Journal of Organometallic Chemistry 983 (2023) 122555

Contents lists available at ScienceDirect

Journal of Organometallic Chemistry

journal homepage: www.elsevier.com/locate/jorganchem

Synthesis and biological properties of palladium(II) cyclometallated compounds derived from (*E*)-2-((4-hydroxybenzylidene)amino)phenol

Joan Albert[a,b,*], Basma Al Janabi[a], Jaume Granell[a,b], Mojdeh Sadat Hashemi[a,c], Daniel Sainz[a], M. Kaleem Khosa[d], Carme Calvis[e], Ramon Messeguer[e], Laura Baldomà[b,f], Josefa Badia[b,f], Mercè Font-Bardia[g,h]

[a] *Departament de Química Inorgànica i Orgànica, Secció de Química Inorgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1, 08028 Barcelona, Spain*
[b] *Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*
[c] *Faculty of Chemistry, Bu-Ali Sina University, Hamedan 65174, Iran*
[d] *Department of Chemistry, Sir Syed Block, New Campus, Government College University, Jhang Road, Faisalabad, Pakistan*
[e] *Biomed Division LEITAT Technological Center, Parc Científic, Edifici Hèlix, Baldiri Reixach 15-21, 08028 Barcelona, Spain*
[f] *Departament de Bioquímica i Fisiologia, Secció de Bioquímica i Biologia Molecular, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII 27-31, 08028 Barcelona, Spain*
[g] *Departament de Mineralogia, Petrologia i Geologia Aplicada, Facultat de Ciències de la Terra, Universitat de Barcelona, Martí i Franqués s/n, 08028 Barcelona, Spain*
[h] *Unitat de Difracció de RX, Centre Científic i Tecnològic de la Universitat de Barcelona, Solé i Sabarís 1-3, 08028 Barcelona, Spain*

ARTICLE INFO

Article history:
Received 29 August 2022
Revised 7 November 2022
Accepted 8 November 2022
Available online 12 November 2022

Keywords:
Cyclometallation
Palladium(II)
Iminophenol
Anticancer
Antibacterial
Antioxidant

ABSTRACT

(*E*)-2-((4-hydroxybenzylidene)amino)phenol (iminophenol **a**) reacted with $Pd(OAc)_2$ giving place to compound **1a**, in which the iminophenol was bonded to palladium(II) in a κ^3-C_{ortho},*N*,O_{ortho} tridentate chelating mode. Thus, **1a** was formed by neutral mononuclear units of schematic formula Pd(C,N,O), consisting of two fused five-membered metallacycles. Self-assembly of the Pd(C,N,O) units gave place to the polynuclear structure of **1a**. Treatment of **1a** with PPh_3 or $PPh_2CH_2CH_2PPh_2$ in molar ratio Pd(II)/PPh_3 = 1/1 or Pd(II)/$PPh_2CH_2CH_2PPh_2$ = 2/1 produced the mononuclear or dinuclear compound of schematic formula [Pd(C,N,O)(PPh_3)] (**2a**) or {[Pd(C,N,O)]$_2$(μ_2-$PPh_2CH_2CH_2PPh_2$)} (**3a**), respectively. Compounds **a** were characterized by elemental analysis, high resolution ESI-(+) mass spectrometry, IR, and NMR. In addition, the crystal structure of the adducts **2a**·2(CH_2Cl-CH_2Cl) and **3a**·5(dmso) was determined by single crystal X-ray diffraction analysis. Most compounds **a** were noncytotoxic or poorly cytotoxic. Nonetheless, **2a** was moderately cytotoxic against the MCF-7 breast and HCT-116 colon human cancer cell lines, and presented very low cytotoxicity towards normal skin human BJ cells. Compounds **a** showed moderate antibacterial activity against some Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*E. coli*) bacterial strains, and displayed also moderate antioxidant activity, producing **3a** the best antioxidant activity. **1a** changed the electrophoretic mobility of the pBluescript SK+ plasmid DNA. This change followed the pattern of *cisplatin*, but it started at a concentration twenty times higher than with *cisplatin*. Moreover, compounds **1a** - **3a** inhibited topoisomerase IIα at concentrations of 10, 50 and 25 μM, respectively.

1. Introduction

Potential applications of palladium(II) compounds in pharmacology have been extensively studied in recent years [1–6]. This is because there is a great interest for finding new metallodrugs capable of overcoming the issues of toxicity and intrinsic or acquired resistance produced by the coordination square-planar platinum(II) compounds *cisplatin, carboplatin* and *oxaliplatin* nowadays in clinical use worldwide for the treatment of different cancers in humans [7–8], and also because several palladium(II) compounds present *in vivo*, using mice as models, good profiles as anticancer [9–23], antiparasitic [24–26] and anticonvulsant agents [27].

Most of the palladium(II) compounds with good pharmacological activities *in vivo* are mononuclear [9,12,21,26] or dinuclear [14,16,18-19,22-25,27] palladium(II) cyclometallated compounds, and in this latter case, bridging diphosphane [14,16,18-

* Corresponding author: Dr. Joan Albert, Departament de Química Inorgànica i Orgànica, Universitat de Barcelona, Martí i Franquès, 1 - 11, 08028 Barcelona, Spain.
E-mail address: joan.albert@qi.ub.es (J. Albert).

https://doi.org/10.1016/j.jorganchem.2022.122555

19,22-24], acetato [27], chlorido [27] or azido [25] ligands connect the palladium(II) centres. Furthermore, these *in vivo* pharmacological active palladium(II) cyclometallated compounds present bidentate k^2-*C,N* [12,14,16,18-19,21-27] or tridentate k^3-*C,N,N* [9] chelating ligands, which form five-membered metallacycles and present an *ortho*-palladated carbon atom, and other ligands, such as acetato [27], chlorido [14,16,18-19,22-24,27], azido [25], diphosphanes [12,14,16,18-19,21-24,26] or a heterocyclic carbene [9], complete the coordination sphere of the palladium(II) centres. In addition, a k^1-C_{ortho}-phenylpalladium(II) compound with a chelating diphosphane ligand and a terminal *N*-thiocyanato ligand [20], and a few ionic or neutral mononuclear palladium(II) coordination compounds containing k^3-*N,N,N* [10-11,17], k^2-*N,N* [13] or k^2-*O,O* [15] chelating ligands also present a good anticancer activity *in vivo*. In these latter compounds, the chelating ligands form very stable five- or six-membered metallacycles.

These reports reinforce the original idea that palladium(II) compounds presenting chelating ligands, forming five- or six-membered metallacycles, good sigma donor or π-acceptors ligands, and steric hindrance around of palladium(II) centres, are sufficiently stable in the biological medium in relation to substitution reactions, and therefore they can enter quite unaltered inside the cancer cells, parasites or microbes and produce their pharmacological effect [28].

The studies on the mechanism of the anticancer activity of palladium(II) compounds point to their permeabilization effect over the mitochondria and lysosome membranes, as the main event that triggers a series of processes that ultimate end with the death of the cancer cells, in many cases, by apoptosis [12,16,21,29-30]. Furthermore, some of these compounds are efficient inhibitors of the cysteine protease cathepsin B [31] and strongly bind to CT-DNA [32]. These results suggest that they could present antimetastatic activity [31] and that the nuclear DNA could be a primary target for them [32]. The topoisomerase 1B inhibitory effect or the antioxidant activity displayed by some of these compounds has also been related with their antiparasitic [25] or anticancer activity [33], respectively.

In previous articles [34–39], we studied the anticancer activity *in vitro* of palladium(II) cyclometallated compounds derived from benzophenone imines (first series) [35,38–39], diimines (second series) [36], primary amines (third series) [37] and imine masked primary amines (fourth series) [34]. The effect of the compounds on the electrophoretic mobility of the pBluescript SK+ plasmid DNA was also analysed. In addition, and for some selected compounds, we tested their inhibitory effect over topoisomerase I [35,36] and cathepsin B [34,38], and their antibacterial and antioxidant activity [34,38].

The results in relation to the preceding studies are summarized in Table 1. Compounds **A** and **B** were the palladium(II) cyclometallated compounds studied in our research group with the best profile as anticancer drugs *in vitro* [35,38-39]. In the three first series studied, a clear relationship was established between the lipophilicity of the compounds and their anticancer activity [35–37], and several compounds from the first, third and fourth series showed moderate antibacterial and antioxidant activity [34,38]. Furthermore, none of the compounds interacted strongly with the pBluescript SK+ plasmid DNA at a concentration in which a strong binding to the pBluescript SK+ plasmid DNA was observed for *cisplatin*, a strong covalent binder to DNA, or ethidium bromide, a strong intercalator binder to DNA, according to electrophoretic mobility experiments. These results suggested that DNA was not the primary target for the tested compounds [34–39]. Furthermore, for a few selected compounds, their inhibitory effect on topoisomerase I and cathepsin B was analysed, and they were neither efficient cathepsin B inhibitors (compounds **A**, **B**, **I**, **J** and **K**) [34,38] nor topoisomerase I inhibitors (compounds **L**, **M** and **N**) [35,36].

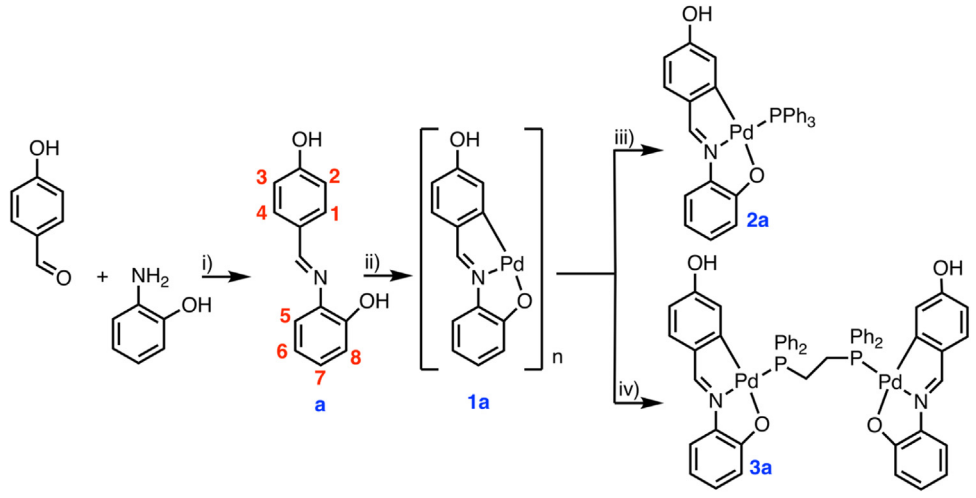
Following the preceding studies [34–39], we describe here the preparation of a new series of palladium(II) cyclometallated compounds containing a k^3-C_{ortho},*N*,O_{ortho} iminophenol chelating ligand, and their anticancer, antibacterial, and antioxidant activity. Furthermore, the interaction of the compounds with the pBluescript SK+ plasmid DNA, and their ability to inhibit the topoisomerases I or IIα by electrophoretic experiments are also addressed.

