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Cyclopalladated benzophenone imines: Synthesis, cytotoxicity against human breast adenocarcinoma cell lines and DNA interaction

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ABSTRACT

Treatment of benzophenone imine with the stoichiometric amount of $Pd(OAc)_2$ in acetic acid at 60 °C produced the corresponding acetato-bridged five-membered *ortho*-cyclopalladated dimer $[Pd\{C_6H_4CPh{=}NH\}(\mu\text{-}OAc)]_2$ (**1**), which was isolated in pure form in a 79% yield. Reaction of **1** with an excess of LiCl in acetone gave rise to the corresponding chlorido-bridged cyclopalladated dimer $[Pd\{C_6H_4CPh{=}NH\}(\mu\text{-}Cl)]_2$ (**2**) in a 78% yield. Compounds **1**–**2** reacted with an excess of py-d_5 or the stoichiometric amount of PPh_3 to give the mononuclear compounds *trans*-***N,L***-$[Pd\{C_6H_4CPh{=}NH\}(X)(L)]$ [**3** (X = OAc, L = py-d_5); **4** (X = Cl, L = py-d_5); **5** (X = OAc, L = PPh_3) and **6** (X = Cl, L = PPh_3)]. Compounds **2**–**3** were prepared in $CDCl_3$/py-d_5 solution and were studied by ^1H NMR, but were not isolated. In contrast, compounds **5**–**6** were prepared in acetone and were isolated in pure form in 43 and 79% yields, respectively. Compounds **1**, **2**, **5** and **6** were characterized by elemental analyses, mass spectrometry, IR, NMR and electronic spectroscopy. Compounds **1**, **2**, **5** and **6** showed high antiproliferative activity against MDA-MB231 and MCF7 human breast cancer cell lines, especially, compounds **5**–**6**. These two latter compounds presented greater antiproliferative activity than *cisplatin* and produced IC_{50} values in the range 1–5 μM. The interaction of compounds **1**, **2**, **5** and **6** with DNA was also studied by the DNA electrophoretic migration, DNA-ethidium bromide fluorescence quenching and viscometry techniques.

1. Introduction

Despite the tremendous success of *cisplatin* – *cis*-$[PtCl_2(NH_3)_2]$ – as an anticancer drug, this compound suffers from two main disadvantages; it is inefficient against *cisplatin*-resistant tumours, and it has severe side effects such as nephrotoxicity [1]. Therefore, the modification of *cisplatin* or the development of new anticancer drugs based on other transition metal compounds other than those of platinum(II) are themes that arouse great interest in medicinal chemistry [2].

It has been reported that the organopalladium(II) compounds **A**–**F** shown in Fig. 1, either alone or combined with other therapies, give positive results for the treatment of primary and metastatic cancers in animal models [3]. Organopalladium(II) compounds with a structural formula related to that of compounds **A**, **D**, **E** and **F** are under a WO patent because of their possible role as active inhibitors of enzymes, such Catepsin B, and their ability to modulate the immunological system due to their action on these enzymes and their interaction with DNA [4]. These findings [3,4] add to a few more [5], which point to the use of cyclopalladated compounds as alternative chemotherapeutic drugs for *cisplatin* in view of their *in vitro* cytotoxicity against different cancer cell lines.

The cell killing mechanism of the diphosphane-organodipalladium(II) compound **A** has been investigated [3a,5f,m]. These studies showed that this compound interacted with thiol groups from proteins of the mitochondrial membrane. This

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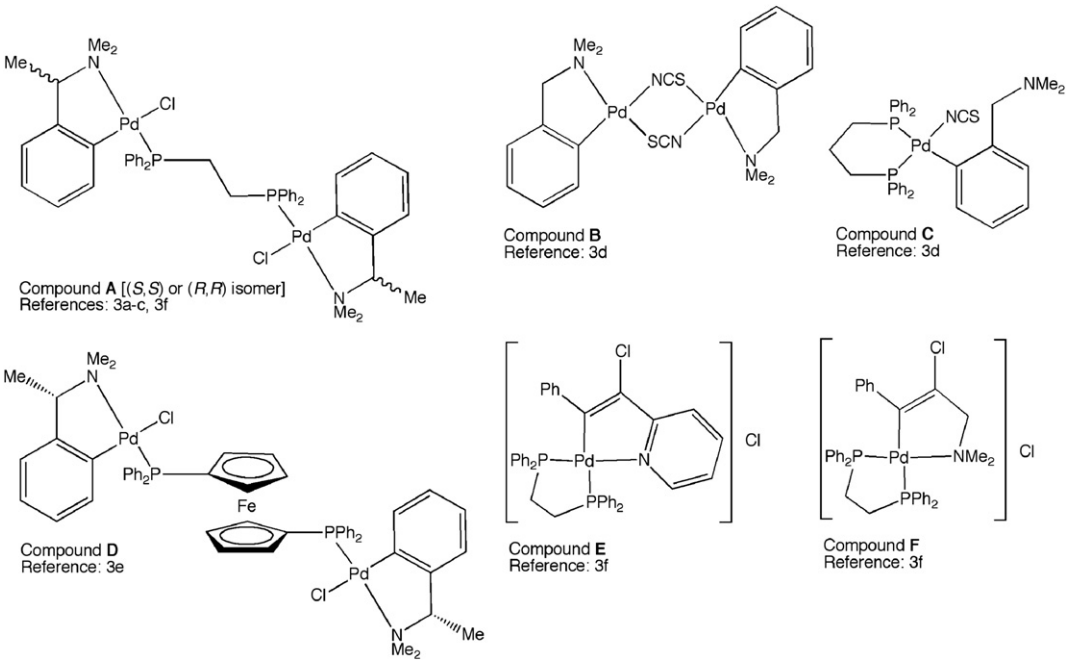


Fig. 1. Organopalladium(II) compounds used as chemotherapeutic drugs with positive results for the treatment of different cancers in animal models.

interaction caused dissipation of the mitochondrial membrane potential and Bax translocation from the cytosol to the mitochondria. Compound **A** also produced an increase in cytosolic calcium concentration, a decrease in ATP levels, and the activation of caspases, which induced chromatin condensation and DNA degradation. These results suggested that compound **A** induced the activation of the apoptotic intrinsic pathway in murine melanoma B16F10-Nex2 cells. Interestingly, the cell killing mechanism of organopalladium(II) compounds may be highly dependent on their structure [3f].

In our ongoing work on the cyclopalladation of imines [6], we realized that the cyclopalladation reaction of benzophenone imine ($Ph_2CH{=}NH$) had not been reported even though it is a relatively non-expensive commercial chemical. Its cyclometallation with platinum(II) compounds has recently been described and its cyclometallated platinum(II) derivatives were found to be luminescent in solution and in solid state at room temperature [7]. Fe(II), Ir(III), Rh(III), Ru(II), Os(II) and Os(IV) metallic centres have also been bonded to benzophenone imine by cyclo-*ortho*-metallation reactions [8].

The cyclopalladation reaction of *N*-benzylideneamines of formula Ar–CH=N–R (R = aryl, benzyl, …) has been extensively studied [9] and their cyclopalladated derivatives have found application in organic synthesis [10] and as precatalysts for Heck and Suzuki reactions [11]. Furthermore, some cyclopalladated *N*-benzylideneamines with adequate substituents at the imine ligand and coligands at the palladium atom form mesophases [12] or present non-linear optical properties [13]. Fig. 2 shows the most usual structural formulae for cyclopalladated *N*-benzylideneamines. It should be noted that no previous studies on the cytotoxic activity of simple cyclopalladated imines such as those shown in Fig. 2 or in Scheme 1 have been reported. Cyclopalladated α-benzoyl-*N*-benzylideneamines are the compounds most closely structurally related with this work for which the antiproliferative activity has been studied [5p,t–v].

