesting to note in the same spectrum the disappearance of the singlet attributable to the NCH_2CONEt group, due to isotope exchange between hydrogen and deuterium.

The ^{13}C NMR resonance values of the $C_{carbene}$ signals in the silver(I) carbene complexes spectra fall over a wide range (213.7−163.2 ppm).[67] Silver(I) has two naturally occurring isotopes, ^{107}Ag and ^{109}Ag, with percent abundances of 51.839(7)% and 48.161(7)%, respectively. Both of these isotopes are NMR-active, and each has a nuclear spin of $^{1}/_{2}$. Given this information, one would expect a complex splitting pattern for the $C_{carbene}$ (doublet of doublets) based upon the coupling constants of each silver isotope. Carbene coupling to silver is observed in a few complexes, but most of them show no splitting pattern. In the ^{13}C NMR spectrum of complex **3** recorded in a $CDCl_3$ solution, no splitting is observed for the 2-C_{Im} resonance. It appears as a very sharp singlet at 183.84 ppm; analogously, no splitting is observed in the D_2O spectrum of complex **6**, where the 2-C_{Im} resonance is found to be a singlet at 167.9 ppm. Lin and co-workers speculated that the absence of such a splitting pattern is due to the fluxional behavior of AgNHC complexes on the NMR time scale.[77] It is possible that the $C_{carbene}$−Ag bonds in complexes **3** and **6** are labile and dissociate (fully or in dynamic equilibrium) in solution. In the ^{13}C NMR spectra of the gold(I) complexes **4** and **7** in a $CDCl_3$ solution, the 2-C_{Im} resonance appeared as singlets at 174.34 and 172.88 ppm, respectively; the chemical shift values for $C_{carbene}$

dx.doi.org/10.1021/ic3013188 | Inorg. Chem. 2012, 51, 9873−9882

Table 1. In Vitro Antitumor Activity[a]

compound	IC_{50} (μM) ± SD			
	A375	A549	HCT-15	MCF-7
{[Im^A]AgCl} (**3**)	24.65 ± 2.81	22.14 ± 1.73	20.32 ± 1.08	21.54 ± 1.97
{[Im^A]AuCl} (**4**)	44.64 ± 3.22	42.37 ± 2.97	41.33 ± 4.72	38.53 ± 3.08
{[Im^B]$_2$Ag}Cl (**6**)	24.46 ± 2.75	16.23 ± 2.31	14.11 ± 2.11	15.31 ± 3.44
{[Im^B]AuCl} (**7**)	46.33 ± 2.98	43.24 ± 3.52	49.25 ± 3.36	52.08 ± 2.25
[HIm^ACl] (**1**)	>100	>100	>100	84.35 ± 2.74
[HIm^BCl] (**2**)	>100	>100	>100	>100
cisplatin	3.11 ± 0.98	13.10 ± 1.23	15.25 ± 2.24	8.78 ± 1.32

[a]SD = standard deviation. Cells [$(3-8) \times 10^4$ mL^{-1}] were treated for 72 h with increasing concentrations of tested compounds. The cytotoxicity was assessed by the MTT test. IC_{50} values were calculated by a four-parameter logistic model ($P < 0.05$).

are in good agreement with those of similar monocarbene gold species.[58]

The usefulness of electrospray ionization mass spectrometry (ESI-MS) for characterization of the Ag-, Au-, and CuNHC complexes has been shown to be relatively poor. However, examination of the mass spectra has led to several interesting observations. This study was conducted by dissolving the ligands **1** and **2** in water and methanol, respectively, and the corresponding silver(I) and gold(I) complexes **3**–**7** in acetonitrile and recording the spectra in positive- and negative-ion mode. The fragmentation voltage was maintained at the minimum to reduce dissociation of the complex and ensure that the maximum amount of analyte reaches the detector without significant fragmentation.

The negative-ion ESI-MS spectra of ligands **1** and **2** showed the major peaks at m/z 312 and 365, respectively, corresponding to the $[HIm^A + 2Cl]^-$ and $[HIm^B + 2Cl]^-$ fragments. In the spectra recorded in positive-ion mode, the peaks attributable to the $[HIm^A]^+$ and $[HIm^B]^+$ species are present at m/z 241 and 295 at high abundance, with minor peaks attributable to the $[2HIm^A + Cl]^+$ and $[HIm^B + Cl]^+$ species.

The positive-ion ESI-MS spectra of compounds **3**–**5** showed peaks corresponding to the $[2Im^A + Ag]^+$, $[2Im^A + Au]^+$, and $[Im^ACu + CH_3CN]^+$ fragments at m/z 588, 677, and 344, respectively. The formation of the gold(I) carbene complex was also confirmed by analysis of the spectrum recorded in negative-ion mode, where the peak attributable to the $[Im^AAuCl + Cl]^-$ species is present at m/z 508 at high abundance. The positive-ion ESI-MS spectra of compounds **6** and **7** showed peaks corresponding to the $[2Im^B + Ag]^+$ and $[2Im^B + Au]^+$ fragments at m/z 695 and 785, respectively. The formation of the gold(I) carbene complex **7** was also confirmed by analysis of the spectrum recorded in negative-ion mode, where the peak attributable to the $[Im^BAuCl + Cl]^-$ species is present at m/z 561 at high abundance. These data are in accordance with literature studies[67] where Ag^INHC complexes with C–Ag–X and C–Ag–X_2 solid-state motifs form bis-carbenes (C_2–Ag) in the gas phase. An interesting behavior of complex **6** in water is also apparent from the ESI-MS study in D_2O. In the ESI-MS spectra recorded in H_2O, only peaks due to the free ligand are present at m/z 295 and 365. The positive-ion ESI-MS spectrum of compound **6**, recorded in D_2O 6 h after dissolution of the sample, showed peaks corresponding to the $[HIm^B - 5H + 5D]^+$ and $[2(HIm^B - 4H + 4D) + Ag]^+$ aggregates at m/z 300 and 705, respectively. This study confirms the isotope exchange between hydrogen and deuterium atoms, which was also observed in the ^{1}H NMR spectrum of **6** in D_2O recorded 6 h after dissolution of the sample.

