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Short communication

Cyclopalladated primary amines: A preliminary study of antiproliferative activity through apoptosis induction

Joan Albert [a, b, *], Ramon Bosque [a, b], Margarita Crespo [a, b], Graciela García [a], Jaume Granell [a, b], Concepción López [a, *], María Victoria Lovelle [a], Romana Qadir [a], Asensio González [b, c], Anusha Jayaraman [b, d], Enric Mila [b, d], Roldán Cortés [b, d], Josefina Quirante [b, c], Carme Calvis [e], Ramon Messeguer [e], Josefa Badía [b, f], Laura Baldomà [b, f], Marta Cascante [b, d, g]

[a] Departament de Química Inorgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain
[b] Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain
[c] Laboratori de Química Orgànica, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII, s/n, 08028 Barcelona, Spain
[d] Department of Biochemistry and Molecular Biology, Faculty of Biology, Universitat de Barcelona, Unit Associated with CSIC, Diagonal 643, 08028 Barcelona, Spain
[e] Biomed Division LEITAT Technological Center, Parc Científic, Edifici Hèlix, C/ Baldiri Reixach, 15-21, 08028 Barcelona, Spain
[f] Departament de Bioquímica i Biologia Molecular, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan XXIII s/n, 08028 Barcelona, Spain
[g] Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Spain

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ABSTRACT

Twelve cyclometallated palladium(II) complexes containing primary aromatic amines [benzylamine (**a**), (*R*)-1-(1-naphthyl)ethylamine (**b**) and 2-phenylaniline (**c**)] as anionic bidentate $(C,N)^-$ ligands have been evaluated against a panel of human adenocarcinoma cell lines (A549 lung, MDA-MB231 and MCF7 breast, and the *cisplatin* resistant HCT116 colon). The results revealed a remarkable antiproliferative activity of the triphenylphosphane mononuclear compounds **3**–**4** (series **a**, **b**, **c**) and the best inhibition was provided for **3c** and **4c** with the 2-phenylaniline ligand and a six membered chelate ring. Interestingly, **3c** and **4c** were 14 and 19 times more potent than *cisplatin* for the inhibition of the *cisplatin* resistant HCT116 human adenocarcinoma cell line, respectively. Cyclopalladated complexes **3c** and **4c** exercise their antiproliferative activity over A549 cells mainly through the induction of apoptosis (38 and 31-fold increase in early apoptotic cells, respectively).

1. Introduction

It is estimated that 50–70% of cancer patients are treated with platinum(II) drugs. Despite the therapeutic benefit of the approved platinum based drugs [1,2] (*cisplatin*, *carboplatin* and *oxaliplatin*), their efficacy is still limited due to side effects [3,4] and resistances (intrinsic or acquired) [5,6]. Hence, great efforts have been undertaken to develop novel metal-based drugs. Multinuclear platinum(II) complexes [7], platinum(IV) prodrugs [8–11], platinum photoactivable drugs [12,13], and platinum(II) drug delivery strategies (polymers, nanoparticles and oligonucleotides) [14,15] have emerged. Other metallodrugs based on ruthenium [16], gold [17,18], titanium [19], copper [20], iron [21,22], have also attracted great interest.

Palladium(II) derivatives have also been explored as an alternative to platinum(II) based drugs. Palladium(II) displays similar chemistry to platinum(II) in many ways, however a significant difference between them is the high kinetic lability of palladium(II) compared to platinum(II). The cytotoxic nature of cyclopalladated compounds has been studied with the rationale that palladium(II) centers could have good activity because of their restricted lability due to the use of a chelating ligand [23,24].

Abbreviations: DMEM, Dulbecco's Modified Eagle Medium; DMSO, dimethylsulfoxide; EB, Ethidium bromide; ELISA, Enzyme-Linked ImmunoSorbent Assay; FACS, Fluorescence-Activated Cell Sorting; FITC, Fluorescein Isothiocyanate; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, Propidium Iodide; PS, Phosphatidylserine.

* Corresponding authors. Departament de Química Inorgànica, Facultat de Química, Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain.
E-mail addresses: joan.albert@qi.ub.es (J. Albert), conchi.lopez@qi.ub.es (C. López).

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Following previous studies of our group on the anticancer activity of cyclopalladated compounds [25–27], we report herein a SAR analysis of the inhibition of cell proliferation activity of the cyclopalladated primary amine complexes shown in Fig. 1, against a panel of human adenocarcinoma cell lines (A549 lung, MDA-MB231 and MCF7 breast, and HCT116 colon). To gain insight into the action mechanism of the investigated complexes, studies of electrophoretic shift DNA-migration, cell cycle arrest and apoptosis were performed.

2. Results and discussion

2.1. Synthesis of the compounds

Fig. 1 shows the structural formula of the cyclopalladated primary amines under study. These compounds have been prepared as previously reported by cyclopalladation, metathesis and splitting reactions [28–36]. The purity of compounds **1**–**4** has been checked by IR and ^{1}H NMR and that of compounds **3** and **4** also by ^{31}P-{^{1}H} NMR. Compound **3b** is new and has been prepared by a splitting reaction between cyclopalladated dimer **1b** and PPh_3. Synthesis of **3b** together with optimized methods of preparation of **1b** and **2b** and some characterization data for these compounds are included as supplementary materials. Elemental analyses of **3b** agree with the proposed formula and its IR spectrum indicated the presence of a terminal acetato ligand. ^{1}H NMR studies for compound **3b** revealed that the NH_2 protons were diastereotopic, thus indicating the binding of the amino group to the palladium(II) atom. The chemical shift of the ^{31}P of compound **3b** (41.50 ppm) was in the range expected for compounds of formula *trans*-***N,P***-[Pd(C,N)X(PPh_3)] (X = OAc or Cl) with a five membered palladacycle and a palladated phenyl or naphthyl carbon atom [37].