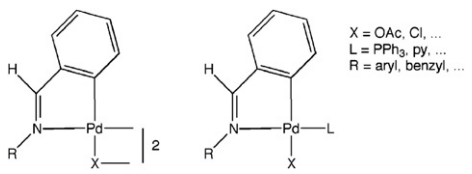
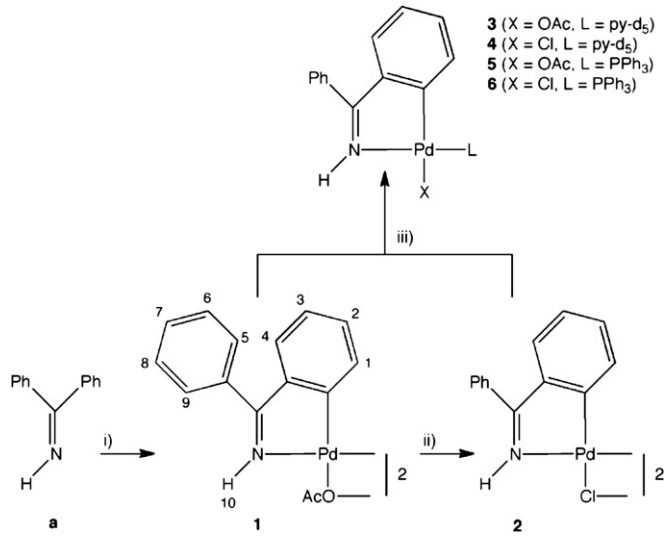


Fig. 2. Most usual structural formulae of cyclopalladated *N*-benzylideneamines.

Finally, it should be noted that the cyclopalladation of a few benzophenone imines derived from aniline, benzylamine, cyclohexylamine, 3,3-bis(isopropylthio)prop-2-en-1-amine, α-amino-acid and peptide esters has been reported [9f,h,14].

The precedent studies [3–14] prompted us to investigate: *i*) the preparation and characterization of several new cyclopalladated compounds derived from benzophenone imine ($Ph_2C{=}NH$), including their photophysical properties, *ii*) their cytotoxicity towards MDA-MB231 and MCF7 human breast adenocarcinoma



Scheme 1. i) $Pd(OAc)_2$ (stoichiometric), HOAc, 60 °C, 24 h; ii) LiCl (excess), acetone, r.t., 2 h; iii) **3** and **4**: py-d_5 (excess), $CDCl_3$, r.t.; **5** and **6**: PPh_3 (stoichiometric), acetone, r.t., 2 h.

cell lines and *iii*) their interaction with DNA using the DNA electrophoretic migration, DNA-ethidium bromide fluorescence quenching and viscometry techniques.

2. Results and discussion

2.1. Organopalladium compounds: synthesis and characterization

Scheme 1 shows the structural formula of the new compounds prepared in this study and the numbering of their protons for the discussion that follows.

Treatment of $Ph_2CH{=}NH$ (compound **a** in Scheme 1) with the stoichiometric amount of $Pd(OAc)_2$ in acetic acid at 60 °C produced the corresponding acetato-bridged five-membered *ortho*-cyclopalladated dimer **1**. This compound was easily converted by a metathesis reaction with LiCl into the chlorido-bridged cyclopalladated dimer **2** (Scheme 1). Compounds **1**–**2** were isolated in pure form in 79% and 78% yields respectively. The details of the preparation and isolation of these compounds are given in the Experimental section. Compound **1** was a deep yellow solid which was highly soluble in $CDCl_3$ and compound **2** was a pale yellow solid which was moderately soluble in $CDCl_3$. The $CDCl_3$ solutions of compounds **1**–**2** were stable on contact with air. Compounds **1**–**2** produced satisfactory elemental analyses, ESI-(+) mass spectrum, IR and ^1H NMR spectra.

The ESI-(+) mass spectrum produced intense peaks for the cations $[(M/2) + Na]^+$ for compound **1** and $[M - Cl]^+$ and $[(M/2) - Cl + CH_3CN]^+$ for compound **2**. These results were in agreement with their proposed dinuclear structure with acetato- and chlorido-bridged ligands respectively [6a].

The asymmetric and symmetric stretching of the carboxylate functions of **1** produced broad intense bands at 1565 and 1431 cm^{-1} respectively, indicating that the acetato ligands of compound **1** presented a bridging coordination mode [15]. The N–H st and the C=N stretching of compound **1** were not observed since they were occluded inside the broad and strong signals corresponding to the water O–H stretching and to the asymmetric stretching of the carboxylate functions of **1** respectively. For compound **2**, the N–H and the C=N stretching were assigned to sharp signals of medium intensity at 3309 and 1584 cm^{-1}, and were shifted 43 and 26 cm^{-1}, respectively, to lower wave numbers in relation to the N–H and C=N stretching of free benzophenone imine. The 26 cm^{-1} shift of the C=N stretching of compound **2** to lower wave numbers in relation to free benzophenone imine was an indication of its k^1-***N*** coordination to the palladium(II) centre [9h].

The principal feature of the ^1H NMR at 400 MHz of compound **1** in $CDCl_3$ solution was the clear separation between non-palladated and *ortho*-palladated phenyl proton signals. Thus, the non-palladated phenyl produced a triplet of triplets for proton 7 (the *para* proton) at 7.86 ppm, a broad triplet for protons 8 and 6 (the *meta* protons) at 7.24 ppm, and a doublet of doublets for protons 9 and 5 (the *ortho* protons) at 6.83 ppm. The protons of the *ortho*-palladated phenyl appeared in the interval 7.21 and 6.83 ppm and afforded the expected multiplicity pattern for a 1,2-disubstituted phenyl ring. Thus, protons 1 and 4 afforded doublets of doublets at 7.21 and 6.83 ppm, while protons 2 and 3 afforded triplets of doublets at 7.11 and 6.88 ppm. In addition, the protons of the acetato ligands of **1** produced a singlet at 2.22 ppm. This result indicated that the dinuclear compound **1** with acetato ligands bridging the cyclopalladated units adopted a *trans* configuration. Finally, proton 10 (the NH proton) of compound **1** afforded a broad singlet centred at 7.86 ppm. This proton for free benzophenone imine in $CDCl_3$ solution produced a broad signal at 8.4 ppm.

The ^1H NMR at 400 MHz of compound **2** in $CDCl_3$ solution was less informative than that of compound **1**. For compound **2**, the NH proton appeared as a broad singlet centred at 8.38 ppm and the aromatic protons produced three groups of signals in the interval 7.61–7.02 ppm integrating six, one and two protons respectively.

Interestingly, the aromatic protons of compound **1** appeared in the interval 7.41–6.70 ppm at lower frequencies than those of compound **2**. This shift to lower frequencies of the aromatic protons of compound **1** in relation to compound **2** indicates a quite important change in the structure on going from compound **1** to compound **2**. Overall, comparison of the ^1H NMR data of compounds **1** and **2** suggests that compound **1** adopted a *trans*-folded (open book) structure and compounds **2** a *trans*-planar structure (Fig. 3). Most of the acetato and chlorido bridged cyclopalladated dimers adopt these structures in $CDCl_3$ solution and in the solid state [16].

Compounds **1**–**2** reacted with an excess of py-d_5 or a stoichiometric amount of PPh_3 giving the mononuclear compounds **3**–**6**. Compounds **3**–**4** were prepared in $CDCl_3$/py-d_5 solution and studied by ^1H NMR, but were not isolated. The ^1H NMR of the solution resulting from the reaction of dinuclear cyclopalladated compounds with acetato or halogeno bridging ligands with py-d_5 in $CDCl_3$ is a fast test for determining their cyclopalladated structure [6a]. Compounds **3**–**4** were highly soluble in $CDCl_3$/py-d_5. Compounds **5**–**6** were prepared in acetone and isolated in pure form as pale yellow solids in 43% and 79% yields, respectively, and were quite soluble in $CDCl_3$. These latter $CDCl_3$ solutions and those of compounds **3**–**4** in $CDCl_3$/py-d_5 were stable on contact with air. Compounds **5**–**6** produced satisfactory elemental analysis, mass spectra, IR and ^1H and $^{31}P\{^1H\}$ NMR.

