Titanocene—Gold Complexes Containing N-Heterocyclic Carbene Ligands Inhibit Growth of Prostate, Renal, and Colon Cancers in Vitro

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ABSTRACT: We report on the synthesis, characterization, and stability studies of new titanocene complexes containing a methyl group and a carboxylate ligand (mba = −OC(O)-p-C6H4-S−) bound to gold(I)−N-heterocyclic carbene fragments through the thiolate group: [(η5-C5H5)2TiMe(μ-mba)Au(NHC)]. The cytotoxicities of the heterometallic compounds along with those of novel monometallic gold−N-heterocyclic carbene precursors [(NHC)Au(mbaH)] have been evaluated against renal, prostate, colon, and breast cancer cell lines. The highest activity and selectivity and a synergistic effect of the resulting heterometallic species was found for the prostate and colon cancer cell lines. The colocalization of both titanium and gold metals (1:1 ratio) in PC3 prostate cancer cells was demonstrated for the selected compound 5a, indicating the robustness of the heterometallic compound in vitro. We describe here preliminary mechanistic data involving studies on the interaction of selected mono- and bimetallic compounds with plasmid (pBR322) used as a model nucleic acid and the inhibition of thioredoxin reductase in PC3 prostate cancer cells. The heterometallic compounds, which are highly apoptotic, exhibit strong antimigratory effects on the prostate cancer cell line PC3.

INTRODUCTION

The potential of heterometallic complexes as cancer chemotherapeutics has been recently highlighted.1 The improved activity of heteronuclear complexes as antitumor agents by incorporation of two different cytotoxic metals within the same molecule has been demonstrated. The beneficial influence may be due to a synergistic or cooperative effect. Bimetallic and trimetallic compounds with anticancer properties have been described. There have been reports on titanocenes incorporating Ru(II), Pt(II), and Pd(II) centers2,3 and a number of complexes containing ferrocene moi eties and other metals.4 Bimetallic systems based on Ru(II)—Pt(II)5 or Ru(II)—Ir(III)6 have also been described. Ferrocenyl phosphanes were incorporated in the iminophosphorane skeleton of gold(III) and palladium(II) coordination complexes.4 Heterometallic compounds based on gold(I) fragments have been reported for titanocene,1,3,8 ruthenium(II),9–12 platinum(II),13 rhenium(II),14 and copper(II)15 derivatives.

We have reported a number of titanocene−gold derivatives with potential as anticancer agents (zero-, first-, and second-generation derivatives 1–3 in Chart 1).1,3,8 We described cytotoxic species in which gold fragments coordinate to cyclopentadienyl−phosphate ligands that displayed a synergistic effect (such as 1 in Chart 1).3 In order to improve
stability in physiological media and prevent the loss of cyclopentadienyl–gold fragments, first-generation derivatives (such as 2) were developed. While Ti–Cp hydrolysis still occurs at pH 7, the gold fragment remains linked to titanium by the carboxylate ligand. These first-generation compounds showed excellent activity against renal cancer cell lines.

In addition, the compounds were more selective toward cancerous cells and lacked systemic toxicity in mice models.

A further and successful modification was the introduction of a bifunctional ligand, mba (−OC(O)-p-C6H4-S−; derived from 4-mercaptobenzoic acid (H2mba)). We reported on compounds of the type [(η5-C5H5)2TiMe(μ-mba)Au(PR3)] (second generation such as 3 in Chart 1). Compound 3 was able to block renal cancer growth both in vitro and in vivo by pathway(s) that involve the inhibition of thioredoxin reductase and decreased expression of protein kinases known to drive cell migration. Preliminary evidence indicated that compound 3 may have appreciable anti-invasive properties. In addition, its robustness was demonstrated in cellular uptake experiments on Caki-1 cells by colocalization of Ti and Au metals in a 1:1 ratio.

We aimed to exchange the gold(I)–phosphate fragments by gold(I)−N-heterocyclic carbene moiety to improve or modify the pharmacological profile of the previously reported heterometallic complexes [(η5-C5H5)2TiMe(μ-mba)Au(PR3)] (such as 3). NHC–gold(I) complexes are usually more stable than gold(I)–phosphate compounds, display strong anti-mitochondrial effects,15,16 and have demonstrated excellent inhibitory properties of certain enzymes such as thioredoxin reductase.3−23 They have also relevant anticancer effects in vitro15–25 (including some recent examples on heterometallic complexes9,10), and in vivo effects on melanoma have recently been described.26 We report here on the synthesis, characterization, and stability studies of the novel monometallic gold−N-heterocyclic carbene precursors [(NHC)Au(mbaH)] and heterometallic titanocenes of the type [(η5-C5H5)2TiMe(μ-mba)Au(NHC)]. We describe their in vitro activity against human renal, prostate, colon, and breast cancer cell lines and nontumorigenic human embryonic kidney cell lines HEK-293T. In addition, we present studies of cellular uptake, the effect of compounds on cell death, interactions with DNA, inhibitory effects on thioredoxin reductase in vitro, and antimitogenic properties on PC3 prostate cancer cells.