It should be noted that the palladium(II) compounds described here, once their target biomolecules could be coordinated to their palladium(II) centres, could establish secondary hydrogen bonds with them through their phenoxido and phenol functions. This could give place to stronger and more selective interactions with their target biomolecules and therefore an improvement of their pharmacological activities.

2. Results and discussion

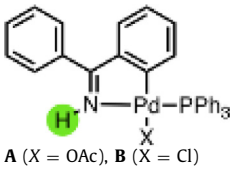
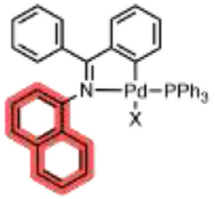
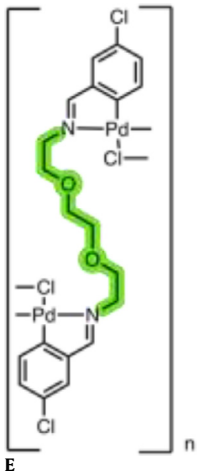
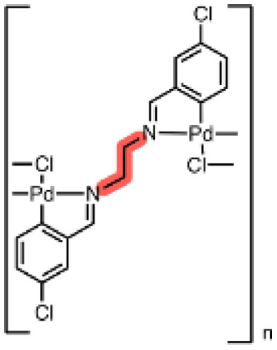
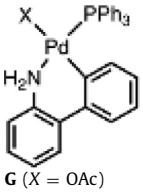
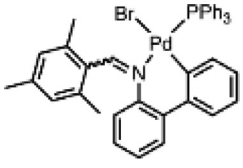
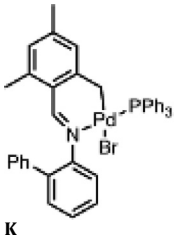
2.1. Synthesis and characterization of the compounds

Scheme 1 shows the preparation method and numbering of the compounds under study, together with the numbering of their aromatic protons, for the discussion that follows.



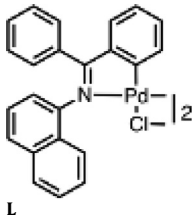
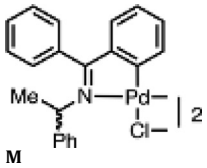
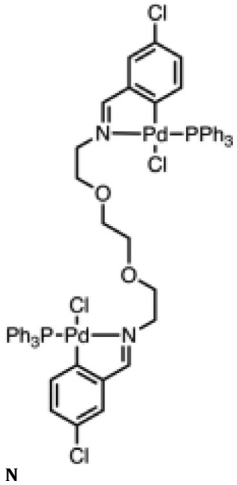
Scheme 1. i) Ethanol, reflux, 4 h, ii) $Pd(OAc)_2$, HOAc, 24 h, 60 °C, iii) PPh_3, acetone, rt, 1 h, molar ratio **1a**(n = 1)/PPh_3 = 1, iv) $PPh_2CH_2CH_2PPh_2$, acetone, rt, 1 h, molar ratio **1a**(n = 1)/$PPh_2CH_2CH_2PPh_2$ = 2.

Table 1
In vitro pharmacological activities of palladium(II) cyclometallated compounds reported by our research group [34–39].

Compound	*In vitro* pharmacological activity [1,2,3]
 **A** (X = OAc), **B** (X = Cl)	*a*) **A** and **B** were four times more cytotoxic than *cisplatin* against the MDA-MB-231 and the MCF-7 breast human cancer cell lines [39]. **A** was three times less cytotoxic towards quiescent healthy HUVEC cells than *cisplatin* [35]. *b*) **C** and **D** were noncytotoxic towards the MDA-MB-231 and the MCF-7 breast human cancer cell lines [35]. *c*) **B** presented moderate antibacterial and antioxidant activity compared with the commercial antibiotic cefixime, and the natural antioxidant ascorbic acid, respectively [38].
 **C** (X = OAc), **D** (X = Cl)	
 **E**	*a*) **E** presented similar cytotoxicity to *cisplatin* against MDA-MB-231 and MCF-7 breast and HCT-116 colon human cancer cell lines, but **F** was noncytotoxic against these cancer cell lines [36].
 **F**	
 **G** (X = OAc) **H** (X = Cl) **I** (X = Br)	*a*) **G**, **H**, and **I** were very cytotoxic toward different cancer cell lines [34,37] and **G** and **H** produced the death of the A549 human lung cancer cells mainly by early apoptosis [34]. *b*) **I** was extremely cytotoxic towards normal skin human BJ cells, and presented moderate antibacterial and antioxidant activity [34].
 **J**	*a*) **J** (an imine masked form of **I**) afforded a similar cytotoxicity to *cisplatin* for the HCT-116 human colon cancer cell line, but it was almost noncytotoxic for the normal skin human BJ cells, and produced moderate antibacterial and antioxidant activity [34].
 **K**	*a*) **K** (a structural isomer of **J**) was almost noncytotoxic, but it presented moderate antibacterial and antioxidant activity [34].

(*continued on next page*)

Table 1 (*continued*)

Compound	*In vitro* pharmacological activity [1,2,3]
L 	*a*) **L**, **M** and **N** were not efficient inhibitors of topoisomerase I [35–36].
M 	
N 	

[1] The precursor imine of **J** and **K** of formula (*E*)−2,4,6-$Me_3C_6H_2$–CH=N-2-PhC_6H_4 displayed strong antibacterial and antioxidant activity [34].
[2] **A** - **N** were not efficient covalent or intercalator binders for the pBluescript SK+ plasmid DNA [34–39].
[3] **A, B, I, J** and **K** were not efficient inhibitors of cathepsin B [34,38].

Iminophenol **a** and compounds **1a** - **3a** were obtained by an adaptation of known procedures [40]. Thus, iminophenol **a** was synthesized by a condensation reaction between 4-hydroxybenzaldehyde and 2-aminophenol, **1a** by concatenation in acetic acid of cyclopalladation and *ortho* phenol deprotonation reactions between iminophenol **a** and $Pd(OAc)_2$, and **2a** and **3a** by splitting reactions in acetone at room temperature between compound **1a** and the phosphanes PPh_3 or $PPh_2CH_2CH_2PPh_2$, respectively.

Compounds **a** were yellowish (iminophenol **a**), brownish (**1a**) and reddish (**2a** and **3a**) solids, which afforded satisfactory elemental analysis. Iminophenol **a**, and compounds **2a** and **3a**, were soluble in dmso, and compound **1a** in acetone. Therefore, the NMR experiments for the iminophenol **a**, and compounds **2a** and **3a** were recorded in dmso-d_6, and in acetone-d_6 for compound **1a**. Iminophenol **a** protons were assigned by bidimensional ^1H – ^1H COSY and NOESY experiments. The same bidimensional experiments were used for the proton assignation of the κ^3-C_{ortho},*N*,O_{ortho} coordinated iminophenol of **1a** and **2a**. Furthermore, the proton assignation of the κ^3-C_{ortho},*N*,O_{ortho} coordinated iminophenol of **2a** served as a guide for the assignation of these protons for **3a**.

Mono- and bidimensional ^1H NMR experiments for **1a** - **3a** were consistent with the lack of the proton in *ortho* position of the phenyl group bonded to the iminic carbon, and the deprotonation of the *ortho* phenol function of the coordinated iminophenol. Furthermore, the shift of the iminic proton of **1a** - **3a** to a lower chemical shift in relation to the free iminophenol by 0.98 ppm for **1a**, 0.26 ppm for **2a**, and 0.25 ppm for **3a**, was consistent with the coordination of the iminic nitrogen to the palladium(II) centre and an *endo* structure [40]. Thus, iminophenol **a** was bonded to the palladium(II) centre in an *endo*-κ^3-C_{ortho},*N*,O_{ortho} coordination mode in **1a** - **3a**. It should be noted that for an iminophenol coordinated to palladium(II) in an *exo*-κ^3-C_{ortho},*N*,O_{ortho} coordination mode, its iminic proton appeared shifted to a higher chemical shift in relation to its precursor ligand by 0.11 ppm [41]. The precedent *endo* and *exo* descriptors refer to compounds having the iminic function inside (*endo* compounds) or outside (*exo* compounds) of the metallacycle containing the Pd-C bond [42].

In addition, the iminic proton appeared as a doublet with a $^4J_{HP}$ coupling constant of 12 Hz for **2a**, which agreed with the *trans* arrangement between the iminic proton and the phosphorous atom in this compound [40]. Furthermore, the iminic protons and the phosphorus atoms of **3a** produced an apparent AA'XX' (A = A' = ^1H and X = X' = ^{31}P) spin system, which afforded an apparent triplet for the iminic protons. Similarly, the CH_2 protons of **3a** were part of an apparent $A_2A'_2XX'$ (A = A' = ^1H and X = X' = ^{31}P) spin system, which afforded a somewhat broad apparent singlet at 2.74 ppm for them. These results agreed with an apparent high symmetry for **3a** in dmso solution due to a fast rotation through its single bonds. The $^{31}P\{^1H\}$ NMR of **2a** and **3a** in dmso-d_6 solution afforded a singlet at 34.06 and 34.33 ppm, respectively. Similar spin systems to that observed for **3a** have been previously reported in our research group [43]. The above discussed NMR experiments are given in the Supplementary Material (Figures S1 - S12).

It is worth to note that the nuclearity of compound **1a** is not known at present, and that the attempts to grow single crystals of **1a** to determine its nuclearity by X-Ray diffraction analysis were unsuccessful. Similar compounds to **1a**, which are formed by neutral Pd(C,N,O) units derived from an iminophenol and whose crystal structure has been determined, presented in the selected crystals for the X-ray diffraction analysis, a tetranuclear structure with the phenoxido functions bridging the palladium(II) centres, see for instance references [44–45]. In addition, these tetranuclear compounds with phenoxido bridges in dmso solution experience splitting reactions, which convert them into mononuclear compounds of general formula [Pd(C,N,O)(dmso)], in which the dmso ligand is located in *trans* position to the iminic nitrogen [46].