The LDI-TOF (+) mass spectrum for compound **5** produced intense peaks corresponding to $[M + Na]^+$ and $[M - OAc]^+$ and the MALDI-TOF(+) of compound **6** produced an intense signal at $[M - Cl]^+$ in accordance with their proposed structures [6a]. For compound **5** the N–H stretching appeared at 3319 cm^{-1}, and the asymmetric and symmetric stretching of the carboxylate function of the acetato ligand produced intense signals at 1574 and 1374 cm^{-1}, in agreement with its unidentate coordination mode [15]. The C=N stretching of compound **5** was not observed since it was occluded inside the broad and strong band corresponding to the asymmetric stretching of the carboxylate function of **5**. For compound **6**, the N–H and C=N stretching produced sharp signals

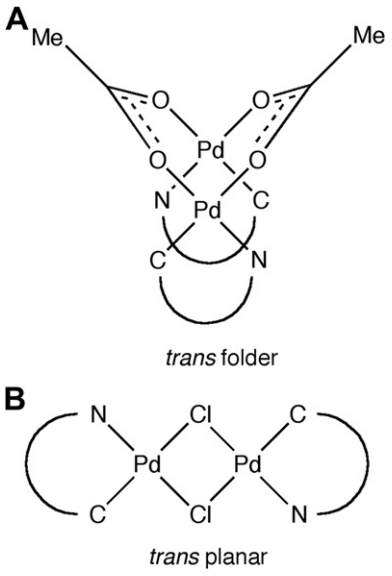


Fig. 3. A) *trans* folded and B) *trans* planar structures proposed for compounds **1** and **2** respectively. C–N=C_6H_4PhC=NH.

of medium intensity at 3310 and 1592 cm^{-1}, respectively. Furthermore, the *q* X-sensitive mode of the coordinated PPh_3 appeared at 1103 and 1098 cm^{-1} for compounds **5** and **6** respectively [17].

^1H NMR data for the mononuclear compounds **3**–**6** were consistent with their proposed stereochemistry, in which the L ligand is located *trans* to the iminic nitrogen and the X ligand is *trans* to the palladated carbon atom (see Scheme 1). We refer to this arrangement as *trans*-***N,L*** stereochemistry. Accordingly to this stereochemistry, the aromatic protons of the *ortho*-palladated ring of compounds **3**–**6** appeared at lower frequencies (between 7.20 and 6.40 ppm) in relation to the other aromatic protons, because they were located in the shielding zone of the aromatic ring of the py-d_5 ligand for compounds **3**–**4** and in that of the phenyl substituents of the PPh_3 ligand for compounds **5**–**6** [6a]. Protons of the *ortho*-palladated ring of compounds **3**–**6** afforded the expected multiplicity pattern for an 1,2 disubstituted phenyl. In addition, for compounds **5**–**6**, the 1 protons were coupled with the phosphorus nuclei. The value of this coupling constant of *ca.* 8 Hz was also an indication of their *trans*-***N,P*** stereochemistry [6a,17]. The NH proton for compounds **3**, **4**, **5** and **6** appeared at 10.18 (broad singlet), 8.73 (broad singlet), 9.44 (broad doublet, $^3J_{HP} \approx 4$) and 9.21 (broad doublet, $^3J_{HP} \approx 4$) respectively. In addition, the protons of the unidentate acetato ligands of compounds **3** and **5** afforded a singlet at 1.98 and 1.44 ppm respectively.

A further indication of the *trans*-***N,P*** stereochemistry of compounds **5**–**6** was given by the chemical shift of their ^{31}P-{^1H} NMR, which were 40.73 and 41.34 ppm, respectively. These chemical shifts were in the range expected for mononuclear cyclopalladated compounds of general formula *trans*-***N,P***-[Pd(C–N)(X)(PPh_3)] (X = OAc, Cl, Br, I) that contain a five-membered palladacycle and a phenyl or a naphthyl metallated carbon atom (43–40 ppm) [6a,18].

In order to complete the characterization of compounds **1**, **2**, **5** and **6**, their absorption and emission electronic spectra were recorded. The absorption spectra of the complexes in dichloromethane at 298 K showed high intensity superimposed $\pi \rightarrow \pi^*$ intraligand transitions in the range 280–350 nm. In addition, a lower energy band at 466 nm was observed for compound **1**. This band was attributed to a MLCT [4d(Pd) $\rightarrow \pi^*$ C=N] transition. This assignation is based on similarities in energy and on theoretical calculations carried out with analogous platinum cyclometallated complexes [7]. This transition is probably occluded under the intraligand transitions and displayed as a shoulder for the other compounds. The absorption observed at longer wavelength for compound **1** is also present in the electronic spectrum of other acetato bridged cyclopalladated complexes [19].

Compounds **1**, **2**, **5** and **6** were not luminescent in solution but a weak emission was observed in the solid state for the acetato compounds **1** and **5** upon excitation of their powders at the lower energy absorption band. No emission was observed when the samples were excited at the intraligand transitions. This is consistent with an involvement of the MLCT on this emission band. This transition is more favoured for compound **1** and give rise to a red-shifted emission band. The large Stokes' shift observed is in agreement with a triplet origin (^{3}MLCT). Table 1 gives a summary of the absorption and emission electronic spectra of compounds **1**, **2**, **5** and **6**.

Table 1
Absorption and emission electronic spectra for compounds **1**, **2**, **5** and **6**.

Compound	λ absorption in nm (ε·10^{-3}, M^{-1} cm^{-1})	λ emission in nm (solid)
1	275 (22.6), 322 (12.4), 466 (3.0)	670 (λ_{exc} = 450 nm)
2	285 (31.1), 311 (19.9), 330 (19.3)	–
5	280 sh (14.8), 313 sh (7.1), 336 sh (3.3)	615 (λ_{exc} = 370 nm)
6	287 sh (10.7), 312 sh (6.32), 340 sh (3.1)	–

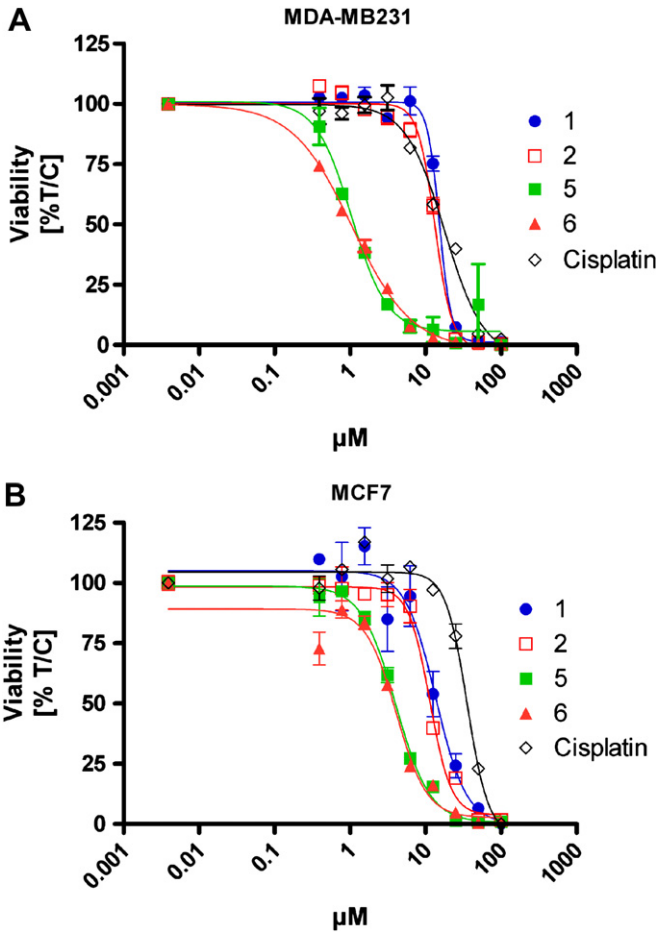


Fig. 4. Inhibition of cell growth proliferation in MDA-MB231 and MCF7 breast human cancer cell lines, after 72 h of exposure to compounds **1**, **2**, **5**, **6** and *cisplatin*.

