Heterodi- (Fe, Pd/Pt) and Heterotrimetallic (Fe2, Pd) Complexes Derived from 4-(Ferrocenylmethyl)-N-(2-methoxyethyl)-3,5-diphenylpyrazole as Potential Antitumoral Agents

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ABSTRACT:

The study of the reactivity of the pyrazole derivative 1-[MeO-(CH2)2]-3,5-Ph2-4-(CH2Fc)–(C3N2) (1, Fc = ferrocenyl) with Na2[PdCl4], Pd(OAc)2, and [MCl2(dmso)2] (M = Pd or Pt, dmso = dimethyl sulfoxide) has allowed us to isolate trans-[Pd{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}2Cl2] (2), [Pd{κ2-C,N(1-{MeO(CH2)2}-3-{C6H4}-5-Ph-{C3N2})}{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl] (3), [Pd{κ2-C,N(1-{MeO(CH2)2}-3-{C6H4}-4-{CH2Fc}-5-Ph-{C3N2})}Cl-(PPh3)] (4), and the trans (5) and cis (6) isomers of [Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2(dmso)]. Compound 1 acts as a N (in 2, 5, and 6) or (C,N)– donor ligand (in 4) and shows both binding modes in 3. The cytotoxic assessment of 1–6 against MCF7, MDA-MB231 (breast), and HCT-116 (colon) cancer cell lines reveal that (1) 1 is more potent than 1-[MeO(CH2)2]-3,5-Ph2-(C3HN2) (V), (2) 2–6 have cytotoxic activity, (3) 2 and 3 are less active than 4–6, and (4) 6 is the most potent compound against the three cancer cell lines.

INTRODUCTION

Pyrazole derivatives are valuable cores for the design and synthesis of compounds with interesting applications in different fields, such as catalysis, agrochemistry, supra- and macromolecular chemistry, as well as biomedicine.[1] Compounds of this type with outstanding anticancer, antiviral, antimicrobial, antiglycemic, or even antiallergenic activities have been described.[2–5] Moreover, pyrazoles are useful ligands in coordination and organometallic chemistry.[6] The nature and position of the substituents on the ring, the metal ion (its oxidation number and environment), and even the ancillary ligands (donor atoms, electron-donor ability, bulk, and relative orientation in the complex) modify the properties and activities of the complexes.[7] Among the wide variety of transition metal complexes derived from pyrazole, those containing PdII or PtII ions are probably the most attractive mainly owing to their potential biological activity.[8,9] Compounds of this kind with greater antitumor activity and lower toxicity than cis-[PtCl2(NH3)2] (cisplatin) or antibacterial activity have been reported.[8,9]

On the other hand, bioorganometallic chemistry is a fast developing area of increasing interest, and the idea of using organometallic complexes in drug discovery is becoming more and more popular.[10–12] Antimalarials, antibacterials, and neuroprotectors based on organometallic compounds have been described.[10,11] In this context, ferrocene derivatives are probably among those with the best prospects.[13–15] One of the most promising strategies used to achieve new therapeutic agents consists of the incorporation of a ferrocenyl (Fc) unit on the scaffold of a biologically active molecule.[10–15] Commonly, this change increases the biological activity of the molecule and widens its range of application. Owing to the increasing interest in pyrazole derivatives and new ferrocene-based drugs or prodrugs, several groups have centered their attention on the design and synthesis of mixed ferrocenyl–pyrazole derivatives.[16] Unfortunately, studies on their coordination ability to PdII or Previous studies on the cytotoxic activities of pyrazoles 1-R1-3,5-(R2)2–(C3HN2) (types IV and V in Figure 1) and their PdII or PtII complexes against MCF7 and MDAMB231 breast cancer cell lines have shown that compounds with R2 = H or Me are less active than those with R2 = Ph.[9b] Moreover, the replacement of the Me2N(CH2)2 [9b] group by a MeO(CH2)2 unit increases their cytotoxic activity.[9a] In view of these findings and the increasing interest in new ferrocene derivatives with bioactive moieties, now we present the hybrid Fc/pyrazole 1-[MeO(CH2)2]-3,5-Ph2-4-(CH2Fc)–(C3N2) (1, Figure 1) and report its utility as a ligand for the synthesis of heterodi- or heterotrimetallic complexes containing FeII and PtII or PdII ions and a study of the antitumoral activity of the compounds against breast (MDA-MB231 and MCF7) and colon (HCT-116) cancer cell lines.

RESULTS AND DISCUSSION

Synthesis and Characterization of Ligand 1 Ligand 1 was prepared in good yield (80%) by the alkylation of 3,5-Ph2-4-(CH2Fc)–(C3HN2) (IIa)[18] with 2-chloroethyl methyl ether (Scheme 1) by using the same procedure as that described for indigo[19] or the N-methylated derivative 1-Me-3,5-Ph2-4-(CH2Fc)–(C3N2).[18]

Compound 1 is a stable yellow solid at 298 K and exhibits high solubility in CH2Cl2, CHCl3, and acetone. The characterization data (Supporting Information) agreed with the proposed formula, and NMR spectroscopy studies confirmed the presence of the MeO(CH2)2 unit attached to the nitrogen atom of the pyrazole ring.

Palladium(II) Complexes

In a first attempt to evaluate the potential coordination abilities of 1 to PdII ions, its reactivity with Na2[PdCl4], [PdCl2(dmso)2] (dmso = dimethyl sulfoxide), and Pd(OAc)2 was studied under different experimental conditions (Table 1, Entries 1–3; Scheme 2).

The treatment of 1 with Na2[PdCl4] (molar ratios 1:1 or 1:2) in MeOH at 298 K gave a yellow solid (Table 1, Entry 1; Scheme 2, Step A). Its characterization data (Supporting Information) agreed with those expected for the heterotrimetallic product 2, and its crystal structure confirmed the presence of [Pd{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}2Cl2] (Figure 2).

The PdII ion is located on an inversion center and, consequently, the relative disposition of the two identical ligands is trans. The Pd–Cl bond length [2.3013(17) Å] is similar to those reported for most trans-[Pd(κ-N-1-R1-3,5-Ph2–pyrazole) 2Cl2] complexes[9a,20,21] and its analogue Va [Figure 1; Pd1–Cl1 2.3044(5) Å].[9a] The Pd–N1 bond length [2.007(4) Å] is slightly shorter than that in Va [Pd1–N1 2.020(19) Å] but only by an insignificant amount.[9a] The Pd1···H5 separation of 2.9 Å suggests a weak agostic interaction.[22] The pyrazolyl rings are planar and nearly orthogonal to the "PdCl2" unit. The phenyl rings form angles of 53.3 and 58.9° with the heterocycle. In the crystal, the molecules are assembled through C–H···π intermolecular contacts[23] involving the H24 atom and the C5H5 ring (the separation between H24 and the centroid of the C1–C5 ring is 2.604 Å).

Compound 2 was also isolated from the reaction of ligand 1 and [PdCl2(dmso)2] (in molar ratios of 1:1 or 2:1) in MeOH under reflux (Table 1, Entry 2; Scheme 2, Step A). It should be noted that no evidence of the formation of PdII complexes with a Pd–OMe bond or arising from the cyclometalation of any of the rings was detected when Na2[PdCl4] or [PdCl2(dmso)2] was used (Table 1, Entries 1 and 2).

As (1) cyclopalladated complexes are attracting great interest in a wide variety of areas,[24,25] especially in view of their potential biological activity as cytotoxic, antimalarial, and antioxidants agents,[25a,26,27] and (2) Pd(OAc)2 is a more potent metallating agent than Na2[PdCl4] or [PdCl2(dmso)2],[17,26,29] we also studied the reactivity of 1 with Pd(OAc)2. When equimolar amounts of Pd(OAc)2 and 1 were heated to reflux in toluene for 24 h, the formation of Pd0 was detected on the walls of the flask. Subsequent filtration though a Celite pad followed by column chromatography gave a brownish solid (3; Table 1, Entry 3; Scheme 2, Step B).

The IR spectrum of 3 exhibited the typical bands for a monodentate OAc– ligand.[28] The 1H NMR spectrum of 3 showed two sets of superimposed signals with relative intensities 1:1 and, thus, suggested the presence of two different and inequivalent units of ligand 1. Moreover, in one of the sets of resonances, the signals for 3a-H and 4a-H of the phenyl ring at the 3-position of the pyrazole ring appeared high-field shifted in relation to the signals of the free ligand and to those of 2. This trend, also

observed for the cyclopalladated compounds derived from V,[9a] is typical of palladacycles containing bidentate [C(sp2, phenyl), N]– ligands.[30,31] These findings suggested one of the units of 1 adopted this binding mode in 3, whereas the other acted as a N donor.