In the IR, iminophenol **a** produced a broad band at 3212 and a medium sharp signal at 1623 cm^{-1}, which were assigned to the stretching of its phenol and iminic functions, respectively. In the IR of compounds **1a** - **3a**, a broad band at 3260 for **1a**, 3227 for **2a** or 3212 cm^{-1} for **3a**, and a medium sharp band at 1579 for **1a**, 1586 for **2a** or 1586 cm^{-1} for **3a** were assigned to the stretching of their phenol and iminic functions, respectively [46]. Furthermore, **2a** or **3a** presented in the IR a sharp medium band at 1098 or 1107 cm^{-1}, respectively, which was assigned to the q X-sensitive band of the coordinated phosphane ligand [43].

In the high-resolution ESI-(+) mass spectrum, iminophenol **a** afforded an intense peak at 214.0862 *m/z*, which was assigned to $[M_{\mathbf{a}} + H^+]$ (calc. 214.0862). M represents the mass of the most abundant isotopologue of the cation under discussion. Compound

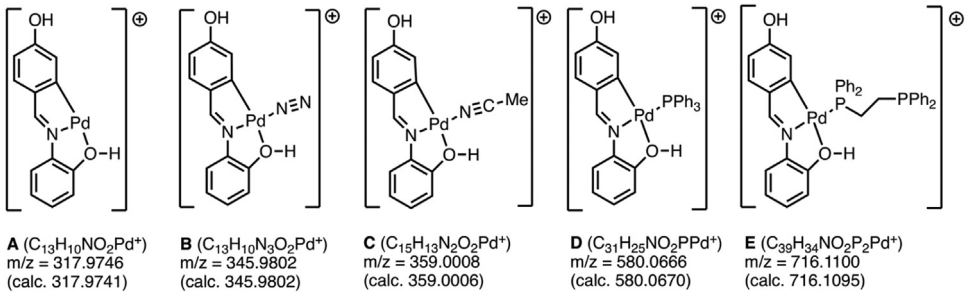


Fig. 1. Proposed structures for the most intense cations **A** – **E** observed in the ESI-(+) experiments. Cations **A** – **C** derived from **1a**, cation **D** from **2a** and cation **E** from **3a**. The observed and calculated *m/z* values correspond to the most abundant isotopologue.

1a produced a quite complex high-resolution ESI-(+) mass spectrum with intense signals for the cations $[M_{\mathbf{1a}(n\,=\,1)} + H^+]$ (**A**) at *m/z* 317.9746 (calc. 317.9741), $[M_{\mathbf{1a}(n\,=\,1)} + N_2 + H^+]$ at *m/z* 345.9802 (calc. 345.9802) (**B**) and $[M_{\mathbf{1a}(n\,=\,1)} + MeCN + H^+]$ at *m/z* = 359.0008 (calc. 359.0006) (**C**). The precedent cations were formed in the ionization process in the ESI experiment since a mixture of water and acetonitrile was used for transporting the sample to the spray needle and N_2 was utilized as drying gas. Furthermore, the high-resolution ESI-(+) mass spectrum of **1a** produced signals of low intensity, containing two and four palladium atoms, but their molecular formula could not be assigned unambiguously. In the high-resolution ESI-(+) mass spectrum, **2a** produced an intense signal corresponding to the cation $[M_{\mathbf{2a}} + H^+]$ (**D**) at *m/z* 580.0666 (calc. 580.0670), while **3a** produced an intense signal for the cation $[M_{\mathbf{1a}(n\,=\,1)} + PPh_2CH_2CH_2PPh_2 + H^+]$ at *m/z* 716.1100 (calc. 716.1095) (**E**). Fig. 1 gives the proposed structures for the cations **A** – **E**.

2.2. Crystal structures of the adducts 2a·2(CH_2Cl-CH_2Cl) and 3a·5(dmso)

Single crystals for the X-ray diffraction analysis of the adducts **2a**·2(CH_2Cl-CH_2Cl) and **3a**·5(dmso) were obtained by slow evaporation of the solvents of a solution of **2a** in acetone and 1,2-dichloroethane, and by diffusion of 1,2-dichloroethane into a solution of **3a** in dmso, respectively. Details on the crystal data and structure refinement of the adducts **2a**·2(CH_2Cl-CH_2Cl) and **3a**·5(dmso) are given in the Supplementary Material (Tables S1 and S2). The adducts commented above constituted the asymmetric residue units of the studied single crystals.

The adducts **2a**·2(CH_2Cl-CH_2Cl) and **3a**·5(dmso) crystalized in the orthorhombic space groups Pbca with $Z = 8$ and $Pca2_1$ with $Z = 4$, respectively. The phenol and the phenoxido functions of neighbouring molecules of **2a** in the crystal of **2a**·2(CH_2Cl-CH_2Cl) were bonded by hydrogen bond. This hydrogen bond gave place to a chain in the direction [100] in the crystal of the adduct **2a**·2(CH_2Cl-CH_2Cl). In the crystal of the adduct **3a**·5(dmso), two hydrogen bonds per molecule of **3a** were observed, each one formed by a phenol function and the corresponding neighbouring dmso molecule.

Figs. 2 and 3 show the X-ray molecular structure of **2a** and **3a**, together with some selected bond distances and angles. The X-ray molecular structure of **2a** and **3a** confirmed their proposed structure by NMR spectroscopy. Thus, iminophenol **a** was bonded to the palladium(II) centres of **2a** and **3a** in an *endo*-κ^3-C_{ortho},*N*,O_{ortho} coordination mode. Furthermore, the phosphorous atom of the PPh_3 ligand in **2a** and those of the $PPh_2CH_2CH_2PPh_2$ ligand in **3a**, were located in *trans* position to the iminic nitrogen of the Pd(C,N,O) units. Moreover, the $PPh_2CH_2CH_2PPh_2$ ligand acted as a bridging ligand between the Pd(C,N,O) units of **3a**. The distances and angles around of the palladium(II) centres and for the atoms determining

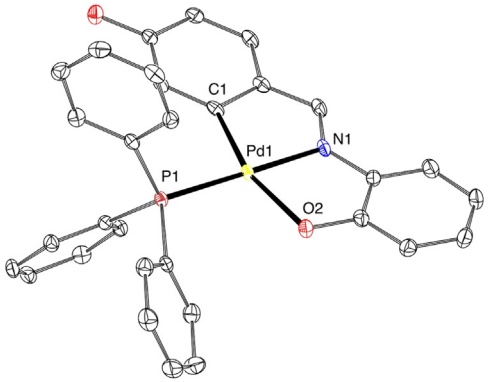


Fig. 2. Crystal structure of **2a**. Solvent molecules and hydrogen atoms have been omitted for clarity. Selected bond distances (Å) and angles (°): Pd(1)-C(1) = 2.004(3), Pd(1)-N(1) = 2.026(2), Pd(1)-O(2) = 2.1210(18), Pd(1)-P(1) = 2.2529(7), C(1)-Pd(1)-N(1) = 82.54(10), C(1)-Pd(1)-O(2) = 162.50(9), N(1)-Pd(1)-O(2) = 80.21(8), C(1)-Pd(1)-P(1) = 95.85(8), N(1)-Pd(1)-P(1) = 177.91(6), O(2)-Pd(1)-P(1) = 101.46(5).

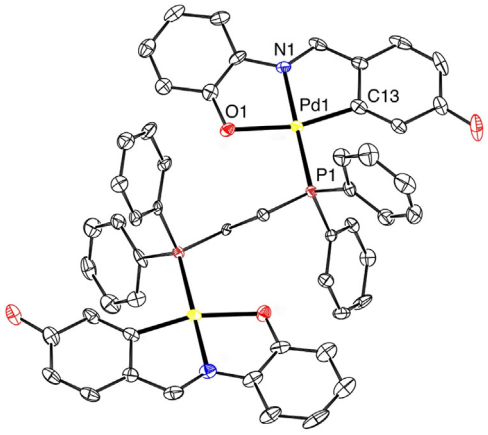


Fig. 3. Crystal structure of **3a**. Solvent molecules and hydrogen atoms have been omitted for clarity. Selected bond distances (Å) and angles (°): Pd(1)-C(13) = 1.992(10), Pd(1)-N(1) = 2.013(8), Pd(1)-O(1) = 2.087(7), Pd(1)-P(1) = 2.254(2), C(13)-Pd(1)-N(1) = 82.4(4), C(13)-Pd(1)-O(1) = 163.5(3), N(1)-Pd(1)-O(1) = 81.2(3), C(13)-Pd(1)-P(1) = 97.6(3), N(1)-Pd(1)-P(1) = 177.8(2), O(1)-Pd(1)-P(1) = 98.90(19).

the two fused five-membered metallacycles were in the usual intervals for this type of molecules [44–46]. It should be noted that the two halves of the molecule of **3a** were not equivalent in the crystal of the adduct **3a**·5(dmso). Therefore, small differences in their bond distances and angles were observed between them.

The two fused five-membered metallacycles formed by iminophenol **a** bonded in an *endo*-κ^3-C_{ortho},*N*,O_{ortho} coordination mode to the palladium(II) centre of **2a** were almost planar.

Table 2
IC_{50} (μM) cell viability. Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation.

Compound	Trial	MDA-MB-231	MCF-7	HCT-116	BJ
a	1	> 100	64 ± nd	> 100	> 100
1a	1	> 100	> 100	> 100	> 100
2a	1	29 ± nd	7.8 ± 1.7	31 ± 5	86 ± nd
cisplatin[a]	1	4.4 ± 0.5	3.6 ± 1.7	19 ± 2	3 ± nd
3a	2	> 100	> 100	22 ± 5	15 ± 6
cisplatin[a]	2	13 ± 3	13 ± 2	3.6 ± 0.5	5.3 ± 0.7

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

The maximum deviated atoms from the planar metallacycles were C(13) = 0.023(3) Å for the metallacycle Pd(1)-N(1)-C(8)-C(13)-O(2) (plane 1) and N(1) = −0.025(2) Å for the metallacycle Pd(1)-C(1)-C(6)-C(7)-N(1) (plane 2). The angle between planes 1 and 2 for **2a** was 2.74(11) °. Furthermore for **2a**, the angles of the coordination plane, which is formed by the atoms directly bonded to the palladium(II) centre, with planes 1 and 2 were 2.13(9) and 2.19(10) °, respectively. Thus, the coordination plane and the two fused five-membered metallacycles of **2a** were almost co-planar. In spite of this, the coordination sphere of the palladium(II) centre in **2a** was quite distorted due to the chelate bite angles of its two fused five-membered metallacycles, which were 82.54(10) and 80.21(8) ° for C(1)-Pd(1)-N(1) and N(1)-Pd(1)-O(2), respectively. This gave place to an angle C(1)-Pd(1)-O(2) of 162.50(9) ° for **2a**, quite far from 180 ° that should be expected for an ideal square-planar geometry of coordination. Similar arguments can be applied to describe the structure of the molecule of **3a** and in this case the angles C(13)-Pd(1)-O(1) and C(26)-Pd(2)-O(3) were 163.5(3) and 162.8(3) °, respectively.

