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Tuning the coordination properties of phenothiazine by regioselective introduction of diphenylphosphanyl groups†

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The palladium and platinum complexes of the newly synthesized 1-(diphenylphosphino)-10-methyl-10*H*-phenothiazine (**1**) and the previously reported 3-(diphenylphosphino)-10-alkyl-10*H*-phenothiazine [alkyl = Me (**2**), Et (**3**)] and 4-(diphenylphosphino)-10-ethyl-10*H*-phenothiazine (**4**) were prepared. Density functional calculations were carried out to explain the electronic properties of compounds **1**, **3** and **4**. Compounds **1**, **3** and **4** can interact with DNA, as was observed in agarose gel electrophoresis experiments. In addition, the cytotoxicity of the platinum complexes of ligands **2** and **4** towards breast, colorectal and hepatocarcinoma cell lines was studied.

Introduction

Research in the field of bis-phosphine ligands based on heterocyclic aromatic backbones has evolved rapidly in the last few decades, mainly because of their applications in a great variety of catalytic processes. Since the synthesis of Xantphos[1] many similar ligands have been developed and successfully used in catalysis (*e.g.*, hydroformylation[2,3] or asymmetric allylic alkylation[4]) due to their large bite angles. The electronic and steric properties of these ligands were tuned to study the effects on the geometry of the palladium(II) complexes and their catalytic activity.[5] There are only a few examples of monophosphines with a xanthene-type backbone,[6–8] and they have found applications in telomerisation of buta-1,3-diene and methanol.

Phenothiazine ligands with diphenylphosphanyl groups are good candidates for the preparation of transition metal complexes not only with catalytic properties but also with biological activity. Therefore, we decided to combine the well-known properties of phenothiazine with the coordination ability of the phosphanyl group towards transition metals already used in cancer therapy as a continuation of our search for new palladium and platinum compounds with antitumor activity.[9–11] The first examples of phenothiazine-based ligands containing phosphorus are the reaction products obtained by phosphorylation of 10-methyl-phenothiazine with phosphorus tribromide used for the preparation of phosphorus(V) ligands.[12] We have previously reported the preparation of some mono- and bis-(diphenylphosphino)phenothiazines: 4-(diphenylphosphino)-10-alkyl-10*H*-phenothiazine (alkyl = methyl, ethyl, isopropyl), 2- and 3-(diphenylphosphino)-10-methyl-10*H*-phenothiazine, bis-phosphines 3,7- and 4,6-bis(diphenylphosphino)-10-methyl-10*H*-phenothiazine.[13,14]

Herein we report the coordination behaviour of a new phenothiazinyl-phosphine regioisomer, 1-(diphenylphosphino)-10-methyl-10*H*-phenothiazine (**1**), and the previously reported 3-(diphenylphosphino)-10-alkyl-10*H*-phenothiazine [alkyl = Me (**2**), Et (**3**)] and 4-(diphenylphosphino)-10-ethyl-10*H*-phenothiazine (**4**)[13,14] towards transition metals (Pd, Pt) and the biological activity of **1**, **3** and **4** and of transition metal complexes **11**, **14** and **15**.

Results and discussion

The diphenylphosphino-phenothiazines **1–3** were prepared by lithiation of the corresponding bromo-10-alkyl-phenothiazine with ^{n}BuLi in diethyl ether at −78 °C (according to a slightly modified procedure by Katritzky *et al.*[15]), followed by electrophilic substitution with diphenylchlorophosphine. The lithia-

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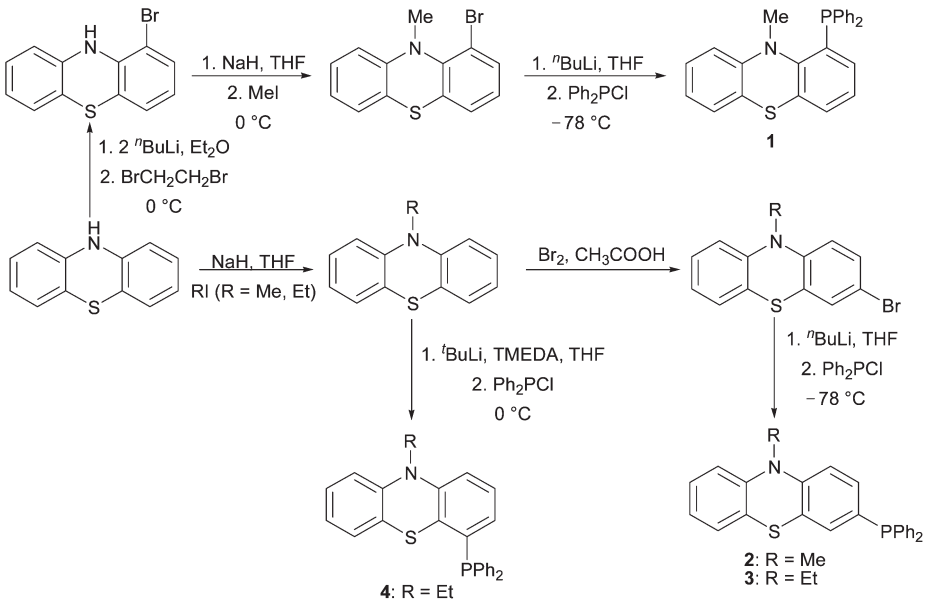


Scheme 1 Synthesis of diphenylphosphino-phenothiazines **1–4**.

tion of 10-ethylphenothiazine with ^{t}BuLi in THF at 0 °C in the presence of TMEDA (TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine) and the reaction with Ph_2PCl afforded **4** (Scheme 1).

Compounds **1–4** were identified by ^{1}H, ^{13}C and ^{31}P NMR spectroscopy and elemental analysis. The signals in the ^{31}P NMR spectra are in the range of −6.9 to −13.2 ppm [−8.9 (**1**), −6.9 (**2**), −7.0 (**3**) and −13.2 ppm (**4**)]. The C–P coupling constants ($^1J_{PC}$ = 5.6–10.9 Hz; $^2J_{PC}$ = 17.9–30.8 Hz; $^3J_{PC}$ = 5.7–8.9 Hz) have values similar to those of other phosphorus-aryl containing compounds.[16]

The electronic properties of phenothiazinyl-phosphines **1**, **3** and **4** were investigated by absorption/emission UV/Vis spectroscopy and cyclic voltammetry. UV absorption and emission data of dilute solutions are listed in Table 1.

The UV/Vis spectra of compounds **1**, **3** and **4** exhibit two absorption maxima around 253 and 310 nm, in accordance with the TD-DFT calculated UV/Vis spectra. A strong contributor to the absorption band around 250 nm is a formal excitation of the lone pair of electrons at the phosphorus atom into the π* orbitals of the phenothiazine with some delocalization onto the phenylphosphine substituents (ESI, Table S1 and Fig. S1†). The absorption around 310 nm is mainly related to excitations of phenothiazine orbitals with little participation of the diphenylphosphanyl group and with important contributions from the heteroatoms into LUMOs dominated by the rest of the π* system.

The similarity of the UV/Vis spectra of **1**, **3** and **4** is in contrast to the dramatic difference in fluorescence quantum yields. To provide more details on this issue, DFT calculations [B3LYP/6-311G(2d,2p)] were performed on the ground states and excited states of **1**, **3** and **4**. The resulting geometries are shown in Fig. 1. In the ground state, **1**, **3** and **4** display typical phenothiazine geometries, folded along the N–S axis. The first excited singlet (S_1) state of **3** features a planar phenothiazine,

Table 1 Absorption and emission data of compounds **1**, **3–7**, and **10–15**

Compound	Absorption $\lambda_{max.abs}$ (nm) exptl./calcd	Emission $\lambda_{max.em}$ (nm)	Quantum yield Φ (%)	Stokes shift (cm^{-1})
1	253/242; *313*/292	464	0.5[a]	10 397
3	262/255; *311*/282, 289	458	4.6[a]	10 320
4	252/242; *305*/296	456	0.5[a]	10 857
5	*254*, 309, 565	366	5.2[b]	12 048
6	*256*, 310, 571	363	4.1[b]	11 514
7	228, *281*, 356	375	0.9[b]	8921
10	227, *263*, 335, 400	375	4.8[b]	11 356
11	234, *268*, 318	371	3.5[b]	10 359
12	229, *263*, 336, 404	368	2.8[b]	10 849
13	227, *267*, 318	366	2.9[b]	10 131
14	228, *287*, 417	370	0.3[b]	7816
15	229, 327	No fluorescence observed		

[a] Relative to perylene. [b] Relative to naphthalene. The excitation wavelength is italicized. The concentrations of the solutions used for fluorescence measurements were 5.1×10^{-7} M (**1**), 2.1×10^{-7} M (**3**), 9.2×10^{-7} M (**4**), 10^{-10} M (**5–7**, **10–13**), 10^{-8} M (**14**) and 10^{-5} to 10^{-12} M (**15**). Quantum yields were calculated according to ref. 17.