To confirm this hypothesis, further reactivity studies were performed. The treatment of 3 with LiCl first and then an equimolar amount of PPh3 gave a deep brown solid after concentration of the reaction solution (Table 1, Entry 4; Scheme 2, Step C). The TLC and 1H NMR spectrum of the raw material revealed the presence of the free ligand (1) and a new PdII complex (4). The two products (1 and 4) were separated in a molar ratio of ca. 1:1 by SiO2 column chromatography. The elemental analysis and mass spectrum of 4 (Supporting Information) agreed with those expected for [Pd{κ2-C,N-(1-{MeO(CH2)2}-3-{C6H4}-4-{CH2Fc}-5-Ph-{C3N2})}Cl(PPh3)] (Scheme 2, Step C), in which the PPh3 ligand is in a cis arrangement to the metallated carbon atom.[32] The position of the singlet in the 31P{1H} NMR spectrum of 4 (δ = 43.6 ppm) is very similar to that of [Pd{κ2-C,N-(1-{MeO(CH2)2}-3-{C6H4}-5-Ph-{C3HN2})}-Cl(PPh3)][9a] and falls in the range reported for other palladacycles with "Pd{C(sp2 phenyl),N}Cl(PPh3)" cores.[17,18,26,29]

Platinum(II) Complexes

To compare the effect produced by the binding of the MII atom to ligand 1, we also studied the reactivity of 1 with cis-[PtCl2(dmso)2][33] under different experimental conditions (Table 1, Entries 5 and 6). The treatment of these two reagents (in molar ratios 1/PtII = 1 or 2) in MeOH under reflux for 1 h followed by the partial evaporation of the solvent gave a yellow solid (Table 1, Entry 5; Scheme 2, Step D), which was identified as trans-[Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2(dmso)] (5). These results are different from those obtained with [PdCl2(dmso)2] (Table 1, Entry 2), which gave the heterotrimetallic complex 2.

In view of the increasing interest in the cytotoxic activities of the trans and cis isomers of PtII complexes containing mono- (L) or bidentate (L,L) N-donor ligands,[34,35] we also attempted the preparation of the cis isomer of 5. Several strategies were used to achieve this aim. In a first attempt, the reaction period (t) was increased gradually from 1 to 72 h. Under these experimental conditions, two products were isolated by SiO2 column chromatography. The major component was the trans isomer (5); the characterization data of the minor product (6) agreed with those expected for cis-[Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2(dmso)] (Scheme 1, Step E), and its crystal structure (see below) confirmed this result. These two isomers were isolated in a 5/6 molar ratio of 1.0:0.65, but larger reaction periods did not produce significant variations in their relative abundance (for t = 72 h, the 5/6 molar ratio was 1.0:0.70).

When the reaction was performed in the presence of NaOAc (molar ratio OAc–/PtII = 2) and in a toluene/MeOH mixture (5:1) under reflux for 24 h, the 5/6 molar ratio decreased (Table 1, Entry 6), and the cis isomer (6) became the major product.

Isomers 5 and 6 were separated by SiO2 column chromatography and characterized by elemental analysis, mass spectrometry, IR spectroscopy, and NMR spectroscopy (Supporting Information). Their 1H NMR spectra showed (1) one (for 5) or two singlets (for 6) of identical intensity for the protons of the dmso ligand and (2) two well-resolved triplets (for 5) or four multiplets (for 6) for the –(CH2)2– protons of the pendant arm. The 195Pt{1H} NMR spectra showed a singlet in both cases, and the chemical shifts were consistent with those of PtII compounds with a "N,Cl2,S(dmso)" set of donor atoms. The magnitude of the shift [Δδ = δ(6) – δ(5) = 123 ppm] is similar to those reported for related cis and trans isomers of PtII compounds with "Pt(N-donor ligand)Cl2(dmso)" cores.[17,27a,36]

The slow evaporation of a CH2Cl2 solution of 6 layered with MeOH at 298 K produced crystals suitable for X-ray diffraction. The crystal structure confirmed the existence of [Pt{κ-N-(1-{MeO(CH2)2}-3,5-

Ph2-4-{CH2Fc}–{C3N2})}Cl2-(dmso)] (6), CH2Cl2, and MeOH molecules in a 1:1:1 ratio. In the heterodimetallic molecules of 6 (Figure 3), the PtII atom is bound to nitrogen atom N1 of the pyrazolyl unit, the sulfur atom S1 of the dmso ligand, and two Cl– ligands (Cl1 and Cl2) in a cis arrangement [Cl1–Pt–Cl2 bond angle: 89.86(5)°]. The bond lengths and angles around the PtII center are similar to those of cis-[Pt{κ-N-1-(CH2Fc)-3,5-Ph2–(C3HN2)}Cl2(dmso)], which contains pyrazole I as a N-donor ligand.[17a]

The three rings of the "3,5-Ph2-C3N2" backbone are planar. The main plane of the heterocycle forms an angle of ca. 74.01° with the coordination plane of the PtII ion, and the phenyl rings (C15–C20 and C21–C26) are not coplanar with the pyrazole ring (the angles between the main planes are 66.9 and 63.6°). The orientation of these two phenyl rings allows intramolecular C–H···π interactions between (1) the C9–H9 bond of the C5H4 ring and the C21–C26 phenyl ring and (2) the other phenyl ring and the hydrogen atom (H30) of the dmso ligand.

In the crystal, the separation between the centroids of the cyclopentadienyl (Cp) ring of a molecule at (x, y, z) and the C5H4 ring of a proximal unit is 3.295 Å, which indicate the existence of π–π stacking. Moreover, two of the hydrogen atoms of the OMe unit (H29A and H29B) are involved in intermolecular C–H···π interactions with the substituted ring of the Fc group and the pyrazolyl unit, respectively, of another molecule at (1/2 – x, 1/2 – y, 1 – z).

Study of Cytotoxic Activities

In a first stage, two human breast cancer cell lines (MCF7 and MDA-MB231) were used to test the cytotoxic activity of ligand 1 and complexes 2–6. Cisplatin (as a positive control) was also evaluated under the same experimental conditions. A summary of the results obtained for the inhibition concentrations (IC50 in μm) of ligand 1, pyrazoles IV and V (Figure 1), and complexes 2–6 is presented in Table 2. For comparison, the data for related PdII complexes derived from V are also included.

Ligand 1 exhibited outstanding activity (IC50 ≈ 5.8 μm, greater than that of cisplatin) against these two breast cancer cell lines. A comparison of the data presented in Table 2 for 1 and IV allows us to conclude that the replacement of the hydrogen atom at the 4-position of the pyrazole ring (in IV) by the –CH2Fc unit to give 1 enhances the cytotoxic activity against these two (MCF7 and MDA-MB231) cell lines. Moreover, as shown in Figure 4, the framework and substitution pattern of the pyrazole has a great influence on its cytotoxic activity. For the studied pyrazoles 1, IV, and V, the cytotoxicity increases in the order V ≪ IV ≪ cisplatin ≈ 1 (against MCF7 and MDA-MB231 cell lines)

To evaluate the effect produced by the binding of the PdII or PtII atoms to the ligand, we also investigated the cytotoxic activity of the new complexes against these cancer cell lines. The new PdII complexes 2–4 exhibit cytotoxic activity (Figure 5 and Table 2) but are less active than the free ligand 1. However, in general, they are more potent than their analogues derived from V. This suggests once more that the –CH2Fc group at the 4-position of the pyrazole ring increases their activity.

Better results were obtained for the trans (5) and cis (6) isomers of: [Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2(dmso)]. These compounds exhibited outstanding activity (IC50 ≈ 8.3 μm) against the MCF7 and in the MDA-MB231 breast cancer cell lines. The cytotoxic activities increase according to sequences:

3<2<4<cisplatin<5<6 (for MCF7 cell line)

3<2<4<5<cisplatin<6 (for MDA-MB231 cell line)

These trends are practically identical except for the position of cisplatin and reveal that the heterotrimetallic compounds 2 and 3 are less potent than the heterodimetallic derivatives 4–6.