2.3. Biological studies

2.3.1. Cytotoxic activity

Table 2 shows the results of the cell viability assay regarding the cell lines MDA-MB-231 and MCF-7 breast and HCT-116 colon human cancer cell lines, and the normal skin human BJ cells. The effects of the assayed compounds **a** on the selected cell lines were assessed after 72 h and the tabulated IC_{50} values are the average of two experiments. *Cisplatin* was incorporated in the trials as a reference anticancer drug.

The results of the cell viability assay showed that most compounds **a** were noncytotoxic or poorly cytotoxic against the cell lines studied. For instance, iminophenol **a** and compound **1a** were noncytotoxic at concentrations of 100 μM for most of the cell lines studied. Nonetheless, iminophenol **a** was somewhat cytotoxic against the MCF-7 cell line (IC_{50} = 64 μM). It should be noted that in the same trial and cell lines, *cisplatin* presented IC_{50} values in the interval between 3 and 19 μM. **3a** was noncytotoxic as well for the MDA-MB-231 and MCF-7 breast cancer cell lines, and poorly cytotoxic towards the HCT-116 colon cancer cell line (IC_{50} = 22 ± 5 μM). In fact, **3a** was six times less cytotoxic than *cisplatin* against the HCT-116 colon cancer cell line. Furthermore, **3a** was a nonselective compound since it was more cytotoxic towards the normal human skin BJ cells (IC_{50} = 15 ± 6) than for the HCT-116 cancer cells (IC_{50} = 22 ± 5 μM).

Compound **2a** displayed the best profile as anticancer drug. Thus, **2a** was moderately cytotoxic against the MCF-7 breast (IC_{50} = 7.8 ± 1.7 μM) and HCT-116 colon (IC_{50} = 31 ± 5 μM) human cancer cell lines, and presented very low cytotoxicity towards normal skin human BJ cells (IC_{50} = 86 ± nd μM). Therefore, **2a** was approximately two times less cytotoxic than *cisplatin* against the MCF-7 breast and HCT-116 colon human cancer cell lines, but it was almost noncytotoxic against the normal skin human BJ cells.

The results obtained on the cell viability assay for **1a** - **3a** are in agreement with previous findings in our research group about the relationship between the lipophilicity of palladium(II) cyclometallated compounds derived from imines or primary amines and their cytotoxicity [35–37]. The phenol functions of the iminophenol **a** or the phenol and phenoxido functions of compound **1a** could explain their noncytotoxic or low cytotoxic character against the cell lines studied due to the high hydrophilic character that these functions impart to them. Therefore, their transport by passive diffusion inside the cells could not be efficient due to their non-well-balanced lipophilicity. In compound **2a**, the PPh_3 ligand increases the lipophilia, and this could allow a better passive transport of this compound through the cell membrane, explaining its better profile as anticancer drug in relation to iminophenol **a** and compound **1a**. Ongoing from compound **2a** to compound **3a**, two phenyl groups are formally replaced by a CH_2–CH_2 group. This should increase the hydrophilic character of compound **3a** and difficult its passive transport inside the cells in relation to **2a**. Therefore, the cytotoxic activity of compound **3a** should decrease in relation to that of compound **2a**, which is in fact what it is observed.

2.3.2. Antibacterial activity

Table 3 summarizes the results obtained in the antibacterial assay for iminophenol **a** and compounds **1a** - **3a** by the disk diffusion method. Interestingly, **1a** - **3a** presented a moderate antibacterial activity towards the gram positive *B. subtilis* and *S. aureus* and the gram negative *E. Coli* bacterial strains. The palladium(II) cyclometallated imine compounds **J** and **K** in Table 1 presented a somewhat better antibacterial activity than compounds **1a** - **3a**. Moreover, **J** and **K** were active against all the antibacterial strains given in Table 3 [34]. In addition, a benzophenone imine palladium(II) cyclometallated compound (**B** in Table 1) afforded also moderate antibacterial activity in relation to the commercial antibiotic cefixime against the gram positive *B. subtilis* and the gram negative *E. areogenes* bacterial strains [38]. Futhermore, a palladium(II) cyclometallated primary amine quite related to **J** (compound **I** in Table 1) produced a somewhat higher antibacterial activity and greater selectivity than **J** [34].

Iminophenol **a** was the most active antibacterial compound **a**. In a similar way, the precursor imine of compounds **J** and **K** of formula (*E*)−2,4,6-$Me_3C_6H_2$–CH=N-2-PhC_6H_4 was also more active than them, regarding its antibacterial activity. Noteworthy, this latter imine presented an antibacterial activity approaching that of the commercial antibiotics cefixime and ciprofloxacin for several bacterial strains [34]. Surprisingly, the iminophenol **a** or the imine (*E*)−2,4,6-$Me_3C_6H_2$–CH=N-2-PhC_6H_4 presented the same antibacterial selectivity than compounds **1a** - **3a** or compounds **J** and **K**, respectively.

The antibacterial activity of palladium(II) organometallic or coordination compounds has not been extensively studied [47]. Nevertheless, related with the antibacterial activity of the palladium(II) cyclometallated compounds discussed in this section, a κ^1-C_{ortho} iminophenylpalladium(II) compound displayed a high antitubercular activity [48], and one palladium(II) cyclometallated compound containing a κ^3-*P,C,P* diphosphane chelating ligand, a high antibacterial activity [49].

2.3.3. Antioxidant activity

Table 4 shows the results of the DPPH radical scavenging assay for iminophenol **a** and compounds **1a** - **3a**. In previous works, we studied the antioxidant activity of: 1) a few palladium(II) cyclometallated compounds derived from benzophenone imine, including compound **B** in Table 1 [38], 2) the palladium(II) cyclometallated compound **I** derived from the primary amine 2-phenylaniline [34], and 3) compounds **J** and **K** derived from the imine (*E*)−2,4,6-$Me_3C_6H_2$–CH=N-2-PhC_6H_4 [34]. The structural formula of com-

Table 3
Antibacterial activity (*in vitro*) given as inhibition zone (mm).

Compound	B. subtilis[a]	S. aureus[a]	S. pyogenes[a]	E. coli[b]	P. aeruginosa[b]	S. typhi[b]
a	24 ± 1.5	26 ± 0.5	–	22 ± 0.5	–	20 ± 1
1a	22 ± 1	18 ± 1	–	19 ± 1	–	18 ± 1
2a	18 ± 0.5	20 ± 0.5	–	21 ± 1.5	–	–
3a	20 ± 0.5	16 ± 1.5	–	16 ± 1.25	–	–
cefixime	33 ± 1.5	31 ± 1	35 ± 1.5	29 ± 0.5	36 ± 1.25	31 ± 2

5 - 10 mm = activity. 11 - 25 mm = moderate activity. 26 - 40 mm = strong activity [50].
[a] Gram-positive.
[b] Gram-negative. - = No activity.

Table 4
Antioxidant activity expressed as the% ± sd of DPPH free radical scavenging.

Compound	200[a]	100[a]	40[a]	20[a]	10[a]	5[a]	IC_{50}[a]	IC_{50}[b]
a	79 ± 1	67 ± 1	58 ± 1	39 ± 2	25 ± 1	16 ± 1	32 ± 1	0.15
1a	73 ± 1	60 ± 1	48 ± 1	36 ± 1	23 ± 1	10 ± 1	50 ± 1	0.16
2a	66 ± 1	52 ± 2	41 ± 2	30 ± 2	20 ± 1	5 ± 1	87 ± 1	0.15
3a	81 ± 1	73 ± 2	64 ± 1	50 ± 1	33 ± 1	20 ± 1	82 ± 1	0.08
ascorbic acid	87 ± 0.5	84 ± 1	80 ± 0.25	70 ± 0.5	56 ± 1	35 ± 1	8.75 ± 0.5	0.05

[a] μg/mL.
[b] mM.

pounds **B**, **I**, **J** and **K** is given in Table 1. The antioxidant activity in the DPPH radical scavenging assay for these latter compounds can be classified as moderate with IC_{50} values in the interval between 0.22 and 0.13 mM related to the ascorbic acid (IC_{50} = 0.05 mM). Ascorbic acid was used as a reference antioxidant agent in these experiments. The antioxidant activity of **1a** and **2a** was also in the interval 0.22 and 0.13 mM. **3a** was the compound **a** with the best antioxidant activity (IC_{50} = 0.08 mM), approaching that of the ascorbic acid.

It should be noted that the precursor imine of compounds **J** and **K** presented a strong antioxidant activity (IC_{50} = 0.06 mM) quite similar to that of the ascorbic acid (IC_{50} = 0.05 mM) [34], while the iminophenol **a** was only a moderate antioxidant compound.

The antioxidant activity of the precedent palladium(II) compounds could be related with the redox properties of their palladium(II) centre. Thus, an accessible Pd(III) oxidation state for these compounds could explain the reduction of the DPPH radicals when they are used as scavengers in this assay [51]. Then, the dinuclear structure of compound **3a** could explain its better antioxidant activity in relation to compound **2a**, with a mononuclear structure. Moreover, the presence of HN-Pd(II) bonds in compounds **B** or **I** or the phenol function in compounds **1a** or **2a** did not increase their antioxidant activity in relation to the palladium(II) imine cyclometallated compounds **J** or **K** without N–H or O–H bonds in their structure. These results suggest that the transference of a hydrogen atom from compounds **B**, **I**, **1a**, **2a** and **3a** to the DPPH radical could not be their main mechanism for the scavenging of DPPH radicals [52].

