Inorganic Chemistry

Article

pubs.acs.org/IC

Synthesis, Structural Characterization, Solution Behavior, and in Vitro Antiproliferative Properties of a Series of Gold Complexes with 2-(2′-Pyridyl)benzimidazole as Ligand: Comparisons of Gold(III) versus Gold(I) and Mononuclear versus Binuclear Derivatives

Maria Serratrice,[†] Maria A. Cinellu,*[,†] Laura Maiore,[†] Maria Pilo,[†] Antonio Zucca,[†] Chiara Gabbiani,[‡] Annalisa Guerri,[§] Ida Landini,[∥] Stefania Nobili,[∥] Enrico Mini,[∥] and Luigi Messori*[,§]

[†]Department of Chemistry and Pharmacy, University of Sassari, Via Vienna 2, 07100 Sassari, Italy.

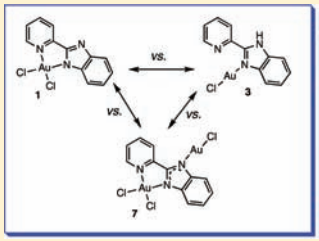
[‡]Department of Chemistry and Industrial Chemistry, University of Pisa, Via Risorgimento 35, 56126 Pisa, Italy

[§]Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino (FI), Italy

[∥]Department of Pharmacology, University of Florence, Viale Pieraccini 6, 50139 Firenze, Italy

Supporting Information

ABSTRACT: A variety of gold(III) and gold(I) derivatives of 2-(2′-pyridyl)benzimidazole (pbiH) were synthesized and fully characterized and their antiproliferative properties evaluated in a representative ovarian cancer cell line. The complexes include the mononuclear species [(pbi)AuX$_2$] (X = Cl, **1**; OAc, **2**), [(pbiH)AuCl] (**3**), [(pbiH)Au(PPh$_3$)][PF$_6$] (**4**-PF$_6$), and [(pbi)Au(L)] (L = PPh$_3$, **5**; TPA, **6**), and the binuclear gold(I)/gold(I) and gold(I)/gold(III) derivatives [(PPh$_3$)$_2$Au$_2$(μ_2-pbi)][PF$_6$] (**10**-PF$_6$), [ClAu(μ_3-pbi)AuCl$_2$] (**7**), and [(PPh$_3$)Au(μ_3-pbi)AuX$_2$][PF$_6$] (X = Cl, **8**-PF$_6$; OAc, **9**-PF$_6$). The molecular structures of **6**, **7**, and **10**-PF$_6$ were determined by X-ray diffraction analysis. The chemical behavior of these compounds in solution was analyzed both by cyclic voltammetry in DMF and absorption UV−vis spectroscopy in an aqueous buffer. Overall, the stability of these gold compounds was found to be acceptable for the cellular studies. For all complexes, relevant antiproliferative activities in vitro were documented against A2780 human ovarian carcinoma cells, either resistant or sensitive to cisplatin, with IC$_{50}$ values falling in the low micromolar or even in the nanomolar range. The investigated gold compounds were found to overcome resistance to cisplatin to a large degree. Results are interpreted and discussed in the frame of current knowledge on cytotoxic and antitumor gold compounds.



IC_{50} values (μM)

Compound	A2780/S	A2780/R
pbiH	45.30 ± 1.40	60.00 ± 3.40
[(pbi)AuCl$_2$] **1**	6.60 ± 4.01	5.31 ± 0.66
[(pbi)Au(OAc)$_2$] **2**	1.90 ± 0.20	4.40 ± 1.10
[(pbiH)AuCl] **3**	6.70 ± 1.40	8.30 ± 1.40
[(pbiH)Au(PPh$_3$)](PF$_6$) **4**-PF$_6$	1.50 ± 0.10	2.00 ± 0.10
[(pbi)Au(PPh$_3$)] **5**	0.60 ± 0.05	0.90 ± 0.02
[(pbi)Au(TPA)] **6**	13.30 ± 2.61	28.83 ± 1.04
[ClAu(μ-pbi)AuCl$_2$] **7**	2.90 ± 0.60	9.70 ± 1.60
[(PPh$_3$)Au(μ-pbi)AuCl$_2$] **8**-PF$_6$	0.60 ± 0.09	1.25 ± 0.06
[(PPh$_3$)Au(μ-pbi)Au(OAc)$_2$] **9**-PF$_6$	0.60 ± 0.05	1.50 ± 0.10
[(PPh$_3$)$_2$Au$_2$(μ-pbi)] **10**-PF$_6$	0.60 ± 0.01	3.57 ± 0.80
CDDP	2.10 ± 0.40	18.60 ± 3.70

INTRODUCTION

Gold compounds are drawing increasing attention within the bioinorganic and medicinal chemistry communities for their very encouraging antiproliferative and antitumor properties.[1] Indeed, a number of interesting compounds were recently described and characterized showing favorable pharmacological profiles among which we like to highlight gold(III) porphyrins,[2] gold(III) dithiocarbamates,[3] and a variety of gold(I) complexes with phosphine and/or *N*-heterocyclic carbene ligands.[4,5] A limited number of binuclear gold(III)[6−8] as well as gold(I)[9,10] complexes were also reported which display quite exciting biological properties.

It was established that the relevant antiproliferative actions of these metallodrugs depend on the presence of either a gold(III) or a gold(I) center although the respective cytotoxic mechanisms seem to be rather distinct.[11] On the whole, gold compounds constitute a very promising family of potential anticancer agents and deserve further and deeper investigations.

Remarkably, during the past decade, we have prepared and evaluated several gold(III) complexes, mainly of nitrogen donor ligands, and some organogold(III) derivatives, that revealed very attractive antiproliferative profiles in vitro.[12] Very recently, a series of gold(III) and gold(I) derivatives of the saccharinato ligand, both homoleptic and heteroleptic, were prepared and their reactions with model proteins and cytotoxic activity against cancer cell lines studied in depth, allowing a comparison to be made between derivatives of the same ligand with gold in its two different oxidation states.[13] Therefore, relying on the promising results obtained for both gold(III) and gold(I) complexes with nitrogen donor ligands, and considering it of interest to compare the different biological profiles displayed by gold in its two different oxidation states, yet linked to the same organic carrier, we looked for a polytopic nitrogen ligand which might give stable gold(III) and gold(I) complexes and, hopefully, afford binuclear species. Spurred by our previous experience with gold(III) derivatives of substituted 2,2′-bipyridines and of phenanthrolines,[7] this latter objective was specifically pursued.

Received: December 7, 2011
Published: February 17, 2012

   dx.doi.org/10.1021/ic202639t | *Inorg. Chem.* 2012, 51, 3161−3171

Indeed, we observed that the binuclear derivatives featured higher performances than the corresponding mononuclear species, both in terms of stability in solution and of biological activity. Here, our main goal was to join within the same chemical entity a gold(III) and a gold(I) center with the idea that it might result in innovative medicinal chemistry and hopefully in a synergism between the biological actions of the two different gold centers.

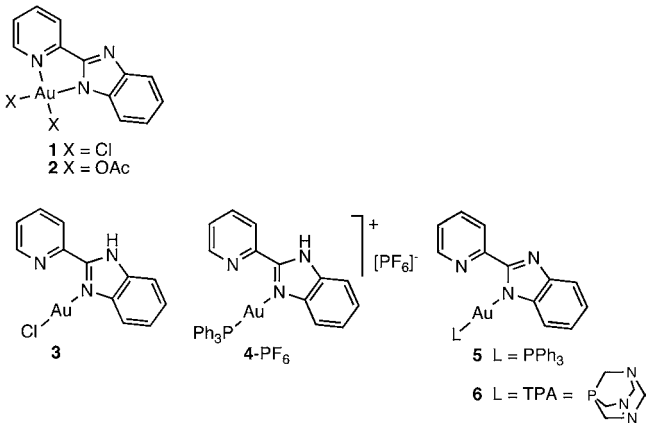
2-(2′-Pyridyl)benzimidazole, pbiH, a potentially tridentate ligand featuring two sp^2-hybridized and one sp^3-hybridized nitrogen atoms, seemed to us particularly appropriate for reaching the above goals. Moreover, this ligand displays valuable pharmacological activities in its own right, being, for example, an anti-inflammatory agent[14] and an inhibitor of *Escherichia coli* methionine aminopeptidase.[15] Metal complexes of 2-(2′-pyridyl)benzimidazole and related ligands are the subject of intensive research,[16] not only owing to their rich coordination chemistry but also due to a number of established and potential application areas, including medicinal chemistry.[16h,j,k] Gold(III) and gold(I) derivatives of this ligand were reported several years ago by Dash: they were tentatively formulated, respectively, as five- and three-coordinated complexes of the neutral ligand on the basis of analytical and spectroscopic (IR and Mössbauer) data.[17] Later on, the gold(I) derivative $[(PPh_3)Au(pbiH)][ClO_4]$ was structurally characterized showing a linear coordination at the gold atom.[18]

We report here the synthesis and structural and spectroscopic characterization of mononuclear gold(III) and gold(I) complexes and binuclear gold(I)/gold(I) or gold(III)/gold(I) complexes of 2-(2′-pyridyl)benzimidazole, pbiH, and of its deprotonated form pbi. The solution chemistry of the various complexes and their in vitro cytotoxic actions toward two established tumor cell lines are investigated in detail.

RESULTS AND DISCUSSION

Synthesis and Characterization of Mononuclear Complexes. The ligand 2-(2′-pyridyl)benzimidazole, pbiH, was synthesized by condensation between pyridine-2-carboxylic acid and 1,2-phenylenediamine in the presence of polyphosphoric acid at 180 °C, according to a literature method.[19] The gold(III) derivatives $[(pbi)AuX_2]$ (X = Cl, **1**; OAc, **2**) (see Chart 1) have been obtained by reaction of pbiH with

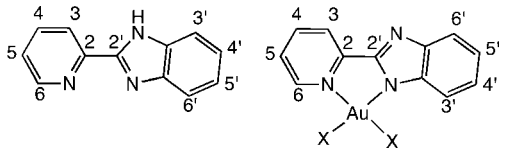
Chart 1. Gold(III) (1 and 2) and Gold(I) (3, 4-PF_6, 5, and 6) Mononuclear Derivatives



$Na[AuCl_4]\cdot 2H_2O$ in H_2O–MeCN at room temperature and with $Au(OAc)_3$ in AcOH at reflux, respectively. The two gold(III) complexes are quite stable both in solid state and in solution; complex **1** is very insoluble in most organic solvents likely due to intermolecular stacking. In both complexes the ligand is in its deprotonated form as shown by the IR and ^1H NMR spectra. This is surprising, in particular for complex **2**, having been obtained in AcOH. Notably, under various reaction conditions, reaction of pbiH with palladium(II) and platinum(II) chlorides gives $[(pbiH)MCl_2]$.[16g–k] Attempts to protonate complex **1** with protic acids HX (X = BF_4, PF_6) failed to give the cationic complex $[(pbiH)AuCl_2][X]$ (**1**-X); nevertheless, "protonated" forms of **1** and **2** were easily obtained by reaction of the complexes with $[(L)Au]^+$ (L = PPh_3, TPA = 1,3,5-triaza-7-phosphaadamantane), i.e., the isolobal analogues[20] of H^+ (vide infra). The IR spectrum of **1**, in the low frequency region, is characterized by the presence of two strong bands at 374 and 359 cm^{-1} due to Au–Cl stretching vibrations; the different stretching frequencies are indicative of the different *trans*-influence exhibited by the sp^2 and sp^3 N atoms.[21] In the IR of **2** the two acetato ligands give rise to very strong bands at 1685(sh), 1672 ($\nu_{asym}(CO_2)$), and 1255 cm^{-1} ($\nu_{sym}(CO_2)$); two medium intensity bands at 322 and 280 cm^{-1} are attributed to Au–O vibrational modes.[22]

A ^1H NMR spectrum of **1** could be obtained only in DMSO-d_6: it shows six resonances, with integral ratio of 2:1:1:2:1:1, most of which shifted downfield with respect to those of free pbiH in the same solvent (see Experimental Section and Table S1), the most affected by coordination being those of the H^6 and $H^{3'}$ protons (see Scheme 1 for the numbering scheme), with $\Delta\delta$ ($\delta_{coord} - \delta_{free}$) of 0.52 and 0.86 ppm, respectively, as a consequence of the neighborhood of the Cl^- ligands. At variance with **1**, complex **2** is very soluble in most organic solvents; its proton spectrum in $CDCl_3$ shows two signals at δ 2.29 and 2.36 ppm for the methyl protons of the two acetato ligands. In the aromatic region, the H^6 resonance is found at slightly higher fields ($\Delta\delta$ = −0.23 ppm) while that of $H^{3'}$ is shifted downfield with respect to the free ligand; in both complexes the chemical shift of $H^{4'}$ and $H^{5'}$ is almost unchanged. This is a common feature of most of the other complexes, although the $H^{4'}$, $H^{5'}$ multiplet structure changes its appearance dramatically as a consequence of the different environment experienced by the ortho protons $H^{3'}$ and $H^{6'}$, depending on coordination to gold of the adjacent nitrogen atoms.

