Pd(II) complexes with N-substituted pyrazoles as ligands. The influence of the R group [OMe versus NMe2] of [1-{Re(CH2)2e}-3,5-Ph2e(C3HN2)] on their cytotoxic activity on breast cancer cell lines

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ABSTRACT

The study of the reactivity of the novel pyrazole derivative [1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)] (1) with Na2[PdCl4] or Pd(OAc)2 under different experimental conditions has allowed us to isolate and characterize the trans-isomers of [Pd{[1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)]}2(X)2] [X = Cl (2) or OAc (3)] and the di-m-ligand bridged cyclopalladated complexes [Pd{k2,C,N[1-{MeOe(CH2)2e}-3-(C6H4),5-Ph-(C3HN2)]}(m-X)]2 [X = OAc (4) or Cl (5)]. Further treatment of compounds 4 or 5 with PPh3 in CH2Cl2 produced the bridge splitting and the formation of [Pd{k2,C,N[1-{MeOe(CH2)2e}-3-(C6H4),5-Ph-(C3HN2)]}X(PPh3)] [X = OAc (6) or Cl (7)]. The cytotoxic assessment of the free ligand (1) and the Pd(II) complexes on the two breast cancer cell lines MCF7 and MDA-MB231 reveals that: a) compound 1 is less active than its analogue [1-{Me2Ne(CH2)2e}-3,5-Ph2e(C3HN2)] (Ic) and b) palladacycles 4e7 showed a remarkable cytotoxic activity in the MDA-MB231 cell line (with IC50 values in the range 9.1e14.4 mM).

Introduction

Pyrazoles are widely used as core motifs for a large number of compounds of significant relevancy and they have a variety of applications (i.e. as agrochemicals, dyes, food additives, catalysts and pharmaceuticals) [1e2]. Moreover, N-substituted derivatives are particularly attractive in view of their antiproliferative properties against cancer cells [3,4]. Furthermore, this type of heterocycles have a rich and versatile coordination ability with transition metals that affects their properties [5e6]. This fact, together with the increasing interest in the design of novel antitumoral drugs with greater efficiency, lower toxicity and less undesirable side effects than the current Pt-based drugs [7], have triggered the development of novel Pd(II) and Pt(II)-pyrazole containing complexes [8e13]. Substituted pyrazoles in which there is a s(CeH) bond with the proper orientation are valuable substrates to achieve cyclopallada- or platinated complexes [11e13]. This sort of metallacycles are relevant due to their chemical, physical or optical properties and their applications in different areas (i.e. as precursors in organic, organometallic synthesis, homogeneous catalysis and as building blocks in supramolecular chemistry) [14,15].

On the other hand, bioorganometallic chemistry is a rapid developing area of increasing interest and the idea of using organometallic complexes in drug discovery is becoming more and more popular [16,17]. Antimalarials, antibacterials, neuroprotectors, based on organometallic compounds have been described [16,17]. Moreover, recent contributions suggest that cyclopallada- and cycloplatinated compounds are probably amongst the best candidates for the design of newanticancer drugs [18e20].

Despite the increasing interest in both Pd(II)-pyrazole complexes [21,22] and cyclometallated compounds [14,15,18e20], only a few articles on cyclopalladated-pyrazole derivatives have been published [11,13]. We have recently reported three N-substituted pyrazoles of general formulae 1-{Me2Ne(CH2)2e}-3,5-R2e(C3HN2) (I) (Fig. 1), their Pd(II) and Pt(II) complexes of general formulae ([M {1-[Me2Ne(CH2)2e]-3,5-R2-(C3HN2)}Cl2] [(II) and (III), in Fig. 1) and the cyclometallated derivatives (IV and V) [11b]. The comparison of their antitumoral activity in three different cell lines (MDAMB231, MCF7 and A549) revealed that: a) pyrazoles (I) with R = Ph were more potent than their analogues with R = H or Me, b) the binding of these ligands to Pd(II) or Pt(II) produced an enhancement of their activity, and c) cyclometallated products (IV and V) were more potent than complexes IIc and IIIc in which the pyrazole Ic acts as a bidentate (N,N0) ligand.

In view of these findings and in order to elucidate the effect produced by the functional group on the pendant arm of the heterocyclic nitrogen, now we report a parallel study on the novel pyrazole: [1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)] (1), that has the OMe group attached at the end of the e(CH2)2e chain instead of the -NMe2 of Ic.

Experimental

Materials and methods

All the reagents were obtained from commercial sources and used as received. Solvents were distilled and dried before use [23]. Elemental analyses were carried out at the Serveis Científico-Tècnics (Universitat Barcelona). Mass spectra (ESIþ) were performed at the Servei d'Espectrometria de Masses (Universitat de Barcelona). Infrared (IR) spectra were obtained with a Nicolet 400FTIR instrument using KBr pellets. A selection of the most relevant absorptions observed in the IR spectra are presented in the following sections. Ultravioletevisible (UVevis) spectra of CH2Cl2 solutions of the free ligand 1 and the Pd(II) compounds (5e7) were recorded at 298 K with a Cary 100 scan 388 Varian UV spectrometer. Thewavelengths (l, in nm) and the extinction coefficients (ε, in M-1 cm-1) of the bands observed in the UVevis spectra are specified in the characterization section of the corresponding product. The retention coefficients (Rf) were obtained using SiO2 (Merck silica gel 60 F254) plates. High resolution 1H NMR spectra and the two-dimensional [{1He1H} NOESY and COSY and {1He13C}-heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond coherence (HMBC)] experiments were registered with a Varian VRX-500 or a Bruker Avance DMX-500 MHz instruments. 13C{1H} NMR spectra and 31P{1H} spectra of compounds 6 and 7 obtained with a Mercury-400 and a Varian 300 MHz instrument, respectively. NMR studies of 1e3 and 5e7 were carried out at 298 K, using CDCl3 (99.9%) as solvent and SiMe4 [for 1H and 13C{1H} NMR] and P(OMe)3 [d(31P) = 140.17 ppm for 31P NMR] as references. Due to the low solubility of complex 5 in CDCl3 its NMR studies were performed in DMSO-d6 (99.8%). In all cases, chemical shifts (d) are given in ppm and the coupling constants (J) in Hz. In the characterization section of each product the assignment of signals detected in their NMR spectra refers to the labelling patterns presented in Scheme 1 (Abbreviations for the multiplicities of the signals observed in the NMR spectra: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad).

Synthesis

Preparation of 1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2) (1)

Potassium hydroxide (0.800 g, 14 x 10-3 mol) was treated with 9.0 mL of DMSO and the resulting mixturewas magnetically stirred at room temperature (ca. 298 K) for 30 min. After this period 3,5-diphenylpyrazole (0.788 g, 3.58 x 10-3 mol) was added and the stirring was maintained for 30 min at 298 K. Then, 1-chloro 2-methoxyethane (4.0 mL, 45 x 10-3 mol) was gradually added and the reaction mixture was left overnight under stirring at 298 K. After this period, 50 mL of iced cold water was added to eliminate the unreacted KOH; the organic phase was then extracted with diethylether and washed repeatedly with water, dried over Na2SO4 and filtered. Then, the filtrate was concentrated to dryness on a rotary evaporator. The solid formedwas collected and later on dried in vacuum for 3 days. [Yield: 0.880 g, (88%)]. Characterization data: Anal (%). Calc. for C18H18N2O (MW = 278.35): C, 77.67; H, 6.52; N, 10.06%; found: C, 77.8; H, 6.6 and N, 10.1. MS (ESIþ): m/z = 279.15 {[M] þ H}þ. IR selected data: 3059e3015 [n(CeH)] and 2987e2952 [n(CeH)], 1481(m), 1462(s), 1441(m), 1363(m), 1300(m), 1115(s), 1012(m), 762(s), 691(s) cm-1. UVevis data (c = 5.74 x 10-5 M in CH2Cl2): l (ε) = 223 (1.5 x 104) and 253 (3.1 x 104). Rf (in CHCl3) = 0.24. 1H NMR data: d = 3.32 (s, 3H, OMe); 3.91 [t, 3JH,H = 7.2, 2H, (eCH2ed)]; 4.34 [t, 3JH,H = 7.2, 2H, (eCH2ec)]; 6.65 (s, 1H, H4); 7.35 (t, 3JH,H = 7.7, 1H, H4b); 7.47e7.60 (m, 5H, H3a, H4a, H5a, H3b and H5b); 7.70 (d, 2H, 3JH,H = 7.6, H2b and H6b), 7.92 (d, 2H, 3JH,H = 7.5, H2a and H6a). 13C{1H} NMR data: 49.1 (Cd); 58.9 (OMe); 71.4 (Cc); 103.4 (C4);

