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Platinum(II) and palladium(II) complexes with (N,N′) and (C,N,N′)$^-$ ligands derived from pyrazole as anticancer and antimalarial agents: Synthesis, characterization and in vitro activities

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ABSTRACT

The study of the reactivity of three 1-(2-dimethylaminoethyl)-1*H*-pyrazole derivatives of general formula [1-$(CH_2)_2NMe_2$}-3,5-R_2-pzol] {where pzol represents pyrazole and R═H (**1a**), Me (**1b**) or Ph (**1c**)} with [$MCl_2(DMSO)_2$] (M═Pt or Pd) under different experimental conditions allowed us to isolate and characterize *cis*-[M{κ^2-*N,N′*-{[1-$(CH_2)_2NMe_2$}-3,5-R_2-pzol])}Cl_2] {MM═PtPt (**2a**–**2c**) or Pd (**3a**–**3c**)} and two cyclometallated complexes [M{κ^3-*C,N,N′*-{[1-$(CH_2)_2NMe_2$}-3-(C_5H_4)-5-Ph-pzol])}Cl] {M═Pt(II) (**4c**) or Pd(II) (**5c**)}. Compounds **4c** and **5c** arise from the orthometallation of the 3-phenyl ring of ligand **1c**. Complex **2a** has been further characterized by X-ray crystallography. Ligands and complexes were evaluated for their in vitro antimalarial against *Plasmodium falciparum* and cytotoxic activities against lung (A549) and breast (MDA MB231 and MCF7) cancer cellular lines. Complexes **2a**–**2c** and **5c** exhibited only moderate antimalarial activities against two *P. falciparum* strains (3D7 and W2). Interestingly, cytotoxicity assays revealed that the platinacycle **4c** exhibits a higher toxicity than cisplatin in the three human cell lines and that the complex **2a** presents a remarkable cytotoxicity and selectivity in lung (IC_{50} = 3 μM) versus breast cancer cell lines (IC_{50} >20 μM). Thus, complexes **2c** and **4c** appear to be promising leads, creating a novel family of anticancer agents. Electrophoretic DNA migration studies in presence of the synthesized compounds have been performed, in order to get further insights into their mechanism of action.

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1. Introduction

Platinum drugs have played a key role among the metal-based anticancer agents [1]. The discovery of the antitumor properties of *cis*-[$PtCl_2(NH_3)_2$] *cisplatin* [2] (Fig. 1) in 1965 was rapidly followed by clinical trials and finally in 1978, FDA granted approval. Platinum(II) complexes such as cisplatin and carboplatin (Fig. 1) are widely used to treat cancers such as testicular, ovarian, urinary bladder, melanoma, etc. The cytotoxicity of Pt-based drugs is mainly attributed to their ability to bind DNA and to induce DNA damage leading then to apoptosis [3–5].

Unfortunately, the use of cisplatin is restricted due to dose-limiting toxicity, including nephrotoxicity, neurotoxicity and ototoxicity [6,7]. Additional side effects such as blood pressure increase, severe nausea, vomiting and diarrhea have also been reported. Moreover, the biochemical resistance mode limits the clinical utility of the Pt-based drugs in current use [1].

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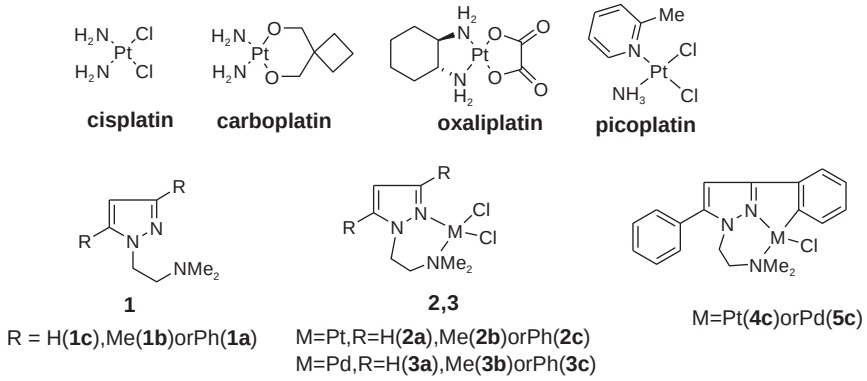


Fig. 1. Chemical structure of some platinum(II) based anticancer agents and compounds used in this study.

In the search of new metallodrugs avoiding toxicity and resistance, special attention has been paid to the replacement of one or both NH_3 ligands of *cisplatin* by other *N*-donor ligand(s). This strategy gave rise to oxaliplatin and picoplatin [8] (Fig. 1). In oxaliplatin, which is active in patients with colorectal cancer, both NH_3 units have been replaced by (1*R*,2*R*)-cyclohexane-1,2-diamine (*R*,*R*-dach); while picoplatin, that is in clinical development for the treatment of patients with solid tumors, contains a 2-methylpyridine instead of one NH_3 ligand.

The search of novel *N*-donor ligands (i.e. amines, oximes, imines or azoles) for the synthesis of optimized platinum(II) and palladium(II) drugs is still in progress [9,10]. In addition, it is well-known that azoles are valuable reagents in coordination chemistry and that their binding to a transition metal ion affects their properties and activities. Few complexes with pyrazole ligands showing an antitumor activity similar to that of cisplatin have been reported [11–20], but the effect produced by the substituents on the heterocycle has not been clarified so far. Within this therapeutic context, and in order to clarify this point and to elucidate the influence of mode of binding of this family of ligands in the biological activity of the complexes, we decided to synthesize the new pyrazole derivatives **1a**–**1c** (Fig. 1) and to study their reactivity with Pt(II) and Pd(II). Due to the relative disposition of the two nitrogen atoms, compounds **1a**–**1c** may bind to the M(II) center as a neutral (*N*) or (*N*,*N*′) ligand. Moreover, for **1c**, the presence of the phenyl ring on position 3 of the heterocycle may also allow the formation of metallacycles containing **1c** as a *mer*-terdentate $(C,N,N')^-$ ligand.

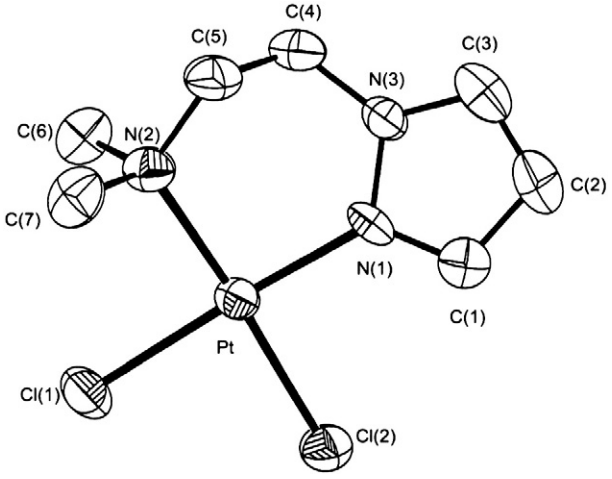


Fig. 2. ORTEP plot of complex **2a**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (in Å) and angles (in deg.) for **2a**: Pt–N(1), 2.010(3); Pt–N(3), 2.091(4); Pt–Cl(1), 2.3056(13); Pt–Cl(2), 2.3058(13); N(1)–C(1),1.339(6); N(1)–N(2), 1.370(6); N(3)–C(7), 1.499(5); N(3)–C(6), 1.499(7); N(3)–C(8), 1.506(6); C(1)–C(2), 1.409(8); C(2)–C(3), 1.367(10); C(3)–N(2), 1.349(7); N(2)–C(5), 1.453(7); C(5)–C(6), 1.483(7); N(1)–Pt–N(3), 93.57(15); N(1)–Pt–Cl(1), 176.60(11), N(3)–Pt–Cl(1), 89.61(12); N(1)–Pt–Cl(2), 89.32(12); N(3)–Pt–Cl(2), 177.03(12); Cl(1)–Pt–Cl(2), 87.52(5); C(1)–N(1)–N(2), 105.3(4); C(1)–N(1)–Pt, 129.5(3); N(2)–N(1)–Pt, 125.2(3); C(7)–N(3)–C(6), 108.2(4); C(7)–N(3)–C(8), 108.3(4); C(6)–N(3)–C(8), 106.6(4); C(7)–N(3)–Pt, 109.7(3); C(6)–N(3)–Pt, 113.5(3); C(8)–N(3)–Pt, 110.4(3); N(1)–C(1)–C(2), 111.0(5); C(3)–C(2)–C(1), 104.7(5); N(2)–C(3)–C(2), 108.5(6); C(3)–N(2)–N(1), 110.6(4); C(3)–N(2)–C(5), 129.1(5); N(1)–N(2)–C(5), 120.1(4); N(2)–C(5)–C(6), 111.5(4) and C(5)–C(6)–N(3), 113.8(4).