Scheme 1. Numbering Scheme of pbiH and of Complexes 1 and 2

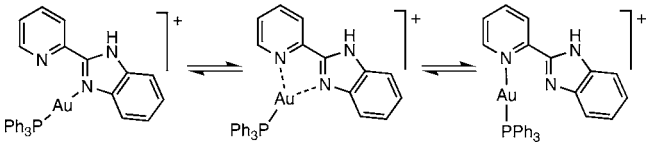


Reaction of pbiH with (THT)AuCl and $[(PPh_3)Au][PF_6]$ afforded, respectively, [(pbiH)AuCl] (**3**) and $[(pbiH)Au(PPh_3)][PF_6]$ (**4**-PF_6), both containing the neutral ligand. Deprotonation of the coordinated ligand of **4**-PF_6 with KOH in MeOH gave $[(pbi)Au(PPh_3)]$ (**5**), while [(pbi)Au(TPA)] (**7**) was obtained one-pot from reaction of pbiH with (TPA)AuCl in the presence of KOH. Any attempt to deprotonate the ligand of the chloride complex **3** failed to give the expected anionic derivative $[(pbi)AuCl]^-$: large decomposition was instead observed.

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161–3171

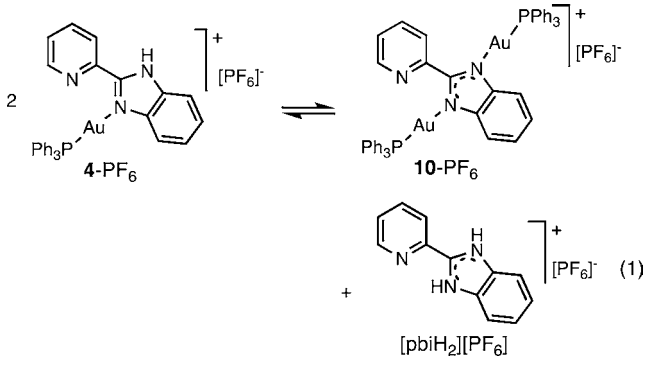
The IR and ^{1}H NMR spectra of **3** and **4**-PF_6 give evidence of N−H groups (see Experimental Section). In complex **3** the ν(Au−Cl) is observed at 347 cm^{-1}. In both cases the N−H resonance is strongly deshielded with respect to the free ligand, which suggests that the complexes are more acidic than the ligand. In the proton spectrum of **3** ($CDCl_3$) two sets of signals are found with a 3:1 integral ratio; the signals of the minor species, with the exception of the N−H resonance which is found at a lower frequency, are superimposable with those of pbiH in the same solvent. The signals of **3** are well resolved multiplets shifted downfield with respect to those of the uncoordinated ligand, with the H^3 proton being the most deshielded with a $\Delta\delta$ of 1.29 ppm. Treatment of **3**·$^1/_3$pbiH with diethyl ether failed to give the free complex, which suggests that pbiH is either clathrated and/or hydrogen-bonded to [(pbiH)AuCl] units.[23] At variance with **3**, only one set of signals is found for complex **4**-PF_6: the spectrum is characterized by broad poorly resolved signals. The main feature of the spectrum of **4**-PF_6, as that of the other PPh_3 derivatives here described, is the upfield shift of H^6 ($\Delta\delta$ = −0.34 ppm), while the resonance of the $H^{6'}$ proton could not be attributed with certainty. The $^{31}P\{^1H\}$ NMR spectrum of **4**-PF_6 exhibits two signals: a multiplet centered at −143.9 ppm is assigned to the PF_6^- ion, and a singlet resonance at 31.4 is consistent for linear gold(I) PPh_3 derivatives having a phosphorus *trans* to a nitrogen atom. The broad signals in the ^{1}H NMR spectrum suggest a fluxionality process involving positional exchange of the $(PPh_3)Au^+$ group between the two iminic nitrogen coordination sites. The site-exchange mode is represented in Scheme 2.

Scheme 2



A similar behavior was previously observed for **4**-ClO_4 as well as for other $(PPh_3)Au^+$ derivatives of bidentate nitrogen ligands.[18] Ligand redistribution to give the homoleptic cations $[(PPh_3)_2Au]^+$ and $[(pbiH)_2Au]^+$ was ruled out by the absence in the ^{31}P NMR spectrum of any signal ascribable to the $[(PPh_3)_2Au]^+$ species, typically at ca. 45 ppm.[24]

Attempts to grow crystals of **4**-PF_6, by slow diffusion of diethyl ether into a concentrated chloroform solution of the complex, afforded crystals of the dinuclear complex $[(PPh_3)_2Au_2(\mu\text{-pbi})][PF_6]$ (**10**-PF_6) (vide infra) and a white powder identified as $[pbiH_2][PF_6]$. These products very likely originate from an equilibrium reaction according to eq 1:



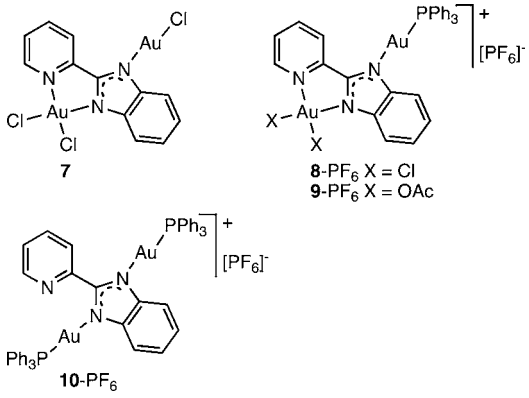
(1)

Note that **4**-PF_6, **10**-PF_6, and $[pbiH_2][PF_6]$ are connected with each other owing to the isolobal analogy between H^+ and $(PPh_3)Au^+$.[20]

The ^{1}H NMR spectrum of **5** in $CDCl_3$ shows one well resolved set of signals; as in the case of **4**-PF_6, the H^3 proton at δ 8.57 is the most deshielded and downfield shifted of 0.12 ppm with respect to the free ligand, while the resonance of the H^6 proton is shifted upfield of 0.44 ppm which suggests some involvement of the pyridinic nitrogen in the PPh_3Au-pbi bonding. The $H^{4'}$, $H^{5'}$, $H^{3'}$, $H^{6'}$ protons give rise to a well separated AA′BB′ spin system with $\Delta\delta$ ($\delta_{BB'} - \delta_{AA'}$) of 0.65 ppm. An analogous pattern for these protons was found also in the proton spectrum (in acetone-d_6) of complex **6** ($\Delta\delta$ = 0.56 ppm). In this case the H^6 proton is the most deshielded, although slightly upfield shifted ($\Delta\delta$ = −0.06 ppm) with respect to the free ligand. The N−CH_2−N protons of the TPA ligand give rise to an AB spin system centered at δ 4.67 ppm (J_{AB} = 12.8 Hz) and the N−CH_2−P a singlet resonance at δ 4.58 ppm. In the $^{31}P\{^1H\}$ NMR spectra of **5** and **6** only one resonance is found for the coordinated P atom of the phosphane ligand, respectively, at δ 33.8 and −65.1 ppm. Single crystals of **6** were obtained by slow diffusion of diethyl ether into an acetone solution. The X-ray structure analysis of compound **6** allowed us to establish that the isolobal (TPA)Au unit replaces the H atom of the N−H group in the imidazole ring.

Synthesis and Characterization of Binuclear Complexes. Binuclear derivatives **7**, **8**-PF_6, **9**-PF_6, and **10**-PF_6 (Chart 2)

Chart 2. Gold(III)/Gold(I) (7, 8-PF_6, and 9-PF_6) and Gold(I)/Gold(I) (10-PF_6) Binuclear Derivatives



have been obtained by reaction of the metalloligands **1**, **2**, and **5** with (THT)AuCl, **7**, or $[(PPh_3)Au][PF_6]$, **8**-PF_6, **9**-PF_6, and **10**-PF_6; the latter complex was also obtained in a crystalline form from a $CHCl_3$−Et_2O solution of **4**-PF_6 as a result of a redistribution of the H^+ and $(PPh_3)Au^+$ cations according to eq 1. The IR spectrum of **7** is almost unchanged with respect to that of its parent compound **1** both in the high and low frequencies region. The ^{1}H NMR spectra of **7** and **1** in the same solvent (DMSO-d_6) show differences either in the chemical shift and in the appearance of the signals; e.g., the resonance of the H^6 proton, the most deshielded in both compounds, in **7** is less downfield shifted with respect to the free ligand, with $\Delta\delta$ of 0.18 ppm (0.52 ppm in **1**). The $H^{4'}$, $H^{5'}$, $H^{3'}$, $H^{6'}$ protons give rise to a well resolved AA′BB′ spin system, while in **1** the same protons display an ABCD pattern. In the IR spectra of

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171

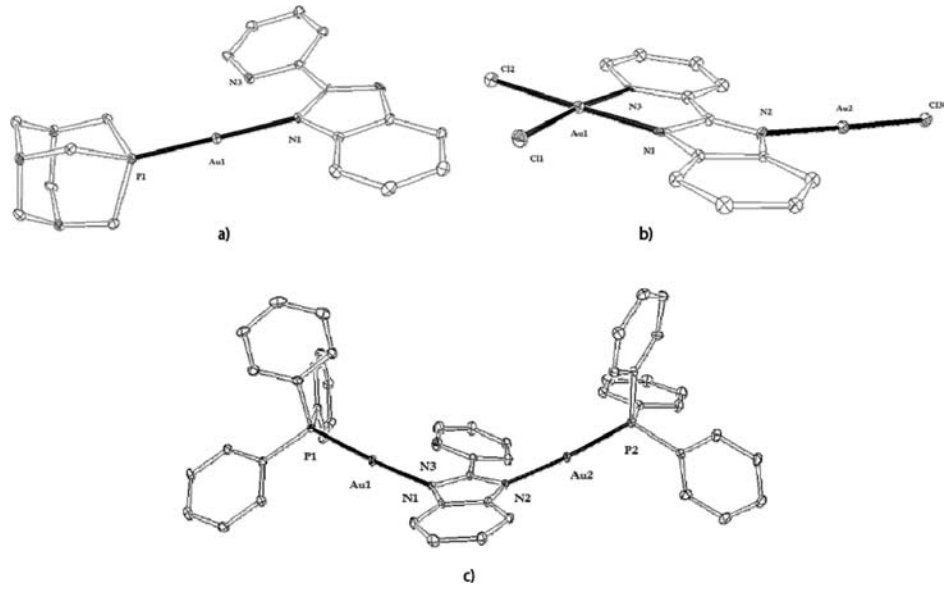


Figure 1. ORTEP drawings (5% probability ellipsoids) of complex **6** (a), **7** (b), and **10** (c).

compounds **8**-PF_6 and **9**-PF_6 new vibrational bands relative to the PPh_3 ligand and to the PF_6^- anion are found, in addition to those exhibited by their parent compounds **1** and **2**. Compound **8**-PF_6 is much more soluble than **1** and **6**, and ^1H NMR spectra have been recorded in various solvents; with the obvious exception of the signals of the PPh_3 protons, the spectrum in DMSO-d_6 is almost superimposable with that of **1**. At variance, the proton spectrum of **9**-PF_6 shows for the pbi protons different chemical shifts from that of the parent compound **2**; moreover, the $H^{4'}$, $H^{5'}$, $H^{3'}$, $H^{6'}$ protons give rise to an AA′BB′ spin system, while in **2** the same protons display an ABCD pattern. In the ^{31}P NMR spectrum of **8**-PF_6 and **9**-PF_6, one resonance is observed for the PPh_3 ligand, respectively, at δ 32.8 and 30.8 ppm; the resonance of the PF_6^- ion is observed at −143.5 and −144.3 ppm, respectively.