while in the S_1 states of **1** and **4** this planarity appears to be hampered by steric repulsion between the nitrogen and phosphorus atoms in **1** and the sulfur and phosphorus atoms in **4**; the N···P distance of 3.3 Å and the S···P distance of 3.3 Å are shorter than the sums of the van der Waals radii (3.4 and 3.6 Å, respectively).[18] These distortions from planarity are also coupled with a deviation from planarity of the substituted benzene unit in the phenothiazine, both of which thus offer reasons for a clear difference in excited-state properties between isomer **3** on the one hand and isomers **1** and **4** on the other hand, including fluorescence: in the first excited singlet states of **1** and **4**, the aromatic π system is disrupted and the system is destabilised by the steric repulsion, so that these

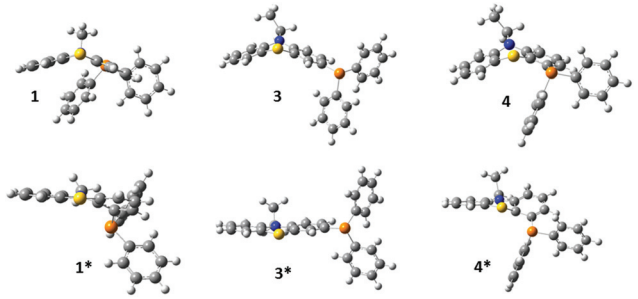


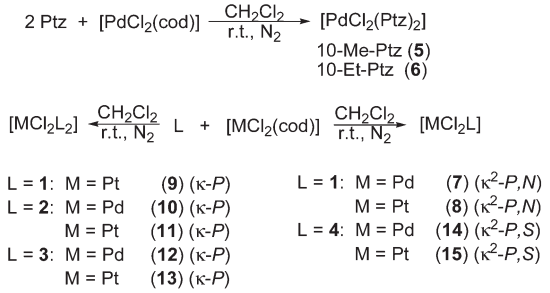
Fig. 1 Optimised geometries for **1**, **3**, and **4** and their first excited singlet states (**1***, **3***, and **4***), viewed along the N–S axis (N: blue, S: yellow, P: orange).

excited states are expected to have a shorter lifetime as well as supplementary mechanisms for dissipating energy, *e.g.* due to the additional conformational space provided by the distortion (hence the reduced availability for fluorescence).

Cyclic voltammetry (CV) was carried out in dichloromethane, with ferrocene/ferrocenium (Fc/Fc^{+}) as the internal standard, scanning in the anodic (up to 1.8 V) and cathodic (up to −0.2 V) region. In the case of compounds **3** and **4**, the first oxidation potential is due to the phenothiazine moiety[19] and is quasireversible for both compounds, while the second oxidation potential for compound **3** (1.109 V) and the second and third oxidation potentials (1.112 V and 1.446 V, respectively) for compound **4** are generated by phosphorus oxidation and the processes are irreversible (ESI, Fig. S2†). Compound **1** shows only one oxidation potential (Table 2).

Table 2 Cyclic voltammetric data *E* (V) for **1**, **3** and **4**

Compound	$E_{1/2(1)}$	$E_{1/2(2)}$	$E_{1/2(3)}$
1	0.976	—	—
3	0.827	1.109	—
4	0.825	1.112	1.446



Scheme 2 Synthesis of transition metal complexes **7**–**15** (cod = 1,5-cyclooctadiene; Ptz = phenothiazine).

Transition metal complexes

Phenothiazine, its *N*-alkyl derivatives and metal phenothiazine complexes are known to be biologically active compounds. Although many transition metal phenothiazine complexes have been synthesised and characterised, there are only a few reports of their crystal and molecular structures.[20–25] In order to investigate the coordination chemistry of N-substituted phenothiazines, palladium(II) complexes were prepared by reaction of *N*-alkyl-Ptz (alkyl = methyl and ethyl; Ptz = phenothiazine) with $[PdCl_2(cod)]$ (cod = 1,5-cyclooctadiene) in 2 : 1 molar ratio (Scheme 2). Complexes **5** and **6** were obtained as dark purple solids. The formation of the two complexes was demonstrated by X-ray diffraction measurements; the ^{1}H and ^{13}C NMR spectra are similar to those of the starting materials. Complex **6** has already been reported in the literature[26] but its solid-state structure was not confirmed.

Suitable crystals of **5** and **6** for X-ray diffraction measurements were obtained from dichloromethane at room temperature in one week. The solid-state structures are presented in Fig. 2 (Table 3).

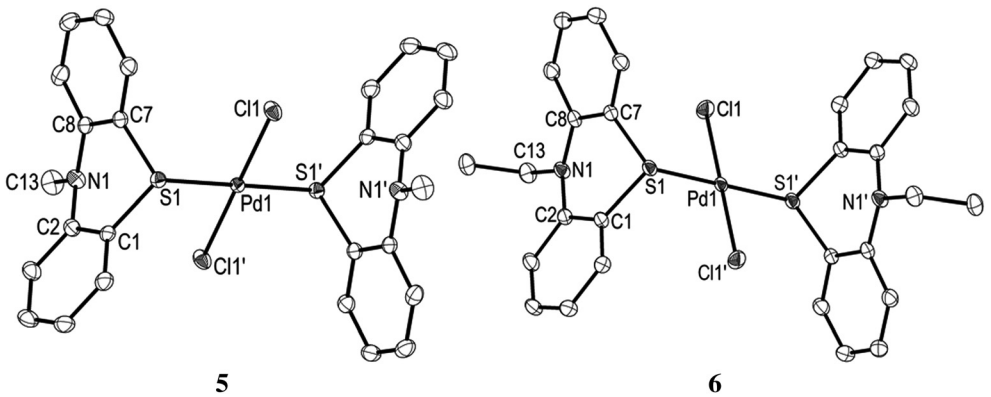


Fig. 2 Molecular structures of complexes **5** and **6**. Thermal ellipsoids are drawn at 50% probability. All hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°) for **5**: Pd(1)–Cl(1) 2.3083(6), Pd(1)–S(1) 2.3267(6), S(1)–C(7) 1.770(2), S(1)–C(1) 1.756(2), N(1)–C(2) 1.396(3), N(1)–C(8) 1.395(3), N(1)–C(13) 1.452(3), Cl(1)–Pd(1)–S(1) 84.35(2), Cl(1′)–Pd(1)–S(1) 95.65(2), C(1)–S(1)–C(7) 99.00(1), Pd(1)–S(1)–C(7) 103.06 (8), Pd(1)–S(1)–C(1) 111.26(8), C(2)–N(1)–C(8) 119.3(2), C(8)–N(1)–C(13) 119.2(2); for **6**: Pd(1)–Cl(1) 2.2921(5), Pd(1)–S(1) 2.3338(5), S(1)–C(7) 1.749(2), S(1)–C(1) 1.761(2), N(1)–C(2) 1.400(2), N(1)–C(8) 1.401(2), N(1)–C(13) 1.472(2), C(13)–C(14) 1.528(3), Cl(1)–Pd(1)–S(1) 95.36(2), Cl(1′)–Pd(1)–S(1) 84.64(2), C(1)–S(1)–C(7) 98.32(9), Pd(1)–S(1)–C(7) 110.19(7), Pd(1)–S(1)–C(1) 106.76(7), C(2)–N(1)–C(8) 120.4(2), C(8)–N(1)–C(13) 118.1(2).

Table 3 Summary of data collection, structure solution and refinement details for **5–7**, **10**, **14** and **15**