Cyclometallated complexes derived from *N*-donor ligands have attracted great interest during the last decade due to their properties and applications in a wide variety of fields [10,20–36] and compounds of this kind containing Pd(II), Pt(II), Ru(II), Ir(III) Rh(III) and Au have shown promising cytotoxic activities [10,20,27–36]. Few cyclopalladated complexes derived from pyrazole are known [37–41] and, to the best of our knowledge, their cytotoxicity has only been reported once [41]. On the other hand despite the interest for Pt(II) complexes, platinacycles with pyrazolyl ligands are scarce and studies on their biological activity have not been performed so far.

In this work, we present the study of the reactivity of ligands **1a**–**1c** with syntheses of *cis*-$[MCl_2(DMSO)_2]$, the new compounds **2a**–**2c** and **3a**–**3c** and the cyclometallated complexes **4c** and **5c**, together with a comparative study of their antineoplastic activity against lung (A549) and breast (MDA MB231 and MCF7) human cancer cell lines. As our interests lie also in the area of antiparasitic drugs [42,43] and since a few Pt(II) [44] and Pd(II) based complexes [45] with antimalarial activity (in the micromolar range) have been reported, we also tested the potential of the free ligands (**1a**–**1c**) and the complexes **2a**–**5c** against the chloroquine-susceptible strain (3D7) and the chloroquine-resistant strain (W2) of *Plasmodium falciparum*.

2. Experimental

2.1. Chemistry

2.1.1. Materials and methods

Reagents were obtained from commercial sources and used as received. *Cis*-$[MCl_2(DMSO)_2]$ (M=Pd or Pt) were prepared according to literature protocols [46,47]. All reactions were carried out with dry and freshly distilled solvents. Column chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel 60, SDS, 230–240 mesh) or Al_2O_3 (neutral alumina, 63–200 μm). Analytical TLC was performed on SiO_2 (Merck silica gel 60 F_{254}) plates. Elemental analyses were carried out at the Serveis Cientifico-Tècnics (*Universitat Barcelona*). Mass spectra (electrospray ionization, ESI^+) were performed at the Servei d'Espectrometria de Masses (*Universitat de Barcelona*). Infrared spectra were obtained with a Nicolet 400FTIR instrument using KBr pellets. Only noteworthy IR absorptions are listed. Unless otherwise noted ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 298 K with a Mercury-400 MHz. ^1H and ^{13}C chemical shifts (δ) are reported in ppm downfield and referred to $SiMe_4$ and to the resonance of $CDCl_3$, respectively and coupling constants (*J*) are given in Hz. All NMR assignments were

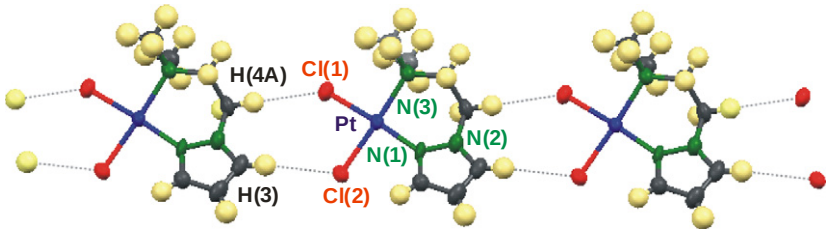


Fig. 3. Assembly of molecules of **2a** in the crystal by multiple weak C–H···Cl intermolecular interactions involving: a) the two chlorides {Cl(1) and Cl(2)} the unit at (*x*, *y*, *z*) (represented as the central unit) and the H(4B) and H(3) atoms, respectively of another and close molecule at (*x*, *1 + y*, *z*) and b) these two hydrogen atoms and the two Cl^- ligands of the molecule located at (*−1 + x*, *y*, *z*).

made on the basis of two dimensional NMR experiments (gradient correlation spectroscopy gCOSY, gradient heteronuclear single quantum correlation gHSQC, and gradient Heteronuclear Single Quantum Correlation gHMBC). In all cases the atom labeling scheme for the assignment of the signals corresponds to that shown in Scheme 1. If not specified, ^{195}Pt {^1H} NMR spectra of **2a**, **2c** and **4c** were recorded in $CDCl_3$ with a Bruker 250DXR instrument using $CDCl_3$ as solvent and $H_2[PtCl_6]$ {$\delta^{195}Pt(H_2[PtCl_6]) = 0.0$ ppm} as reference. The splitting of proton resonances in the reported ^1H NMR spectra is defined as s = singlet, d = doublet, dd = doublet of doublet, t = triplet, td = triplet of doublet and m = multiplet.

*2.1.2. Synthesis of ligands [1-$(CH_2)_2NMe_2$}-3,5-R_2-pzol] {R=H (**1a**), Me (**1b**) or Ph (**1c**)}*

*2.1.2.1. Preparation of 1-(2-dimethylaminoethyl)-1H-pyrazole (**1a**).* A mixture of 1*H*-pyrazole (1.92 g, 2.8×10^{-3} mol) and sodamide (1.56 g, 4×10^{-3} mol) in dry toluene (20 mL) was heated at reflux temperature with stirring for 2.5 h. The mixture was cooled, 2-dimethylaminoethyl chloride hydrochloride (2.6 g, 2.8×10^{-3} mol) was added, and the mixture was heated under reflux for 4 h. Water (20 mL) was added, the toluene layer was separated and the aqueous phase was extracted with toluene (20 mL). The joined organic phases was extracted with aqueous 0.1 M HCl solution, basified with aqueous saturated Na_2CO_3, extracted with CH_2Cl_2, dried with Na_2SO_4 and finally evaporated to dryness to give **1a** as a colorless oil (2.6 g, 67%). IR (cm^{-1}): 3423, 2946, 2772, 1461, 1397, 1283, 1092, 1054, 751. ^1H NMR (gCOSY): 2.27 (s, 6 H, NMe_2), 2.76 (t, *J* = 7 Hz, 2 H, $-CH_2-^b$), 4.24 (t, *J* = 7 Hz, 2 H, $-CH_2-^a$), 6.24 (m, 1 H, H^4), 7.45 (d, *J* = 2 Hz, 1 H, H^5), 7.50 d, *J* = 1.6 Hz, 1 H, H^3). ^{13}C NMR (gHSQC, gHMBC): 45.6 (NMe_2), 50.2 (C^a), 59.2 (C^b), 105.3 (C^4), 129.3 (C^5), 139.2 (C^3). EM (ESI^+): m/z = 140.12 (Calc. 140.11) $\{[M]+H\}^+$. $C_7H_{13}N_3$ (FW = 139.11).

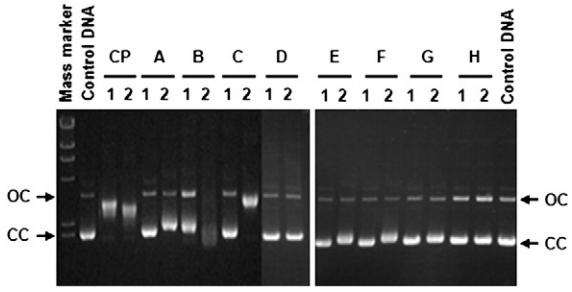


Fig. 4. DNA unwinding assay of supercoiled pBluescript SK+ (40 μg/mL) by platinum and palladium drugs at 5 μM (lanes 1) or 50 μM (lanes 2). A, **2c**; B, **5c**; C, **2a**; D, **4c**; E, **3b**; F, **3a**; G, **2b**, H, **1c**. Cisplatin (CP) was analyzed in parallel for comparison. The molecular mass marker was lambda DNA digested with HindIII. cc, closed circular DNA form (supercoiled form); oc, open circular DNA form.