With the exception of a new band relative to the PF_6^- anion, no significant differences are found in the IR spectrum of **10**-PF_6 with respect to that of **5**. Comparison of the ^1H NMR spectrum of **10**-PF_6 (in $CDCl_3$) with those of the mononuclear derivatives **4**-PF_6 and **5** in the same solvent showed significant differences of chemical shifts of most of the pbi protons; e.g., the H^3 proton, the most deshielded in all complexes, in **10**-PF_6 is further downfield shifted ca. 0.2 ppm with respect to that of **4**-PF_6 and **5**, likely due to coordination of the neighboring nitrogen atom. This suggests that also in solution in **4**-PF_6 and **5** the pyridinic N atom is oriented toward the PPh_3Au group bound to one of the imidazolic N atom, as found in the solid state for **4**-ClO_4[18] and for the (TPA)Au group in complex **6** (vide infra). This is in line with what is observed for some gold(I) complexes with other N/N ligands.[25] The ^{31}P NMR of **10**-PF_6 shows only one singlet resonance at 32.6 ppm for the two PPh_3 ligands, which indicates equivalence of the two nitrogen atoms of the imidazolato anion; it is noteworthy that this value is exactly half way between 31.4 ppm of **4**-PF_6 (PPh_3 *trans* to the iminic N) and 33.8 ppm of **5** (PPh_3 *trans* to the amidic N).

Crystal Structures of Complexes 6, 7, and 10-PF_6. Complexes **6**, **7**, and **10**-PF_6 were characterized by X-ray diffraction; perspective views of the complexes are shown in Figure 1a−c, respectively and selected bond parameters collected in Table 1. All these complexes feature an imidazolate unit acting in all possible coordination modes, i.e., as a monodentate ligand in complex **6**, as a μ_2-bridging form in **10**-PF_6, and as a μ_3-bridging form in **7**. The latter form, also featured by complexes **8**-PF_6 and **9**-PF_6, is a rare phenomenon in the literature.[26] The asymmetric units **6** and **7** contain only the metal complex while for **10**-PF_6 one hexafluorophosphate anion is also present. The gold(I) ion is arranged in all complexes in the usual linear coordination geometry, and the gold(III) atom has the common square planar geometry. Bond lengths and angles (Table 1) are in agreement with those found in the CSD (v 5.32 + update[27]) for fragments similar to the complexes under study.

Table 1. Bond Lengths (Å) and Angles (deg) for Compounds 6, 7, and 10-PF_6

	6	**7**	**10**-PF_6
Au(1)—P(1)	2.205(4)		2.222(2)
Au(1)—N(1)	2.06(1)	1.97(3)	2.092(5)
Au(1)—N(3)	2.70(1)	2.05(1)	2.691(9)
Au(1)—Cl(1)		2.26(1)	
Au(1)—Cl(2)		2.24(1)	
Au(2)—Cl(3)		2.236(5)	
Au(2)—N(2)		2.086(4)	2.065(5)
Au(2)—P(2)			2.237(2)
P(1)—Au(1)—N(1)	177.6(3)		173.7(2)
P(1)—Au(1)—N(3)	107.8(2)		113.2(2)
N(1)—Au(1)—N(3)	69.9(4)	78.6(8)	68.8(2)
N(1)—Au(1)—Cl(1)		96.8(9)	
N(1)—Au(1)—Cl(2)		175.4(9)	
N(3)—Au(1)—Cl(1)		174.6(4)	
N(3)—Au(1)—Cl(2)		96.8(4)	
Cl(1)—Au(1)—Cl(2)		87.8(4)	
N(2)—Au(2)—Cl(3)		178.5(2)	
N(2)—Au(2)—P(2)			175.0(2)

In complexes **6** and **10**-PF_6 the relative rigidity of the 2-(2′-pyridyl)benzimidazolate ligand automatically brings atom N(3) to a distance from the gold atom, ca. 2.7 Å, which is well below

dx.doi.org/10.1021/ic202639t | *Inorg. Chem.* 2012, 51, 3161−3171

the sum of the van der Waals radii of Au and N (2.2 + 1.5 = 3.7 Å)[28] but much longer than the usual $Au^{III}-N_{py}$ single bonds [for instance, it is 2.05(1) Å in **7**]. A longer distance of 2.930 Å was found in **4**-ClO_4 between the same atoms.[18] Similar Au−N interactions have been previously observed in a number of cases.[25,29] They have generally been considered fairly weakly bonding; however, it has been pointed out that they take place only when sterically enforced by the presence of relatively rigid multidentate ligands. Moreover, the dihedral angles formed by the plane containing the pyridyl residue and that containing the benzimidazolate moiety are different in **6** and **10**-PF_6 compared to the following latter value: 12.7(5)°, 18.2(4)°, and 2.81°, respectively. These differences could be ascribed to the different steric hindrance present in the three complexes: in **6** the less hindered TPA ligand allows the tilting of the ring, and in **10**-PF_6 the μ_2-bridging arrangement constrains the conformation as it is. Moreover, in the crystal lattice of this complex, an intramolecular C−H···π interaction can be detected which contributed to the conformation of the molecule: the hydrogen atom H(12) of the pyridyl ring points to the centroid of one of the phenyl rings being this distance Ct···H(12) 3.09 Å and the angle Ct···H(12)−C(12) 157°. In addition, the lines defined by the atoms P(1)−Au(1)−N(1) and P(2)−Au(2)−N(2) draw an angle of 50.2(2)°. Concerning compound **7**, the planes including the pyridyl moiety and that containing the benzimidazolate residue form an angle of only 2.3(3)°. This different behavior could be attributed to the μ_3-bridging role played by the pbi ligand in this complex. Moreover, the mean planes defined by this moiety and the atoms coordinating the gold metal ion [Cl(1), Cl(2), N(1), and N(3)] are almost coplanar.

It can be pointed out that complex **6** piles up in the crystal lattice in a head-to-tail fashion. The distance between the mean planes containing the pbi residue is 3.6 Å; ca. C−H···π interactions can be found between the hydrogen atoms of the adamantane ring and the phenyl ring. Moreover, some hydrogen bond interactions are detected in the crystal lattice which contribute to the packing of the compound in the crystal.

In the crystal lattice, complex **7** also stacks in head to tail mode. Different M−Cl···H interactions were detected in the crystal lattice involving Cl(1) and Cl(3) and hydrogen atoms belonging to molecules of different asymmetric units. In addition the chlorine atom Cl(3) weakly interacts with Au(1) reported by $-x$; $-y + 1$; $-z + 2$ (3.282(4) Å).

Also, complex **10** stacks along the x direction in the crystal lattice: the distance between the mean planes containing the pbi moieties is 3.5 Å ca. Many C−H···π interactions can be detected, involving both the aromatic rings of the PPh_3 moieties and the benzimidazolate ring and the hydrogen atoms belonging to these same rings.

Solution Studies. Compounds **1**−**10**-PF_6 are poorly soluble in water but very soluble in DMSO and MeCN; the gold(I) derivatives and the dinuclear derivatives containing the Au(PPh_3) unit are also soluble in chlorinated solvents. The solution chemistry of the complexes was analyzed by absorption UV−vis spectrophotometry. Spectra have been recorded in some cases in various solvents (see Experimental Section and Table S2). The gold(III) derivatives **1** and **2** and the mixed-valence complexes **7**, **8**-PF_6, and **9**-PF_6 exhibit, in MeCN, intense transitions in the range 345−358 nm which are assigned as LMCT bands characteristic of the gold(III) chromophore.[30] Additional bands at ca. 290 nm are attributed to a metal-perturbed intraligand (IL) $\pi-\pi^*$ transition within the pbi ligand. The gold(I) derivatives **3**−**6** and the mixed-valence complexes **7** and **10**-PF_6 show in MeCN a common absorption band at 306−308 nm (at 313 nm in DMSO), assigned to $\pi-\pi^*$ transitions located in the heteroaromatic rings.[31] Complexes **4**-PF_6, **5**, **8**-PF_6, **9**-PF_6, and **10**-PF_6, all containing the ancillary ligand PPh_3, display additional bands at 268 and 275 nm; complex **6**, with TPA as ancillary ligand, and the mixed-valence derivative **7**, with all chlorides as ancillary ligands, show in addition a band at 321 nm. In DMSO the latter band is observed for almost all complexes at 325 nm.

For the stability tests as well as for the biological studies, concentrated DMSO solutions of each gold complex (1×10^{-2}) were diluted in the reference phosphate buffer (PB), at pH 7.4, to final concentrations of 10^{-5} to 10^{-4} M, and the samples were monitored over 24 h at 25 °C.

The resulting spectral profiles are shown in Figure S1 in the Supporting Information. In this medium the absorption maxima of the characteristic chromophores observed in CH_3CN are either blue- or red-shifted (see Table S2). In most cases the observed transitions remain substantially unmodified over 24 h observation, implying a substantial stability of the respective chromophore under the present solution conditions. Nevertheless, a slight progressive decrease in intensity, of the characteristic bands, is noticed with time, although without significant shape modifications. Later, these effects could be ascribed to the occurrence of some precipitation phenomena. Only in the case of complex **5** are significant spectral changes observed which suggest its conversion into $\mathbf{4}^+$.[32] All complexes exhibit high stability toward reduction: negligible amounts of colloidal gold, revealed by a broad absorption band around 550 nm, are observed in a few cases.

Finally, the stability of the various compounds toward biologically relevant reducing agents was evaluated. Sodium ascorbate (Asc) was selected as the reference reducing agent. Asc was added to freshly prepared solutions of the various compounds in a 10:1 molar ratio, and UV−vis spectra were recorded. The resulting spectral profiles are shown in Figure S2. We found that most of the complexes are fairly stable to reduction, with complexes **6** and **1** being, respectively, the most and the least stable compounds.

Electrochemical Properties. The electrochemical behavior of compounds **1**−**10**-PF_6 was investigated in DMF−TBAPF_6 0.1 M solvent system through cyclic voltammetry. The voltammetric curves of the chloro complexes **1**, **3**, and **7** are shown in Figure 2, and Table 2 summarizes the voltammetric data of all the study compounds. The ligand (pbiH) appears not to be electroactive in this solvent system.

The gold(I) derivatives (**3**−**6**) show one or no reduction process. In particular, the voltammetric response of **3** (Figure 2A) evidences a cathodic process attributable to the gold(I)→gold(0) reduction, as confirmed by the appearance of a gold film on the working electrode surface when the potential is scanned over the peak value (−1.09 V). The deposition of a gold film is not evident in the case of **4**-PF_6, maybe due to the very cathodic value of the reduction process (−1.93 V), near to the solvent system reduction. The voltammetric responses of **5** and **6** do not show cathodic or anodic processes, probably due to a stabilizing effect of PPh_3 and TPA ligand on the neutral complexes.

