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Cyclopalladated Benzophenone Imines: Synthesis, Antitumor Activity, Cell Accumulation, DNA Interaction, and Cathepsin B Inhibition

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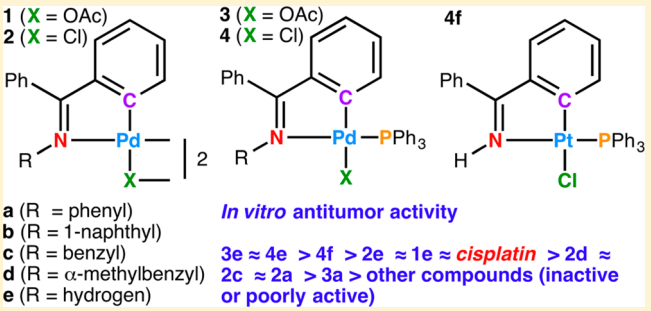
Supporting Information

ABSTRACT: The synthesis of the *endo* five-membered cyclo-*ortho*-palladated benzophenone imines $[Pd\{C_6H_4(Ph)C{=}NR\}]_2(\mu\text{-}X)_2$ [**1** (X = OAc), **2** (X = Cl), **a** (R = phenyl), **b** (R = 1-naphthyl), **c** (R = benzyl), **d** (R = α-methylbenzyl)], and *trans-N,P*-$[Pd\{C_6H_4(Ph)C{=}NR\}X(PPh_3)]$ [**3** (X = OAc), **4** (X = Cl), **a** (R = phenyl), **b** (R = 1-naphthyl), **c** (R = benzyl), **d** (R = α-methylbenzyl)] and the X-ray molecular structure of **1a**, **1c**, **1d**, **4a**, **4b**, and **4c** are reported. The antitumor activity, DNA interaction, and cathepsin B inhibition of palladium compounds **a**–**d** were studied and compared with those previously reported for palladium compounds **e** with R = H and compound **4f** analogous to **4e** but with a platinum(II) center. The IC_{50} values against a panel of human cancer cell lines allowed the establishment of a qualitative relationship between their structure and antitumor activity. Compounds **3e**, **4e**, and **4f** were the most active ones in relation to their in vitro anticancer activity. Compounds **3e** and **4e** were about 4 times more active than cisplatin against the MDA-MB-231 and MCF-7 breast human cancer lines, and compound **4f** was about 4 times more active than cisplatin against the cisplatin-resistant HCT-116 colon human cancer cell line. In addition, compound **3e** was 3 times less cytotoxic than cisplatin toward the quiescent HUVEC cells. Accumulation of palladium compounds **e** and **b** in the MDA-MB-231 cell line was considerably greater than that of cisplatin in the same cell line, but palladium compounds **b** were noncytotoxic. Some of these complexes altered the DNA tertiary structure in a similar way to cisplatin but at higher concentration, and most cytotoxic ones did not present a high efficiency as cathepsin B inhibitors.



INTRODUCTION

In recent years, coordination and organometallic palladium(II) compounds have been explored as an alternative to platinum(II) compounds as anticancer drugs, in part due to the similarity of their substitution reactions.[1,2] A significant difference between the two metal centers is the higher kinetic lability of palladium(II) compared to that of platinum(II). This renders palladium(II) less attractive for anticancer studies since hydrolysis of Pd−Cl bonds is quite fast and gives way to very reactive species that are unable to reach the target biomolecules into the cancer cells.[3] In spite of this, the use of bulky monodentate ligands or chelate bidentate or terdentate ligands has provided palladium(II) compounds that are less reactive and with promising cytotoxicity against different cancer cell lines.[2]

The cytotoxicity of a significant number of cyclopalladated compounds has been explored against a variety of cancer cell lines with interesting outcomes and with some chances of progressing for evaluation as drug candidates.[4−20] For instance, compounds **A** and **B** in Figure 1 presented IC_{50} values against the B16-next2 murine melanoma cell line less than 1.25 μM.

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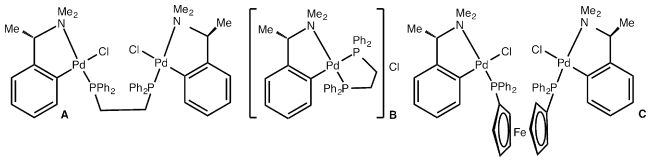
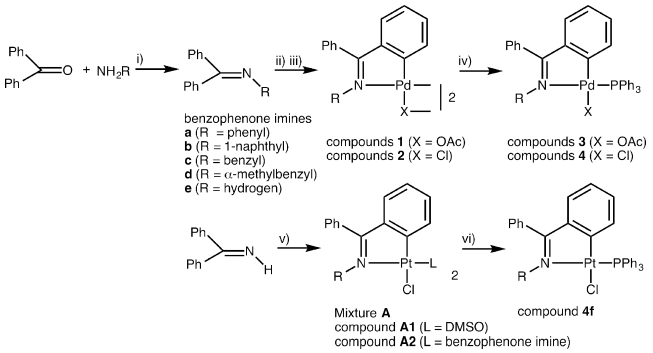


Figure 1. Structural formula of cyclopalladated compounds with chances of progressing for evaluation as drug candidates.

Compound **B** was the most potent in vivo, delaying tumor growth and prolonging animal survival in mice subcutaneously inoculated with the B16F10-Nex2 melanoma cell line. On the other hand, cyclopalladated compound **C** with a bridging 1,1′-bis(diphenylphosphane)ferrocene showed a notable cathepsin B inhibitory activity, with the potential to treat metastatic cancers.[10−18] It should be noted that cyclopalladated compounds **A**−**C** shown in Figure 1 were patented as antitumor drugs.[18]

Recently, we have reported the synthesis of the cyclopalladated benzophenone imines **1e**−**4e** and the cycloplatinated benzophenone imine **4f** (see Scheme 1 for their structural formula), and we have studied their (i) antitumor activity toward MDA-MB-231 and MCF-7 breast and cisplatin-resistant HCT-116 colon human cancer cell lines, (ii) interaction with pBluescript SK + plasmid DNA, (iii) cathepsin B inhibition test, and (iv) antibacterial and antioxidant activity.[19,20] Noteworthy, compounds **3e** and **4e** were about 4 times more active than cisplatin against the MDA-MB-231 and MCF-7 human breast cancer lines, and compound **4f** was also about 4 times more active than cisplatin against the cisplatin-resistant HCT-116 human colon cancer cell line. In addition, compounds **1e**−**4e** and **4f** presented a moderate antibacterial and antioxidant activity.[20]

Scheme 1[a]



[a]Reactions and conditions: (i) $TiCl_4$ (Lewis acid promoter and drying agent) + benzophenone + amine, toluene, 24 h, reflux, molar ratio $TiCl_4$/benzophenone/amine = 1:1:5; (ii) benzophenone imine + $Pd(OAc)_2$, HOAc, 60 °C, 24 h, molar ratio $Pd(OAc)_2$/benzophenone imine = 1:1; (iii) compound **1** + LiCl excess, acetone, room temperature, 2 h; (iv) compounds **1** or **2** + PPh_3, acetone, room temperature, 2 h, molar ratio **1** or **2**/PPh_3 = 1:2; (v) *cis*-$[PtCl_2(DMSO)_2]$, NaOAc (stoichiometric), benzophenone imine (excess), methanol, reflux, 24 h, under N_2; (vi) mixture **A** + PPh_3, acetone, room temperature, 2 h, molar ratio mixture **A**/PPh_3 = 1:1.

Following these studies, we report herein the preparation of cyclopalladated benzophenone imines **a**−**d** (Scheme 1) and the study of (i) the antitumor activity against MDA-MB-231 and MCF-7 human breast and cisplatin-resistant HCT-116 human colon cancer cell lines for compounds **a**−**d**, (ii) the cellular accumulation of the palladium compounds **b** and **e**, (iii) the interaction of compounds **a**−**d** with the pBluescript SK + plasmid DNA, (iv) the interaction of compounds **2b** and **2d** with the pBluescript SK + plasmid DNA in the presence of topoisomerase I, and (v) the cathepsin B inhibition test for the most cytotoxic palladium compounds **a**−**d**. The cytotoxicity of compound **3e**, one of the most antiproliferative complexes, was also studied toward the human umbilical vein endothelial cells (HUVEC) in quiescent and normal culture conditions. It should be noted that these cells in quiescent conditions are used as a model for nonproliferative normal cells. These analyses are compared with those previously reported by our group for palladium compounds **e** and for the platinum compound **4f**.[19,20]

The aim of these studies is to establish structure−activity relationships upon the structure of the proposed compounds and their anticancer activity and the identification of plausible primary target biomolecules for them.