In view of the outstanding results obtained for some of the new products against the two breast cancer cell lines, we decided to study their effect on the cisplatin-resistant HCT-116 colon cell line. A comparison of the data (Table 2 and Figure 5) shows that their cytotoxic activity against this cell line increases as follows:

$3 \leq 4 < 2 < 5 < \text{cisplatin} < 1 < 6$

Among the new products prepared in this work, complex 6 and the free ligand 1 are the most active ones. Their potency against the HCT-116 cell line is greater (10 and 14 times, respectively) than that of cisplatin under identical conditions.

CONCLUSIONS

The new hybrid ferrocenyl–pyrazole derivative 1-[MeO-(CH2)2]-3,5-Ph2-4-(CH2Fc)–(C3N2) (1) has been prepared. The study of its reactivity with different PdII and PtII salts and complexes allowed us to isolate and characterize three PdII compounds 2–4 and the trans (5) and cis (6) isomers of [Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2(dmso)]. We have also proved that 1 is a versatile and valuable ligand. It may (1) adopt two different binding modes {N donor (in 2, 5, and 6), [C(sp2, phenyl), N]– (in 3), or even both simultaneously in the same complex (4)} and (2) produce heterotri- (2 and 3) or heterodimetallic complexes 4–6.

The new products 1–6 exhibit growth inhibitory activity against breast (MDA-MB231 and MCF7) and colon (HCT- 116) human cancer cell lines. We have also demonstrated that the presence of the CH2Fc moiety at the 4-position of the pyrazole ring plays a key role in determining the activity of the products. Against the three cell lines, the PdII complexes 2 and 3 are less potent than the PtII derivatives 5 and 6 and the free ligand 1. Complex 6 is clearly more active than its trans isomer (5) against the three cell lines assayed.

The free ligand and the cis isomer of [Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-{C3N2}-4-{CH2Fc}–{C3N2})}Cl2-(dmso)] (6) are the most potent against the three cell lines. Their activities against the cisplatin-resistant colon cell line (HCT-116) are especially relevant [ca. 10 (for 1) and 14 (for 6) times bigger than that of cisplatin].

Although 6 has a slightly greater inhibitory effect than 1 against the cell lines MDA-MB231 and HCT116, the new ligand is particularly attractive because it does not contain PtII and consequently might not produce the typical and undesirable side effects of conventional PtII-based drugs. Thus, among the new products presented here, compounds 1 and 6 appear to be excellent candidates for further studies mainly centered on (1) the study of their cytotoxic activities against other cancer cell lines (i.e., lung, ovarian, etc.), (2) the investigation of their effect on normal nontumor cells (i.e., fibroblasts), (3) additional studies (cell cycle arrest, induction of apoptosis, etc.) to gain further insights into their mechanism of action, and (4) additional studies on their potential utility in combined therapies. Preliminary studies in these fields are underway.

EXPERIMENTAL SECTION

Materials and Methods: 3,5-Ph2-4-(CH2Fc)–(C3HN2), Na2[PdCl4], and complexes [MCl2(dmso)2] (M = Pd or Pt) were prepared as described previously,[17,31,36–38] and the remaining reagents were obtained from commercial sources and used as received. The success of the synthesis of the PtII complexes (5 and 6) is strongly dependent on the quality of the methanol; the presence of water results in the formation of metallic platinum, other undesirable minor byproducts, and a significant decrease in the yield. Thus, the use of high quality MeOH (HPLC grade) is required. The remaining solvents used were dried and distilled before use.[39] Elemental analyses were performed at the Serveis de Cientifico-Tècnics (Universitat de Barcelona). Mass spectrometry (ESI+) was performed at the Servei d'Espectrometria de Masses (Universitat de Barcelona). The infrared spectra in the range ν˜ = 4000–400 cm–1 were obtained with a Nicolet 400 FTIR instrument with samples as KBr pellets, and far-IR spectra were recorded with a Bomen-DA3 instrument by using polyethylene discs. Thin layer chromatography (TLC) was performed with SiO2 plates (Merck silica gel 60 F254). The UV/Vis spectra of solutions of the compounds in CH2Cl2 were recorded at 298 K with a Cary 100 scan Varian UV spectrometer. The routine 1H NMR spectra were recorded with a Mercury 400 MHz instrument at the Indian Institute of Chemical Biology of Kolkata and at the Serveis Cientifico-Tècnics (Universitat de Barcelona). The high-resolution 1H NMR spectra of the compounds dissolved in CDCl3 (99.9%) containing SiMe4 as an internal reference were obtained with a Varian VRX-500 instrument or a Bruker Avance DMX 500 MHz instrument at 298 K. The 31P{1H} (of 4) and 195Pt{1H} NMR spectra (of CDCl3 solutions of 5 and 6) were recorded with a Varian 300 MHz instrument with P(OMe)3 [δ(31P) = 140.17 ppm] and H2[PtCl6] [δ(195Pt) = 0.0 ppm], respectively, as references. The coupling constants (J) are given in Hz, and chemical shifts (δ) are given in ppm.

1-[MeO(CH2)2]-3,5-Ph2-4-(CH2Fc)–(C3N2) (1): To a solution con taining 3,5-Ph2-4-(CH2Fc)–(C3HN2) (IIa, 1.0 g, 2.39 10–3 mol)[17a] and toluene (30 mL), benzyltriethylammonium chloride (BTAC; 136 mg, 6.0 10–4 mol) and an aqueous NaOH (40%) solution (7 mL) were added. Then, a threefold excess of ClCH2CH2OMe (0.66 mL, 7.2 10–3 mol) was added dropwise to the mixture with stirring. Once this process had finished, the resulting mixture was kept at 370 K for 24 h. After this period, H2O (50 mL) was added, and then the mixture was extracted with toluene (2 25 mL). The organic layers were combined, dried with Na2SO4, and then filtered. The resulting filtrate was concentrated to dryness with a rotary evaporator. The residue was then dissolved in the minimum amount of CH2Cl2 (5 mL) and passed through a short SiO2 column (5.0 cm 2.0 cm). Elution with CH2Cl2 produced a deep yellow band, which was collected and concentrated to dryness with a rotary evaporator to give 1. The solid formed was then dried in vacuo for 2 d (yield: 911 mg, 80%).

trans-[Pd{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}2Cl2] (2): This product can be obtained with Na2[PdCl4] or cis-[PdCl2- (dmso)2] (methods a and b, respectively).

Method a: Ligand 1 (146 mg, 3.1 10–4 mol) was introduced to an Erlenmeyer flask, and then a solution containing Na2[PdCl4] (45 mg, 1.5 10–4 mol) and MeOH (5 mL) was added. The resulting mixture was protected from the light with aluminium foil and stirred at 298 K for 1 h. The yellow solid formed was collected, washed with methanol (2 2 mL portions), air-dried, and then dried in vacuo for 3 d (yield: 121 g, 70 %).

Method b: [PdCl2(dmso)2] (50 mg, 1.5 10–4 mol) was suspended in methanol (30 mL), and the suspension was heated under reflux until the solid dissolved completely. Then, the hot solution was filtered, and the filtrate was poured into an Erlenmeyer flask containing ligand 1 (143 mg, 3.0 10–4 mol). The container was protected from the light with aluminium foil, and the reaction mixture was heated under reflux for 2 h. The solid formed was collected, washed (as in method a), air-dried, and then dried in vacuo for 2 d (yield: 137 mg, 80 %).

[Pd{κ2-C,N-(1-{MeO(CH2)2}-3-{C6H4}-4-{CH2Fc}-5-Ph–{C3N2})}-(κ-N-1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})(OAc)] (3): A mixture of 1 (238 mg, 5 10–3 mol) , Pd(OAc)2 (113 mg, 5 10–3 mol), and toluene (20 mL) was protected from the light with aluminium foil and heated under reflux for 24 h. After this period, the hot mixture was carefully filtered through a Celite pad, and the filtrate was cooled and kept aside. The Celite was then washed with small portions of CHCl3 until colorless mother liquors were obtained. These washings were combined with the filtrate and concentrated to dryness with a rotary evaporator. The residue was then treated with the minimum amount of CHCl3 (ca. 5 mL) and passed through a SiO2 chromatographic column (120 mm 18 mm). Elution with CHCl3 produced a wide yellow band that gave small amounts of 1 (ca. 15 mg) after concentration. Once this band was collected, a CHCl3/MeOH mixture (100:1) produced the release of a brownish band, which gave 3 after concentration by rotary evaporation. The solid formed was first air-dried and dried in vacuo for 3 d (yield: 208 mg, 74%).