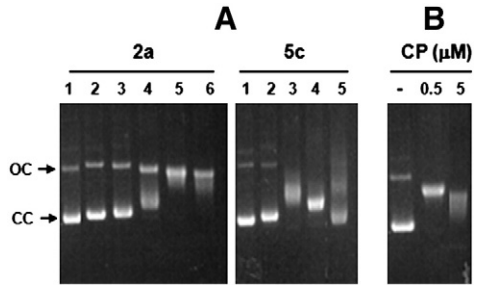


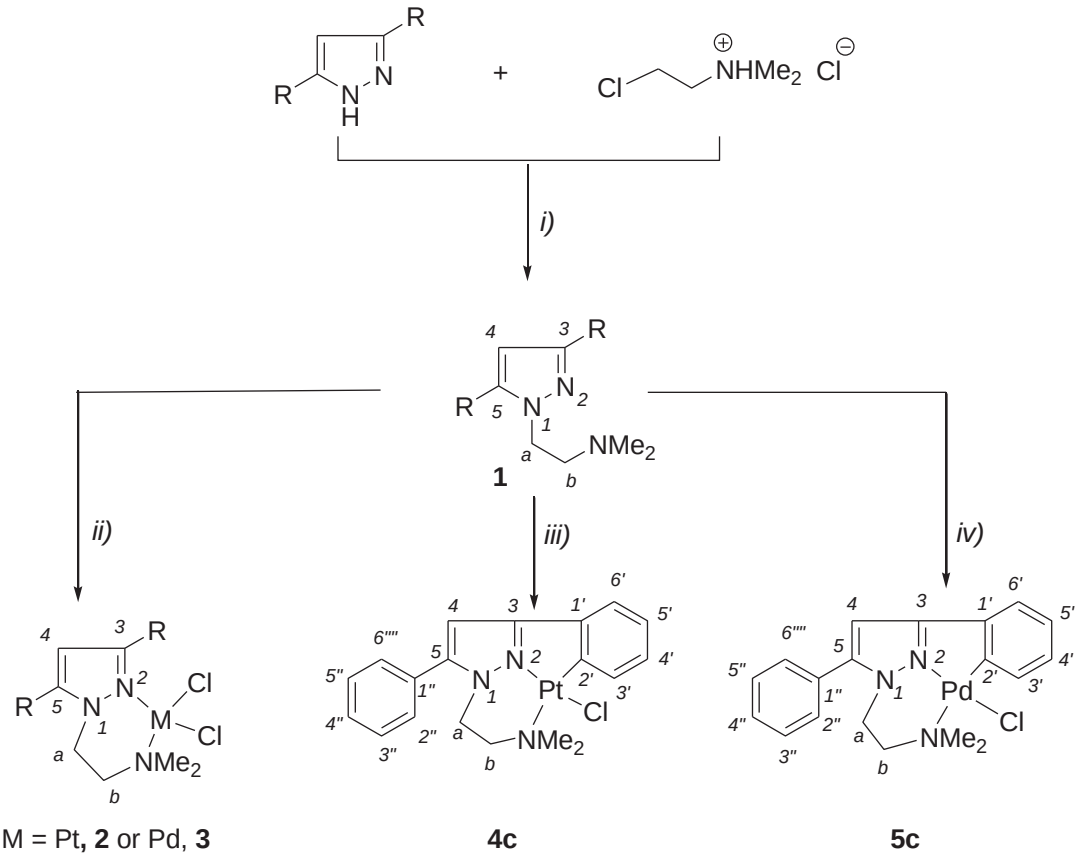
Fig. 5. (A) Interaction of pBluescript SK+ plasmid DNA (40 μg/mL) with increasing concentrations of the platinum(II) complex **2a** and the palladium(II) complex **5c**. Lanes 1, DNA only; lanes 2, 5 μM drug; lanes 3, 10 μM drug, lanes 4, 25 μM drug; lanes 5, 50 μM drug; and lanes 6, 100 μM drug. (B) Unwinding assay with cisplatin (CP) at the indicated concentrations for comparison. cc, closed circular DNA form; oc, open circular DNA form.

*2.1.2.2. Preparation of 1-(2-dimethylaminoethyl)-3,5-dimethyl-1H-pyrazole (**1b**).* This product **1b** was obtained as a colorless oil following the same procedure as described for **1a**. Yield 75%. IR (cm^{-1}): 3423, 2945, 2771, 1554, 1461, 1425, 1386, 1042, 1018, 773. ^1H NMR (gCOSY): 2.21 (s, 3H, Me), 2.21 (s, 3H, Me), 2.28 (s, 6H, $-NMe_2$), 2.68 (t, *J* = 3.2, 2H, $-CH_2-^b$), 4.06 (t, *J* = 3.4, 2H, $-CH_2-^a$), 5.77 (s, 1H, H^4). ^{13}C NMR (gHSQC, gHMBC): 11.0 (Me), 22.0 (Me), 45.7 ($-NMe_2$), 46.0 (C^a), 59.1 (C^b), 104.9 (C^4), 140.0 (C^5), 147.4 (C^3). EM (ESI^+): m/z = 168.15 (Calc. 168.14) $\{[M]+H\}^+$. $C_9H_{17}N_3$ (FW = 167.14).

*2.1.2.3. 1-(2-dimethylaminoethyl)-3,5-diphenyl-1H-pyrazole (**1c**).* To a suspension of 3,5-diphenylpyrazole (1.26 g, 5.74×10^{-3} mol) in toluene (100 mL) Aliquat 336 (1 g), 40% aqueous NaOH solution (50 mL) and $ClCH_2CH_2NMe_2\cdot HCl$ (2.5 g, 23.04×10^{-3} mol) were added and the resulting mixture was stirred at 95 °C for 24 h. After this period water (50 mL) was added, the organic phase was extracted, dried with Na_2SO_4 and finally evaporated to dryness. The crude material was chromatographed on silica gel (Et_2O to Et_2O:CH_2Cl_2 1:1), to yield the ligand **1c** as a clear viscous oil (1.28 g, 77%). An analytical sample was recrystallized from hexane/CH_2Cl_2 to obtain colorless crystals. IR (cm^{-1}): 3463, 3040, 2945, 2819, 2775, 1483, 1460, 1438, 1299, 763, 694. ^1H NMR (gCOSY): 2.17 (s, 6H, NMe_2), 2.79 (t, *J* = 7.2, 2H, $-CH_2-^b$), 2.25 (t, *J* = 7.2, 2H, $-CH_2-^a$), 6.57 (s, 1H, H^4), 7.25–7.48 (m, 8H, ArH), 7.83 (dd, *J* = 8 and 1.4, 2H, $H^{2'}$ and $H^{6'}$). ^{13}C NMR (gHSQC): 45.6 (NMe_2), 47.8 (C^a), 59.1 (C^b), 103.4 (C^4), 125.6, 127.5, 128.5, 128.7, 129.0 (Ar) 130.8, 135.5 ($C^{1'}$ and $C^{1''}$), 145.1 (C^5), 150.7 (C^3). EM (ESI^+): m/z = 292.18 (Calc. 292.17) $\{[M]+H\}^+$. Anal (%) Calc. for $C_{19}H_{21}N_3.1/4\ H_2O$: C, 77.12; H, 7.32; N, 14.20. Found: C, 77.5; H, 7.4; N, 14.4. $C_{19}H_{21}N_3$ (FW = 291.17).

*2.1.3. Synthesis of compounds cis-[Pt{κ^2-N,N′-[1-$(CH_2)_2NMe_2$}-3,5-R_2-pzol}Cl_2] (**2**)*

*2.1.3.1. Cis-[Pt{κ^2-N,N′-[1-$(CH_2)_2NMe_2$]pzol}Cl_2] (**2a**).* A suspension of *cis*-[$PtCl_2(DMSO)_2$] (52.8 mg, 125×10^{-3} mol) and ligand **1a** (17.4 mg, 125×10^{-3} mol) in toluene (10 mL) was heated at reflux temperature for 2 h. The reaction mixture was evaporated to dryness and the obtained



Scheme 1. Synthesis of ligands (**1a**–**1c**) and their palladium(II) and platinum(II) derivatives with **1** acting as a bidentate (N,N′) ligand (**2**,·**3**) and the cyclometallated compounds **4c** and **5c**. In order to ease the visualization of the reactions, letters **a**, **b** and **c** refer to the substituents on positions 3 and 5 of the pyrazole {R═H (**a**), Me (**b**) or Ph (**c**)}. Reagents and conditions: i) in toluene, in the presence of sodamine (for R═H or Me) or using Aliquat 336 and NaOH (40%) for R═Ph. ii) *Cis*-[$MCl_2(DMSO)_2$] (M═Pt or Pd for **2** and **3**, respectively) (1:1) toluene, reflux; iii) *Cis*-[$PtCl_2(DMSO)_2$] and NaOAc in toluene:MeOH (12:1) mixture, reflux 3 h. iv) *Cis*-[$PdCl_2(DMSO)_2$] and NaOAc in toluene:MeOH (12:1) mixture, reflux 1 h.