Finally, it should be noted that attempts to grow crystals suitable for X-ray diffraction analysis for compounds **1**, **2**, **5** and **6** were not successful.

2.2. Antitumour activity

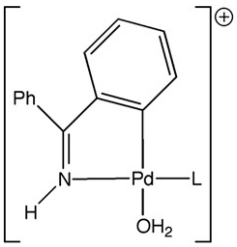
Compounds **1**, **2**, **5** and **6** were evaluated *in vitro* for inhibition of cell proliferation against the MDA-MB231 and MCF7 human breast cancer cell lines, using *cisplatin* as a positive control. The effects of the assayed palladacycles on the growth of the selected cell lines were assessed after 72 h and the results are displayed in Fig. 4. The IC_{50} values of compounds **1**, **2**, **5** and **6** resulting from an average of two experiments are listed in Table 2.

Table 2 and Fig. 4 show that compounds **5**–**6** exhibit a high antiproliferative activity, showing IC_{50} values in the range 1–5 μM below those of *cisplatin* in both cellular lines. Compounds **5**–**6** were

Table 2
IC_{50} (μM) values for compounds **1**, **2**, **5** and **6**. Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation.

Compound	Tumour cell line	
	MDA-MB231	MCF-7
1	15 ± 1.2	14 ± 4.2
2	13 ± 1	11 ± 1.5
5	1.1 ± 0.3	4 ± 0.5
6	1.1 ± 0.1	4.1 ± 0.9
cisplatin[a]	6.5 ± 2.4	19 ± 4.5

[a] *cis*-[$PtCl_2(NH_3)_2$] is taken as reference compound.



I (L = H_2O)
II (L = PPh_3)

Fig. 5. Aqua cations **I** and **II**.

approximately 6- and 4-fold times more potent than *cisplatin* in the MDA-MB231 and MCF7 human breast cancer cells respectively. In addition, the IC_{50} of compounds **1**–**2** was on the low side of the interval reported for the IC_{50} values of cyclopalladated compounds towards different cancer cell lines, which varies between 0.5 and 450 μM for these compounds [3–5]. In addition, compound **2** was more potent than *cisplatin* against the MCF7 tumour cell line.

The similarity in the IC_{50} values of compounds **1**–**2** and **5**–**6** for the MDA-MB231 and MCF7 cell lines (see Table 2) can be understood if we consider that in the biological media these compounds may be present as the aqua cations shown in Fig. 5. The aquatization of the Pd–OAc and Pd–Cl bonds of compounds **1**–**2** should give the aqua cation **I** and that of compounds **5**–**6** the aqua cation **II** [20].

2.3. Interaction with DNA

The interaction of compounds **1**–**2** and **5**–**6** with 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. The ccc form usually moves faster due to its compact structure. When the test compounds were incubated with plasmid DNA at 37 °C, they coordinated to DNA molecule, which in some extend was cleaved into fragments, and the brightness of band diminished in gel. In spite of their high antiproliferative activity, compounds **1**–**2** and **5**–**6** were 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 of their cytotoxicity [3a,5f,m].

An unwinding experiment was performed with increasing concentration of compounds **1**–**2** and **5**–**6** ranging from 2.5 μM to 200 μM and 40 μg/mL pBluescript (Fig. 6). At 50 μM, only a slight decrease in the rate of migration of the supercoiled closed circular form was observed for the dinuclear compound **2** (compound **2**, Lane 5) and at 100 μM all the compounds greatly altered the mobility of the plasmid DNA (compounds **1**–**2** and **5**–**6**, Lane 6).

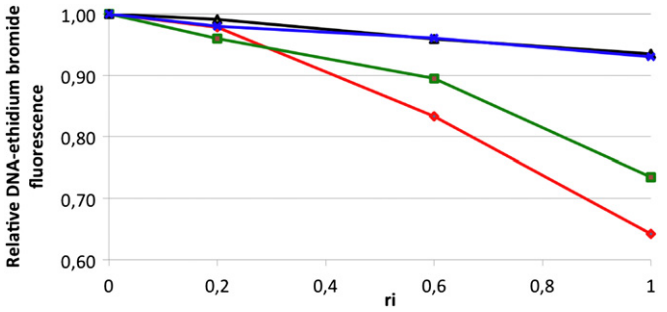


Fig. 7. Relative *Calf thymus* DNA-ethidium bromide fluorescence. r_i = molar ratio between drug/DNA nucleotides. Red rhombus = compound **1**, green square = compound **2**, blue cross = compound **5**, black triangle = compound **6**. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The rate of migration of the supercoiled band (ccc) decreased and tended to approach that of the nicked relaxed band (oc). At high concentrations of compounds **1**–**2**, a strong unwinding of negative to positive supercoiled DNA was displayed in the electrophoretogram (Compounds **1**–**2**, Lane 7). The same effect was observed for *cisplatin* (Fig. 6, *cisplatin*, Lanes 3 and 4).

On the basis of the alteration of the electrophoretic mobility of pBluescript plasmid DNA it is hypothesized that compounds **1**–**2** and **5**–**6** alter the DNA tertiary structure by the same mechanism as the standard reference, *cisplatin*. In addition, the interaction of compounds **5**–**6** with supercoiled plasmid DNA seems to be stronger than that of compounds **1**–**2** since Lanes 6–7 for compounds **5**–**6** are less brilliant than Lanes 6–7 for compounds **1**–**2** [5j].

DNA-ethidium bromide fluorescence quenching and viscometry experiments were performed in order to explore an intercalating mode of binding to DNA for compounds **1**–**2** and **5**–**6**. In the DNA-ethidium bromide fluorescence quenching technique, the quenching of the emission maximum of the fluorescence of ethidium bromide intercalated between DNA base pairs can be taken as an indication of a competition between the drug and the ethidium bromide for the intercalating binding sites in DNA [21]. This experiment (Fig. 7) suggested that compounds **1**–**2** could be intercalating agents since their DNA-ethidium bromide fluorescence was 0.63 and 0.72 respectively, at $r_i = 1$ (r_i = molar ratio between drug/DNA nucleotides [22]) relative to the DNA-ethidium bromide initial fluorescence, taken as 1. However, viscometry experiments ruled out this possibility since no increase in viscosity was observed when compounds **1**–**2** were incubated with DNA [23].

The DNA-ethidium bromide fluorescence quenching for compounds **1**–**2** and **5**–**6** followed the Stern–Volmer equation: $I_0/I = 1 + K_q[Q]$. Where I_0 and I represent the emission intensity in the absence and presence of quencher respectively, is a linear

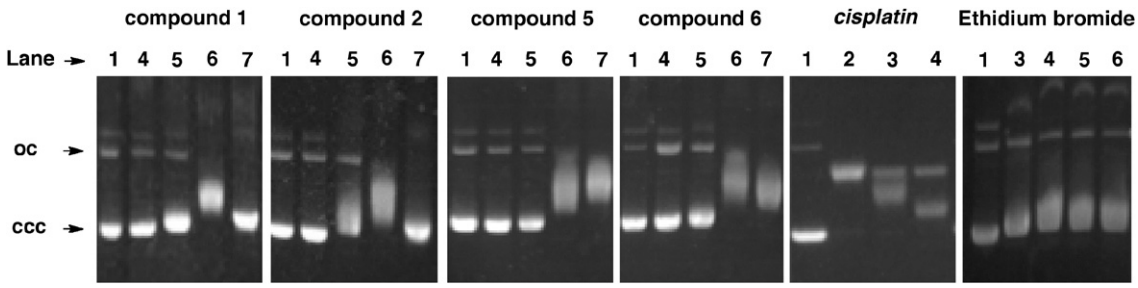


Fig. 6. Interaction of pBluescript SK + plasmid DNA (40 μg/mL) with increasing concentrations of compounds **1**, **2**, **5**, **6**, *cisplatin* and ethidium bromide. Lane 1: DNA only. Lane 2: 2.5 μM drug. Lane 3: 5 μM drug. Lane 4: 25 μM drug. Lane 5: 50 μM drug. Lane 6: 100 μM drug. Lane 7: 200 μM drug. ccc = supercoiled closed circular DNA. oc = open circular DNA.