Compounds **2**, **4**, and **7** were also characterized by X-ray crystallography, and the molecular structures are illustrated in Figures 4, 2, and 5, respectively. These molecular structures confirm the identity of the compounds, as suggested by spectroscopic results. The Au^INHC adducts **4** and **7** feature linear gold atoms, which is fairly common. The Au–C distances of 1.992(4) and 1.989(4) Å of **4** and **7** (which have hydrophilic esters or amides on the imidazole ring nitrogen atoms) are essentially identical with 1.998(5) Å observed for AuCl(IMes) [IMes = 1,3-bis(2,4,6-trimethylphenyl)imidazol-2-ylidene] or 1.989(2) Å of AuCl(IAd) [IAd = 1,3-bis(adamantyl)imidazol-2-ylidene].[78] The Au–Cl distances of 2.2886(11) and 2.2871(11) Å of **4** and **7** are also very similar to the corresponding distances in AuCl(IMes) of 2.2756 (12) Å and AuCl(IAd) of 2.2761(6) Å. The intermolecular Au···Au distances in **4** and **7** of 4.02 and 5.40 Å, respectively, are longer than the sum of the van der Waals radii of two gold atoms (3.32 Å).

Growth Inhibition Assays. Ag^INHC complexes **3** and **6** and Au^INHC complexes **4** and **7** were evaluated for their cytotoxic activity against human tumor cell lines, including examples of lung (A549), colon (HCT-15), and breast (MCF-7) cancers and melanoma (A375). Cytotoxicity was evaluated by means of the MTT test after 72 h of treatment with increasing concentrations of metal complexes. For comparison purposes, the cytotoxicity of cisplatin, the most widely used anticancer metallodrug, was evaluated in the same experimental conditions. IC_{50} values, calculated from dose–survival curves, are summarized in Table 1.

MTT assay data for complex **5** were not reproducible probably because of the high instability of copper derivatives in physiological media, thus avoiding IC_{50} calculation. Uncoordinated NHC ligand salts **1** and **2** were not effective in decreasing cancer cell viability over all cell lines (IC_{50} values > 100 μM), confirming that the metal center was necessary to obtain bioactive compounds. Both gold(I) complexes **4** and **7** showed comparable cytotoxic activity against all cancer cell lines, suggesting that the different types of functional groups on these imidazole rings did not have a considerable effect on the cytotoxicity. Silver(I) derivatives **3** and **6** were more effective, although their cytotoxicities were not superior to those of cisplatin [IC_{50} average values of 22.16 μM (20.32–24.65), 17.52 μM (14.11–24.46), and 10.06 μM (3.11–15.25)]. Noticeably, the cationic AgNHC complex **6** bearing two amide-functionalized imidazole rings proved to be the most promising derivative, affording activity similar to that of cisplatin in HCT-15 colon and A549 lung cancer cells. This in vitro antitumor activity gains importance in light of the

dx.doi.org/10.1021/ic3013188 | Inorg. Chem. 2012, 51, 9873–9882

tolerance of the silver complex **6** by the nonmalignant epithelial cells, where the improvement over the parent drug cisplatin was noteworthy. Actually, as shown in Table 2 summarizing IC_{50} values obtained by exposing human embryonic kidney HEK293 cells (human noncancerous cells in rapid proliferation) to NHC complexes, compound **6** elicited a cytotoxic activity roughly 3-fold lower than that recorded after cisplatin treatment, suggesting a preferential cytotoxicity of complex **6** versus neoplastic cells.

Table 2. Cytotoxicity in Nontumor Cells[a]

compound	IC_{50} (μM) ± SD for HEK293
{[Im^A]AgCl} (**3**)	65.96 ± 3.17
{[Im^A]AuCl} (**4**)	>100
{[Im^B]$_2$Ag}Cl (**6**)	77.36 ± 3.95
{[Im^B]AuCl} (**7**)	>100
[HIm^ACl] (**1**)	>100
[HIm^BCl] (**2**)	>100
cisplatin	19.56 ± 3.47

[a]SD = standard deviation. Cells (5×10^4 mL^{-1}) were treated for 72 h with increasing concentrations of tested compounds. The cytotoxicity was assessed by the MTT test. IC_{50} values were calculated by a four-parameter logistic model ($P < 0.05$).

To evaluate the NHC complex ability to induce delayed reproductive cellular death, classical clonogenic assays were performed in 2008 human ovarian cancer cells. Cell survival curves, obtained after treating 2008 cells with increasing concentrations of NHC derivatives for 6 h, are shown in Figure 6.