125.3 (C2b and C6b); 125.7 (C2a and C6a); 126.0 (C4b); 126.8 (C4a); 128.7 (C3a and C5a); 129.2 (C3b and C5b); 130.7 (C1b); 133.6 (C1a); 145.9 (C5) and 150.9 (C3).

Preparation of trans-[Pd{[1-{MeOe(CH2)2e}-3,5-Ph2e (C3HN2)]2Cl2] (2)

To a solution formed by Na2[PdCl4] (100 mg, 3.4 x10-4 mol) and methanol (10 mL), ligand 1 (189 mg, 6.8 x 10-4 mol)was added and the reaction mixture was stirred at 298 K for 2 h. The yellow solid formed was filtered and dried overnight. Afterwards, it was dissolved in the minimum amount of CH2Cl2 and passed through a SiO2 (2.0 cm x 8.0 cm) column. Elution with CH2Cl2 produced a wide yellow band which was collected and concentrated to dryness on a rotary evaporator and the bright yellow solid formed was collected and finally dried in vacuum for 2 days [Yield: 196 mg (78%)]. Characterization data: Anal. (%) Calc for C36H36Cl2N4O2Pd: (MW = 734.0): C, 58.91; H, 4.94; N, 7.63; Found: C, 59.0; H, 5.0; N, 7.5. MS (ESIþ): m/z = 697.16 {[M] - Cl}+. IR selected data: 3047e 3015 [n(CeH)] and 2980e2925 [n(CeH)], 1483(m), 1461(m), 1448(m), 1124(s), 765(s) and 700(s) cm-1. UVevis data: (c = 8.17 x 10-6 M in CH2Cl2): l (ε) = 238 (3.8 x 105), broad band. Rf (in CHCl3) = 0.19. 1H NMR data: d = 3.20 [s, 6H, 2 (OMe)]; 3.96 [t, 3JH,H = 6.9, 4H, 2 (eCH2-d)]; 4.89 [t, 3JH,H = 6.9, 4H, 2 (eCH2ec)]; 6.36 [s, 2H, 2 (H4)]; 7.40e7.58 [br. m, 12H, 2(H3a, H4a, H5a, H3b, H4a and H5b)]; 7.62 [d. 3JH,H = 7.6, 4H, 2 (H2b and H6b)] and 8.20 [d, 3JH,H = 7.6, 4H, 2 (H2a and H6a)]. 13C{1H} NMR data: 50.31 (2 Cd); 58.7 (2 OMe); 69.9 (2 Cc); 108.5 (2C4); 125.5 [2 (C2b and C6b)]; 125.9 (2C4b); 126.4 (2C4a); 128.5 [2 (C2a and C6a)]; 129.4 (2C1b), 129.8 [2 (C3b and C5b)]; 130.1 [2 (C3a and C5a)]; 132.7 (2C1a); 149.7 (2C5) and 154.7 (2C3).

Preparation of trans-[Pd{[1-{MeOe(CH2)2e}-3,5-Ph2e (C3HN2)]2(OAc)2] (3) and [Pd{k2,C,N)[1-{MeO-(CH2)2}-3-(C6H4),5-Ph-(C3HN2)]}(m-OAc)]2 (4)

76 mg (3.4 x 10-4 mol) of Pd(OAc)2 was dissolved in 15 mL of toluene at 298 K, then ligand 1 (95 mg, 3.4 x 10-4 mol) was added. The reaction flask was protected from the light with aluminium foil and refluxed for 12 h. After this period the black solution was concentrated to dryness on a rotary evaporator and left overnight in a dessicator. The residue was then treated with CH2Cl2 (15 mL) and passed through a Celite pad to remove the metallic palladium. This was repeated until colourless mother liquors were obtained. The solutionwas dried with Na2SO4 and the filtratewas concentrated to dryness on a rotary evaporator. The brownish solid formed consisted on a mixture of compounds 3 and 4 in a molar ratio (3:4 = 1:10). These products were isolated by SiO2 column chromatography. Elution with CHCl3 produced a pale yellow band that gave after concentration 11 mg of the minor product (3). Afterwards the use of a CHCl3:MeOH (100:0.4) mixture released another band that was collected and concentrated to dryness on a rotary evaporator. The yellowish solid formed (4) was collected, air-dried and dried in vacuum for 2 days (yield: 112 mg). Characterization data for 3: Anal. (%) Calc. for C40H42N4O6Pd (MW = 780.21): C, 61.59; H, 5.4; N, 7.17; Found: C, 61.4; H, 5.5; N, 7.0. MS (ESIþ): m/z = 721.2 {[M] - (OAc)}+. IR selected data: 3067e3023 [n(CeH)] and 2984e 2940 [n(CeH)], 1566 [nas(COO)] and 1386 [ns (COO)] cm-1. UVe vis data: (c = 1.43 x10-5 M in CH2Cl2): l (ε) = 234 (4.1 x105) broad band. Rf (in CHCl3) = 0.21. 1H NMR data: d = 1.83 [br. s, 6H, 2 (OAc)]; 3.15 (s, 6H, 2 OMe); 3.95 [t, 3JH,H = 7.1, 4H, 2 (eCH2ed)]; 4.75 [t, 3JH,H = 7.1, 4H, 2 (eCH2ec)]; 6.40 (s, 2H, 2H4); 7.37e7.58 [m, 16H, 2(H3a, H4a, H5a, H2b, H3b, H4b, H5b and H6b)] and 8.16 [d, 3JH,H = 7.6, 4H, 2 (H2a and H6a)]. 13C{1H} NMR data: 23.3 [2Me(OAc)]; 50.2 (2Cd); 58.4 (2OMe); 70.1 (2Cc); 108.7 (2C4); 125.7 [2(C2b and C6b)]; 126.1 (2C4b); 126.4 (2C4a); 128.6 [2(C2a and C6a)]; 129.5 [2(C3b and C5b)]; 130.0 [2(C3a and C5a)]; 130.4 (2C1b); 132.6 (2C1a); 149.5 (2C5); 154.3 (2C3) and 180.9 [2 > COO(OAc)]. For 4: Anal. (%) Calc. for C40H40N4O6Pd2 (MW = 886.1): C, 54.25; H, 4.55; N, 6.32; Found: C, 54.1; H, 4.5; N, 6.0. MS (ESIþ): m/z = 425.06 {[M] - 2(OAc) þ 2(CH3CN)}2þ/2. IR selected data: 3060e3018 [n(Ce H)] and 2984e2940 [n(CeH)], 1560 [nas(COO)] and 1413 [ns(COO)] cm-1. UVevis data: (c =