2.3.4. Electrophoretic DNA migration

The results of the electrophoretic gel mobility shift assay of the pBluescript SK+ plasmid DNA incubated with iminophenol **a** or compounds **1a** - **3a** are presented in Fig. 4. In this assay, *cisplatin* and ethidium bromide were included as positive controls for an alkylating and intercalator agent for DNA, respectively. **1a** was the unique compound of the series that changed the electrophoretic mobility of the pBluescript SK+ plasmid DNA. This change followed the pattern of *cisplatin*, but it started to take place at a concentration twenty times higher than that of *cisplatin*. In addition, a comparison with ethidium bromide, revealed that none of the compounds **a**, including iminophenol **a**, acted as a DNA intercalator since no retardation in the mobility of pBluescript SK+ plasmid DNA bands was observed when the pBluescript SK+ plasmid DNA was incubated with compounds **a**, contrary to what was observed with ethidium bromide. These results suggest that the genomic DNA is not the primary target for iminophenol **a** or compounds **1a** - **3a**. Similar results have been obtained in the other series of palladium(II) cyclometallated compounds studied in our research group [34–39].

2.3.5. Topoisomerase I inhibition

To evaluate the ability of iminophenol **a** or compounds **1a** - **3a** to inhibit topoisomerase I activity, pBluescript SK+ plasmid DNA was incubated with topoisomerase I in the presence of compounds **a** at a concentration of 100 μM. Then, the incubated samples were subjected to agarose gel electrophoresis to evaluate the extent of relaxed DNA. The results are presented in Fig. 5 and show that none of the compounds **a** prevented the unwinding of DNA by the action of the topoisomerase I. Therefore, these results indicated that compounds **a** were not topoisomerase I inhibitors, thus pointing out to another biological target for them.

2.3.6. Topoisomerase IIα inhibition

To test the putative inhibitory activity of iminophenol **a** or compounds **1a** - **3a** on the topoisomerase IIα, pBluescript SK+ plasmid DNA was incubated at 37 °C with topoisomerase IIα in the presence of increasing concentrations of the preceding compounds. The results are presented in Fig. 6 and show that **1a** - **3a** were able to inhibit topoisomerase IIα activity. Compound **1a** was the most active since inhibition of topoisomerase IIα was observed at 10 μM, while **2a** and **3a** produced the inhibition of the topoisomerase IIα at 50 and 25 μM, respectively. The inhibition *in vitro* of topoisomerase IIα by compounds **1a** - **3a** suggest that topoisomerase IIα could be a biological target for them.

Topoisomerases I and II are important enzymes, which are involved in the mechanism of DNA replication, and have been identified as the primary target for drugs in clinical use for the treatment of cancer. Other Ru(II), Pd(II), Pt(II), Cu(II), Au(III) and Zn(II), between other metallic centres, coordination or organometallic compounds have been reported to be very efficient inhibitors of topoisomerases I or II [53].

3. Final remarks

Although the pharmacological activities reported in this work are in most cases, low or moderate, they can be useful to understand the relationship between the structure of palladium(II)

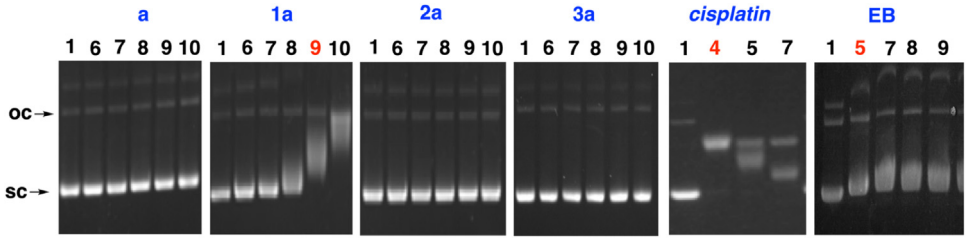


Fig. 4. Interaction of pBluescript SK+ plasmid DNA (0.8 μg). **1**: DNA (0.8 μg). **4**: 1 + 2.5 μM of the tested compound. **5**: 1 + 5 μM of the tested compound. **6**: 1 + 10 μM of the tested compound. **7**: 1 + 25 μM of the tested compound. **8**: 1 + 50 μM of the tested compound. **9**: 1 + 100 μM of the tested compound. **10**: 1 + 200 μM of the tested compound. sc = supercoiled closed circular DNA; oc = open circular DNA.

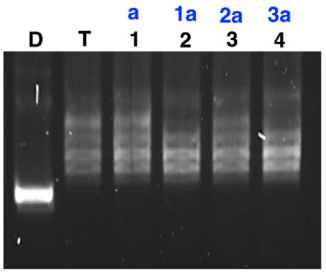


Fig. 5. Analysis of the compounds as topoisomerase I inhibitors. **D**: supercoiled pBluescript SK+ DNA (0.8 μg). **T**: D + topoisomerase I (3 units). **1**: T + 100 μM of **a**. **2**: T + 100 μM of **1a**. **3**: T + 100 μM of **2a**. **4**: T + 100 μM of **3a**.

molecular compounds and their pharmacological properties. Accumulated data suggest a clear relationship between the cytotoxicity of these compounds and their lipophilicity. Thus, a well-balanced lipophilicity seems to be crucial for their incorporation by passive transport inside the cancer cells, parasites, or microbes to produce then their pharmacological activities [[35–37], 54 and this work]. In addition, the antioxidant activity of **3a** and the inhibitory effect of **1a** on the topoisomerase IIα reported in this work are remarkable.

4. Experimental part

C, H, N microanalyses were performed with a Carlo-Erba EA 1108 instrument. High resolution ESI(+) mass spectra were acquired with an LC/MSD-TOF mass spectrometer using H_2O/CH_3CN (1:1) as eluent. IR spectra were collected with a Nicolet Avatar 300 FT-IR spectrometer using KBr discs. ^1H and $^{31}P\{^1H\}$ NMR spectra were recorded in a Varian Mercury 400 and Bruker 400 Avance III, respectively. Chemical shifts were measured relative to $SiMe_4$ for ^1H and to trimethylphosphate (δ = 2.39 ppm) for ^{31}P Chemical shifts are reported in ppm and coupling constants in Hz. Chemical compounds were commercial and were used as received.

The crystal structures of the adducts **2a**·2(CH_2Cl-CH_2Cl) and **3a**·5(dmso) have been deposited at The Cambridge Crystallographic Data Centre (CCDC) and they have been assigned with the deposition numbers: CCDC 2,192,023 and CCDC 2,192,024, respectively.

4.1. Preparation of compounds

a: A suspension formed by 2.442 g (20 mmol) of 4-hydroxybenzaldehde, 2.182 g (20 mmol) of 2-aminophenol and 20 mL of ethanol was stirred under reflux for 4 h. The solution was concentrated in vacuum and 5 mL of ethanol was added to the residue. Then, the suspension was stirred at room temperature for 1 h, and the yellow solid was filtered and dried in vacuum. Yield: 3.436 g (82%). Anal. Calc. for $C_{13}H_{11}NO_2$ (%): C, 73.23; H, 5.20; N, 6.57. Found: C, 73.2; H, 5.3; N, 6.5. High resolution ESI-(+) (*m/z*): 214.0862 (calculated for $C_{13}H_{12}NO_2$ = 214.0862) $[M_{\mathbf{a}} + H]^+$. IR (cm^{-1}): 3212 (st OH), 1623 (st C = N). ^1H NMR (400 MHz, dmso-d_6): 10.03 (br s, OH), 8.72 (br s, OH), 8.54 (s, 1H, CH=N), 7.86 (d, $^3J_{HH}$ = 8, 2H, 1 and 4), 7.15 (dd, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 5), 7.04 (td, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 7), 6.88 (d, $^3J_{HH}$ = 8, 2H, 2 and 3), 6.87 (dd, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 8), 6.81 (td, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 6).

1a: A suspension formed by 0.250 g (1.113 mmol) of palladium(II) acetate, 0.237 g (1.113 mmol) of iminophenol **a** and 5 mL of acetic acid was stirred at 60 °C for 24 h. The solution was concentrated in vacuum and the residue was purified by column chromatography, using silica gel-60 as the stationary phase and ethyl acetate as the mobile phase. The red band was collected and concentrated in vacuum. Addition of 7 mL of CH_2Cl_2 to the residue and stirring for 1 h afforded a reddish solid, which was filtered and dried in vacuum. Yield: 0.230 g (65%). Anal. Calc. for $C_{13}H_9NO_2Pd$ (%): C, 49.16; H, 2.86; N, 4.41. Found: C, 49.2; H, 2.9; N, 4.4. High resolution ESI-(+) (*m/z*): 317.9746 (calculated for $C_{13}H_{10}NO_2Pd$ = 317.9741) $[M_{\mathbf{1a}(n\,=\,1)} + H^+]$, 345.9802 (calculated for $C_{13}H_{10}N_3O_2Pd$ = 345.9802) $[M_{\mathbf{1a}(n\,=\,1)} + N_2 + H^+]$, 359.0008 (calculated for $C_{15}H_{13}N_2O_2Pd$ = 359.0006) $[M_{\mathbf{1a}(n\,=\,1)} + MeCN + H^+]$. IR ($cm^{-1}$): 3260 (st OH), 1579 (st C = N). ^1H NMR (400 MHz, acetone-d_6): 7.92 (s, OH), 7.56 (s, 1H, CH=N), 7.32 (dd, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 8), 7.00 (d, $^3J_{HH}$ = 6.9, 1H, 4), 6.85 (td, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 7), 6.69 (dd, $^3J_{HH}$ = 8,

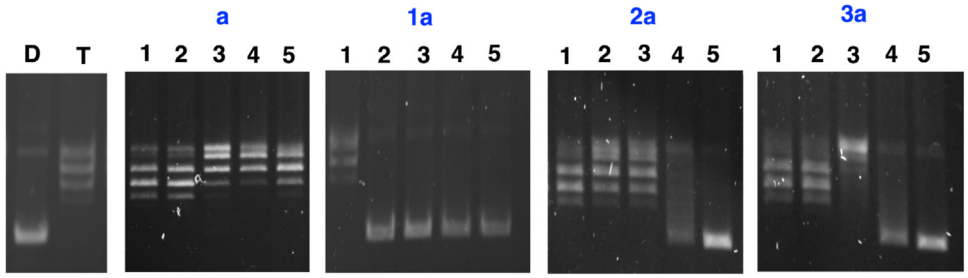


Fig. 6. Analysis of compounds as topoisomerase IIα inhibitors. **D**: supercoiled pBluescript DNA (0.3 μg). **T**: D + Topoisomerase IIα (4 units). **1**: T + 5 μM of tested compound. **2**: T + 10 μM of tested compound. **3**: T + 25 μM of tested compound. **4**: T + 50 μM of tested compound. **5**: T + 100 μM of tested compound.

