Highly selective metal mediated *ortho*-alkylation of phenol. First platinum containing organometallic chromane analogues†‡

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We were able, for the first time, to synthesize and characterize Pt derivatives with a structural shape similar to vitamin E, having a metalla-chromane core. The formation reaction mechanism includes an unexpected highly selective *ortho* aromatic electrophilic substitution on phenol, operated by [PtCl(η¹-C_2H_4OR)(N–N)], R = Me or Ph, and a final cyclization step. The X-ray structure of one of the new metalla-chromane complexes [Pt(**EtPh**)(**phen**)], **1a**, (**EtPh** = 2-(ethan-2′-yl-kC^1)-1-phenolato-kO^1, **phen** = 1,10-phenanthroline) is reported. Cytotoxicity and Pt uptake measurements, performed on HeLa cancer cells, show an interesting structure–activity correlation for the new metalla-chromane analogues **1a** and [Pt(**MeOEtPh**)(**phen**)], **1b**, (**MeOEtPh** = 2-(ethan-2′-yl-kC^1)-4-(methoxy)-1-phenolato-kO^1), being the structurally closest to vitamin E and also the most active.

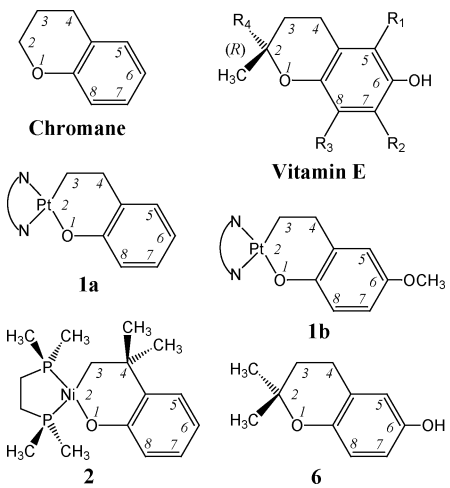
Introduction

Bioorganometallic chemistry, a rapidly emerging area of organometallic chemistry, has recently provided significant advancements in structural diversity and molecular recognition studies.[1] Complex systems, characterized by the presence of both coordination and organometallic bonds, considerably increase the number of available structures for biomimetic and molecular recognition investigations. The presence of a metal in a biomimetic molecule may be useful, in principle, for a number of reasons. Among them, one should consider the possibility to send a particular metal to a specific target for therapeutic (platinum derivatives are currently used in the chemotherapy of many cancer forms[2]) and/or imaging purposes. Moreover, metal containing biomimetic molecules, allow a wider range of structural modification and screening possibilities (with respect to plane organic compounds) in host guest related Quantitative Structure–Activity Relationships (QSAR) analysis. In particular, cyclometallated Pt(II) and Pd(II) compounds have been recently synthesized and tested for their cytotoxic activity due to possible interactions with DNA.[3–8]

Among cyclometallated complexes organometallic metalla-chromane systems, of many metals,[9–19] are widely reported in the literature. It should be noted that, due to the structural analogies with vitamin E, such complexes, could also be used to address a metal to specific targets. Here we discuss the synthesis, characterization and biological activity of the first Pt(II) metalla-chromane complexes obtained by a novel highly selective metal mediated *ortho*-alkylation of phenols.

Results and discussion

In this work we report the synthesis of [Pt(**EtPh**)(**phen**)], **1a**, and [Pt(**MeOEtPh**)(**phen**)], **1b**, the first Pt containing organometallic species having the same structural core of vitamin E, a remarkable family of biologically active chromane derivatives, Scheme 1. Compounds **1a–b** are characterized by substitution of the carbon atom in the 2 position within the chromane pseudo-pyran heterocycle with a square planar Pt(II) centre, which also carries a chelate nitrogen ligand, Scheme 1. Compounds **1a–b**, are unprecedented in Pt chemistry and represent, together with previously reported [Ni(**Me₂EtPh**)(N–N)], **Me₂EtPh** = 2-(ethan-1′,1′-dimethyl-2′-yl-kC^1)-1-phenolato-kO^1, N–N = **phen**, **dip** = 2,2′-dipyridyl, and [Ni(**Me₂EtPh**)(**dmpe**)], **2**, **dmpe** = 1,2-bis-(dimethylphosphino)ethane complexes,[9] all the available metalla-chromanes, of d^8 metals, at present, Scheme 1.



Scheme 1 Structure of chromane, vitamin E (tocopherols and tocotrienols; R_1, R_2, R_3 = H or Me; R_4 = phytol side chain) and the chromane derivatives **1a**, **1b** (N–N = 1,10-phenanthroline), **2** and **6**. The conventional numbering scheme of chromane is also reported.