1.91 x 10-5 M in CH2Cl2): l (ε) = 223 (3.2 x 105), 247 (2.5 x 105) and 341(broad, z1.9 x 103). Rf (in CHCl3) = 0.14. 1H NMR data: d = 2.01 [br. s, 6H, 2(OAc)]; 3.17 [s, 6H, 2(OMe)]; 3.30e3.40 [(br. m, 4H, 2(eCH2ed)]; 3.67 [m, 2H, 2 (one of the two protons of the eCH2ec unit)]; 3.93 [m, 2H, 2 (the other proton of the eCH2ec unit)]; 6.04 [s, 2H, 2(H4)]; 6.74 [m, 2H, 2(H3a)]; 6.81 [m, 2H, 2(H4a)]; 7.31e7.80 [m, 10H, 2(H5a, H6a, H3b, H4b and H5b)] and 8.51 [br., 4H, 2(H2b and H6b)]. 13C{1H} NMR data: 23.9 [2Me(OAc)]; 49.7 (2Cd); 58.2 (2OMe); 70.6 (2Cc); 109.3 (2C4); 125.5 [2 (C2b and C6b)]; 125.9 (2C4b); 126.3 (2C4a); 127.6 (2C5a); 128.3 (2C6a); 129.0 [2 (C3b and C5b)]; 129.7 (2C3a); 130.2 (2C1b); 132.3 (2C1a); 148.9 (2C2a), 150.6 (2C5); 153.9 (2C3) and 178.9 [2 > COO (OAc)].

Preparation of [Pd{k2,C,N)[1-{MeOe(CH2)2}-3-(C6H4),5-Phe (C3HN2)]}(m-Cl)]2 (5)

Compound [Pd{k2,C,N[1-{MeOe(CH2)2}-3-(C6H4),5-Phe (C3HN2)]}(m-OAc)]2 (4) (40 mg, 4.37 x 10-5 mol) was suspended in acetone (20 mL), then LiCl (5 mg, 1.1 x 10-4 mol) was added. The resulting mixture was stirred overnight at 298 K. The solid formed was collected by filtration and dried in vacuum for 2 days [Yield: 32 mg, (81%)]. Characterization data: Anal. (%) Calc. for C36H34Cl2N4O2Pd2 (MW = 838.43): C, 51.7; H, 4.09; N, 6.68; Found: C, 51.6; H, 4.2; N, 6.8. MS (ESIþ): m/z = 383.761 {[M] - 2Cl}2þ/2. IR selected data: 3055e3022 [n(CeH)] and 2988e2924 [n(CeH)]. UVe vis data: (c = 1.91 x10-5 Min CH2Cl2): l (ε) = 240 (br. 2.5 x105) and 338 (z3.6 x 103). 1H NMR data (in DMSO-d6): d = 3.22 [s, 6H, 2(OMe)]; 3.30 [br. m, 2H, {one of the protons of the (eCH2ed) unit}], 4.03 [m, 2H, 2 {one of the protons of the (eCH2ec) moiety]; the signals due the remaining protons the (eCH2e)c and d moieties were masked by the broad signal (at d = 3.50 ppm) of the residual water present in the DMSO-d6, their chemical shifts [ca. 3.45 (e CH2ed) and 3.85 for that of the eCH2ec unit] were obtained from the HSQC experiment; 6.07 [s, 2H, 2(H4)]; 6.74 [m, 2H, 2(H3a)]; 6.81 [m, 2H, 2(H4a)] and 7.18e8.10 [br. m, 14H, 2(H5a, H6a, H2b, H3b, H4b, H5b and H6b)]. 13C{1H} NMR data (in DMSO-d6): 50.1 (2Cd); 58.0 (2OMe); 70.3 (2Cc); 109.1 (2C4); 125.0 [2(C2b and C6b)]; 125.7 (2C4b); 126.0 (2C4a); 127.9 (2C5a); 128.4 (2C6a); 128.9 [2(C3b and C5b)]; 129.6 (2C3a); 130.4 (2C1b); 131.8 (2C1a); 151.2 (2C5); 152.0 (2C2a) and 153.7 (2C3)].

Preparation of [Pd{k2,C,N)[1-{MeOe(CH2)2e}-3-(C6H4),5-Phe (C3HN2)]}(X)(PPh3)] {X = OAc (6) or Cl (7)}

To a mixture containing 3.8 x 10-5 mol of the corresponding dimeric complex 4 (35 mg) {or 5 (34 mg)} and 20 mL of CH2Cl2, PPh3 (20 mg, 7.6 x 10-5 mol) was added. The resulting mixturewas kept under stirring for 20 min and then filtered. The pale yellowish filtrate was concentrated to dryness on a rotary evaporator and the solid formed was collected, air-dried and recrystallized in a CH2Cl2:n-hexane (1:1) mixture [Yields: 38 mg (70%) and 40 mg (77%) for 6 and 7, respectively]. Characterization data for 6: Anal. Calc for C38H35N2O3PPd (MW = 705.9): C, 64.73; H, 5.00; N, 3.97%; Found: C, 64.6; H, 5.1 and N, 4.0; MS (ESIþ): m/z = 645.14 {[M] (OAc)}þ. IR selected data: 3050e3018 [n(CeH)] and 2985e 2925 [n(CeH)]; 1590 [nas(COO)] and 1381 [ns(COO)] and 1094 (PPh3, X-sensitive), cm-1. UVevis (c = 1.42 x 10-5 M in CH2Cl2): l (ε) = 229 (4.0 x 10-5), 246 (3.0 x 10-4) and 348 (3.5 x 10-3). Rf (in CHCl3) = 0.20. 1H NMR data: d = 1.85 (s, 3H, OAc); 3.12 (s, 3H, OMe); 3.67 (t, 3JH,H = 7.3, 2H, eCH2ed); 4.37 (t, 2H, 3JH,H = 7.0, eCH2ec); 6.07 (s, 1H, H4); 6.44 (br. 1H, H3a); 6.96 (m, 1H, H4a); 7.28e7.96 [br. m, 22H, (H5a , H6a, H2b-H6b, and aromatic protons of the PPh3 ligand)]. 13C{1H} NMR data: 23.3 [Me(OAc)], 51.4 (Cd); 58.5 (OMe); 70.2 (Cc); 110.2 (C4); 125.3 (C4b); 126.5 (C4a); 127.4 (C2b and C6b); 128.0 (C6a); 128.4 (C5a); 129.1 (C3b and C5b); 129.5 (C3a); 129.8 (C1b); 132.5 (C1a); 150.2 (C5); 151.2 (C2a); 154.7 (C3), 180.4 [>COO(OAc)] and four additional doublets due to four types of carbon-13 nuclei (ipso, ortho, meta and para) of the PPh3 ligand. 31P{1H} NMR data: d = 40.3. For 7: Anal. Calc. for C36H32ClN2OPPd (MW = 680.1): C, 63.45; H, 4.73; N, 4.11%; Found: C, 63.2; H, 4.8; N, 4.1; MS (ESIþ): m/z = 645.14 {[M] - Cl}þ. IR selected data: 3049e3018 [n(CeH)]

and 2982e2925 [n(CeH)] and 1094 (X-sensitive of the phosphine ligand) cm-1. UVevis data: (c = 1.8 x 10-5 M in CH2Cl2): l (ε) = 242 (2.0 x 10-5); and 341 (3.9 x 10-3). 1H NMR data: d = 3.11 (s, 3H, OMe); 3.82 (br., 2H, eCH2ed); 4.50 (br., 2H, eCH2ec); 6.35 (s, 1H, H4); 6.58 (m, 1H, H4a); 6.84 (m, 1H, H3a) and 7.30e8.10 [br. m, 22H, (H5a, H6a, H2b-H6b and aromatic protons of the PPh3 ligand)]. 13C {1H} NMR data: 51.2 (Cd); 58.4 (OMe); 70.1 (Cc); 110.1 (C4); 125.4 (C4b); 126.3 (C4a); 127.3 (C2b and C6b); 127.8 (C6a); 128.6 (C5a); 129.0 [(C3b and C5b); the {1He13C}-HSQC spectra showed that the signal due to C3a was overlapped by this one]; 129.6 (C1b); 132.8 (C1a); 149.9 (C5); 152.6 (C2a); 153.8 (C3), and four additional doublets due to the aromatic 13C nuclei of the PPh3. 31P{1H} NMR data: d = 40.1.