$^4J_{HH}$ = 2, 1H, 5), 6.29 (td, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 6), 6.25 (dd, $^3J_{HH}$ = 8, $^4J_{HH}$ = 2, 1H, 3), 5.80 (d, $^4J_{HH}$ = 2, 1H, 2).

2a: A suspension formed by 0.100 g (0.314 mmol) of **1a**, 0.082 g (0.314 mmol) of PPh_3 and 5 mL acetone was stirred at room temperature for 1 h. The garnet solid was filtered, washed with 5 mL of acetone and dried in vacuum. Yield: 0.130 g (73%). Anal. Calc. for $C_{31}H_{24}NO_2PPd$ (%): C, 64.20; H, 4.05; N, 2.42. Found: C, 64.2; H, 4.2; N, 2.5. High resolution ESI-(+) (*m/z*): 580.0666 (calculated 580.0670 for $C_{31}H_{25}NO_2PPd$) [$M_{\mathbf{2a}} + H^+$]. IR (cm^{-1}): 3227 (st OH), 1586 (st C = N), 1098 (q X-sensitive of PPh_3). ^1H NMR (400 MHz, dmso-d_6): 9.45 (br, OH), 8.28 (d, $^4J_{HP}$ = 12, CH=N), 7.63 - 7.55 (m, 6H, o-pH), 7.54 - 7.50 (m, 9H, p- and m-pH), 7.23 (d, $^3J_{HH}$ = 8, 1H, 5), 7.04 (d, $^3J_{HH}$ = 8, 1H, 4), 6.78 (t, $^3J_{HH}$ = 8, 1H, 6), 6.27 - 6.17 (m, 3H, 7, 3, and 8), 5.51 (s, 1H, 2). $^{31}P\{^1H\}$ NMR (162 MHz, dmso-d_6): 34.06 (s).

3a: A suspension formed by 0.100 g (0.317 mmol) of compound **1a**, 0.062 g, (0.157 mmol) of $PPh_2CH_2CH_2PPh_2$ and 5 mL of acetone was stirred at room temperature for 2 h. The garnet solid formed was filtered, washed with 5 mL of acetone and dried in vacuum. Yield: 0.144 g (89%). Anal. Calc. for (%): C, 60.42; H, 4.10; N, 2.71. Found: C, 60.4; H, 4.2; N, 2.8. High resolution ESI-(+) (*m/z*): 716.1100 (calculated 716.1095 for $C_{39}H_{34}NO_2P_2Pd$) [$M_{\mathbf{1a}(n\,=\,1)} + PPh_2CH_2CH_2PPh_2 + H^+$]. IR ($cm^{-1}$): 3213 (st OH), 1582 (st C = N), 1107 (q X-sensitive of $PPh_2CH_2CH_2PPh_2$). ^1H NMR (400 MHz, dmso-d_6): 8.29 (apparent triplet, 2H, CH=N), 7.97–7.94 (m, 8H, o-pH), 7.48–7.44 (m, 12H, m-pH and p-pH), 7.26 (d, $^3J_{HH}$ = 8, 2H, 5), 7.05 (d, $^3J_{HH}$ = 8, 2H, 4), 6.89 (t, $^3J_{HH}$ = 8, 2H, 6), 6.38–6.19 (m, 6H, 3, 7 and 8), 5.49(s, 2H, 2), 2.74 (br s, 4H, CH_2P). $^{31}P\{^1H\}$ NMR (162 MHz, dmso-d_6): 34.33 (s).

4.2. Cell culture

MBA-MD-231 and MCF-7 breast, and HCT-116 colon human cancer cells and primary normal fibroblasts human BJ cells were grown as a monolayer culture in Dulbecco's Modified Eagle Medium-High Glucose (450 mM) (DMEM-HG), with L-glutamine and without sodium pyruvate, in presence of 10% heat-inactivated foetal calf serum, and 0.1% streptomycin/penicillin, in standard culture conditions (humidified air with 5% CO_2 at 37 °C).

4.3. Cell viability assay

A stock solution (50 mM) of each tested compound was prepared in high purity DMSO. Serial dilutions were made with DMSO/DMEM-HG (1:1) and finally a 1:500 dilution on culture medium was prepared. The final assay concentration of DMSO was 0.2% in all experiments. In the case of *cisplatin*, a stock solution in water of *cisplatin* (1 mg/mL) was diluted with water until final assay concentrations. The assay was performed as described [55]. MDA-MB-231, MCF-7, and HCT-116 cells were plated at 5000 cells/well and BJ cells at 10,000 cells/well in 100 µL media in tissue culture 96-well plates. After 24 h, the media 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 for 72 h. Hexosaminidase activity was measured according to the following protocol. The media were 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 absorbence was recorded at 410 nm.

4.4. Antibacterial assay

Tested compounds were screened to determine their antibacterial activity against six bacterial strains, three gram positive *Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes*, and three gram negative *Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella typhi* by using the disc diffusion method [56–57]. The organisms were cultured in nutrient broth at 37 °C for 24 h. One per cent broth culture containing approx. 10^6 colony-forming units (CFU/mL) of test strain were added to nutrient agar medium at 45 °C and poured into sterile petri plates. The medium was allowed to solidify. Five microliters of the test compound (10 mg/mL in DMSO) were poured on 4-mm diameter sterile paper disks and placed on nutrient agar plates respectively. In each plate, 5 microliters of DMSO served as negative control and 50 microliters of standard antibacterial drug cefixime (1 mg/mL) served as a positive control. Triplicate plates of each bacterial strain were prepared. The plates were incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm).

4.5. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The scavenging activity of tested compounds for DPPH radicals was determined according to the method reported earlier with minor modifications [58]. A stock solution (5 mg/mL) of each tested compound was prepared in DMSO. Serial dilutions were carried out to obtain concentrations of 200, 100, 40, 20, 10 and 5 μg/mL. 15 µL of each test sample or DMSO in the case of the negative control was mixed with 2985 µL of 0.1 mM methanolic solution of DPPH in glass vials so that the final volume was 3 mL. The vials were capped, and the reaction mixture was incubated for 30 min at 37 °C in dark. After incubation the change in colour (from deep-violet to light-yellow) of DPPH solution was measured by taking absorbence of reaction mixtures at 517 nm on a PDA (photo diode array) spectrophotometer (Agilent 8453). A mixture of 2985 µL of methanol and 15 µL of DMSO was used as a blank for spectrophotometric measurements. Each concentration was assayed in triplicate. Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg/mL). A control was prepared containing the same volume without any test solution and reference ascorbic acid. The antioxidant activity of the synthesized compounds is expressed comparing with standard ascorbic acid. Lower absorbence of the reaction mixture indicates higher free radical scavenging activity. The% scavenging of the DPPH radical was calculated by using the following formula:

$$\%\,scavenging\,activity = \frac{absorbance\,of\,control - absorbance\,of\,test\,sample}{absorbance\,of\,control} \times 100$$

4.6. DNA migration studies

All compounds under study were prepared at 10 mM concentration in DMSO and afterwards, serial dilutions in MilliQ water (1:1) were made. For DNA interaction studies, plasmid pBluescript SK+ (Stratagene) was used at a concentration of 40 μg/mL. Plasmid DNA (0.8 μg) was incubated with different concentrations of compounds ranging from 0 to 200 μM at 37 °C for 24 h in a final reaction volume of 20 μL. The final DMSO concentration in the reactions was always lower than 1%. DNA-drug interaction was analysed by agarose gel electrophoresis following a modification of the method described [59]. *Cisplatin* (1–10 μM) was used as a reference control. The gel was stained at the end of the electrophoretic

process with ethidium bromide at 0.5 mg/mL for 1 hour and DNA bands were visualized under UV light.

4.7. Topoisomerase I inhibition assay

Topoisomerase I assay was performed as previously described [60]. Supercoiled pBluescript DNA was treated with topoisomerase I in the absence or presence of the compounds under analysis. Assay mixtures contained supercoiled pBluescript DNA (0.8 μg), calf thymus topoisomerase I (3 units), and the compounds under analysis at 100 μM in 20 μL of relaxation buffer Tris–HCl buffer (pH 7.5), containing 175 mM KCl, 5 mM $MgCl_2$ and 0.1 mM EDTA. 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 stained with ethidium bromide as described in the DNA migration studies experimental part.

4.8. Topoisomerase IIα inhibition assay

Topoisomerase IIα inhibitory activity was measured as previously described [60]. Briefly, pBluescript DNA was incubated with topoisomerase IIα in the absence or presence of compounds under analysis. Assay mixtures contained supercoiled pBluescript DNA (0.3 μg), tested compounds at concentrations 5 - 100 μM in 20 μL of Topo II reaction buffer and Topoisomerase IIα (4 units). Reactions were incubated for 45 min at 37 °C and stopped by the addition of 2 μL of agarose gel loading buffer. After agarose gel electrophoresis, DNA bands were visualized by ethidium bromide staining as described in the DNA migration studies experimental part.

Declaration of Competing Interest

The authors neither have financial interests nor personal relationships that could have influenced the work reported in this paper.

Data Availability

Data will be made available on request.

Acknowledgements

We are grateful to the Ministerio de Economía y Competitividad for financial support (grant number CTQ2015–65040-P) and to the Centres Científics i Tecnològics de la Universitat de Barcelona for the facilities given for the structural determination of the compounds.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2022.122555.