residue was purified by column chromatography (Al_2O_3, hexane: acetone 50:50 to 25:75) to yield 33 mg (65%) of the title complex as a yellow solid. An analytical sample was recrystallized from CH_2Cl_2/MeOH. IR (cm^{-1}): 3430, 3085, 2921, 1740, 1627, 1517, 1447, 1422, 1156, 1020, 756. ^1H NMR (DMSO-d_6, 500 MHz, gCOSY): 2.85 (m, 2H, $-CH_2-^b$), 2.94 (s, 6H, NMe_2), 4.53 (m, 2H, $-CH_2-^a$), 6.45 (m, 1H, H^4), 8.07 (m, 2H, H^3 and H^5). ^{13}C NMR (DMSO-d_6, 125.9 MHz, gHSQC, gHMBC): 47.5 (C^a), 52.4 ($-NMe_2$), 62.6 (C^b), 105.8 (C^4), 133.8 (C^5), 141.3 (C^3). $^{195}Pt\{^1H\}$ NMR (DMSO-d_6, 54 MHz): −2178 (s).[1] EM (ESI^+): m/z = 422.05 (Calc. 422.05) $\{[M]+NH_4\}^+$. Anal (%) Calc. for $C_7H_{13}Cl_2N_3Pt$ (FW = 404.01): C, 20.75; H, 3.23; N, 10.37. Found: C, 20.9; H, 3.3; N, 10.3.

2.1.3.2. Cis-[Pt{κ²-N,N′-[1-(CH₂)₂NMe₂]-3,5-Me₂pzol}Cl₂] (**2b**). A suspension of *cis*-[$PtCl_2(DMSO)_2$] (52.8 mg, 125×10^{-3} mol) and ligand **1b** (20.9 mg, 125×10^{-3} mol) in toluene (10 mL) was heated at reflux temperature for 3 h. The reaction mixture was evaporated to dryness and the residue was treated with MeOH (10 mL). The obtained suspension was stirred for 2 h and filtered out. The solid was washed with two 5 mL portions of CH_2Cl_2 to remove the MeOH, and finally dried under vacuum to yield 32.5 mg (60%) of complex **2b** as a yellow solid. IR (cm^{-1}): 3427, 2916, 2342, 1553, 1465, 1450, 1156, 987, 808. ^1H NMR (DMSO-d_6, 500 MHz, gCOSY): 2.33 (s, 3H, Me), 2.43 (s, 3H, Me), 2.45 (m, 2H, $-CH_2-^b$), 2.83 (s, 6H, NMe_2), 4.40 (m, 2H, $-CH_2-^a$), 6.09 (s, 1H, H^4). ^{13}C NMR (DMSO-d_6, 125.9 MHz, gHSQC, gHMBC): 11.1 (Me), 14.7 (Me), 45.4 (C^a), 53.1 (NMe_2), 64.1 (C^b), 107.8 (C^4), 143.5 (C^5), 153.1 (C^3). EM (ESI^+): m/z = 450.10 (Calc. 450.08) $\{[M]+NH_4\}^+$. Anal (%) Calc. for $C_9H_{17}Cl_2N_3Pt$ (FW = 432.04): C, 24.95; H, 3.96; N, 9.70. Found: C, 25.0; H, 4.0; N, 9.4.

2.1.3.3. Cis-[Pt{κ²-N,N′-[1-(CH₂)₂NMe₂]-3,5-Ph₂-pzol}Cl₂] (**2c**). A suspension of *cis*-[$PtCl_2(DMSO)_2$] (26.4 mg, 62.5×10^{-3} mol) and ligand **1c** (18.2 mg, 62.5×10^{-3} mol) in toluene (5 mL) was heated at reflux for 1.5 h. The solid formed was filtered out and purified by column chromatography {Al_2O_3, hexane: CH_2Cl_2 (25:75)} to yield 23 mg (66%) of the title complex as a yellow solid. IR (cm^{-1}): 3430, 2925, 1628, 1480, 1023, 768, 698. ^1H NMR (gCOSY): 2.66 (b m, 2H, $-CH_2-^b$), 3.18 (s, 6H, $-NMe_2$), 4.6 (b m, 2H, $-CH_2-^a$), 6.65 (s, 1H, H^4), 7.45 (m, 2H, $H^{2''}$ and $H^{6''}$), 7.44–7.54 (m, 6H,), 8.30 (d, J = 8.4, 2H, $H^{2'}$ and $H^{6'}$). ^{13}C NMR (gHSQC, gHMBC): 48.7 ($-NMe_2$), 54.0 (C^a), 64.8 (C^b), 107.3 ($C^{4'}$), 127.6 (C^4), 128.4 ($C^{3''}$ and C''), 128.4 ($C^{2''}$ and $C^{6''}$), 128.8 ($C^{2'}$ and $C^{6'}$), 129.3 ($C^{1'}$), 129.4 ($C^{3'}$ and $C^{5'}$), 130.4 ($C^{4''}$), 131.5 ($C^{1''}$), 148.8 (C^5), 157.1 (C^3). $^{195}Pt\{^1H\}$ NMR (54 MHz): −2165 (s). EM (ESI^+): m/z = 521.11 (Calc. 520.58) $\{[M]-Cl\}^+$. Anal (%) Calc. for $C_{19}H_{21}Cl_2N_3Pt.1/2\ H_2O$: C, 40.29; H, 3.92; N, 7.42. Found: C, 40.25; H, 3.8; N, 7.4. $C_{19}H_{21}Cl_2N_3Pt$ (FW = 556.08).

2.1.4. Synthesis of compounds cis-[Pd{κ²-N,N′-[1-(CH₂)₂NMe₂]-3,5-R₂-pzol}Cl₂] (**3**)

2.1.4.1. Cis-[Pd{κ²-N,N′-[1-(CH₂)₂NMe₂]pzol}Cl₂] (**3a**). A suspension of *cis*-[$PdCl_2(DMSO)_2$] (41.7 mg, 125×10^{-3} mol) and ligand **1a** (17.4 mg, 125×10^{-3} mol) in toluene (10 mL) was heated at reflux temperature for 2 h. The reaction mixture was evaporated to dryness and the obtained residue was purified by column chromatography {Al_2O_3, CH_2Cl_2: MeOH

[1] During the acquisition time (more than 24 h) of the $^{195}Pt\{^1H\}$ NMR data the presence of an additional signal at δ = −2982 ppm was also observed in the spectrum. Its intensity increased with time, thus suggesting the formation of a new species in solution.

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(from 2% to 5%)} to yield 28 mg (71%) of the title complex as an orange solid. IR (cm^{-1}): 3447, 3086, 2920, 1628, 1421, 1277, 1109, 1078, 796, 760. ^{1}H NMR (acetone-d_6, 500 MHz, gCOSY): 2.82 (masked m, 2H, $-CH_2-^b$), 2.91 (s, 6 H, NMe_2), 4.70 (m, 2H, $-CH_2-^a$), 6.39 (t, $J=2.5$, 1H, H^4), 7.91 (dd, $J=2.5$, 1H, H^5), 8.17 (d, $J=2.5$, 1H, H^3). ^{13}C NMR (acetone-d_6, 125.9 MHz, gHSQC, gHMBC): 48.4 (C^a), 52.0 (NMe_2), 63.2 (C^b), 106.5 (C^4), 134.2 (C^5), 143.9 (C^3). EM (ESI^+): m/z = 321.0 (320.45) $\{[M]\text{-}Cl+CH_3CN\}^+$. Anal (%) Calc. for $C_7H_{13}Cl_2NPd\cdot 1H_2O\cdot 1/4C_7H_8$: C, 29.40; H, 4.76; N, 11.70. Found: C, 29.5; H, 4.9; N, 11.0.[2] $C_7H_{13}Cl_2NPd$ (FW = 314.95).