The gold(III) complexes (**1** and **2**) undergo two irreversible reduction processes, the first one at −0.41 and −0.70 V and the second one at −0.75 and −0.87 V, respectively. By comparison with the gold(I) derivatives, we ascribed the more cathodic process to the gold(I)→gold(0) reduction, and as a consequence

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171

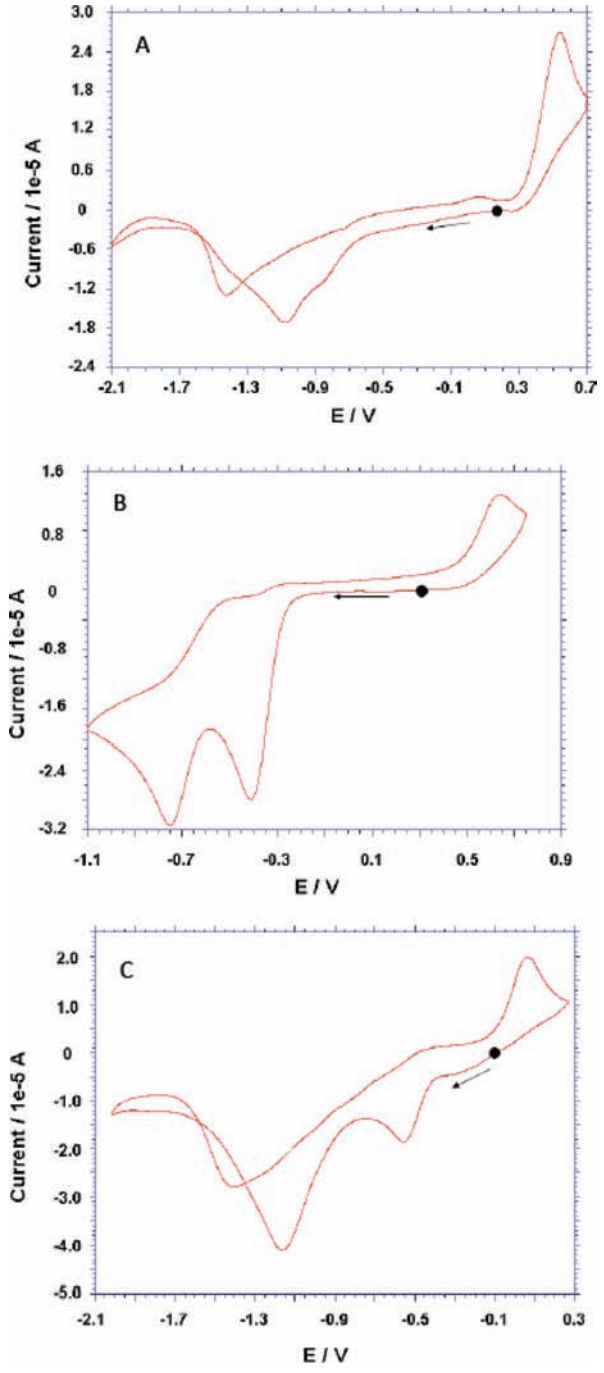


Figure 2. Cyclic voltammograms of **3** (A), **1** (B), and **7** (C) in DMF/$TBAPF_6$ 0.1 M at Pt; potential scan rate = 100 mV s^{-1}; ● indicates starting point.

the less cathodic one to the gold(III)→gold(I) process. This pattern is in line with that featured by analogous gold(III) complexes which in nonaqueous solvents undergo a two-step reduction, where the first is a gold(III)→gold(I) and the second a gold(I)→gold(0) process.[7b] As previously observed for analogous gold(III) complexes, substitution of the chloride ligands with the O-donor ligands acetato results in a more stable complex.[33]

Similarly to the gold(III) complexes, also in the case of the mixed-valence gold(III)−gold(I) species (**7**−**9**-PF_6), we ascribe the two cathodic processes to the gold(III)→gold(I) and gold(I)→gold(0) reduction, respectively. As observed for complex **3**, also in the case of **7** the reduction to gold(0) is confirmed by the presence of a thin gold layer on the electrode surface reaching potential values more cathodic than the second reduction process (Figure 2C). The dinuclear gold(I)−gold(I) derivative (**10**-PF_6) appears not electroactive under the experimental conditions.

Voltammetric results, on the whole, suggest that these compounds are quite stable toward gold(I)→gold(0) reduction. The electrochemical data are in agreement with the absorption spectra, suggesting that TPA and PPh_3 ligands stabilize the gold(I) derivatives (**4**-PF_6, **5**, **6**, **10**-PF_6).

Table 2. Voltammetric Data (E in V) in DMF/$TBAPF_6$ 0.1 M Solvent System

compd	$E_{pc,1}$	$E_{pc,2}$	E_{pa}
[(pbi)$AuCl_2$], **1**	−0.41[a]	−0.75	
[(pbi)$Au(OAc)_2$], **2**	−0.70	−0.87	0.5[b]
[(pbiH)AuCl], **3**		−1.09	0.51[c]
[(pbiH)$Au(PPh_3)$]PF_6, **4**-PF_6		−1.93[d]	
[(pbi)$Au(PPh_3)$], **5**			
[(pbi)Au(TPA)], **6**			
[ClAu(pbi)$AuCl_2$], **7**	−0.56	−1.17[e]	
[(PPh_3)Au(pbi)$AuCl_2$]PF_6, **8**-PF_6	−0.47	−0.83	
[(PPh_3)Au(pbi)Au$(OAc)_2$]PF_6, **9**-PF_6	−0.74	−1.15	
[(PPh_3)Au(pbi)Au(PPh_3)]PF_6, **10**-PF_6			

[a]Associated backward peak at +0.64 V. [b]Broad, with an associated backward peak at −0.35 V. [c]Associated backward peak at −0.18 V. [d]Associated backward peak at −0.58 V. [e]Associated backward peaks at +0.09 V and about 0.5 V (broad). When reversing again the scan after the backward peaks, another cathodic peak is evident at −0.15 V.

Antiproliferative Properties of Study Compounds. The antiproliferative effects of the study compounds have been determined according to established protocols, after 72 h of exposure in three independent experiments.

Most of the gold compounds were found to show remarkable antiproliferative effects when tested in vitro against the cisplatin-sensitive A2780 ovarian cancer cell line and its cisplatin-resistant counterpart. The resulting IC_{50} values are shown in Table 3 in comparison to those of cisplatin and of the free pbiH ligand.

Table 3. IC_{50} Values Determined after 72 h of Exposure to pbiH and Gold Complexes 1−10-PF_6 (μM)[a]

compd	A2780/S	A2780/R	RI
pbiH	45.30 ± 1.40[b]	60.00 ± 3.40[c]	1.3
[(pbi)$AuCl_2$], **1**	6.60 ± 4.01[d]	5.31 ± 0.66	0.8
[(pbi)$Au(OAc)_2$], **2**	1.90 ± 0.20	4.40 ± 1.10[e]	2.3
[(pbiH)AuCl], **3**	6.70 + 1.40	8.30 + 1.40	1.2
[(pbiH)$Au(PPh_3)$]PF_6, **4**-PF_6	1.50 ± 0.10	2.00 ± 0.10	1.3
[(pbiH)$Au(PPh_3)$], **5**	0.60 ± 0.05	0.90 ± 0.02	1.5
[(pbi)Au(TPA)], **6**	13.30 ± 2.61	28.83 ± 1.04	2.2
[ClAu(pbi)$AuCl_2$], **7**	2.90 ± 0.60	9.70 ± 1.60[f]	3.3
[(PPh_3)Au(pbi)$AuCl_2$]PF_6, **8**-PF_6	0.60 ± 0.09	1.25 ± 0.06	1.9
[(PPh_3)Au(pbi)Au$(OAc)_2$]PF_6, **9**-PF_6	0.60 ± 0.05	1.50 ± 0.10	2.6
[(PPh_3)Au(pbi)Au(PPh_3)]PF_6, **10**-PF_6	0.60 ± 0.01	3.57 ± 0.80	6.0
CDDP	2.10 ± 0.40	18.60 ± 3.70	9.1

[a]Mean ± ES of three independent experiments performed with triplicate cultures at each concentration tested; % of DMSO inhibition at IC_{50} concentrations listed in the following footnotes. [b]15%. [c]10%. [d]10%. [e]2%. [f]2%.

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171

The latter was found to be poorly cytotoxic with IC_{50} values of 45.30 and 60.00 μM against the two cell lines, respectively. Notably, 4 compounds out of 10, namely, **5**, **8**-PF_6, **9**-PF_6, and **10**-PF_6, show high cytotoxic activity against the cisplatin sensitive cell line, with IC_{50} values falling in the nanomolar range, and are also good in the cisplatin sensitive cell line (IC_{50} in the range 0.9−3.57 μM). Overall, all investigated compounds, with the exception of complex **6**, are more active than cisplatin in the A2780/R cell line with resistance index (RI) value ranging from 0.8 to 6.0. In particular, complex **1** was more active in the resistant cell line than in the sensitive one (RI = 0.8). These findings may suggest that these gold compounds might partially overcome some of the mechanisms of resistance to cisplatin. At variance, in the heteroleptic complex **6** the presence of the TPA ligand causes a substantial decrease of biological activity in comparison to the other compounds of this series.

Structure−function relationships, although still preliminary, may be proposed for this series of gold compounds characterized by the presence of the pbiH or pbi ligand. Notably, within the series of the mononuclear species, the chloro complexes [(pbi)$AuCl_2$], **1**, and [(pbiH)AuCl], **3**, despite the different oxidation state, display similar antiproliferative effects, with IC_{50} values of 6.60 and 6.70 μM in A2780/S cells, and 5.31 and 8.30 μM in A2780/R cells, respectively. This suggests that gold(III) in complex **1** may undergo reduction to gold(I) in the biological fluids and then act through the same molecular mechanism of gold(I) complex **3**. Notably, when changing from the dichloro complex **1** to the bis(acetato) complex **2**, an increase in the cytotoxic potency (from 6.60 to 1.90 μM in A2780/S cells) was observed. An even more substantial increase of the antiproliferative activity against both cell lines was found when changing from the gold(I) chloro complex **3** to the triphenylphosphine derivatives **4**-PF_6 and **5**. Notably, the neutral complex **5**, i.e., the deprotonated counterpart of **4**-PF_6, is more than 2-fold more active than its protonated analogue.

As far as binuclear complexes are concerned, they manifest in general quite remarkable antiproliferative properties. Compared to the parent mononuclear compounds **1** and **3**, only a modest improvement of the cytotoxic activity against the sensitive cell line was found for the mixed valence complex [ClAu(μ-pbi)$AuCl_2$], **7**. The mixed valence complexes **8**-PF_6 and **9**-PF_6 appear to be the most effective of the series, although differences with the gold(I)/gold(I) derivative **10**-PF_6 are not very large. In fact, all these complexes have in common a pbiAuPPh_3 moiety (corresponding to compound **5**) which displays per se a very high antiproliferative potential. Thus, the high antiproliferative properties typically observed for most of the binuclear complexes might be ascribed to the remarkable biological actions of the pbiAuPPh_3 moiety.

CONCLUSIONS

In conclusion, we have designed and obtained here a series of novel gold complexes, either mononuclear or binuclear, based on the pbiH ligand, and explored some aspects of their biological behavior. The pbiH ligand, mainly in its deprotonated form pbi, turned out to be a very appropriate and a very versatile one and allowed us to obtain the designed compounds, either mononuclear or binuclear. It is notable that pbi may serve as a bridge connecting two gold centers, even in different oxidation states; owing to this favorable property it might be at the basis of even more complex molecular architectures.

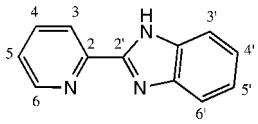
The biological properties of the studied compounds are promising both in terms of significant stability under physiological-like conditions and of antiproliferative potency effects. Remarkably, all tested compounds turned out to cause relevant growth inhibition of two representative ovarian cancer cell lines with IC_{50} values falling in the low micromolar and even nanomolar range. In addition, most compounds turned out to overcome resistance to cisplatin to a great extent. Though we reported that the pbiH ligand manifests as such some minor antiproliferative effects it is evident that the remarkable cytotoxicity observed for the tested compounds is to be ascribed primarily to the presence of the gold center. Comparative analysis of the biological behavior of the various compounds allowed us to draw some initial structure−activity relationships. Notably, similar cytotoxic properties were observed in parent complexes only differing in the oxidation state of the gold center, possibly implying that gold(III) to gold(I) reduction occurs in the cellular milieu. Also, we could establish that the pbiAuPPh_3 moiety displays the highest antiproliferative effects and that there is no particular advantage in terms of cytotoxicity in joining two gold centers within the same binuclear species. Further studies will be carried out in the near future to expand the knowledge of the biological effects of these compounds, and to identify the mechanism of interaction with proteins and the nature of their actual biological targets.