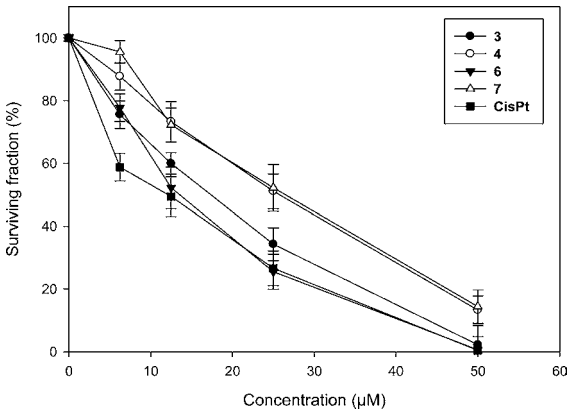

Figure 6. Clonal growth in 2008 cells. Cells were treated for 6 h with increasing concentrations of metal complexes or cisplatin, and the cell capacity to form clones was assayed.

For comparisons, cisplatin was evaluated in the same experimental conditions. The inhibitory effect on cell proliferation was concentration-dependent for all metal complexes. In particular, complex **6** was able to abrogate the clonogenic ability of cancer cells similarly to cisplatin. The antiproliferative activity of NHC complexes was also investigated on human ovarian cancer cells endowed with cisplatin resistance, C13* cells.[79] In Table 3, IC_{50} values calculated for C13* cells have been compared with those recorded in parental 2008 human ovarian cancer cells sensitive to cisplatin. Again, the cationic complex **6** was found to be the most active of the two series, with IC_{50} values about 2-fold lower than those achieved with cisplatin in C13*cells. For both silver(I) and gold(I) complexes, the resistance factors (defined as the ratio between IC_{50} values calculated for the resistant cells and for the sensitive ones) were more than 10 times lower than that of cisplatin, clearly confirming the ability of these species to overcome the acquired cisplatin resistance.

Table 3. Cross-Resistance Profiles[a]

	IC_{50} (μM) ± SD		
compound	2008	C13*	RF
{[Im^A]AgCl} (**3**)	26.65 ± 2.84	26.52 ± 1.85	1.0
{[Im^A]AuCl} (**4**)	44.14 ± 2.09	55.53 ± 4.37	1.3
{[Im^B]$_2$Ag}Cl (**6**)	14.22 ± 2.82	12.33 ± 3.62	0.9
{[Im^B]AuCl} (**7**)	51.17 ± 3.50	50.06 ± 3.11	1.0
[HIm^ACl] (**1**)	88.58 ± 3.24	98.31 ± 3.42	1.1
[HIm^BCl] (**2**)	99.12 ± 3.24	>100	
cisplatin	2.24 ± 1.32	22.54 ± 2.16	10.1

[a]SD = standard deviation. Cells (3×10^4 mL^{-1}) were treated for 72 h with increasing concentrations of tested compounds. The cytotoxicity was assessed by the MTT test. IC_{50} values were calculated by a four-parameter logistic model ($P < 0.05$). RF = IC_{50} resistant/IC_{50} sensitive.

In Vitro Inhibition of Purified TrxR. In order to achieve information concerning the putative biological target, NHC complexes were in vitro tested for their ability to inhibit TrxR. Although it is generally accepted that gold(I,III) complexes act as efficient inhibitors of TrxR,[2] there is little evidence that this reductase may represent a target for silver compounds.[28] However, a selective antimitochondrial activity has been described for several silver derivatives,[80] thus supporting the hypothesis of an interaction between silver compounds and redox enzymes involved in mitochondria stability. On this basis, the inhibitory effects of NHC compounds toward TrxR were measured according to standard procedures.[81] NHC complexes were tested at increasing concentrations, and the dose−effect curves are reported in Figure 7.

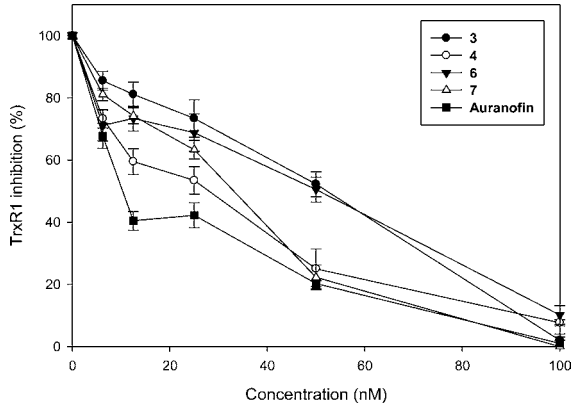

Figure 7. Inhibitory effect of metal compounds on TrxR. The TrxR activity was assayed by measuring NADPH-dependent reduction of DTNB at 412 nm.

TrxR appeared markedly inhibited by both the gold(I) and silver(I) derivatives at nanomolar concentrations. Despite different enzyme inhibition trends, both Ag^I- and Au^INHC complexes were able to decrease the TrxR activity by approximately 80% at a concentration of 90 nM. Overall, these results point out the selenoenzyme TrxR as a protein target for silver(I) in addition to gold(I) complexes.

