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Titanocene−Phosphine Derivatives as Precursors to Cytotoxic Heterometallic $TiAu_2$ and TiM (M = Pd, Pt) Compounds. Studies of Their Interactions with DNA

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Supporting Information

ABSTRACT: A series of tri- and bimetallic titanium−gold, titanium−palladium, and titanium−platinum derivatives of the general formulas $[Ti\{\eta^5\text{-}C_5H_4(CH_2)_nPPh_2(AuCl)\}_2]\cdot 2THF$ [n = 0 (**1**); n = 2 (**2**); n = 3 (**3**)] and $[TiCl_2\{\eta^5\text{-}C_5H_4\kappa\text{-}(CH_2)_nPPh_2\}_2(MCl_2)]\cdot 2THF$ [M = Pd, n = 0 (**4**); n = 2 (**5**); n = 3 (**6**) ; M = Pt, n = 0 (**7**); n = 2 (**8**); n = 3 (**9**)] have been synthesized and characterized by different spectroscopic techniques and mass spectrometry. The molecular structures of compounds **1**−**9** have been investigated by means of density functional theory calculations. The calculated IR spectra of the optimized structures fit well with the experimental IR data obtained for **1**−**9**. The stability of the heterometallic compounds in deuterated solvents [$CDCl_3$, dimethyl sulfoxide (DMSO)-d^6, and mixtures 50:50 DMSO-d^6/D_2O and 1:99 DMSO-d^6/D_2O at acidic and neutral pH] has been evaluated by ^{31}P and ^1H NMR spectroscopy showing a higher stability for these compounds than for Cp_2TiCl_2 or precursors $[Ti\{\eta^5\text{-}C_5H_4(CH_2)_nPPh_2\}_2]$. The new compounds display a lower acidity (1−2 units) than Cp_2TiCl_2. The decomposition products have been identified over time. Complexes **1**−**9** have been tested as potential anticancer agents, and their cytotoxicity properties were evaluated in vitro against HeLa human cervical carcinoma and DU-145 human prostate cancer cells. $TiAu_2$ and TiPd compounds were highly cytotoxic for these two cell lines. The interactions of the compounds with calf thymus DNA have been evaluated by thermal denaturation (**1**−**9**) and by circular dichroism (**1**, **3**, **4**, and **7**) spectroscopic methods. All of these complexes show a stronger interaction with DNA than that displayed by Cp_2TiCl_2 at neutral pH. The data are consistent with electrostatic interactions with DNA for $TiAu_2$ compounds and for a covalent binding mode for TiM (M = Pd, Pt) complexes.

INTRODUCTION

Metallocene dihalides (Cp_2MCl_2, where Cp = cyclopentadienyl and M = Ti, V, Nb, Mo, Re) were the first organometallic compounds with antitumor properties to be identified.[1,2] Titanocene dichloride (Cp_2TiCl_2; Chart 1) was the first nonplatinum metal complex to enter clinical trials in 1993.[3] Cp_2TiCl_2 exhibited considerable antitumor activity in in vitro and in vivo experimental models with significant cytotoxicity even in cisplatin-resistant cells and tumors generally difficult to treat.[4,5] However, the efficacy of Cp_2TiCl_2 in phase II clinical trials in patients with metastatic renal cell carcinoma[6] or metastatic breast cancer was too low to be pursued.[7] Nevertheless, the absence of any effect on the proliferative activity on the bone marrow, which is the usual dose-limiting side effect of organic drugs, was a promising result that suggested Cp_2TiCl_2 may have significant potential for possible use in combination therapy. The hydrolysis chemistry and mode of action of Cp_2TiCl_2 have been investigated.[4,5,8] Cp_2TiCl_2 hydrolyzes at pH above 4, and there is protonation and a loss of Cp ligands and the formation of insoluble titanium oxo $(Ti-O-Ti)_n$ species. This instability in solution and lack of standard formulation were contributing factors to discontinuing Cp_2TiCl_2 from clinical trials (although promising results have been recently obtained with different mixtures of solvents and aged solutions[9] or by a controlled-release system by a electrospun fiber[10]). Titanium derived from administered Cp_2TiCl_2 accumulates in the nucleic acid rich regions of tumor cells and exhibits pronounced inhibition of nucleic acid synthesis. An interaction with biomolecules may occur to facilitate the transport, uptake, and delivery of titanium to the nucleus. It was demonstrated that Cp_2TiCl_2 and other titanium compounds interact with transferrin at blood plasma pH values, taking the vacant Fe^{III} binding sites in transferrin. The bound Ti^{IV} is released to adenosine triphosphate (ATP) at cellular endosomal pH values.[11] It was proposed that ATP would facilitate the transport of Ti^{IV} to the nucleus and interaction with deoxyribonucleic acid (DNA).[12] Recent studies on the interaction of titanium compounds with transferrin[13] point to a nonredox release of titanium from transferrin into the cell (different from that of iron).[13a] Computational and some experimental studies have provided information about titanocene binding sites for human serum albumin[14] and DNA.[15] pH-dependent hydrolysis

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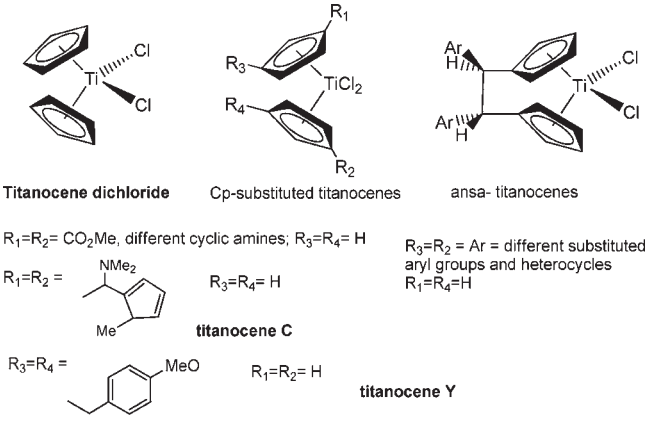
Chart 1. Selected Cytotoxic Titanocene Derivatives

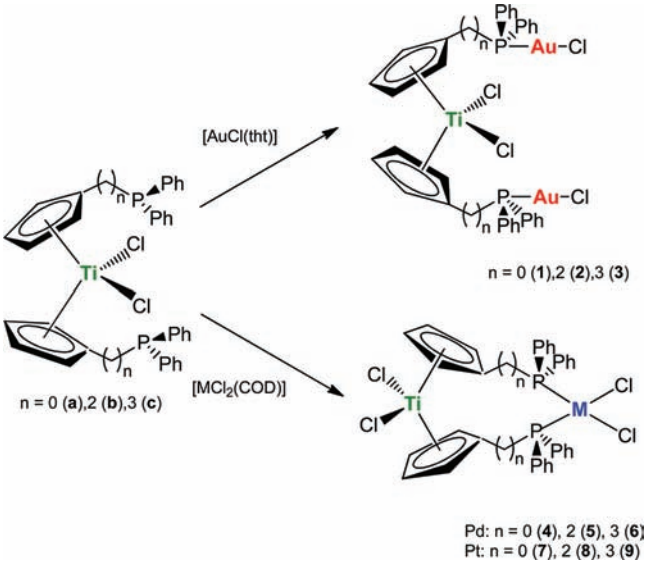


of the Cp ligands is a critical property required for activity. Slow hydrolysis permits the titanium(IV) species to be maintained in a lipophilic environment on a time scale that allows uptake of titanium by transferrin. Cytotoxic titanium derivatives, which have more stable Cp—Ti bonds, may have a different mechanism of action.[4] During the last 10 years, new cytotoxic Cp-derived and other coordination titanium complexes have been reported (selected examples in Chart 1).[1,4,5,16−20]