## RESULTS AND DISCUSSION

### Synthesis and Characterization.

The synthesis of the new monometallic gold(I)−NHC compounds bearing the bifunctional ligand Hmba (Scheme 1) was carried out following the same strategy reported for the synthesis of species [Au(Hmba)(phosphate)].

The thiol group on the bifunctional ligand Hmba (1 equiv) was deprotonated by reaction with 1 equiv of KOH for 20 min at room temperature. Subsequent addition of 1 equiv of the gold(I) N-heterocyclic carbene complexes a–d in situ, in a mixture of ethanol and water (4/1), led to the formation of the corresponding monometallic gold complexes [Au(NHC)-(Hmba)] (NHC = SIPr (4a), IPr (4b), IMes (4c), ICy (4d)). Compounds 4a–d were isolated as pale orange solids in the case of compound 4c in tetrahydrofuran.

The crystals of compound 4c were determined to be triclinic (space group P1) with Z = 4 formula units in the unit cell. The environment of the gold atoms is close to linear (C−Au−S 177.76(18)°) (Figure 1). A selection of structural parameters is given in Table 1. The individual monomeric units (Figure 1) show hydrogen bonds (~1.83 Å) between the carboxylic groups of two neighboring units (Figure 2).

![Figure 1. ORTEP view of the molecular structure of 4c showing the labeling scheme. The labels for hydrogen and some carbon atoms are omitted for clarity. A drawing of the molecular structure containing all labeled carbon atoms is provided in the Supporting Information.](image)

### Table 1. Selected Structural Parameters of Complex 4c Obtained from X-ray Single-Crystal Diffraction Studies

| Bond | Length (Å) | Angle (deg) |
|------|------------|-------------|
| Au(1)−C(1) | 1.995(6) | 1.341(7) |
| Au(1)−C(2) | 2.2790(17) | 1.381(8) |
| S(1)−C(1) | 1.746(6) | 1.453(7) |
| C(1)−N(1) | 1.333(7) | 1.382(7) |
| C(1)−N(2) | 1.341(7) | 1.456(7) |
| C(1)−Au−S(1) | 177.76(18) | 122.7(7) |
| N(HC)=SiPr (4a) | 111.4(5) | N(HC)=IPr (4b) | 111.1(5) |
| N(HC)=IMes (4c) | 123.4(5) | N(HC)=ICy (4d) | 124.8(5) |
| N(2)−C(1)−N(3) | 104.2(5) | N(2)−C(2)−N(3) | 106.2(5) |

*Bond lengths are given in Å and angles in deg.*
mononuclear gold(I) complexes 4a–d with 1 equiv of \((\eta^5-C_5\text{H}_5)_2\text{TiMe}_2\) afforded the corresponding hetereobimetallic complexes \([(\eta^5-C_5\text{H}_5)_2\text{TiMe}(\mu\text{-mwa})\text{Au(NHC)}]\) (NHC = SIPr (5a), IPr (5b), IMes (5c), ICy (5d)), with concomitant elimination of 1 equiv of methane. Compounds 5a–d were obtained in moderate to high yields as air- and moisture-stable yellow solids. These compounds are less acidic than titanocene dichloride and soluble in DMSO/H$_2$O, DMSO/PBS, or DMSO/media (1/99) mixtures at micromolar concentrations, which is relevant for subsequent biological testing. Moreover, they are more soluble at higher concentrations in DMSO/PBS mixtures than the previously described \([(\eta^5-C_5\text{H}_5)_2\text{TiMe}(\mu\text{-mba})\text{Au}(\text{NHC})]\) counterparts. Compounds 5a–d were stable in DMSO-\(\text{d}_6\) solution for at least 3 days. They are stable in DMSO-\(\text{d}_6\) solution for weeks.

The structures of complexes 5a–d depicted in Scheme 1 have been proposed on the basis of NMR and UV–vis spectroscopy, mass spectrometry, and elemental analysis (see the Experimental Section). Moreover, IR experiments and DFT calculations were carried out in order to shed light on the coordination mode of the carboxylate groups. The differences found between the symmetric and antisymmetric stretching bands for the carboxylate groups in the solid state IR spectra (ranging from 210 to 351 cm$^{-1}$) are greater than 200 cm$^{-1}$, indicating a monodentate coordination mode.\(^{30,31}\) DFT calculations (e.g., Figure 3) also confirmed the monodentate nature of the carboxylate functionality. In the Supporting Information, data on different optimizations are provided. All calculations performed led to the species containing a monodentate carboxylate. Similar difference values (ranging from 200 to 328 cm$^{-1}$) were found between the symmetric and antisymmetric stretching bands for the carboxylate groups in the IR calculated on the basis of the DFT studies.