EXPERIMENTAL SECTION

General. All starting materials were used as received from commercial sources: Na[$AuCl_4$]·$2H_2O$ and [$Au(OAc)_3$] were purchased from METALOR and AlfaAesar, respectively; 2-(2-pyridyl)benzimidazole,[19] [(Ph_3P)AuCl],[34] and [(THT)AuCl][35] were prepared according to literature methods. Buffer solutions were freshly prepared before use. Elemental analysis was performed with a Perkin-Elmer elemental analyzer 240B by Mr. A. Canu (Dipartimento di Chimica, Università di Sassari). Conductivity measurements were performed with a Philips PW 9505 conductivity meter. Infrared spectra were recorded with a Jasco FTIR 480 Plus spectrophotometer using Nujol mulls. UV−vis spectra were recorded on a Varian Cary 50 or on a Hitachi U-2010 UV−vis spectrophotometer. ^{1}H and ^{31}P{^{1}H} NMR spectra were recorded at room temperature (20 °C) with a Varian VXR 300 spectrometer operating at 300.0 and 121.4 MHz, respectively. Chemical shifts are given in ppm relatively to internal TMS (^{1}H), and external H_3PO_4 (^{31}P). Cyclic voltammetric tests were performed using a CHI650 computerized instrument, in a single-compartment three-electrode cell, at room temperature, under Ar atmosphere, at a potential scan rate of 100 mV s^{-1}. A 3 mm diameter Pt disk electrode (CH Instruments) was used as working electrode, an aqueous Ag/AgCl (Amel) with suitable salt bridge was the reference electrode, and a graphite rod was the auxiliary electrode. All the experiments were carried out in DMF (Sigma-Aldrich, anhydrous, 99.8%) using 0.1 M tetrabutylammonium hexafluorophosphate ($TBAPF_6$, Sigma-Aldrich, for electrochemical analysis, ≥99.0%) as supporting electrolyte. The tests have been performed at room temperature, and the reported potential values are referred to bis-cyclopentadienyliron(III) iron(II) couple ($Fc^{+/0}$, $E_{1/2,r}$ = +0.50 V versus Ag/AgCl in DMF solvent).

Syntheses. *Spectroscopic Data of 2-(2-Pyridyl)benzimidazole (pbiH).* Selected IR bands (ν_{max}/cm^{-1}): 3060 ν(N−H), 1593, 1568, 1400, 1314, 1280, 744, 703. UV−vis (CH_3CN): λ_{max} (ε) 222sh, 240, 308 (74 405) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 7.30 (m, 2H, J = 9.3, 6.7, 5.4, 1.2, 0.8 Hz; $H^{4'}$, $H^{5'}$), 7.38 (ddd, 1H, J = 7.5, 4.8, 1.2 Hz; H^5), 7.49 (m, 1H, J = 9.3, 5.6, 1.9 Hz; $H^{3'}$), 7.86 (m, 1H, J = 9.1, 1.6, 1.3 Hz; $H^{6'}$), 7.88 (td, 1H, J = 8.4, 1.7 Hz; H^4), 8.44 (dt, 1H, J = 7.9, 1.1 Hz; H^3), 8.64 (dd, 1H, J = 4.8, 1.7 Hz; H^6), 10.77 (broad s, 1H; NH).

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171



*[Au(pbi)Cl$_2$] (**1**).* To a stirred solution of pbiH (195.2 mg, 1.0 mmol) in CH_3CN (2 mL) was added an aqueous solution (50 mL) of $NaAuCl_4{\cdot}2H_2O$ (397.9 mg, 1.0 mmol); the resulting suspension was stirred in the dark for 24 h at room temperature. The brown solid which formed was filtered off; washed with water, EtOH, and Et_2O; and dried under vacuum. Recrystallization from CH_3NO_3/Et_2O gave **1** as a brown solid. Yield 423.8 mg, 92%; mp 220 °C. Anal. Calcd for $C_{12}H_8AuCl_2N_3$: C 31.19; H 1.75, N 9.09%. Found: C 31.27; H 1.61; N 8.91%. Selected IR bands (ν_{max}/cm^{-1}): 1612, 1564, 1531, 740, 553, 432 ν(Au−N), 374 and 359 ν(Au−Cl). UV−vis (CH_3CN): λ_{max} (ε) 292 (7087), 358 (11 424) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (DMSO-d_6): δ 7.27 (m, 2H, J = 9.3, 6.3, 4.8, 2.0 Hz; H$^{4'}$, H$^{5'}$), 7.75 (m, 1H, J = 9.3, 6.2, 3.0 Hz; H$^{6'}$), 7.8 (td, 1H, J = 7.7, 1.8 Hz; H^5), 8.35 (d, 1H, J = 7.9 Hz; H^3), 8.38 (m, 1H, J = 9.3, 4.8, 3.0 Hz; H$^{3'}$), 8.46 (t, 1H, J = 7.8 Hz; H^4), 9.24 (d, 1H, J = 6.0 Hz; H^6).

*[Au(pbi)(OAc)$_2$] (**2**).* A solution of pbiH (97.6 mg, 0.5 mmol) and [Au(OAc)$_3$] (187.6 mg, 0.5 mmol) in acetic acid (30 mL) was refluxed for 3 h. After cooling, the yellow solution was filtered through Celite and the solvent removed under reduced pressure. The crude product was recrystallized from CH_2Cl_2/Et_2O to give **2** as a yellow solid. Yield 155.3 mg, 61%; mp 170 °C. Anal. Calcd for $C_{16}H_{14}AuN_3O_4$: C 37.73; H 2.77, N 8.25%. Found: C 37.52; H 2.69; N 8.05%. Selected IR bands (ν_{max}/cm^{-1}): 1685sh and 1672 ν_a(COO), 1614, 1562, 1525, 1255 ν_s(COO), 746, 553, 438 ν(Au−N), 322, and 280 ν(Au−O). UV−vis (CH_3CN): λ_{max} (ε): 290 (12 411), 350 (17 603) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H; Me), 2.36 (s, 3H; Me), 7.30 (m, 2H, J = 9.3, 4.8, 3.3 Hz; H$^{4'}$, H$^{5'}$), 7.57 (m + m, 2H, H$^{6'}$ + H^5), 7.76 (m, 1H, J = 9.3, 4.8, 3.3 Hz; H$^{3'}$), 8.24 (td, 1H, J = 7.8, 1.3 Hz; H^4), 8.36 (d, 1H, J = 8.1 Hz; H^3), 8.41 (d, 1H, J = 6.0 Hz; H^6). ^{1}H NMR (acetone-d_6): δ 2.14 (s, 3H; Me), 2.23 (s, 3H; Me), 7.28 (m, 2H, J = 8.0, 7.1, 1.3 Hz; H$^{4'}$, H$^{5'}$), 7.56 (m, 1H, J = 7.2, 1.8, 1.3 Hz; H$^{6'}$), 7.70 (m, 1H, J = 7.2, 1.3 Hz; H$^{3'}$), 7.86 (ddd, 1H, J = 7.7, 6.0, 1.6 Hz; H^5), 8.39 (dd, 1H, J = 7.8, 1.2 Hz; H^3), 8.54 (td, 1H, J = 7.8, 1.3 Hz; H^4), 8.67 (d, 1H, J = 6.0 Hz; H^6).

*[(pbiH)AuCl]·$^1/_3$pbiH (**3**·$^1/_3$pbiH).* A solution of pbiH (195.2 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added to a solution of (THT)AuCl (321.1 mg, 1 mmol) in the same solvent (10 mL); the resulting colorless solution was stirred in the dark for 24 h at room temperature. After removal of the solvent under reduced pressure, the residue was taken up with $CHCl_3$ and filtered through Celite and the solution concentrated to a small volume; addition of diethyl ether afforded a white precipitate which was fitered off, washed with diethyl ether, and dried under vacuum to give the analytical sample. The elemental analyses and ^{1}H NMR spectra indicated the presence of a clathrated pbiH molecule with a **3**/pbiH molar ratio of 3/1. Yield 229.8 mg, 70%; mp 220 °C. Anal. Calcd for $C_{16}H_{12}AuClN_4$: C 39.00; H 2.45, N 11.37%. Found: C 38.87; H 2.37; N 11.15%. Λ_M (acetone, 5 × 10^{-4} mol.L^{-1}): 8 Ω^{-1} cm^2 mol^{-1}. Selected IR bands (ν_{max}/cm^{-1}): 3174 (broad) ν(N−H), 1589, 736, 553, 497, 428 ν(Au−N), 399, 347 ν(Au−Cl). UV−vis (CH_3CN): λ_{max} (ε): 240, 295sh, 307 (24 604), 319sh nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 7.32 (m, 2H, J = 9.0, 6.3, 2.7 Hz; H$^{4'}$, H$^{5'}$ pbiH), 7.38 (dd, 1H, J = 7.5, 4.2; H^5 pbiH), 7.48 (m, 2H, J = 9.3, 6.3, 3.0 Hz; H$^{4'}$, H$^{5'}$ **3**), 7.56 (dd, 1H, J = 7.8, 4.8 Hz; H^5 **3**), 7.61 (m, 1H, J = 9.3, 6.3, 3.0 Hz; H$^{3'}$ **3**), 7.85 (m, 1H; H$^{6'}$ pbiH), 7.87 (t, 1H, J = 7.5 Hz; H^4 pbiH), 8.00 (td, 1H, J = 8.4, 1.8 Hz; H^4 **3**), 8.08 (m, 1H, J = 9.3, 6.3, 3.0 Hz; H$^{6'}$ **3**), 8.43 (d, 1H, J = 8.1 Hz; H^3 pbiH), 8.65 (d, 1H, J = 5.7 Hz; H^6 pbiH), 8.73 (d, 1H, J = 4.5 Hz; H^6 **3**), 9.72 (d, 1H, J = 7.8 Hz, H^3 **3**), 10.50 (broad s, 1H, NH pbiH), 11.12 (broad s, 1H, NH **3**).

*[(pbiH)Au(Ph$_3$P)][PF$_6$] (**4**-PF$_6$).* A solution of [(Ph$_3$P)AuCl] (247.4 mg, 0.5 mmol) and $AgPF_6$ (127.0 mg, 0.5 mmol) in dichloromethane (40 mL) was stirred in the dark until AgCl precipitation was completed. Then, the filtered solution was added to a solution of pbiH (97.6 mg, 0.5 mmol) in the same solvent (15 mL) and stirred in the dark for 3 h at room temperature. After this period, the solution was concentrated to a small volume; addition of diethyl ether afforded the precipitation of **4**-PF_6 as a white solid. Yield 221.5 mg, 55%; mp 160 °C. Anal. Calcd for $C_{30}H_{24}AuF_6N_3P_2$: C 45.07; H 3.03, N 5.26%. Found: C 45.26; H 2.97; N 5.12%. Λ_M (acetone, 5 × 10^{-4} mol.L^{-1}): 115 Ω^{-1} cm^2 mol^{-1}. Selected IR bands (ν_{max}/cm^{-1}): 3323 ν(N−H), 1587, 1439, 1105 (PPh_3), 841 (PF_6^-), 748, 694, 557, 548, 505. UV−vis (CH_3CN): λ_{max} (ε): 268 (9566), 275 (10 340), 308 (19 147) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 7.44 (m, 2H; J = 8.7, 6.0, 2.6 Hz; H$^{4'}$, H$^{5'}$), 7.50 (broad m, 1H; H^5), 7.58−7.70 (m, 15H; H PPh$_3$), 7.86 (broad m, 1H; H$^{6'}$), 7.94 (broad m, 1H; H$^{3'}$), 7.98 (broad t, 1H, J = 7.0 Hz; H^4), δ 8.27 (broad d, 1H, J = 5.0 Hz; H^6), 8.53 (broad d, 1H, J = 7.0 Hz; H^3), 13.11 (s, 1H, NH). ^{31}P NMR ($CDCl_3$): δ 31.4 (s, PPh_3), δ −143.9 (sept, J_{P-F} = 711.8 Hz; PF_6^-).