The majority of derivatives have incorporated electron-donating substituents. One of the main advances in the field came from the introduction of a basic group (alkylammonium substituents) in the Cp ring through a general route patented by McGowan and co-workers in 2004.[21] Substitution of the Cp groups with new protonated cyclic amines and arylamines[19] and some other aryl and alkyl side chains (extensive work by Strohfeldt and Tacke[16] and some others[22]) increased the solubility in water of titanocene dichloride or improved the cytotoxicity. From these Cp-substituted titanocenes, those named titanocene C and titanocene Y (Chart 1) were the more successful. Titanocene Y was tested against a range of freshly explanted human tumors, and its sensitivity was highly remarkable in the case of renal cell, ovarian, nonsmall-cell lung, colon, and prostate cancer,[19,23] as well as in MCF-7 xenograft models[24] and A431 xenografts in vivo.[25] More impressive were the studies in vivo on human renal cancer cells (Caki-1) in mice,[26] which may lead to clinical tests against metastatic renal cancer.[26,27] Titanocene Y was highly cytotoxic also in prostate cancer cell lines and induced more apoptosis (specifically DNA-damage-induced apoptosis)[19] than cisplatin for these cell lines by overexpression of Bcl-2.[28] Modifications of the substituents of titanocenes based on Y[29−31] or substitution of chloride ligands by fluoride,[32] oxalate,[33] or other anions[34] may be another approach to increase the cytotoxicity of titanocenes. More recently, cytotoxic steroid-functionalized titanocenes have been described.[35] The mode of action of Cp_2TiCl_2 and titanocene C has been investigated, but only limited information has been obtained dealing with the transport of titanium inside the cells, accumulation in the nucleus, and DNA damage.[5]

The number of polymetallic complexes (homo- or heterometallic) that have been evaluated as anticancer agents is more limited,[36] especially for titanium.[37] The hypothesis is that the incorporation of two different cytotoxic metals in the same molecule may improve their activity as antitumor agents because of interaction of the different metals with multiple biological

Scheme 1. Synthesis of Titanocene—Phosphine Trimetallic $TiAu_2$ and Bimetallic TiM (M = Pd, Pt) Complexes (1—9)



targets. We have chosen gold, palladium, and platinum as second cytotoxic metals. While the cytotoxicity of platinum derivatives has long been known,[38] the number of reports with phosphine ligands is much more limited.[39] Gold compounds are currently considered as a class of metallodrugs with great potential for cancer treatment[40] (including some examples from our laboratories[41]), and cytotoxic palladium derivatives have also been reported.[42] As far as we are aware, trimetallic $TiAu_2$ complexes have not been investigated before.[43]

We have used previously reported titanocene—phosphine derivatives (Scheme 1, **a**—**c**)[44] as backbones to obtain heterometallic titanocene compounds. While this work was in progress, cytotoxic bimetallic Ti—Ru compounds incorporating a similar skeleton with only one phosphine-containing Cp were reported.[37b]

We describe here the preparation, characterization, stability in solution, and study of the cytotoxic properties of trinuclear $TiAu_2$ and dinuclear TiM (M = Pd, Pt) heterometallic titanocene compounds (Scheme 1). The study of their interactions with calf thymus DNA (CT-DNA) by spectroscopic techniques is also reported.

RESULTS AND DISCUSSION

1. Chemistry. The new heterometallic compounds can be obtained in moderate-to-high yields following the procedure depicted in Scheme 1. The titanocene—phosphine derivatives **a**—**c** had been described before.[44] All of the heterometallic compounds obtained are stabilized with two molecules of tetrahydrofuran (THF; see the Experimental Section). Gold(I) compounds (**1**—**3**) have a linear configuration for the Au^I center, whereas Pd^{II} (**4**—**6**) and Pt^{II} (**7**—**9**) centers have a square-planar arrangement with a cis disposition of the phosphine and chloro ligands, as demonstrated by their ^{31}P chemical shifts (δ) and $^{195}Pt—^{31}P$ coupling constants (J).[45] When starting materials $MCl_2(NCPh)_2$ (M = Pd, Pt) were reacted with titanocene—phosphine **a** instead of $MCl_2(cod)$, a mixture of cis and trans isomers in a 50:50 ratio were obtained: δ(cis) = 30.8 (s) ppm (**4**),

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Table 1. Structural Parameters and Frequencies of Selected Normal Modes (IR Spectroscopy) of Complexes 1−9 Obtained from DFT Calculations[a]

	Ti−Au			Ti−Pd			Ti−Pt		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
average bond length Ti−Cp (Å)	2.451	2.420	2.420	2.425	2.436	2.435	2.425	2.437	2.435
average bond length Ti−Cl (Å)	2.319	2.361	2.363	2.345	2.344	2.347	2.343	2.344	2.353
angle Cl−Ti−Cl (deg)	98.15	94.91	94.88	95.40	96.78	94.66	95.40	96.75	93.36
average bond length M−Cl (Å)	2.309	2.309	2.310	2.398	2.365	2.370	2.367	2.379	2.382
angle P1−M−Cl1 (deg)	177.31	179.28	179.28	88.13	87.09	86.74	89.28	87.85	87.28
angle P2−M−Cl2 (deg)[c]	179.28	179.28	179.28	84.72	81.70	82.58	85.38	82.38	82.69
average angle Cl1−M−Cl2 (deg)				88.31	90.15	90.05	86.63	87.94	87.30
average bond length M−P (Å)	2.294	2.289	2.290	2.338	2.356	2.349	2.302	2.319	2.317
average angle P1−M−P2 (deg)				98.77	103.89	103.45	98.65	103.57	104.15
calcd IR M−Cl (cm^{-1})	339	337	337	A[b] 304	A[b] 296	A[b] 296	A[b] 299	A[b] 294	A[b] 291
				S[b] 327	S[b] 317	S[b] 317	S[b] 324	S[b] 316	S[b] 315
exptl IR M−Cl (cm^{-1})	303	308	307	281	283	285	270	281	273
calcd IR M−P (cm^{-1})	526	538	529	A[b] 448	A[b] 479	A[b] 431	A[b] 524	A[b] 456	A[b] 530
				S[b] 455	S[b] 495	S[b] 437	S[b] 559	S[b] 487	S[b] 540
exptl IR M−P (cm^{-1})	477	477	472	420	450	423	448	480	477

[a] *Gaussian 09* program package (see the Experimental Section). [b] A = asymmetric vibration; S = symmetric vibration. [c] In the case of $TiAu_2$ derivatives: P1−Au1−Cl1 and P2−Au2−Cl2. Compounds **2** and **3** are symmetric and values are identical; in the case of TiM (M = Pd, Pt) derivatives: P1−M−Cl1 and P2−M−Cl2.

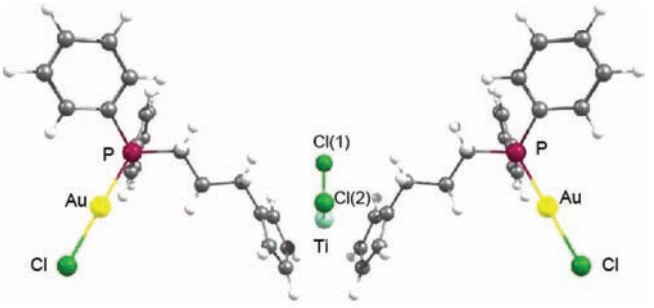

Figure 1. Optimized structure of $[Ti\{\eta^5\text{-}C_5H_4(CH_2)_3PPh_2(AuCl)\}_2]$ (**3**) obtained from DFT calculations.

8.0 ppm (s), J_{P-Pt} = 3642 Hz (**7**); δ(trans) = 16.9 (s) ppm (Pd), 12.2 ppm (s), J_{P-Pt} = 2396 Hz (Pt). Thus, titanocene−phosphine derivatives **b** and **c** were reacted with MCl_2(cod) (M = Pd, Pt) for the preparation of pure cis compounds **5**, **6**, **8**, and **9**. Related complexes $[TiCl_2\{\eta^5\text{-}C_5H_4\kappa PPh_2\}_2M(C_6F_5)_2)]$ (M = Pd, Pt) were obtained as the trans isomers although no specification was made and they were not characterized by crystallographic methods.[46] In bimetallic $[Cp_2Ti(OSO_2(CH_2)_2\text{-}PPh_2\}_2MCl_2]$ (M = Pd, Pt; where the Ti is bonded to two phosphines through the oxygen of the sulfonato group), the Pd and Pt atoms are also in a cis environment.[37a] All of the new compounds (**1**−**9**) have been fully characterized (see the Experimental Section), and they are air-stable solids for short periods of time (2 days) but can be stored under nitrogen for months. Unfortunately, we could not get single crystals of enough quality to perform an X-ray diffraction study of these compounds.