[Pd{κ2-C,N-(1-{MeO(CH2)2}-3-{C6H4}-4-{CH2Fc}-5-Ph–{C3N2})}Cl(PPh3)] (4): Compound 3 (115 mg, 1 10–4 mol) was dissolved in acetone (5 mL). Then, an excess of LiCl (6.5 mg, 1.5 10–4 mol) was added. The reaction mixture was protected from the light, stirred at 298 K for 1 h, and then filtered. The filtrate was concentrated to dryness with a rotary evaporator, the residue was stored in a SiO2 desiccator overnight. The residue was then dissolved in the minimum amount of CH2Cl2 (ca. 15 mL) and treated with PPh3 (27 mg, 1.0 10–4 mol); the mixture was stirred at 298 K for 1 h and then filtered. The deep yellow solution was concentrated to ca. 5 mL with a rotary evaporator. After cooling to room temperature, the solution was purified by SiO2 column chromatography (3.0 1.2 cm2). Elution with CH2Cl2 produced a pale yellow band, which gave the free ligand 1 (41 mg) after concentration to dryness. Once this band was collected, a CH2Cl2/MeOH mixture (100:0.5) was used as the eluent. This produced an additional band, which was collected and concentrated with a rotary evaporator to give 4 as a brownish solid. This product was collected, air-dried, and finally dried in vacuo for 2 d (yield: 79 mg, 90 %). trans-[Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}-Cl2(dmso)] (5): cis-[PtCl2(dmso)2] (225 mg, 5.3 10–4 mol) was treated with methanol (20 mL). The resulting suspension was protected from the light with aluminium foil, and the suspension was heated under reflux until the solid dissolved completely. Then, the hot solution was carefully filtered, and the filtrate was poured into an Erlenmeyer flask containing ligand 1 (269 mg, 5.3 10–4 mol). The resulting mixture was heated under reflux for 1 h. After this period, the bright yellow solution was concentrated with a rotary evaporator until a solid was obtained. The flask was cooled to ca. 298 K, and the solid that formed was collected by filtration and dried in vacuo for 2 d (yield: 0.233 mg, 76%).

cis-[Pt{κ-N-(1{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}-Cl2(dmso)] (6)·CH2Cl2·MeOH: Ligand 1 (254 mg, 5.3 10–4 mol) and cis-[PtCl2(dmso)2] (225 mg, 5.3 10–4 mol) were dissolved in toluene (20 mL). Then, a solution containing an equimolar amount of NaOAc (87 mg, 1.06 10–3) dissolved in MeOH (5 mL) was added dropwise with stirring at 298 K. The reaction mixture was heated under reflux for 24 h. After this period, the deep orange solution that formed was filtered, and the filtrate was concentrated to dryness with a rotary evaporator and kept in a desiccator overnight. The residue was then dissolved in the minimum amount of CH2Cl2. The undissolved products were removed by filtration, and the filtrate was passed through a short SiO2 column (4.0 cm 1.0 cm). Elution with CH2Cl2 produced a yellowish band, which gave the trans isomer 5 (67 mg) after concentration. Next, a mixture of CH2Cl2/MeOH (100:1) was used, and the orange band was concentrated to dryness by rotary evaporation. The solid formed, 6, was collected, air-dried for 24 h, and then dried in vacuo for 2 d (259 mg). Crystals of 6·CH2Cl2·MeOH were obtained at 298 K by the slow evaporation of a saturated solution of 6 in CH2Cl2 layered with an identical volume of MeOH.

Crystallography: Prismlike crystals of 2 and 6·CH2Cl2·MeOH (sizes in Table 3) were used for the X-ray crystallographic analysis. The X-ray intensity data were measured with a D8 Venture system equipped with a multilayer monochromator and a Mo high-brilliance Microfocus Source (λ = 0.71073 Å). The frames were integrated with the Bruker SAINT software package by using a narrow-frame

algorithm. The final cell constants (Table 3) are based upon the refinement of the XYZ centroids of 3561 reflections above 2σ(I) with 5.11° ≤ 2θ ≤ 50.26°. The data were corrected for absorption effects by the multiscan method (SADABS). The ratio of minimum to maximum apparent transmission was 0.779.

For 2, the integration of the data with a monoclinic unit cell yielded a total of 10506 reflections to a maximum θ angle of 25.13° (0.84 Å resolution), of which 4519 were independent (average redundancy 2.325, completeness: 98.8%, Rint = 4.42%) and 3146 (69.62%) were greater than 2σ(F2). For 6·CH2Cl2·MeOH, the integration of the data with a monoclinic unit cell yielded a total of 28067 reflections to a maximum θ angle of 25.11° (0.84 Å resolution), of which 6261 were independent (average redundancy 4.483, completeness: 99.4%, Rint = 8.20%, Rsig = 4.65%) and 5497 (87.80%) were greater than 2σ(F2).

These structures were solved and refined with the Bruker SHELXTL software package[40] in the space groups P21/c (for 2) and C2/c (for 6·CH2Cl2·MeOH). The final anisotropic full-matrix least-squares refinement on F2 with 302 (for 2) or 413 (for 6·CH2Cl2·MeOH) variables converged at R1 = 6.58 (for the observed data and wR2 = 17.62% for all data) and 3.94% (for the observed data and wR2 = 10.92% for all data), respectively. The goodness-of-fit values were 1.044 (for 2) and 1.028 (for 6·CH2Cl2·MeOH).

For 2, the largest peak in the final difference electron density synthesis was 2.237 e/Å3 and the largest hole was –1.473 e/Å3 with a root mean square (RMS) deviation of 0.128 e/Å3. On the basis of the final model, the calculated density was 1.465 g/cm3, and F(000) was 1160 e. For 6·CH2Cl2·2MeOH, the largest peak in the final difference electron density synthesis was 2.521 e/Å3, and the largest hole was –2.829 e/Å3 with an RMS deviation of 0.182 e/Å3. On the basis of the final model, the calculated density was 1.792 g/cm3, and F(000) was 3760 e–.

CCDC-1014331 (for 2) and -1014332 (for 6·CH2Cl2·MeOH) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. **Cell Cultures:** Breast cancer (MCF7 and MDA-MB231) cells (from European Collection of Cell Cultures, ECACC) and colon adenocarcinoma (HCT116) cells (from the American Type Culture Collection) were used in all of the experiments. The cells were grown as monolayer cultures in minimum essential medium (DMEM with l-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum, 10 mm d-glucose, and 0.1% streptomycin/penicillin under standard culture conditions.

Cell Viability Assays: For these studies, the compounds were dissolved in 100% DMSO at 50 mm as stock solutions; then, serial dilutions were performed with DMSO (1:1; in this way, the DMSO concentrations in the cell media were always the same); finally, 1:500 dilutions of the serially diluted solutions of the compounds on the cell media were performed. The assays were performed as described by Givens et al.[41] In brief, MDA-MB231 and MCF7 cells were plated at 5000 and 10000 cells/well, respectively, in media (100 µL) in tissue culture 96-well plates (Cultek). After 24 h, the media was replaced by 100 µL/well of serial dilutions of the drugs. Each point concentration was run in triplicate. Reagent blanks, containing media plus colorimetric reagent without cells were run on each plate. The blank values were subtracted from the test values and were routinely 5–10% of the uninhibited control values. The plates were incubated for 72 h. The hexosaminidase activities were measured according to the following protocol: the media containing the cells were removed, and the cells were washed once with phosphate-buffered saline (PBS), and substrate solution [60 µL; pnitrophenyl N-acetyl-β-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 1–2 h; after this incubation time, a bright yellow color appeared; then, the plates were developed by the addition of developer solution [90 µL; Glycine 50 mm, pH 10.4; ethylenediaminetetraacetic acid (EDTA) 5 mm], and the absorbance was recorded at 410 nm.

Supporting Information (see footnote on the first page of this article): Characterization data (elemental analyses, MS, IR, UV/Vis, and NMR spectroscopy) for 1–6 and crystal structures of 2 and 6·CH2Cl2·MeOH.

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582 **Legends to figures**

583

584 **Figure 1**. Selected 3,5-disubstituted pyrazoles (I–V) described previously, the new ligand used in this
585 work (1), and the PdII complex trans-[Pd{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-{C3HN2})}2Cl2] (Va).

586

587 **Scheme 1.** i) Toluene, benzyltriethylammonium chloride (BTAC), NaOH (40 %), and MeO(CH2)2Cl at
588 370 K for 24 h.