2.1.4.2. Cis-[Pd{κ²-N,N′-[1-(CH₂)₂NMe₂]-3,5-Me₂-pzol}Cl₂] (**3b**). A suspension of *cis*-$[PdCl_2(DMSO)_2]$ (41.7 mg, 125×10^{-3} mol) and ligand **1b** (20.9 mg, 125×10^{-3} mol) in toluene (10 mL) was heated at reflux temperature for 2 h. The reaction mixture was evaporated to dryness and the obtained residue was purified by column chromatography (Al_2O_3, CH_2Cl_2: MeOH 1% to 8%) to yield 30 mg (70%) of complex **3b** as an orange solid. IR (cm^{-1}): 3449, 3003, 2916, 2860, 1151, 1465, 1430, 1310, 1091, 1026, 991, 810. ^{1}H NMR (acetone-d_6, 500 MHz, gCOSY): 2.36 (s, 3H, Me), 2.52 (s, 3H, Me), 2.56 (m, 2H, $-CH_2-^b$), 2.80 (s, 6H, NMe_2), 4.68 (m, 2H, $-CH_2-^a$), 6.09 (s, 1H, H^4). ^{13}C NMR (acetone-d_6, 125.9 MHz, gHSQC, gHMBC): 11.1 (Me), 15.6 (Me), 48.9 (C^a), 53.1 (NMe_2), 64.1 (C^b), 106.5 (C^4), 134.0 (C^5), 142.6 (C^3). EM (ESI^+): m/z = 377 (Calc. 375.0) $\{[M]+MeOH\}^+$. Anal (%) Calc. for $C_9H_{17}Cl_2N_3Pd\cdot 3/2H_2O\cdot 1/4C_7H_8$: C, 32.71; H, 5.51; N, 10.65. Found: C, 32.7; H, 5.2; N, 10.7. $C_9H_{17}Cl_2N_3Pd$ (FW = 342.98).

2.1.5. Synthesis of the cyclometallated compounds

2.1.5.1. [Pt{κ²-C,N,N′-{[1-(CH₂)₂NMe₂}-3-(C₅H₄)-5-Ph-pzol]}Cl₂] (**4c**). To a suspension of *cis*-$[PtCl_2(DMSO)_2]$ (52.8 mg, 125×10^{-3} mol) and ligand **1c** (18.2 mg, 125×10^{-3} mol) in toluene (5 mL), a MeOH (1 mL) solution of NaOAc (11.2 mg, 138×10^{-3} mol). The resulting mixture was heated at reflux for 3 h and later on concentrated to dryness. The solid formed was dissolved in CH_2Cl_2 and filtered very slowly through a short pad of celite. The filtrate was evaporated and the obtained residue was submitted to column chromatography {Al_2O_3, hexane:CH_2Cl_2 (75:25)}, to yield 47 mg (72%) of the title complex as a yellow solid. IR (cm^{-1}): 3438, 3046, 2923, 1506, 1448, 1335, 1022, 799, 759, 695. ^{1}H NMR (gCOSY): 2.95 (s, 6H, NMe_2), 3.27 (m, 2H, $-CH_2-^b$), 4.31 (m, 2H, $-CH_2-^a$), 6.50 (s, 1 H, H^4), 6.97–7.08 (m, 2H, $H^{4'}$ and $H^{5'}$), 7.24 (m, 1H, $H^{3'}$), 7.40–7.47 (m, 2 H, $H^{2''}$ and $H^{6''}$), 7.48–7.57 (m, 2H, $H^{3''}$ and $H^{5''}$), 7.71 (dd, $J=5.6$, 3.2 Hz, 1H, $H^{4''}$), 7.89 (dd, $J=7.2$ and 1.6 Hz, 1H, $H^{6'}$). ^{13}C NMR (gHSQC, gHMBC): 45.7 (C^a), 49.2 ($-NMe_2$), 61.7 (C^b), 101.3 (C^4), 121.7 ($C^{3'}$), 123.7 ($C^{5'}$), 127.5 ($C^{4'}$), 128.8 ($C^{2''}$, $C^{4''}$ and $C^{6''}$), 129.3 ($C^{3''}$ and $C^{5''}$), 129.8 ($C^{1''}$), 135.0 ($C^{6'}$), 137.5 ($C^{1'}$), 144.6 (C^5), 160.5 (C^3 and $C^{2'}$). ^{195}Pt{^{1}H} NMR (54 MHz): −3555 (s). EM (ESI^+): m/z = 521.11 (Calc. 521.10){[M] + H}$^+$. Anal (%) Calc. for $C_{19}H_{20}ClN_3Pt$ (FW = 520.10): C, 43.81; H, 3.87; N, 8.07. Found: C, 43.81; H, 3.84; N, 7.77.

2.1.5.2. [Pd{κ²-C,N,N′-{[1-(CH₂)₂NMe₂}-3-(C₅H₄)-5-Ph-pzol]}Cl₂] (**5c**). To a suspension of *cis*-$[PdCl_2(DMSO)_2]$ (41.7 mg, 125×10^{-3} mol) and ligand **1c** (36.4 mg, 125×10^{-3} mol) in toluene (12 mL), a MeOH (1 mL) solution of NaOAc (11.2 mg, 138×10^{-3} mol) was added. The resulting mixture was heated at reflux for 1 h. Afterwards the solvent was evaporated to dryness and the residue formed was dissolved in CH_2Cl_2 and filtered very slowly through a short pad of celite. The filtrate was evaporated and the obtained residue was passed through a Al_2O_3 column using CH_2Cl_2:MeOH (100:1) mixture as eluent. The band collected gave, after concentration to dryness, **5c** as a white solid (38 mg, 70%). IR (cm^{-1}): 3448, 3038, 2924, 2856, 1528, 1449, 1387, 796, 758, 698. ^{1}H NMR (gCOSY): 2.77 (s, 6H, NMe_2), 2.99 (m, 2H, $-CH_2-^b$), 4.25 (m, 2H, $-CH_2-^a$), 6.45 (s, 1 H, H^4), 6.95 (td, J = 7.6 and 1.6, 1H, $H^{4'}$), 7.01 (td, J = 7.2 and 1.2, 1H, H5′), 7.23(dd, J = 7.6 and 1.6, 1H, $H^{3'}$), 7.40–7.46 (m, 2H, $H^{2''}$ and $H^{6''}$), 7.50–7.56 (m, 3H, $H^{3''}$, $H^{4''}$ and $H^{5''}$), 7.87 (d, J = 7.2, 1H, $H^{6'}$). ^{13}C NMR (gHSQC, gHMBC): 45.2(C^a), 48.5 ($-NMe_2$), 60.9 (C^b), 101.0 (C^4), 122.1 ($C^{3'}$), 124.5 ($C^{5'}$), 127.1 ($C^{4'}$), 128.9 ($C^{2''}$, $C^{4''}$ and $C^{6''}$), 129.2 ($C^{3''}$ and $C^{5''}$), 129.8 ($C^{1''}$), 136.6 ($C^{6'}$), 137.5 ($C^{1'}$), 145.1 (C^5), 148.4 ($C^{2'}$), 160.5 (C^3). EM (ESI^+): m/z = 396.07 $\{[M]\text{-}Cl\}^+$. Anal (%) Calc. for $C_{19}H_{20}ClN_3Pd$ (FW = 431.04): C, 52.80; H, 4.66; N, 9.72. Found: C, 52.55; H, 4.6; N, 9.9.

2.2. Crystallography

A prismatic crystal of **2a** (sizes in Table 1) was selected and mounted on a MAR345 diffractometer with an image plate detector. Unit-cell parameters were determined from 5843 reflections ($3°<\theta<31°$) and refined by least-squares method. Intensities were collected with graphite monochromatized Mo K_α radiation. 9754 reflections were measured (in the range $2.39°\le\theta\le32.38°$) of which 2861 were non-equivalent by symmetry $\{R_{int}(\text{on } I)=0.072\}$ and 2741 reflections were assumed as observed applying the condition $I>2\sigma(I)$. Lorentz-polarization and absorption corrections were made.

The structure was solved by Direct methods using SHELXS computer program [48] and refined by full-matrix least-squares method with SHELX97 computer program [49] using 9754 reflections, (very negative intensities were not assumed). The function minimized was $\Sigma w|\,|Fo|^2-|Fc|^2\,|^2$, where $w=[\sigma^2(I)+(0.0518P)^2+0.5576P]^{-1}$, and $P=(|Fo|^2+2|Fc|^2)/3$; f, f' and f'' were taken from *International Tables of X-Ray Crystallography* [50]. All H atoms were computed and refined, using a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the atom to which is linked. The final R(on F) factor was 0.033, wR(on $|F|^2$) = 0.085 and goodness of fit = 1.062 for all observed reflections. Number of refined parameters was 118. Max. shift/esd = 0.00, Mean shift/esd = 0.00. Max. and min. peaks in final difference synthesis were 2.767 and −2.054 eÅ^{-3}, respectively.

Table 1
Crystal data and details of the structure refinement for **2a**. Standard deviations are given in parentheses.