We were able to investigate the molecular structures of **1**−**9** by means of density functional theory (DFT) calculations (see the Experimental Section). The optimized structures show a distorted

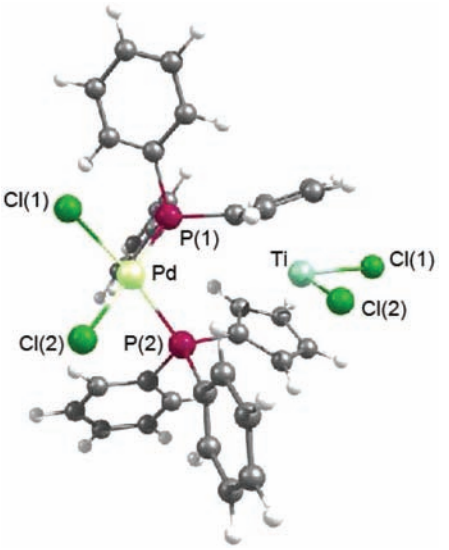

Figure 2. Optimized structure of $[TiCl_2\{\eta^5\text{-}C_5H_4\kappa PPh_2\}_2(PdCl_2)]$ (**4**) obtained from DFT calculations.

tetrahedral arrangement of the Cp rings and chloro ligands around the Ti centers in all cases (Table 1). The calculated distances Ti−Cl and Ti−C are within the range for those in a related heterometallic Ti−Rh sandwich complex whose crystal structure is known as $[Ti\{\eta^5\text{-}C_5H_4(CH_2)_2PPh_2RhCl(CO)\}_2]$ [Ti−Cl 2.382(2), 2.375(2) Å; Ti−C 2.321(6)−2.435(5) Å].[47] The Au centers are in a nearly linear arrangement (see the structure of **3** in Figure 1), whereas the Pd and Pt centers are in a distorted square-planar arrangement with the ligands in a cis disposition (see the structure of **4** in Figure 2). The two parts in the molecule of $TiAu_2$ containing the Cp rings and phosphine−gold−chloride

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fragments are symmetrical (C_s symmetry for the molecule) in the case of the compounds with an alkyl spacer (**2** and **3**).

When there is no alkyl spacer between the phosphorus and the Cp ring, the molecule has a degree of distortion and there is no symmetry in this case (**1**).

The distortion in the square-planar arrangement of the palladium and platinum compounds is weaker for the compounds with a phosphine ligand incorporating an alkyl spacer (**5**, **6**, **8**, and **9**). The angles P1−M−P2 are wider than the expected 90° (Table 1) in all cases (**4**−**9**), indicating a wide "bite angle" of the titanium-containing bidentate phosphine ligands. The distances M−Cl and M−P are within the expected range for gold(I), palladium(II), and platinum(II) complexes and do not merit further explanation.

Frequencies of selected normal modes (in IR spectroscopy) were also determined by DFT methods (Table 1) for the optimized structures of compounds **1**−**9**. The calculated and the experimental frequencies of M−Cl and M−P stretching modes are in reasonable agreement to validate the calculated structures for these complexes. It is worth mentioning that reported DFT frequencies are not refined by any empirical scaling procedures and, therefore, are expected to be slightly overestimated (by about 10−15%).[48] This is partly due to computational limitation, such as basis set truncation or electron correlation effects and, to a lesser extent, to the anharmonicity of a given vibration.

All of the complexes are completely soluble in dimethyl sulfoxide (DMSO) and in mixture 1:99 DMSO/H_2O. DMSO is necessary to exchange the coordinated THF and favor their solubility in H_2O. The pH values of the new heterometallic complexes have been measured in 1:99 DMSO/H_2O, and they are less acidic than titanocene dichloride under the same conditions (pH = 3.12). The more acidic gold derivatives are those without a spacer (n = 0: **1**, pH = 4.16), whereas the introduction of a spacer or chain between the phosphine atom and the Cp ring increases the basicity of the resulting $TiAu_2$ compound (n = 2, **2**, pH = 5.05; n = 3, **3**, pH = 5.09). In the case of palladium and platinum, different effects were observed (n = 0, **4**, pH = 4.40; **7**, pH = 4.25; n = 2, **5**, pH = 3.90; **8**, pH = 4.55; n = 3, **6**, pH = 4.06; **9**, pH 4.15), although the pH values are very similar in these cases. The fact that they are less acidic than titanocene dichloride is of importance in their future application as anticancer agents. As commented on before, Cp_2TiCl_2 hydrolyzes at pH above 4 and there is protonation and a loss of Cp ligands and the formation of insoluble titanium oxo $(Ti-O-Ti)_n$ species. This instability in solution and lack of standard formulation were contributing factors to discontinuing Cp_2TiCl_2 from clinical trials.

By ^{31}P NMR spectroscopy, we have been able to quickly evaluate their stability in solution. Compounds **1**−**3** are stable for months in $CDCl_3$ at 0 °C, and palladium and platinum complexes **4**−**9** are also stable at room temperature. Importantly, for biological testing, the compounds are stable in DMSO-d_6 solutions at room temperature for at least 2 days. For titanocene dichloride, DMSO resulted in a faster Cp loss than water or other organic solvents.[49] Studies in mixtures 50:50 DMSO-d_6/D_2O have shown that all derivatives are stable for at least 24 h (most of them for even 2−3 weeks). Detailed studies in mixtures of DMSO-d_6/D_2O (1:99) have shown that the compounds are stable for 24 h, and after that time, decomposition takes place (see a detailed table and selected spectra in the Supporting Information). The heterometallic compounds are in all cases more stable than the parent phosphine-containing titanocenes (**a**−**c**), which are totally decomposed after 1 week (**a**) or just 36 h (**b** and **c**). Compounds of TiPd and TiPt containing titanium fragments **a**−**c** (**4**−**9**) are still present (60%) after 1 week, while the $TiAu_2$ derivatives are less stable (**1**−**3** present at 30−40% after 1 week). The main products of decomposition are phosphine oxides, indicating a cleavage of the phosphine−second metal (Au, Pd, or Pt) and a cleavage of the Ti−Cp bonds. The first cleavage seems to be for the Ti−Cp bond as species that can be assigned to *cis*-$Pt(PPh_2Cp)_2Cl_2$ are identified as the first decomposition products by $^{31}P\{^1H\}$ NMR spectroscopy for the TiPt derivatives (see the table and selected spectra in the Supporting Information). The second cleavage M−P occurs faster for $TiAu_2$ and TiPd compounds. For all these complexes, decoordination of the Cp ring from the Ti center can be observed in ^1H NMR when the products start to decompose. The stabilities of $[Ti\{\eta^5\text{-}C_5H_4PPh_2\}_2]$ (**a**) and its heterometallic complexes $[Ti\{\eta^5\text{-}C_5H_4(CH_2)_nPPh_2(AuCl)\}_2]$ (**1**) and $[TiCl_2\{\eta^5\text{-}C_5H_4\kappa\text{-}(CH_2)_nPPh_2\}_2(MCl_2)]$ [M = Pd (**4**), Pt (**7**)] were studied in a mixture of DMSO-d^6/D_2O at physiological pH = 7.39 [5 mM Tris/$NaClO_4$ buffer (50 mM $NaClO_4$)] and compared with the stability of Cp_2TiCl_2 in D_2O at pH = 7.39. While the titanocene−phosphine derivative **a** decomposed to phosphine oxide almost completely upon the addition of a deuterated buffer, decomposition of the heterometallic complexes was slower, and after 24 h, a 54−60% of the $TiAu_2$ and TiM species was still present at neutral pH (see the Supporting Information). Titanocene dichloride was totally decomposed after 16 h. Thus, the stabilities of the new heterometallic compounds are higher than that of titanocene dichloride or the starting titanocene−phosphine derivatives at neutral pH.

Table 2. IC_{50} of Titanium Complexes Obtained after Exposure to Human Cancer Cells[a]

compound		HeLa	DU-145
$[Ti\{\eta^5\text{-}C_5H_5\}_2Cl_2]$ titanocene dichloride (**C1**)		*b*	*b*
$[Ti\{\eta^5\text{-}C_5H_4PPh_2\}_2Cl_2]$ (**a C2**)		16.87	*b*
$[\{AuCl\}_2(\mu\text{-dppe})]$ (**C3**)		13.17	3.87
$[PdCl_2(dppe)]$ (**C4**)		*b*	*b*
$[PtCl_2(dppe)]$ (**C5**)		*b*	*b*
$TiAu_2$ derivatives	**1**	2.44	27.26
	2	35.58	*b*
	3	1.12	14.16
TiPd derivatives	**4**	37.53	49.84
	5	19.16	*b*
	6	4.25	*b*
TiPt derivatives	**7**	33.58	*b*
	8	*b*	*b*
	9	*b*	73.51

[a] All values in the table are in micromolar. All compounds were dissolved in 1% DMSO and diluted with water. Titanocene dichloride, Cp_2TiCl_2, was diluted in water. [b] Complexes show low toxicity against human cancer cells. When the maximum toxicity observed experimentally was less than 30%, it was considered risky to calculate IC_{50}; therefore, the titanium compound was categorized as low toxic. For these cases, see tables with values in the Supporting Information.