589

590 **Scheme 2.** i) Na2[PdCl4] in MeOH (298 K, 1 h) or [PdCl2(dmso)2] in MeOH, reflux, 1 h (molar ratios
591 1/PdII = 1 or 2, see text and Table 1, Entries 1 and 2); ii) Pd(OAc)2, toluene, reflux, 24 h; iii) SiO2
592 column chromatography; iv) LiCl (50% excess) in acetone at 298 K (1 h) followed by treatment with
593 PPh3 (molar ratio 3/PPh3 = 1:1) in CH2Cl2 at 298 K for 1 h; v) cis-[PtCl2(dmso)2] in MeOH, reflux;
594 vi) cis-[PtCl2(dmso)2] and NaOAc (molar ratio 1/PtII/OAc– = 1:1:2), toluene/methanol (5:1), reflux, 24
595 h.

596

597 **Figure 2.** Molecular structure of 2. Hydrogen atoms have been omitted for clarity. Selected bond
598 lengths [Å] and angles [°]: Pd1–N1 2.007(4), Pd1–Cl1 2.3013(17), C22–O1 1.465(10), N2–C13,
599 1.354(8), N2–N1 1.364(7), N2–C20 1.460(8), O1–C21 1.390(9), N1–C24 1.336(8), C20–C21 1.495(11),
600 C13–C12 1.387(9), C13–C14 1.479(9), Fe–C(average value of Fc moiety) 2.037(9), C–C(average for
601 Fc) 1.41(4), N1–Pd1–Cl1 88.95(14).

602

603 **Figure 3** Molecular structure of the dimetallic molecules in the crystal structure of 6·CH2Cl2·MeOH.
604 Solvation molecules and hydrogen atoms have been omitted for clarity. Selected bond lengths [Å] and
605 angles [°]: Pt1–N1 2.0127(4), Pt1–S1 2.2037(13), Pt1–Cl1 2.2868(12), Pt1–Cl2 2.3184(13), S1–O1
606 1.466(4), S1–C30 1.766(6), S1–C31 1.778(6), O2–C28 1.409(6), O2–C29 1.429(6), N1–C13 1.338(6),
607 N1–N2 1.364(5), N2–C14 1.362(6), N2–C(27) 1.455(6), Fe–C (Fc unit) 2.044(6), C–C (average value
608 for the Fc moiety) 1.42(1), N1–Pt1–S1 91.89(12), S1–Pt1–Cl1 90.79(5), N1– Pt1–Cl2 87.34(12).

609

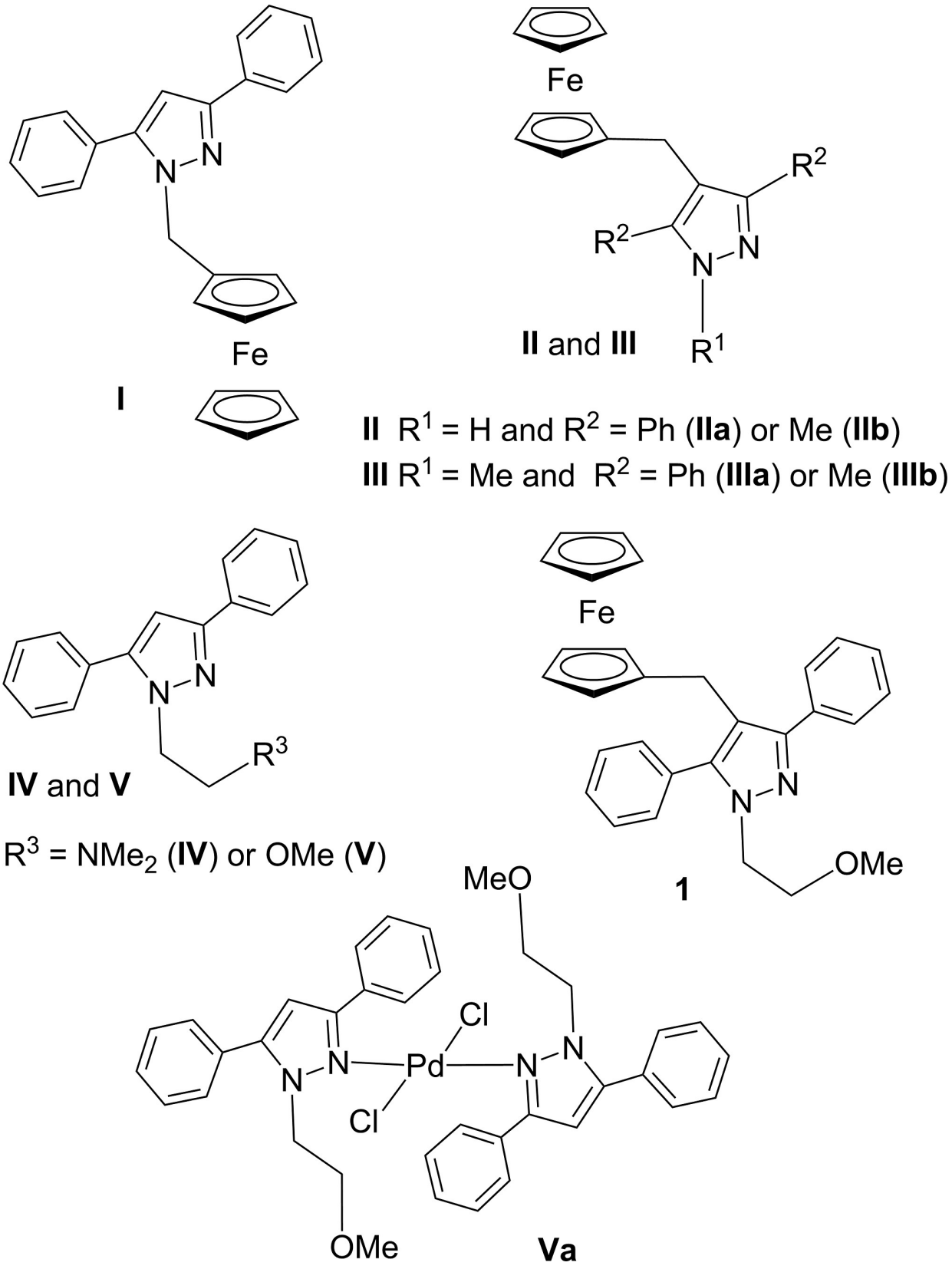
610 **Figure 4**. Comparative plot of the IC50 values [μm] of 1, V, their closely related derivative IV, and
611 cisplatin against the MCF7 and MDA-MB231 breast cancer cell lines.

612

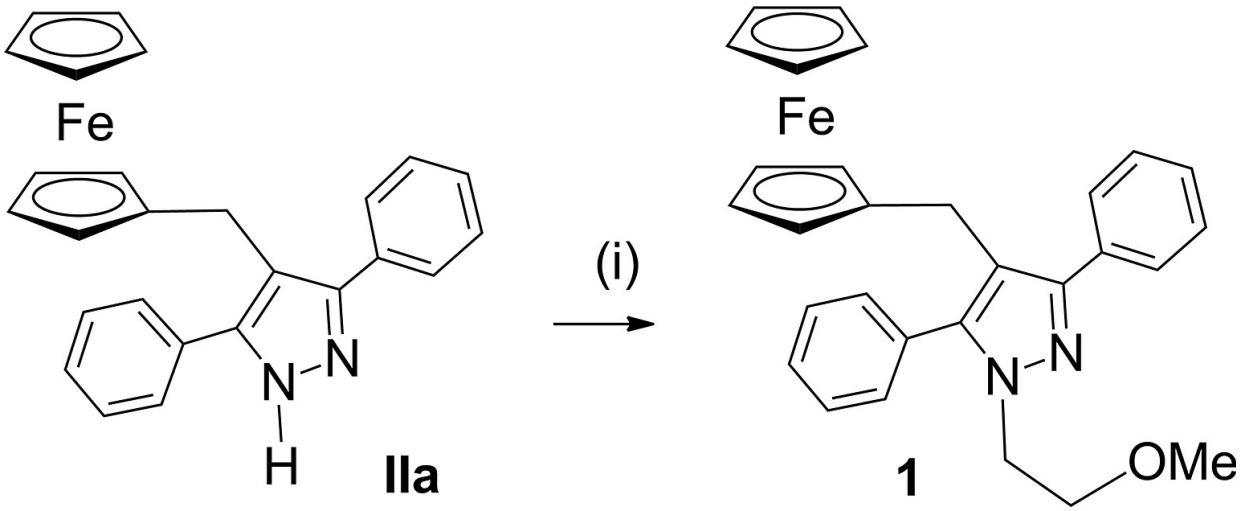
613 **Figure 5** Comparative plot of the IC50 values [μm] of the free ligand 1, the PdII complexes 2–4, and the
614 trans and cis isomers of [Pt{κ-N-(1-{MeO(CH2)2}-3,5-Ph2-4-{CH2Fc}–{C3N2})}Cl2- (dmso)] (5 and
615 6, respectively) against the MCF7 and MDAMB231 breast cancer cell lines and the cisplatin-resistant
616 colon cancer cell line (HCT-116). For comparison, the data obtained for cisplatin under identical
617 experimental conditions are also included.