Crystallography

A plaque-like specimen of compound 2 (dimensions in Table 1), was used for the X-ray crystallographic analysis. The X-ray intensity data were measured and integration of the data using a triclinic unit cell yielded a total of 11,383 reflections to a maximum q angle of 28.34° (0.75 Å resolution), of which 4182 were independent (average redundancy 2.722, completeness = 99.7%, Rint = 2.43%, Rsig = 2.81%) and 3912 (93.54%) were greater than 2s(F2). The final cell constants (Table 1) are based upon the refinement of the XYZcentroids of reflections above 20 s(I).

The structure was solved and refined using the Bruker SHELXTL Software Package, [24] using the space group P-1, with Z = 1 for the formula unit, C36H36Cl2N4O2Pd. The final anisotropic full-matrix least-squares refinement on F2 with 205 variables converged at R1 = 3.10%, for the observed data and wR2 = 8.03% for all data. The goodness-of-fit was 1.055. The largest peak in the final difference electron density synthesis was 0.562 e-/Å3 and the largest hole was -0.269 e-/Å3 with an RMS deviation of 0.065 e-/Å3. Final details concerning the resolution and refinement of the crystal structure are presented in Table 1.

CCDC 951054 contains the crystallographic information file for compound 2.

Biological studies

Cell culture

Breast cancer MCF7 and MDA-MB231 cells (from European Collection of Cell Cultures, ECACC) 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 of D-glucose and 0.1% streptomycin/penicillin in standard culture conditions.

Cell viability assays

For these studies, compounds were dissolved in 100% DMSO at 50 mM as stock solution; then, serial dilutions have been done in DMSO (1:1) (in this way DMSO concentration in cell media was always the same); finally, 1:500 dilutions of the serial dilutions of compounds on cell media were done. The assay was performed as described by Givens et al. [25]. In brief, MDA-MB231 and MCF7 cells were plated at 5000 cells/well or 10,000 cells/well respectively, in 100 mL media in tissue culture 96 well

plates (Cultek). After 24 h, media was replaced by 100 mL/well of serial dilution of drugs. Each point concentration was run in triplicate. Reagent blanks, containing media plus colorimetric reagent without cells were run on each plate. Blank values were subtracted from test values and were routinely 5e10% of uninhibited control values. Plates were incubated for 72 h. Hexosamidase activity was measured according to the following protocol: the media containing the cells was removed and cells werewashed once with PBS 60 mL of substrate solution (pnitrophenol-N-acetyl-b-D-glucosamide 7.5 mM [Sigma N-9376], sodium citrate 0.1 M, pH = 5.0, 0.25% Triton X-100) was added to each well and incubated at 37 °C for 1e2 h; after this incubation time, a bright yellow colour appeared; then, plates could be developed by adding 90 mL of developer solution (Glycine 50 mM, pH = 10.4; EDTA 5 mM), and absorbance was recorded at 410 nm.

Results and discussion

Synthesis

Compound 1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2) (1) was obtained in a fairly good yield (88%) by alkylation of 3,5-diphenylpyrazole with a three-fold excess of 1-chloro 2-methoxyethane [Scheme 1, step (i)] [26]. Treatment of 1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2) (1) with Na2[PdCl4] (in a 1:1 or 2:1Mratio) in methanol at 298 K produced a yellow solid that was identified as [Pd{[1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)]}2Cl2] (2) [Scheme 1, step (ii)]. Its X-ray crystal structure (see below) confirmed that it was the trans isomer. Whenligand 1was reacted with Pd(OAc)2 in toluene under reflux for 12 h a black solution was obtained. The subsequent chromatography on silica gel gave two products 3 and 4 [Scheme 1, step (iii)] in a molar ratio 4:3 =10:1. The minor component (3) was identified as trans-[Pd{[1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)]}2(OAc)2] and characterization data of the major product (4) were consistent with those expected for the di-m-acetato-bridged cyclopalladated complex [Pd{k2,C,N[1-{MeOe(CH2)2e}-3-(C6H4),5-Phe(C3HN2)]}(m-OAc)]2 that arises form the activation of the s(CeH) bond of the phenyl ring in position 3 of the pyrazole. The reaction of 4 with an excess of LiCl in acetone at 298 K gave the dinuclear compound [Pd{k2,C,N[1-{MeOe(CH2)2e}-3-(C6H4),5-Phe(C3HN2)]}(m-Cl)]2 (5) as a pale brownish solid [Scheme 1, step (iv)]. Further treatment of di-m-ligand bridged cyclopalladated products (4 or 5) with PPh3 (in a 1:2 M ratio) in CH2Cl2 produced the splitting of the "Pd(m-X)2Pd" units and the formation of the monomeric derivatives [Pd{k2,C,N)[1-{MeOe(CH2)2e}-3-(C6H4),5-Phe(C3HN2)]}(X)(PPh3)] with X = OAc (6) or Cl (7) [Scheme 1, step (v)] respectively.

Characterization

The new ligand (1) and its palladium(II) derivatives (2e7) were characterized by elemental analyses, mass spectra and infrared spectroscopy. In all cases the elemental analyses were consistent with the proposed formulae.

The IR spectra of the complexes 3, 4 and 6 showed the typical bands due to the symmetric and asymmetric stretchings of the e COO- unit [27] and the separation between them was consistent with the values reported for related Pd(II)-compounds containing OAc- ligands acting as monodentate group (in 3 and 6) or as a bridging ligand (in 4) [27]. For compounds 6 and 7, with the PPh3 ligand, X-sensitive bands were also detected in their IR spectra.

Compound 2 was also characterized by X-ray diffraction. Its molecular structure and the atom labelling scheme is presented in Fig. 2. The crystal contains molecules of [Pd{[1-{MeOe(CH2)2e }-3,5-Ph2e(C3HN2)]2Cl2] in which, the Pd(II) atom is bound to two nitrogen atoms of two units of 2 and two chlorido ligands. Bond lengths and angles around the palladium(II) are similar to those reported for related trans-[Pd(L)2Cl2] complexes (with L = Nsubstituted pyrazoles) [28e30].

The Pd(II) atom is located on an inversion centre and as a consequence of this: a) the two Cl- ligands are in a transarrangement and b) the "(CH2)2OMe" pendant arms are on opposite sides of the coordination plane. In addition, "PdCl2" moiety is nearly orthogonal to the heterocycle and the phenyl rings are not coplanar with the pyrazolyl ring (angles between their main planes = 64.4 and. 36.0°). As a consequence of these arrangements the distance between the Cl- ligands and one of the protons of the eCH2eO00 unit [2.787 Å] is smaller than the sum or the van derWaals radii of these atoms [31]; thus suggesting an intramolecular CeH/Cl interaction [32].

In the crystal the molecules are assembled by CeH/p interactions (Fig. 3) involving: the centroids of the phenyl rings (Cg1 and Cg2) {defined by the sets of atoms [C1eC6] and [C10eC15], respectively} and

329 one of the hydrogen atoms of the OMe unit (H18C) and the H5 atom, [distances Cg1/H18C = 3.440 Å
330 and Cg2/H5 = 3.477 Å].