dx.doi.org/10.1021/ic3013188 | Inorg. Chem. 2012, 51, 9873−9882

CONCLUSIONS

To explore the possible use of NHC ligands as alternatives to phosphines in the synthesis of biologically active group 11 metal complexes, NHC precursors **1** (1,3-bis(2-ethoxy-2-oxoethyl)-1*H*-imidazol-3-ium chloride) and **2** (1,3-bis[2-(diethylamino)-2-oxoethyl]-1*H*-imidazol-3-ium chloride) have been synthesized and used in the preparation of Cu^{I}-, Ag^{I}-, and Au^{I}NHC adducts. In the literature, there are few NHCs with amide and ester groups, and to the best of our knowledge, hydrophilic and water-soluble Au^{I}NHC complexes have not been accomplished so far. **2** and the gold(I) complexes **4** and **7** were also characterized by X-ray crystallography. The cytotoxic properties of the newly synthesized NHC complexes have been assessed in various human cancer cell lines, including cisplatin-sensitive and -resistant cells. Silver(I) derivatives **3** and **6** were found to be more effective than the gold adducts. In particular, the cationic AgNHC complex **6** bearing two amide-functionalized imidazole rings proved to be the most promising derivative, affording activity similar to that of cisplatin in HCT-15 colon and A549 lung cancer cells. Conversely, against human embryonic kidney noncancerous cells, compound **6** elicited a cytotoxic activity roughly 3-fold lower than that recorded after cisplatin treatment, suggesting a preferential cytotoxicity of complex **6** versus neoplastic cells. Growth-inhibitory effects evaluated in human cisplatin-resistant cells revealed that both gold(I) and silver(I) species were able to overcome the acquired cisplatin resistance. Studies on the inhibition of the selenoenzyme TrxR evidenced that Ag^{I}- and Au^{I}NHC complexes **3**, **4**, **6**, and **7** were able to decrease the TrxR activity by approximately 80% at a concentration of 90 nM. These results support our previous findings pointing out this disulfide reductase as a protein target for silver(I) in addition to gold(I) complexes.[22]

EXPERIMENTAL SECTION

General Procedures. All reagents were purchased from Aldrich and used without further purification. Some syntheses and handling were carried out under an atmosphere of dry oxygen-free dinitrogen, using standard Schlenk techniques. All solvents were dried, degassed, and distilled prior to use. Elemental analyses (C, H, N, and S) were performed in-house with a Fisons Instruments 1108 CHNS-O elemental analyzer. Melting points were taken on an SMP3 Stuart Scientific Instrument. IR spectra were recorded as neat from 4000 to 600 cm^{-1} with a Perkin-Elmer Spectrum One system. IR annotations used are as follows: br = broad, m = medium, s = strong, sbr = strong broad, sh = shoulder, w = weak, wbr = weak broad. ^{1}H and ^{13}C NMR spectra were recorded on a Oxford 400 Varian spectrometer (400.4 MHz for ^{1}H and 100.1 MHz for ^{13}C). Chemical shifts (δ) are quoted relative to internal $SiMe_4$. NMR annotations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. ESI-MS spectra were obtained in positive- or negative-ion mode on a Series 1100 MSD detector HP spectrometer, using an acetonitrile or a water mobile phase. The compounds were added to reagent-grade methanol to give solutions of approximate concentration 0.1 mM. These solutions were injected (1 μL) into the spectrometer via a HP 1090 Series II high-performance liquid chromatograph fitted with an autosampler. The pump delivered the solutions to the mass spectrometer source at a flow rate of 300 μL min^{-1}, and nitrogen was employed both as a drying and nebulizing gas. Capillary voltages were typically 4000 and 3500 V for positive- and negative-ion mode, respectively. Confirmation of all major species in this ESI-MS study was aided by a comparison of the observed and predicted isotope distribution patterns, with the latter calculated using the *IsoPro 3.0* computer program.

Synthetic Procedures. *$HIm^{A}Cl$ (1).* Ethyl chloroacetate (4.89 g, 0.040 mol) was added at room temperature to 1-(trimethylsilyl)-imidazole (2.80 g, 0.020 mol) in a two-necked batch, under oxygen-free dinitrogen. The mixture was gradually heated to 60 °C and stirred for 24 h to obtain a white solid. The solid was cooled to room temperature, washed with diethyl ether (3 × 20 mL), filtered off, and dried under reduced pressure to yield a white solid **1** in 98% yield. Mp: 133−134 °C. IR (cm^{-1}): 3110w, 3050w, 2984w, 2950w, 2907w, 2841w (CH); 1749s, 1732s (ν_{asym}COOEt); 1629wbr, 1577w, 1561m (ν_{sym}COOEt); 1484w, 1468w, 1439w, 1397m, 1376m, 1355w, 1302w, 1215s, 1197sh, 1170s, 1111w, 1090m, 1030w, 1011s, 985m, 938w, 890m, 872w, 861m, 810w, 778w, 749s, 669m. ^{1}H NMR (D_2O, 293 K): δ 1.08 (t, ^{3}J = 6.8 Hz, 6H, CH_2CH_3), 4.10 (q, ^{3}J = 6.8 Hz, 4H, CH_2CH_3), 5.01 (s, 4H, NCH_2COOEt), 7.39 (s, 2H, 4,5-CH_{Im}), 8.78 (s, 1H, 2-CH_{Im}). ^{1}H NMR ($CDCl_3$, 293 K): δ 1.30 (t, ^{3}J = 7.2 Hz, 6H, CH_2CH_3), 4.25 (q, ^{3}J = 7.2 Hz, 4H, CH_2CH_3), 5.41 (s, 4H, NCH_2COOEt), 7.63 (s, 2H, 4,5-CH_{Im}), 10.48 (s, 1H, 2-CH_{Im}). $^{13}C\{^{1}H\}$ NMR (D_2O, 293 K): δ 13.31 (CH_2CH_3), 50.33 (CH_2CH_3), 63.78 (NCH_2COOEt), 123.71 (4,5-CH_{Im}), 138.55 (2-CH_{Im}), 168.06 (*C*OOEt). ESI-MS (major negative ions, H_2O): m/z 312 (100%) $[HIm^{A} + 2Cl]^{-}$, 589 (55%) $[2HIm^{A} + 3Cl]^{-}$. ESI-MS (major positive ions, H_2O): m/z 241 (100%) $[HIm^{A}]^{+}$, 518 (55%) $[2HIm^{A} + Cl]^{+}$. Anal. Calcd for $C_{11}H_{17}ClN_2O_4$: C, 47.75; H, 6.19; N, 10.12. Found: C, 46.45; H, 6.08; N, 10.31.