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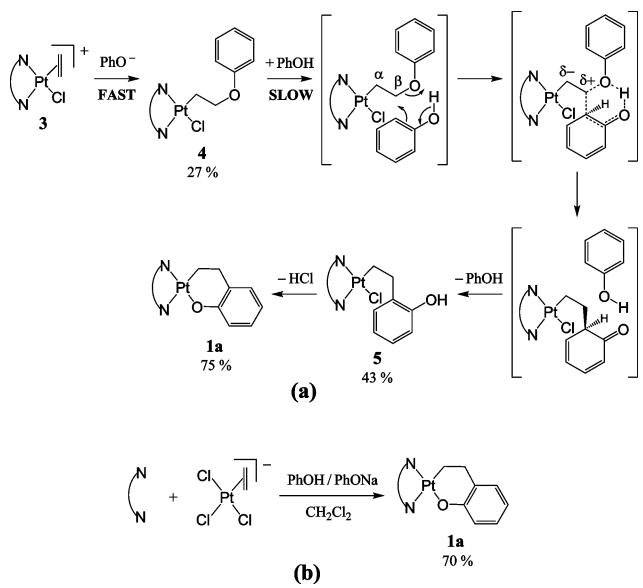
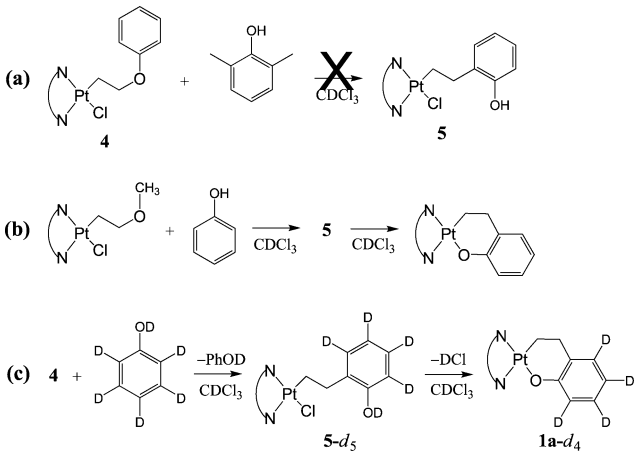
‡ Electronic supplementary information (ESI) available: Selected bond lengths and angles for complex **1a**. See DOI: 10.1039/b712248a

Step-by-step synthesis of **1a** suggests that the formation reaction mechanism occurs according to Scheme 2, eqn (a). The cationic species $[PtCl(\eta^2\text{-}C_2H_4)(\mathbf{phen})]^+$, **3**,[20] undergoes a fast nucleophilic attack of phenolate oxygen, to give $[PtCl(\eta^1\text{-}C_2H_4OC_6H_5)(\mathbf{phen})]$, **4**, which can be obtained as the sole reaction product (isolated yield 27%, based on **3**), if only phenolate is used (see Experimental). In the presence of a phenol/phenolate mixture, compound **4** undergoes a transposition which gives $[PtCl\{o\text{-}(\eta^1\text{-}C_2H_4)\text{-phenol}\}(\mathbf{phen})]$, **5**, *via* the concerted mechanism, depicted in Scheme 2, eqn (a). This requires that the same phenol which protonates the ether oxygen of **4** undergoes electrophilic attack on the *ortho* position by the incipient carbocation formed at the β carbon of **4**. The overall transposition can therefore be considered as an electrophilic substitution at the *ortho* position of the phenol which occurs in unusually very mild conditions. The phenol/phenolate ratio must be carefully set in the reaction since excess acidity inhibits cyclization of **5** to give **1a**. Indeed compound **5** can be obtained (isolated yield 43% based on **3**) if the phenol/phenolate ratio is strongly increased in favour of phenol. The possibility of an alternative direct intramolecular rearrangement of **4**, to give **5**, has been excluded since: (i) the rate of formation of **5**, starting from isolated **4**, strongly depends on the concentration of the phenol, according to Scheme 2, eqn (a); (ii) in the presence of an acid, different from phenol, but with a similar pK_a (*i.e.* 2,6-dimethylphenol or 2,4,6-trimethylphenol), **4** does not give any observable reaction (after 48 h), Scheme 3, eqn (a); (iii) compound **5** could also be obtained in the reaction of $[Pt(\eta^1\text{-}C_2H_4OCH_3)Cl(\mathbf{phen})]$ with phenol, Scheme 3, eqn (b); (iv) in the presence of excess phenol-d_6, **4** gives **5**-d_5 and therefore **1a**-d_4, fully deuterated at the phenolate ring, Scheme 3, eqn (c).



Scheme 2 Step-by-step (a) and one-pot (b) synthesis of complex **1a** (N–N = 1,10-phenanthroline). Isolated yields are referenced to Pt.