331 The new products were also characterized in solution by UVe vis spectroscopy and NMR. The
332 absorption spectra of the new compounds were registered in CH2Cl2 at 298 K. The UVevis spectra of
333 the Pd(II) complexes showed a band in the range 320 nm < l3 < 360 nm {not present in the spectrum of
334 the free ligand (1)} with extinction coefficients between 2.0 x 103 and 4.0 x 103 M-1 cm-1. This band
335 that has also been observed for related Pd(II) complexes with pyrazole ligands [11] is assigned to a
336 metal-to-ligand charge transfer (MLCT) transition from the 4d orbitals of the Pd(II), to a p orbital of the
337 ligand. The remaining bands detected at lower wavelengths (220 nm < l < 370 nm) are attributed to
338 metal perturbed intraligand transitions (MPILT).

339 In the 1H NMR spectra of the Pd(II) complexes (2e7), the chemical shifts of the OMe protons were very
340 similar to that of the free ligand, thus suggesting that the oxygen of the methoxy group was not bound
341 to the Pd(II) centre. NMR spectra of 3, 4 and 7 showed the typical resonances due to the OAc- ligand
342 bound to the Pd(II) atom. The low-field region of these 1H NMR spectra was complex and the
343 assignment of the signals detected was achieved with the aid of 2D NMR experiments [NOESY, COSY,
344 {1He13C}-HSQC and HMBC].

345 For the palladacycles (4e7): a) the signal due to H2a proton was not observed in their 1H NMR spectra
346 and b) the resonances of the H3a and H4a nuclei, appeared at higher fields than for the free ligand (1).
347 Carbon-13 NMR and the 2D {1He13C}-HMBC spectra showed a downfield shift of the signal due to
348 the metallated carbon (C2a). All these findings are consistent with previous NMR studies of
349 cyclopalladated complexes [11a,14b,19b].

350 The 31P{1H}-NMR spectra of 6 and 7 showed a singlet at around 40 ppm. According to the bibliography
351 [11], this is indicative of a cis-arrangement of the PPh3 ligand and the metallated carbon, in good
352 agreement with the transphobia effect [33].

353

354 *Study of the antitumor activity of the free ligand and the complexes*

355 The cytotoxic activity of the new ligand (1) and the palladium(II) complexes (2, 4e7) against two human
356 breast cancer cell lines (MCF7 and MDA-MB231) was tested using cisplatin as positive control. The
357 effects of these products on the growth of two cell lines were assessed after 72 h and the results are
358 displayed in Fig. 4. The IC50 values corresponding to the inhibition of cancer cell growth at 50% level
359 are listed in Table 2. For comparison purposes the IC50 parameters for the free ligand Ic and the
360 palladacycle [Pd{k3,C,N,N0 [1-{Me2Ne(CH2)2e}-3,5-Ph2e(C3HN2)]2Cl] (V in Fig. 1) are also
361 included [11b] (Fig. 5).

362 The comparison of the in vitro cytotoxic activities of the free ligands (Ic and 1) against the MCF7 and
363 MDA-MB231 cell lines shows (Table 2 and Fig. 4) that the presence of the NMe2 group on the pendant
364 arm of the two 3,5-diphenyl pyrazole: [1-{R0e(CH2)2e }-3,5-Ph2e(C3HN2)] produces a greater
365 cytotoxic effect than the OMe. This could be related to several factors such as their different lipophilicity
366 and their proclivity to protonate in biological media [18,20].

367 Complex 2 did not show any significant effect on the MCF7 cell line. This result is similar to those
368 obtained for compound: [Pd {k2,N,N0[1-{Me2Ne(CH2)2e}-3,5-Ph2e(C3HN2)]Cl2] (IIc shown in Fig.
369 1). Except for 6, the remaining cyclopalladated complexes were more active in the MCF7 cell line than
370 the free ligand (1) and compound 2. Among all these palladacycles, the di-m-acetatobridged complex 4
371 was the most potent. Its effect was ca. 1.4 times greater than the palladacycle V (shown in Fig. 1) with
372 a (C,N,N0)-pincer ligand [11b]. Unfortunately, none of these products exhibited IC50 values smaller
373 than cisplatin.

374 More encouraging were the results obtained for MDAMB231 cell line. Compound 2 exhibited a
375 moderate activity and for the remaining products, the IC50 values [in the range: 9.1 mM (for 6)e14.4
376 mM (for 5)] were a bit smaller, if significant, than that of V [IC50 = 16.2 ± 4.6 mM] [11b] and closer to
377 that of cisplatin [IC50 = 6.5 ± 2.4 mM]. Thus, suggesting that the new Pd(II) compounds were more
378 effective in the triple negative MDA-MB231 cancer cell line than in the MCF7 line.

379

Conclusions

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The study of the reactivity of the novel N-substituted pyrazole derivative: [1-{MeOe(CH2)2e}-3,5-Ph2e(C3HN2)] (1) with Na2[PdCl4] or Pd(OAc)2 has allowed us to isolate and characterize a family of palladium(II) complexes (2e7). In two of them (2 and 3) the Pd(II) atom is bound to the heterocyclic nitrogen. The remaining four complexes (4e7) contain one (in 6 and 7) or two (in 4 and 5) five-membered palladacycles formed by the binding of the same nitrogen and the metallation of the phenyl ring on position 3 of the pyrazole. These findings indicate that ligand 1 may adopt two binding modes [(N) or (C,N)-] in front of the Pd(II) atom. However, no evidences of the formation of complexes containing 1 as a (C,N,O)- ligand were detected.

The comparison of the results obtained from in vitro studies of their cytotoxic activity against the MCF7 cell line and the MDAMB231 {triple negative (ER, PR and no HER2 over expression)} breast cancer cell line showed that the free ligand (1) was less active than its analogue Ic (Fig. 1) with the NMe2 unit at the end of the pendant arm. We have also proved that compounds (4e7) exhibit greater cytotoxic activity than the free ligand (1) and complex 2 (with two units of 1 acting as a monodentate N-donor ligand). The new cyclopalladated complexes (4e7) have an outstanding antiproliferative effect in the MDA-MB231 cell line. For all of them the IC50 values (Table 2) are a bit smaller than that reported for palladacycle V previously reported [11b]; but closer to that of cisplatin (under identical conditions). These results are especially outstanding because they open up new possibilities in the design of Pd(II)-pyrazole based complexes as promising alternatives to cisplatin and related Pt(II) drugs.

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402

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590 **Legends to figures**

591

592 **Figure 1.** General formulae of three N-substituted pyrazoles (I) and their Pd(II) and Pt(II) complexes
593 recently reported [11b]. {In compounds IeIII, the R group represents H (a), Me (b) or Ph (c)}.

594

595 **Scheme 1.** Key reagents and conditions: i) KOH, in DMSO, followed by treatment with 1-chloro 2-
596 methoxyethane, 298 K; ii) Na2[PdCl4] in MeOH at 298 K; iii) Pd(OAc)2 in refluxing toluene, (12 h),
597 followed by SiO2 column chromatography; iv) LiCl in acetone, at 298 K; v) PPh3 in CH2Cl2 at 298 K.

598

599 **Figure 2.** ORTEP plot of the molecular structure of trans-[Pd{[1-{MeOe(CH2)2e}-3,5-
600 Ph2e(C3HN2)]2Cl2] (2). Selected bond lengths (in Å) and angles (in deg.): Pd1eN1, 2.020(19);
601 Pd1eCl1, 2.3044(5), N1eC9, 1.339(2); N1eN2, 1.362(2); N2eC7, 1.535(4); N2eC16, 1.460(3); C9eC10,
602 1.472(3); C16eC17, 1.500(3); C17eO1, 1.404(3); O1eC18, 1.387(4); N1ePdeCl, 87.17(5); N1eN2eC16,
603 120.98(16); N1eN2eC7, 110.24(16); N2e C7eC6,124.11(19); N2eC7eC8, 107.02(17); C7eC8eC9,
604 106.76(18) and N1eC9eC10, 124.11(17).