The stability of compounds 5a–d was evaluated by $^1$H NMR spectroscopy in DMSO-\(\text{d}_6\) and DMSO-\(\text{d}_6\)/PBS-D$_2$O (3/2) and by mass spectrometry over time (see the Supporting Information). NMR experiments were performed in DMSO-\(\text{d}_6\) and in DMSO-\(\text{d}_6\)/PBS-D$_2$O mixtures. The stability study of compounds 5a–d by $^1$H NMR in DMSO-\(\text{d}_6\) showed half-life values of 1, 3, 2, and 2 h, respectively: i.e., shorter than those for the corresponding phosphane derivatives\(^1\) (3, Chart 1). However, as shown in Table 2, compounds 5a–d exhibited longer half-lives in 3/2 of DMSO-\(\text{d}_6\)/PBS-D$_2$O mixtures. Titanocene dichloride is also known to hydrolyze with a higher rate in DMSO than in water.\(^{12}\) Mass spectrometry further supports the presence of species containing both titanium and gold in 1% DMSO/PBS solution after 24 h (see the Supporting Information).

**Biological Activity. Assays of Cytotoxicity and Cell Death.** The cytotoxicity of the heterometallic complexes \([(\eta^5-C_5\text{H}_5)_2\text{TiMe}(\mu\text{-mwa})\text{Au(NHC)}]\) (NHC = SIPr (5a), IPr (5b), IMes (5c), ICy (5d)), monometallic gold(I) complexes \[(\eta^5-C_5\text{H}_5)_2\text{TiMe}(\mu\text{-mba})\text{Au}(\text{NHC})(\text{Hmba})]\] (NHC = SIPr (4a), IPr (4b), IMes (4c), ICy (4d)) in Scheme 1, and titanocene Y\(^{33}\) used as control was assayed by monitoring their ability to inhibit cell growth using the PrestoBlue Cell Viability assay (see the Experimental Section). The cytotoxic activity of the compounds was determined as described in the Experimental Section. In this assay, human cancer cell lines such as prostate PC3 and DU145, renal Caki-1, colon DLL1, triple negative breast MDA-MB-231, and nontumorigenic human embryonic kidney cell lines HEK-293T were incubated with the indicated compound for 72 h. The results are summarized in Table 3.

The heterometallic compounds are considerably more toxic to the prostate cancer cell lines (PC3 and DU145) and the colon cancer cell line (DLD1) than titanocene Y. In addition, the heterometallic compounds 5a–d are more toxic in all the cell lines (excluding the triple negative breast cancer cell lines)
Table 3. IC₅₀ Values (μM) in Human Cell Lines Determined with Heterometallic Ti–Au Compounds 5a−d, Monometallic Au Compounds 4a−d, and Titanocene Y as Control

| Compound       | PC3    | DU-145 | Caki-1  | DLD1    | MDA-MB-231 | HEK-293T |
|----------------|--------|--------|--------|---------|------------|----------|
| Titanocene Y   | 58.1 ± 11.2 | 55.2 ± 7.9 | 29.4 ± 4.2 | 56.2 ± 9.8 | 18.0 ± 3.6 | >200     |
| 5a             | 9.8 ± 2.2  | 11.8 ± 3.0 | 21.0 ± 1.9 | 13.9 ± 1.7 | >100       | 58.8 ± 6.7 |
| 5b             | 10.3 ± 2.8 | 18.9 ± 2.9 | 51.5 ± 3.7 | 30.4 ± 4.1 | >100       | >100     |
| 5c             | 17.1 ± 2.9 | 13.76 ± 2.7 | 29.11 ± 4.1 | 19.9 ± 4.1 | >100       | 69.7 ± 9.9 |
| 5d             | 11.8 ± 1.6 | 16.7 ± 2.0 | 42.9 ± 5.8 | 21.5 ± 2.0 | >100       | >100     |
| 4a             | 66.3 ± 6.4 | 74.8 ± 4.4 | 81.4 ± 2.9 | 78.2 ± 6   | >100       | >100     |
| 4b             | 70.4 ± 6.8 | 60.9 ± 5.2 | 79.2 ± 11.7 | 82.6 ± 5.9 | >100       | >100     |
| 4c             | 57.1 ± 5.1 | 67.6 ± 7.1 | 97.2 ± 8.6 | 73.1 ± 9.6 | >100       | 87.9 ± 6.4 |
| 4d             | 65.1 ± 4.4 | 59.9 ± 4.7 | 88.9 ± 5.1 | 77.5 ± 8.1 | >100       | 97.2 ± 5.1 |

*aAll compounds were dissolved in 1% of DMSO and diluted with water before addition to cell culture medium for a 72 h incubation period.