$HIm^{B}Cl$ (2). 2-Chloro-*N*,*N*-diethylacetamide (2.35 g, 0.016 mol) was added at room temperature to 1-(trimethylsilyl)imidazole (1.00 g, 0.007 mol) in a two-necked batch, under oxygen-free dinitrogen. The mixture was gradually heated up to 60 °C and stirred for 24 h to obtain a white solid. The solid was cooled to room temperature, washed with diethyl ether (3 × 20 mL), filtered off, and recrystallized from a $CHCl_3/Et_2O$ solution in 75% yield. Crystals of **2** suitable for X-ray diffraction analysis were obtained from a CH_2Cl_2/THF solution (1:5) of compound **2**. Mp: 97−99 °C. IR (cm^{-1}): 3531w, 3388w, 3107w, 3066w, 2979w, 2936w (CH); 1643s (ν_{asym}CO); 1569m (ν_{sym}CO); 1470w, 1451w, 1417w, 1384w, 1361m, 1341m, 1302w, 1266sh, 1218sh, 1179m, 1143s, 1102m, 1083m, 1072m, 1042w, 1018w, 949m, 907m, 876w, 814m, 788s, 744w. ^{1}H NMR (D_2O, 293 K): δ 0.95 (t, ^{3}J = 6.8 Hz, 6H, CH_2CH_3), 1.09 (t, ^{3}J = 6.8 Hz, 6H, CH_2CH_3), 3.23−3.28 (m, 8H, CH_2CH_3), 5.18 (s, 4H, NCH_2CONEt_2), 7.32 (s, 2H, 4,5-CH_{Im}), 8.64 (s, 1H, 2-CH_{Im}). ^{1}H NMR ($CDCl_3$, 293 K): δ 1.13 (t, ^{3}J = 7.2 Hz, 6H, CH_2CH_3), 1.29 (t, ^{3}J = 7.2 Hz, 6H, CH_2CH_3), 3.36−3.45 (m, 8H, CH_2CH_3), 5.40 (s, 4H, NCH_2CONEt_2), 7.45 (s, 2H, 4,5-CH_{Im}), 10.41 (s, 1H, 2-CH_{Im}). ^{1}H NMR (DMSO, 293 K): δ 1.02 (t, ^{3}J = 6.8 Hz, 6H, CH_2CH_3), 1.19 (t, ^{3}J = 6.8 Hz, 6H, CH_2CH_3), 3.24−3.37 (m, 8H, CH_2CH_3), 5.38 (s, 4H, NCH_2CONEt_2), 7.66 (s, 2H, 4,5-CH_{Im}), 9.06 (s, 1H, 2-CH_{Im}). $^{13}C\{^{1}H\}$ NMR (D_2O, 293 K): δ 11.97, 12.90 (CH_2CH_3), 41.63, 42.07 (CH_2CH_3), 50.44 (NCH_2CONEt_2), 123.73 (4,5-CH_{Im}), 138.72 (2-CH_{Im}), 165.59 ($CONEt_2$). ESI-MS (major negative ions, CH_3OH): m/z 365 (100%) $[HIm^{B} + 2Cl]^{-}$. ESI-MS (major positive ions, CH_3OH): m/z 295 (100%) $[HIm^{B}]^{+}$, 625 (20%) $[2HIm^{B} + Cl]^{+}$. Anal. Calcd for $C_{15}H_{27}ClN_4O_2$: C, 54.45; H, 8.23; N, 16.93. Found: C, 53.86; H, 8.20; N, 17.01.

{[Im^{A}]AgCl} (3). **1** (2.00 g, 7.24 mmol) was dissolved in hot degassed acetonitrile (40 mL); the solution was cooled to room temperature, Ag_2O (0.84 g, 3.62 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The precipitate was filtered off and washed with hot chloroform; the filtrate was dried under reduced pressure to obtain gray-brown solid **3** in 85% yield. Mp: 134−136 °C. IR (cm^{-1}): 3170w, 3136w, 2980w, 2945w, 2926w (νCH); 1739s, 1730sh (ν_{asym}COOEt); 1623wbr, 1571br (ν_{sym}COOEt); 1469w, 1446m, 1396m, 1376m, 1357w, 1296w, 1198s, 1171sh, 1098m, 1044m, 1013s, 966w, 934w, 870m, 790m, 734s, 707s, 673sh. ^{1}H NMR ($CDCl_3$, 293 K): δ 1.32 (t, 6H, CH_2CH_3), 4.26 (q, 4H, CH_2CH_3), 4.93 (s, 4H, NCH_2COOEt), 7.13 (s, 2H, 4,5-CH_{Im}). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$, 293 K): δ 14.28 (CH_2CH_3), 52.89 (CH_2CH_3), 62.73 (NCH_2COOEt), 122.85 (4,5-CH_{Im}), 167.30 (COOEt), 183.84 (2-CH_{Im}). ESI-MS (major positive ions, CH_3CN): m/z 241 (75%) $[HIm^{A}]^{+}$, 588 (100%) $[2Im^{A} + Ag]^{+}$. Anal. Calcd for $C_{11}H_{16}AgClN_2O_4$: C, 34.44; H, 4.20; N, 7.30. Found: C, 34.62; H, 4.19; N, 7.20.