2. Cytotoxicity Studies. Cytotoxicity results for the heteronuclear compounds are collected in Table 2 and in tables in the Supporting Information. The cytotoxicity (by a live-cell imaging

method; see the Experimental Section) was evaluated against two selected cell lines: HeLa human cervical carcinoma and DU-145 human prostate cancer cell line. The new compounds are much more cytotoxic than titanocene dichloride (Cp_2TiCl_2) for the HeLa cell line, with the best values obtained in the case of the $TiAu_2$ and TiPd derivatives [especially for ligands with the spacer $(CH_2)_3$, $n = 3$]. For HeLa cells, the gold compounds **1** and **3** and the palladium derivative **6** are also more cytotoxic than cisplatin ($IC_{50} = 14.9\ \mu M$).[41a] More importantly, some of the compounds [like $TiAu_2$(**1** and **3**) and TiPd with no spacer (**4**)] are quite cytotoxic for the DU-145 human prostate cell line. The more cytotoxic titanocene compounds against this cell line described to date (titanocene Y and C) had values of IC_{50} of 30−50 μM[19] (cisplatin for this cell line was less cytotoxic,[19] and we have obtained a value of 70 μM[50]). TiPd compound **4** and especially $TiAu_2$ compounds **1** and **3** are more cytotoxic for this cell line than the titanocene derivatives and cisplatin. The TiPt compound with a propylic spacer (**9**) displays a cytotoxicity similar to that of cisplatin. The compounds that do not show values had a much lower cytotoxicity. They did not kill half of the cells at the highest soluble concentrations tested, and at lower concentrations, their IC_{50} values were too low. Unfortunately, at high concentrations, the compounds precipitated in the media and thus their IC_{50} values could not be calculated. However, it seems reasonable to assume that their cytotoxicity is quite low (values in tables in the Supporting Information).

We studied the cytotoxicity of the titanocene−phosphine starting material **a** (**C2** in Table 2) as well as the cytotoxicity of complexes of gold(I), palladium(II), and platinum(II) with a bidentate phosphine [1,2-bis(diphenylphosphino)ethane, dppe] in order to make comparisons with the new heterometallic complexes. However, these highly lipophilic dppe compounds are only soluble in DMSO and precipitate easily in concentrations above 4−15 μM (depending on the metal; see the Supporting Information) while added to the culture media. Compound **a** (**C2**) and the gold derivative [$\{AuCl\}_2(\mu$-dppe)] (**C3**) display cytotoxicities in HeLa cells that are similar to that of cisplatin (Table 2). The new compounds **1**, **3**, and **6** are more cytotoxic than **C2** and **C3**. The palladium and platinum controls (**C4** and **C5**) have a very low cytotoxicity (Table 2 and the Supporting Information). Besides, we studied the cytotoxicity of combinations of titanocene dichloride Cp_2TiCl_2 and the bidentate phosphine metal complexes to assess a possible additive or synergistic effect (see the Supporting Information). Combinations of Cp_2TiCl_2 (**C1**) and [$\{AuCl\}_2(\mu$-dppe)] (**C3**), **C1** and [$PdCl_2$(dppe)] (**C4**, and **C1**) and [$PtCl_2$(dppe)] (**C5**; see details in the Supporting Information) showed in all cases a low cytotoxicity (nonadditive or synergistic effect) in HeLa cells.

In DU-145 cells, the cytotoxicity was low for combinations of titanium and palladium or titanium and platinum compounds (**C1** and **C4** or **C1** and **C5**) and a little higher for the combination of **C1** and the gold compound (**C3**). In these cases, an additive effect was observed for the gold and palladium combinations and no effect was observed for the platinum compounds. The gold compound**C3** itself was surprisingly quite cytotoxic for this cell line (more cytotoxic than the more cytotoxic $TiAu_2$ and TiPd compounds reported here). Some gold(III) compounds seem to hold excellent potential as drugs for prostate cancer,[40b] but we are not aware so far of reports on gold(I) derivatives.

These results are consistent with what was found for bimetallic platinum titanocene derivatives[37b] and for the recently reported bimetallic ruthenium titanocene complexes.[37a] In both cases, the cytotoxicity of the bimetallic species is higher than that of monometallic titanium, platinum, and ruthenium species.[37] It could be argued that the cytotoxicity could come from degraded species (after 24 h in physiological media at neutral pH, 55−60% of the heterometallic species are present in solution). The main decomposition product (phosphine oxide) is not cytotoxic. NMR data indicate that the heterometallic complexes decompose to species containing the second metal (Au, Pd, or Pt) prior to total decomposition. However, from experiments done with equivalent second metal-containing species (**C3**−**C5**), we have demonstrated that the heterometallic species are, in general, more cytotoxic.

It seems clear that the new trinuclear and dinuclear compounds possess peculiar chemicophysical properties with respect to their precursors responsible for the observed biological effects (especially in HeLa cell lines). These results also strongly support the main hypothesis of our proposal: that the incorporation of a second different cytotoxic metal in titanocenes can improve their activity as antitumor agents.

Table 3. Changes in T_m of CT-DNA after Incubation with Complexes Cisplatin,[a] Cp_2TiCl_2,[a] and 1−9[b] for 1 h in 5 mM Tris/$NaClO_4$ Buffer (50 mM $NaClO_4$) at pH = 7.39 and $r = 0.5$

compound	ΔT_m (°C)/1 h	ΔT_m (°C)/24 h
DNA−cisplatin	−7.08	- 6.35
DNA−titanocene dichloride	0.65	0.20
DNA−**1** ($TiAu_2$, $n = 0$)	8.65	5.67
DNA−**2** ($TiAu_2$, $n = 2$)	6.80	3.04
DNA−**3** ($TiAu_2$, $n = 3$)	4.25	2.05
DNA−**4** (Ti−Pd, $n = 0$)	5.91	−1.10
DNA−**5** (Ti−Pd, $n = 2$)	4.15	−0.60
DNA−**6** (Ti−Pd, $n = 3$)	2.15	−0.35
DNA−**7** (Ti−Pt, $n = 0$)	6.51	−1.97
DNA−**8** (Ti−Pt, $n = 2$)	5.05	−1.43
DNA−**9** (Ti−Pt, $n = 3$)	4.25	−1.01

[a] Compounds dissolved in buffer. [b] Compounds dissolved in a 1:99 DMSO/buffer solution.

3. Interactions with CT-DNA. Because the DNA biopolymer is involved in cellular replication, it becomes an interesting target in cancer chemotherapy. It is well-known that most cytotoxic platinum drugs present strong covalent bonds with the DNA bases,[51] although a variety of platinum compounds act as DNA intercalators while coordinated to the appropriate ancillary ligands.[52] There are also reports on palladium derivatives interacting with DNA in covalent[42a,53] and noncovalent ways.[54] While most gold(III) and gold(I) compounds display reduced affinity for DNA,[40,41a] gold(I) phosphine derivatives with weakly bound ligands (such as halides) bind in a nondenaturing fashion to DNA.[55] Although initial studies suggested that the effect of titanocene derivatives might be related to DNA interaction,[56] later investigations indicate that metallocene dihalides neither bind strongly to DNA at neutral pH nor suppress DNA-processing enzymes.[57] More recent reports provide experimental evidence of titanium being accumulated in the cellular nucleic acid rich regions, particularly in chromatin.[58] Moreover, it has been suggested that Cp_2TiCl_2 or, more likely, Ti^{IV} ions can interact weakly with the phosphate groups of nucleotides at neutral pH.[15b,59,60] The DNA binding ability of the nine heterometallic complexes was investigated by the use of two different techniques