Interestingly, the metalla-chromanes **1a–b** could also be directly obtained, starting from Zeise's salt, phenol, phenolate and the bidentate nitrogen donor, Scheme 2, eqn (b), or **3** and the phenol, phenolate mixture in a one-pot reaction. The phenol/phenolate ratio strongly affects the yield of complex **1** also in the one-pot reactions starting either from Zeise's salt or **3**. Optimized



Scheme 3

experimental conditions for the synthesis of **1** require that phenolate should be almost twice the stoichiometric amount with respect to the Zeise's salt or **3**. This is because phenolate is also needed to neutralize excess HCl produced in the final cyclization of **5**. Phenol/phenolate ratios in the range 5–10 gave both high yields and reaction rates.

According to organic chemistry rules, alkylation on phenols should take place at the activated both *ortho* and *para* positions. This is the case for the reported formation of ring alkylated products, from alkyl phenyl ethers, in the presence of Brønsted/Lowry acids, although the reaction conditions are considerably more drastic in that case.[21] The total absence of *para* alkylated derivatives in the reaction of **4** with phenol, which selectively gives **5**, further supports the concerted mechanism of Scheme 2, eqn (a). The high concentration of phenol, required only to speed up the reaction, is consistent with the need of such a suitable acid in the isomerization step which gives **5**. This excludes *a priori* a possible mechanism involving an intramolecular Claisen-type rearrangement, previously reported for the formation of Si and Ge chromane derivatives.[19] Noteworthy the present highly selective metal mediated *ortho*-alkylation of the phenol also represents a new synthetic application of olefin containing cationic species,[22] $[PtCl(\eta^2\text{-olefin})(N\text{–}N)]^+$, since in their derivatives $[Pt(\eta^1\text{-}CR_1R_2\text{-}CR_3R_4\text{-}Nu)Cl(N\text{–}N)]$, $R_{1\text{–}4}$ = H or alkyl, one end of the former olefin proved to be characterized (at least in the cases of $[Pt(\eta^1\text{-}C_2H_4OR)Cl(\mathbf{phen})]$, R = Me or Ph) by an incipient carbo-cationic character.

The molecular structure of compound **1a**, obtained by single crystal X-ray diffraction is reported in Fig. 1. Relevant crystallographic parameters are given in Table 1, while selected bond lengths and angles are given in Table 1s.‡

Comparison of the X-ray structure data of 2,2-dimethyl-6-chromanol, **6**, (X2 = C)[23] with the previously described **2** (X2 = Ni)[9] and the here reported **1a** (X2 = Pt) metalla-chromane compound shows a progressive size and distortion increase of the pseudo-pyrane ring on passing from X2 = C to X2 = Pt. Structural markers for this behaviour are the reduction of O1–X2–C3 angle and the lengthening of O1–X2 and X2–C3 distances for the increase of ring size and both θ (torsion angle between the O1–X2 bond and the aromatic ring) and $\theta\gamma$ (torsion angle between the C3–C4 bond and the aromatic ring) as a measure of the ring distortion, Table 2.

Table 1 Crystallographic data† for complex [Pt(**EtPh**)(**phen**)], **1a**

Formula	$C_{20}H_{16}N_2OPt$
Formula weight	495.44
Temperature/K	293(2)
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	7.506(1)
b/Å	20.254(2)
c/Å	10.733(1)
β/°	102.473(6)
Volume/Å^3	1593.2(3)
Z, calculated density/Mg m^{-3}	4, 2.066
Absorption coefficient/mm^{-1}	8.815
F(000)	944
Crystal colour and shape	Red, prism
Crystal size	0.02 × 0.03 × 0.7 mm
θ range for data collection/°	5.04 to 27.52
Limiting indices	$-9 \le h \le 9$, $-26 \le k \le 24$, $-13 \le l \le 12$
Reflections collected/unique	9761/3471 [$R_{int} = 0.0377$]
Completeness	94.6% ($\theta = 27.52$)
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2467/0/281
Goodness-of-fit on F^2	1.122
Final R indices [$I > 2\sigma(I)$]	$R1 = 0.0484$, $wR2 = 0.0848$
R indices (all data)	$R1 = 0.0800$, $wR2 = 0.0934$
Largest diff. peak and hole	2.287 and −1.020 e Å^{-3}

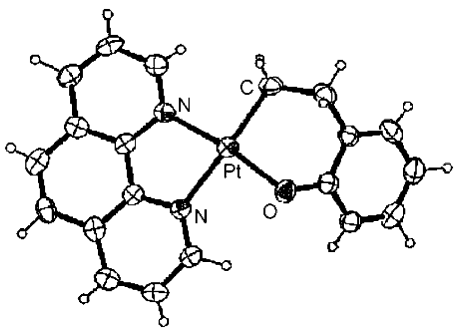


Fig. 1 Single crystal X-ray diffraction molecular structure of [Pt(**EtPh**)(**phen**)], **1a**.