Stern–Volmer quenching constant and [Q] is the quencher concentration, the concentration of cyclopalladated compound in our study. The I_0/I vs. [Q] followed a linear pattern from which K_q was calculated. K_q was 1.1×10^4 M^{-1} and 7.0×10^3 M^{-1} for **1** and **2**, respectively, and 1.3×10^3 M^{-1} for **5** and **6**.

3. Conclusions

Four new cyclopalladated benzophenone imines (**1**–**2** and **5**–**6**) were prepared by cyclopalladation, metathesis and splitting reactions and were fully characterized by the standard techniques. These compounds showed a high antiproliferative activity against the MDA-MB231 and MCF7 human breast cancer cell lines. Compounds **5**–**6** were approximately 6- and 4-fold times more potent than *cisplatin* in the MDA-MB231 and MCF7 human breast cancer cells and presented IC_{50} values in the range 1–5 μM. The study of the interaction of compounds **1**–**2**, and **5**–**6** with DNA by agarose gel electrophoresis showed that they modify the helicity of the DNA as the alkylating agent *cisplatin*, although in a less extensive way. In addition, DNA-ethidium bromide fluorescence quenching and viscometry experiments ruled out an intercalating binding mode to DNA for these kind of compounds.

4. Experimental

4.1. Instruments and reagents

Elemental analyses of C, H and N were performed with an Eager 1108 microanalyzer. Infrared spectra were recorded on a Nicolet Impact-400 spectrophotometer using pressed discs of dispersed samples of the compounds in KBr. ^{1}H NMR at 400 MHz and $^{31}P\{^1H\}$ NMR at 121.4 MHz spectra were recorded in Varian 400 and 300 instruments, respectively. Chemical shifts are reported in δ values (ppm) relative to $SiMe_4$ ($\delta = 0.00$) for ^{1}H NMR and to $PO(OMe)_3$ ($\delta = 2.39$ ppm) for $^{31}P\{^1H\}$ NMR, and coupling constants are giving in Hz. MALDI-TOF (+) and LDI-TOF (+) spectra were registered on a VOYAGER-DE-RP spectrometer. 2,5-dihydroxybenzoic acid (DHB) was used as matrix for the MALDI-TOF (+) experiment. ESI-(+) spectra were acquired on a LC/MSD-TOF mass spectrometer, using 1:1H_2O:CH_3CN as eluent. The mass of the most abundant isotopic peak is given for the different molecular fragments [24]. UV–vis were recorded at 298 K with a Cary 100 scan 388 Varian UV spectrometer. The fluorescence emission spectra were carried out on a Horiba-Jobin-Yvon SPEX Nanolog-TM at 25 °C. All chemicals and solvents were of commercial grade and used as received. Viscosity experiments were carried out with an AND-SV-1 viscometer in a water bath using a water jacket accessory and maintained at a constant temperature of 25 °C.

4.2. Preparation of compound *1*

A suspension formed by 497 mg (2.21 mmol) of $Pd(OAc)_2$, 400 mg (2.21 mmol) of benzophenone imine and 20 mL of acetic acid was stirred at 60 °C for 24 h. The resulting brown suspension was concentrated under vacuum. Addition of acetone (10 mL) to the residue produced the precipitation of **1** as a yellow powder, which was filtered and dried under vacuum. Yield: 79% (605 mg). Characterization data: Anal. Calc for $C_{30}H_{26}N_2O_4Pd_2$: C, 52.12; H, 3.79; N, 4.05%. Found: C, 52.06; H, 3.72; N, 4.02%. ESI-(+): Intense peak at $[(M/2) + Na]^+ = 368.0395$ (calc. 367.9885). IR (cm^{-1}): 3145 N–H st, 1565 (st as carboxylate function, acetate), 1431 (st s carboxylate function, acetate). ^{1}H NMR (400 MHz, $CDCl_3$): 7.86 (br s, 1H, H_{10}), 7.41 (tt, 1H, H_7, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 1$), 7.24 (t, 2H, H_6 and H_8, $^3J_{HH} \approx 8$), 7,21 (dd, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 1$), 7.11 (td, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.88 (td, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.83 (dd, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.70 (dd, 2H, H_9 and H_5, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 1$), 2.22 (s, 3H, OAc).

4.3. Preparation of compound *2*

A suspension formed by 100 mg (0.15 mmol) of **1**, 25 mg (0.59 mmol) of LiCl and 30 mL of acetone was stirred at room temperature for 2 h. The resulting pale yellow solution was concentrated under vacuum and the residue was eluted through a silica-60 gel column chromatography with a solution of methanol in chloroform in a 4–100 volume ratio. The yellow band was collected and concentrated under vacuum. Addition of diethyl ether (5 mL) to the residue led to the precipitation of **2** as a pale yellow solid, which was filtered and dried under vacuum. Yield: 73 mg (78%). Characterization data: Anal. Calc for $C_{26}H_{26}Cl_2Pd_2$: C, 48.48; H, 3.13; N, 4.35%. Found: C, 48.52; H, 3.18; N, 4.57%. ESI-(+): intense peaks at $[M - Cl]^+ = 608.9392$ (calc. 608.9395), $[(M/2) - Cl + CH_3CN]^+ = 327.0114$ (calc. 327.0120) and $[(M/2) - Cl]^+ = 285.9851$ (calc. 285.9853). IR (cm^{-1}): 3309 st N–H, 1584 st C=N. ^{1}H NMR (400 MHz, $CDCl_3$): 8.38 (br s, 1H, H_{10}), 7.61–7.52 (m, 6H), 7.15 (td, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 7.09–7.02 (m, 2H).

4.4. NMR tube reactions

ca. 10 mg of compound **1**–**2** were treated with 0.7 mL of $CDCl_3$ and two drops of py-d_5. The formation of a pale yellow solution was an indication of the quantitative transformation of compounds **1**–**2** into the mononuclear compounds **3**–**4**. Characterization data: Compound **3**: ^{1}H NMR (400 MHz, $CDCl_3$ + py-d_5): 10.18 (br s, 1H, H_{10}); 7.47–7.57 (m, 5H, H_5–H_9); 7.16 (m, 1H, H_4); 7.02 (m, 2H, H_2 - H_3); 6.47 (m, 1H, H_1); 1.98 (s, 3H, OAc). Compound **4**: ^{1}H NMR (400 MHz, $CDCl_3$ + py-d_5): 8.73 (br s, 1H, H_{10}); 7.51–7.60 (m, 5H, H_5–H_9); 7.17 (dd, 1H, H_4, 1H, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$); 7.04–7.08 (m, 2H, H_2–H_3); 6.44 (d, 1H, H_1, $^3J_{HH} \approx 8$).