RESULTS AND DISCUSSION

Synthesis and Characterization of the Compounds. The structural formula and the numbering of the compounds under study are given in Scheme 1.

Benzophenone imines **a**−**d** were prepared by a condensation reaction between benzophenone and the corresponding amine using $TiCl_4$ as Lewis acid promoter and drying agent in a molar ratio of 1:1:5 $TiCl_4$/benzophenone/amine by an adaptation of a previously reported method.[21] Imines **a**−**d** were obtained in moderate to high yield and were characterized by EM, IR, and ^1H and $^{13}C\{^1H\}$ NMR. Imine **e** ($Ph_2C{=}NH$) was available commercially and was used as received.

Compounds **1e**−**4e** and **4f** were prepared according to the procedures previously described in our research group.[19,20] Thus, treatment of imine **e** ($Ph_2C{=}NH$) with $Pd(OAc)_2$ in a 1:1 molar ratio in acetic acid at 60 °C for 24 h generated the dinuclear cyclopalladated compound **1e** with acetato bridging ligands in high yield. A subsequent metathesis reaction of compound **1e** with LiCl in acetone at room temperature produced the dinuclear cyclopalladated compound **2e** with chlorido bridging ligands. The mononuclear compounds **3e** and **4e** were obtained by bridge-splitting reactions of the dinuclear compounds **1e** and **2e** with PPh_3 in acetone at room temperature. Mixture **A**, the precursor of compound **4f**, was prepared by reaction of *cis*-$[PtCl_2(DMSO)_2]$ and NaOAc in a 1:1 molar ratio with an excess of benzophenone imine in dry methanol under nitrogen and at reflux for 24 h. A subsequent reaction of mixture **A** with PPh_3 yielded compound **4f**. The purity of compounds **1e**−**4e** and **4f** was checked by IR and ^1H NMR, and purity of compounds **3e**, **4e**, and **4f** was checked in addition by $^{31}P\{^1H\}$ NMR.

Compounds **1**−**4** from series **a**−**d** were obtained by an adaptation of the procedures given above for compounds **1e**−**4e**. It should be noted that the synthesis of compounds **2a**, **2c**, and **4c** was previously claimed,[22,23] but their detailed preparative method and their characterization data were not reported, except for the C, H, and N elemental analyses of **2a**.[23] According to the literature,[24] we propose an *endo* five-membered *ortho*-cyclopalladated structure for the palladium compounds **a**−**d**. The *endo* descriptor denotes that the C=N bond is included in the metalacycle.[25] This assumption was established unambiguously by the XRD molecular crystal structure determination of **1a**·2MeOH, **1c**, **1d**, **4a**, **4b**·MeOH, and **4c**·MeOH (see below). Compounds **1**−**4** from series **a**−**d**

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afforded satisfactory EM, IR, and ^{1}H NMR. In addition, the $^{31}P\{^{1}H\}$ NMR spectra of compounds **3** and **4** from series **a**–**d** were also consistent with their proposed structure.[26]

Details of the preparative method and isolation in pure form of the benzophenone imines **a**–**d** and their cyclopalladated derivatives **1**–**4** and selected characterization data for these compounds are given in the Supporting Information.

XRD Molecular Structures of 1a, 1c, 1d, 4a, 4b, and 4c. Figures 2–7 show ball-and-stick models of the molecular structure of **1a**, **1c**, **1d**, **4a**, **4b**, and **4c** determined by single-crystal X-ray diffraction analysis. Single crystals of **1a**·2MeOH, **1c**, **1d**, **4a**, **4b**·MeOH, and **4c**·MeOH were grown by slow evaporation of the solvents of a solution of the corresponding compound **1** in CH_2Cl_2, MeOH, and acetone in 1:1:1 volume ratio or by the slow evaporation of the solvents of a solution of the corresponding compound **4** in CH_2Cl_2 and MeOH in a volume ratio of 1:1. The crystal structure reports and CIF files for these X-ray molecular crystal structure determinations are

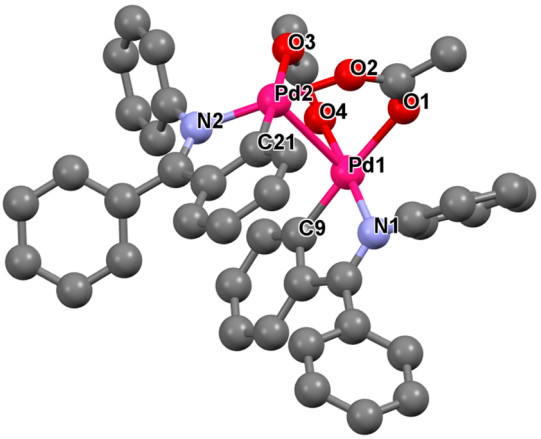

Figure 2. XRD molecular structure of compound **1a**. Selected bond distances (Å) and angles (deg): Pd1–C9 1.950(2), Pd1–N1 2.0191(19), Pd1–O4 2.0576(17), Pd1–O1 2.1247(16), Pd1–Pd2 2.9069(3), Pd2–C21 1.943(2), Pd2–N2 2.0177(19), Pd2–O2 2.0374(16), Pd2–O3 2.1573(16), C9–Pd1–N1 80.77(9), C9–Pd1–O4 93.80(8), N1–Pd1–O4 173.36(7), C9–Pd1–O1 176.17(8), N1–Pd1–O1 95.47(7), O4–Pd1–O1 89.89(7), C21–Pd2–N2 81.27(9), C21–Pd2–O2 92.79(8), N2–Pd2–O2 173.53(7), C21–Pd2–O3 176.31(8), N2–Pd2–O3 95.15(7), O2–Pd2–O3 90.83(7).

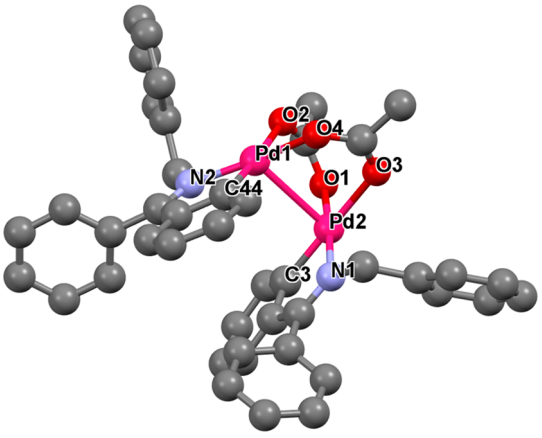

Figure 3. XRD molecular structure of compound **1c**. Selected bond distances (Å) and angles (deg): Pd1–C44 1.968(3), Pd2–C3 1.947(3), Pd1–O2 2.155(2), Pd2–O3 2.147(3), Pd1–N2 2.010(3), Pd2–N1 2.022(3), Pd1–O4 2.055(2), Pd2–O1 2.042(2), Pd1–Pd2 2.8698(4), O2–Pd1–C44 176.26(12), O3–Pd2–C3 176.91(12), O4–Pd1–N2 173.65(11), O1–Pd2–N1 174.78(11), C44–Pd1–N2 81.66(14), C3–Pd2–N1 81.26(13), C44–Pd1–O4 93.70(12), C3–Pd2–O1 93.56(12), O4–Pd1–O2 89.13(10), O1–Pd2–O3 89.42(10), N2–Pd1–O2 95.33(12), N1–Pd2–O3 95.78(10).