Vitamin E vitamers (α-, β-, δ-, and γ-tocopherols and tocotrienols), Scheme 1, are naturally occurring *O*-heterocycles, with a chromane structural core, characterized by useful pharmacological properties. These vitamers are involved in processes such as cellular signalling, gene expression, immune response, apoptosis and protection of biological systems from oxidation. They have also been proposed for the treatment of many diseases (*i.e.* mental disorders,[24] inflammations,[25,26] cancer,[27] cardiovascular[28] and diabetic[29] diseases). For this reason, considerable interest has been devoted to the synthesis of chromane derivatives, characterized by the presence of functional groups or heteroatoms bonded to or inside the chromane skeleton.[30]

Table 2 Significant structure parameters of **6**, **2** and **1a** molecules[a]

X2	O1–X2/Å	X2–C3/Å	O1–X2–C3/°	θ/° [b]	$\theta\gamma$/° [c]
C (**6**)	1.456(3)	1.507(4)	108.5(2)	18.0	14.1
Ni (**2**)	1.872(3)	1.936(5)	94.4(2)	30.6	46.1
Pt (**1a**)	2.013(6)	2.041(10)	92.2(4)	50.8	60.2

[a] The conventional numbering scheme of chromane is adopted even in the case of metal complexes. [b] Torsion angle between the O1–X2 bond and the aromatic ring. [c] Torsion angle between the C3–C4 bond and the aromatic ring.

Biological activity evaluation, by standard protocols, of the metalla chromane complexes **1a** and **1b**, on HeLa cancer cells, gave interesting preliminary results. The short term cellular platinum uptake, measured by atomic absorption,[31] resulted clearly in favour of **1b** with respect to **1a**, by more than an order of magnitude, moreover a MTT test[32] showed a much higher cytotoxicity of compound **1b**, $IC_{50} = 11.3 \pm 0.6$ μM, with respect to **1a**, $IC_{50} \approx 40 \pm 5$ μM (IC_{50} evaluated with a ∼20% cells killed at the maximal administered dose of 10 μM), Fig. 2. It is well known that the 6-hydroxo chromane unit plays a key role for the biological activity of vitamin E derivatives. The 6-hydroxo substituent in the chromane core of Vitamin E derivatives is required for their activity as free radical scavengers.[33] Moreover, together with the hydrophobic phytol chain, both the endocyclic and exocyclic 6-hydroxo chromane's oxygens are known to constitute essential binding sites recognized by a range of proteins in living tissues[25,34–44] (*i.e.* tocopherol transfer proteins,[35,38–41] phospholipase A_2,[25] tocopherol associated protein,[37] protein kinases,[44] supernatant protein factor,[36,42] proteins involved in regulation of gene expression[37] and cell signalling[34]). It is very interesting to note that the only molecular difference between **1a** and **1b** responsible for the higher both cytotoxicity, Fig. 2, and cellular uptake, Fig. 3, of

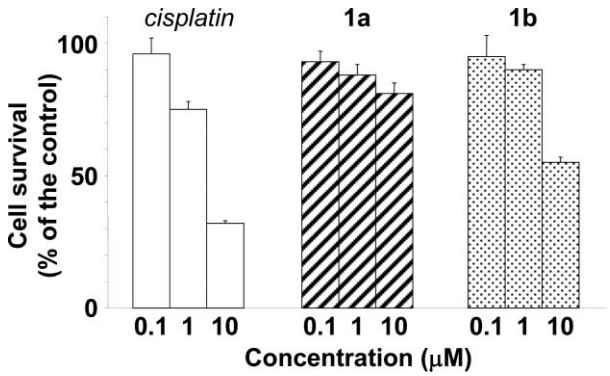


Fig. 2 HeLa cell survival measured by MTT test after 48 h of incubation. Cisplatin, **1a** and **1b** were administered in three different concentrations: 0.1, 1 and 10 μM. An ethanol concentration equal to **1a** and **1b** solutions was opportunely added to the cisplatin solution. The data were the results of three different experiments presented as means ± S.D.

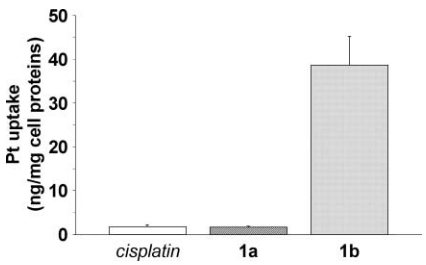


Fig. 3 Platinum intracellular accumulation in HeLa cells after 3 h continuous exposure to cisplatin, **1a** and **1b**, administered at the same concentration (1 μM). An ethanol concentration equal to **1a** and **1b** solutions was opportunely added to the cisplatin solution. The data were the results of three independent experiments presented as means ± S.D.

1b with respect to **1a**, is the presence of the 6-methoxo substituent on the metalla-chromane core.