References

[1] T. Scattolin, V.A. Voloshkin, F. Visentin, S.P. Nolan, A critical review of palladium organometallic anticancer agents, Cell Rep. Phy. Sci. 2 (6) (2021) 100446.
[2] T.J. Carneiro, A.S. Martins, M.P.M. Marques, A.M. Gil, Metabolic Aspects of Palladium(II) Potential Anti-Cancer Drugs, Front. Oncol. 10 (2020) 590970.
[3] A.R. Kapdi, I.J.S. Fairlamb, Anti-cancer palladium complexes: a focus on PdX2L2, palladacycles and related complexes, Chem. Soc. Rev. 43 (2014) 4751–4777.
[4] A.S.S.H. Elgazwy, N.S.M. Ismail, S.R. Atta-Allah, M.T. Sarg, D.H.S. Soliman, M.Y. Zaki, M.A. Elgamas, Palladacycles as Antimicrobial Agents, Curr. Med. Chem. 19 (2012) 3967–3981.
[5] E. Gao, C. Liu, M. Zhu, H. Lin, Q. Wu, L. Liu, Current Development of Pd(II) Complexes as Potential Antitumor Agents. Anti-Cancer Agents Med, Chem. 9 (2009) 356–368.
[6] A. Garoufis, S.K. Hadjikakou, N. Hadjiliadis, Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents, Coord. Chem. Rev. 253 (2009) 1384–1397.
[7] R. Oun, Y.E. Moussa, N.J. Wheate, The side effects of platinum-based chemotherapy drugs: a review for chemists, Dalton Trans 47 (2018) 6645–6653.
[8] J. Zhou, Y. Kang, L. Chen, H. Wang, J. Liu, S. Zeng, L. Yu. The Drug-Resistance Mechanisms of Five Platinum-Based Antitumor Agents. Front. Pharmacol. 11:343. doi:10.3389/fphar.2020.00343.
[9] T.T.H. Fong, C.N. Lok, C.Y.S. Chung, Y.M.E. Fung, P.K. Chow, P.K. Wan, C.M. Che, Cyclometalated Palladium(II) N-Heterocyclic Carbene Complexes: anticancer Agents for Potent In Vitro Cytotoxicity and In Vivo Tumor Growth Suppression, Angew. Chem. Int. Ed. 55 (2016) 11935–11939.
[10] E.I. Ikitimur-Armutak, E. Ulukaya, E. Gurel-Gurevin, I. Yaylim, B. Isbilen-Basok, G. Sennazli, G. Yuzbasioglu-Ozturk, K. Sonmez, F. Celik, O. Kucukhuseyin, G. Korkmaz, V.T. Yilmaz, S.Umit Zeybek, Apoptosis-inducing Effect of a Palladium(II) Complex-[*PdCl*(*terpy*)](sac)·2H_2O] on Ehrlich Ascites Carcinoma (EAC) in Mice, In Vivo (Brooklyn) 30 (2016) 457–464.
[11] E.I. Ikitimur-Armutak, K. Sonmez, K. Akgun-Dar, G. Sennazli, A. Kapucu, F. Yigit, V. Turan Yilmaz, E. Ulukaya, Anticancer Effect of a Novel Palladium–Saccharinate Complex of Terpyridine by Inducing Apoptosis on Ehrlich Ascites Carcinoma (EAC) in Balb-C Mice, Anticancer Res 35 (2015) 1491–1498.
[12] A. Bechara, C.M.V. Barbosa, E.J. Paredes-Gamero, D.M. Garcia, L.S. Silva, A.L. Matsuo, F.D. Nascimento, E.G. Rodrigues, A.C.F. Caires, S.S. Smaili, C. Bincoletto, Palladacycle (BPC) antitumour activity against resistant and metastatic cell lines: the relationship with cytosolic calcium mobilisation and cathepsin B activity, Eur. J. Med. Chem. 79 (2014) 24–33.
[13] M. Tanaka, H. Kataoka, S. Yano, H. Ohi, K. Kawamoto, T. Shibahara, T. Mizoshita, Y. Mori, S. Tanida, T. Kamiya, T. Joh, Anti-cancer effects of newly developed chemotherapeutic agent, glycoconjugated palladium (II) complex, against cisplatin-resistant gastric cancer cells, BMC Cancer 13 (2013) 237.
[14] S. Aliwaini, A.J. Swarts, A. Blanckenberg, S. Mapolie, S. Prince, A novel binuclear palladacycle complex inhibits melanoma growth *in vitro* and *in vivo* through apoptosis and autophagy, Biochem. Pharmacol. 86 (2013) 1650–1663.
[15] Y. Wang, J. Hu, Y. Cai, S. Xu, B. Weng, K. Peng, X. Wei, T. Wei, H. Zhou, X. Li, G. Liang, An Oxygen-Chelate Complex, Palladium Bis-acetylacetonate, Induces Apoptosis in H460 Cells via Endoplasmic Reticulum Stress Pathway Rather than Interacting with DNA, J. Med. Chem. 36 (2013) 9601–9611.
[16] 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, A cyclopalladated complex interacts with mitochondrial membrane thiol-groups and induces the apoptotic intrinsic pathway in murine and cisplatin-resistant human tumor cells, BMC Cancer 11 (2011) 296.
[17] E. Ulukaya, F. Ari, K. Dimas, E.I. Ikitimur, E. Guney, V.T. Yilmaz, Anti-cancer activity of a novel palladium(II) complex on human breast cancer cells *in vitro* and *in vivo*, Eur. J. Med. Chem. 46 (2011) 4957–14963.
[18] 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, E.G. Rodrigues, S.J. Marriott, C7a, a Biphosphinic Cyclopalladated Compound, Efficiently Controls the Development of a Patient-Derived Xenograft Model of Adult T Cell Leukemia/Lymphoma, Viruses 3 (2011) 1041–1058.
[19] F. Hebeler-Barbosa, E.G. Rodrigues, R. Puccia, A.C.F. Caires, L.R. Travassos, Gene Therapy against Murine Melanoma B16F10-Nex2 Using IL-13Rα2-Fc Chimera and Interleukin 12 in Association with a Cyclopalladated Drug, Transl. Oncol. 1 (3) (2008) 110–120.
[20] M.C. da Rocha, A.M. Santana, S.R. Ananias, E.T. de Almeida, A.E. Mauro, M.C.P. Placeresa, I.Z. Carlos, Citotoxicity and Immune Response induced by Organopalladium(II) Compounds in Mice bearing Ehrlich Ascites Tumour, J. Braz. Chem. Soc. 18 (8) (2007) 1473–1480.
[21] 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, Biphosphinic palladacycle complex mediates lysosomal-membrane permeabilization and cell death in K562 leukaemia cells, Eur. J. Pharmacol. 542 (2006) 37–47.
[22] C. Bincoletto, I.L.S. Tersariol, C.R. Oliveira, S. Dreher, D.M. Fausto, M.A. Soufen, F.D. Nascimento, A.C.F. Caires, Chiral cyclopalladated complexes derived from N,N-dimethyl-1-phenethylamine with bridging bis(diphenylphosphine)ferrocene ligand as inhibitors of the cathepsin B activity and as antitumoral agents, Bioorg. & Med. Chem. 13 (2005) 3047–3055.
[23] 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, Cyclopalladated compounds as chemotherapeutic agents: antitumor activity against a murine melanoma cell line, Int. J. Cancer 107 (2003) 498–504.
[24] I.B. dos Santos, D.A.M. da Silva, F.A.C.R. Paz, D.M. Garcia, A.K. Carmona, D. Teixeira, I.M. Longo-Maugéri, S. Katz, C.L. Barbiéri, Leishmanicidal and Immunomodulatory Activities of the Palladacycle Complex DPPE 1.1, a Potential Candidate for Treatment of Cutaneous Leishmaniasis, Front. Microbiol. 9 (1427), doi:10.3389/fmicb.2018.01427.
[25] A.M. Arenas Velásquez, W. Campos Ribeiro, V. Venn, S. Castelli, M. Santoro de Camargo, R. Pires de Assis, R. Alves de Souza, A. Rimoldi Ribeiro, T. Gaban Passalacqua, J. Aristeu da Rosa, A. Martins Baviera, A.E. Mauro, A. Desideri, E.E. Almeida-Amaral, M.A.S. Graminha. Efficacy of a binuclear cyclopalladated com-