618

FIGURE 1.


Fe
Fe
I
II and III
II R1 = H and R2 = Ph (IIa) or Me (IIb)
III R1 = Me and R2 = Ph (IIIa) or Me (IIIb)
IV and V
R3 = NMe2 (IV) or OMe (V)
Fe
OMe
1
MeO
Cl
Pd
Cl
OMe
Va

SCHEME 1.



SCHEME 2.

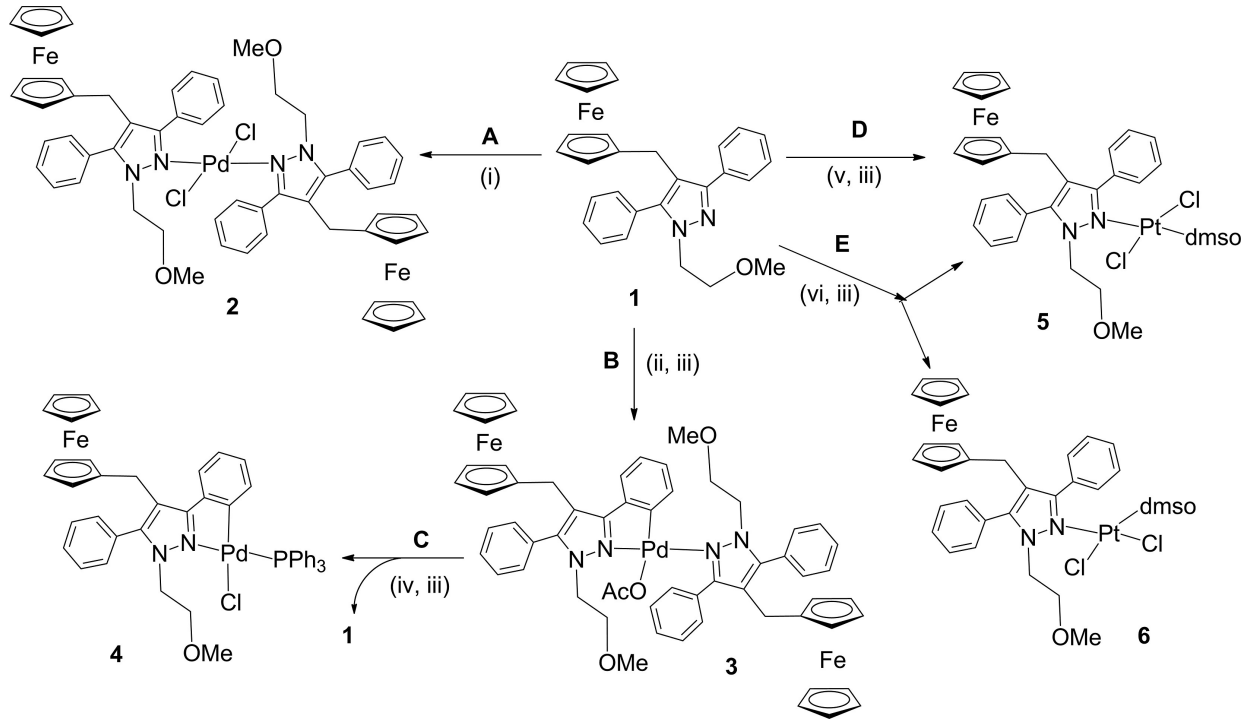
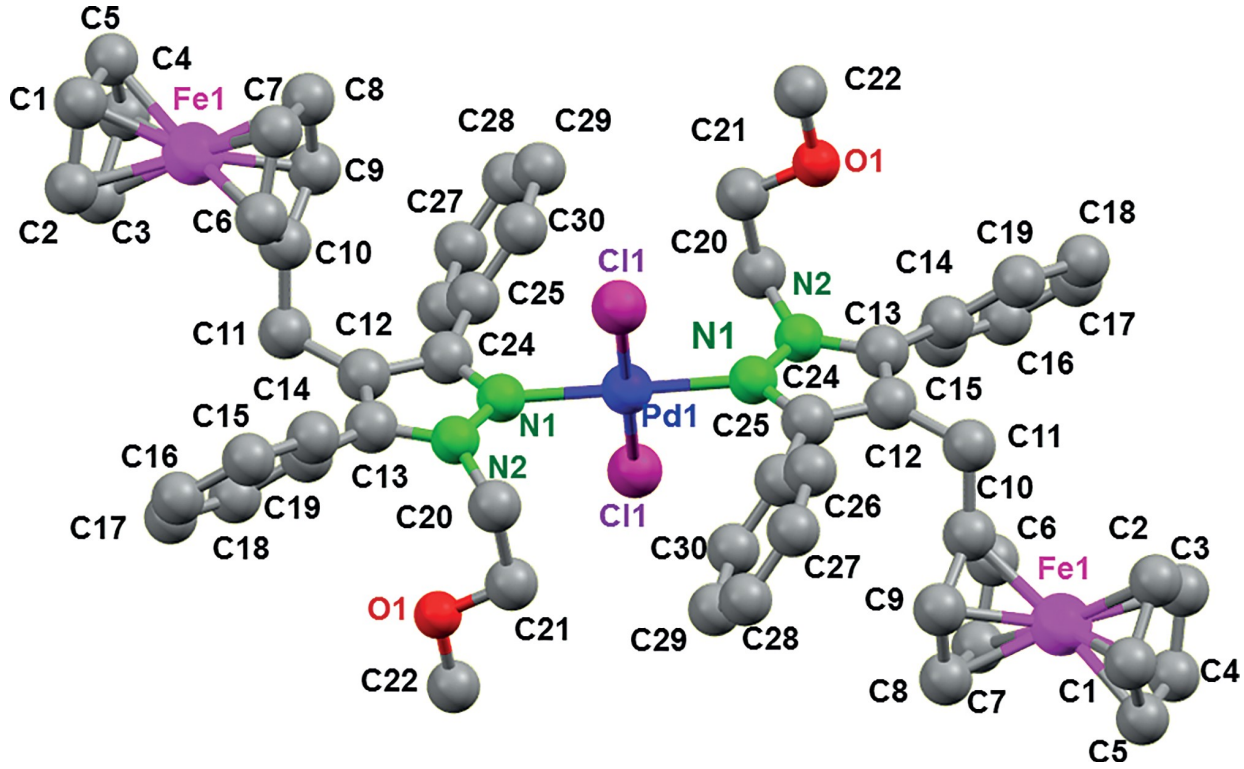

Fe
MeO
Cl
Pd
N
Cl
OMe
2
A
(i)
Fe
N
N
OMe
1
D
(v, iii)
Fe
Cl
Pt
dmso
Cl
OMe
5
E
(vi, iii)
B
(ii, iii)
Fe
MeO
Pd
N
AcO
OMe
3
Fe
C
(iv, iii)
1
Fe
Pd
PPh3
Cl
OMe
4
Fe
dmso
Pt
Cl
Cl
OMe
6

FIGURE 2.