Conclusions

We were able, for the first time, to synthesize and characterize Pt derivatives with a structural shape similar to vitamin E, having a metalla-chromane core. The new complexes were obtained by a novel, highly selective, metal mediated *ortho*-alkylation of phenols which takes place in unusually very mild conditions, by a concerted mechanism. Cytotoxicity and Pt uptake measurements, performed on HeLa cancer cells, showed an interesting structure–activity correlation for the new metalla-chromane analogues **1a** and **1b**, being the structurally closest to vitamin E and also the most active.

Experimental

All solvents and reagents, except otherwise stated, were purchased from Aldrich Chemical Company and used as received. Zeise's salt was prepared from potassium tetrachloroplatinate and ethylene gas as previously described.[45] NMR classical experiments were recorded on a Bruker Avance DPX 400 instrument, using deuterated solvents. ^{1}H and ^{13}C chemical shifts were referenced to TMS by using the residual protic solvent peak as internal reference; ^{195}Pt chemical shifts were referenced to Na_2PtCl_6, δ(Pt) = 0 ppm, in D_2O as an external reference. [PtCl(η^2-C_2H_4)(**phen**)]BF_4, **3**, and [PtCl(η^1-$C_2H_4OCH_3$)(**phen**)] were synthesized with a previously described method.[20]

[Pt{2-(ethan-2′-yl-kC^1)-1-phenolato-kO^1}(phen)], [Pt(EtPh)(phen)], 1a, and [Pt{2-(ethan-2′-yl-kC^1)-4-methoxy-1-phenolato-kO^1}(phen)], [Pt(MeOEtPh)(phen)], 1b

In a typical synthetic procedure the **phen** ligand (90 mg, 0.50 mmol) is added to a mixture of phenol (470 mg, 5.0 mmol), sodium phenolate (170 mg, 1.0 mmol) (**1a**) or 4-methoxyphenol (745 mg, 6.0 mmol), NaOH (40 mg, 1.0 mmol) (**1b**) and the Zeise's salt (195 mg, 0.5 mmol), partially dissolved in CH_2Cl_2 (2–3 mL) and kept under stirring at 0 °C. After one hour, stirring in the dark, the colour of the reaction mixture turns from pale yellow to orange–yellow. The solvent is then evaporated under vacuum and the residual oil, stirred at room temperature for a further 8 h, undergoes a colour change from orange–yellow to dark-red. The dark-red oil is then diluted with CH_2Cl_2 (5 mL) and flash chromatographed on silica gel (≈1 g) eluting, first with CH_2Cl_2 to obtain a pale yellow eluate containing intermediate **5**, which is discarded, then with CH_2Cl_2/acetone = 90/10, to obtain a red fraction which is collected, concentrated to 2 mL, under vacuum, and added with Et_2O (*ca.* 100 mL) to obtain a red precipitate. This is separated from the solution by filtration and recrystallized from CH_2Cl_2/Et_2O to give the final product **1** (yield 175 mg, 0.35 mmol, 70%, **1a**; 160 mg, 0.3 mmol, 60%, **1b**, with respect to the starting Zeise's salt).

Compounds **1a** and **1b** were also obtained starting from [PtCl(η^2-C_2H_4)(**phen**)]BF_4, **3**,[20] as a substitute of Zeise's salt and **phen**. In a typical synthetic procedure we partially dissolve phenol (941 mg, 10.0 mmol), sodium phenolate (170 mg, 1.0 mmol) (**1a**) or 4-methoxyphenol (745 mg, 6.0 mmol), NaOH (40 mg, 1.0 mmol) (**1b**) in CH_2Cl_2 (20 mL) and kept under stirring at 0 °C. After 10 min, complex **3** is added (525 mg, 1.0 mmol), obtaining a pale yellow suspension. After 1 h, the temperature is increased to 25 °C. After 3 h stirring, the solution is concentrated under vacuum until a pale yellow oil is obtained. The residual oil, stirred at room temperature for a further 8 h, undergoes a colour change to dark-red. Isolation of pure compounds **1a** or **1b** from the oil mixtures was performed with the above described method. Since the reaction yields of **1a** and **1b** based on **3** are very similar to those reported above for the one-pot reaction, this latter was preferred in our routine synthetic procedure.