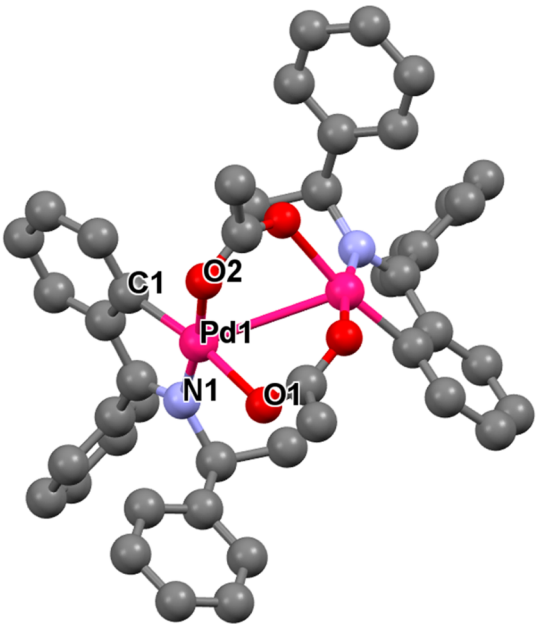

Figure 4. XRD molecular structure of compound **1d**. Selected bond distances (Å) and angles (deg): Pd1–C1 1.957(4), Pd1–N1 2.038(3), Pd1–O1 2.141(3), Pd1–O2 2.050(3), Pd(1)–Pd(1) 3.2037(6), O1–Pd1–C1 171.07(13), N1–Pd1–O2 172.97(12), C1–Pd1–N1 81.19(14), C1–Pd1–O2 92.50(15), O2–Pd1–O1 87.34(11), N1–Pd1–O1 98.40(12).

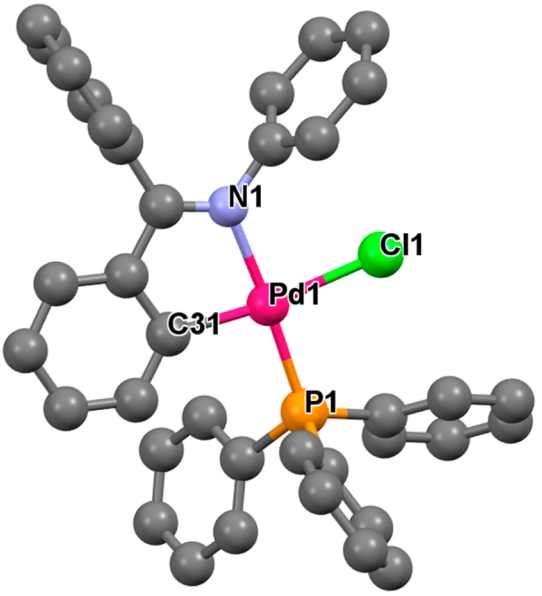

Figure 5. XRD molecular structure of compound **4a**. Selected bond distances (Å) and angles (deg): Pd1–C31 2.015(2), Pd1–Cl1 2.3508(6), Pd1–N1 2.1028(19), Pd1–P1 2.2695(6), N1–Pd1–P1 176.59(5), C(31)–Pd(1)–Cl(1) 170.94(7), C31–Pd1–N1 80.70(8), N1–Pd1–Cl1 90.86(6), P1–Pd1–Cl1 92.42(2), C31–Pd1–P1 96.09(7).

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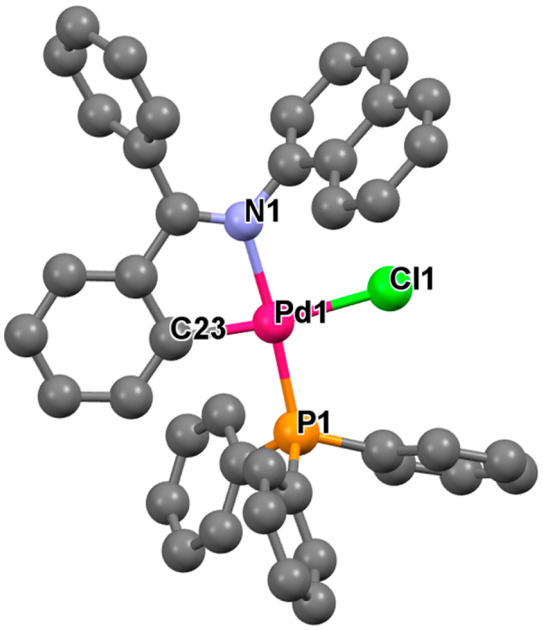

Figure 6. XRD molecular structure of compound **4b**. Selected bond distances (Å) and angles (deg): Pd1−C23 2.0226(19), Pd1−Cl1 2.3647(10), Pd1−N1 2.1162(17), Pd1−P1 2.2651(9), Cl1−Pd1−C23 172.91(6), P1−Pd1−N1 175.62(5), N1−Pd1−C23 81.11(7), Cl1−Pd1−N1 91.83(5), Cl1−Pd1−P1 92.54(3), P1−Pd1−C23 94.52(6).

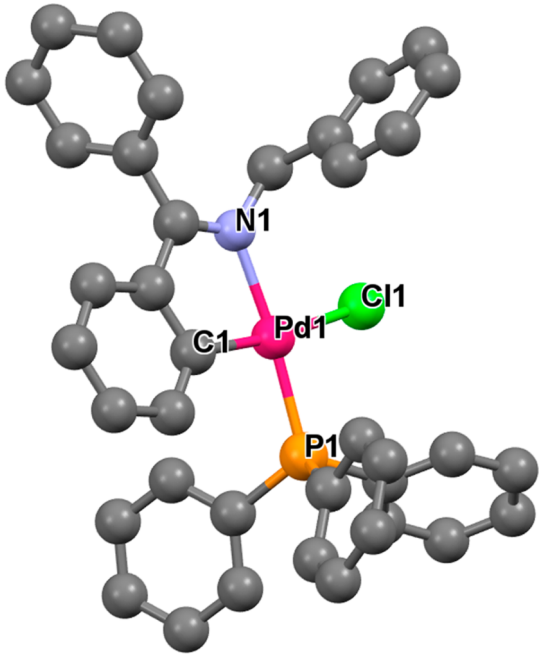

Figure 7. XRD molecular structure of compound **4c**. Selected bond distances (Å) and angles (deg): Pd(1)−C(1) 2.0135(19), Pd(1)−N(1) 2.0964(16), Pd(1)−P(1) 2.2639(6), Pd(1)−Cl(1) 2.3837(5), C(1)−Pd(1)−N(1) 80.69(7), C(1)−Pd(1)−P(1) 93.70(6), N(1)−Pd(1)−P(1) 174.15(5), C(1)−Pd(1)−Cl(1) 173.10(6), N(1)−Pd(1)−Cl(1) 92.62(5), P(1)−Pd(1)−Cl(1) 92.942(19).

given in the Supporting Information. These structures have been also deposited in the Cambridge Crystallographic Data Centre with the following deposition numbers: 1025622 (**4b**·MeOH), 1025623 (**1c**), 1025624 (**1d**), 1025625 (**1a**·2MeOH), 1025626 (**4a**), and 1025627 (**4c**·MeOH).

These molecular structures confirm the proposed structural formula for the compounds under study. Thus, all these molecules can be classified as *endo* five-membered *ortho*-cyclopalladated benzophenone imines. In addition, molecules **1a**, **1c**, and **1d** are dinuclear with the acetato ligands bridging two cyclopalladated units and present a *trans*-folded structure, and molecules **4a**, **4b**, and **4c** are mononuclear and present a *trans-N,P* configuration.

In the crystal of compound **1d**, both halves of the molecules were equivalent because these molecules were situated over crystallographic binary proper rotation axes, but in the crystals of **1a**·2MeOH and **1c**, the molecules of compounds **1a** and **1c** presented two nonequivalent halves, which differed between them in small differences in distances, angles, and torsion angles.