None of the heterometallic or monometallic gold compounds are toxic in the triple negative breast cancer cell lines in concentrations lower than 100 μM, as opposed to the reference compound titanocene Y. While the heterometallic compounds are toxic on both the Caki-1 cancer cell lines, the IC₅₀ values are only comparable to those for titanocene Y for compounds 5a,c. These IC₅₀ values are larger than those found for the first-generation titanocene–gold compounds previously described by us and larger than the IC₅₀ value of the second-generation compounds of the type [(η⁵-C₅H₅)₂TiMe(μ-mba)Au(PR₃)]²⁻ (especially 3). In terms of selectivity, the heterometallic compounds exhibit selectivity for the cancer cell lines (excluding the triple negative breast cancer cell line, MDA-MB-231), with compound 5b having a better selectivity in comparison to nontumorigenic human embryonic kidney cell lines HEK-293T. The new compounds display a better selectivity toward the HEK-293T cell line than the phosphane [(η⁵-C₅H₅)₂TiMe(μ-mba)Au(PR₃)] derivatives described before.

We did not find a strong correlation between the type of NH ligand employed and the biological activity. Lysates of PC3 cells treated with 5a were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) to determine the colocalization and amounts of Au and Ti metals in these prostate cancer cell lines (see the Experimental Section for details). Lysate of untreated cells was employed as a control. It was observed that these cells have some basal levels of Au and Ti present (0.1 μg Au per mg cell protein). The cellular uptake of these cells increases with the increase in drug concentration in the media, indicating a dose-dependent uptake of compound 5a by PC3 cells (Figure 4).

Increasing the drug concentration from 10 to 20 μM resulted in a 2.6-fold increase in the cellular levels of compound 5a. More importantly, on correction for background levels of Au and Ti in cell lysates, the stoichiometric ratios of these elements were close to unity, suggesting that the compound remains stable in the intracellular environment after 72 h (or that at least uptake of both metals occurs concurrently).

We had found that the compound [(η⁵-C₅H₅)₂TiMe(μ-mba)Au(PPh₃)] (3) exerted cell death by inducing apoptosis. Titanocenes C, X, and Y are also known to induce apoptosis in different cancer cell lines. In order to gain some insight into the nature of the cytotoxicity of the new heterometallic compounds containing N-heterocyclic carbenes, we performed some experiments. The effect of titanocene Y, selected monometallic compounds 4a,b, and bimetallic 5a,b on necrosis and apoptosis on Caki-1 and PC3 was assessed by measuring protease activity using non-cell-permeable substrates and cell-permeable substrates and by measuring the total caspase-3 and -7 activities with the ApoTox-Glo triplex assay (see the Experimental Section). The effect of each treatment was determined by comparing treated and untreated cells after 72 h incubation. The results for each treatment were expressed as fold changes between nontreated (0.1% DMSO) and treated samples. ApoTox-Glo triplex assays were repeated twice (n = 2), and each repetition was run in quadruplicate.

The average of the four values was used for statistical calculations. The data (Figure S) are presented as the mean value. It is important to note that, while all compounds are represented at once in graphs in Figure S, the amounts of compound used to run the assays is different (according to their different IC₅₀ values). The lower amounts used (20 μM) correspond to the new compounds 5a,b.

From these data, it can be deduced that compounds 5a,b mainly induce apoptosis both in the renal Caki-1 and prostate PC3 cancer cell lines in a way similar to that for titanocene Y (highly apoptotic in vitro and in vivo) and in the case of compound 5a in Caki-1 and both 5a and 5b in PC3 with a lower IC₅₀ value. Titanocene Y was specially apoptotic on PC3 cancer cell lines. Monometallic compounds 4a,b were also mainly apoptotic, but their IC₅₀ values are considerably higher than those of the new compounds 5a,b.

Migration Studies. In advanced tumors, increased cell migration is a hallmark of cancer cell invasion and meta-
The effect of titanocenes on the migratory capability of cancer cells has been scarcely studied.38 We previously found that first-generation \([(\eta^5-C_5H_5)2Ti(OC(O)p-C_6H_4−PPh_2AuCl)}2\)](2)8 and second-generation \([(\eta^5-C_5H_5)2TiMe−(\mu-mba)Au(PPh_3)](3)1\) titanocene−gold complexes displayed relevant antimigratory properties.1 We evaluated the anti-invasive properties of the most active heterometallic complexes \([((\eta^5-C_5H_5)2TiMe(\mu-mba)Au(NHC)](NHC = SIPr (5a), IPr (5b))\) and titanocene Y, by using the same wound-healing scratch assay (Experimental Section) on prostate cancer PC3 cell lines. Twenty-four hours following a scratch through an in vitro confluent monolayer of prostate carcinoma PC3, cells treated with 15 μM titanocene Y invaded 69% of the scratch and cells treated with 5 μM of 5a or 5b invaded 42% or 33% of the scratch, respectively, while cells treated with 0.1% DMSO control invaded 88% of the scratch (Figure 6A). Figure 6B shows a comparison in terms of total reduction of migration among compounds 5a,b and titanocene Y. A similar assay was performed with gold monometallic compounds 4a,b (see Figure S60 in the Supporting Information), and it was found that under the same conditions (in vitro confluent monolayer of prostate carcinoma PC3 cells treated with 15 μM of the gold monometallic compounds) 4a,b invaded 73% and 80% of the scratch, respectively.