Table 4. Changes in T_m of CT-DNA after Incubation with Complexes 1−3 for 1 and 24 h with Different Ionic Strengths of 5 mM Tris/$NaClO_4$ Buffer at pH = 7.39 and r = 0.5

compound	ΔT_m (°C)/1 h of incubation/ 25 mM $NaClO_4$	ΔT_m (°C)/1 h of incubation/ 50 mM $NaClO_4$	ΔT_m (°C)/24 h of incubation/ 25 mM $NaClO_4$	ΔT_m (°C)/24 h of incubation/ 50 mM $NaClO_4$
DNA−**1** ($TiAu_2$, n = 0)	11.39	8.65	7.35	5.67
DNA−**2** ($TiAu_2$, n = 2)	9.75	6.80	6.31	3.04
DNA−**3** ($TiAu_2$, n = 3)	7.75	4.25	5.73	2.05

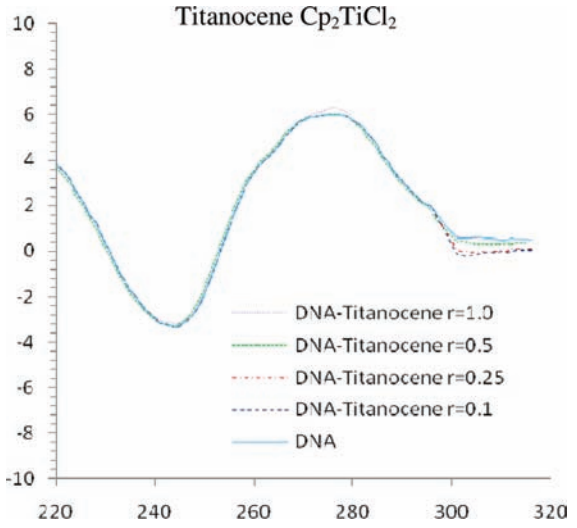

Figure 3. CD spectra of CT-DNA incubated for 24 h at 37 °C with Cp_2TiCl_2 at 0, 0.1, 0.25, 0.5, and 1 ratios.

and directly compared to that of cisplatin and titanocene dichloride (Cp_2TiCl_2).

3.1. Thermal Denaturation Experiments. The melting point technique is a sensitive and easy tool to detect even slight DNA conformational changes. It is known that a destabilizing interaction with the double helix (typically, covalent) is observed as a decrease in the melting point (T_m), while a stabilizing interaction (usually by intercalation or by electrostatic attraction) induces an increase of T_m. Bearing that in mind, CT-DNA was incubated for different periods of time (1 or 24 h) with each drug at a ratio of 2:1 DNA/drug (see the Experimental Section for details). The results are summarized in Table 3. These experiments indicate that all of the compounds tested (including $TiCp_2Cl_2$ and cisplatin) have interactions with DNA, as demonstrated by a change in T_m.

Complexes Cp_2TiCl_2 and **1**−**9** produce a stabilizing effect (increase in T_m) on DNA. This increase is especially pronounced for the gold compounds and for the compounds that do not contain an alkylic spacer between the Cp ring and phosphine (**1**, **4**, and **7**). The increase is significantly higher than that of titanocene dichloride (Table 3).

Because the studies of cytotoxicity are performed at 20 h and subsequent more detailed DNA conformational studies (circular dichroism, CD) are also performed after 20 h of incubation with DNA, we studied the behavior of DNA after 24 h of incubation with the complexes and controls. We have found that compounds **1**−**9** are stable in a solution of DMSO/H_2O (1:99) for 24 h as studied by NMR spectroscopy (see the Results and Discussion Section and Supporting Information). At physiological neutral pH, we have found that the compounds are less stable, but they are more stable than Cp_2TiCl_2 or the starting titanocene−phosphine, and after 24 h, there is still 60% $TiAu_2$ or 55% Ti−M (M = Pd, Pt) heterometallic compounds. However, when DNA is present in the neutral solutions, it interacts with the drugs (Table 3), but we cannot anticipate if decomposition of the complexes or Cp cleavage from titanium may be accelerated or slowed down.

After 24 h of incubation with DNA, the stabilizing effect is observed only for the three $TiAu_2$ derivatives. A very small effect is observed for titanocene dichloride, indicating a small interaction with DNA. The complexes TiM (M = Pd, Pt) show a decrease in T_m, indicating covalent bonding to DNA similar to that produced by cisplatin (Table 3). The increase or decrease of T_m is quite similar at 24 h for complexes with an ethyl or propyl spacer between the Cp and phosphine ligands for all of the heterometallic complexes. It seems that the stabilizing effect observed for all of the heteronuclear compounds after 1 h of incubation with DNA is due to an initial electrostatic interaction with the Ti center (which hydrolyses very quickly, exchanging Cl^- ligands with OH^- groups).[49] When the Cp−Ti bonds start to be cleaved (in less than 24 h), Ti ions may be released and interaction of DNA with the metal may be due, at least in part, to the second metal-containing (Au, Pd, or Pt) degraded species.

In order to assess whether the $TiAu_2$ complexes are stabilized by electrostatic interaction (a most plausible interaction for these complexes because its structure does not seem suitable for an intercalation effect), a different ionic strength of 5 mM Tris/HCl buffer was employed (25 mM $NaClO_4$; Table 4). At low Na^+ concentrations, the increase of T_m is higher, most likely because of the complexes stabilizing DNA through electrostatic binding. On the other hand, at high salt concentration, the stabilizing effects are reduced because the electrostatic component is lowered by an increase of the concentrations of Na^+ counterions. T_m is lower in all cases after incubation for 24 h, which better resembles the conditions for the cytotoxicity experiments. In the case of the compounds with an alkyl spacer (**2** and **3**) at high salt concentration (double the amount of Na^+), T_m is reduced to half after 24 h. This effect is smaller for compound **1** but still noticeable.

From these experiments, it seems clear that all new compounds show interaction with DNA. This interaction (which is stronger for the heterometallic compounds than for titanocene dichloride) seems to be covalent in nature for TiM (M = Pd, Pt) complexes and mostly of electrostatic nature for the $TiAu_2$ derivatives.

3.2. CD Spectroscopy Studies. More detailed DNA conformational alterations can be detected by means of CD spectroscopy. We selected the $TiAu_2$ derivatives (**1**) and TiM complexes [M = Pd (**4**), Pt (**7**)], which display stronger interaction with DNA by T_m experiments (Table 3), to carry out these studies. Additionally, we studied the interaction of the most cytotoxic compound

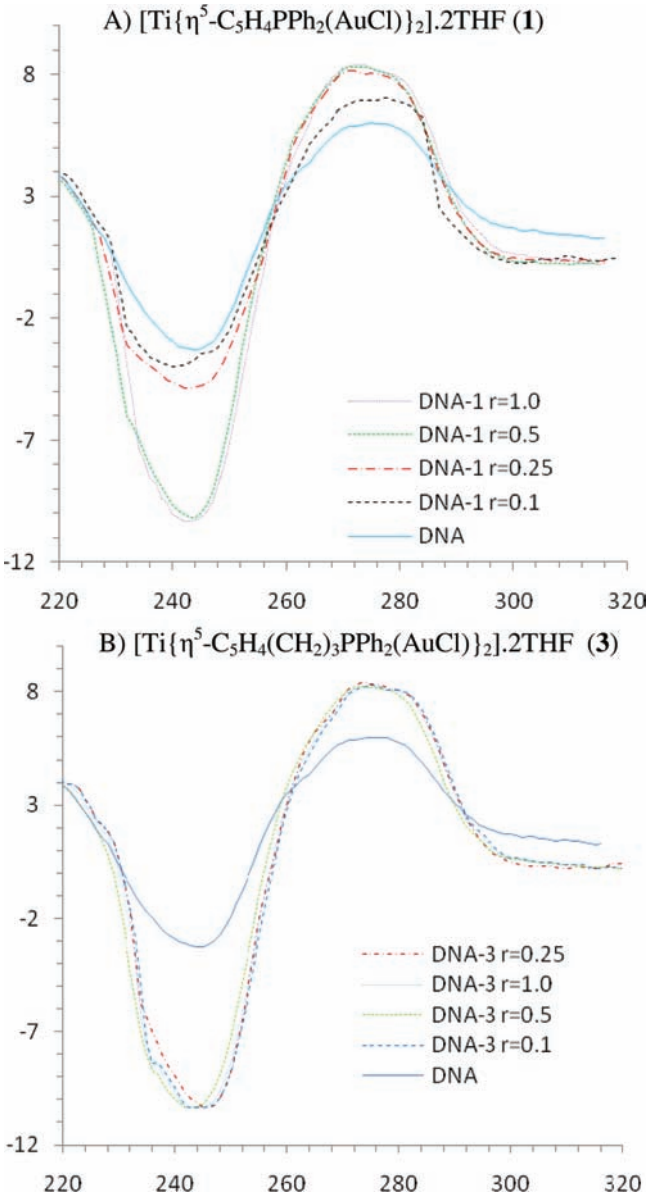

Figure 4. CD spectra of CT-DNA incubated for 24 h at 37 °C with $TiAu_2$ derivatives **1** (A) and **3** (B) at 0, 0.1, 0.25, 0.5, and 1 ratios.