*[(pbi)Au(PPh$_3$)] (**5**).* A methanol solution of KOH (14.0 mg, 0.24 mmol) was added to a solution of **4**-PF_6 (191.0 mg, 0.24 mmol) in the same solvent (25 mL); the resulting mixture was stirred in the dark for 1 h at room temperature. Then the solvent was removed under vacuum, the residue taken up with CH_2Cl_2 and filtered through Celite, and the solution concentrated to a small volume; addition of diethyl ether afforded **5** as a white solid. Yield 100.3 mg, 64%; mp 110 °C. Anal. Calcd for $C_{30}H_{23}AuN_3P$: C 55.14; H 3.55, N 6.43%. Found: C 54.87; H 3.48; N 6.28%. Selected IR bands (ν_{max}/cm^{-1}): 1606, 1587, 1566, 1101 (PPh_3), 739, 694, 546, 509. UV−vis (CH_3CN): λ_{max} (ε): 236 (26941), 268 (17 682), 275 (18 372), 296 (19 640), 306 (19 993) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR ($CDCl_3$): δ 7.19 (m, BB′ part of an AA′BB′, 2H, J = 9.3, 6.0, 3.2 Hz, H$^{4'}$, H$^{5'}$), 7.21 (ddd, 1H, J = 7.7, 4.8, 1.3 Hz, H^5), 7.49−7.59 (m + m, 9H; H^m, H^p PPh_3), δ 7.71 (m, 6H; H^o PPh_3), 7.77 (td, 1H, J = 8.0, 1.7 Hz, H^4), 7.84 (m, AA′ part of an AA′BB′, 2H, J = 9.1, 5.9, 3.2 Hz; H$^{3'}$, H$^{6'}$), 8.20 (dd, 1H, J = 4.8, 0.9 Hz, H^6), δ 8.57 (d, 1H, J = 8.1 Hz, H^3). ^{31}P NMR ($CDCl_3$): δ 33.8 (s, PPh_3).

*[(pbi)Au(TPA)] (**6**).* An aqueous solution of KOH (26.5 mg, 0.5 mmol) (20 mL) was added to a solution of pbiH (97.61 mg, 0.5 mmol) in CH_3CN (3 mL); the resulting solution was added to an aqueous suspension of [(TPA)AuCl] (195.0 mg, 0.5 mmol) (20 mL). The resulting suspension was stirred in the dark for 24 h at room temperature. Afterward, the white solid was collected by filtration under vacuum and washed with H_2O, EtOH, Et_2O. Recrystallization from acetone/Et_2O gave the analytical sample. Yield 135.1 mg, 49%; mp 235 °C. Anal. Calcd for $C_{18}H_{20}AuN_6P$: C 39.43; H 3.68; N 15.33%. Found: C 39.28; H 3.48; N 15.19%. Selected IR bands (ν_{max}/cm^{-1}): 1590, 1281, 1241, 1094, 1011, 970, 949, 738, 585. UV−vis (CH_3CN): λ_{max} (ε): 308 (14 878), 321 (16 800) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (acetone-d_6): δ 4.58 (s, 6H; N−CH_2−P), 4.67 (AB q, 6H, J = 12.8 Hz; N−CH_2−N), 7.03 (m, BB′ part of an AA′BB′, 2H, J = 9.3, 6.0, 3.2 Hz; H$^{4'}$, H$^{5'}$), 7.40 (ddd, 1H, J = 7.5, 4.8, 1.2 Hz; H^5), 7.59 (m, AA′ part of an AA′BB′, 2H, J = 9.1, 5.9, 3.2 Hz; H$^{3'}$, H$^{6'}$), 7.88 (td, 1H, J = 7.9, 1.8 Hz; H^4), 8.43 (dt, 1H, J = 7.9, 1.2 Hz; H^3), 8.61 (ddd, 1H, J = 4.8, 1.8, 0.9 Hz; H^6). ^{31}P NMR (acetone-d_6): δ −65.1 (s, TPA). X-ray quality crystals of **6** were obtained by slow diffusion of diethyl ether into an acetone solution.

*[ClAu(μ-pbi)AuCl$_2$] (**7**).* A solution of [(THT)AuCl] (112.2 mg, 0.35 mmol) in CH_3CN (10 mL) was added to a suspension of **1** (230.2 mg, 0.35 mmol) in CH_3CN (50 mL); the resulting mixture was stirred for 24 h at room temperature. Afterward, the unreacted insoluble adduct was removed by filtration and the solution concentrated to a small volume; addition of diethyl ether afforded **7** as an orange solid. Yield 138.1 mg, 57%; mp 193−195 °C. Anal. Calcd for $C_{12}H_8Au_2Cl_3N_3$: C 20.75; H 1.16, N 6.05%. Found: C 20.54; H 0.98; N 5.95%. Selected IR bands (ν_{max}/cm^{-1}): 1612, 1566, 1531, 822, 777, 761, 740, 434 ν(Au−N), 376 and 359 ν(Au−Cl). UV−vis (CH_3CN): λ_{max} (ε): 307 (22 050), 321 (17 145), 350sh nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (DMSO-d_6): δ 7.53 (m, BB′ part of an AA′BB′, 2H, J = 9.3, 6.2, 3.2 Hz; H$^{4'}$, H$^{5'}$), 7.74 (ddd, 1H, J = 7.7, 4.8, 1.2 Hz; H^5), 7.81 (m, AA′ part, 2H, J = 9.3, 6.2, 3.2 Hz; H$^{3'}$, H$^{6'}$), 8.20 (td, 1H, J = 7.8, 1.5 Hz, H^4), 8.40 (d, 1H, J = 7.8 Hz, H^3), 8.90 (d, 1H, J = 4.8 Hz; H^6). Crystals were obtained by slow diffusion of diethyl ether into a CH_3CN solution. The quality for X-ray measurement was not very good, and despite various attempts, we were not able to improve them.

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171

*[(PPh$_3$)Au(μ-pbi)AuCl$_2$][PF$_6$] (**8**-PF$_6$).* A solution of [(Ph$_3$P)AuCl] (247.4 mg, 0.5 mmol) and AgPF$_6$ (127.0 mg, 0.5 mmol) in acetone (30 mL) was stirred in the dark until AgCl precipitation was completed. Then, the filtered solution was added to a suspension of **1** (230.0 mg, 0.5 mmol) in 20 mL of acetone and the resulting mixture stirred for 2 h at room temperature; during this time the color of the suspension turned to orange. Then, the orange solid was collected by filtration and recrystallized from MeCN/Et$_2$O to give the analytical sample. Yield 341.2 mg, 64%; mp 225 °C. Anal. Calcd for $C_{30}H_{23}Au_2Cl_2F_6N_3P_2$: C 33.79; H 2.17, N 3.94%. Found: C 33.52; H 2.04; N 3.88%. Λ_M (acetone, 5×10^{-4} mol L^{-1}): 120 Ω^{-1} cm^2 mol^{-1}. Selected IR bands (ν_{max}/cm^{-1}): 1721, 1698, 1608, 1103 (PPh$_3$), 841 (PF$_6^-$), 750, 694, 558, 546, 380 ν(Au−Cl). UV−vis (CH$_3$CN): λ_{max} (ε): 267 (5205), 275 (5589), 291 (6118), 353 (10 926) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (CDCl$_3$): δ 7.32 (m, 2H, J = 9.1, 4.8, 4.3 Hz; H$^{4'}$, H$^{5'}$), 7.47−7.56 (m, 15H; H PPh$_3$), 7.64 (dd, 1H, J = 7.5, 6.0 Hz; H^5), 7.80 (m, 1H, J = 6.1, 4.8 Hz, H$^{6''}$), 8.25 (pseudo t, 1H, J = 7.8, 7.5 Hz, H^4), δ 8.42 (d, 1H, J = 7.8 Hz; H^3), 8.53 (m, 1H, J = 9.3, 5.1, 4.2 Hz; H$^{3'}$), 9.40 (d, 1H, J = 6.0 Hz; H^6). ^{31}P NMR (acetone-d_6): δ 32.7 (s, PPh$_3$), −143.5 (sept, J_{P-F} = 706.3 Hz; PF$_6^-$).

*[(PPh$_3$)Au(μ-pbi)Au(OAc)$_2$][PF$_6$] (**9**-PF$_6$).* A solution of [(Ph$_3$P)-AuCl] (227.5 mg, 0.46 mmol) and AgPF$_6$ (116.3 mg, 0.46 mmol) in CH$_2$Cl$_2$ (30 mL) was stirred in the dark until AgCl precipitation was completed. Then, the filtered solution was added to a solution of **2** (116.3 mg, 0.46 mmol) in the same solvent (20 mL). The resulting yellow solution was stirred for 2 h at room temperature, and then concentered to a small volume; addition of diethyl ether afforded a yellow precipitate of **9**-PF$_6$ which was filtrated and dried in vacuo. Yield 268.9 mg, 52%; mp 160 °C. Anal. Calcd for $C_{34}H_{29}Au_2F_6N_3O_4P_2$: C 36.67; H 2.63; N 3.77%. Found: C 36.46; H 2.53; N 3.65%. Λ_M (acetone, 5×10^{-4} mol L^{-1}): 112 Ω^{-1} cm^2 mol^{-1}. Selected IR bands (ν_{max}/cm^{-1}): 1716sh and 1676 ν_a(COO), 1614, 1265 ν_s(COO), 1103 (PPh$_3$), 841 (PF$_6^-$), 750, 694, 546, 499, 401. λ_{max} (ε): 267 (13 788), 275 (13 932) 345 (14 833) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (CDCl$_3$): δ 2.29 (s, 3H; CH$_3$), 2.34 (s, 3H; CH$_3$), 7.39 (m, BB′ part of an AA′BB′, 2H, J = 9.3, 6.0, 3.0 Hz; H$^{4'}$, H$^{5'}$) 7.59−7.66 (m, 15H; H PPh$_3$), 7.81 (m, AA′ part, 2H, J = 9.3, 6.0, 3.0 Hz; H$^{3'}$,H$^{6'}$), 7.85 (pseudo t, 1H, J = 7.2, 6.9 Hz; H^5), 8.47 (t, 1H, J = 8.1 Hz; H^4), 8.49 (d, 1H, J = 5.4 Hz; H^6), 8.73 (d, 1H, J = 7.5 Hz, H^3). ^{31}P NMR (CD$_2$Cl$_2$): δ 30.8 (s, PPh$_3$), −144.3 (sept, J_{P-F} = 711.1 Hz; PF$_6^-$).