We can conclude from this experiment that heterometallic compounds 5a,b possess antimigratory properties in PC3 cells in comparison to the control and are twice as powerful in inhibiting migration as titanocene Y. Recently enantiopure cyclopentadienyl Ti(IV) oximato compounds have been shown to affect the cell adhesion and migration of PC3 cancer cell

Interaction with Plasmid pBR322 DNA. We and others have previously found that titanocene−gold compounds interact weakly with calf thymus DNA or do not interact with plasmid pBR322 DNA, as is the case for many other gold compounds.1,3,7,8 Recent reports on titanocene dichloride and titanocene Y also indicate a weak interaction with DNA40,41 and the lack of suppression for DNA-processing enzymes.40 DNA interactions were tested with heterometallic compounds 5a−d or cisplatin by using plasmid (pBR322) DNA (Figure 7). This plasmid has two main forms: OC (open circular or relaxed form, form II) and CCC (covalently closed or supercoiled form, form I). Agarose gel electrophoresis assays were performed whereby decreased electrophoretic mobilities of both forms were taken as evidence of metal−DNA binding.
Generally, the slower the mobility of supercoiled DNA (CCC, form I), the greater the DNA unwinding produced by the drug. For example, binding of cisplatin to plasmid DNA results in decreased mobility of the CCC form and increased mobility of the OC form. Treatment of plasmid DNA with increasing amounts of the new heterometallic compounds 5a–d did not affect the mobility of the faster-running supercoiled form (form I) even at the highest molar ratios (d). This result is in accordance with the lack of interaction shown by titanocene–gold compounds (zero, first, and second generation) and the lack of interaction displayed by monometallic gold compounds 4a–d (see Figure S59 in the Supporting Information).

**Inhibition of Thioredoxin Reductase in PC3 Cancer Cells.**
Many chemoresistant cancers produce changes in the cell antioxidant capacity. The overexpression of thioredoxin reductase (TrRx) is among the key defense and survival mechanisms of cisplatin-resistant cells. Thioredoxin reductase has become a potential target in cancer chemotherapy. We have reported on the inhibition of TrRx in Caki-1 cells by aurano and the heterometallic titanocene–gold complex \([\left\{\eta^2-C_2H_4\right\}_2TiMe(\mu-mba)Au(PR_3)\}] (3). Since Au–NHC compounds are known to inhibit TrRx, we measured the activity of thioredoxin reductase in PC3 prostate cancer cells, following incubation with monometallic compounds [Au(NHC)(Hmba)] (NHC = SIPr (4a), IPr (4b)) and bimetallic compounds \([\left\{\eta^2-C_2H_4\right\}_2TiMe(\mu-mba)Au(NHC)\}] (NHC = SIPr (5a), IPR (5b)). We found thioredoxin reductase activity to be lower in cells treated with 5 μM of 5a and 5b with observed inhibitions of 31% and 30%, respectively, after a 5 h incubation period (Figure 8). The inhibition was 61% (5a) and 76% (5b) after 24 h incubation. In the case of PC3 cells treated with 30 μM of monometallic gold compounds (4a,b) there was inhibition of thioredoxin reductase but to a lower extent (30% and 36% for 4a and 4b, respectively, after 24 h incubation). Surprisingly, we found that titanocene Y (15 μM) was also a strong TrRx inhibitor in PC3 cells (with a 57% or 80% reduction after 5 or 24 h of treatment, respectively, see Figure S61 in the Supporting Information). This experiment showed that the inhibition of TrRx is involved in the cell death mechanism of the new compounds and that the titanocene component has an influence on this target, although other cellular targets may not be excluded. In the past years, a number of other targets (such as glutathione reductase, cysteine proteases such as cathepsins K and S, protein thyrosine phosphatases, glutathione peroxidase (GPx), iodothyronine deiodinase (ID), and IkB kinase) have been identified for gold(I) complexes. Helicases/topoisomerases and HIST1H4 core histones have been pointed out as targets of titanocene C5, and we reported on the strong inhibitory effect of titanocene dichloride against PI3 protein kinases from a panel of 35 kinases of oncological interest.

For titanocene–gold(I) heterometallic complexes, we have shown that the compound \([\left\{\eta^2-C_2H_4\right\}_2TiMe(\mu-mba)Au(PR_3)\}] (3) not only inhibited TrRx in Caki-1 renal cancer cells with an IC50 value very similar to that of Aurano but also was considerably more cytotoxic than aurano in this cell line due to a more potent inhibition of the specific protein kinases AKT, p90-RSK, and MAPKAPK3 in vitro. All of these results warrant further studies on the mode of action of the new heterometallic compounds.