(**1a**) (Found: C, 48.0; H, 3.2; N, 5.6%. $C_{20}H_{16}N_2OPt$ requires C, 48.5; H, 3.3; N, 5.7%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.61 (t, J_{H-H} = 6.4 Hz, $^{3}J_{Pt-H}$ = 51 Hz, 2H, Pt–CH_2–CH_2–), 2.76 (t, J_{H-H} = 6.4 Hz, $^{2}J_{Pt-H}$ = 81 Hz, 2H, Pt–CH_2–CH_2–), 6.56 (m, 1H, CH), 7.02 (m, 2H, CH), 7.10 (d, J_{H-H} = 6.9 Hz, 1H, CH), 7.57 (dd, J_{H-H} = 5.4, 8.2 Hz, 1H, CH), 7.85 (s, 1H, CH), 7.87 (s, 1H, CH), 8.02 (dd, J_{H-H} = 4.8, 8.2 Hz, 1H, CH), 8.48 (d, J_{H-H} = 8.2 Hz, 1H, CH), 8.52 (d, J_{H-H} = 8.2 Hz, 1H, CH), 9.31 (d, J_{H-H} = 5.4 Hz, $^{3}J_{Pt-H}$ = 54 Hz, 1H, CH), 9.73 (d, J_{H-H} = 4.8 Hz, $^{3}J_{Pt-H}$ = 13 Hz, 1H, CH) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$) δ 15.8 (Pt–*C*H_2-CH_2-), 29.9 (Pt–CH_2–*C*H_2–), 117.0, 119.0, 126.6, 128.7, 134.4 and 163.7 (6C, phenyl), 125.2, 125.5, 126.9, 127.1, 129.6, 130.8, 134.9, 136.5, 145.4, 146.5, 148.3 and 149.6 (12C, **phen**) ppm. ^{195}Pt–NMR (400 MHz, $CDCl_3$) δ −2948 ppm. (**1b**) (Found: C, 47.5; H, 3.9; N, 5.4%. $C_{21}H_{18}N_2O_2Pt$ requires C, 48.0; H, 3.5; N, 5.3%) ^{1}H–NMR (400 MHz, $CDCl_3$) δ 2.62 (t, J_{H-H} = 6.2 Hz, $^{3}J_{Pt-H}$ = 62 Hz, 2H, Pt–CH_2–CH_2–), 2.84 (t, J_{H-H} = 6.2 Hz, $^{2}J_{Pt-H}$ = 91 Hz, 2H, Pt–CH_2–CH_2–), 3.74 (s, 3H, CH_3), 6.60 (dd, J_{H-H} = 2.9, 8.5 Hz, 1H, CH), 6.71 (d, J_{H-H} = 2.9, 1H, CH), 6.93 (d, J_{H-H} = 8.5 Hz, 1H, CH), 7.61 (dd, J_{H-H} = 5.4, 7.9 Hz, 1H, CH), 7.90 (m, 1H, CH), 7.91 (m, 1H, CH), 8.05 (dd, J_{H-H} = 5.1, 8.2 Hz, 1H, CH), 8.54 (d, J_{H-H} = 5.3 Hz, 1H, CH), 8.56 (d, J_{H-H} = 7.7 Hz, 1H, CH), 9.40 (d, J_{H-H} = 5.4 Hz, $^{3}J_{Pt-H}$ = 52 Hz, 1H, CH), 9.79 (d, J_{H-H} = 4.7 Hz, $^{3}J_{Pt-H}$ = 14 Hz, 1H, CH) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$) δ 16.1 (Pt–*C*H_2–CH_2–), 30.5 (Pt–CH_2–*C*H_2–), 56.2 (OCH_3), 111.7, 115.0, 117.0, 135.0, 150.2 and 158.0 (6C, phenyl), 125.2, 125.5, 126.8, 127.0, 129.5, 130.9, 134.8, 136.5, 145.8, 146.5, 148.4 and 149.7 (12C, **phen**) ppm. ^{195}Pt-NMR (400 MHz, $CDCl_3$) δ −2937 ppm.

[PtCl(ethan-2-yl-1-phenolato-kC^1)(phen)], [PtCl(η^1-$C_2H_4OC_6H_5$)(phen)], 4

In a typical synthetic procedure, sodium phenolate (120 mg, 0.7 mmol) and [PtCl(η^2-C_2H_4)(**phen**)]BF_4, **3**, (460 mg, 0.9 mmol) are reacted, suspended in CH_2Cl_2 (200 mL) by strongly stirring, for 1 h at 0 °C and a further 2 h at room temperature. A yellow–orange solution is collected by filtration of the suspension. This solution is concentrated to 20 mL, by evaporation under vacuum. The yellow precipitate **4** is then collected by filtration after addition of abundant Et_2O (≈400 mL). To obtain a pure product, the solid **4** is washed with abundant Et_2O (≈100 mL) and dried (yield 130 mg, 0.24 mmol, 27%). (Found: C, 45.3; H, 3.2; N, 5.4%. $C_{20}H_{17}ClN_2OPt$ requires C, 45.2; H, 3.2; N, 5.3%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.58 (m, $^{2}J_{Pt-H}$ = 94 Hz, 2H, Pt–CH_2–CH_2–), 4.32 (m, $^{3}J_{Pt-H}$ = 28 Hz, 2H, Pt–CH_2–CH_2–), 6.85 (m, 1H, CH), 6.96 (m, 2H, CH), 7.23 (m, 2H, CH), 7.80 (m, 1H, CH), 7.97 (m, 2H, CH), 7.99 (m, 1H, CH), 8.57 (dd, J_{H-H} = 4.8, 8.2 Hz, 1H, CH), 8.66 (dd, J_{H-H} = 5.2, 8.3 Hz, 1H, CH), 9.57 (d, J_{H-H} = 5.2 Hz, $^{3}J_{Pt-H}$ = 58 Hz, 1H, CH), 9.85 (d, J_{H-H} = 4.8 Hz, $^{3}J_{Pt-H}$ = 16 Hz, 1H,