dx.doi.org/10.1021/ic3013188 | *Inorg. Chem.* 2012, 51, 9873−9882

*{[Im^A]AuCl} (**4**).* $(CH_3)_2SAuCl$ (0.50 g, 1.70 mmol) was added to a degassed chloroform solution (40 mL) of **3** (0.65 g, 1.70 mmol).The reaction mixture was stirred at room temperature for 4 h. The suspension was filtered through Celite, and the solution was concentrated under vacuum to afford an oil that was purified by recrystallization from $CHCl_3$/diethyl ether and dried at reduced pressure to give compound **4** in 62% yield. The crude complex **4** was recrystallized from $CHCl_3$/*n*-hexane to obtain crystals suitable for X-ray diffraction analysis. Mp: 124−126 °C. IR (cm^{-1}): 3170w, 3129w, 2983w, 2938w (CH); 1737sbr (ν_{asym}COOEt); 1626wbr, 1573w (ν_{sym}COOEt); 1528w, 1457m, 1417w, 1396w, 1374m, 1348w, 1202s, 1180sh, 1113w, 1096m, 1052w, 1017s, 964w, 939w, 871m, 788w, 742w, 714w, 677m. ^{1}H NMR ($CDCl_3$, 293 K): δ 1.32 (t, 6H, CH_2CH_3), 4.26 (q, 4H, CH_2CH_3), 5.00 (s, 4H, NCH_2COOEt), 7.11 (s, 2H, 4,5-CH_{Im}). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 293 K): δ 14.28 (CH_2CH_3), 52.13 (CH_2CH_3), 62.74 (NCH_2COOEt), 122.32 (4,5-CH_{Im}), 166.89 (COOEt), 174.34 (2-C_{Im}). ESI-MS (major negative ions, CH_3CN): *m*/*z* 508 (100%) [Im^{A}AuCl + Cl]$^-$. ESI-MS (major positive ions, CH_3CN): *m*/*z* 677 (100%) [2Im^A + Au]$^+$. Anal. Calcd for $C_{11}H_{16}AuClN_2O_4$: C, 27.95; H, 3.41; N, 5.93. Found: C, 28.01; H, 2.91; N, 5.72.

*{[Im^A]CuCl} (**5**).* **1** (0.55 g, 2.00 mmol) was added to a dry THF solution of lithium bis(trimethylsilyl)amide (0.37 g, 2.20 mmol) in a two-necked flask. The yellow reaction mixture was stirred at room temperature for 1 h under oxygen-free dinitrogen. Then an acetonitrile solution (30 mL) of CuCl (0.045 g, 2.00 mmol) was added to give a green reaction mixture. After 12 h, the solution was filtered off and concentrated under vacuum to afford a green oil. IR (cm^{-1}): 3139w, 2984w, 2941w (CH); 1740s (ν_{asym}COOEt); 1528m (ν_{sym}COOEt); 1465m, 1397m, 1377m, 1294m, 1202sbr, 1095m, 1019s, 960m, 870m, 791m, 758m, 655m. ^{1}H NMR ($CDCl_3$, 293 K): δ 1.29 (t, 6H, CH_2CH_3), 4.22 (q, 4H, CH_2CH_3), 4.41 (s, 4H, NCH_2COOEt), 6.31 (s, 2H, 4,5-CH_{Im}). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 293 K): δ 14.39 (CH_2CH_3), 44.80 (CH_2CH_3), 61.98 (NCH_2COOEt), 111.62 (4,5-CH_{Im}), 154.08 (2-C_{Im}), 168.91 (COOEt). ESI-MS (major positive ions, CH_3CN): *m*/*z* 344 (100%) [Im^{A}Cu + CH_3CN]$^+$. Anal. Calcd for $C_{11}H_{16}CuClN_2O_4$: C, 38.94; H, 4.75; N, 8.26. Found: C, 40.02; H, 4.95; N, 8.62.