Empirical formula	$C_7H_{13}Cl_2N_3Pt$
Formula weight	405.19
Temperature (K)	293 (2)
λ (Å)	0.71073
Crystal size (mm × mm × mm)	0.2 × 0.1 × 0.1
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	7.162 (3)
b (Å)	8.725 (3)
c (Å)	17.478 (4)
α = γ (deg.)	90
β (deg.)	102.76 (2)
Volume (Å^3)	1065.2 (6)
Z	4
$D_{calc.}$ (Mg × m^{-3})	2.527
μ (mm^{-1})	13.633
F(000)	752
Θ for data collection (deg.)	From 2.39 to 32.38
N. of reflections collected	9754
N. of unique reflections, [R(int)]	2861[0.0721]
N. of parameters	118
Completeness to Θ = 25.00°	94.2%
Goodness-of-fit on F^2	1.062
Final R indices [$I>2\sigma(I)$]	$R_1=0.0327$, $wR_2=0.0850$
R indices (all data)	$R_1=0.0338$, $wR_2=0.0859$
Largest diff. peak and hole	2.767 and −2.054 e.Å^{-3}

[2] Compounds **3a** and **3b** retain solvents. Evidence of the presence of small amounts of toluene is in the ^{1}H NMR spectra (a singlet at 2.3 ppm). Despite the fact that the two compounds were dried in the vacuum for a week, it was impossible to evaporate the solvents. The best analytical results are presented above.

2.3. Biological studies

2.3.1. Cell culture

Human lung carcinoma A549 cells (from the American Type Culture Collection), MBA MD231 and MCF7 (from the European Collection of Cell Cultures — ECACC) were used in all the experiments. Cells were grown as a monolayer culture in minimum essential medium (DMEM with L-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum, 10 mM of D-glucose and 0.1% streptomycin/penicillin in standard culture conditions.

2.3.2. Cell proliferation assay

The assay was performed by a variation of the method described by Mosmann et al. [51] as specified by Matito and coworkers [52]. In brief, 3×10^3 A549 cells/well were cultured in 96 well plates. Concentrations that inhibited cell growth by 50% (IC_{50}) after 72 h of treatment were calculated based on the survival rate compared with untreated cells. Relative cell viability was measured by the absorbance on an ELISA (enzyme-linked immunosorbent assay) plate reader (Tecan Sunrise MR20-301, TECAN, Salzburg, Austria) at 550 nm.

2.3.3. Cell viability assay

The 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 concentrations in cell media were 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. [53]. In brief, MDA MB231 and MCF7 cells were plated at 5000 and 10,000 cells/well, respectively, in 100 μL media in tissue culture 96 well plates (Cultek). After 24 h, media was replaced by 100 μL/well of serial dilution of drugs. Control wells did not contain compounds. 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 5–10% of uninhibited control values. Plates were incubated 72 h. Hexosaminidase activity was measured according to the following protocol: the media containing was removed and cells were washed once with PBS 60 μL of substrate solution (*p*-nitrophenol-*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 appears; then, plates could be developed by adding 90 μL of developer solution (glycine 50 mM, pH 10.4; EDTA 5 mM), and absorbance was recorded at 410 nM.

2.3.4. DNA migration studies

Plasmid pBluescript SK+ was obtained using a QIAGEN plasmid midi kit as described by the manufacturer. Interaction of drugs with pBluescript SK+ plasmid DNA (Stratagene) was analyzed by agarose gel electrophoresis following a modification of the method described by Abdullah et al.[54]. In brief, plasmid DNA aliquots (40 μg/mL) were incubated with different concentrations of the platinum and palladium compounds (ranging from 5 μM to 100 μM) at 37 °C for 24 h. For comparison, cisplatin was used as a positive control. Aliquots of 20 μL of compound: DNA complexes containing 0.8 μg of DNA were subjected to 1% agarose gel electrophoresis in TAE buffer (40 mM Tris–acetate, 2 mM EDTA, pH 8.0). The gel was stained in the same buffer containing ethidium bromide (0.5 $mg.mL^{-1}$) and visualized and photographed under UV light.

2.3.5. In vitro antimalarial assay

The 3D7 chloroquine-susceptible *P. falciparum* clone (Africa) and the W2 chloroquine resistant clone (Indochina) were maintained in culture in RPMI 1640 (Invitrogen, Paisley, United Kingdom), supplemented with 10% human serum (Abcys S.A., Paris, France) and buffered with 25 mM HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 25 mM $NaHCO_3$. Parasites were grown in A-positive human blood under controlled atmospheric conditions that consisted of 10% O_2, 5% CO_2 and 85% N_2 at 37 °C with a humidity of 95%. All strains were synchronized twice with sorbitol before use. Clonality was verified using PCR genotyping of polymorphic genetic markers, *msp1*, *msp2* and microsatellite loci [55,56].

Chloroquine (CQ) diphosphate was purchased from Sigma (Saint Louis, MO). CQ was suspended in water in concentrations ranging from 5 to 3200 nM. Compounds **1**–**5** were suspended in methanol and then diluted in RPMI to obtain final concentrations ranging from 0.01 μM to 500 μM.

For in vitro isotopic microtests, 25 μL/well of antimalarial drug and 200 μL/well of the parasitized red blood cell suspension (final parasitemia, 0.5%; final hematocrit, 1.5%) were distributed into 96 well plates. Parasite growth was assessed by adding 1 μCi of tritiated hypoxanthine with a specific activity of 14.1 Ci/mmol (Perkin-Elmer, Courtaboeuf, France) to each well at time zero. The plates were then incubated for 48 h in controlled atmospheric conditions. Immediately after incubation, the plates were frozen and thawed to lyse erythrocytes. The contents of each well were collected on standard filter microplates (Unifilter GF/B; Perkin-Elmer) and washed using a cell harvester (Filter-Mate Cell Harvester; Perkin-Elmer). Filter microplates were dried, and 25 μL of scintillation cocktail (Microscint O; Perkin-Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured with a scintillation counter (Top Count; Perkin-Elmer).

The IC_{50}, the drug concentration able to inhibit 50% of parasite growth, was assessed by identifying the drug concentration corresponding to 50% of the uptake of tritiated hypoxanthine by the parasite in the drug-free control wells. The IC_{50} value was determined by nonlinear regression analysis of log-based dose–response curves (Riasmart™, Packard, Meriden, USA). IC_{50} are expressed as means of 3 to 4 experiments ± standard deviation.

3. Results and discussion

3.1. Synthesis and characterization

The preparation of ligands **1a** and **1b** was carried out by a modification of former literature protocols [57,58] consisting in the alkylation of 1*H*-pyrazole and 3,5-dimethyl-1*H*-pyrazole respectively with 2-dimethylaminoethyl chloride using sodamide as a base (Scheme 1). The best yield for the synthesis of **1c** was obtained by alkylation of 3,5-diphenyl-1*H*-pyrazole using Aliquat 336 as a phase transfer catalyst. The three ligands were fully characterized by NMR, IR and mass spectroscopy. To the best of our knowledge, these data were found to be absent or incomplete in the literature.

The reactivity of ligands **1a**–**1c** with *cis*-$[MCl_2(DMSO)_2]$ {M=Pt(II) or Pd(II)} was studied under different experimental conditions. Treatment of equimolar amounts of the corresponding ligand (**1a**–**1c**) and of *cis*-$[PtCl_2(DMSO)_2]$ in toluene under reflux gave after work up yellow solids that were identified as the corresponding *cis*-$[Pt\{\kappa^2\text{-}N,N'\text{-}\{[1\text{-}(CH_2)_2NMe_2\}\text{-}3,5\text{-}R_2\text{-}pzol]\}Cl_2]$ (**2a**–**2c** in Scheme 1). The palladium(II) analogs (**3a**–**3c**) were isolated using the same strategy.

However, treatment of **1c** with *cis*-$[PtCl_2(DMSO)_2]$ in a toluene: methanol (12:1) mixture in the presence of a slight excess (~10%) of NaOAc, produced the activation of the *ortho* σ(C–H) bond of the phenyl ring on position 3 giving the platinacycle $[Pt\{\kappa^3\text{-}C,N,N'\text{-}[1\text{-}\{(CH_2)_2NMe_2\}\text{-}3\text{-}(C_6H_4)\text{-}5\text{-}Ph\text{-}pzol]\}Cl]$ **4c**. When *cis*-$[PtCl_2(DMSO)_2]$ was replaced by its palladium(II) analog, the palladacycle $[Pd\{\kappa^3\text{-}C,N,N'\text{-}[1\text{-}\{(CH_2)_2NMe_2\}\text{-}3\text{-}(C_6H_4)\text{-}5\text{-}Ph\text{-}pzol]\}Cl]$ **5c** was achieved. It should be noted that this product could also be isolated in the absence of the base in the reaction medium.