Compound	**5**	**6**	**7**	**10**	**14**	**15**
Formula	$C_{26}H_{22}Cl_2N_2PdS_2$	$C_{28}H_{26}Cl_2N_2PdS_2$	$C_{25}H_{20}Cl_2NPPdS{\cdot}CH_2Cl_2$	$C_{50}H_{40}Cl_2N_2P_2PdS_2$	$C_{26}H_{22}Cl_2NPPdS$	$C_{26}H_{22}Cl_2NPPtS$
Formula weight	603.87	631.93	659.67	972.20	588.78	677.47
Temperature [K]	130(2)	130(2)	180(2)	293(2)	293(2)	293(2)
Crystal system	Monoclinic	Monoclinic	Triclinic	Triclinic	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P\bar{1}$	$P\bar{1}$	$P2_1/n$	$P2_1/n$
Unit cell						
a [Å]	10.1581(3)	9.9826(3)	10.4334(2)	8.453(1)	10.8921(7)	10.8975(7)
b [Å]	8.0485(2)	15.5847(4)	11.1888(3)	10.438(1)	19.511(1)	19.615(1)
c [Å]	15.1612(4)	8.2241(2)	13.1883(3)	12.960(2)	11.2482(7)	11.2702(8)
α [°]	90	90	78.881(2)	97.823(2)	90	90
β [°]	109.362(3)	105.269(3)	87.795(2)	97.519(2)	97.910(1)	97.995(1)
γ [°]	90	90	62.256(2)	97.248(2)	90	90
V [Å^3]	1169.44(6)	1234.31(6)	1334.66(6)	1110.9(3)	2367.7(3)	2385.6(3)
Z	2	2	2	1	4	4
$\rho_{calcd.}$ [g cm^{-3}]	1.715	1.700	1.641	1.453	1.652	1.886
μ [mm^{-1}]	1.220	1.160	1.251	0.742	1.181	6.276
F(000)	608	640	660	496	1184.0	1312.0
Crystal size [mm]	0.15 × 0.15 × 0.02	0.3 × 0.15 × 0.02	0.3 × 0.25 × 0.2	0.32 × 0.25 × 0.20	0.32 × 0.20 × 0.18	0.30 × 0.26 × 0.23
Θ_{Min}–Θ_{Max} [°]	2.848–30.507	2.881–30.508	2.863–33.728	1.60–25.00	2.09–25.02	2.08–25.02
Collected reflections	8742	14 390	36 807	10 581	22 472	22 638
Indep. reflections (R_{int})	3575 (0.0406)	3759 (0.0364)	10 662 (0.0269)	3890 (0.0332)	4176 (0.0374)	4203 (0.0442)
Completeness to Θ_{Max}	100.0%	99.9%	99.8%	99.5%	100%	99.9%
$T_{max.}/T_{min}$	1/0.99342	1/0.97637	1/0.98005	0.866/0.797	0.816/0.704	0.326/0.255
Restraints/parameters	0/152	0/161	7/313	0/268	0/290	0/290
GooF (F^2)	1.032	1.069	1.042	1.075	1.141	1.162
R_1, wR_2 ($I > 2\sigma(I)$)	0.0353, 0.0689	0.0301, 0.0640	0.0344, 0.0830	0.0401, 0.0876	0.0364, 0.0783	0.0358, 0.0720
R_1, wR_2 (all data)	0.0519, 0.0743	0.0376, 0.0669	0.0414, 0.0868	0.0475, 0.0906	0.0414, 0.0804	0.0409, 0.0739
Residual electron density [e·Å^{-3}]	0.476/−0.748	0.626/−0.598	2.188/−1.471	0.498/−0.262	0.490/−0.384	1.263/−1.037

The bond lengths and angles have similar values to those observed in dichlorobis(phenothiazine-κ*S*)palladium(II)[20] (**I**), that is they are not affected by alkylation of the nitrogen atom [*cf.* Pd–S 2.3267(6) Å (in **5**), 2.3338(5) Å (in **6**), 2.3378(5) Å (in **I**); Cl–Pd–S 84.35(2)° (in **5**), 84.64(2)° (in **6**), 84.05(2)° (in **I**)]. The palladium atom has a slightly distorted square-planar geometry with the sulfur atoms in a *trans* arrangement. The dihedral angle between the phenylene planes of the phenothiazine moiety is 141.8° in **5** and 145.9° in **6**.

The reaction of *N*-alkyl-phenothiazines (alkyl = methyl and ethyl) with [$PtCl_2$(cod)] was attempted, but formation of the corresponding platinum complexes could not be observed, even upon increasing the temperature or the reaction time, although the synthesis of a similar platinum(II) complex, namely [$PtCl_2(SPh_2)_2$], has already been reported.[27]

In order to study the coordination mode (monodentate, bidentate, and preferred donor atom(s)) of the phosphanyl-substituted phenothiazine ligands **1–4**, reactions with [MCl_2(cod)] (M = Pd, Pt) in dichloromethane were performed (1 : 1 for **1** and **4**, 2 : 1 (L : M) for **2** and **3**, Scheme 2). In the resulting metal(II) complexes **7–15**, the signal of the phosphorus atom in the $^{31}P\{^1H\}$ NMR spectra is shifted to low field compared to the free ligand [**1**: −8.9 ppm, **7**: 42.6 ppm, **8**: 14.8 ppm ($^1J_{PtP}$ = 3808 Hz), **9**: 14.9 ppm ($^1J_{PtP}$ = 2714 Hz); **2**: −6.9 ppm, **10**: 22.3 ppm, **11**: 13.2 ppm ($^1J_{PtP}$ = 3699 Hz); **3**: −7.0 ppm, **12**: 22.1 ppm, **13**: 13.0 ppm ($^1J_{PtP}$ = 3672 Hz); **4**: −13.2 ppm, **14**: 59.5 ppm, **15**: 36.6 ppm ($^1J_{PtP}$ = 3490 Hz)].

Depending on the position of the phosphanyl substituent (1-, 3-, or 4-position), different bonding and coordination modes are observed. 1-PPh_2- (**1**) and 4-PPh_2-substituted phenothiazine (**4**) prefer a bidentate P,N (**1**) or P,S (**4**) bonding mode resulting in five-membered chelate rings, while 3-PPh_2-substituted phenothiazines (**2**, **3**) act only as monodentate phosphine ligands.

An example of the preferred bidentate P,N bonding mode of 1-PPh_2-substituted phenothiazine (**1**) is the dark orange palladium(II) dichloride complex **7**. The molecular structure of compound **7** was determined by X-ray diffraction measurements. A summary of data collection, structure solution and refinement details is presented in Table 3. Compound **7** crystallises in the triclinic space group $P\bar{1}$ with one molecule in the asymmetric unit. The asymmetric unit also contains an additional dichloromethane molecule.

Compound **1** acts as a bidentate P,N ligand towards Pd in complex **7** (Fig. 3). The palladium atom is situated in a slightly distorted square-planar geometry (P–Pd–Cl(1) 176.74(2)° and N–Pd–Cl(2) 171.27(4)°). The angles around the P atom indicate a tetrahedral environment and are in the range of observed bond angles for similar compounds.[28] The Pd–N (2.161(1) Å) and Pd–P (2.1829(5) Å) bond lengths are similar to the values reported for other palladium complexes with bidentate P,N ligands (*i.e.*, Pd–N is 2.100(5) Å in diiodo-{2-[bis(2-methylphenyl)phosphinomethyl]pyridine}palladium(II), 2.131(9) Å in diiodo-{*N*-[(*Z*)-2-(diphenylphosphine)-1-phenylethylidene]-2,6-diisopropylaniline}palladium(II); the Pd–P bond lengths are 2.230(2) Å and 2.223(3) Å, respectively, in the aforementioned complexes).[28] The Pd–P and Pd–Cl bonds are shorter in the chelate complex **7** (2.1829(5) Å and 2.2936(4) Å, respectively) compared to the same bonds in *trans*-[$PdCl_2(PPh_3)_2$][29] (2.346(2) Å and 2.379(4) Å, respectively) or in di-μ-chlorobis-[bis(triphenylphosphine)palladium(II)]bis(tetrafluoroborate)[30] (2.290(2) Å and 2.386(3) Å, respectively). The dihedral angle between the phenylene rings is smaller in complex **7** (132.1°) compared to 10-methylphenothiazine (143.7°),[31] due to the rigid structure obtained after chelation. Two platinum complexes were obtained (**8** and **9**) by the 1 : 1 reaction between **1** and [$PtCl_2$(cod)] as a 1 : 1.4 mixture (**8** : **9**) in solution. As **8** and **9** could not be separated, their structures were elucidated on the basis of the NMR spectra of the mixture. Two signals with the appropriate platinum satellites were observed in the $^{31}P\{^1H\}$ NMR spectrum at 14.8 ppm ($^1J_{PtP}$ = 3808 Hz) and 14.9 ppm ($^1J_{PtP}$ = 2714 Hz). Based on the *trans* influence on platinum complexes[32] and taking into account the linear correlation between the Pt–P coupling constant and the Pt–P bond length,[33] the first signal was assigned to complex **8** (isostructural to palladium complex **7**) with a bidentate P,N ligand, and the second signal was assigned to complex **9**, in which the ligand is coordinated only through the phosphorus atom (Fig. 4).

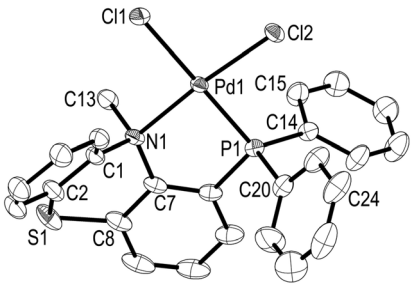


Fig. 3 Molecular structure of complex **7**. Thermal ellipsoids are drawn at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°): Pd(1)–N(1) 2.161(1), Pd(1)–P(1) 2.1829(5), Pd(1)–Cl(2) 2.2936(4), Pd(1)–Cl(1) 2.3925(5), N(1)–C(1) 1.473(2), N(1)–C(7) 1.479(2), N(1)–C(13) 1.519(2), N(1)–Pd(1)–P(1) 86.18(4), P(1)–Pd(1)–Cl(2) 86.99(2), N(1)–Pd(1)–Cl(1) 94.68(4), P(1)–Pd(1)–Cl(1) 176.74(2), Cl(2)–Pd(1)–Cl(1) 92.44(2), C(12)–P(1)–C(14) 105.52(9), C(14)–P(1)–C(20) 108.01(9), C(12)–P(1)–Pd(1) 102.51(7), C(14)–P(1)–Pd(1) 119.60(6), C(20)–P(1)–Pd(1) 113.02(7), C(1)–N(1)–Pd(1) 117.70(1), C(7)–N(1)–Pd(1) 112.50(1), C(13)–N(1)–Pd(1) 98.90(1).