C5
C4
C1
Fe1
C7
C8
C28
C29
C21
C22
O1
C9
C27
C30
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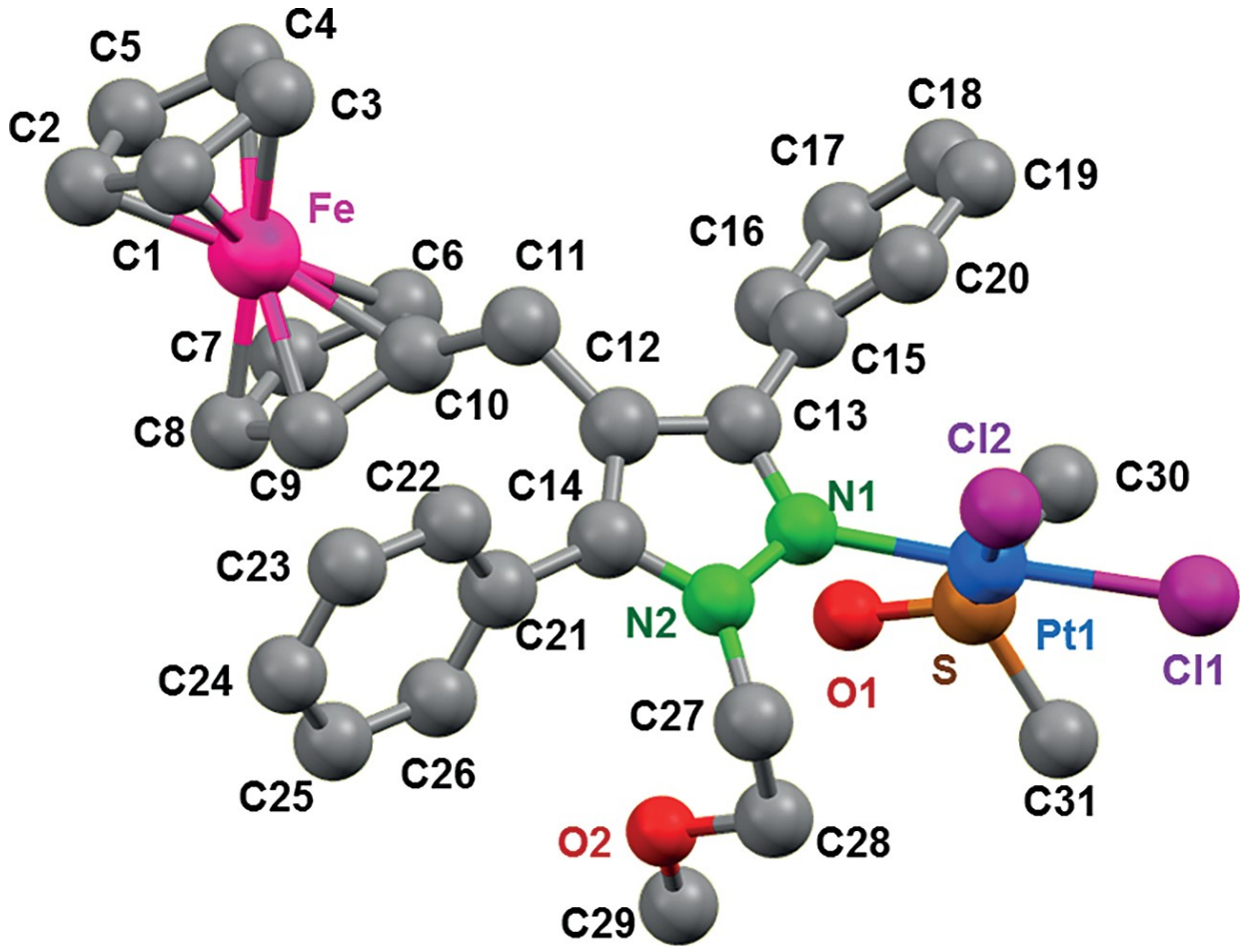
C3
C6
C10
Cl1
C20
N2
C19
C18
C14
C25
C11
C12
C24
N1
C13
C17
C16
C14
C15
N1
Pd1
C25
C15
C16
N2
C12
C11
C13
C19
C20
Cl1
C10
C17
C18
C26
C6
C2
C3
C30
C27
Fe1
O1
C21
C9
C29
C28
C22
C8
C7
C1
C4
C5

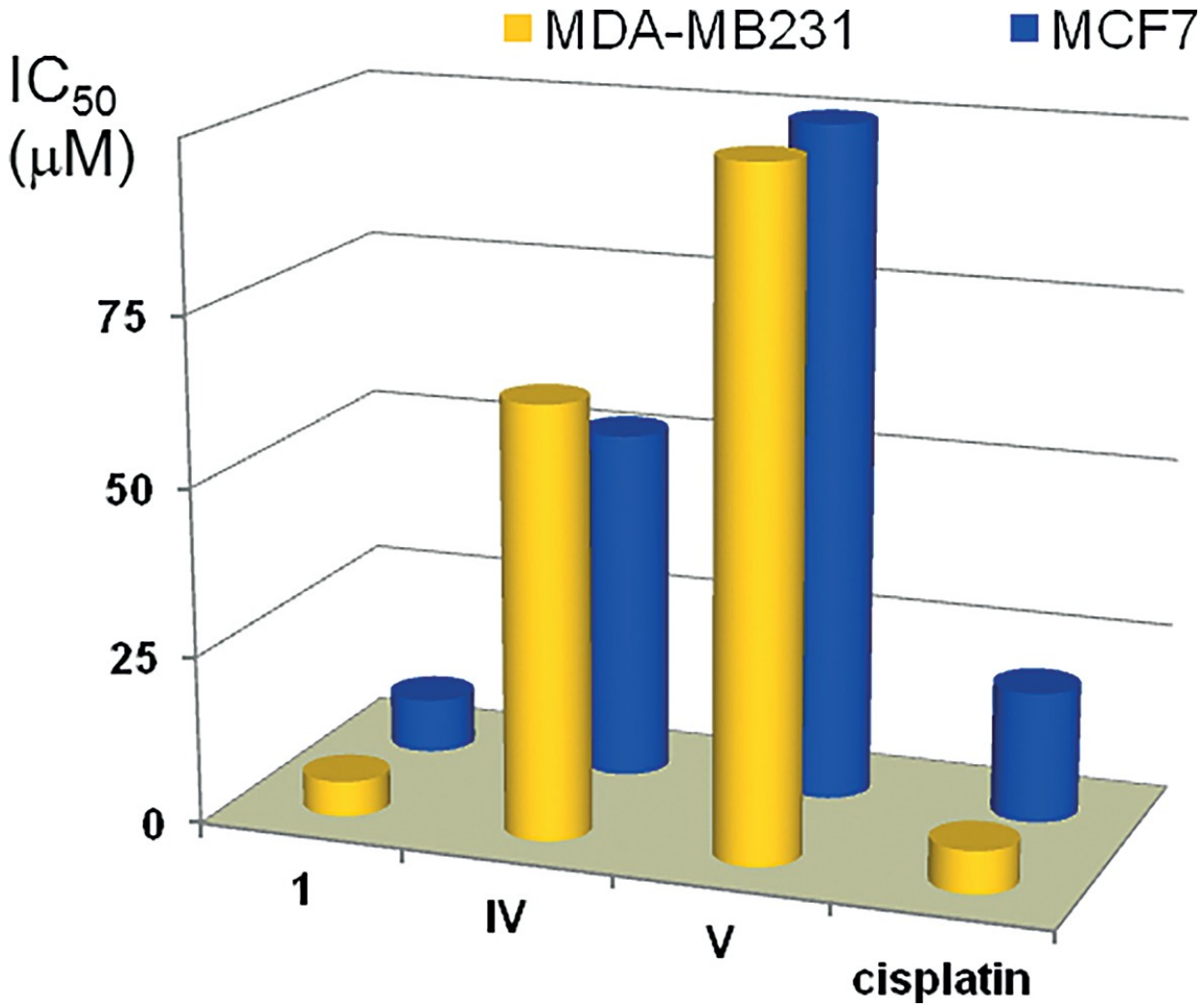
FIGURE 3.


C4
C5
C3
C2
C18
C17
C19
Fe
C16
C1
C6
C11
C20
C7
C12
C15
C10
C13
C8
Cl2
C30
C9
C22
C14
N1
C23
N2
Pt1
C21
S
Cl1
C24
O1
C27
C26
C31
C25
O2
C28
C29

644 **FIGURE 4.**

645

646



647

648

FIGURE 5.