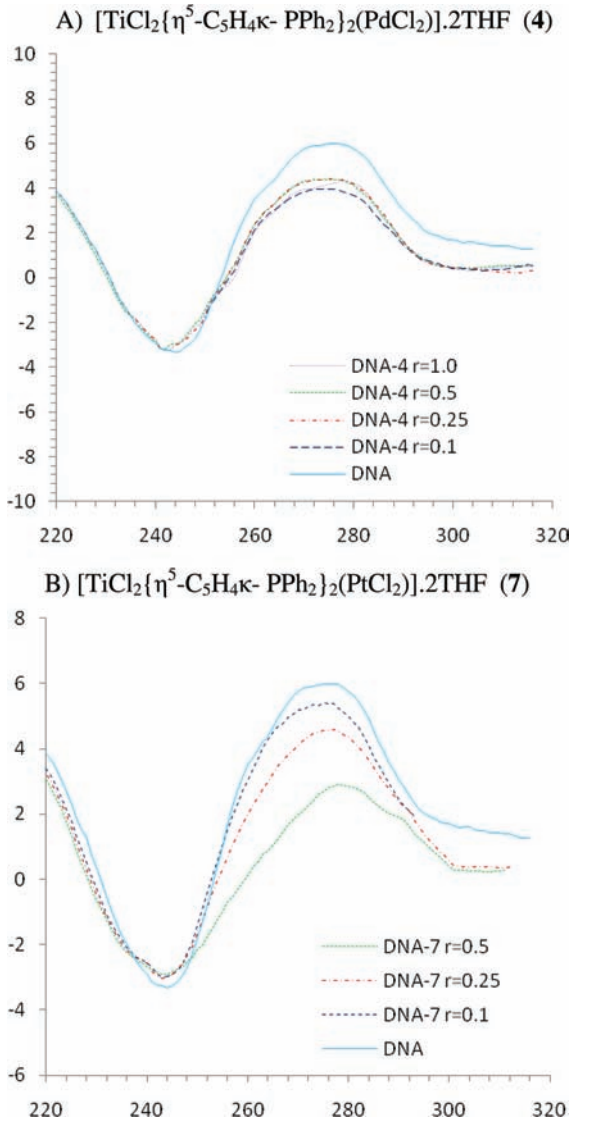

Figure 5. CD spectra of CT incubated for 24 h at 37 °C with Ti−Pd **4** (A) and Ti−Pt **7** (B) derivatives at 0, 0.1, 0.25, 0.5, and 1 ratios.

$TiAu_2$ (**3**). Figure 3 shows a CD spectrum of DNA in its B conformation treated with increasing amounts of titanocene dichloride Cp_2TiCl_2. The band of the CD spectrum of DNA at 275 nm is due to stacking between base pairs, while the band at 248 nm originated because of the right-handed helicity.[61] In the CD technique, interaction of the drug with DNA in the region between 275 and 248 nm and the changes in the curve can be attributed to conformational modifications in the double helix.

When CT-DNA is incubated with increasing amounts of Cp_2TiCl_2, there are no noticeable changes at small DNA/drug ratios (up to r = 0.5), and only at ratios of 1 is there a small increase of the positive band, indicating a stabilizing effect most plausibly by intercalation or electrostatic effects. We have not found publications describing CD spectroscopic studies for DNA−titanocene dichloride interactions. The results are in agreement with what we found for the melting point experiments with titanocene dichloride. It seems that the interaction of titanocene dichloride with DNA is not very strong and does not seem to be covalent in nature. This is in agreement with the recent investigations of titanocene dichlorides, which indicate they do not interact strongly with DNA at neutral pH.[57]

When CT-DNA is incubated with increasing amounts of $TiAu_2$ derivatives (**1** and **3**), slight increases of the intensities of the positive and especially the negative bands are observed (Figure 4). These changes in the wavelength and ellipticity of free DNA indicate modifications on the secondary structure of DNA as a consequence of interaction of the complexes with DNA.[62] This type of change has been previously observed in similar experiments with gold(I) phosphine halide derivatives such as AuCl(PEt$_3$).[55] For the positive bands, there is also a shift to lower energy when the amount of drug added is increased (from 278 to 274 cm^{-1} for **1** and to 271 cm^{-1} for **3**). The negative bands show a shift to higher energy when the amount of drug added is increased (from 238 to 242 cm^{-1} for **1** and to 244 cm^{-1} for **3**). This interaction has been reported before as the binding of $AuPR_3^+$ cations to the heterocyclic bases of DNA in a nondenaturating

and reversible fashion. This only happens for compounds with a weakly bound ligand such as chlorine or bromine.[55] In these cases, increasing the ionic strength of the medium served to decrease the extent of interaction between the gold(I) complex and DNA.[55] This is totally in agreement with what we observed in the T_m studies and indicates an interaction of the $TiAu_2$ compounds with DNA of electrostatic nature. When CT-DNA is incubated with increasing amounts of TiM derivatives [TiPd (**4**) and TiPt (**7**); Figure 5], slight decreases of the intensities of the negative and especially the positive bands are observed. Those changes in the stacking and helicity of CT-DNA are consistent with a covalent binding mode to DNA in a cis-bidentate fashion.[63] These changes are more noticeable for the TiPt derivative **7** (as indicated by the T_m experiment; see Table 3). In both cases, there is a bathochromic shift for the positive (from 278 to 270 cm^{-1} for **4** and to 274 cm^{-1} for **7**) and negative (from 238 to 243 cm^{-1} for **4** and to 243 cm^{-1} for **7**) bands.

In conclusion, the experiments of DNA−drug interactions have shown that Cp_2TiCl_2 seems not to interact strongly with DNA at physiological pH in vitro and that this interaction is electrostatic in nature. The new heterometallic compounds interact more strongly with DNA. In the same period of time that cytotoxicity studies are carried out, this type of drug−DNA interaction depends partly on the second metal-containing degraded species. Thus, interaction of $TiAu_2$ derivatives with DNA is dominated by electrostatic effects, while interaction of DNA with d^8 metals (Pd^{II} and Pt^{II}) seems to be covalent in nature. The length of the alkyl chain (n = 0, 2, 3) between the phosphorus and the Cp ring is also an important factor, and the strength of the interaction decreases with increasing n (more flexibility of the "bidentate titanocene phosphine" in compounds with n = 2 and 3). However, the cytotoxicity of the compounds may not be related in all cases to these metal−DNA interactions. In the case of $TiAu_2$, interactions of the Au cations with thiol or selenol groups of transport and mitochondrial proteins may play a major role.[40,51b]

CONCLUSIONS

Organometallic compounds are currently being studied as potential anticancer agents because they exhibit chemicophysical properties intermediate between organic drugs and metallic coordination compounds. We have prepared trinuclear $TiAu_2$ and binuclear TiM (M = Pd, Pt) complexes based on a titanocene−phosphine backbone. Their stability in different deuterated solvents (including buffer solutions) can be quickly evaluated by $^{31}P\{^1H\}$ NMR spectroscopy. These complexes are less acidic and more stable in DMSO/D_2O (1:99) solutions at acidic and neutral pH than titanocene dichloride. TiPd and especially $TiAu_2$ complexes are highly cytotoxic in vitro against HeLa human cervical carcinoma and DU-145 human prostate cancer cells. In general, the most cytotoxic compounds incorporate the propyl spacer between the P atom and Cp ring. They are more active than their parent titanocene dichloride and titanocene−phosphine derivatives and second metal-related precursors (with the exception of Au for the DU-145 cell line).