pound therapy for cutaneous leishmaniasis in the murine model of infection with Leishmania amazonensis and its inhibitory effect on topoisomerase 1B Antimicrob. Agents Chemother. 61:e00688--7. doi:10.1128/AAC.00688-17.
[26] C. de Siqueira Paladi, I.A. Salerno Pimentel, S. Katz, R.L.O.R. Cunha, W. Alves de Souza Judice, A.C.F. Caires, C.L. Barbiéri, Vitro and In Vivo Activity of a Palladacycle Complex on Leishmania (Leishmania) amazonensis, PLoS Negl. Trop. Dis. 6 (5) (2012) e1626.
[27] W.B.Z. Galvão Barros, A. Haide Queiroz da Silva, A.S. Lima Barbosa, Á.Magalhães Nunes, J.R. Machado Reys, H. Gomes de Araújo-Filho, J. de Souza Siqueira Quintans, L.J. Quintans-Júnior, M. Pfeffer, V. Rodrigues dos Santos Malta, M.R. Meneghetti, Palladium–benzodiazepine derivatives as promising metallodrugs for the development of antiepileptic therapies, J. Inorg. Biochem. 155 (2016) 129–135.
[28] A.C.F. Caires, Recent Advances Involving Palladium (II) Complexes for the Cancer Therapy, Anti-Cancer Agents Med. Chem. 7 (5) (2007) 484–491.
[29] D.P. Santana, P.A. Faria, E.J. Paredes-Gamero, A.C.F. Caires, I.L. Nantes, T. Rodrigues, Palladacycles catalyse the oxidation of critical thiols of the mitochondrial membrane proteins and lead to mitochondrial permeabilization and cytochrome c release associated with apoptosis, Biochem. J. 417 (2009) 247–256.
[30] T. Keswani, S. Chowdhury, S. Mukherjee, A. Bhattacharyya, Palladium(II) complex induces apoptosis through ROS-mediated mitochondrial pathway in human lung adenocarcinoma cell line (A549), Curr. Sci. 107 (10) (2014) 1711–1719.
[31] J. Spencer, R.P. Rathnam, M. Motukuri, A.K. Kotha, S.C.W. Richardson, A. Hazrati, J.A. Hartley, L. Malec, M.B. Hursthouse, Synthesis of a 1,4-benzodiazepine containing palladacycle with *in vitro* anticancer and cathepsin B activity, Dalton Trans (2009) 4299–4303.
[32] Z. Mehri Lighvan, H. Ali Khonakdar, A. Akbari, M. Dehdashti Jahromi, A. Ramezanpour, A. Kermagoret, A. Heydari, E. Jabbari, Synthesis and biological evaluation of novel tetranuclear cyclopalladated complex bearing thiosemicarbazone scaffold ligand: interactions with double-strand DNA, coronavirus, and molecular modeling studies, Appl. Organomet. Chem. (2021) e6502.
[33] S.Z. Khan, I.S.Butler Zia-ur-Rehman, F. Bélanger-Gariepy, New ternary palladium(II) complexes: synthesis, characterization, *in vitro* anticancer and antioxidant activities, Inorg. Chem. Commun. 105 (2019) 140–146.
[34] J. Albert, J. Granell, J.A. Durán, A. Lozano, A. Luque, A. Mate, J. Quirante, M.K. Khosa, C. Calvis, R. Messeguer, L. Baldomà, J. Badía, Endo and exo cyclopalladated (E)-N-([1,1′-*biphenyl*]-2-yl)-1-mesitylmethanimines: anticancer, antibacterial and antioxidant activities, J. Organomet. Chem. 839 (2017) 116–125.
[35] J. Albert, J. Granell, R. Qadir, J. Quirante, C. Calvis, R. Messeguer, J. Badía, L. Baldomà, M. Font-Bardia, T. Calvet, Cyclopalladated Benzophenone Imines: synthesis, Antitumor Activity, Cell Accumulation, DNA Interaction, and Cathepsin B Inhibition, Organometallics 33 (2014) 7284–7292.
[36] J. Albert, R. Bosque, M. Cadena, L. D'Andrea, J. Granell, A. González, J. Quirante, C. Calvis, R. Messeguer, J. Badía, L. Baldomà, T. Calvet, M. Font-Bardia, A New Family of Doubly Cyclopalladated Diimines. A Remarkable Effect of the Linker between the Metalated Units on Their Cytotoxicity, Organometallics 33 (2014) 2862–2873.
[37] J. Albert, R. Bosque, M. Crespo, G. García, J. Granell, C. López, M.V. Lovelle, R. Qadir, A. González, A. Jayaraman, E. Mila, R. Cortés, J. Quirante, C. Calvis, R. Messeguer, J. Badía, L. Baldomà, M. Cascante, Cyclopalladated primary amines: a preliminary study of antiproliferative activity through apoptosis induction, Eur. J. Med. Chem. 84 (2014) 530–536.
[38] J. Albert, L. D'Andrea, J. Granell, P. Pla-Vilanova, J. Quirante, M.K. Khosa, C. Calvis, R. Messeguer, J. Badía, L. Baldomà, M. Font-Bardia, T. Calvet, Cyclopalladated and cycloplatinated benzophenone imines: antitumor, antibacterial and antioxidant activities, DNA interaction and cathepsin B inhibition, J. Inorg. Biochem. 140 (2014) 80–88.
[39] J. Albert, S. García, J. Granell, A. Llorca, M.V. Lovelle, V. Moreno, A. Presa, L. Rodríguez, J. Quirante, C. Calvis, R. Messeguer, J. Badía, L. Baldomà, Cyclopalladated benzophenone imines: synthesis, cytotoxicity against human breast adenocarcinoma cell lines and DNA interaction, J. Organomet. Chem. 724 (2013) 289–296.
[40] A. Fernández, D. Vázquez-García, J.J. Fernández, M. López-Torres, A. Suárez, S. Castro-Juiz, J.M. Vila, Synthesis and reactivity of novel cyclometallated complexes derived from [C,N,O] terdentate ligands. Crystal structure of [Pd{2,3,4-$(MeO)_3C_6HC(H)$=N[2-$(O)C_6H_4$]}(PPh_3)], New. J. Chem. 26 (2002) 398–404.
[41] J. Albert, J. Granell, J. Sales, M. Font-Bardía, X. Solans, Optically active exocyclic cyclopalladated derivatives of benzylidene-(R)-(1-phenylethyl)amines: syntheses and X-ray molecular structures of [Pd(2-{(E)-(R)-CHMeN=CH-2′,6′-$Cl_2C_6H_3$)C_6H_4)Cl(PPh_3)] and [Pd(2-{(Z)-(R)-CHMeN=CH-2′,6′-$F_2C_6H_3$)C_6H_4)I(PPh_3)], Organometallics 14 (1995) 1393–1404.
[42] P.W. Clark, S.F. Dyke, G. Smith, C.H.L. Kennard, The cyclopalladation of benzylidenebenzylamines, J. Organomet. Chem. 330 (1987) 447–460.
[43] J. Albert, R. Bosque, L. D'Andrea, J. Granell, M. Font-Bardia, T. Calvet, Synthesis of Cyclopalladated Derivatives of (E)-N-Benzylidene-2-(2,6-dichlorophenyl)ethanamine and Their Reactivity towards Monodentate and Symmetric Bidentate Lewis Bases, Eur. J. Inorg. Chem. (2011) 3617–3631.
[44] H. Yang, M.A. Kan, K.M. Nicholas, Formation of a Novel Pd4 Cluster from the Acetolysis of Bis[(R)-N-*α*-*methylbenzylsalicylideneaminato*-N,O]palladium(II), J. Chem. Soc. Chem. Commun. (1992) 210–212.
[45] C. López, A. Caubet, S. Pérez, X. Solans, M. Font-Bardia, Assembly of cyclopalladated units: synthesis, characterisation, X-ray crystal structure and study of the reactivity of the tetrametallic cyclopalladated complex [Pd{C_6H_4-CH=N-C_6H_4-2-O}]4·2$CHCl_3$, J. Organomet. Chem. 681 (2003) 82–90.
[46] J.J. Fernández, A. Fernández, D. Vázquez-García, M. López-Torres, A. Suárez, N. Gómez-Blanco, J.M. Vila, Tetranuclear complexes of PdII with Tridentate [C,N,O] and [O,N,O] Ligands: synthesis, reactivity and structural isomerism, Eur. J. Inorg. Chem. (2007) 5408–5418.
[47] A.M. Nassar, Bioactive palladium azomethine chelates, a review of recent research. Synthesis and reactivity in inorganic, Metal-Organ. Nano-Metal Chem. 46 (2016) 1349–1366.
[48] J.G. Ferreira, A. Stevanato, A.M. Santana, A.E. Mauro, A.V.G. Netto, R.C.G. Frem, F.R. Pavan, C.Q.F. Leite, R.H.A. Santos, Structure and antimycobacterial activity of the novel organometallic [Pd(C-bzan)(SCN)(dppp)] compound, Inorg. Chem. Commun. 23 (2012) 63–66.
[49] H.J. Lee, S.H. Lee, H.C. Kim, Y.E. Lee, S. Park, Pseudohalide complexes of palladium(II) containing PCP pincer: synthesis, characterization, and their antimicrobial activities, J. Organomet. Chem. 717 (2012) 164–171.
[50] S. Arikan, V. Paetznick, J.H. Rex, Comparative Evaluation of Disk Diffusion with Microdilution Assay in Susceptibility Testing of Caspofungin against Aspergillus and Fusarium Isolates, Antimicrob. Agents Chemother. 46 (2002) 3084–3087.
[51] B.N. Nguyen, L.A. Adrio, T. Albrecht, A.J.P. White, M.A. Newton, M. Nachtegaal, S.J.A. Figueroa, K. Kuok (Mimi) Hii, Electronic structures of cyclometalated palladium complexes in the higher oxidation states, Dalton Trans 44 (2015) 16586–16591.
[52] A. Siddeeg, N.M. AlKehayez, H.A. Abu-Hiamed, E.A. Al-Sanea, A.M. Al-Farga, Mode of action and determination of antioxidant activity in the dietary sources: an overview, Saudi J. Biol. Sci. 28 (2021) 1633–1644.
[53] X. Liang, Q. Wu, S. Luan, Z. Yin, C. He, L. Yin, Y. Zou, Z. Yuan, L. Li, X. Song, M. He, C. Lv, W. Zhang, A comprehensive review of topoisomerase inhibitors as anticancer agents in the past decade, Eur. J. Med. Chem. 171 (2019) 129–168.
[54] S.K. Bjelogrlića, T.R. Todorović, M. Kojić, M. Senćanski, M. Nikolić, A. Višnjevac, J. Araškov, M. Miljković, C.D. Muller, N.R. Filipović, Pd(II) complexes with N-heteroaromatic hydrazone ligands: anticancer activity, in silico and experimental target identification, J. Inorg. Biochem. 199 (2019) 110758.
[55] K.T. Givens, S. Kitada, A.K. Chen, J. Rothschiller, D.A. Lee, Proliferation of Human Ocular Fibroblasts. An Assessment of In Vitro Colorimetric Assays, Investig. Ophthalmol. Vis. Sci. 31 (1990) 1856–1862.
[56] A. Felten, B. Grandry, P.H. Lagrange, I. Casin, Evaluation of Three Techniques for Detection of Low-Level Methicillin-Resistant Staphylococcus aureus (MRSA): a Disk Diffusion Method with Cefoxitin and Moxalactam, the Vitek 2 System, and the MRSA-Screen Latex Agglutination Test, J. Clin. Microbiol. 40 (2002) 2766–2771.
[57] M. Jamil, I.U. Haq, B. Mirza, M. Qayyum, Isolation of antibacterial compounds from Quercus dilatata L. through bioassay guided fractionation, Ann. Clin. Microbiol. Antimicrob. 11 (2012) 1–11.
[58] H. Nawaz, Z. Akhter, S. Yasmeen, H.M. Siddiqi, B. Mirza, A. Rifat, Synthesis and biological evaluations of some Schiff-base esters of ferrocenyl aniline and simple aniline, J. Organomet. Chem. 694 (2009) 2198–2203.
[59] A. Abdullah, F. Huq, A. Chowdhury, H. Tayyem, P. Beale, K. Fisher, Studies on the synthesis, characterization, binding with DNA and activities of two cis-planaramineplatinum(II) complexes of the form: cis-Pt$L(NH_3)Cl_2$ where L = 3-hydroxypyridine and 2,3-diaminopyridine, BMC Chem. Biol. 6 (2006) 3, doi:10.1186/1472-6769-6-3.
[60] D.S. Sappal, A.K. McClendon, J.A. Fleming, V. Thoroddsen, K. Connolly, C. Reimer, R.K. Blackman, C.E. Bulawa, N. Osheroff, P. Charlton, L.A. Rudolph-Owen, Biological characterization of MLN944: a potent DNA binding agent, Mol. Cancer Ther. 3 (2004) 47–58.