4.5. Preparation of compound *5*

A suspension formed by 100 mg (0.14 mmol) of **1**, 76 mg of PPh_3 (0.29 mmol) and 10 mL of acetone was stirred at room temperature for 2 h. The resulting solution was concentrated under vacuum and the residue was eluted trough a silica-60 gel column chromatography with a solution of methanol in chloroform in a 4–100 volume ratio. The second coloured band was collected and concentrated under vacuum. Addition of diethyl ether (5 mL) to the residue led to the precipitation of **5** as a white solid, which was filtered and dried under vacuum. Yield: 59 mg (43%). Characterization data: Anal. Calc for $C_{33}H_{28}NO_2PPd$: C, 65.19; H, 4.64; N, 2.30%. Found: C, 65.08; H, 4.62; N, 2.31%. LDI-TOF(+): Intense peaks at $[M + Na]^+ = 630.1$ (calc. 630.0803) and $[M - OAc]^+ = 548.1$ (calc. 548.0771). IR (cm^{-1}): 3319 st N–H, 1574 (st as carboxylate function, acetate), 1374 (st s carboxylate function, acetate), 1103 *q* X-sensitive mode of the coordinated PPh_3. ^{1}H NMR (400 MHz, $CDCl_3$): 9.44 (br d, 1H, H_{10}, $^3J_{HP} \approx 4$), 7.82–7.77 (m, 6H, *o*-PPh_3), 7.57–7.37 (m, 14 H, *m*- and *p*-PPh_3 and H_5–H_9), 7.10 (dd, 1H, H_4, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.86 (td, 1H, H_3, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 1$), 6.62 (td, 1H, H_1, $^4J_{HP} \approx {}^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.55 (td, 1H, H_1, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 1.44 (s, 3H, OAc). $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): 40.73.

4.6. Preparation of compound *6*

A suspension formed by 51 mg (0.08 mmol) of **2**, 43 mg of PPh_3 (0.16 mmol) and 30 mL of acetone was stirred at room temperature for 2 h. The resulting white suspension was concentrated under vacuum and the residue was eluted trough a silica-60 gel column chromatography with a solution of methanol in chloroform in a 4–

100 volume ratio. The yellowish band was collected and concentrated under vacuum. Addition of diethyl ether (5 mL) to the residue led to the precipitation of **6** as a white solid, which was filtered and dried under vacuum. Yield: 74 mg (79%). Characterization data: Anal. Calc for $C_{31}H_{25}ClNPPd$: C, 63.71; H, 4.31; N, 2.40%. Found: C, 62.92; H, 4.04; N, 2.50%. MALDI-TOF(+): Intense peak at $[M - Cl]^+ = 548.3$ (calc. 548.0771). IR (cm^{-1}): 3310 st N–H, 1592 st C=N, 1098 *q* X-sensitive mode of the coordinated PPh_3. ^1H NMR (400 MHz, $CDCl_3$): 9.21 (d, 1H, H_{10}, $^3J_{HP} \approx 4$); 7.75–7.80 (m, 5H, H_5–H_9); 7.37–7.57 (m, 15H, PPh_3); 7.14 (dd, 1H, H_4, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.90 (td, 1H, H_3, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 1$), 6.62 (td, 1H, H_2, $^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$), 6.54 (td, 1H, H_1, $^4J_{HP} \approx {}^3J_{HH} \approx 8$, $^4J_{HH} \approx 2$). $^{31}P\{^1H\}$ NMR (121.4 MHz, $CDCl_3$): 41.34.

4.7. Cell culture

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, 10 mM D-glucose and 0.1% streptomycin/penicillin, in standard culture conditions (humidified air with 5% CO_2 at 37 °C).

4.8. Cell viability assay

A stock solution (50 mM) of each compound was prepared in high purity DMSO. Then, serial dilutions were made with DMSO (1:1) and finally a 1:500 dilution of the diluted solutions of compounds on cell media was prepared. In this way DMSO concentration in cell media was always the same. The assay was performed as described by Givens et al. [25]. MDA-MB231 and MCF7 cells were plated at 5000 cells/well, respectively, in 100 μL media in tissue culture 96-well plates (Cultek). After 24 h, media was replaced by 100 μL/well of drug serial dilutions. Control wells did not contain compounds **1**, **2**, **5** and **6**. 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 media was removed and cells were washed once with PBS. 60 μL of substrate solution (*p*-nitrophenol-*N*-acetyl-β-D-glucosamide 7.5 mM, sodium citrate 0.1 M 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 appeared. Then, the plates were developed by adding 90 μL of developer solution (Glycine 50 mM, pH 10.4; EDTA 5 mM) and the absorbance was recorded at 410 nm.

4.9. DNA migration studies

A stock solution (10 mM) of each compound was prepared in high purity DMSO. Then, serial dilutions were made in MilliQ water (1:1). Plasmid pBluescript SK+ (Stratagene) was obtained using a QIAGEN plasmid midi kit as described by the manufacturer. Interaction of drugs with pBluescript SK + plasmid DNA was analysed by agarose gel electrophoresis following a modification of the method described by Abdullah et al. [26]. 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 compounds **1**, **2**, **5** and **6** ranging from 0 μM 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 ethidium bromide (0.5 mg mL^{-1}) and visualized and photographed under UV light.

4.10. DNA-ethidium bromide fluorescence quenching

To a 50 μM *Calf thymus* (CT) DNA solution in Milli-Q water (3 mL total volume), 30 μL of a 5 mM ethidium bromide solution was added to get a 3:1 DNA:ethidium bromide molar ratio. The mixture was incubated for 30 min at 37 °C. Increasing amounts of a 1.5 mM DMSO/Milli-Q water (1:1) stock solutions of compounds **1**, **2**, **5** and **6** were added to reach the following final concentrations of the compounds in the DNA:ethidium bromide solution: 0, 10, 20, 30, 40 and 50 μM. After incubation for 4–5 h at 37 °C, fluorescence emission spectra (λ_{ex} = 514 nm) were recorded at room temperature in the wavelength range 530–670 nm. For each compound, three blanks were prepared and measured: 1) 100 μL of 1.5 mM of compound solution in 3 mL TE buffer (to rule out autofluorescence of the compound), 2) 100 μL of 1.5 mM solution of compound in 3 mL of the CT DNA solution (to verify that the compound does not fluoresce with DNA), and 3) 100 μL of the 1.5 mM solution of compound and 30 μL of ethidium bromide 5 mM solution in TE buffer (to check any fluorescent interaction between the compound and ethidium bromide). FluorEssence software for Windows was used in data treatment.

4.11. Viscometry

All compounds were dissolved in high purity DMSO (1 mg compound/1 mL). The stock solutions were freshly prepared, just before use. The samples were prepared by addition of aliquots of these stock solutions to the appropriate volume of *Calf thymus* DNA in a TE buffer solution (50 mM NaCl, 10 mM tris-(hydroxymethyl) aminomethane hydrochloride (Tris–HCl), 0.1 mM H4edta, pH 7.4) (3 mL). The amount of complex added to the DNA solution was designated as r_i. As a blank, a solution in TE of free native DNA was used. The viscosity spectra of DNA in the presence or absence of complexes (DNA concentration 20 μM, molar ratios $r_i = 0.2$, 0.6 and 1) were recorded at 25 °C, after 5 h incubation at 37 °C.