Distances and angles around of the palladium(II) centers for all these molecules were between the normal intervals.[26−29] The chelate bite angles were those from the coordination sphere of the palladium(II) centers with the largest deviations from the ideal angles of 90 and 180° and were 80.77(9) and 81.27(9)° for **1a**, 81.66(14) and 81.26(13)° for **1c**, 81.19(14)° for **1d**, 80.70(8) Å for **4a**, 81.11(7)° for **4b**, and 80.69(7) Å for **4c**. The five-membered metalacycles in all these molecules were planar, and maximum deviated atoms from the five-membered planar metalacycles were C1 0.064 Å and C21 −0.78 Å for **1a**, C39 −0.011(3) Å and N1 0.068(3) for **1c**, C6 0.008(4) Å and C1 −0.008(4) for **1d**, N1 −0.038(2) for **4a**, N1 0.021(2) for **4b**, and N1 0.049 Å for **4c**.

In compounds **1**, Pd−O bonds *trans* to the metalated carbon atoms were longer than Pd−O bonds *trans* to the iminic nitrogen atom [Pd1−O1 2.1247(16) Å and Pd2−O3 2.1573(16) Å but Pd1−O4 2.0576(17) Å and Pd2−O2 2.0374(16) Å for **1a**, Pd1−O2 2.155(2) Å and Pd2−O3 2.147(3) Å but Pd1−O4 2.055(2) and Pd2−O1 2.042(2) Å for **1c**, and Pd1−O1 2.141(3) but Pd1−O2 2.050(3) Å for **1d**]. These results were in agreement with the greater *trans* influence of the palladated carbon atom in relation to the iminic nitrogen atom.[30] Also, the greater *trans* influence of the phosphorus atom of PPh_3 in relation to the oxygen atom of the acetato ligand explained the longer Pd−N bonds in compounds **4** in relation to compounds **1** [Pd1−N1 2.1028(19) Å for **4a**, Pd1−N1 2.1162(17) Å for **4b**, Pd1−N1 2.0964(14) Å for **4c** but Pd1−N1 2.0191(19) Å and Pd2−N2 2.0177(19) Å for **1a**, Pd1−N2 2.010(3) Å and Pd2−N1 2.022(3) Å for **1c**, and Pd1−N1 2.038(3) Å for **1d**].[30]

The angle between the palladacycles in compounds **1a**, **1c**, and **1d** were 25.11, 27.66(14), and 54.16°, respectively, and the distances between palladium atoms were 2.90693(3), 2.8698(4), and 3.204 Å, respectively. These distances were too long to be considered a palladium−palladium single bond.[31]

The angles between the palladacycles and the phenyls bonded to the methinic carbon atoms in these molecules were 59.38 and 58.78° for **1a**, 78.4(2) and 62.42(18)° for **1c**, 86.1(2)° for **1d**, 85.11(11)° for **4a**, 66.69(12)° for **4b**, and 76.75° for **4c**. In compounds **1a** and **4a**, the angles between the phenyls bonded to the iminic nitrogen atoms and the palladacycles were 61.57 and 61.24° for **1a** and 79.75(11)° for **4a**, and the angle between the 1-naphthyl group bonded to the iminic nitrogen atom and the palladacycle was 75.23(10)° in compound **4b**.

Finally, in all these molecules, the metalated phenyl and the metalacycle were almost coplanar, with the angles between

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them being 7.05 and 5.82° for **1a**, 1.73(16) and 7.23(15)° for **1c**, 4.15(18)° for **1d**, 5.50(11)° for **4a**, 1.00(10)° for **4b**, and 4.52° for **4c**.

Biological Studies. *Antiproliferative Activity.* The cytotoxicity of palladium compounds **a**–**d** against MDA-MB-231 and MCF-7 breast and cisplatin-resistant HCT-116 colon adenocarcinoma human cell lines was studied, and their IC_{50} values against the precedent cell lines are collected in Table 1.

Table 1. IC_{50} (μM) Values against the Indicated Cancer Cell Lines and clog *P* for the Compounds under Study[a]

compound	MDA-MB-231	MCF-7	HCT-116	clog *P*[b]
a	> 100	> 100	> 100	5.11
1a	>100	> 100	82 ± 19	12.10
2a	40 ± 2	30 ± 9	47 ± 8	10.35
3a	12 ± 2	> 100	66 ± 10	11.89
4a	> 100	> 100	> 100	11.01
b	> 100	> 100	> 100	6.28
1b	> 100	> 100	> 100	14.45
2b	> 100	> 100	> 100	12.69
3b	> 100	> 100	> 100	13.06
4b	> 100	> 100	> 100	12.19
c	> 100	> 100	94	4.58
1c	> 100	> 100	> 100	12.77
2c	20 ± nd	44 ± 10	31 ± 3	10.62
3c	> 100	> 100	> 100	12.22
4c	91 ± nd	> 100	65 ± 8	11.35
d	> 100	80 ± nd	57 ± 5	4.89
1d	> 100	> 100	98 ± 28	12.99
2d	16 ± nd	37 ± 9	27 ± 2	11.24
3d	> 100	> 100	> 100	12.53
4d	> 100	> 100	> 100	11.65
e	> 100	> 100	> 100	2.69
1e	15.0 ± 1.2[c]	14.0 ± 4.2[c]	33 ± 3[c]	7.48
2e	13 ± 1[c]	11.0 ± 1.5[c]	20.0 ± 0.7	5.73
3e	**1.1 ± 0.3**[c]	**4.0 ± 0.5**[c]	18 ± 2[c]	9.58
4e	**1.1 ± 0.1**[c]	**4.1 ± 0.9**[c]	20 ± 5[c]	8.70
4f	5.0 ± 1.2[c]	13.4 ± 1.5[c]	**11.0 ± 0.6**[c]	8.70
cisplatin[a]	5.0 ± 1.2	19 ± 4.5	40.0 ± 4.4	-2.5

[a]Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation. *cis*-$[PtCl_2(NH_3)_2]$ is taken as a reference compound. [b]clog *P* is the calculated logarithmic value of the *n*-octanol/water partition coefficient and was estimated using the program ChemBioDraw Ultra 12.0 (ChemBioOffice 2010). [c]Previously reported.[19,20] Cells color code: green = more cytotoxic than cisplatin, yellow = less cytotoxic than cisplatin, red = inactive, white = poorly active or inactive, blue = cisplatin.

For comparison, the IC_{50} values against the above cited cell lines for cisplatin, free imines, palladium compounds **e**, and platinum compound **4f** are also included in Table 1. It should be noted that the IC_{50} values for palladium compounds **e** and compound **4f** against the cancer cell lines cited above have been previously reported by our research group.[19,20] Table 1 gives also the clog *P* for the compounds under study. This is the calculated logarithmic value of the *n*-octanol/water partition coefficient of the compound under consideration and can be related to its lipophilicity.[32]

For a better understanding of Table 1, we have included a color code for the cells. Thus, green ones mean that the compounds under consideration are more cytotoxic than cisplatin against the specified tumor cell lines; yellow ones are compounds that are cytotoxic but less cytotoxic than cisplatin; red ones are compounds that are not cytotoxic with IC_{50} values greater than 100 μM, and cells not filled with color indicate compounds that are poorly cytotoxic (IC_{50} > 50 μM) or noncytotoxic. Cells corresponding to cisplatin are filled with blue color.

From data given in Table 1, the following trend for the in vitro antitumor activity of compounds **1**–**4** from series **a**–**e** and compound **4f** can be derived: **3e** ≈ **4e** (more cytotoxic than cisplatin) > **4f** (more cytotoxic than cisplatin) > **2e** ≈ **1e** (less cytotoxic than cisplatin) > **2d** ≈ **2c** ≈ **2a** (less cytotoxic than cisplatin) > **3a** (less cytotoxic than cisplatin) > the other compounds (inactive or poorly active).