605

606 **Figure 3.** Schematic view of assembly of vicinal molecules of trans-[Pd{[1-{MeO-(CH2)2e }-3,5-
607 Ph2e(C3HN2)]2Cl2] (2) by C-H···π interactions. Red balls represent the centroids of the rings defined
608 by the sets of atoms [C1eC6] and [C10eC15] (Cg1 and Cg2, respectively). (For interpretation of the
609 references to colour in this figure legend, the reader is referred to the web version of this article.)

610

611 **Figure 4.** Inhibition of cell growth proliferation in MCF7 and MDA-MB231 human breast cancer cell
612 lines, after 72 h of exposure to the free ligand 1 and compounds 2, 4e7.

613

614 **Figure 5.** Comparative plot of the IC50 values (in mM) of the free ligands [1-{R'-(CH2)2e]-3,5-
615 Ph2e(C3HN2)] {R'= NMe2 (Ic) or MeO (1)}, the Pd(II) complex trans-[Pd{[1-{MeOe(CH2)2e}-3,5-
616 Ph2e(C3HN2)]2Cl2] (2), the cyclopalladated compounds (4e7 and V) and cisplatin in front of the MCF7
617 and MDA-MB231 cancer cell lines.

618

619

620 **Table 1** Crystal data and structure refinement for trans-[Pd{[1-{MeOe(CH2)2e]-3,5-Ph2e
621 (C3HN2)}2Cl2] (2).

Crystal dimensions/mm × mm × mm	0.2 × 0.08 × 0.08
Empirical formula	$C_{36}H_{36}Cl_2N_4O_2Pd$
Formula weight	733.99
T/K	293(2)
λ/Å	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
a/Å	9.1567(5)
b/Å	9.5825(5)
c/Å	10.6315(5)
α/°	108.171(2)
β/°	104.895(2)
γ/°	95.986(2)
Volume/Å^3	839.09(7)
Z	1
Calculated density/Mg × m^{-3}	1.453
μ/mm^{-1}	0.750
F(000)	376
Θ range for data collection/°	2.12 to 28.34
Limiting indices	$-12 \le h \le 8$, $-12 \le k \le 12$, $-13 \le l \le 14$
Reflections collected/unique	11383/4182 [R(int) = 0.0243]
Completeness to Θ = 28.34°	99.7%
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.7457 and 0.6541
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4182/0/205
Goodness-of-fit on F^2	1.055
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0310, wR_2 = 0.0763
R indices (all data)	R_1 = 0.0349, wR_2 = 0.0803
Largest diff. peak and hole/e Å^{-3}	0.562 and −0.269

622

623

624 **Table 2** Cytotoxic activities (IC50 values) on MCF7 and MDA-MB231 cancer cell lines for ligands 1-
625 [R0e(CH2)2e]-3,5-Ph2e(C3HN2) with R0 = NMe2 (Ic) or OMe (1), the palladium(II) complex (2), the
626 cyclometallated products (4e7 and V) and cisplatin under identical experimental conditions are also
627 included.

628

IC_{50} (μM) for the cell lines[a]					
Compound	R	Mode of binding	MCF7	MDA-MB231	Ref.
Free ligands					
Ic	NMe_2	–	52 ± 10	64 ± 24	[11b]
1	OMe	–	100	100	This work
Palladium(II) complexes					
2	OMe	(N)	>100	75 ± nd	This work
4	OMe	$(C,N)^-$	28 ± 5	9.5 ± 2.1	This work
5	OMe	$(C,N)^-$	44 ± nd	14.4 ± 4	This work
6	OMe	$(C,N)^-$	>100	9.1 ± nd	This work
7	OMe	$(C,N)^-$	67 ± nd	13 ± nd	This work
V	NMe_2	$(C,N,N')^-$	38.4 ± 16.5	16.2 ± 4.6	[11b]
Cisplatin	–	(N)	19 ± 4.5	6.5 ± 2.4	This work

[a] Data are shown as the mean values obtained of two or more experiments performed in triplicate with the corresponding standard deviation.

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632 **Figure 1**

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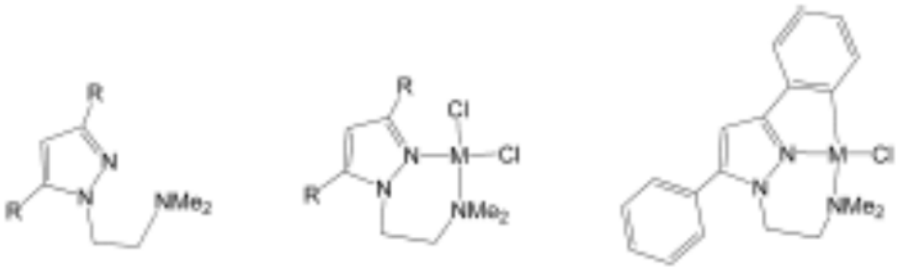

635 I M = Pd (**II**) or Pt (**III**) M = Pt (**IV**) or Pd (**V**)

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641 **Scheme 1.**

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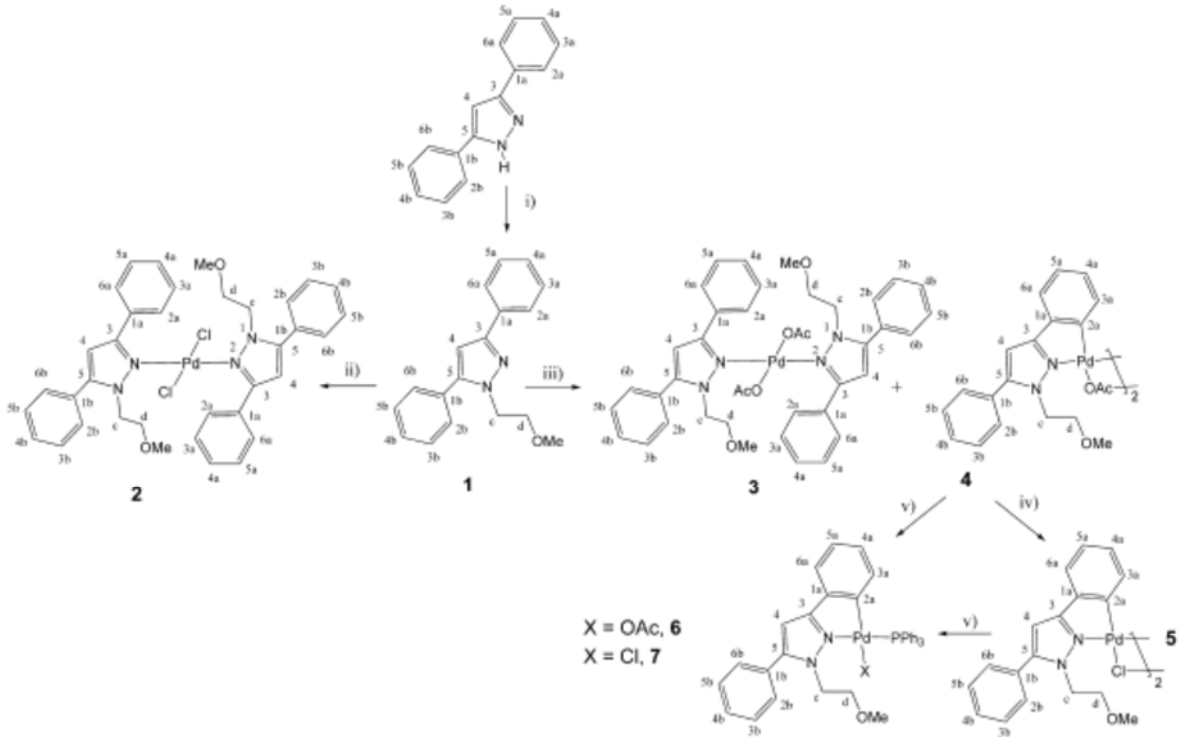