Interactions of the compounds with CT-DNA have been evaluated by thermal denaturation and CD spectroscopic methods. All of these complexes show a stronger interaction with DNA than that displayed by Cp_2TiCl_2 at neutral pH. The data are consistent with electrostatic interactions with DNA for $TiAu_2$ compounds and for a covalent binding mode for TiM (M = Pd, Pt) complexes. However, with these data, we cannot propose that DNA is the ultimate biomolecular target for these compounds. These preliminary results strongly support that incorporation of a second different cytotoxic metal in titanocenes can improve their activity as antitumor agents, as has been recently reported for a related family of Ti−Ru bimetallic complexes.[37b] We are currently working on ligand/anion modifications for the most cytotoxic compounds to increase their stability as well as in the study of reactions of the compounds with transport and mitochondrial proteins to gain insight into the plausible mode of action of these types of heteronuclear complexes.

EXPERIMENTAL SECTION

1. Synthesis and Characterization of the Heterometallic Complexes. All manipulations involving syntheses of titanium complexes (**a**−**c**) and heterometallic complexes were performed at an argon/vacuum manifold using standard Schlenk-line techniques under an argon atmosphere or in a glovebox MBraun MOD System. Solvents were purified by use of a PureSolv purification unit from Innovative Technology, Inc. The phosphine substrates were purchased from Aldrich and used without further purification. Titanocene−diphenylphosphine complexes (**a**−**c**),[44] [AuCl(tht)],[64] [$PdCl_2$(cod)],[65] and [$PtCl_2$(cod)][66] were prepared as previously reported. NMR spectra were recorded in a Bruker AV400 (^1H NMR at 400 MHz, ^{13}C NMR at 100.6 MHz, ^{31}P NMR at 161.9 MHz, and ^{195}Pt NMR at 86.1 MHz). Chemical shifts (δ) are given in ppm using $CDCl_3$ as the solvent, unless otherwise stated. ^1H and ^{13}C NMR resonances were measured relative to solvent peaks considering tetramethylsilane = 0 ppm, and $^{31}P\{^1H\}$ NMR was externally referenced to H_3PO_4 (85%). Coupling constants J are given in hertz. IR spectra (4000−250 cm^{-1}) were recorded on a Nicolet 6700 Fourier transform infrared spectrophotometer on KBr pellets. Elemental analyses were performed on a Perkin-Elmer 2400 CHNS/O series II analyzer. Mass (MS) spectra (electrospray ionization, ESI) were performed on an Agilent analyzer and on a Bruker analyzer. DNA thermal denaturation experiments were performed with a Cary 100 Bio UV−visible spectrophotometer. CD spectra were taken in a Chirascan CD spectrometer equipped with a thermostatted cuvette holder. The pH was measured in an OAKTON pH conductivity meter in 1:99 DMSO/H_2O solutions.

*[Ti{η^5-$C_5H_4(CH_2)_nPPh_2(AuCl)$}$_2$]·2THF [n = 0 (**1**), 2 (**2**), 3 (**3**)].* [AuCl(tht)] (0.06 g, 0.20 mmol) was added to a dichloromethane solution (15 mL) of **a** (0.07 g, 0.10 mmol, for the preparation of **1**), **b** (0.07 g, 0.10 mmol, **2**), or **c** (0.08 g, 0.10 mmol, **3**) at 0 °C (ice bath). The reaction mixture was stirred for 20 min afterward, the ice bath was removed, and the mixture was stirred for 10 min at room temperature. The yellow oil formed was collected after evaporation, and the volatiles were removed under vacuum. The residue was washed with *n*-hexane (10 mL) and diethyl ether (10 mL) and dried under reduced pressure to afford **1** as a white solid, or **2** and **3** as pale-yellow solids. **1**. Yield: 0.070 g, 60%. Anal. Calcd for $C_{42}H_{44}Au_2Cl_4O_2P_2Ti$ (1226.38): C, 41.13; H, 3.62. Found: C, 41.02; H, 3.64. MS(ESI+) [m/z (%)]: 1225 $[M]^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 30.8 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 2.73 (m, 16H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([8.15×10^{-4} M] in 1:99 DMSO/H_2O) = 4.16. **2**. Yield: 0.064 g, 50%. Anal. Calcd for $C_{46}H_{52}Au_2$ $Cl_4O_2P_2Ti$ (1282.49): C, 43.08; H, 4.09. Found: C, 43.28; H, 4.01. MS(ESI+) [m/z (%)]: 1281 $[M]^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 29 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 1.66 (m, 8H,

THF), 2.13 (t 2H, PCH_2, $^3J_{HH}$ = 8), 2.45 (t 2H, $C_5H_4CH_2$, $^3J_{HH}$ = 8), 3.66 (m, 8H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, C_{ipso}-C_5H_4), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([7.79 × 10^{-4} M] in 1:99 DMSO/H_2O) = 5.05. **3**. Yield: 0.08 g, 59%. Anal. Calcd for $C_{48}H_{56}Au_2Cl_4O_2P_2Ti$ (1310.54): C, 43.99; H, 4.31. Found: C, 44.01; H, 4.27. MS(ESI+) [*m*/*z* (%)]: 1309 $[M]^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 28.0 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ (m, 8H, THF), 3.15 (dd, 2H, CH_2), 4.62 (dd, 1H, $^3J_{HH}$ = 10, $^3J_{HH}$ = 5), 5.47 (m, 1H), 6.68 (m, 1H, C_5H_4), 6.90 (m, 2H, C_5H_4), 7.18 (m, 1H, C_6H_4), 7.33 (m, 1H, C_6H_4), 7.42 (m, 1H, C_6H_4), 8.09 (m, 1H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, CH_2), 46.8 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([7.67 × 10^{-4} M] in 1:99 DMSO/H_2O) = 5.09.

*[TiCl$_2${η^5-C$_5$H$_4$κ-(CH$_2$)$_n$PPh$_2$}$_2$(PdCl$_2$)]·2THF [n = 0 (**4**), 2 (**5**), 3 (**6**)].* [$PdCl_2$(cod)] (0.03 g, 0.10 mmol) was added to a dichloromethane solution (15 mL) of **a** (0.07 g, 0.10 mmol, for the preparation of **4**), **b** (0.07 g, 0.10 mmol, **5**), or **c** (0.08 g, 0.10 mmol, **6**) at room temperature. The reaction mixture was stirred for 30 min. The red (**4**), yellow (**5**), or orange-yellow (**6**) oil formed was collected by evaporation, and the volatiles were removed under vacuum. The residue was washed with cold *n*-hexane (10 mL) at 0 °C and dried under reduced pressure to afford **4** as a red solid, **5** as a pale-yellow solid, or **6** as a pale-orange solid. **4**. Yield: 0.058 g, 62%. Anal. Calcd for $C_{42}H_{44}PdCl_4O_2P_2Ti$ (938.86): C, 53.73; H, 4.72. Found: C, 53.99; H, 4.80. MS(ESI+) [*m*/*z* (%)]: 937 $[M]^-$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 30.8 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 2.73 (m, 16H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([9.77 × 10^{-4} M] in 1:99 DMSO/H_2O) = 4.40. **5**. Yield: 0.051 g, 51%. Anal. Calcd for $C_{46}H_{50}PdCl_4O_2P_2Ti$ (994.97): C, 55.53; H, 5.25. Found: C, 55.22; H, 5.09. MS(ESI+) [*m*/*z* (%)]: 993 $[M]^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 30.8 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 1.66 (m, 8H, THF), 2.13 (t, 2H, PCH_2, $^3J_{HH}$ = 8), 2.45 (t, 2H, $C_5H_4CH_2$, $^3J_{HH}$ = 8), 3.66 (m, 8H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([9.72 × 10^{-4} M] in 1:99 DMSO/H_2O) = 3.90. **6**. Yield: 0.073 g, 72%. Anal. Calcd for $C_{48}H_{54}PdCl_4O_2P_2Ti$ (1023.03): C, 56.35; H, 5.52. Found: C, 56.21; H, 5.68. MS(ESI+) [*m*/*z* (%)]: 1022 $[M]^+$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 30.8 (s). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 2.73 (m, 16H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, CH_2), 46.8 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, C_{ipso}-C_6H_4). Value of pH ([9.75 × 10^{-4} M] in 1:99 DMSO/H_2O) = 4.06.