This trend seems to indicate that an accurate balance between hydrophilicity and lipophilicity could be regulating the in vitro antitumor activity of these cyclometalated benzophenone imines. For instance, for palladium compounds **e**, which should be the most hydrophilic ones due to the presence of the N–H function in their structural formula, when passing from **3e** and **4e** to **1e** and **2e**, a substantial drop in activity is observed. This should be ascribed to the decrease in lipophilicity on going from **3e** and **4e** to **1e** and **2e** (see clog *P* values in Table 1). On the other hand, for palladium compounds **a**, **c**, and **d**, which are more lipophilic than palladium compounds **e**, since in this series the group bonded to the iminic nitrogen is phenyl, benzyl, or α-methylbenzyl, respectively, the maximum activity corresponds to compounds **2a**, **2c**, and **2d**, which are the ones with the lowest lipophilicity of these series (see clog *P* values in Table 1). Finally, compound **2b** is not active at all since this compound is more lipophilic (R = 1-naphthyl) than compounds **2a** (R = phenyl), **2c** (R = benzyl), and **2d** (R = α-methylbenzyl). At present, we cannot find a reliable explanation for the IC_{50} value of compound **3a** (12 μM) toward the MDA-MB-231 cell line. In addition, the great cytotoxicity observed for palladium compounds **e** could also be related to their better solubility and/or to the ability of their NH moiety to establish hydrogen bonds with a target biomolecule.[33]

It should be noted that water solubility for the organometallic compounds under study is in fact very low, but we expect that, once in solution in the biological media, palladium compounds **a**–**d** should be converted into the ionic aqua complexes $[Pd(C,N)(H_2O)_2]X$ (X = OAc or Cl) (compounds **I**) and *trans-N,P*-$[Pd(C,N)(PPh_3)(H_2O)]X$ (X = OAc or Cl) (compounds **II**) by substitution of their chlorido ligands by water molecules. Therefore, we propose that the responsible species for the biological activities studied here are the ionic aqua complexes **I** and **II** commented above. Similar aqua complexes were proposed to be the responsible species for the biological activities observed for palladium compound **e** and compound **4f**.[19,20]

The cytotoxicity of compound **3e**, one of the most potent complexes in the cancer cell lines evaluated, and cisplatin was also tested against HUVEC cells in starving culture conditions, in which cells are almost quiescent or in low proliferative conditions, and in normal cell culture conditions, in which cells are in highly proliferative conditions (see Experimental Section for the details). Table 2 gives the IC_{50} values for these studies. Interestingly, compound **3e** was 2 times and 1.5 times less cytotoxic than cisplatin toward the HUVEC cells in starving and in normal cell culture conditions, respectively.

Cell Accumulation. The cellular accumulation of platinum drugs in vivo is well-documented, and it is believed to be dependent on both passive diffusion and active transport. Active transport can involve the entry into the cell with the help of copper transporters and organic cation transporters, as well as active efflux of the drug by copper efflux transporters.[34] The relationship between lipophilicity, cellular accumulation, and cytotoxicity of oxaliplatin derivatives with different substituents

Table 2. IC_{50} (μM) Values for Compound 3e toward HUVEC Cells in Starving and Normal Culture Conditions[a]

compound	HUVEC starving[b]	HUVEC normal[c]
3e	49 ± nd	29 ± 7
cisplatin[a]	16 ± 3	18 ± 2

[a]Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation. *cis*-$[PtCl_2(NH_3)_2]$ is taken as a reference compound. [b]Low proliferative culture conditions. [c]Highly proliferative culture conditions.

at the 4 position of the cyclohexane ring has been investigated. In this study, the more lipophilic compounds revealed a lower cytotoxicity, although the early influx rate (passive diffusion) was increased in the case of more lipophilic platinum complexes. Alterations in the reactivity in the intracellular environment of the platinum compounds with bulky substituents or increased sequestration of the most lipophilic platinum complexes in cellular organelles such as lysosomes could be responsible for their reduced cytotoxicity in the investigated cell lines.[35] This explanation could account for the lower cytotoxicity observed for palladium compounds **a**−**d** with regard to that of palladium compounds **e** and the absence of antiproliferative activity of palladium compounds **b**, which present the bulkier and more lipophilic substituent in series **a**−**d**.

To get more insight on the antitumor activity of the benzophenone cyclopalladated imines, the cellular accumulation of palladium compounds **b** and **e** was studied and compared with that of cisplatin. In these studies, palladium was used as a measure of the cellular accumulation of the palladium compounds, and platinum was used as a measure of the cellular accumulation of cisplatin. Mole of palladium or platinum per cell was determined by ICP-MS. Table 3 gives the cellular accumulation for the tested compounds and for cisplatin after 4 h of incubation on the breast cancer cell line MDA-MB-231.

Table 3. Palladium and Platinum Accumulation in the MDA-MB-231 Cell Line

compound	cell accumulation[a]
1b	0.40 ± 0.15
2b	0.56 ± 0.09
3b	0.7 ± 0.4
4b	0.02 ± 0.01
1e	0.2 ± 0.1
2e	0.072 ± 0.006
3e	0.9 ± 0.2
4e	1.6 ± 0.6
cisplatin	0.015 ± 0.002

[a]Cell accumulation for compounds **b** and **e** and for cisplatin is given as (mol of Pd or of Pt per cell ± SD) × 10^{-15} and was measured by ICP-MS after 4 h of treatment at 50 μM with the indicated compounds. Experiments were performed in duplicate. SD = standard deviation.

The accumulation of the tested compounds in the cell line studied was considerably greater than that of cisplatin, and compounds **3e** and **4e**, which are the compounds with the highest antitumor activity against the MDA-MB-231 cell line, were those with the highest amounts of palladium accumulated per cell. The accumulation of these latter compounds was about 50 and 100 times higher than that of cisplatin. Interestingly, the noncytotoxic compounds **1b**−**4b** were accumulated on the MDA-MB-231 cell line in quite high amounts. This result suggests that these compounds could be noncytotoxic because of their bulky 1-naphthyl group, which should render their palladium(II) centers less reactive, or because of their accumulation in cellular organelles such as lysosomes due to their elevated lipophilicity.[35]

DNA Interaction. The binding of palladium compounds **a**−**d** to DNA was studied by their ability to modify the electrophoretic mobility of the supercoiled closed circular (ccc) and the open circular (oc) forms of pBluescript SK + plasmid DNA.

Figure 8 shows the electrophoretic mobility of pBluescript SK + plasmid DNA incubated with the free ligands **a**−**d** and palladium compounds **a**−**d** at increasing concentrations. To provide a basis for comparison, incubation of DNA with cisplatin and ethidium bromide (EB) was also performed using the same concentrations and conditions. The electrophoretic mobility of pBluescript SK + plasmid DNA incubated with palladium compounds **e** and compound **4f** was previously reported by our research group and has also been included in Figure 8 for a comparative purpose.[19,20]

As expected, cisplatin greatly altered the electrophoretic mobility of pBluescript SK + plasmid DNA at 2.5 μM, but for EB, only a very slight decrease in the electrophoretic mobility of DNA was detected between 25 and 100 μM.[19] For the free ligands **a**−**d**, no shift in the rate of migration of the supercoiled band was observed.

Palladium compounds **a**−**e** were less efficient than cisplatin in removing the supercoils from DNA, in spite of the fact that some were more cytotoxic than cisplatin. Only compound **3b** produced at 10 μM concentration (lane 4), a significant effect on the mobility of pBluescript DNA. At 25 μM concentration (lane 5), complexes **1a**−**1c** induced significant changes in plasmid DNA mobility. Complexes **1a**−**1e**, **2e**, and **3a**−**3d** showed the same effect of coalescence and positive supercoiling observed for cisplatin but at a higher concentration. Compound **4f** was also less efficient than cisplatin for removing the supercoils from pBluescript SK + plasmid DNA, suggesting that the unwinding of the DNA is not the key factor responsible for its cytotoxicity.[20]

Compounds with the greatest effect on DNA mobility did not necessarily correlate to those with the lowest IC_{50} values. For instance, modifications induced on DNA mobility for **1a** were greater than those produced for **2a**, but **2a** was more potent than **1a** in the three cancer cell lines assayed. On the other hand, noncytotoxic cyclopalladated compounds (i.e., **1b** and **3b**, IC_{50} values >100 μM) induced significant changes on DNA mobility. It is hypothesized that, in the conditions of the gel mobility assay, compounds such as **1b**, **3b**, or **4f** interacted with DNA in a similar way as that of cisplatin but at a higher concentration.

To evaluate the ability of the investigated complexes to intercalate into DNA, a topoisomerase-based gel assay was performed on **2b** and **2d**. Supercoiled pBluescript plasmid DNA was incubated in the presence of topoisomerase I at increasing concentrations of compounds **2a** and **2b**. The results are given in Figure 9, and they show that **2b** and **2d** do not prevent unwinding of DNA by the action of topoisomerase I, indicating that these compounds are neither intercalators nor inhibitors of topoisomerase I.[8]

The precedent results suggest that DNA is not a primary target biomolecule for the cyclopalladated and cycloplatinated benzophenone imines under study.