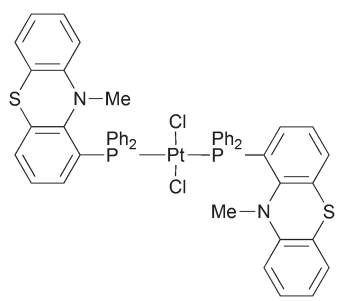


Fig. 4 Proposed structure for complex **9**.

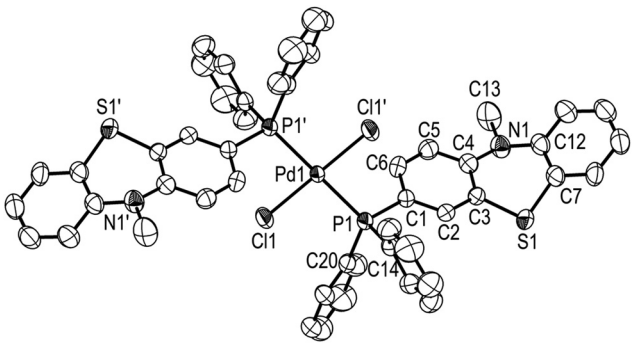


Fig. 5 ORTEP drawing of **10**. Thermal ellipsoids are drawn at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°): Pd(1)–P(1) 2.3233(8), Pd(1)–P(1′) 2.3233(8), Pd(1)–Cl(1) 2.2878(9), Pd(1)–Cl(1′) 2.2878(9), P(1)–C(1) 1.811(3), P(1)–C(14) 1.813(3), P(1)–C(20) 1.825(3), S(1)–C(3) 1.759(3), S(1)–C(7) 1.759(3), N(1)–C(13) 1.464(4), N(1)–C(4) 1.404(4), N(1)–C(12) 1.414(4), P(1)–Pd(1)–P(1′) 180.0(3), P(1)–Pd(1)–Cl(1) 91.55(3), Cl(1)–Pd(1)–Cl(1′) 180.0, C(1)–P(1)–C(14) 105.6(1), C(1)–P(1)–Pd(1) 114.0(1), C(14)–P(1)–Pd(1) 110.3(1), C(14)–P(1)–C(20) 103.6(2), C(3)–S(1)–C(7) 98.9(2), C(4)–N(1)–C(12) 119.6(3), C(4)–N(1)–C(13) 117.0(3), P(1)–C(1)–C(2) 122.1(2).

Similar coupling constants were observed in the case of $[PtCl_2(PPh_3)_2]$ ($^1J_{Pt-P}$ = 3679 Hz for the *cis* isomer and 2630 for the *trans* isomer).[34] The ESI mass spectrum of compound **9** shows a peak corresponding to the $[M - Cl]^+$ fragment at m/z = 1025.

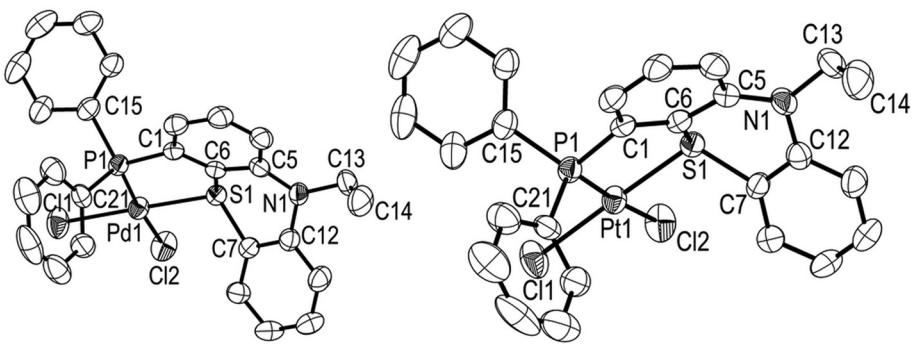
In palladium(II) and platinum(II) complexes, the two 3-PPh_2-substituted phenothiazines **2** and **3** act as monodentate ligands and have a *trans* arrangement due to steric reasons. Dark orange crystals of palladium complex **10** were obtained from dichloromethane/diethyl ether, and the molecular structure was determined by X-ray crystallography (Fig. 5).

In **10**, the palladium atom is located on a crystallographic inversion centre, is coordinated by two phosphine ligands in a *trans* arrangement, and exhibits a slightly distorted square-planar geometry (P(1)–Pd(1)–Cl(1) 91.55(3)°, P(1′)–Pd(1)–Cl(1) 88.45(3)°). The P–Pd bond is shorter in complex **7** (2.1829(5) Å), in which a five-membered ring is formed by bidentate coordination of **1** to the palladium atom, compared to 2.3233(8) Å in **10**, which is similar to 2.347(1) Å in bis[1,1′-bis(diphenylphosphino)ferrocenyl]palladium dichloride.[35] The dihedral angle between the phenylene planes in **10** (143.0°) is similar to the dihedral angles observed in non-chelate complexes **5** and **6**, but larger than that in the chelate complex **7** (132.1°). The phosphorus atoms are coordinated in a distorted tetrahedral fashion in **10** (*i.e.*, C(1)–P(1)–C(14) 105.6(1)°, C(1)–P(1)–Pd(1) 114.0(1)°, C(14)–P(1)–Pd(1) 110.3(1)°, C(14)–P(1)–C(20) 103.6(2)°).

Ligand **4** reacts with $[PdCl_2(cod)]$ and $[PtCl_2(cod)]$ in 1 : 1 molar ratio to give complexes which could be isolated from the dichloromethane solution.

Crystals of palladium(II) complex **14** (orange) and platinum(II) complex **15** (yellow) with the 4-PPh_2-substituted phenothiazine **4** suitable for X-ray diffraction measurements were obtained from dichloromethane/diethyl ether at room temperature in one week (Fig. 6).

4-PPh_2-substituted phenothiazine **4** acts as a bidentate ligand coordinating *via* the phosphorus(III) and sulfur atoms to Pd or Pt forming a five-membered chelate ring. A distorted square-planar environment is observed (P–M–Cl(2) 173.69(4)° for **14** and 174.69(6)° for **15**, and S–M–Cl(1) 175.28(3)° for **14** and 177.55(6)° for **15**). Complexes **14** and **15** are isostructural. The M–S (2.2575(8) Å for **14** and 2.239(1) Å for **15**) and M–P bonds (2.2236(9) Å for **14** and 2.215(1) Å for **15**) and S–M–P bond angles (88.21(3)° for **14** and 88.71(5)° for **15**) have comparable values to other five-membered P,S chelates like *cis*-[Pd{(1-P(Biph)-2-S-C_6H_4)-κ^2S,P}$_2$][16] (Biph = 1,1′-biphenyl-2,2′-diyl), *cis*-[Pt{(1-P(Biph)-2-S-C_6H_4)-κ^2S,P}$_2$][16] and [$PtCl_2${(1-SPh-8-PPh_2-Nap)-κ^2S,P}][36] (Nap = naphthalene-1,8-diyl) [M–S:



14 **15**

Fig. 6 Molecular structures of complexes **14** and **15**. Thermal ellipsoids are drawn at 50% probability. Hydrogen atoms are omitted for clarity. Selected bond lengths (Å) and angles (°) for **14**: Pd(1)–Cl(1) 2.310(1), Pd(1)–P(1) 2.2236(9), Pd(1)–S(1) 2.2575(8), Pd(1)–Cl(2) 2.3552(9), S(1)–C(6) 1.764(3), S(1)–C(7) 1.783(3), P(1)–Pd(1)–S(1) 88.21(3), P(1)–Pd(1)–Cl(1) 88.47(3), P(1)–Pd(1)–Cl(2) 173.69(4), Cl(1)–Pd(1)–Cl(2) 94.62(4), C(1)–P(1)–C(15) 107.4(2), C(1)–P(1)–Pd(1) 107.1(1), C(21)–P(1)–Pd(1) 114.9(1), C(15)–P(1)–C(21) 106.7(2), C(1)–P(1)–C(21) 105.0(2), C(6)–S(1)–Pd(1) 105.4(1), C(7)–S(1)–Pd(1) 114.3(1), C(6)–S(1)–C(7) 96.15(1), C(1)–C(6)–S(1) 121.0(2); for **15**: Pt(1)–Cl(1) 2.313(2), Pt(1)–P(1) 2.215(1), Pt(1)–S(1) 2.239(1), Pt(1)–Cl(2) 2.357(2), S(1)–C(6) 1.767(5), S(1)–C(7) 1.791(5), P(1)–Pt(1)–S(1) 88.71(5), P(1)–Pt(1)–Cl(1) 90.10(5), P(1)–Pt(1)–Cl(2) 174.69(6), Cl(1)–Pt(1)–Cl(2) 91.94(6), C(1)–P(1)–C(15) 107.4(3), C(1)–P(1)–Pt(1) 107.1(2), C(21)–P(1)–Pt(1) 115.4(2), C(15)–P(1)–C(21) 105.7(3), C(1)–P(1)–C(21) 105.5(2), C(6)–S(1)–Pt(1) 105.4(2), C(7)–S(1)–Pt(1) 114.3(2), C(6)–S(1)–C(7) 95.7(2), C(1)–C(6)–S(1) 120.7(4).