2.2. Biological studies

2.2.1. Antiproliferative assay

Human lung, breast and colon cancer cell lines (A549, MDA-MB231 and MCF7 and HCT116, respectively) were used to test the cytotoxic activity of the palladium(II) cyclometallated complexes **1**–**4**. For comparison purposes, the free ligands **a**–**c** and *cisplatin* were tested under the same experimental conditions in the four cells lines selected. The effects of the assayed metalacycles on the growth of the selected cell lines were evaluated after 72 h and the obtained IC_{50} values are listed in Table 1.

Table 1
IC_{50} (μM) values[a] for compounds under study and clog *P*[b] values.

	IC_{50} (μM)				clog *P*
Compound	A549	MDA-MB-231	MCF-7	HCT116	
a	>100	>100	>100	>100	1.09
1a	>50	≈100	>100	≈100	5.75
2a	98 ± 3.5	≈100	>100	≈100	4.00
3a	40 ± 8.7	4.6 ± 1.2	11 ± 0.8	28 ± 21	8.91
4a	43.7 ± 11	13 ± 2.7	41 ± 19	16 ± 1.7	8.04
b	>100	>100	>100	>100	2.58
1b	>100	40 ± 15	51 ± 19	39 ± 6.1	9.14
2b	88 ± 2.8	32 ± 6.7	44 ± nd	35 ± 4.5	7.39
3b	23.7 ± 5.5	5.2 ± 0.9	11 ± 2.1	5.4 ± 0.4	10.61
4b	45 ± 5	2.7 ± 0.3	7.3 ± 1.7	3.2 ± 0.4	9.73
c	>100	>100	>100	>100	2.80
1c	45 ± 11	25 ± 18	18 ± 5	8.7 ± 1.1	8.01
2c	35 ± 7	17 ± 9	17 ± 5	7.4 ± 0.6	6.26
3c	20 ± 2	1.4 ± 0.26	4.2 ± 0.5	2.1 ± 0.1	10.04
4c	18 ± 1.0	1.0 ± 0.1	7.8 ± 1.2	2.9 ± 0.3	9.17
Cisplatin[c]	9.3 ± 3	6.5 ± 2.4	19 ± 4.5	40 ± 4.4	−2.5

[a] Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation.
[b] *clog P* is the calculated logarithmic value of the n-octanol/water partition coefficient, and was calculated using the computer program ChemBioDraw Ultra 12.0 (ChemBioOffice 2010).
[c] *Cis*-[$PtCl_2(NH_3)_2$] is taken as reference compound.

For SAR analysis some elements could be highlighted when comparing the cytotoxicity of the palladium(II) complexes with general structures **a**, **b** and **c**.

1) With regard to the dinuclear/mononuclear character of the investigated complexes, in general the mononuclear complexes **3** and **4** exhibit lower IC_{50} values than their corresponding parent dinuclear compounds **1** and **2.** Factors such as lipophilicity, stability in biological medium, molecular size, flexibility, and influx or efflux through cellular membranes, may account for the greater cytotoxicity of the mononuclear complexes versus the dinuclear ones. Despite of this, several dinuclear compounds showed a remarkable potency, even greater than *cisplatin* in some of the selected cellular lines. For instance, in MCF7 breast and the *cisplatin* resistant HCT116 colon cancer

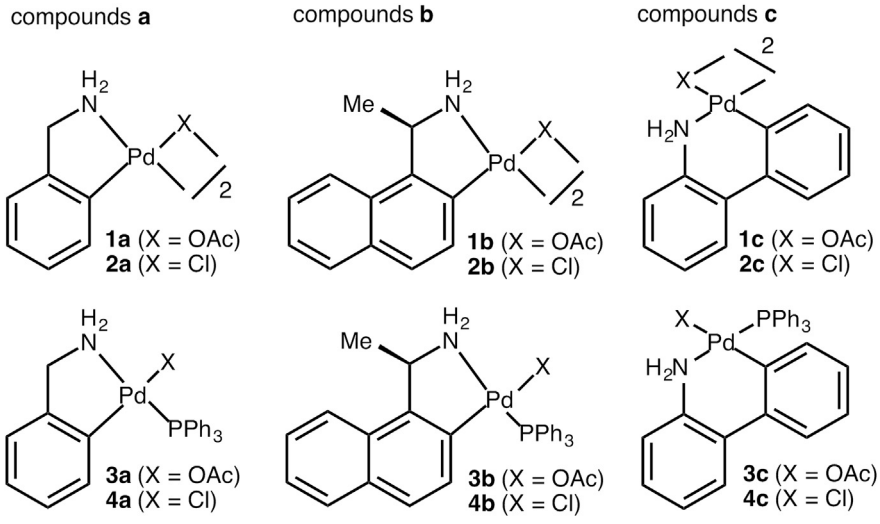

Fig. 1. Compounds selected for this study.