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References

[1] L. Kelland, Nat. Rev. Cancer 7 (2007) 573.
[2] G. Gasser, I. Ott, N. Metzler-Nolte, J. Med. Chem. 54 (2011) 3.
[3] (a) F.A. Serrano, A.L. Matsuo, P.T. Monteforte, A. Bechara, S.S. Smaili, D.P. Santana, T. Rodrigues, F.V. Pereira, L.S. Silva, J. Machado Jr., E.L. Santos, J.B. Pesquero, R.M. Martins, L.R. Travassos, A.C.F. Caires, E.G. Rodrigues, BMC Cancer 11 (2011) 296;
(b) A.B. Guimaraes-Correa, L.B. Crawford, C.R. Figueiredo, K.P. Gimenes, L.A. Pinto, M.F. Rios Grassi, G. Feuer, L.R. Travassos, A.C.F. Caires, Elaine G. Rodrigues, Susan J. Marriott, Viruses 3 (2011) 1041;
(c) F. Hebeler-Barbosa, E.G. Rodrigues, R. Puccia, A.C.F. Caires, L.R. Travassos, Transl. Oncol. 1 (2008) 110;
(d) M.C. da Rocha, A.M. Santana, S.R. Ananias, E.T. de Almeida, A.E. Mauro, M.C.P. Placeresa, I.Z. Carlos, J. Braz. Chem. Soc. 18 (2007) 1473;
(e) C. Bincoletto, I.L.S. Tersariol, C.R. Oliveira, S. Dreher, D.M. Fausto, M.A. Soufen, F.D. Nascimento, A.C.F. Caires, Bioorg. Med. Chem. 13 (2005) 3047;
(f) E.G. Rodrigues, L.S. Silva, D.M. Fausto, M.S. Hayashi, S. Dreher, E.L. Santos, J.B. Pesquero, L.R. Travassos, A.C.F. Caires, Int. J. Cancer 107 (2003) 498.
[4] A.C.F. Caires, C.B. Trindade, I.L.d.S. Tersariol, WO/2004/019924.
[5] (a) M. Carreira, R. Calvo-Sanjuán, M. Sanaú, I. Marzo, M. Contel, Organometallics 31 (2012) 5772;
(b) E. Budzisz, R. Bobka, A. Hauss, J.N. Roedel, S. Wirth, I.-P. Lorenz, B. Rozalska,

M. Więckowska-Szakiel, U. Krajewskad, M. Rozalskid, Dalton Trans. 41 (2012) 5925;
(c) N.C. Campanella, M. da Silva Demartini, C. Torres, E. Tonon de Almeida, C.M. Cação Paiva Gouvêa, Genet. Mol. Biol. 35 (2012) 159;
(d) J. Quirante, D. Ruiz, A. Gonzalez, C. López, M. Cascante, R. Cortés, R. Messeguer, C. Calvis, L. Baldomà, A. Pascual, Y. Guérardel, B. Pradines, M. Font-Bardía, T. Calvet, C. Biot, J. Inorg. Biochem. 105 (2011) 1720;
(e) M.S. Subhas, S.S. Racharlawar, B. Sridhar, P.K. Kennady, P.R. Likhar, M.L. Kantama, S.K. Bhargavad, Org. Biomol. Chem. 8 (2010) 3001;
(f) D.P. Santana, P.A. Faria, E.J. Paredes-Gamero, A.C.F. Caires, I.L. Nantes, T. Rodrigues, Biochem. J. 417 (2009) 247;
(g) J. Spencer, A. Casini, O. Zava, R.P. Rathnam, S.K. Velhanda, M. Pfeffer, S.K. Callear, M.B. Hursthoused, P.J. Dyson, Dalton Trans. (2009) 10731;
(h) J. Spencer, R.P. Rathnam, M. Motukuri, A.K. Kotha, S.C.W. Richardson, A. Hazrati, J.A. Hartley, L. Malec, M.B. Hursthouse, Dalton Trans. (2009) 4299;
(i) A.C. Moro, A.E. Mauro, A.V.G. Netto, S.R. Ananias, M.B. Quilles, I.Z. Carlos, F.R. Pavan, C.Q.F. Leite, M. Hörner, Eur. J. Med. Chem. 44 (2009) 4611;
(j) J. Ruiz, J. Lorenzo, C. Vicente, G. López, J.M. López-de-Luzuriaga, M. Monge, F.X. Avilés, D. Bautista, V. Moreno, A. Laguna, Inorg. Chem. 47 (2008) 6990;
(k) A.I. Matesanz, P. Souza, J. Inorg. Biochem. 101 (2007) 1354;
(l) X. Riera, V. Moreno, C.J. Ciudad, V. Noe, M. Font-Bardia, X. Solans, Bioinorg. Chem. Appl. (2007) 98732;
(m) C.M.V. Barbosa, C.R. Oliveira, F.D. Nascimento, M.C.M. Smith, D.M. Fausto, M.A. Soufen, E. Sena, R.C. Araújo, I.L.S. Tersariol, C. Bincoletto, A.C.F. Caires, Eur. J. Pharmacol. 542 (2006) 37;
(n) D. Pucci, V. Albertini, R. Bloise, A. Bellusci, A. Cataldi, C.V. Catapano, M. Ghedini, A. Crispini, J. Inorg. Biochem. 100 (2006) 1575;
(o) G.L. Edwards, D.St. C. Black, G.B. Deacon, L.P.G. Wakelin, Can. J. Chem. 83 (2005) 980;
(p) M.A. Peláez, T. Ramírez, M. Martínez, P. Sharma, C. Álvarez, R. Gutiérrez, Z. Anorg. Allg. Chem. 630 (2004) 1489;
(q) A.C.F. Caires, E.T. Almeida, A.E. Mauro, J.P. Hemerly, S.R. Valentini, Quim. Nova 22 (1999) 329;
(r) A.G. Quiroga, J.M. Pérez, I. López-Solera, J.R. Masaguer, A. Luque, P. Román, A. Edwards, C. Alonso, C. Navarro-Ranninger, J. Med. Chem. 41 (1998) 1399;
(s) F. Zamora, V.M. Gónzalez, J.M. Pérez, J.R. Masaguer, C. Alonso, C. Navarro-Ranninger, Appl. Organomet. Chem. 11 (1997) 659;
(t) C. Navarro-Ranninger, I. López-Solera, V.M. González, J.M. Pérez, A. Alvarez-Valdés, A. Martín, P.R. Raithby, J.R. Masaguer, C. Alonso, Inorg. Chem. 35 (1996) 5181;
(u) C. Navarro-Renninger, I. López-Solera, J.M. Pérez, J.R. Masaguer, C. Alonso, Appl. Organomet. Chem. 7 (1993) 57;
(v) C. Navarro-Ranninger, I. López-Solera, J.M. Perez, J. Rodriguez, J.L. Garcla-Ruano, P.R. Raithby, J.R. Masaguer, C. Alonso, J. Med. Chem. 36 (1993) 3795;
(w) J.D. Higgins III, L. Neely, S. Fricker, J. Inorg. Biochem. 49 (1993) 149;
(x) A. Yoneda, M. Ouchi, T. Hakushi, G.R. Newkome, F.R. Fronczek, Chem. Lett. (1993) 709.

[6] See for instance: (a) J. Albert, R. Bosque, L. D'Andrea, J. Granell, M. Font-Bardia, T. Calvet, Eur. J. Inorg. Chem. (2011) 3617;
(b) J. Albert, J.M. Cadena, A. González, J. Granell, X. Solans, M. Font-Bardia, Chem. Eur. J. 12 (2006) 887;
(c) J. Albert, J. Granell, Trends Organomet. Chem. 3 (1999) 99.

[7] (a) S.U. Pandya, K.C. Moss, M.R. Bryce, A.S. Batsanov, M.A. Fox, V. Jan kus, H.A. Al Attar, A.P. Monkman, Eur. J. Inorg. Chem. (2010) 1963;
(b) Y.Y. Scaffidi-Domianello, A.A. Nazarov, M. Haukka, M. Galanski, B.K. Keppler, J. Schneider, P. Du, R. Eisenberg, V.Y. Kukushkin, Inorg. Chem. 46 (2007) 4469.

[8] (a) S. Camadanli, R. Beck, U. Flörke, H.-F. Klein, Organometallics 28 (2009) 2300;
(b) F. Lorenzini, P. Marcazzan, B.O. Patrick, B.R. James, Can. J. Chem. 86 (2008) 253;
(c) M.B. Ezhova, B.O. Patrick, B.R. James, Organometallics 24 (2005) 3753;
(d) G. Barea, M.A. Esteruelas, A. Lledós, A.M. López, E. Oñate, J.I. Tolosa, Organometallics 17 (1998) 4065;
(e) C. Bohanna, M.A. Esteruelas, A.M. López, L.A. Oro, J. Organomet. Chem. 526 (1996) 73;
(f) M.A. Esteruelas, F.J. Lahoz, A.M. López, E. Oñate, L.A. Oro, Organometallics 14 (1995) 2496;
(g) H. Werner, T. Daniel, T. Braun, O. Nürnberg, J. Organomet. Chem. 480 (1994) 145.