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646 **Figure 2**

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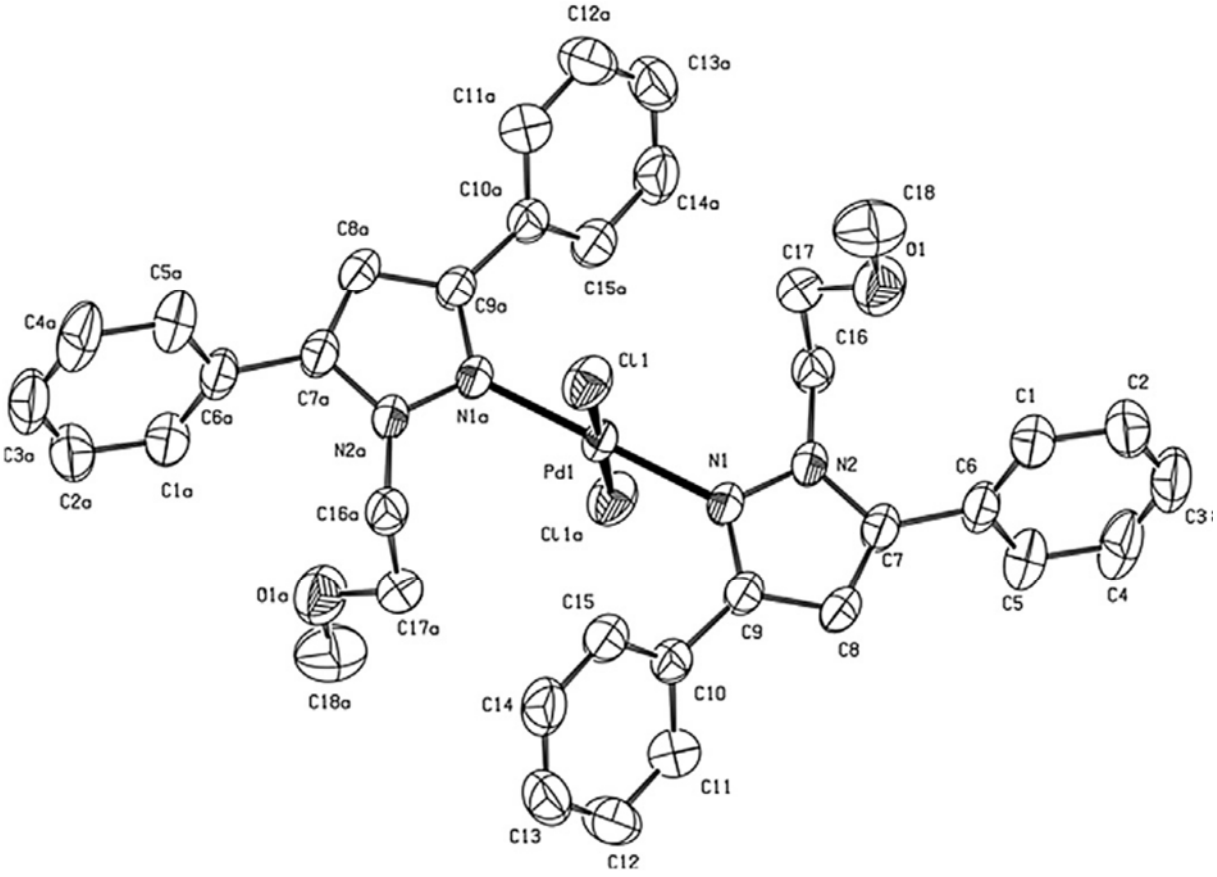

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654 **Figure 3**

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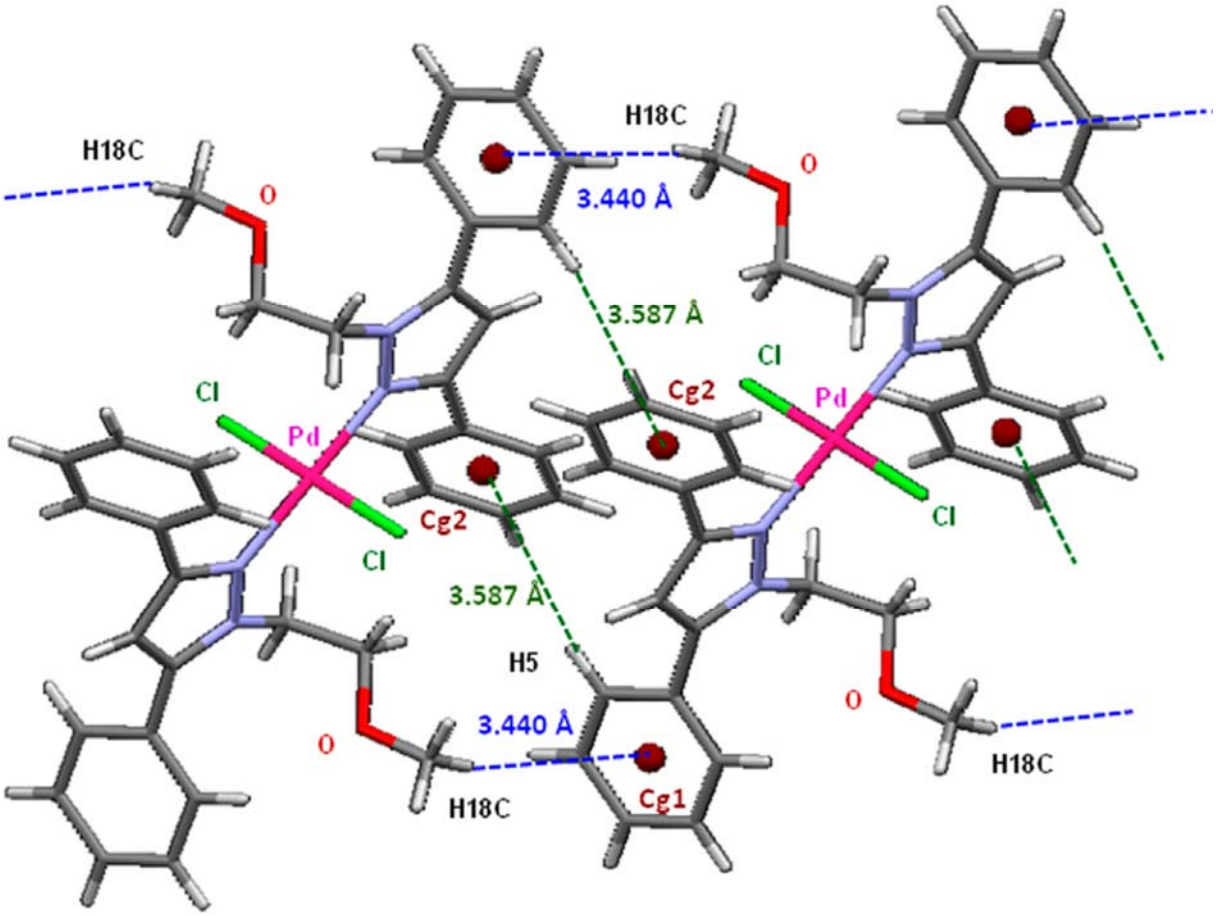