2.3110(5) Å, 2.3098(8) Å, 2.261(4) Å; M–P: 2.2567(4) Å, 2.2365(7) Å, 2.221(4) Å; S–M–P: 87.37(2)°, 87.63(3)°, 90.5(2)°, respectively]. The Pd–P (2.2236(9) Å) bond length in complex **14** is similar to that observed in complex **7** (2.1829(5) Å), but the deviation from square-planar geometry is smaller in the case of **7** [P(1)–Pd(1)–Cl(1) 176.74(2)° in **7**, P(1)–Pd(1)–Cl(2) 173.69(4)° in **14**]. The phenothiazine unit is folded along the N–S axis in the complexes in which five-membered rings are formed through coordination. For example, in complex **10**, in which two molecules of the ligand coordinate to the metal centre and there are no structural restraints, the N(1)–C(4)–C(5) and S(1)–C(3)–C(2) bond angles are 122.7(3) and 118.4(2)°, respectively, while in complex **7**, in which the nitrogen atom participates in chelation, the N(1)–C(7)–C(12) bond angle is smaller (120.4(2)°) and the S(1)–C(8)–C(9) bond angle is larger (119.4(2)°). However, in complex **14**, in which P,S coordination is observed, both the N(1)–C(5)–C(4) (124.1(3)°) and the S(1)–C(6)–C(1) bond angles (121.0(2)°) are larger than in **10**. The dihedral angle between the phenylene rings of the phenothiazine moiety is 133.3° in **14** and 132.2° in **15**, indicating a similar deviation from planarity compared to complex **7** (132.1°) and a smaller deviation compared to non-chelate complex **10** (143.0°).

Interactions with DNA

Chemotherapeutic anticancer drugs are known to interact with DNA by intercalation or groove binding.[37] Intercalation of molecules with DNA may lead to frameshift mutagenesis and chromosomal breakage.[38] DNA intercalators and groove-binding compounds have found clinical applications as anticancer and antibacterial agents.[39] To evaluate the potential activity of phenothiazines in this biological context, the interaction of phenothiazinyl-phosphines **1**, **3** and **4** and metal complexes **14** and **15** with DNA was investigated by agarose gel electrophoresis (ESI, Fig. S3†).

These experiments indicated that phenothiazinyl-phosphines **1** and **3** interact with DNA and change its physical properties, as the modified DNA does not migrate during electrophoresis and can be observed at the starting line, whereas there was no significant interaction for phenothiazinyl-phosphine **4** at low concentrations. For the highest tested concentrations of all three compounds, the disappearance of some relaxed form of DNA and an agglomeration of DNA molecules that did not migrate and remained in the wells was observed, similar to other phenothiazine-containing compounds, such as 2-amino-10-methyl-10*H*-phenothiazine and a phenothiazine analogue of Tröger's base.[19]

The Pd^{II} and Pt^{II} complexes of 4-PPh_2-substituted phenothiazine **4** are capable of binding DNA to generate molecular aggregates with different migration capacities in the electrophoresis experiment compared to the free DNA.

To evaluate the intercalation capacity of complexes **14** and **15** in DNA, the electronic absorption spectral titration in the presence of increasing amounts of CT-DNA was performed (ESI, Fig. S4†). CT-DNA absorption increased during titration, but no other relevant hypochromic or hyperchromic effect could be observed at 343 nm for complex **15**. In the case of complex **14**, a clear distinction between the absorption maxima from 289 nm of complex **14** and 260 nm of DNA cannot be made. The ability of complex **15** to displace CT-DNA-bound ethidium bromide was investigated by fluorescence measurements (ESI, Fig. S5†). Only a small increase in the ethidium bromide emission could be observed upon addition of the complexes to DNA-bound ethidium bromide so an intercalative binding of the studied complexes to DNA is unlikely.

DNA melting experiments were carried out based on the intensity of the absorption maximum CT-DNA at 260 nm at different temperatures, in the absence and presence of complexes **14** and **15** (ESI, Fig. S6†). According to this, the T_m of free CT-DNA was 70.6 °C, while for DNA treated with complexes **14** and **15** (at a DNA–complex ratio of 1.5 : 1) T_m values of 66.8 °C and 68.6 °C, respectively, were observed. The T_m of DNA treated with complexes **14** and **15** should increase compared to free DNA if intercalation would occur. The observed T_m values were not significantly different for the free DNA and the two tested complexes and none of the performed experiments show intercalation of the tested complexes in DNA.

Cytotoxicity of complexes 11 and 15

Due to the proven antiproliferative effects of platinum(II) complexes against diverse tumour types, reports on new platinum complexes and their cytotoxic activity are constantly increasing.[40–43]

The cytotoxicity of platinum(II) complexes **11** and **15** was assessed against breast, colorectal and hepatocarcinoma cell lines. The platinum(II) complex **8** could not be obtained in pure form, and complex **13** is similar to its methyl analogue **11**; therefore, these complexes were not tested. The viability of all three cell lines decreased proportionally with the increase in the concentration of both compounds (Fig. 7). The concentrations of compound **15** which reduced the viability by 50% (IC_{50}) were 28.07 μM for the breast carcinoma (MCF7) cell line, 94.22 μM for the hepatocarcinoma (HepG2) cell line, and 110 μM for colorectal carcinoma (DLD1). For compound **11**, the toxicity against all three cell lines was similar, with no significant differences: $IC_{50\ MCF7}$ = 46.75 μM; $IC_{50\ DLD1}$ = 51.58 μM; $IC_{50\ HepG2}$ = 48.49 μM.

When compared with the two-way ANOVA test, the toxicity of compound **15** was found to be significantly higher towards MCF7 cells compared to DLD1 and HepG2 (Table 4). Compound **11** had similar toxicities against all tumor cell lines tested. When comparing the two compounds, the toxicity of compound **11** was significantly higher than that of compound **15** for the HepG2 and DLD1 ($p < 0.001$), while against the MCF7 compound **15** was more effective ($p < 0.01$). The IC_{50} values for both compounds were comparable to those obtained for cisplatin and oxaliplatin for the MCF7 cell line (ESI, Fig. S7†).

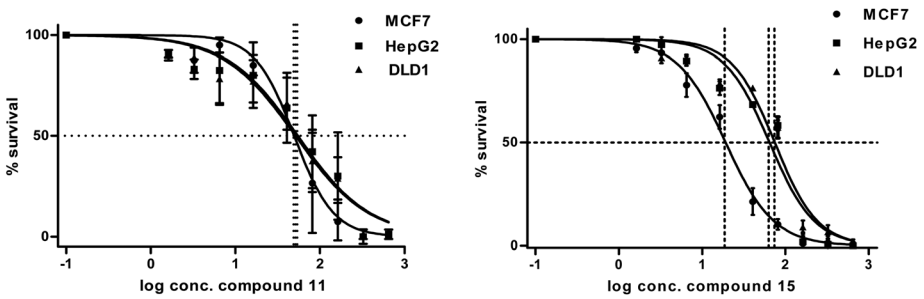


Fig. 7 The cytotoxicity of platinum(II) complexes **11** and **15** against breast (MCF7), colorectal (DLD1) and hepatocarcinoma (HepG2) cell lines. The IC_{50} values were calculated using a nonlinear regression and a four-parameter sigmoidal curve fit, each point representing the mean ± SEM from three separate measurements.