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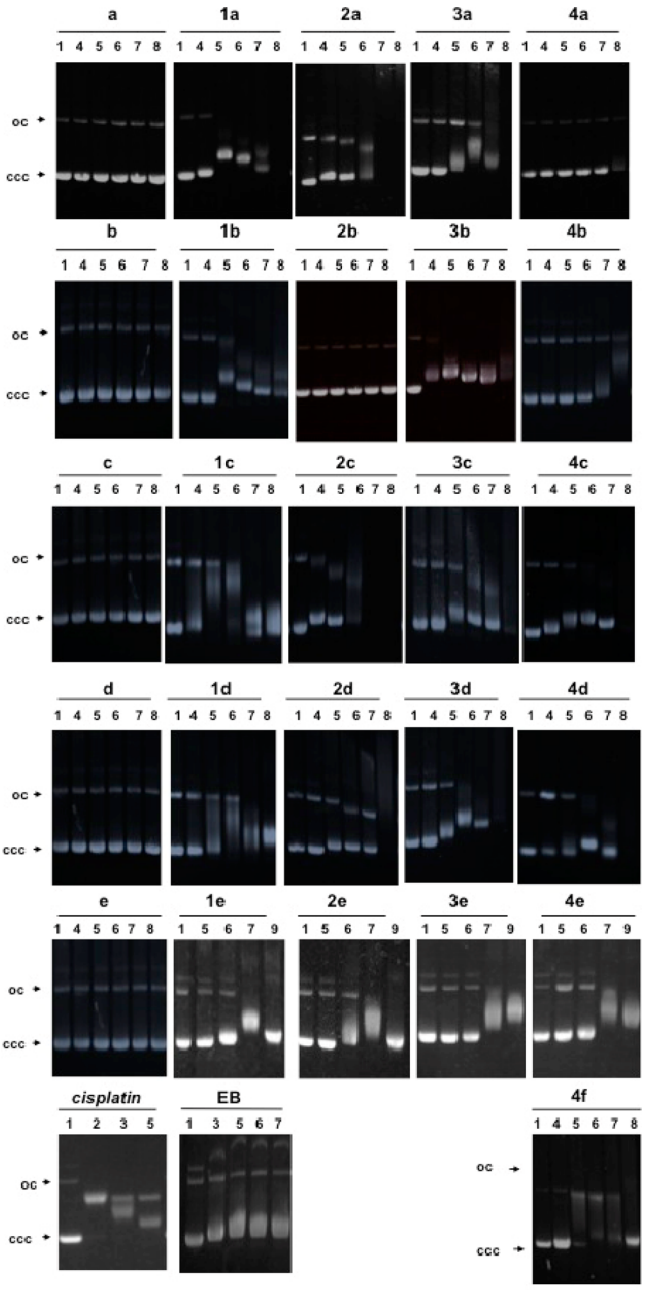

Figure 8. Interaction of pBluescript SK + plasmid DNA (0.8 μg) with increasing concentrations of compounds under study, cisplatin and ethidium bromide. Lane 1: DNA only. Lane 2: 2.5 μM. Lane 3: 5 μM. Lane 4: 10 μM. Lane 5: 25 μM. Lane 6: 50 μM. Lane 7: 100 μM. Lane 8: 150 μM. Lane 9: 200 μM; ccc = supercoiled closed circular DNA; oc = open circular DNA.

Cathepsin B Inhibition. Cathepsin B is a cysteine metalloprotease highly upregulated in a wide variety of cancers by mechanisms ranging from gene amplification to post-transcriptional modification. The exact role of cathepsin B in solid tumors has yet to be defined, but it has been proposed to participate in metastasis, angiogenesis, and tumor progression. Recently, compounds based on palladium, platinum, ruthenium, rhenium, gold, and tellurium were shown to be effective inhibitors of cathepsin B.[36] In addition, an excellent correlation between cathepsin B inhibition and cytotoxicity for some dinuclear biphosphane palladacycles[37] and mononuclear platinacycles containing a fluorinated phosphane[38] has been

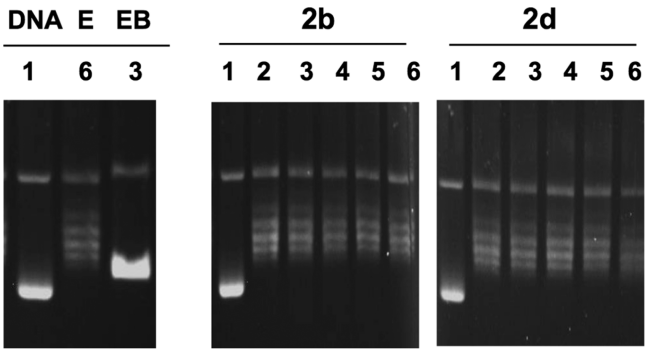

Figure 9. Analysis of **2b** and **2d** as putative DNA intercalators or topoisomerase I inhibitors. Conversion of supercoiled pBluescript plasmid DNA (0.8 μg) to relaxed DNA by the action of topoisomerase I (3 units) in the absence or in the presence of increasing amounts of compounds **2b** and **2d** was analyzed by agarose gel stained with ethidium bromide. EB was used as a control of intercalating agent and etoposide (E) as a control of nonintercalating agent. Lane 1: DNA only. Lane 2: 0 μM drug. Lane 3: 10 μM drug. Lane 4: 25 μM drug. Lane 5: 50 μM drug. Lane 6: 100 μM drug. Except for lane 1, all lanes included topoisomerase I; ccc = supercoiled closed circular DNA; oc = open circular DNA.

reported. In spite of these results, we have recently reported[20] that compounds **1e**−**4e** and **4f** are not efficient inhibitors of cathepsin B although they are quite cytotoxic in vitro.

Following these studies, we have determined the cathepsin B inhibition activity for compounds **2a**, **2c**, **2d**, and **3a**, which are the more active ones from series **a**−**d** in relation to their in vitro antitumor activity. The results are given in Table 4. This table also includes our previously reported results for the cathepsin B inhibition test for palladium compounds **e** and compound **4f** for a comparative purpose.[20]

Table 4. IC_{50} (μM) Values and Percent of Residual Activity of Cathepsin B at 100 μM for Compounds 2a, 2c, 2d, and 3a[a]

compound	IC_{50} (μM) vs cathepsin B	% of residual activity at 100 μM
2a	>100	58.0 ± 0.8
2c	>50	47.0 ± 1.1
2d	>50	45.0 ± 1.9
3a	>50	47.0 ± 0.9
1e[b]	>100	62.0 ± 2.1
2e[b]	>100	60.0 ± 1.7
3e[b]	>100	70.0 ± 1.5
4e[b]	>50	50.0 ± 0.9
4f[b]	>100	72.0 ± 1.0

[a]The enzyme was preincubated for 2 h with 50 or 100 μM of each compound. The activity is given as a percentage of the enzyme activity determined in the absence of the test compound. Data are shown as the mean values of the experiment performed in triplicate with the corresponding standard deviation. [b]Previously reported.[20]

Compounds **2a**, **2c**, **2d**, and **3a** inhibited cathepsin B in a dose-dependent manner, but they were not efficient as cathepsin B inhibitors since 50% of residual activity of the enzyme is not observed until quite high concentrations, greater than 50 or 100 μM depending on the compound. These results confirm that cathepsin B is not a primary target biomolecule for the cyclopalladated and cycloplatinated benzophenone imines studied here.