[9] See for instance: (a) L. Naya, D. Vázquez-García, M. López-Torres, A. Fernández, J.M. Vila, N. Gómez-Blanco, J.J. Fernández, J. Organomet. Chem. 693 (2008) 685;
(b) J. Albert, R. Bosque, J. Granell, R. Tavera, Polyhedron 20 (2001) 3225;
(c) M. Gómez, J. Granell, M. Martínez, Organometallics 16 (1997) 2539;
(d) J. Albert, J. Granell, J. Mínguez, Quím. Int. Ed. 92 (1996) 399;
(e) J. Albert, J. Granell, J. Sales, Synth. React. Inorg. Met. Org. Chem. 19 (1989) 1009;
(f) W. Clark, S.F. Dyke, G. Smith, C.H.L. Kennard, J. Organomet. Chem. 330 (1987) 447;
(g) J. Granell, D. Sainz, J. Sales, X. Solans, M. Font-Altaba, J. Chem. Soc. Dalton Trans. (1986) 1785;
(h) H. Onoue, I. Moritani, J. Organomet. Chem. 43 (1972) 431.

[10] See for instance: (a) J. Albert, M. Crespo, J. Granell, J. Rodríguez, J. Zafrilla, T. Calvet, M. Font-Bardia, X. Solans, Organometallics 29 (2010) 214;
(b) L. Adrio, J.M. Antelo, J.M. Ortigueira, J.J. Fernández, A. Fernández, M.T. Pereira, J.M. Vila, J. Organomet. Chem. 694 (2009) 1273;
(c) J. Albert, L. D'Andrea, J. Granell, R. Tavera, M. Font-Bardia, X. Solans, J. Organomet. Chem. 692 (2007) 3070;
(d) C. Zuccaccia, G. Bellachioma, G. Cardaci, A. Macchioni, B. Binotti, C. Carfagna, Helv. Chim. Acta 89 (2006) 1524;
(e) J.J.H. Diederen, H.W. Frühauf, H. Hiemstra, K. Vrieze, M. Pfeffer, Tetrahedron Lett. 39 (1998) 4111;
(f) J. Albert, J. Granell, J. Mínguez, G. Muller, D. Sainz, P. Valerga, Organometallics 16 (1997) 3561.

[11] See for instance: (a) A. Yu, Y. Wu, B. Cheng, K. Wei, J. Li, Adv. Synth. Catal. 351 (2009) 767;
(b) R. Huang, K.H. Shaughnessy, Organometallics 25 (2006) 4105;
(c) K. Takenaka, M. Minakawa, Y. Uozumi, J. Am. Chem. Soc. 127 (2005) 12273;
(d) R.B. Bedford, C.S.J. Cazin, S.J. Coles, T. Gelbrich, M.B. Hursthouse, V.J.M. Scordia, Dalton Trans. (2003) 3350;
(e) J. Bravo, C. Cativiela, R. Navarro, E.P. Urriolabeitia, J. Organomet. Chem. 650 (2002) 157;
(f) H. Weissman, D. Milstein, Chem. Commun. (1999) 1901;
(g) M. Ohff, A. Ohff, D. Mielstein, Chem. Commun. (1999) 357.

[12] See for instance: (a) A.S. Mocanu, M. Ilis, F. Dumitrascu, M. Ilie, V. Cîrcu, Inorg. Chim. Acta 363 (2010) 729;
(b) S. Coco, C. Cordovilla, P. Espinet, J.-L. Gallani, D. Guillon, B. Donnio, Eur. J. Inorg. Chem. (2008) 1210;
(c) O.N. Kadkin, J. An, H. Han, Y.G. Galyametdinov, Eur. J. Inorg. Chem. (2008) 1682;
(d) A.C. Tenchiu, M. Ilis, F. Dumitrascu, A.C. Whitwood, V. Cîrcu, Polyhedron 27 (2008) 3537;
(e) J. Arias, M. Bardají, P. Espinet, J. Organomet. Chem. 691 (2006) 4990;
(f) B.B. Eran, D. Singer, K. Praefcke, Eur. J. Inorg. Chem. (2001) 111;
(g) D.P. Lydon, J.P. Rourke, Chem. Commun. (1997) 1741.

[13] (a) P. Espinet, J.A. Miguel, J.M. Martín-Alvarez, B. Villacampa, J. Organomet. Chem. 695 (2010) 437;
(b) U. Caruso, A. Di Matola, B. Panunzi, A. Roviello, A. Sirigu, Polymer 42 (2001) 3973;
(c) J. Buey, S. Coco, L. Díez, P. Espinet, J.M. Martín-Alvarez, J.A. Miguel, S. Gracía-Granda, A. Tesouro, I. Ledoux, J. Zyss, Organometallics 17 (1998) 1750.

[14] (a) S. Jacquot-Rousseau, A. Khaty, G. Schmitt, M. Knorr, M.M. Kubicki, O. Blacque, Inorg. Chem. Commun. 8 (2005) 610;
(b) A. Böhm, W. Beck, J. Organomet. Chem. 588 (1999) 247;
(c) M. Gayoso, M. Alonso, J.M. Vila, E.B. Rivero, W. Hiller, J. Strahle, Acta Cryst. C44 (1988) 929.

[15] K. Nakamoto, Infrared and Raman Spectra of Inorganic and Coordination Compounds, fifth ed., Wiley, New York, 1997.

[16] See for instance: (a) P.G. Evans, N.A. Brown, G.J. Clarkson, C.P. Newman, J.P. Rourke, J. Organomet. Chem. 691 (2006) 1251;
(b) M. Ghedini, A. Crispini, Comment. Inorg. Chem. 21 (1999) 53.

[17] G.D. Deacon, J.H.S. Green, Spectrochim. Acta 24A (1968) 845.

[18] See for instance: (a) J. Albert, L. D'Andrea, G. García, J. Granell, A. Rahmouni, M. Font-Bardia, T. Calvet, Polyhedron 28 (2009) 2559;
(b) S. Pérez, R. Bosque, C. López, X. Solans, M. Font-Bardia, J. Organomet. Chem. 625 (2001) 67;
(c) J. Albert, J. Granell, J. Sales, M. Font-Bardía, X. Solans, Organometallics 14 (1995) 1393;
(d) J. Albert, M. Gómez, J. Granell, J. Sales, X. Solans, Organometallics 9 (1990) 1405.

[19] M. Ghedini, D. Pucci, A. Crispini, I. Aiello, F. Barigelletti, A. Gessi, O. Francescangeli, Appl. Organomet. Chem. 13 (1999) 565.

[20] (a) J. Reedijk, Platin. Met. Rev. 52 (2008) 2;
(b) J. Vicente, A. Arcas, Coord. Chem. Rev. 249 (2005) 1135.

[21] R.N. Jadeja, S. Parihar, K. Vyas, V.K. Gupta, J. Mol. Struct. 1013 (2012) 86.

[22] J. de Mier-Vinué, J. Lorenzo, A.M. Montaña, V. Moreno, F.X. Avilés, J. Inorg. Biochem. 102 (2008) 973.

[23] M.N. Patel, M.R. Chhasatia, D.S. Gandhi, Bioorg. Med. Chem. 17 (2009) 5648.

[24] The mass of the most abundant isotopic peak was calculated online on the website http://www.lfd.uci.edu/~gohlke/molmass/.

[25] K.T. Givens, S. Kitada, A.K. Chen, J. Rothschiller, D.A. Lee, Investig. Ophthalmol. Vis. Sci. 31 (1990) 1856.

[26] A. Abdullah, F. Huq, A. Chowdhury, H. Tayyem, P. Beale, K. Fisher, BMC Chem. Biol. 6 (2006) 3. http://dx.doi.org/10.1186/1472-6769-6-3.