*[TiCl$_2${η^5-C$_5$H$_4$κ-(CH$_2$)$_n$PPh$_2$}$_2$(PtCl$_2$)]·2THF [n = 0 (**7**), 2 (**8**), 3 (**9**)].* [$PtCl_2$(cod)] (0.03 g, 0.10 mmol) was added to a dichloromethane solution (15 mL) of **a** (0.07 g, 0.10 mmol for the preparation of 7), **b** (0.07 g, 0.10 mmol, **8**), or **c** (0.08 g, 0.10 mmol, **9**) at room temperature. The reaction mixture was stirred for 30 min. The yellow oil formed was collected by evaporation, and the volatiles were removed under vacuum. The residue was washed with cold *n*-hexane (10 mL) at 0 °C and dried under reduced pressure to afford **7** as a white solid, **8** as a pale-yellow solid, or **9** as a beige solid. **7**. Yield: 0.080 g, 80%. Anal. Calcd for $C_{42}H_{44}PtCl_4O_2P_2Ti$ (1027.53): C, 49.09; H, 4.32. Found: C, 48.89; H, 4.28. MS(ESI+) [*m*/*z* (%)]: 1026 [M]. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 8.0 (s, J_{P-Pt} = 3642). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 2.73 (m, 16H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([9.72 × 10^{-4} M] in 1:99 DMSO/H_2O) = 4.25. **8**. Yield: 0.087 g, 80%. Anal. Calcd for $C_{46}H_{50}PtCl_4O_2P_2Ti$ (1081.62): C, 50.99; H, 4.84. Found: C, 50.20; H, 4.78. MS(ESI+) [*m*/*z* (%)]: 1080 [M]. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 8.3 (s, J_{P-Pt} = 3648). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 1.66 (m, 8H, THF), 2.13 (t, 2H, PCH_2, $^3J_{HH}$ = 8), 2.45 (t, 2H, $C_5H_4CH_2$, $^3J_{HH}$ = 8), 3.66 (m, 8H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([9.72 × 10^{-4} M] in 1:99 DMSO/H_2O) = 4.55. **9**. Yield: 0.090 g, 82%. Anal. Calcd for $C_{48}H_{54}PtCl_4O_2P_2Ti$ (1109.67): C, 51.86; H, 5.08. Found: C, 51.00; H, 5.06. MS(ESI+) [*m*/*z* (%)]: 1108 $[M]^-$. $^{31}P\{^1H\}$ NMR ($CDCl_3$): δ 8.1 (s, J_{P-Pt} = 3640). ^1H NMR (plus COSY, plus NOESY, $CDCl_3$): δ 2.73 (m, 16H, THF), 7.54 (m, 8H, C_5H_4), 7.56 (m, 8H, C_6H_4), 7.75 (m, 12H, C_6H_4). $^{13}C\{^1H\}$ NMR (plus APT, plus HSQC, $CDCl_3$): δ 22.6 (−, PCH_2), 34.2 (−, CH_2), 46.8 (−, $C_5H_4CH_2$), 122.4, 123.1, 124.9 (all +, C_5H_4), 125.5 (+, C_6H_4), 127.6 (+, C_5H_4), 128.9, 129.3, 129.5 (all +, C_6H_4), 130.0 (−, $C_{ipso}C_5H_4$), 139.7 (−, $C_{ipso}C_6H_4$). Value of pH ([9.73 × 10^{-4} M] in 1:99 DMSO/H_2O) = 4.15.

2. Computational Methods. All calculations reported here were carried out by the *Gaussian 09* program package.[67] All structures are found by full geometry optimization at the B3LYP/6-31G* level of DFT in combination with relativistic effective-core potential cc-pVDZ-PP for heavy metals; palladium, platinum, and gold in appropriate compounds were used to describe their core electrons and valence orbitals with the [4s, 4p, 3d, 1f], contracted Gaussians composition,[68] which were treated explicitly in electronic structure calculations. Computed frequencies of all structures are positive, indicating that the optimized structures are at real minima of their ground-state potential energy surfaces. In addition, a simple visual comparison of computed IR spectra with experimental spectra was performed to further validate the reliability of computed structures.

3. Determination of Compound Cytotoxicity. Cells were seeded on 96-well microplate at 10 000 cells/well density in 200 μL culture media consisting of Dulbecco's modified eagle medium supplemented with antibiotics (100 units/mL of penicillin, 100 μg/mL of streptomycin; Invitrogen, Carlsbad, CA) and 10% heat-inactivated fetal bovine serum (Hyclone, Logan, UT). Plates were incubated overnight at 37 °C in a 5% CO_2 humidified atmosphere to promote cell attachment, followed by cell exposure to experimental chemical compounds for 20 h, essentially as described by Lema et al.[69] A total of 1 h prior to capturing the images in live-cell mode, each well was added with a mixture of two fluorescent dyes, propidium iodide (MP Biomedicals, Solon, OH) and Hoechst 33342 (Invitrogen, Eugene, OR), reaching a final concentration of 1 μg/mL.[69] The fluorescence signal emitted from each individual fluorophore was captured in two separate channels, according with the dye emission properties. Images were acquired directly from the tissue culture microplate utilizing a BD Pathway 855 Bioimager system (BD Biosciences, Rockville, MD). To obtain sufficient numbers of regions of interest, for statistical analysis purposes, images from nine contiguous image fields (3 × 3 montages) were acquired per well utilizing a 20× objective.[67] Data analysis determining the percentage of dead cells from each individual well was achieved by using *BD AttoVision v1.6.2* software.[69] Several controls were included in each experiment. DMSO was used as a solvent control at the same final concentration of

0.5 v/v used with the test compounds. Untreated cells were also used as negative controls, as an indicator of the cell viability during the incubation period. Cells treated with 600 μM hydrogen peroxide were included as positive controls for the cytotoxicity activity. The IC_{50} values were determined as previously described.[70] Briefly, the average expressed as a percentage from triplicates of the two compound concentrations closest to the 50% cytotoxicity value was plotted against the chemical compound concentration in a *xy* (scatter) chart function (Microsoft Excel). The best-fit regression line and its equation was used to calculate the concentration of the chemical compound required to damage the plasma membrane of 50% of the cell population, compared to solvent-treated cells (DMSO). On the basis of this approach, there is not standard deviation because the IC_{50} values were calculated by linear extrapolation. Potentially, the two molecules of THF released by dissolution of the new compounds in DMSO/buffer could have a cytotoxic effect.[71] However, the amount of THF released is within the low-micromolar range or lower. LD50 values for THF have been reported for concentrations in the millimolar ranges.[71]

4. Interaction of Metal Complexes with CT-DNA by Thermal Denaturation (Melting Point) Experiments. Melting curves were recorded in media containing 50 mM $NaClO_4$ or 25 mM $NaClO_4$ (Tables 3 and 4) and 5 mM Tris/HCl buffer (pH = 7.39). The absorbance at 260 nm was monitored for solutions of CT-DNA (70 μM) before and after incubation with a solution of the drug under study (35 μM in Tris/HCl buffer) for 1 or 24 h (Tables 3 and 4) at room temperature. The temperature was increased by 2 °C/min between 25 and 60 °C, by 1 °C/min between 60 and 70 °C, by 0.5 °C/min between 70 and 85 °C, and by 1 °C/min between 85 and 98 °C.

5. Interaction of Metal Complexes with CT-DNA by CD Spectroscopy. Stock solutions (5 mM) of each complex were freshly prepared in DMSO/H_2O prior to use. The right volume of those solutions was added to 3 mL samples of an also freshly prepared solution of CT-DNA (195 μM) in Tris/HCl buffer (5 mM Tris/HCl, 50 mM NaClO4, pH = 7.39) to achieve molar ratios of 0.1, 0.25, 0.5, and 1.0 drug/DNA. The samples were incubated at 37 °C for a period of 20 h. All CD spectra of DNA and of the DNA/drug adducts were recorded at 25 °C over a range of 220−420 nm and finally corrected with a blank and noise reduction. The final data are expressed in molar ellipticity (millidegrees).

■ ASSOCIATED CONTENT

Supporting Information. Tables of the stability of compounds **1**−**9** by $^{31}P\{^1H\}$ NMR spectroscopy (1:99 DMSO-d^6/D_2O) and for Cp_2TiCl_2 at different pHs as well as selected spectra and complete cytotoxicity data for the compounds and controls. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

This paper is dedicated to Prof. Roberto Sánchez-Delgado on the occasion of his 61st birthday.

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