CH) ppm. ^{13}C-NMR (400 MHz, $CDCl_3$) δ 4.1 (Pt–CH_2–CH_2–), 71.2 (Pt–CH_2–CH_2–), 114.6 (2C), 119.4 (1C), 129.0 (2C), 159.4 (1C) (6C, phenyl), 125.5, 125.6, 127.0, 127.6, 130.0, 131.0, 136.3, 136.9, 146.2, 148.3 (2C), and 149.4 (12C, **phen**) ppm. ^{195}Pt-NMR (400 MHz, $CDCl_3$) δ −3230 ppm.

[PtCl{2-(ethan-2′-yl-k*C*^{1})-1-phenol}(phen)], [PtCl{*o*-(η^1-C_2H_4)-phenol}(phen)], 5

In a typical synthetic procedure the **phen** ligand (47 mg, 0.26 mmol) is added to a mixture of phenol (243 mg, 2.6 mmol), sodium phenolate (44 mg, 0.26 mmol) and the Zeise's salt (100 mg, 0.26 mmol), partially dissolved in CH_2Cl_2 (3–4 mL) and kept under stirring at 0 °C. After 1 h, the colour of the reaction mixture turns from pale yellow to orange–yellow. After evaporation of the solvent, under vacuum, the residual oil, stirred at room temperature for a further 4 h, undergoes a colour change from orange–yellow to dark-red. The dark-red oil is then diluted with CH_2Cl_2 (5 mL) and flash chromatographed on silica gel (≈1 g) eluting, with CH_2Cl_2 to obtain a pale yellow eluate of **5**. This is separated from the solution by filtration and recrystallized from CH_2Cl_2/pentane to give the final product **5** (yield 60 mg, 0.11 mmol, 43%). (Found: C, 45.0; H, 3.4; N, 5.1%. $C_{20}H_{17}ClN_2OPt$ requires C, 45.2; H, 3.2; N, 5.3%) ^{1}H-NMR (400 MHz, $CDCl_3$) δ 2.40 (m, $^2J_{Pt-H}$ = 85 Hz, 2H, Pt–C*H*$_2$–CH$_2$–), 2.79 (m, $^3J_{Pt-H}$ = 47 Hz, 2H, Pt–CH$_2$–C*H*$_2$–), 6.53 (m, 1H, CH), 6.62 (s, J_{Pt-OH} = 13 Hz, 1H, OH), 6.84 (m, 1H, CH), 6.93 (m, 1H, CH), 7.29 (m, 1H, CH), 7.47 (dd, J_{H-H} = 5.4, 8.1 Hz, 1H, CH), 7.97 (dd, J_{H-H} = 5.1, 8.3 Hz, 1H, CH), 7.90 (dd, J_{H-H} = 8.1 Hz 1H, CH), 7.92 (dd, J_{H-H} = 8.3 Hz 1H, CH), 8.50 (m, 1H, CH), 8.54 (m, 1H, CH), 8.96 (dd, J_{H-H} = 5.4, $^3J_{Pt-H}$ = 62 Hz, 1H, CH), 9.80 (dd, J_{H-H} = 5.1 Hz, $^3J_{Pt-H}$ = 26 Hz, 1H, CH), ppm. ^{13}C-NMR (400 MHz, $CDCl_3$) δ 7.2 (Pt–*C*H$_2$–CH$_2$–), 34.1 (Pt–CH$_2$–*C*H$_2$–), 116.0 (1C), 120.2 (1C), 126.4 (1C), 129.8 (1C), 131.2 (1C), 153.7 (1C) (6C, phenyl), 125.1, 125.6, 126.7, 127.0, 130.2, 130.9, 136.1, 137.0, 145.7, 146.2, 148.4, and 148.6 (12C, **phen**) ppm. ^{195}Pt–NMR (400 MHz, $CDCl_3$) δ −3311 ppm.

Synthesis of 5 starting from [Pt(η^1-$C_2H_4OCH_3$)Cl(phen)]

In a typical experiment [Pt(η^1-$C_2H_4OCH_3$)Cl(**phen**)] (10 mg, 0.02 mmol) are dissolved in 1 mL of $CDCl_3$ adding a light excess of phenol (3 mg, 0.03 mmol). Formation of **5** is followed by ^{1}H NMR spectroscopy and is complete after a week.