Table 4 IC_{50} of complexes **11** and **15** against the tested cell lines

IC_{50} (μM)	MCF7	HepG2	DLD1
Complex **11**	46.75 ± 3.3	48.49 ± 2.02	51.58 ± 0.6
Complex **15**	28.07 ± 0.24	94.22 ± 1.2	110 ± 0.83
Cisplatin	25.18 ± 0.14	13.09 ± 0.08	32.07 ± 0.54
Oxaliplatin	38.46 ± 0.12	20.46 ± 0.21	51.41 ± 0.07

Conclusions

In the pursuit of new compounds with biological activity we combined the well-known properties of phenothiazine with the coordination ability of the phosphorus towards transition metals already used in cancer therapy. Reactions of phenothiazinyl-phosphine ligands, 1-(diphenylphosphino)-10-methyl-10*H*-phenothiazine (**1**), 3-(diphenylphosphino)-10-alkyl-10*H*-phenothiazine (alkyl = Me (**2**), Et (**3**)) and 4-(diphenylphosphino)-10-ethyl-10*H*-phenothiazine (**4**) with [MCl_2(cod)] (M = Pd, Pt) afforded three coordination patterns for the three regioisomers (**1**, **3** and **4**), characterised by spectroscopy methods and X-ray structure analysis: chelating with nitrogen and phosphorus (the first example in phenothiazine chemistry), [MCl_2{(10-methyl-1-diphenylphosphino-phenothiazine)-κ^2N,P}] (M: Pd, Pt), monodentate through phosphorus, [MCl_2{(10-alkyl-3-diphenylphosphino-phenothiazine)-κP}] (alkyl: Me, Et; M: Pd, Pt) and chelating with sulfur and phosphorus, [MCl_2{(10-ethyl-4-diphenylphosphin-phenothiazinyl)-κ^2S,P}] (M: Pd, Pt). The biological activity of the ligands (**1**, **3** and **4**) and the transition metal complexes **11** ([$PtCl_2$(10-Me-3-PPh_2-Ptz)$_2$]), **14** ([$PdCl_2${(10-Et-4-PPh_2-Ptz)-κ^2S,P}]) and **15** ([$PtCl_2${(10-Et-4-PPh_2-Ptz)-κ^2S,P}]) was also reported. A higher DNA binding capacity was observed for ligand **3** compared to compounds **1** and **4**. Complexes **14** and **15** also interact with DNA, generating molecular aggregates. Compound **15** showed better cytotoxic activity against breast carcinoma, but had a lesser effect on hepatocarcinoma and colorectal carcinoma cell lines than compound **11**. Theoretical calculations (DFT and TD-DFT) explained the better fluorescence quantum yield observed for ligand (**3**) compared to the other regioisomers.

Experimental

General remarks

All manipulations were carried out by standard Schlenk and vacuum-line techniques under an atmosphere of dry argon using anhydrous solvents. 1-Bromo-10-methyl-phenothiazine,[44] 10-methyl-phenothiazine,[45] 10-ethyl-phenothiazine,[45] 3-bromo-10-methyl-phenothiazine,[15] 3-bromo-10-ethyl-phenothiazine,[15] [$PdCl_2$(cod)][46] and [$PtCl_2$(cod)][46] were prepared according to literature procedures. All other chemicals were used as purchased. CT-DNA lyophilized powder was purchased from Sigma-Aldrich. THF was dried over sodium/benzophenone, distilled under an atmosphere of dry argon and stored over molecular sieves. CH_2Cl_2 and TMEDA were refluxed over CaH_2, distilled and kept under argon. $CDCl_3$ was dried over $LiAlH_4$, distilled and stored over molecular sieves. The infrared spectra were recorded on a Bruker Vector 22 IR spectrometer scanning between 4000 and 400 cm^{-1} using KBr pellets. The ^1H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker Avance 300 and Bruker Avance 600 spectrometers. ^1H and ^{13}C chemical shifts are quoted in parts per million (ppm) at 600.13 MHz or 300.11 MHz for ^1H and at 150.90 MHz or 75.46 MHz for ^{13}C relative to tetramethylsilane (TMS). ^{31}P NMR chemical shifts were determined relative to 85% H_3PO_4 as the external standard (at 242.94 MHz or 121.49 MHz). *J* values are given in Hz. EI-MS spectra were recorded on a GC-MS Shimadzu QP-2010 Plus instrument. ESI-MS spectra were recorded on an Orbitrap Thermo mass spectrometer. A Perkin Elmer Lambda 35 UV/Vis spectrometer with 1.0 cm quartz cells was used for absorbance studies. Fluorescence studies were conducted on a Perkin Elmer LS 55 instrument. Thermal denaturation studies were carried out with a CECIL CE7450 spectrophotometer equipped with a Peltier temperature-controlling programmer. Cyclic voltammograms were recorded on a, Gamry Instruments, Reference 600 instrument, using a solution of 0.1 M tetrabutylammonium hexafluorophosphate in CH_2Cl_2 as the supporting electrolyte; starting potential: −2.0 V *vs.* SCE, scan rate ν = 50 mV s^{-1}. The reference electrode consisted of a silver wire (diameter ~1 mm, length ~10 cm) coated with AgCl and dipped into a 1 M KCl solution. A glassy carbon electrode (3 mm) was used as the working electrode and a Pt wire as the

counter electrode. Potentials are reported *vs.* ferrocenium/ferrocene (FcH^+/FcH). The concentration of **1**, **3** and **4** was 1.1×10^{-2} M. The elemental analyses (C, H, N, S) were performed on a Vario EL – Heraeus. The melting points were determined using an Electrothermal 9100 instrument and are uncorrected.

Crystallographic data collection and structural refinement

For compounds **5–7**, the data were collected on a Gemini diffractometer (Agilent Technologies) using $Mo_{K\alpha}$ radiation (λ = 0.71073 Å), and ω-scan rotation. Data reduction was performed with CrysAlis Pro[47] including the program SCALE3 ABSPACK for empirical absorption correction. The structures were solved by direct methods and the refinement was performed with SHELXL-2013.[48] With the exception of one disordered solvent molecule in **7**, all non-hydrogen atoms were refined with anisotropic thermal parameters and hydrogen atoms were calculated on idealised positions. For compounds **10**, **14** and **15**, the data were collected on a Bruker SMART APEX diffractometer, using graphite-monochromated $Mo_{K\alpha}$ radiation (λ = 0.71073 Å). For this purpose the crystals were attached on cryoloops to a glass fibre and data were collected at room temperature (293 K). The structures were refined with anisotropic thermal parameters. The hydrogen atoms were refined with a riding model and a mutual isotropic thermal parameter. For structure solving and refinement the software package SHELX-97 was used.[48] CCDC 1019584 (**5**), 1019585 (**6**), 1019586 (**7**), 1019587 (**10**), 1019588 (**14**) and 1019589 (**15**) contain the supplementary crystallographic data for this paper.

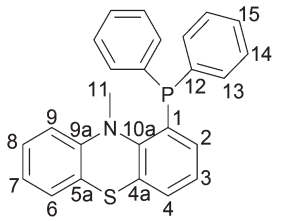
DFT calculations

Geometries for the ground state (S_0) and the first excited singlet state (S_1) of compounds **1**, **3** and **4** were obtained using DFT and TD-DFT, respectively, with the B3LYP functional together with the 6-311G(2d,2p) basis set as implemented in Gaussian 09.[49] Frequency calculations were used to confirm the S_0 and S_1 geometries as local minima on the potential-energy surface. UV/Vis spectra were simulated using TD-DFT with a total number of 50 states.

General procedure for the synthesis of 1-(diphenylphosphino)-10-methyl-10*H*-phenothiazine (1), 3-(diphenylphosphino)-10-methyl-10*H*-phenothiazine (2) and 3-(diphenylphosphino)-10-ethyl-10*H*-phenothiazine (3)

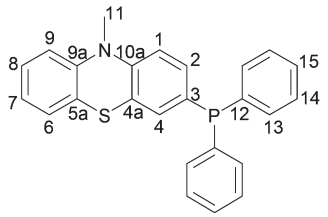
The starting material (1-bromo-10-methyl-10*H*-phenothiazine for **1**, 3-bromo-10-methyl-10*H*-phenothiazine for **2** and 3-bromo-10-ethyl-10*H*-phenothiazine for **3**) was dissolved in dry THF and cooled to −78 °C, and then *n*-butyllithium was added dropwise. After 2 h of stirring at low temperature, a THF solution of chlorodiphenylphosphine was added dropwise and the solution was stirred overnight. The reaction mixture was diluted with 100 ml of dichloromethane and washed with water (3 × 100 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and then the solvent was removed *in vacuo*. The resulting yellow oil was purified by flash chromatography on silica.