*[(Ph$_3$P)Au(μ-pbi)Au(PPh$_3$)][PF$_6$] (**10**-PF$_6$).* A solution of [(Ph$_3$P)-AuCl] (247.4 mg, 0.5 mmol) and AgPF$_6$ (126.3 mg, 0.5 mmol) in acetone (30 mL) was stirred in the dark until AgCl precipitation was completed. Then, the filtered solution was added to a H$_2$O−CH$_3$CN solution of pbiH (97.6 mg, 0.5 mmol) and KOH (26.5 mg, 0.5 mmol). A white solid was instantaneously formed, and the resulting suspension was stirred in the dark for 12 h at room temperature. Afterward, the solvent was removed under vacuum, the residue taken up with CH$_2$Cl$_2$ and filtered through Celite, and the solution concentrated to a small volume; addition of diethyl ether afforded **10**-PF$_6$ as a white solid. Yield 213.7 mg, 34%; mp 240 °C. Anal. Calcd for $C_{48}H_{38}Au_2F_6N_3P_3$: C 45.84; H 3.05, N 3.34%. Found: C 46.02; H 3.14; N 3.31%. Λ_M (acetone, 5×10^{-4} mol L^{-1}): 116 Ω^{-1} cm^2 mol^{-1}. Selected IR bands (ν_{max}/cm^{-1}): 1605, 1587, 1565, 1104 (PPh$_3$), 841 (PF$_6^-$), 749, 693, 558, 556, 511. λ_{max} (ε): 237, 267 (13 788), 275 (13 932), 308 (18 982) nm (mol^{-1} dm^3 cm^{-1}). ^{1}H NMR (CD$_2$Cl$_2$): δ 7.43 (m, BB′ part of an AA′BB′ system, 2H, J = 9.3, 6.0, 3.2 Hz; H$^{4'}$, H$^{5'}$), 7.51 (ddd, 1H, J = 7.5, 4.8, 1.1; Hz, H^5), δ 7.57−7.70 (m, 30H, H PPh$_3$), 7.78 (td, 1H, J = 7.8, 1.8 Hz; H^4), 8.01 (m, AA′ part, 2H, J = 9.3, 6.0, 3.2 Hz; H$^{3'}$, H$^{6'}$), 8.41 (d, 1H, J = 4.8 Hz; H^6), δ 8.93 (d, 1H, J = 7.7 Hz; H^3). ^{31}P NMR (CD$_2$Cl$_2$): δ 32.6 (s, PPh$_3$), −143.4 (sept, J_{P-F} = 710.2 Hz; PF$_6^-$). X-ray quality crystals of **10**-PF$_6$ were obtained by slow diffusion of diethyl ether.

X-ray Crystallography. Reflection intensity data for **6**, **7**, and **10**-PF$_6$ were collected at 293 K on an Oxford diffraction Xcalibur Sapphire3 with Enhance (Mo) X-ray Source (λ = 0.7107) using the ω technique. The program used for this purpose was CrysAlis CCD.[36] Data were reduced with the program CrysAlis RED.[34] Absorption correction was applied through the program ABSPACK implemented in the above-mentioned program. All the structures were solved using direct methods executed by Sir97[37] and refined with the full-matrix least squared on F^2 by SHELX.[38] In all the structure refinements there are some high residual electron density peaks around the gold atom, especially for compound **7**. This can be ascribed to a redundancy of the data not too high (ca. 2 for complex **7**). Moreover, a crystal of **6** showed a very low degree of twinning. An attempt to include it in the refinement of the structure did not lead to a significant improvement both of the R factor and of the thermal parameters. Geometrical calculations were carried out by PARST97,[39] and molecular plots were produced by the program ORTEP3 (ref 5).[40]

Crystal and structure refinement data are reported in Table 4. CCDC files 859567 (compound **6**), 859568 (compound **1**), and 859569 (compound **10**-PF$_6$) contain the supplementary crystallographic data for this paper.

Table 4. Crystal and Structure Refinement Data for Compounds 6, 7, and 10-PF$_6$

	6	**7**	**10**-PF$_6$
empirical formula	$C_{18}H_{20}AuN_6P$	$C_{12}H_8Au_2Cl_3N_3$	$C_{48}H_{38}Au_2F_6N_3P_3$
fw	548.34	694.50	1257.66
T (K)	293	293	293
wavelength (Å)	0.710 69	0.710 69	0.710 69
cryst syst, space group	monoclinic, $P2_1/n$	monoclinic, $P2_1/c$	triclinic, $P\bar{1}$
unit cell dimensions (Å, deg)	a = 7.0844(3) b = 12.0678(5) β = 94.982(4) c = 21.1691(8)	a = 9.923(1) b = 17.850(2) β = 98.92(1) c = 8.531(1)	a = 12.9029(4) α=73.756(3) b = 13.2996(5) β = 77.057(2) c = 17.0278(4) γ = 64.136(3)
V (Å^3)	1802.97(13)	1492.8(3)	2506.73(14)
Z, D_c (mg/cm^3)	4, 2.020	4, 3.090	2, 1.666
μ (mm^{-1})	8.263	20.160	5.998
F(000)	1056	1240	1208
cryst size (mm^3)	0.4 × 0.3 × 0.3	0.3 × 0.2 × 0.07	0.4 × 0.1 × 0.1
θ range (deg)	4.22−29.11	4.19−27.48	4.35−29.32
reflns collected/ unique	7511/4074	5238/2828	37 484/11 979
data/restraints/ params	4074/2/223	2828/0/79	11979/0/475
GOF on F^2	1.086	0.872	0.935
final R indices [$I > 2\sigma(I)$]	R1 = 0.0616, wR2 = 0.1781	R1 = 0.1101, wR2 = 0.1974	R1 = 0.0392, wR2 = 0.1072
R indices (all data)	R1 = 0.0999, wR2 = 0.1858	R1 = 0.2453, wR2 = 0.2287	R1 = 0.0890, wR2 = 0.1131

Cell Growth Inhibition Studies. For cytotoxicity studies the cisplatin-sensitive human ovarian carcinoma cell line (A2780/S) and its cisplatin-resistant cell subline (A2780/R) were used. Cell lines were maintained in RPMI1640 medium supplemented with fetal bovine serum (FBS) and antibiotics at 37 °C in a 5% CO$_2$ atmosphere and subcultured twice weekly.

The cytotoxic effects of studied gold compounds were evaluated against both these cell lines according to the procedure described by Skehan et al.[41] All gold compounds were diluted in DMSO as stock solutions (10 mM).

Exponentially growing cells were seeded in 96-well microplates at a density of 5×10^3 cell/well. After cell inoculation, the microtiter plates were incubated under standard culture conditions (37 °C, 5% CO$_2$, 95% air, and 100% relative humidity) for 24 h prior to the addition of study compounds. After 24 h, the medium was removed and replaced with fresh medium containing drug concentrations ranging from 0.003 to 100 μM for a continuous 72 h exposure for all compounds. For comparison purposes, the cytotoxic effects of cisplatin (CDDP), measured under the same experimental conditions, were also determined.

dx.doi.org/10.1021/ic202639t | Inorg. Chem. 2012, 51, 3161−3171

Then the cells were fixed with 100 μL of ice-cold 10% trichloroacetic acid (TCA) for 60 min at 4 °C, rinsed 6 times with water, and air-dried. Fixed cells were stained with 50 μL of sulforhodamine B (SRB) solution (0.4% SRB/0.1% acetic acid), rinsed with 0.1% acetic acid, and air-dried. At the end of the staining period, SRB was dissolved in 150 μL of 10 mM Tris−HCl solution (pH 10.5) for 10 min in a gyratory shaker. Optical density was read in a microplate reader interfaced with the software Microplate Manager/PV version 4.0 (Bio-Rad Laboratories, Milan, Italy) at 540 nm.

The IC_{50} drug concentration resulting in a 50% reduction in the net protein content (as measured by SRB staining) in drug-treated cells as compared to untreated control cells was determined after 72 h of drug exposure. The IC_{50} data represent the mean of at least three independent experiments.

To evaluate presence or lack of cross-resistance to study compounds of cisplatin-resistant cells, A2780/R as compared to the parental A2780/S cells, the resistance index (RI) was calculated as the ratio of the IC_{50} values in the resistant cell line and the IC_{50} values in the sensitive one.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Crystallographic data in CIF format. NMR data and absorption characteristics of the complexes in various solvents; UV−vis spectral profiles of the complexes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: cinellu@uniss.it (M.A.C.).

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

M.A.C. gratefully acknowledges the Fondazione Banco di Sardegna and the Università degli Studi di Sassari for financial support and the Regione Autonoma della Sardegna (RAS) for the grant "Premialità Regionale 2009". L.M. and C.G. express their gratitude to Nanotreat project (Regione Toscana) and to Beneficentia Stiftung (Vaduz, Liechtenstein) for generous grants.

■ REFERENCES

(1) Berners-Price, S. J.; Filipovska, A. *Metallomics* **2011**, *3*, 863−873.

(2) (a) Sun, R. W.-Y.; Li, C. K.-L.; Ma, D.-L.; Yan, J. J.; Lok, C.-N.; Leung, C.-H.; Zhu, N.; Che, C.-M. *Chem.—Eur. J.* **2010**, *16*, 3097−3113. (b) Sun, R. W.-Y.; Che, C.-M. *Coord. Chem. Rev.* **2009**, *253*, 1682−1691. (c) Lum, C. T.; Yang, Z. F.; Li, H. Y.; Sun, R. W.-Y.; Fan, S. T.; Poon, R. T. P.; Lin, M. C. M.; Che, C.-M.; Kung, H. F. *Int. J. Cancer* **2006**, *118*, 1527−1538. (d) Wang, Y.; He, Q.-H.; Sun, R. W.-Y.; Che, C.-M.; Chiu, J.-F. *Cancer Res.* **2005**, *65*, 11553−11564. (e) Wang, Y.; He, Q.-Y.; Che, C.-M.; Chiu, J.-F. *Proteomics* **2006**, *6*, 131−142. (f) Sun, R. W.-Y.; Yu, W.-Y.; Sun, H.; Che, C.-M. *ChemBioChem* **2004**, *5*, 1293−1298. (g) Che, C.-M.; Sun, R. W.-Y.; Yu, W.-Y.; Ko, C.-B.; Zhu, N.; Sun, H. *Chem. Commun.* **2003**, 1718−1719.

(3) (a) Ronconi, L.; Giovagnini, L.; Marzano, C.; Bettio, F.; Graziani, R.; Pilloni, G.; Fregona, D. *Inorg. Chem.* **2005**, *44*, 1867−1881. (b) Giovagnini, L.; Ronconi, L.; Aldinucci, D.; Lorenzon, D.; Sitran, S.; Fregona, D. *J. Med. Chem.* **2005**, *48*, 1588−1595. (c) Mlacic, V.; Chen, D.; Ronconi, L.; Landis-Piwowar, K. R.; Fregona, D.; Dou, Q. P. *Cancer Res.* **2006**, *66*, 10478−10486. (d) Saggioro, D.; Rigobello, M. P.; Paloschi, L.; Folda, A.; Moggach, S. A.; Parsons, S.; Ronconi, L.; Fregona, D.; Bindoli, A. *Chem. Biol.* **2007**, *14*, 1128−1139.

(4) (a) Ott, I.; Quian, X.; Xu, Y.; Vlecken, D. H. W.; Marques, I. J.; Kubutat, D.; Will, J.; Sheldrick, W. S.; Jesse, P.; Prokop, A.; Bagowski, C. P. *J. Med. Chem.* **2009**, *52*, 763−770. (b) Vergara, E.; Casini, A.; Sorrentino, F.; Zava, O.; Cerrada, E.; Rigobello, M. P.; Bindoli, A.; Laguna, M.; Dyson, P. J. *ChemMedChem* **2010**, *5*, 96−102. (c) Urig, S.; Fritz-Wolf, K.; Reau, R.; Herold-Mende, C.; Toth, K.; Davioud-Charvet, E.; Becker, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 1881−1886. (d) Scheffler, H.; You, Y.; Ott, I. *Polyhedron* **2010**, *29*, 66−69. (e) Bagowski, C. B.; You, Y.; Scheffler, H.; Vlecken, D. H.; Schmitz, D. J.; Ott, I. *Dalton Trans.* **2009**, 10799−10805.