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661 **Figure 4**

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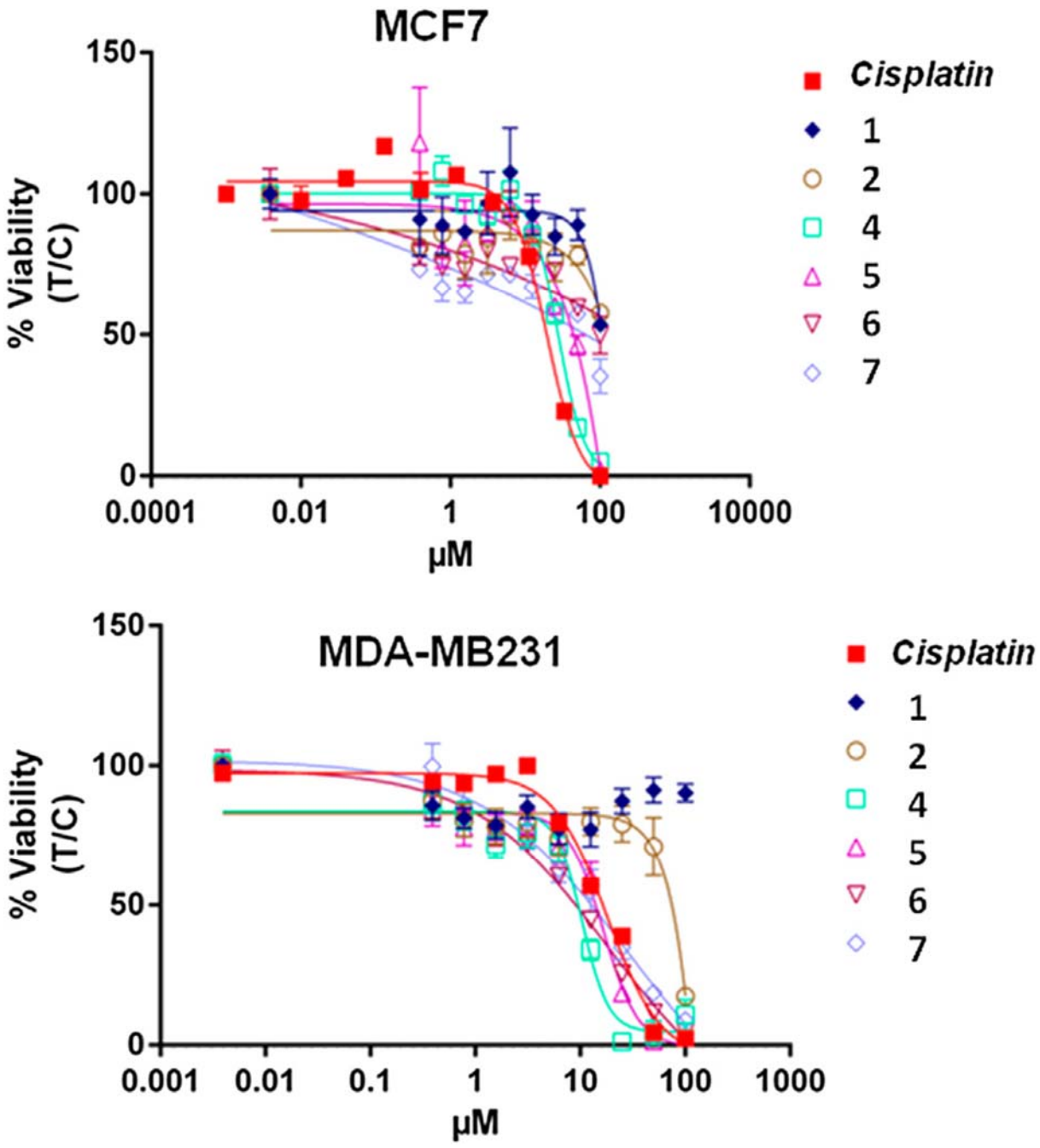

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668 **Figure 5**

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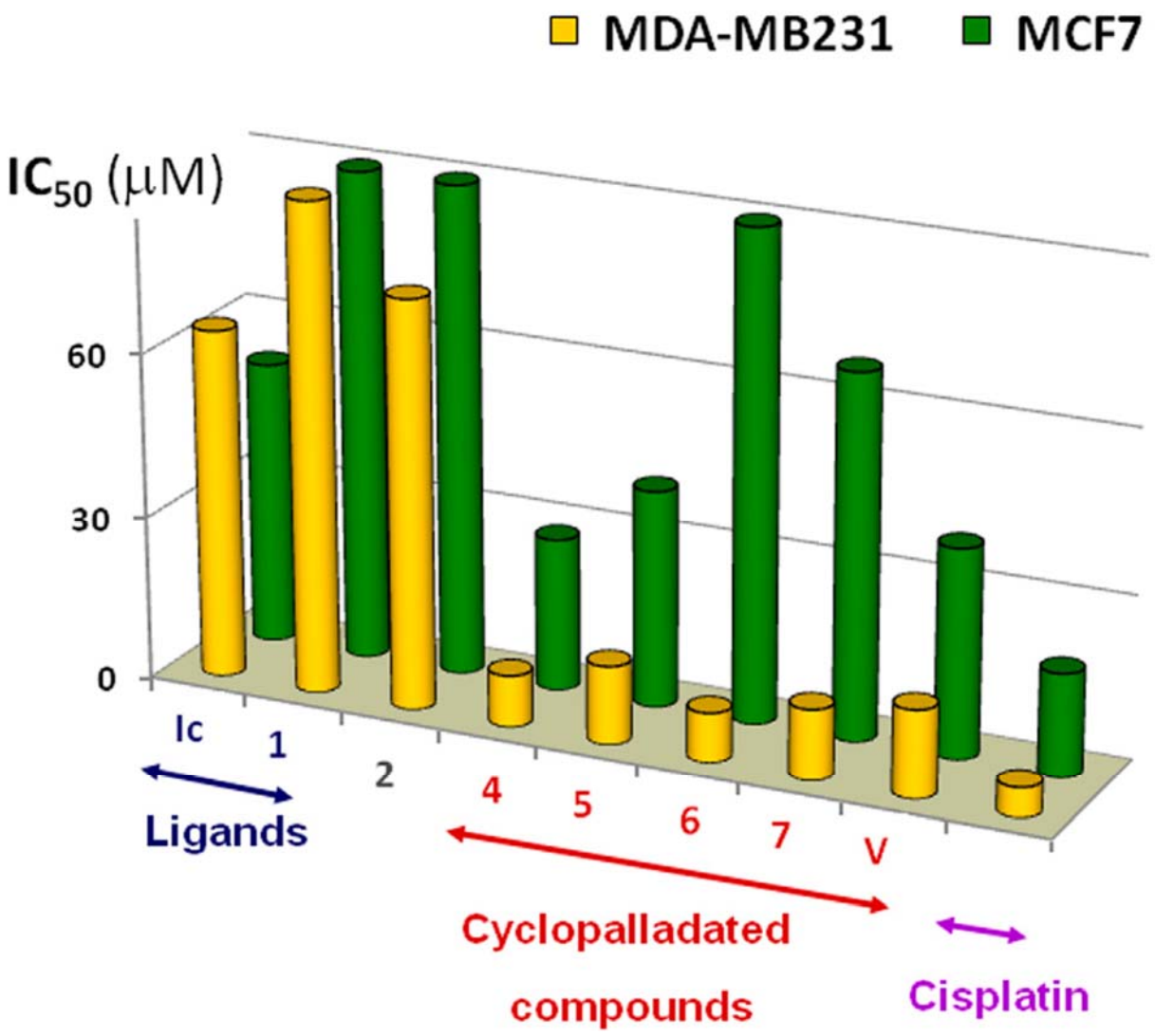

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