The new complexes were characterized by elemental analyses, mass spectrometry, infrared spectroscopy and ^1H, ^{13}C and two-dimensional homo (gCOSY) and heteronuclear (gHSQC and gHMBC) correlations. In the $^{13}C\{^1H\}$ NMR spectra of cyclometallated compounds **4c** and **5c** the intensity of the signal due to the $C^{2'}$ atom decreased substantially and was low-field shifted when compared with that of the free ligand **1c**. In

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addition, no evidence of cross-peak between the resonance of the $C^{2'}$ nuclei and those of the aromatic protons was detected in the [^{1}H–^{13}C]-HSQC spectra of compounds **4c** and **5c**. According to previous studies [59,60], these observations suggested the existence of a σ(M–$C^{2'}$) bond and the presence of five membered ring metallacycle in **4c** and **5c**. ^{195}Pt{^{1}H} NMR spectra not only provided convincing evidence of the coordination sphere of the platinum and structure of **2c** and **4c**, but also explained the variations produced by the different mode of binding of ligand **1c**. The spectrum of **2c** showed a singlet at −2165 ppm, the position of which is consistent with the values reported for related complexes with a "(*N,N′*)Cl_2" environment around the platinum(II) [60–62]. For **2c** the signal appeared at higher fields (δ = −3555 ppm); this trend agrees with those reported for complexes containing R–CH=N–$(CH_2)_n$$NMe_2$ (R = phenyl or ferrocenyl moieties) as bidentate (*N,N′*) or terdentate $(C,N,N')^-$ ligands [61,62]. The ^{195}Pt NMR spectrum of **2b** was not recorded due to the low solubility of this product and for **2a**, two singlets of relative intensities (1.0:0.3) centered at δ = −2178 and −2982 ppm were observed. The chemical shift of the former one is similar to that of **2c** and the presence of the second and less intense signal suggested that this product is less stable in $CDCl_3$ than its analog **2c**.

Complex **2a** was also characterized by X-ray diffraction. Its molecular structure and the atom numbering scheme are presented in Fig. 2. The crystal contains molecules of [Pt{κ^2-*N,N′*-[1-$(CH_2)_2NMe_2$]-Ph_2-pzol}Cl_2] (**2a**) in which the platinum(II) atom is bound to the two nitrogen atoms [N(1) and N(3)] of the pyrazolyl ligand and to two chlorides [Cl(1) and Cl(2)], in a slightly distorted square-planar environment thus confirming the mode of binding of the ligand. The Pt–Cl(1) and Pt–Cl(2) bond lengths are practically identical (the differences do not clearly exceed 3σ) and fall in the range found in Pt(II) complexes having *cis*-coordinated chlorido ligands trans to N-donor ligands.[63–68] The Pt–N(1) [2.010(3) Å] and Pt–N(3) [Pt–N(3) 2.091(4) Å] bond lengths are within the normal distances (1.98–2.06 Å) reported for the same type of complexes and the differences may be attributed to the different basicities of the two donor atoms {N(amine) versus N(heterocycle)}[69].

Each molecule of *cis*-[Pt{κ^2-*N,N′*-[1-$(CH_2)_2NMe_2$]-3,5-Ph_2-pzol}Cl_2] (**2a**) contains a [5.6] bicyclic system formed by the pyrazolyl unit that shares the N(2)–N(3) bond with the six membered chelate ring generated by the coordination of the platinum(II) to the N(1) and N(2) atoms of the ligand. Bond lengths and angles of the pyrazolyl unit agree with those reported for most metal complexes containing this azole. The heterocycle is planar[3] and it forms an angle of ca. 22.3° with the coordination plane of the platinum(II). The puckering analyses of the six-membered chelate ring is formed by the set of atoms Pt, N(1), N(2), C(4), C(5) and N(3) [θ = 106.6(4)° and ϕ = 30.3 (4)° indicates that it adopts a twisted-boat conformation.

In each molecule of *cis*-[Pt{κ^2-*N,N′*[1-{$(CH_2)_2NMe_2$}pzol]}Cl_2], the distances Cl(1)···H(7B) [2.642 Å], Cl(1)···H(6C) [2.699 Å], and Cl(2)···H(1) [2.721 Å] are smaller than the sum of the van der Waals radii of these atoms (Cl, 1.7 Å and H, 1.0 Å) [70] thus suggesting weak C–H···Cl intramolecular interactions.

In the crystal a molecule at (*x*, *y*, *z*) is connected to two different and vicinal ones [at (*x*, *1 + y*, *z*) and (*−1 + x*, *y*, *z*)] by four weak intermolecular C–H···Cl interactions (Fig. 3) forming chains.

3.2. Biological studies

A human lung carcinoma cell line (A549) and two human breast cancer cell lines (MDA MB231 and MCF7) were used to test the cytotoxic activity of the synthesized compounds. Cisplatin was used as a positive control, showing a value of IC_{50} below 20 μM in the three cancer cellular lines (Table 2).

[3] Deviations from the mean plane: N(1), −0.005(4); N(2), 0.008(5); C(1) 0.001(5), C(2), 0.004(6) and C(3), −0.008(7) Å.

Table 2
Cytotoxic activities on A549 human lung carcinoma and MDA-MB231 and MCF7 breast cancer cell lines for the free ligands and their platinum(II) or palladium(II) complexes, using cisplatin as reference. Data are shown as the mean SD of two or more experiments performed in triplicate.

IC_{50} values (μM)			
	A549	MDA-MB231	MCF/
A) Free ligands			
1a	>100	>100	>100
1b	>100	>100	>100
1c	55 ± 14	64 ± 24	52 ± 10
B) Platinum(II) complexes			
2a	3 ± 1	57 ± 8.5	20 ± 3.6
2b	12 ± 5	62 ± 9.3	51 ± 10.4
2c	13 ± 5.8	14 ± 1.7	17 ± 2.2
4c	7 ± 2.8	6.2 ± 1.3	9.3 ± 3.4
C) Palladium(II) complexes			
3a	>100	>100	>100
3b	73.5 ± 2.1	>100	>100
5c	38.5 ± 4.9	16.2 ± 4.6	38.4 ± 16.5
Cisplatin	9.3 ± 3.0	6.5 ± 2.4	19 ± 4.5

For the purely organic ligands **1a**–**1c**, a moderate activity was observed for the 3,5 diphenyl substituted compound **1c** (IC_{50} values between 52 and 64 μM). In all cases, the Pd(II) and Pt(II) complexes were more potent than their corresponding parent ligand and compound **2a** exhibited in human lung A549 cancer cell line the highest potency (IC_{50} = 3 μM) of the synthesized complexes and a notable selectivity for lung cancer cell line versus the two breast cancer cell lines (MDA MB 231 and MCF7) selected. Interestingly, **2a** turned out to be three times more potent than cisplatin in lung A549 cancer cell line. In the three cancer cell lines, the platinacycle **4c** exhibited the highest cytotoxic activity with IC_{50} values in the range of 6.2–9.3 μM. It was more effective against human lung carcinoma (A549) cells and breast cancer (MDA MB231 and MCF7) cells than the reference drug cisplatin. Cytotoxicity effectiveness of **4c** was approximately twice that of the coordination complex **2c** in the three cancer cell lines. Compound **4c** arises from the coordination of the two nitrogen atoms and the formation of a σ(Pt–C) bond, this leads to a [6.5.5.6] tetracyclic system. Consequently, it has a higher degree of rigidity and planarity than **2c**. It is well-known that square-planar metal complexes with aromatic ligands bind to DNA by intercalation [71–74] and consequently compound **4c** may behave not only as alkylating but also as an intercalating agent. This potential dual behavior may account for the increased potency of the platinacycle **4c** when compared with **2c**.

Although compounds **4c** and **5c** only differ by the nature of the M(II) ion, the platinum(II) complex **4c** is (2.6–5.5 times depending on the cell line) more potent than its Pd(II) analog **5c**. This may be due to the greater lability and faster hydrolysis rate of palladium complexes compared to their platinum equivalents [75]. Therefore, higher cytotoxicity of **5c** could be also connected to slower hydrolysis of Pt–Cl bond and to the stability of this compound compared to the palladium(II) derivative **5c**.

The effect of binding of the compounds investigated in this study on supercoiled DNA was determined by their ability to alter the electrophoretic mobility of pBluescript plasmid DNA: supercoiled closed circular (cc) and open circular (oc) forms.