**CONCLUSIONS**
In conclusion, we have described the preparation of novel heterometallic titanocene–gold compounds incorporating gold(I)–N-heterocyclic carbene fragments. The exchange of the phosphate ligands by NHC ligands (L) in complexes of the type \([\left\{\eta^2-C_2H_4\right\}_2TiMe(\mu-mba)Au(\text{NHC})\}] did result in lower IC50 values in renal Caki-1 cancer cell lines, although a significant activity and a considerably higher selectivity with respect to noncancerous cell lines was achieved in prostate and colon cancer cell lines for the new Ti–Au–NHC complexes. As for the analogous titanocene–gold compounds containing phosphanes, the new heterometallic carbene derivatives did not display a significant interaction with plasmid (pBR322) used as a model nucleic acid. Two selected compounds (5a,b) were found to be highly apoptotic and to inhibit TrRx in prostate PC3 cancer cell lines. These complexes also display strong antimigratory properties. The work presented here is the proof of concept that the substitution of PR3 by NHC complexes. As for the analogous titanocene–gold compounds containing phosphanes, the new heterometallic carbene derivatives did not display a significant interaction with plasmid (pBR322) used as a model nucleic acid. Two selected compounds (5a,b) were found to be highly apoptotic and to inhibit TrRx in prostate PC3 cancer cell lines. These complexes also display strong antimigratory properties. The work presented here is the proof of concept that the substitution of PR3 by NHC ligands. Further optimization of the NHC ligands described in this work we did not find a strong SAR correlation. Further optimization of the NHC ligands employed and more detailed mechanistic studies are needed in order to find candidates with improved pharmacological properties. These studies are currently under way in our laboratories.
Chemistry. Synthesis and Characterization: General Procedure. 

Iridodimethyl gold complexes were synthesized from the corresponding iodotris(methyl)gold(III) complexes and the appropriate imidazole. 

**EXPERIMENTAL SECTION**

Organometallics
X-ray Crystallography. Suitable single crystals of compound 4c were obtained by layering pentane over a solution of tetrahydrofuran.

DFT Calculations. The calculations have been performed using the hybrid density functional method B3LYP,57,58 as implemented in Gaussian09.59 Geometries were optimized with the 6-31G(d) basis set for the P and S elements, the 6-31G(dp) basis set for the C, N, P, S, and H elements, and the SDD pseudopotential for the titanium, iron, and gold metal centers.59,60 Frequency calculations have been done at the same level of theory as the geometry optimizations to confirm the nature of the stationary points.

Biology. Interactions of the New Compounds with Plasmid DNA. The Bacteriophage plasmid pBR322 (Gel Electrophoresis Mobility Shift Assay). Ten microliter aliquots of pBR322 plasmid DNA (20 μg/mL) in buffer (5 mM Tris/HCl, 50 mM NaClO4, pH 7.39) were incubated with different concentrations of the compounds (4a–d, 5a–d, and cisplatin as control) (in the range of 0.25 and 4.0 μM) with pBR322 plasmid DNA (20 μg/mL) in buffer (5 mM Tris/HCl, 50 mM NaClO4, pH 7.39) were incubated with different concentrations of the compounds (4a–d, 5a–d, and cisplatin as control) (in the range of 0.25 and 4.0 μM).

Stability of Compound 5a in Vitro and Colocalization of Ti/Au Metals in PC3 Cells. PC3 cells were incubated with 10 and 20 μM compound 5a for 72 h. Postincubation, the cells were washed twice with cold PBS and lysed with cell lysis buffer comprising of 1% (v/v) Triton-X-100, 25 mM HEPES, 100 mM NaCl, 1 mM EDTA, 10% (v/v) glycerol, and protease and phosphatase inhibitors. Lysates from untreated cells incubated with media supplemented with DMSO for the same duration were used as controls. Gold and titanium contents in the cell lysates were determined using ICP-MS. One hundred microliter portions of lysates were transferred into a glass vials, and 1 mL of concentrated acid mixture (comprising of 75% of 16 N nitric acid and 25% of 12 N hydrochloric acid) was added. The mixture was heated at 90 °C for 5 h. After cooling, the samples were diluted with water and 40 ppb of indium internal standard was added and analyzed in a Thermo Scientific XSERIES 2 ICP-MS with ESI PC3 Peltier cooled spray chamber with an SC-FAST injection loop and SC-4 autosampler. All of the elements were analyzed using He/H2 collision-reaction mode. A standard curve (0, 1, 5, 10, and 20 μM) of compound 5a was processed similarly to determine the linearity of extraction efficiency of Au and Ti. The protein contents of the cell lysates were determined using a bicinchoninic acid based protein assay kit (Thermo Scientific). The final levels of either Ti or Au were normalized to the cellular protein levels.