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CONCLUSIONS

The present study and previous ones[19,20] confirm that compounds **3e**, **4e**, and **4f** are the most active in vitro antitumor agents against the evaluated cell lines between a quite large number of cyclometalated benzophenone imines studied. Compounds **3e** and **4e** were about 4 times more active than cisplatin against the MDA-MB-231 and MCF-7 breast human cancer lines, and compound **4f** is about 4 times more active than cisplatin against the cisplatin-resistant HCT-116 colon human cancer cell line. The in vitro cytotoxicity of the cyclopalladated benzophenone imines **a**−**e** against the studied cancer cell lines seems to be regulated by an adequate balance between their hydrophilicity and lipophilicity. In addition, accumulation of palladium compounds **e** and **b** in the MDA-MB-231 cell line was considerably greater than that of cisplatin, but palladium compounds **b** were noncytotoxic. Furthermore, compound **3e**, one of the most potent complexes in the cell lines evaluated, was 2 times and 1.5 times less cytotoxic than cisplatin toward HUVEC cells in starving (quiescent) and normal cell culture conditions, respectively. Concerning the determination of plausible primary target biomolecules for these cyclopalladated and cycloplatinated benzophenone imines, DNA migration studies and cathepsin B inhibition tests suggest that these biomolecules are not their primary target biomolecules.

EXPERIMENTAL SECTION

Cell Culture. Colon HCT-116 and breast cancer MCF-7 and MBA-MD-231 cells were grown as a monolayer culture in minimum essential medium (DMEM with L-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum (FCS), 10 mM D-glucose, and 0.1% streptomycin/penicillin, in standard culture conditions (humidified air with 5% CO_2 at 37 °C).

Cell Viability Assay for HC-T116, MDA-MB-231, and MCF-7 Cell Lines. A stock solution (50 mM) of each compound was prepared in high-purity DMSO. Then, serial dilutions were made with DMSO/DMEM (1:1), and finally a 1:500 dilution on culture medium was prepared. Final assay concentration of DMSO was the same in all experiments and was 0.2%. The assay was performed as described.[39] HCT-116, MDA-MB-231, and MCF-7 cells were plated at 5000 cells/well in 100 μL of media in tissue culture 96-well plates. After 24 h, medium was replaced by 100 μL/well of drug serial dilutions. Control wells did not contain the compounds under study. Each point concentration was run in triplicate. Reagent blanks, containing media and colorimetric reagent without cells, were run on each plate. Blank values were subtracted from test values and were routinely 5−10% of the control values. Plates were incubated 72 h. Hexosaminidase activity was measured according to the following protocol. The medium was removed, and cells were washed once with PBS. Sixty microliters of substrate solution of 7.5 mM *p*-nitrophenol-*N*-acetyl-β-D-glucosamide, 0.1 M sodium citrate at pH 5.0, and 0.25% Triton X-100 was added to each well and incubated at 37 °C for 1−2 h. After this incubation time, a bright yellow color appeared. Then, the plates were developed by adding 90 μL of developer solution (50 mM glycine, pH 10.4; 5 mM EDTA), and the absorbance was recorded at 410 nm.

Cell Viability Assay for the HUVEC Cells. Cytotoxicity in normal cells was assayed using primary human umbilical vein endothelial cells, at passage 6−8. Viability was assayed in two culture conditions: highly proliferative, where 10% of FCS, supplements, and growth factors were included in culture media; low proliferative, where cells were almost quiescent, by restraining FCS at 2% with no supplements or growth factors. Assays were done as described for tumor cell lines, plating 5000 cell/well for each growing condition.

Cell Accumulation. Cell accumulation of compounds was measured in the MDA-MB-231 cell line. A total of 1×10^6 cells was seeded in 6 mm tissue culture dishes for 16 h in DMEM/high glucose plus 10% FCS. Then, compounds were added at 50 μM for 4 h at 37 °C, 5% CO_2. Cisplatin was used as a positive control at the same concentration and DMSO as a vehicle control. After treatment, cells were washed twice with PBS, tripsinized, and harvested in PBS. Cell suspension was centrifuged, and pellets were digested with 12 M HCl and diluted to 1.2 M HCl. Each treatment was done in duplicate. The samples were analyzed by ICP-MS.

DNA Migration Studies. A stock solution (10 mM) of each compound was prepared in high-purity DMSO. Then, serial dilutions were made in Milli-Q water (1:1). Plasmid pBluescript SK+ was obtained using a kit as described by the manufacturer. Interaction of drugs with pBluescript SK+ plasmid DNA was analyzed by agarose gel electrophoresis following a modification of the method previously described.[40] Plasmid DNA aliquots (40 μg mL^{-1}) were incubated in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) with different concentrations of test compounds ranging from 0 to 200 μM at 37 °C for 24 h. Final DMSO concentration in the reactions was always lower than 1%. For comparison, cisplatin and ethidium bromide were used as reference controls. Aliquots of 20 μL of the incubated solutions of compounds containing 0.8 μg of DNA were subjected to 1% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0). The gel was stained in TAE buffer containing EB (0.5 mg mL^{-1}) and visualized and photographed under UV light.

Topoisomerase I-based experiments were performed as described previously.[41] Supercoiled pBluescript DNA, obtained as described above, was treated with topoisomerase I in the absence or presence of increasing concentrations of compounds **2b** and **2d**. Assay mixtures contained supercoiled pBluescript DNA (0.8 μg), calf thymus topoisomerase I (3 units), and complexes **2b** and **2d** (0−100 μM) in 20 μL of Tris-HCl buffer (pH 7.5) containing 175 mM KCl, 5 mM $MgCl_2$, and 0.1 mM EDTA. Ethidium bromide (10 μM) was used as a control of intercalating agents and etoposide (100 μM) as a control of the nonintercalating agent. Reactions were incubated for 30 min at 37 °C and stopped by the addition of 2 μL of agarose gel loading buffer. Samples were then subjected to electrophoresis, and DNA bands were stained with ethidium bromide as described above.

Cathepsin B Inhibition Assay. The colorimetric cathepsin B assay was performed as described by Casini et al.[42] with few modifications. Briefly, the reaction mixture contained 100 mM sodium phosphate (pH 6.0), 1 mM EDTA, and 200 μM sodium *N*-carbobenzoxy-L-lysine *p*-nitrophenyl ester as substrate. To have the enzyme catalytically active before each experiment, the active site of the cysteine was reduced by treatment with dithiothreitol (DTT). For this purpose, 5 mM DTT was added to the cathepsin B sample, before dilution, and incubated 1 h at 30 °C. To test the inhibitory effect of the palladium compounds on cathepsin B, activity measurements were performed in triplicate using fixed concentrations of enzyme (500 nM) and substrate (200 μM). The palladium compounds were used at concentrations ranging from 50 to 100 μM. Previous to the addition of substrate, cathepsin B was incubated with the different compounds at 25 °C for 2 h. The cysteine proteinase inhibitor E-64 was used as a positive control of cathepsin B inhibition. Complete inhibition was achieved at 10 μM concentration of E-64. Activity was measured over 1.5 min at 326 nm on a UV spectrophotometer.