(5) (a) Weaver, J.; Gaillard, S.; Toye, C.; Macpherson, S.; Nolan, S. P.; Riches, A. *Chem.—Eur. J.* **2011**, *17*, 6620−6624. (b) Rubbiani, R.; Kitanovic, I.; Alborzinia, H.; Can, S.; Kitanovic, A.; Onambele, L. A.; Stefanopoulou, M.; Geldmacher, Y.; Sheldrick, W. S.; Wolber, G.; Prokop, A.; Wölfl, S.; Ott, I. *J. Med. Chem.* **2010**, *53*, 8608−8618. (c) Hickey, J. L.; Ruhayel, R. A.; Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Filipovska, A. *J. Am. Chem. Soc.* **2008**, *130*, 12570−12571. (d) Barnard, P. J.; Berners-Price, S. J. *Coord. Chem. Rev.* **2007**, *251*, 1889−1902. (e) Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. *Dalton Trans.* **2006**, 3708−3715. (f) Baker, M. V.; Barnard, P. J.; Berners-Price, S. J.; Brayshaw, S. K.; Hickey, J. L.; Skelton, B. W.; White, A. H. *J. Organomet. Chem.* **2005**, *690*, 5625−5635. (g) Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Skelton, B. W.; White, A. H. *J. Chem. Soc., Dalton Trans.* **2004**, 1038−1047. (h) Ray, S.; Mohan, R.; Singh, J. K.; Samantaray, M. K.; Shaikh, M. M.; Panda, D.; Ghosh, P. *J. Am. Chem. Soc.* **2007**, *129*, 15043−15053.

(6) Li, C. K.-L.; Sun, R. W.-Y.; Kui, S. C.-F.; Zhu, N.; Che, C.-M. *Chem.—Eur. J.* **2006**, *12*, 5253−5266.

(7) (a) Cinellu, M. A.; Maiore, L.; Manassero, M.; Casini, A.; Arca, M.; Fiebig, H.-H.; Kelter, G.; Michelucci, E.; Pieraccini, G.; Gabbiani, C.; Messori, L. *ACS Med. Chem. Lett.* **2010**, *1*, 336−339. (b) Gabbiani, C.; Casini, A.; Messori, L.; Guerri, A.; Cinellu, M. A.; Minghetti, G.; Corsini, M.; Rosani, C.; Zanello, P.; Arca, M. *Inorg. Chem.* **2008**, *47*, 2368−2379. (c) Casini, A.; Cinellu, M. A.; Minghetti, G.; Gabbiani, C.; Coronnello, M.; Mini, E.; Messori, L. *J. Med. Chem.* **2006**, *49*, 5524−5531.

(8) Fan, D.; Yang, C.-T.; Ranford, J. D.; Vittal, J. J. *Dalton Trans.* **2003**, 4749−4753.

(9) Barreiro, E.; Casas, J. S.; Couce, M. D.; Sánchez, A.; Sánchez-González, A.; Sordo, J.; Varela, J. M.; Vázquez López, E. M. *J. Inorg. Biochem.* **2010**, *104*, 551−559.

(10) (a) Barnard, P. J.; Wedlock, L. E.; Baker, M. V.; Berners-Price, S. J.; Joyce, D. A.; Skelton, B. W.; Steer, J. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 5966−5970. (b) Barnard, P. J.; Baker, M. V.; Berners-Price, S. J.; Day, D. A. *J. Inorg. Biochem.* **2004**, *98*, 1642−1647.

(11) (a) Nobili, S.; Mini, E.; Landini, I.; Gabbiani, C.; Casini, A.; Messori, L. *Med. Res. Rev.* **2010**, *30*, 550−580. (b) Ott, I. *Coord. Chem. Rev.* **2009**, *253*, 1670−1681. (c) Bindoli, A.; Rigobello, M. P.; Scutari, G.; Gabbiani, C.; Casini, A.; Messori, L. *Coord. Chem. Rev.* **2009**, *253*, 1692−1707. (d) Milacic, V.; Ping Dou, Q. *Coord. Chem. Rev.* **2009**, *253*, 1649−1660.

(12) (a) Messori, L.; Abbate, F.; Marcon, G.; Orioli, P.; Fontani, M.; Mini, E.; Mazzei, T.; Carotti, S.; O'Connell, T.; Zanello, P. *J. Med. Chem.* **2000**, *43*, 3541−3548. (b) Abbate, F.; Orioli, P.; Bruni, B.; Marcon, G.; Messori, L. *Inorg. Chim. Acta* **2000**, *311*, 1−5. (c) Marcon, G.; Carotti, S.; Coronnello, M.; Messori, L.; Mini, E.; Orioli, P.; Mazzei, T.; Cinellu, M. A.; Minghetti, G. *J. Med. Chem.* **2002**, *45*, 1672−1677. (d) Coronnello, M.; Mini, E.; Caciagli, B.; Cinellu, M. A.; Bindoli, A.; Gabbiani, C.; Messori, L. *J. Med. Chem.* **2005**, *48*, 6761−6765. (e) Messori, L.; Marcon, G.; Cinellu, M. A.; Coronnello, M.; Mini, E.; Gabbiani, C.; Orioli, P. *Bioorg. Med. Chem.* **2004**, *12*, 6039−6043.

(13) Maiore, L.; Cinellu, M. A.; Michelucci, E.; Monetti, G.; Nobili, S.; Landini, I.; Mini, E.; Guerri, A.; Gabbiani, C.; Messori, L. *J. Inorg. Biochem.* **2011**, *105*, 230−237.

(14) Tsukamoto, G.; Yoshino, K.; Kohono, T.; Ohtaka, H.; Kagaya, H.; Ito, K. *J. Med. Chem.* **1980**, *23*, 734−738.

(15) Schiffmann, R.; Neugebauer, A.; Klein, C. D. *J. Med. Chem.* **2006**, *49*, 511−522.

(16) Some recent examples: (a) Mock, C.; Puscasu, I.; Rauterkus, M. J.; Tallen, G.; Wolff, J. E. A.; Krebs, B. *Inorg. Chim. Acta* **2011**, *319*, 109−116. (b) Shen, M.; Huang, W.; Zhang, W.; Hao, X.; Sun, W.-H.; Redshaw, C. *Dalton Trans.* **2010**, *39*, 9912−9922. (c) Huang, W.-K.; Cheng, C.-W.; Chang, S.-M.; Lee, Y.-P.; Diau, E. W.-G. *Chem. Commun.* **2010**, *46*, 8992−8994. (d) Wu, J.; Li, H.-Y.; Kang, L.-C.; Li, D.-P.; Xu, Q.-L.; Zhu, Y.-C.; Tao, Y.-M.; Zheng, Y.-X.; Zuo, J.-L.; You, X.-Z. *J. Organomet. Chem.* **2010**, *695*, 2048−2056. (e) Altaf, M.; Stoeckli-Evans, H. *Transition Met. Chem.* **2009**, *34*, 613−620. (f) Machura, B.; Switlicka, A.; Wolff, M.; Kusz, J.; Kruszynski, R. *Polyhedron* **2009**, *28*, 1348−1354. (g) Haneda, S.; Gan, Z.; Eda, K.; Hayashi, M. *Organometallics* **2007**, *26*, 6551−6555. (h) Casas, J. S.; Castiñeiras, A.; García-Martínez, E.; Parajó, Y.; Pérez-Parallé, M. L.; Sáncez-González, A.; Sordo, J. *Z. Anorg. Allg. Chem.* **2005**, *631*, 2258−2264. (i) Shavaleev, N. M.; Bell, Z. R.; Easun, T. L.; Rutkaite, R.; Swanson, L.; Ward, M. D. *Dalton Trans.* **2004**, 3678−3688. (j) Gümüs, F.; Pamuk, I.; Özden, T.; Yldiz, S.; Diril, N.; Öksüzoglu, E.; Gür, S.; Özkul, A. *J. Inorg. Biochem.* **2003**, *94*, 255−262. (k) Mock, C.; Puscasu, I.; Rauterkus, M. J.; Tallen, G.; Wolff, J. E. A.; Krebs, B. *Inorg. Chim. Acta* **2001**, *319*, 109−116.

(17) Dash, K. C. *Indian J. Chem.* **1986**, *25A*, 552−556.

(18) Munakata, M.; Yan, S.-G.; Maekava, M.; Akiyama, M.; Kitagava, S. *J. Chem. Soc., Dalton Trans.* **1997**, 4257−4262.

(19) (a) Addison, A. W.; Rao, T. N.; Waslgren, C. G. *J. Heterocycl. Chem.* **1983**, *20*, 1481−1484. (b) Alcalde, E.; Dinarés, I.; Pérez-Garzia, L.; Roca, T. *Synthesis* **1992**, 395−398.

(20) (a) Mingos, D. M. P. *J. Chem. Soc., Dalton Trans.* **1976**, 1163−1169. (b) Evans, D. G.; Mingos, D. M. P. *J. Organomet. Chem.* **1982**, *232*, 171−191. (c) Lauher, J. W.; Wald, K. *J. Am. Chem. Soc.* **1981**, *103*, 7648−7650. (c) Liang, X.; Wu, X.; Dong, T.; Qin, Z.; Tan, K.; Lu, X.; Tang, Z. *Angew. Chem., Int. Ed.* **2011**, *50*, 2166−2170.

(21) For example, only one band at 377 cm^{-1} is observed for $[(bipy)AuCl_2]^+$: Cinellu, M. A.; Minghetti, G.; Pinna, M. V.; Stoccoro, S.; Zucca, A.; Manassero, M. *J. Chem. Soc., Dalton Trans.* **2000**, 1261−1265.

(22) Bessonov, A. A.; Basova, T. V.; Kiselev, V. G.; Sheludyakova, L. A.; Morozova, N. B.; Igumenov, I. K. *Vib. Spectrosc.* **2009**, *51*, 283−288.

(23) Bonatti, F.; Felici, M.; Pietroni, B. R.; Burini, A. *Gazz. Chim. Ital.* **1982**, *112*, 5−8.

(24) See, for example: Cinellu, M. A.; Stoccoro, S.; Minghetti, G.; Bandini, A. L.; Demartin, F. *Inorg. Chim. Acta* **1990**, *168*, 33−41.

(25) See for example: Thwaite, S. E.; Schier, A.; Schmidbaur, H. *Inorg. Chim. Acta* **2004**, *357*, 1549−1557.

(26) Tzeng, B.-C.; Chen, B.-S.; Chen, C.-K.; Chang, Y.-P.; Tzeng, W.-C.; Lin, T. Y.; Lee, G.-H.; Chou, P. T.; Fu, Y.-J.; Chang, A. H.-H. *Inorg. Chem.* **2011**, *50*, 5379−5388.

(27) Allen, F. H. *Acta Crystallogr. Sect. B* **2002**, *B58*, 380−388.

(28) Pauling, L. *The Nature of the Chemical Bond*, 3rd ed.; Cornell University Press: Ithaca, NY, 1960.

(29) Cinellu, M. A.; Zucca, A.; Stoccoro, S.; Minghetti, G.; Manassero, M.; Sansoni, M. *J. Chem. Soc., Dalton Trans.* **1996**, 4217−4225 and references therein.

(30) Ivanov, M. A.; Puzyk, M. V.; Tkacheva, T. A.; Balashev, K. P. *Russ. J. Gen. Chem.* **2006**, *76*, 165−169.

(31) Fernández, E. J.; Laguna, A.; López-de-Luzuriaga, J. M.; Monge, M.; Montiel, M.; Olmos, M. E.; Pérez, J.; Rodríguez-Castillo, M. *Gold Bull.* **2007**, *40*, 172−183.

(32) Nevertheless, complex **5** was restored after the solvent was evaporated to dryness (^{1}H NMR criterium).

(33) Sanna, G.; Pilo, M. I.; Minghetti, G.; Cinellu, M. A.; Spano, N.; Seeber, R. *Inorg. Chim. Acta* **2000**, *310*, 34−40.

(34) McAuliffe, C. A.; Parish, R. V.; Randall, P. D. *J. Chem. Soc., Dalton Trans.* **1979**, 1730−1735.

(35) Uson, R.; Laguna, A.; Laguna, M. *Inorg. Synth.* **1989**, *26*, 85.

(36) *CrysAlisPro, Version 1.171.33.41 (release 06−05−2009 CrysAlis171 .NET)*; Oxford Diffraction Ltd., (compiled May 6, 2009,17:20:42).

(37) SIR97: Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115−119.

(38) SHELX: Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112−122.

(39) Nardelli, M. *J. Appl. Crystallogr.* **1995**, *28*, 659.

(40) Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.

(41) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.