Fig. 4 shows the electrophoretic mobility of native pBluescript DNA incubated with the synthesized compounds (**1c** and **2**–**5**) at 5 μM or 50 μM concentration. To provide a basis for comparison, incubation of DNA with cisplatin was also performed using the same concentrations and conditions. As expected, at both 5 μM and 50 μM concentrations, cisplatin greatly altered the electrophoretic mobility of pBluescript DNA. At 5 μM concentration, none of the assayed compounds produced a significant effect on the electrophoretic mobility of native pBluescript DNA. At this concentration, the mobility of the supercoiled closed circular form was only slightly decreased by the platinum(II) compound **2a** and the

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palladium(II) compound **5c**. Consistently, at 50 μM, both compounds greatly alter the mobility of plasmid DNA. For these two compounds (**2a** and **5c**), an unwinding assay was performed with increasing amounts of drugs ranging from 5 μM to 100 μM (Fig. 5). For complex **2a**, the migration rate of supercoiled band decreases until it comigrates with the nicked relaxed band. In this titration experiment of 40 μg/mL pBluescript, the coalescence point, defined as the amount of platinum complex that is necessary for complete removal of all supercoils from DNA, occurs with 50 μM concentration of **2a**. The lowest efficiency of complex **2a** than cisplatin in removing the supercoils from DNA could be related to the shorter incubation time (24 h) of the experiments compared to the incubation time in the experiments with cancer cell lines (72 h). Under these conditions, hydrolysis of the platinum complex should not occur.

For the palladium(II) compound **5c**, the rate of migration of supercoiled band also decreases as drug concentration increases up to 10 μM. At higher concentrations the migration rate begins to increase again in parallel to a decrease in the bands intensity. At higher concentrations of **5c** (more than 100 μM), DNA is no longer visible.

Regarding the other assayed complexes (Fig. 4), at 50 μM concentration, small changes on the migration rate of the supercoiled closed circular plasmid DNA were observed for most of them, with the exception of the free ligand **1c** and the platinum(II) complex **4c**. When assayed at higher concentrations (500 μM), the synthesized compounds dramatically altered plasmid DNA mobility (data not shown). Again, compounds **1c** and **4c** did not produce any effect. Paradoxically, as it was mentioned before, complex **4c** exhibited a great cytotoxicity in the three selected human cancer cell lines.

Overall, these results indicated that most of the compounds investigated in this study interact with DNA, **2a** and **5c** exhibiting the highest effect on plasmid DNA mobility. These two compounds may behave as alkylating agents acting by the same mechanism as cisplatin. However, compound **4c** is hypothesized to act on tumor cells through a different mechanism than cisplatin.

We also evaluated the potential activities of products **1**–**5** against the chloroquine-susceptible strain (3D7) and the chloroquine-resistant strain (W2) of *P. falciparum* (Table 3). The free ligands exhibited poor (for **1a**–**1b**) to moderate (for **1c**) antimalarial activities. In contrast with these results, the platinum(II) complexes (**2a**–**2c**) with a $(N,N')Cl_2$ environment demonstrated higher in vitro activity against *P. falciparum* chloroquine-susceptible and chloroquine-resistant clones than their corresponding parent ligand. The toxicity of platinacycle **4c** was low on *P. falciparum* parasites (IC_{50} of 111.7 μM on 3D7 and 138.0 μM on W2). The cyclopalladated complex **5c** was 10-times more potent than its platinum(II) analog **4c** against *P. falciparum*. Compound **2c** showed similar cytotoxic activity on *P. falciparum* parasites and cancer cells. Compound **5c** was 2 to 3-times more potent against *P. falciparum* parasites than against cancer cells.

In vivo efficiency of platinum derivatives has been already demonstrated in malaria. Cisplatin (Fig. 1) cured mice infected with *Plasmodium berghei* at a dose of 6 mg/kg body weight [76]. The Pd-cyclometallated derivative **5c** was more efficient than Pt-protoporphyrin against *P. falciparum* (38 μM) [77]. However, these compounds were less potent against the two *P. falciparum* clones 3D7 and W2 than Fe-derivatives, such as ferroquine and analogs [78,79], ferrocenic derivatives from ciprofloxacin [80], or Ru, such as ruthenoquine and analogs [42].

Table 3
In vitro activity of ligands **1**, compounds **2**–**5** and chloroquine against chloroquine susceptible *P. falciparum* 3D7 clone and the chloroquine resistant W2 clone. IC_{50} are expressed as means of 3 to 4 experiments ± standard deviation.

IC_{50} values (μM)		
	3D7	W2
A) Free ligands		
1a	182.3 ± 19.6	196.3 ± 13.8
1b	248.0 ± 15.5	183.3 ± 16.6
1c	38.4 ± 4.2	20.2 ± 4.3
B) Platinum(II) complexes		
2a	18.9 ± 1.2	22.5 ± 0.8
2b	18.3 ± 0.7	24.9 ± 2.4
2c	13.8 ± 4.5	16.1 ± 1.1
4c	111.7 ± 18.0	138.0 ± 6.2
B) Palladium(II) complexes		
3a	133.3 ± 11.6	111.0 ± 10.6
3b	132.0 ± 10.1	85.8 ± 3.1
5c	10.3 ± 5.5	19.2 ± 0.8
Chloroquine	0.020 ± 0.004	0.56 ± 0.09

4. Conclusions

The study of the reactivity of the three pyrazolyl ligands [1-$(CH_2)_2NMe_2$-3,5-R_2-pzol] {with R=H (**1a**), Me (**1b**) or Ph (**1c**)} with $[MCl_2(DMSO)_2]$ (M=Pd or Pt) under different experimental conditions reveals that they may act as a bidentate (*N,N′*) (in **2a**–**2c** and **3a**–**3c**) or as terdentate $(C,N,N')^-$ ligand (in **4c** and **5c**). It is noteworthy that **4c** and **5c** are the first examples of platina- and palladacycles with $(C,N_{pyrazole},N')^-$ pincer ligands reported so far. The in vitro antimalarial activity was evaluated against one chloroquine susceptible strain 3D7 and three chloroquine-resistant strains W2 of *P. falciparum*. Compounds **2a**–**2c** and **5c** were found to exhibit significant in vitro activity (with IC_{50} in the microM range). On the contrary, compound **4c** was inactive in similar experimental conditions. The evaluation of the in vitro cytotoxic activity of complexes **2c**–**5c** revealed that they exhibit growth inhibitory activity against lung (A549) and breast (MDA MB 231 and MCF7) human cancer cell lines. The comparison of the results obtained for the three types of complexes in human carcinoma A549 cell line indicates that the obtained IC_{50} values follow the trend **2a**<**4c**<cisplatin<**2b**<**2c**<**5c**<**3b**<**3a**. This means that: a) the platinum(II) derivatives are more potent than their palladium(II) analogs, b) for the platinum(II) complexes the change of binding mode of the ligand from (*N,N′*) in **2c** to (*C,N,N′*) in **4c** increases the cytotoxic activity, and c) compound **2a** is specially relevant due to remarkable potency in lung cancer A549 cell line (twice that of **4c** and three times more potent than cisplatin). It is noteworthy that complex **4c**, the first example of a pyrazole containing platinacycle, shows greater antitumoral in vitro activity than cisplatin. The platinum and palladium complexes evaluated in this study more or less exhibit an effect on DNA electrophoretic mobility. Complexes **2a** and **5c** are those exhibiting the strongest interaction with DNA, and both display moderate to good cytotoxic activities towards different cancer cell lines. In particular complex **2a** is the most potent of the new synthesized compounds in human lung carcinoma A549 cell line (IC_{50} value of 3 μM). An exception occurs in the case of compound **4c** that exhibits a considerable cytotoxicity in several cancer cell lines but has no effect on plasmid DNA mobility. These results underscore the importance of additional factors in predicting anticancer activity.

New Pt- and Pd-cyclometallated derivatives will be synthetized to improve antimalarial activity. Finally it should be noted that the methods and strategies described here, especially those concerning complex **4c**, constitute the first step toward the development of new but closely related platinum(II) complexes with pincer $(C,N;N')^-$ ligands derived from pyrazole. These compounds may exhibit improved biological activities owing to the conversion of **4c** into ionic products of the type [Pt(*C,N,N′*)(L)]X (L = neutral ligand and X = monoanion), which may be more soluble in polar solvents, or [Pt(*C,N,N′*)(CCPh)] that have an additional interest in view of their potential luminescence. The rich chemistry of pyrazole ligands also permits to introduce changes on the pendant arm of the nitrogen N_1 or on the pyrazole ring. These strategies open up a vast array of possibilities.

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Appendix A. Supplementary material

The electronic crystallographic information file (.cif) for **2a**. will be deposited at the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ, UK.

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