Cell Death for Titancene Y, 4a,b, and 5a,b. For apoptosis, viability, and necrosis assays, the Caki-1 and PC3 cells were seeded in 96-well opaque-walled tissue culture plates with clear bottoms (Thermo Scientific Nunc; Somerset, NJ) at an initial density of 5 × 104 red and without antibiotics, supplemented with 10% FBS and 2 mM l-glutamine. Following 24 h incubation, Caki-1 cells were treated with 30 μM of titancene Y, 50 μM of compounds 4a and 4b, and 10 μM of 5a or 5b for 72 h, while PC3 cells were treated with 30 μM of titancene Y, 60 μM of 4a, 70 μM of 4b, 10 μM of 5a or 5b for 72 h. The cells were then assayed using the ApoTox-Glo triplex assay (Promega GmbH, High-Tech-Park, Mannheim, Germany). Twenty microliter portions of viability/cytotoxicity reagent containing both glycyphenylalaninylaminofluorocoumarin (GF-AFC) and bis-alanylan- lanyl-phenylalanyl-rodhamine 110 (bis-AAF-R110) substrates were added to each well, and they were briefly mixed by orbital shaking at 200 rpm for 30 s and then incubated at 37 °C for 2 h. Fluorescence was measured at 400 nm for excitation/505 nm for emission (viability) and 485 nm for excitation/530 nm for emission (cytotoxicity/necrosis) using a BioTek Fluorescence Microplate Reader (BioTek U.S., Winooski, VT)). Next, 100 μL of Caspase-Glo 3/7 reagents was added to each well, and the samples were briefly mixed by orbital shaking at 200 rpm for 30 s and then incubated at room temperature 57,58.

Determination of the Solvent Disorder Area and Removal of its Contribution to the Neutral-Atom Scattering Factors were used. The program SQUEEZE, a routine within the CAMERON software (Gaussian09,52) was used to determine the nature of the stationary points.

Details of the crystallographic data and a complete list of selected structural parameters are given in Tables S1 and S2 in the Supporting Information, respectively. The crystal was mounted on a glass fiber, and the diffraction measurements were performed with a Nonius Kappa CCD area-detector diffractometer with Mo Kα radiation (λ = 0.71073 Å). The structure were solved by direct methods and refined by least-squares techniques on weighted F2 values for all reflections (SHELXTL, 6.14). All non-hydrogen atoms were assigned anisotropic displacement parameters and refined without positional constraints. All hydrogen atoms were calculated with a riding model. Complex neutral-atom scattering factors were used. The program SQUEEZE, a routine within the CAMERON software (Gaussian09,52) was used to determine the nature of the stationary points.

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for 1 h. Luminescence was measured for 1 s and is proportional to the amount of caspase activity present (BioTek U.S., Winooski, VT). The results for each treatment were expressed as fold change between nontreated (0.1% DMSO) and treated samples. ApoTox-Glo trypex assays were repeated twice (n = 2), and each repetition was run in quadruplicate. The average of the four values was used for statistical calculations. The data are presented as the mean values.

In Vitro Migration Assay (Wound Healing Assay). For the assessment of cell migration, confluent PC3 cells maintained in standard medium were wounded with a plastic micropipette tip (tip 20–200 μl). After washing, the medium was replaced by fresh medium containing 5 μM of either 5a or 5b, 15 μM of titancene Y, and 15 μM of 4a or 4b or 0.1% DMSO solution (control). Photographs of the wounded area were taken after 0 and 24 h using phase-contrast microscopy. For evaluation of wound closure, four randomly selected points along each wound area were marked and the horizontal distance of migrating cells from the initial wound was measured (Labomed TCM400 Inverted Phase Microscope Series, equipped with a digital camera (Fisher Scientific Moticam 10). The assays were done twice, and for each trial two images were analyzed per time point.

Method for Thioredoxin Reductase Activity Assay. Whole cell lysates was assayed using PC3 cells treated in vitro with 5 μM of 5a or 5b, 15 μM of titancene Y, 30 μM of 4a or 4b, or 0.1% DMSO solution (control). After 5 or 24 h of treatment, cells were washed three times in PBS and lysed by douncing using scrappers and sheer force through a syringe with a 34 gauge in assay buffer (Abcam Thioredoxin Reductase Assay Kit, ab83463) with 1 mM Protease Inhibitor Cocktail (Abcam, ab65621). The lysates were centrifuged at 10000 rcf for 15 min at 4 °C to isolate insoluble material. The total protein concentrations of soluble lysates were measured using the Bradford assay. The soluble lysates were incubated for 20 min in assay buffer or assay buffer with a proprietary thioredoxin reductase specific inhibitor before adding a specific substrate, DTNB (5,5′-dithiobis(2-nitrobenzoic acid)), and measuring the activity at 1 min intervals for 30 min using a BioTek Fluorescence Microplate Reader (BioTek U.S., Winooski, VT) at λ 412 nm. Lysates were tested in duplicate. TrxR activity was calculated on the basis of the linear amount of TNB (2-nitro-S-thiobenzoic acid) produced per minute per milligram of total protein and adjusted for background activity from enzymes other than TrxR in the lysates.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.6b00051.

NMR, IR and UV–vis spectra of all new compounds, MS ESI+ spectra of all compounds and theoretical isotopic distributions of relevant peaks, DFT calculations for all new compounds, crystallographic data for 4c, interaction of monometallic gold compounds 4a–d with plasmid pBR322 DNA, migration assays with compounds 4a,c, and inhibition of thioredoxin reductase (TrxR) studies of titancene Y at 5 and 24 h (PDF)

Cartesian coordinates for calculated structures (XYZ)

Crystallographic data for 4c (CIF)

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Notes

The authors declare no competing financial interest.