*{[Im^B]$_2$Ag}Cl (**6**).* Ag_2O (0.13 g, 0.56 mmol) was added to a water solution (20 mL) of **2** (0.66 g, 2.00 mmol), and the reaction mixture was stirred at room temperature for 3 h. The precipitate, presumably the excess starting material, was filtered off, and the resulting pale-yellow solution was concentrated under vacuum to afford white solid **6** in 99% yield. Mp: 179−181 °C. IR (cm^{-1}): 3533br, 3395br, 3108w, 3067w, 2976w, 2935w (CH); 1643s (ν_{asym}CO); 1571w (ν_{sym}CO); 1512w, 1471m, 1417m, 1384m, 1361m, 1342m, 1266s, 1219m, 1180m, 1143s, 1101w, 1073m, 1040w, 1019w, 949w, 907w, 876w, 814m, 788s, 744s, 664m. ^{1}H NMR (D_2O, 293 K): δ 0.94 (t, 12H, CH_2CH_3), 1.08 (t, 12H, CH_2CH_3), 3.21−3.29 (m, 16H, CH_2CH_3), 5.18 (s, 8H, NCH_2CONEt), 7.31 (s, 4H, 4,5-CH_{Im}). ^{1}H NMR ($CDCl_3$, 293 K): δ 1.14 (t, 12H, CH_2CH_3), 1.28 (t, 12H, CH_2CH_3), 3.36−3.43 (m, 16H, CH_2CH_3), 4.97 (s, 8H, NCH_2CONEt), 7.15 (s, 4H, 4,5-CH_{Im}). ^{1}H NMR (DMSO, 293 K): δ 1.02 (t, 12H, CH_2CH_3), 1.17 (t, 12H, CH_2CH_3), 3.27−3.37 (m, 16H, CH_2CH_3), 5.08 (s, 8H, NCH_2CONEt), 7.30 (s, 4H, 4,5-CH_{Im}). ^{1}H NMR (DMSO, 323 K): δ 1.04 (t, 12H, CH_2CH_3), 1.19 (t, 12H, CH_2CH_3), 3.30 (q, 8H, CH_2CH_3), 3.38 (q, 8H, CH_2CH_3), 5.07 (s, 8H, NCH_2CONEt), 7.29 (s, 4H, 4,5-CH_{Im}). ^{1}H NMR (DMSO, 363 K): δ 1.14 (br, 24H, CH_2CH_3), 3.36 (q, 16H, CH_2CH_3), 5.05 (s, 8H, NCH_2CONEt), 7.28 (s, 4H, 4,5-CH_{Im}). $^{13}C\{^1H\}$ NMR (D_2O, 293 K): δ 11.96, 12.91 (CH_2CH_3), 41.59, 42.05 (CH_2CH_3), 50.0 (NCH_2CONEt_2), 123.63 (4,5-CH_{Im}), 165.54 ($CONEt_2$), 167.9 (2-CH_{Im}). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 293 K): δ 13.14, 14.78 (CH_2CH_3), 41.19, 42.01 (CH_2CH_3), 52.76 (NCH_2CONEt_2), 123.12 (4,5-CH_{Im}), 165.00 ($CONEt_2$). ESI-MS (major positive ions, CH_3CN): *m*/*z* 295 (100%) [HIm^B]$^+$, 695 (10%) [2Im^B + Ag]$^+$. ESI-MS (major negative ions, H_2O): *m*/*z* 365 (100%) [HIm^B + 2Cl]$^-$. ESI-MS (major positive ions, H_2O): *m*/*z* 295 (100%) [HIm^B]$^+$. ESI-MS (major positive ions, D_2O, 6 h after dissolution of the sample): *m*/*z* 300 (100%) [HIm^B − 5H + 5D]$^+$, 705 (50%) [2(HIm^B − 4H + 4D) + Ag]$^+$. Anal. Calcd for $C_{30}H_{52}AgClN_8O_4$: C, 49.22; H, 7.16, N, 15.31. Found: C, 50.06; H, 7.39; N, 15.75.

*{[Im^B]AuCl} (**7**).* $(CH_3)_2SAuCl$ (0.29 g, 1.00 mmol) was added to a degassed chloroform solution (30 mL) of **6** (0.37 g, 0.50 mmol). The reaction mixture was stirred at room temperature for 4 h. The suspension was filtered through Celite and washed with acetonitrile; the solution was concentrated under vacuum to afford pale-yellow solid **7** in 99% yield. The crude complex **7** was recrystallized from a CH_2Cl_2/*n*-hexane solution (1:5) to obtain single crystals suitable for X-ray diffraction analysis. Mp: 70−73 °C. IR (cm^{-1}): 3500br, 3110w, 2973w, 2933w (CH); 1640s (ν_{asym}CO); 1571w (ν_{sym}CO); 1468s, 1435sh, 1413sh, 1380m, 1362m, 1343m, 1306m, 1263s, 1218m, 1187w, 1142m, 1098m, 1074m, 1018w, 948m, 907w, 872w, 824m, 790m, 737m, 674m. ^{1}H NMR ($CDCl_3$, 293 K): δ 1.14 (t, 6H, CH_2CH_3), 1.30 (t, 6H, CH_2CH_3), 3.37−3.43 (m, 8H, CH_2CH_3), 5.01 (s, 4H, NCH_2CONEt_2), 7.13 (s, 2H, 4,5-CH_{Im}). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 293 K): δ 13.14, 14.80 (CH_2CH_3), 41.25, 42.17 (CH_2CH_3), 51.91 (NCH_2CONEt_2), 122.63 (4,5-CH_{Im}), 164.63 ($CONEt_2$), 172.88 (2-C_{Im}). ESI-MS (major negative ions, CH_3CN): *m*/*z* 561 (100%) [Im^{B}AuCl + Cl]$^-$, 855 (40%) [2Im^B + Au + 2Cl]$^-$. ESI-MS (major positive ions, CH_3CN): *m*/*z* 785 (100%) [2Im^B + Au]$^+$. Anal. Calcd for $C_{15}H_{26}AuClN_4O_2$: C, 34.20; H, 4.97; N, 10.63. Found: C, 35.02; H, 5.33; N, 11.09.