ASSOCIATED CONTENT

Supporting Information

Preparative method and selected characterization data for compounds **a**−**d** and crystal structure reports and CIF files for the X-ray molecular crystal structure determinations of **1a**·2MeOH, **1c**, **1d**, **4a**, **4b**·MeOH, and **4c**·MeOH. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Caires, A. C. F. *Anti-Cancer Agents Med. Chem.* **2007**, *7*, 484−491.
(2) Abu-Surrah, A. S.; Al-Sa'doni, H. H.; Abdalla, M. Y. *Cancer Ther.* **2008**, *6*, 1−10.
(3) Reedijk, J. *Platinum Met. Rev.* **2008**, *52*, 2−11.
(4) Omae, I. *Coord. Chem. Rev.* **2014**, *280*, 84−95.
(5) Cutillas, N.; Yellol, G. S.; de Haro, C.; Vicente, C.; Rodríguez, V.; Ruiz, J. *Coord. Chem. Rev.* **2013**, *257*, 2784−2797.
(6) Quiroga, A. G.; Navarro-Ranninger, C. *Coord. Chem. Rev.* **2004**, *248*, 119−133.
(7) Albert, J.; Bosque, R.; Crespo, M.; García, G.; Granell, J.; López, C.; Lovelle, M. V.; Qadir, R.; González, A.; Jayaraman, A.; Mila, E.; Cortés, R.; Quirante, J.; Calvis, C.; Messeguer, R.; Badía, J.; Baldomà, L.; Cascante, M. *Eur. J. Med. Chem.* **2014**, *84*, 530−536.
(8) Albert, J.; Bosque, R.; Cadena, M.; D'Andrea, L.; Granell, J.; González, A.; Quirante, J.; Calvis, C.; Messeguer, R.; Badía, J.; Baldoma, L.; Calvet, T.; Font-Bardia, M. *Organometallics* **2014**, *33*, 2862−2873.
(9) Aliwaini, S.; Swarts, A. J.; Blanckenberg, A.; Mapolie, S.; Prince, S. *Biochem. Pharmacol.* **2013**, *86*, 1650−1663.
(10) Guimaraes-Correa, A. B.; Crawford, L. B.; Figueiredo, C. R.; Gimenes, K. P.; Pinto, L. A.; Rios Grassi, M. F.; Feuer, G.; Travassos, L. R.; Caires, A. C. F.; Rodrigues, E. G.; Marriott, S. J. *Viruses* **2011**, *3*, 1041−1058.
(11) Serrano, F. A.; Matsuo, A. L.; Monteforte, P. T.; Bechara, A.; Smaili, S. S.; Santana, D. P.; Rodrigues, T.; Pereira, F. V.; Silva, L. S.; Machado, J., Jr.; Santos, E. L.; Pesquero, J. B.; Martins, R. M.; Travassos, L. R.; Caires, A. C. F.; Rodrigues, E. G. *BMC Cancer* **2011**, *11*, 296.
(12) Santana, D. P.; Faria, P. A.; Paredes-Gamero, E. J.; Caires, A. C. F.; Nantes, I. L.; Rodrigues, T. *Biochem. J.* **2009**, *417*, 247−256.
(13) da Rocha, M. C.; Santana, A. M.; Ananias, S. R.; de Almeida, E. T.; Mauro, A. E.; Placeresa, M. C. P.; Carlos, I. Z. *J. Braz. Chem. Soc.* **2007**, *18*, 1473−1480.
(14) Barbosa, C. M. V.; Oliveira, C. R.; Nascimento, F. D.; Smith, M. C. M.; Fausto, D. M.; Soufen, M. A.; Sena, E.; Araújo, R. C.; Tersariol, I. L. S.; Bincoletto, C.; Caires, A. C. F. *Eur. J. Pharmacol.* **2006**, *542*, 37−47.
(15) Bincoletto, C.; Tersariol, I. L. S.; Oliveira, C. R.; Dreher, S.; Fausto, D. M.; Soufen, M. A.; Nascimento, F. D.; Caires, A. C. F. *Bioorg. Med. Chem.* **2005**, *13*, 3047−3055.
(16) Rodrigues, E. G.; Silva, L. S.; Fausto, D. M.; Hayashi, M. S.; Dreher, S.; Santos, E. L.; Pesquero, J. B.; Travassos, L. R.; Caires, A. C. F. *Int. J. Cancer* **2003**, *107*, 498−504.
(17) Hebeler-Barbosa, F.; Rodrigues, E. G.; Puccia, R.; Caires, A. C. F.; Travassos, L. R. *Clin. Transl. Oncol.* **2008**, *1*, 110−120.
(18) Caires, A. C. F.; Trindade, C. B.; Tersariol, I. L. S. Patent Appl. WO/2004/019924, 2014.
(19) Albert, J.; García, S.; Granell, J.; Llorca, A.; Lovelle, M. V.; Moreno, V.; Presa, A.; Rodríguez, L.; Quirante, J.; Calvis, C.; Messeguer, R.; Badía, J.; Baldomà, L. *J. Organomet. Chem.* **2013**, *724*, 289−296.
(20) Albert, J.; D'Andrea, L.; Granell, J.; Pla-Vilanova, P.; Quirante, J.; Khosa, M. K.; Calvis, C.; Messeguer, R.; Badía, J.; Baldomà, L.; Font-Bardia, M.; Calvet, T. *J. Inorg. Biochem.* **2014**, *140*, 80−88.
(21) Dai, W.; Srinivasan, R.; Katzenellenbogen, J. A. *J. Org. Chem.* **1989**, *54*, 2204−2208.
(22) Clark, P. W.; Dyke, S. F.; Smith, G.; Kennard, C. H. L. *J. Organomet. Chem.* **1987**, *330*, 447−460.
(23) Onoue, H.; Moritani, I. *J. Organomet. Chem.* **1972**, *43*, 431−436.
(24) Albert, J.; Gómez, M.; Granell, J.; Sales, J.; Solans, X. *Organometallics* **1990**, *9*, 1405−1413.
(25) Albert, J.; D'Andrea, L.; Granell, J.; Tavera, R.; Font-Bardia, M.; Solans, X. *J. Organomet. Chem.* **2007**, *692*, 3070−3080.
(26) Albert, J.; Bosque, R.; D'Andrea, L.; Granell, J.; Font-Bardia, M.; Calvet, T. *Eur. J. Inorg. Chem.* **2011**, 3617−3631.
(27) Albert, J.; Granell, J.; Sales, J.; Font-Bardía, M.; Solans, X. *Organometallics* **1995**, *14*, 1393−1404.
(28) Albert, J.; Ceder, R. M.; Gómez, M.; Granell, J.; Sales, J. *Organometallics* **1992**, *11*, 1536−1541.
(29) Ghedini, M.; Crispini, A. *Comments Inorg. Chem.* **1999**, *21*, 53−68.
(30) Appleton, T. G.; Clark, H. C.; Manzer, L. E. *Coord. Chem. Rev.* **1973**, *10*, 335−422.
(31) Murahashi, T.; Kurosawa, H. *Coord. Chem. Rev.* **2002**, *231*, 207−228.
(32) See for instance: Fetzer, L.; Boff, B.; Ali, M.; Xiangjun, M.; Collin, J. P.; Sirlin, C.; Gaiddon, C.; Pfeffer, M. *Dalton Trans.* **2011**, *40*, 8869−8878.
(33) Ramos-Lima, F. J.; Moneo, V.; Quiroga, A. G.; Carnero, A.; Navarro-Ranninger, C. *Eur. J. Med. Chem.* **2010**, *45*, 134.
(34) Jung, Y.; Lippard, S. J. *Chem. Rev.* **2007**, *107*, 1387−1407.
(35) Bub, I.; Garmann, D.; Galanski, M.; Weber, G.; Kalayda, G. V.; Keppler, B. K.; Jaehde, U. *J. Inorg. Biochem.* **2011**, *105*, 709−717.
(36) Fricker, S. P. *Metallomics* **2010**, *2*, 366−377.
(37) Spencer, J.; Casini, A.; Zava, O.; Rathnam, R. P.; Velhanda, S. K.; Pfeffer, M.; Callear, S. K.; Hursthoused, M. B.; Dyson, P. J. *Dalton Trans.* **2009**, 10731−10735.
(38) Cutillas, N.; Martínez, A.; Yellol, G. S.; Rodríguez, V.; Zamora, A.; Pedreño, M.; Donaire, A.; Janiak, Ch.; Ruiz, J. *Inorg. Chem.* **2013**, *52*, 13529−13535.
(39) Givens, K. T.; Kitada, S.; Chen, A. K.; Rothschiller, J.; Lee, D. A. *Invest. Ophthalmol. Visual Sci.* **1990**, *31*, 1856−1862.
(40) Abdullah, A.; Huq, F.; Chowdhury, A.; Tayyem, H.; Beale, P.; Fisher, K. *BMC Chem. Biol.* **2006**, *6*, 3.
(41) Sappal, D. S.; McClendon, A. K.; Fleming, J. A.; Thoroddsen, V.; Connolly, K.; Reimer, C.; Blackman, R. K.; Bulawa, C. E.; Osheroff, N.; Charlton, P.; Rudolph-Owen, L. A. *Mol. Cancer Ther.* **2004**, *3*, 47−58.
(42) Casini, A.; Gabbiani, C.; Sorrentino, F.; Rigobello, M. P.; Bindoli, A.; Geldbach, T. J.; Marrone, A.; Re, N.; Hartinger, C. G.; Dyson, P. J.; Messori, L. *J. Med. Chem.* **2008**, *51*, 6773−6781.