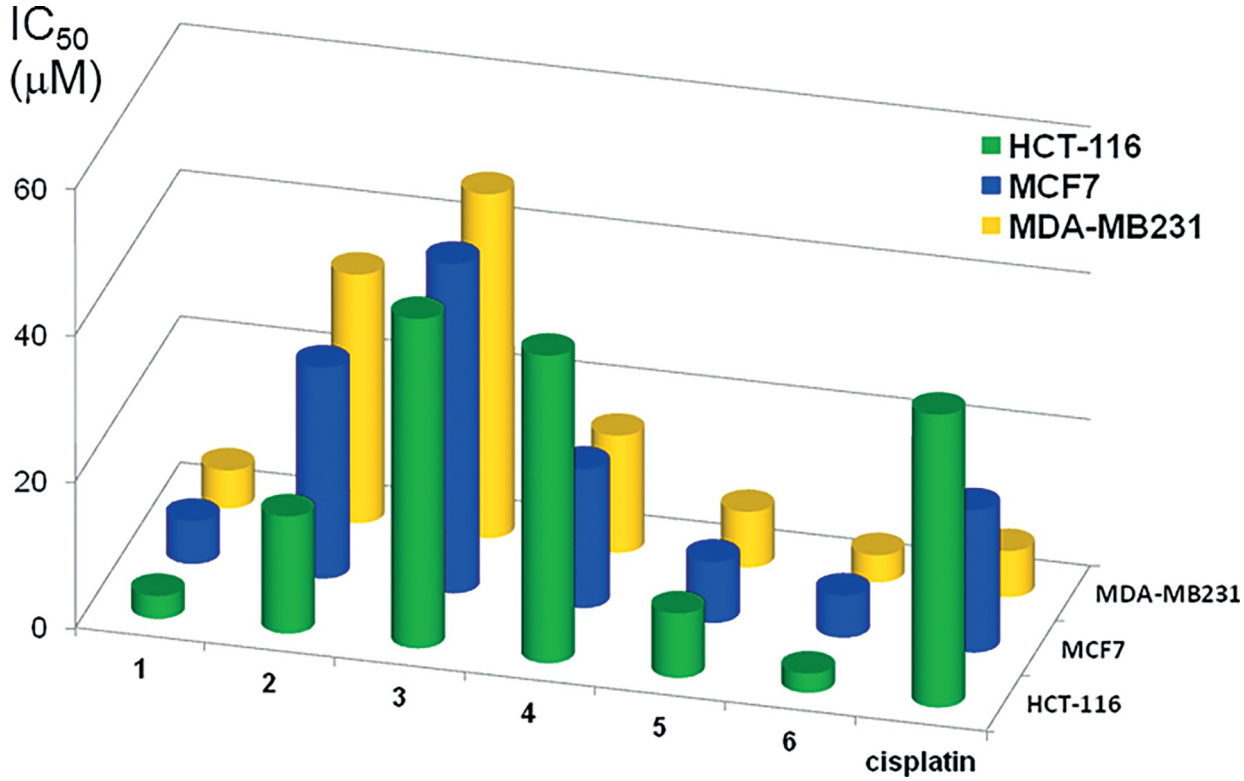

IC50 (μM)
60
40
20
0
HCT-116
MCF7
MDA-MB231
1
2
3
4
5
6
cisplatin
MDA-MB231
MCF7
HCT-116

Table 1. Summary of experimental conditions [reagents, molar ratios, solvents, temperature (T) and reaction time (t)] used to prepare the new PdII complexes 2–4 (Entries 1–4) and PtII compounds 5 and 6 (Entries 5 and 6). In entries 5 and 6, [Pt] represents cis-[PtCl2(dmso)2]. Reagents (molar

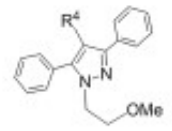
	Reagents (molar ratios)	Solvent	*T*	*t* [h]	Final products
1	**1** and $Na_2[PdCl_4]$ (1:1 or 1:2)	MeOH	298 K	1	**2**
2	**1** and $[PdCl_2(dmso)_2]$ (1:1 or 1:2)	MeOH	reflux	1	**2**
3	**1** and $Pd(OAc)_2$	toluene	reflux	24	**3**
4[a]	(1) **3** and LiCl [b]	acetone	298 K	1	
	(2) PPh_3[c]	CH_2Cl_2	298 K	1	**4**
5	**1** and [Pt]	MeOH	reflux	1	**5**
6	**1**, [Pt], and NaOAc (1:1:2)	toluene/ MeOH[d]	reflux	24	**5** and **6**[e]

[a] Two-step sequence. [b] Molar ratio LiCl/**3** = 1.5. [c] Molar ratio **3**/PPh_3 = 1:1. [d] A 5:1 Mixture. [e] Molar ratio **5**/**6** = 1.0:3.8.

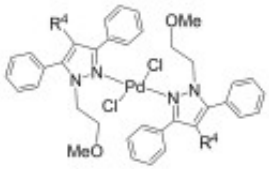
Table 2 Cytotoxic activities (IC50 values in μm) against breast cancer cell lines (MCF7 and MDA-MB231) and the cisplatin-resistant HCT-116 colon cell line for the pyrazoles. To ease the identification of the compounds included in this study, their chemical formulae are shown below.

Compound	R^4	Binding mode	MCF7	MDA-MB231	HCT-116
1	CH_2Fc	–	5.8 ± 0.9	5.2 ± 0.7	3.1 ± 0.5
V	H	–	>100	100	[a]
IV	H	–	52 ± 10	64 ± 24	[a]
2	CH_2Fc	(N)	28 ± 2.3	34 ± 1.7	16 ± 3
Va	H	(N)	>100	75 ± n.d.[b]	[a]
3	CH_2Fc	(N) and (C,N)	45 ± 2.6	47 ± 2.8	45 ± 3.1
Vc	H	(C,N)	67 ± 1.9	13 ± 1.1	[a]
4	CH_2Fc	(C,N)	19.6 ± 1.7	16.1 ± 1.3	42 ± 4.3
5	CH_2Fc	(N)	8.3 ± 1.1	7.6 ± n.d.[b]	8.5 ± 0.9
6	CH_2Fc	(N)	5.7 ± 0.8	3.7 ± n.d.[b]	2.6 ± 0.6
cisplatin			19.4 ± 4.5	6.3 ± 3.4	40 ± 4.4

Ligands

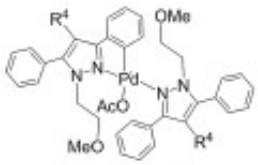


$R^4 = CH_2Fc$ (**1**) or H (**V**)

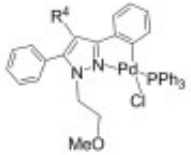


$R^4 = CH_2Fc$ (**2**) or H (**Va**)

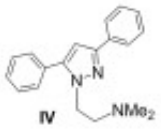
Palladium(II) complexes



$R^4 = CH_2Fc$ (**3**)

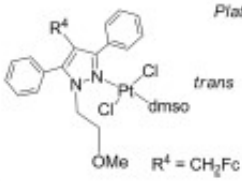


$R^4 = CH_2Fc$ (**4**) or H (**Vc**)



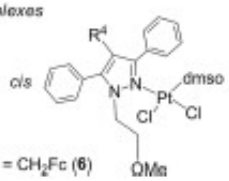
IV

Platinum(II) complexes



trans

$R^4 = CH_2Fc$ (**5**)



cis

$R^4 = CH_2Fc$ (**6**)

[a] Data not available. [b] n.d.: not determined.

668 **Table 3** Crystal data and details of the refinement of the crystal structures of 2 and 6·CH2Cl2·MeOH.

669

	2	6·CH_2Cl_2·MeOH
Empirical formula	$C_{58}H_{56}Cl_2Fe_2N_4O_2Pd$	$C_{33}H_{37}Cl_6FeN_2O_3PtS$
Formula weight	1130.7	936.46
Crystal size [mm]	0.090 × 0.180 × 0.240	0.180 × 0.220 × 0.34
Crystal system	monoclinic	monoclinic
Space group	$P2_1/c$	$C2/c$
a [Å]	11.7349(12)	29.6708(14)
b [Å]	18.7496(17)	14.6180(8)
c [Å]	11.7367	16.4452(10)
$\alpha = \gamma$ [°]	90.0	90.0
β [°]	97.217(4)	98.887(2)
T [K]	100(2)	100(2)
λ [Å]	0.71073	0.71073
V [Å^3]	2561.9(4)	7047.1(7)
Z	2	8
$D_{calcd.}$ [Mg/m^3]	1.465	1.795
F(000)	1149	3704
μ [mm^{-1}]	1.057	4.776
θ range [°]	2.06–25.13	2.507–25.109
Collected reflections	10506	28067
Unique reflections [R(int)]	4519 [0.0442]	626 [0.0820]
Parameters	302	407
R indices [$I > 2\sigma(I)$]	$R_1 = 0.0658$, $wR_2 = 0.1545$	$R_1 = 0.0415$, $wR_2 = 0.1115$
R indices (all data)	$R_1 = 0.1013$, $wR_2 = 0.1762$	$R_1 = 0.0471$, $wR_2 = 0.1168$

670

671

672