X-ray Diffraction Studies. Diffraction data were collected at *T* = 100(2) K. The data sets were collected on a Bruker SMART Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The cell parameters were obtained from a least-squares refinement of the spots (from 60 collected frames) using the *SMART* program. Intensity data were processed using the *Saint Plus* program. All of the calculations for the structure determination were carried out using the *SHELXTL* package (version 6.14).[82] The initial atomic positions were located by direct methods using XS, and the structures of the compounds were refined by the least-squares method using XL. Absorption corrections were applied using *SADABS*. Hydrogen positions were input and refined in a riding manner along with the attached carbon atoms. **2** is a chloride salt that crystallizes in space group $P\bar{1}$. The asymmetric unit contains two HIm^{B}Cl molecules and two water solvent molecules (for a *Z* value of 4). Some residual electron density was observed in close proximity to chloride counterions. However, refinement of these peaks as light atoms yielded no reasonable atom assignments. **4** crystallizes in space group *Pbcn*. The asymmetric unit contains half a molecule, with gold, chlorine, and carbene carbon (C1) all lying on a 2-fold rotation axis (for a *Z* value of 4). **7** crystallizes in space group $P\bar{1}$. The asymmetric unit contains one molecule and one acetonitrile solvent molecule (for a *Z* value of 2). A summary of the refinement details and the resulting factors for the complexes are given in the Supporting Information.

Experiments with Human Cells. *Chemicals.* Metal complexes and the corresponding uncoordinated ligands were dissolved in water just before the experiment. Cisplatin was dissolved in a 0.9% sodium chloride solution. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and cisplatin were obtained from Sigma Chemical Co., St. Louis, MO.

Cell Cultures. Human lung (A549), breast (MCF-7) and colon (HCT-15) carcinoma cell lines along with melanoma (A375) and nontransformed embryonic kidney (HEK293) cells were obtained by the American Type Culture Collection (ATCC; Rockville, MD). 2008 and its cisplatin-resistant variant, C13*, are human ovarian cancer cell lines that were kindly provided by Prof. G. Marverti (Department of Biomedical Science, Modena University, Modena, Italy). Cell lines were maintained in the logarithmic phase at 37 °C in a 5% carbon dioxide atmosphere using the following culture media containing 10% fetal calf serum (Euroclone, Milan, Italy), antibiotics (50 units mL^{-1} penicillin and 50 μg mL^{-1} streptomycin), and 2 mM L-glutamine: (i) RPMI-1640 medium (Euroclone) for MCF-7, HCT-15, 2008, and C13* cells; (ii) F-12 HAM'S (Sigma Chemical Co.) for A549 cells; (iii) DMEM (Sigma Chemical Co.) for A375 and HEK293 cells.

MTT Test. The growth-inhibitory effect toward tumor cell lines was evaluated by means of MTT assay.[83] Briefly, (3−8) × 10^3 cells $well^{-1}$, dependent upon the growth characteristics of the cell line, were seeded

dx.doi.org/10.1021/ic3013188 | *Inorg. Chem.* 2012, 51, 9873−9882

in 96-well microplates in a growth medium (100 μL) and then incubated at 37 °C in a 5% carbon dioxide atmosphere. After 24 h, the medium was removed and replaced with a fresh one containing the compound to be studied at the appropriate concentration. Triplicate cultures were established for each treatment. After 72 h, each well was treated with 10 μL of a 5 mg mL^{-1} MTT saline solution, and following 5 h of incubation, 100 μL of a sodium dodecylsulfate solution in HCl 0.01 M was added. After an overnight incubation, the inhibition of cell growth induced by the tested complexes was detected by measuring the absorbance of each well at 570 nm using a Bio-Rad 680 microplate reader. The mean absorbance for each drug dose was expressed as a percentage of the control untreated well absorbance and plotted versus the drug concentration. IC_{50} values represent the drug concentrations that reduce the mean absorbance at 570 nm to 50% of those in the untreated control wells.

Clonogenic Assay. 2 × 10^5 cells were seeded in Petri dishes (6 cm^2) and incubated overnight. Afterward, cells were treated with an increasing concentration of tested compounds and incubated for 6 h. Cells were washed with phosphate buffered saline and harvested, and aliquots of 500 cells were reseeded in a growth medium in triplicate for 10 days. The colonies were fixed and stained with a crystal violet solution in acetic acid (50%) and ethanol (90%). The colonies were counted, and colonies of fewer than 50 cells were discarded. The efficiency of clonal growth, that is, the ratio between the number of colonies formed and the number of cells seeded, was calculated.

In Vitro TrxR Inhibition. The assay was performed in a 0.2 M Na−K phosphate buffer at pH = 7.4, containing 5 mM EDTA, 0.250 mM NADPH, and 75 nmol of TrxR (IMCO, Sweden). Metal complexes were preincubated for 5 min at room temperature; the reaction started with 1 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), and the increase of the absorbance was monitored at 412 nm over 5 min at 25 °C. The enzyme activity was calculated taking into account that 1 mol of NADPH yields 2 mol of 3-carboxy-4 nitrothiophenolate (reduced DTNB).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary crystallographic data (CIF files) for this paper. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: carlo.santini@unicam.it (C.S.), dias@uta.edu (H.V.R.D.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Consorzio Interuniversitario di Ricerca in Chimica dei Metalli nei Sistemi Biologici. Sanofi-Aventis SpA is acknowledged for financial support. H.V.R.D. is grateful to the National Science Foundation (Grant CHE-0845321) and the Robert A. Welch Foundation (Grant Y-1289) for financial support.

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