Reaction of 4 with phenol-d_6 to give 5-d_5 and 1a-d_4

In a typical experiment complex **4** (10 mg, 0.02 mmol) was dissolved in 10 mL of $CDCl_3$ adding phenol-d_6 (25 mg, 0.25 mmol) and NaOD (≈2 mg, 0.05 mmol). After 1 h, stirring in the dark, the colour of the reaction mixture turns from pale yellow to orange–yellow. The formation of **5**-d_5, and **1a**-d_4 is followed by ^{1}H NMR spectroscopy. After a week, the $CDCl_3$ red solution is flash chromatographed on silica gel (≈1 g) eluting, first with CH_2Cl_2, to obtain a pale yellow eluate containing intermediate **5**-d_5 (isolated yield ≈1 mg, 0.002 mmol, 10%) then with CH_2Cl_2/acetone = 90/10, to obtain a red fraction which is collected, concentrated to 1 mL, under vacuum, and added with Et_2O (*ca.* 10 mL) to obtain a red precipitate. This is separated from the solution by filtration and recrystallized from CH_2Cl_2/Et_2O to give the final product **1a**-d_4 (isolated yield ≈5 mg, 0.01 mmol, 50%).

Atomic absorption determination of cell Pt uptake

Atomic absorption analyses were performed at the central facility of CIRCMSB, Consorzio Interuniversitario di Chimica dei Metalli nei Sistemi Biologici in Bari, Italy. Pt uptake in HeLa cells mediated by **1a** and **1b** was determined by atomic absorption spectroscopy, according to a previously proposed method.[31] After 3 h incubation, treated cells were harvested with a lysis buffer (SDS 0.1%, Na deossicolate 0.1%, Nonidet P40 10 μL mL^{-1}), after which the pellets were digested in 65% nitric acid at room temperature in closed vials. The total amount of intracellular platinum was determined by a Varian SpectrAA-880 Zeeman graphite furnace atomic absorption spectrometer. A five-point calibration curve was prepared for our measurements. The data were expressed as nanogram Pt per milligram of cell proteins (means ± S.D.) and were the result of three independent experiments. The Protein content of the treated cells was determined with the Bio-Rad protein assay kit 1, using lyophilized bovine serum albumin as a standard.

Biological assays

Stability of **1a** and **1b** in the presence of water was checked, before any biological test, recrystallizing the complexes unaltered from ethanol/water mixtures. The cytotoxicity of compounds **1a–b** was evaluated by the MTT biological test, based on the metabolic reduction of a soluble tetrazolium salt *i.e.* MTT = 3-[4,5-dimethyllthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (Sigma),[32] on HeLa cancer cells. This colorimetric assay measures cell survival after compound administration. As they were scarcely water soluble, **1a–b** were previously dissolved in absolute ethanol, obtaining a mother liquor. Aliquots of the mother liquor were added to PBS (phosphate buffered saline solution), in order to treat HeLa cell cultures with ethanol quantities suitably diluted and to avoid the cytotoxic effects of the alcohol (at least 1 : 100). Moreover, control experiments where only ethanol was used were performed. HeLa cells from human tumoral endometrium were grown in DMEM (Euroclone). Culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Euroclone), 0.2 mg mL^{-1} streptomycin, 200 IU mL^{-1} penicillin. Cells were cultured routinely at 37 °C and 5% CO_2 in a humidified incubator. After harvesting, cells were counted and diluted appropriately with the culture medium; 100 μL containing 3000 cells were seeded in each well of a 96-well microtiter plate (Corning). After 24 h of incubation, platinum containing compounds were administrated to each well with a concentration between 0.1 μM and 10 μM. The toxicity of these compounds was tested for 48 h. At the end of every incubation, MTT was added at the final concentration of 500 μg mL^{-1}. After a 4 h incubation, the amount of formazan was spectrophotometrically measured in isopropanol, λ = 550 nm. The optical density was used to calculate cell growth inhibition, as a percentage with respect to the control. For the statistical analysis of the data the Bonferroni–Dunn test was used and a p value < 0.05 was considered significant. All the results are the mean of three different experiments.

X-Ray structure

(Fig. 1) The structure of **1a** was determined by single crystal X-ray diffraction. Suitable crystals of $C_{20}H_{16}N_2OPt$ were selected at the microscope and mounted on a glass fibre. X-Ray data were collected at 293 K from red prismatic crystals using a Nonius Kappa CCD area detector diffractometer, with Mo-Kα radiation ($\lambda = 0.71073$ Å), in ϕ and ω scans mode. The unit-cell parameters were obtained by analyzing 173 reflections with $3.87 \leq \theta \leq 22.47°$. The systematic absences were uniquely consistent for the monoclinic space group $P2_1/c$. The structure was solved by the Direct Method procedure of SIR97,[46] and refined by a full-matrix least-squares technique based on F^2, SHELXL-97.[47] Highest residual peaks in the difference Fourier map were located close to the Pt(II) cation. The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms were found in successive difference-Fourier maps and refined isotropically. Relevant crystallographic parameters are given in Table 1, while selected bond lengths and angles are given in Table 1s.‡

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