1-(Diphenylphosphino)-10-methyl-10*H*-phenothiazine (1). 1.43 g 1-bromo-10-methyl-phenothiazine (4.9 mmol), 6.93 ml *n*-butyllithium (11.09 mmol), and 0.9 ml chlorodiphenylphosphine (11.09 mmol) were added into 8 ml THF. The resulting yellow oil was purified by flash chromatography on silica with *n*-heptane and triethylamine (0.1%) as eluents to afford the product as a white solid. **1** (0.51 g, 26%). mp = 123–125 °C. Found: C 75.8, H 5.15, N 3.4, S 7.7. Calc. for $C_{25}H_{20}NPS$: C 75.5, H 5.1, N 3.5, S 8.1%. UV-vis: λ_{max} (CH_3CN)/nm 253 and 313sh (ε/dm^3 mol^{-1} cm^{-1} 19 738). Fluorescence: λ_{exc} = 313 nm, λ_{em} = 464 nm, Φ = 0.5% in CH_3CN, $c = 5.1 \times 10^{-7}$ M (standard: perylene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3045 (w), 2962 (w), 2861 (w), 1582 (w), 1472 (s), 1432 (s), 1398 (m), 1328 (m), 1260 (s), 1093 (s), 11 020 (s), 869 (w), 799 (s), 747 (s), 693 (s). δ_H (600.13 MHz, $CDCl_3$) 3.44 (3 H, d, $^5J_{PH}$ 1, 11-H), 6.26 (1 H, dd, $^4J_{HH}$ 1, $^3J_{HH}$ 8, 9-H), 6.65 (1 H, ddd, $^4J_{HH}$ 1, $^3J_{HH}$ 8, $^3J_{PH}$ 4, 2-H), 6.89 (1 H, t, $^3J_{HH}$ 8, 7-H), 6.93 (1 H, dd, $^4J_{HH}$ 1, $^3J_{HH}$ 8, 6-H), 6.97 (1 H, td, $^4J_{HH}$ 2, $^3J_{HH}$ 8, 8-H), 7.12 (1 H, dd, $^4J_{HH}$ 2, $^3J_{HH}$ 8, 4-H), 7.28–7.34 (10 H, m, Ph), 7.43 (1 H, td, $^4J_{PH}$ 2, $^3J_{HH}$ 8, 3-H). δ_P (242.94 MHz, $CDCl_3$) −8.9. δ_C (150.90 MHz, $CDCl_3$) 43.2 (d, J_{PC} 17, 11-C), 123.9 (9-C), 126.4 (7-C), 127.2, 127.6 (6-C, 8-C), 127.26 (d, $^3J_{PC}$ 8.9, 4a-C), 127.6 (5a-C), 128.5 (d, $^3J_{PC}$ 6.6, 3-C), 128.6 (d, $^3J_{PC}$ 7.4, 14-C), 128.9 (15-C), 132.3 (d, $^1J_{PC}$ 5.6, 1-C), 132.9 (d, $^2J_{PC}$ 18.1, 2-C), 133.2 (d, $^4J_{PC}$ 1.2, 4-C), 134.2 (d, $^2J_{PC}$ 21, 13-C), 137.0 (d, $^1J_{PC}$ 10.9, 12-C), 147.6 (9a-C), 148.26 (d, $^2J_{PC}$ 17.9, 10a-C). EI-MS: *m*/*z* 397 $[M]^+$.

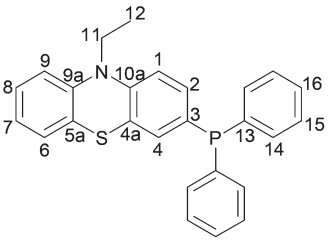


3-(Diphenylphosphino)-10-methyl-10*H*-phenothiazine (2). 1.43 g 3-bromo-10-methyl-phenothiazine (4.9 mmol), 6.93 ml *n*-butyllithium (11.09 mmol), and 0.9 ml chlorodiphenylphosphine (11.09 mmol) were added into 8 ml THF. The resulting yellow oil was purified by flash chromatography on silica with toluene–*n*-heptane = 2 : 3 and triethylamine (0.1%) as eluents to afford the product as a pale yellow solid. **2** (0.60 g, 31%). mp = 141–142 °C (lit.,[18] 112.5 °C). Found: C 75.7, H 5.2, N 3.4, S 8.2. Calc. for $C_{25}H_{20}NPS$: C 75.5, H 5.1, N 3.5, S 8.1%. IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3052 (w), 3006 (w), 2963 (w), 2881 (w), 2817 (w), 1590 (w), 1563 (w), 1457 (vs), 1428 (w), 1370 (m), 1329 (m), 1289 (w), 1256 (m), 1138 (w), 1093 (w), 1020 (m), 997 (w), 814 (m), 745 (vs), 695 (s). δ_H (600.13 MHz, $CDCl_3$) 3.37 (3 H, s, 11-H), 6.79 (1 H, d, $^3J_{HH}$ 8, 9-H), 6.82 (1 H, d, $^3J_{HH}$ 8, 1-H), 6.94 (1 H, t, $^3J_{HH}$ 8, 7-H), 7.08 (1 H, dd, $^3J_{HH}$ 7, $^4J_{HH}$ 2, 6-H), 7.11 (1 H, dd, $^3J_{HH}$ 8, $^4J_{HH}$ 1, 8-H), 7.14–7.19 (2 H, m, 2-H, 4-H), 7.28–7.31 (4 H, m, Ph), 7.32–7.34 (6 H, m, Ph). δ_P (242.94 MHz, $CDCl_3$) −6.9. δ_C (150.90 MHz, $CDCl_3$) 35.5 (11-C), 114.3 (d, $^3J_{PC}$ 8.4, 1-C), 114.3 (9-C), 122.9 (7-C), 123.3 (5a-C),

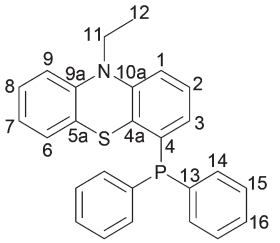
123.9 (d, $^3J_{PC}$ 7.7, 4a-C), 127.4, 127.7 (6-C, 8-C), 128.6 (d, $^3J_{PC}$ 6.8, 14-C), 128.8 (15-C), 130.1 (d, $^1J_{PC}$ 10.7, 3-C), 132.4 (d, $^2J_{PC}$ 20, 2-C), 133.6 (d, $^2J_{PC}$ 19.2, 13-C), 133.8 (d, $^2J_{PC}$ 22.9, 4-C), 137.5 (d, $^1J_{PC}$ 10.5, 12-C), 145.5 (10a-C), 146.7 (9a-C). EI-MS: m/z 397 $[M]^+$.



3-(Diphenylphosphino)-10-ethyl-10*H*-phenothiazine (3). 1.5 g 3-bromo-10-ethyl-phenothiazine (4.9 mmol), 6.93 ml *n*-butyllithium (11.09 mmol), and 0.9 ml chlorodiphenylphosphine (11.09 mmol) were added into 8 ml THF. The resulting yellow oil was purified by flash chromatography on silica with toluene–*n*-heptane = 2 : 3 and triethylamine (0.1%) as eluents to afford the product as a yellow solid. **3** (0.78 g, 39%). mp = 168–169 °C. Found: C 75.5, H 5.5, N 3.7, S 7.6. Calc. for $C_{26}H_{22}NPS$: C 75.9, H 5.4, N 3.4, S 7.8%. UV-vis: λ_{max} (CH_3CN)/nm 262 and 311sh (ε/dm^3 mol^{-1} cm^{-1} 33 570). Fluorescence: λ_{exc} = 311 nm, λ_{em} = 458 nm, Φ = 4.6% in CH_3CN, c = 2.1 × 10^{-7} M (standard: perylene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3068 (w), 3050 (w), 2974 (w), 2930 (w), 2851 (w), 1590 (w), 1565 (m), 1460 (vs), 1433 (w), 1384 (m), 1357 (w), 1326 (m), 1251 (w), 1234 (m), 1160 (w), 1134 (w), 1107 (m), 997 (w), 890 (w), 812 (m), 745 (vs), 695 (s), 637 (vw), 598 (vw). δ_H (300.11 MHz, $CDCl_3$) 1.42 (3 H, t, $^3J_{HH}$ 7, 12-H), 3.92 (2 H, q, $^3J_{HH}$ 7, 11-H), 6.82–6.93 (3 H, m, 1-H, 7-H, 9-H), 7.04–7.20 (4 H, m, 2-H, 4-H, 6-H, 8-H), 7.28–7.35 (10 H, m, Ph). δ_P (121.49 MHz, $CDCl_3$) −7.0. δ_C (75.46 MHz, $CDCl_3$) 13.0 (12-C), 42.0 (11-C), 115.1 (d, $^3J_{PC}$ 8.3, 1-C), 115.3 (9-C), 122.7 (7-C), 124.1 (5a-C), 124.6 (d, $^3J_{PC}$ 7.5, 4a-C), 127.4, 127.5 (6-C, 8-C), 128.6 (d, $^3J_{PC}$ 6.9, 15-C), 128.8 (16-C), 129.9 (d, $^1J_{PC}$ 10.2, 3-C), 132.6 (d, $^2J_{PC}$ 19.9, 2-C), 133.6 (d, $^2J_{PC}$ 22.5, 4-C), 133.6 (d, $^2J_{PC}$ 19.3, 14-C), 137.4 (d, $^1J_{PC}$ 10.4, 13-C), 144.5 (10a-C), 145.8 (9a-C). EI-MS: m/z 411 $[M]^+$.