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**REFERENCES**

(1) (a) Fernández-Gallardo, J.; Elie, B. T.; Sadhukha, T.; Prabha, S.; Sanaí, M.; Rotenberg, S. A.; Ramos, J. W.; Contel, M. Chem. Sci. 2015, 6, 5269–5283. (b) Contel, M.; Fernández-Gallardo, J.; Elie, B. T.; Ramos, J. W. U.S. Pat. Appl. 9,315,531, 2016, 2015.

(2) Pelletier, F.; Comte, V.; Massard, A.; Wenzel, M.; Toulot, S.; Richard, P.; Picquet, M.; Le Gendre, P.; Zava, O.; Edafe, F.; Casini, A.; Dyson, P. J. Med. Chem. 2010, 53, 6923–6933.

(3) González-Pantoja, J. F.; Stern, M.; Jarzecck, A. A.; Royo, E.; Robles-Escajeda, E.; Varela-Ramírez, A.; Aguilera, R. J.; Contel, M. Inorg. Chem. 2011, 50, 11099–11110.

(4) Lease, N.; Naslevski, V.; Carreira, M.; de Almeida, A.; Sanaí, M.; Hirva, P.; Casini, A.; Contel, M. J. Med. Chem. 2013, 56, 5806–5818.

(5) Anderson, C. M.; Taylor, I. R.; Tibbetts, M. F.; Philpott, J.; Hu, Y.; Tanski, J. M. Inorg. Chem. 2012, 51, 12917–1294 references therein.

(6) Tripathy, S. K.; De, U.; Dehury, N.; Pal, S.; Kim, H. S.; Patra, S. Dalton Trans. 2014, 43, 14546–14549

(7) Wenzel, M.; Bertrand, B.; Eymin, M.-J.; Comte, V.; Harvey, J. A.; Richard, P.; Groessl, M.; Zava, O.; Amrouche, H.; Harvey, P. D.; Le Gendre, P.; Picquet, M.; Casini, A. Inorg. Chem. 2011, 50, 9472–9480.

(8) Fernandez-Gallardo, J.; Elie, B. T.; Sulzmaier, F.; Sanaí, M.; Ramos, J. W.; Contel, M. Organometallics 2014, 33, 6669–6681.

(9) Boselli, L.; Carraz, M.; Mazeres, S.; Paleque, L.; Gonzalez, G.; Benoît-Vical, F.; Valentin, A.; Hemmert, C.; Gornitzka, H. Organometallics 2015, 34, 1046–1055.

(10) Bertrand, B.; Citta, A.; Franken, I. L.; Picquet, M.; Folda, A.; Scalcon, V.; Rigobello, M. P.; Le Gendre, P.; Casini, A.; Bodio, E. J. Inorg. Chem. 2015, 20, 1005–1020.

(11) Massai, L.; Fernandez-Gallardo, J.; Guerrì, A.; Arcangeli, A.; Pillozzi, S.; Contel, M.; Messier, L. Dalton Trans. 2015, 44, 11067–11076.

(12) Bjelosevic, H.; Guzei, I. A.; Spencer, L. C.; Persson, T.; Kriel, F. H.; Hewer, R.; Nell, M. J.; Gut, J.; van Resburg, C. E. J.; Rosenthal, P.; Coates, J.; Darkowa, J.; Elmoth, S. K. C. J. Organomet. Chem. 2012, 720, 52–59.

(13) Wenzel, M.; Bigaeva, E.; Richard, E.; Le Gendre, P.; Picquet, M.; Casini, A.; Bodio, E. J. Inorg. Biochem. 2014, 141, 10–16.

(14) Fernandez-Moreira, V.; Marzo, I.; Gimeno, C. Chem. Sci. 2014, 5, 4434–4446.

(15) Zou, T.; Lum, C. T.; Lok, C.-N.; Zhang, J.-J.; Che, C.-M. Chem. Soc. Rev. 2015, 44, 8786–8801 and references therein.

(16) Bertrand, B.; Casini, A. Dalton Trans. 2014, 43, 4209–4219 and references therein.

(17) Oehninger, L.; Ott, I. Dalton Trans. 2013, 42, 3269–3284 and references therein.

(18) Liu, W.; Gust, R. Chem. Soc. Rev. 2013, 42, 755–773 and references therein.

(19) Barnard, P. J.; Berners-Price, S. J. Coord. Chem. Rev. 2007, 251, 1889–1902 and references therein.

(20) Rubbiani, R.; Can, S.; Kitancovic, I.; Alborzinia, H.; Stefanopoulou, M.; Kokoschka, M.; Monchgesang, S.; Sheldrick, W. S.; Wolf, S.; Ott, I. J. Med. Chem. 2011, 54, 8646–8657.