Synthesis of 4-(diphenylphosphino)-10-ethyl-10*H*-phenothiazine (4). To a mixture of 10-ethyl-phenothiazine (1 g, 4.40 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) (1.68 ml, 11.01 mmol) in 30 ml of dry THF, *t*-butyllithium (5.91 ml, 11 mmol) was added dropwise at 0 °C. The reaction mixture was allowed to stir overnight at room temperature and then cooled to 0 °C. Then chlorodiphenylphosphine (2.07 ml, 11.01 mmol) in 5 ml THF was added dropwise. After stirring for 16 h at room temperature, the reaction mixture was diluted with 100 ml dichloromethane and washed with water (3 × 100 ml). The organic phase was dried over anhydrous sodium sulfate, filtered and then the solvent was removed *in vacuo*. The resulting yellow oil was purified by flash chromatography on silica with toluene–*n*-heptane = 1 : 2 and triethylamine (0.1%) as eluents to afford the product as a light yellow crystalline powder. **4** (0.33 g, 18%). mp = 143–145 °C (lit.,[19] 151–152 °C). Found: C 75.7, H 5.5, N 3.4, S 7.6. Calc. for $C_{26}H_{22}NPS$: C 75.9, H 5.4, N 3.4, S 7.8%. UV-vis: λ_{max} (CH_3CN)/nm 252 and 305sh (ε/dm^3 mol^{-1} cm^{-1} 19 935). Fluorescence: λ_{exc} = 305 nm, λ_{em} = 456 nm, Φ = 0.5% in CH_3CN, c = 9.2 × 10^{-7} M (standard: perylene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3075 (vw), 3042 (vw), 2990 (w), 2937 (vw), 2793 (vw), 1586 (w), 1554 (w), 1475 (m), 1442 (s), 1401 (w), 1384 (w), 1322 (w), 1285 (w), 1245 (m), 1195 (vw), 1158 (vw), 1135 (w), 1097 (w), 780 (w), 759 (m), 722 (w), 699 (m), 563 (vw). δ_H (300.11 MHz, $CDCl_3$) 1.43 (3 H, t, $^3J_{HH}$ 7, 12-H), 3.94 (2 H, q, $^3J_{HH}$ 7, 11-H), 6.39 (1 H, dd, $^3J_{HH}$ 7, $^3J_{PH}$ 4, 3-H), 6.85–6.90 (3 H, m, 1-H, 7-H, 9-H), 7.02–7.16 (3 H, m, 2-H, 6-H, 8-H), 7.32–7.35 (10 H, m, Ph). δ_P (121.49 MHz, $CDCl_3$) −13.2. δ_C (75.46 MHz, $CDCl_3$) 13.2 (12-C), 42.1 (11-C), 115.1, 115.5 (1-C, 9-C), 122.5 (7-C), 124.7 (d, $^1J_{PC}$ 6.6, 4-C), 126.8 (6-C, 8-C), 127.4 (3-C), 127.8 (2-C), 128.7 (d, $^3J_{PC}$ 7.2, 15-C), 128.9 (16-C), 130.3 (d, $^2J_{PC}$ 30.8, 4a-C), 134.1 (d, $^2J_{PC}$ 19.9, 14-C), 136.0 (5a-C), 136.2 (d, $^1J_{PC}$ 10.2, 13-C), 145.2 (9a-C), 145.6 (d, $^3J_{PC}$ 5.7, 10a-C). EI-MS: m/z 411 $[M]^+$.



General procedure for the synthesis of [$PdCl_2${(10-alkyl-phenothiazine)-κ*S*}$_2$]

A solution of [$PdCl_2$(cod)] in CH_2Cl_2 was added dropwise with stirring at room temperature to a CH_2Cl_2 solution of N-substituted phenothiazine. The reaction mixture turned dark purple and was stirred for one additional hour. On standing at room temperature crystals suitable for X-ray measurements were obtained.

[$PdCl_2$(10-Me-Ptz)$_2$] (5). 79 mg 10-*H*-10-methyl-phenothiazine (0.37 mmol), 53 mg [$PdCl_2$(cod)] (0.19 mmol). **5** (92 mg, 82%). mp = 257–258 °C. Found: C 51.9, H 3.8, N 4.55, S 10.8. Calc. for $C_{26}H_{22}Cl_2N_2PdS_2$: C 51.7, H 3.7, N 4.6, S 10.6%. UV-vis: λ_{max}(CH_2Cl_2)/nm 254, 309 and 565 (ε/dm^3 mol^{-1} cm^{-1} 29 970, 7957 and 3260). Fluorescence: λ_{exc} = 254 nm, λ_{em} = 366 nm, Φ = 5.2% in CH_2Cl_2, c = 10^{-10} M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 2881 (w), 2854 (w), 2806 (w), 1645 (m), 1599 (m), 1571 (s), 1484 (m), 1455 (s), 1339 (m), 1286 (m), 1256 (m), 1166 (w), 1136 (w), 1098 (w), 1039 (w), 996 (w), 871 (m), 756 (s). δ_H (600.13 MHz, $CDCl_3$)

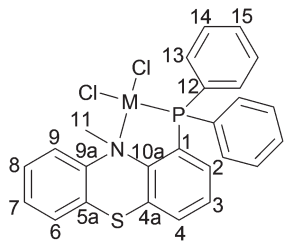
3.38 (6 H, s, CH_3), 6.82 (4 H, d, $^3J_{HH}$ 8, 1-H, 9-H), 6.93 (4 H, t, $^3J_{HH}$ 7, 3-H, 7-H), 7.14 (4 H, d, $^3J_{HH}$ 8, 4-H, 6-H), 7.17 (4 H, t, $^3J_{HH}$ 8, 2-H, 8-H). δ_C (150.90 MHz, $CDCl_3$) 35.4 (*C*H_3), 114.2 (1-C, 9-C), 122.6 (3-C, 7-C), 123.5 (4a-C, 5a-C), 127.3 (2-C, 8-C), 127.6 (4-C, 6-C), 146.0 (9a-C, 10a-C).

[$PdCl_2$(10-Et-Ptz)$_2$] (6). 84 mg 10-*H*-10-ethyl-phenothiazine (0.37 mmol), 53 mg [$PdCl_2$(cod)] (0.19 mmol). **6** (91 mg, 78%). mp = 245–247 °C. Found: C 53.1, H 4.2, N 4.6, S 10.3. Calc. for $C_{28}H_{26}Cl_2N_2PdS_2$: C 53.2, H 4.15, N 4.4, S 10.15%. UV-vis: λ_{max} (CH_2Cl_2)/nm 256, 310 and 571 (ε/dm^3 mol^{-1} cm^{-1} 51 833, 13 473 and 5607). Fluorescence: λ_{exc} = 256 nm, λ_{em} = 363 nm, Φ = 4.1% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3061 (w), 2987 (w), 1571 (s), 1486 (s), 1456 (s), 1369 (m), 1288 (m), 1243 (m), 1172 (w), 1140 (w), 1104 (w), 1041 (w), 932 (w), 893 (m), 749 (s). δ_H (600.13 MHz, $CDCl_3$) 1.42 (6 H, t, $^3J_{HH}$ 7, C*H*$_3$), 3.93 (4 H, br s, C*H*$_2$), 6.88–6.92 (8 H, m, 1-H, 9-H, 3-H, 7-H), 7.13 (4 H, d, $^3J_{HH}$ 7, $^3J_{HH}$ 8, 4-H, 6-H), 7.15 (4 H, t, $^3J_{HH}$ 8, 2-H, 8-H). δ_C (150.90 MHz, $CDCl_3$) 13.0 (*C*H_3), 41.7 (*C*H_2), 115.0 (1-C, 9-C), 122.2 (3-C, 7-C), 123.4 (4a-C, 5a-C), 127.2 (2-C, 8-C), 127.3 (4-C, 6-C), 144.9 (9a-C, 10a-C).

General procedure for the synthesis of complexes 7–15

A solution of [MCl_2(cod)] (M = Pd, Pt) in CH_2Cl_2 was added dropwise to a solution of the ligand in CH_2Cl_2 at room temperature. The reaction mixture was allowed to stir overnight, and then the volume of the obtained solution was reduced to 1/4 of the initial volume by removing the solvent *in vacuo*. Suitable crystals for X-ray measurements were obtained by slow crystallisation from CH_2Cl_2/Et_2O.

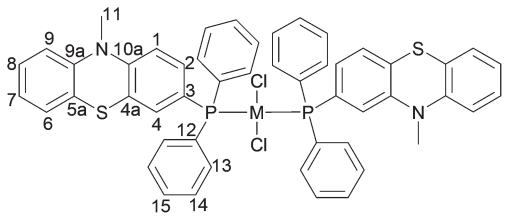
[MCl_2\{(10-methyl-1-diphenylphosphino-phenothiazine)-κ^{2}N, *P*\}] (M = Pd, Pt).



[$PdCl_2$(10-Me-1-PPh_2-Ptz)] (7). 37 mg [$PdCl_2$(cod)] (0.13 mmol), 52 mg **1** (0.13 mmol). **7** (60 mg, 80%). mp > 207 °C (decomp.). Found: C 47.5, H 3.3, N 2.3, S 5.0. Calc. for $C_{25}H_{20}Cl_2NPPdS{\cdot}CH_2Cl_2$: C 47.3, H 3.4, N 2.1, S 4.9%. UV-vis: λ_{max} (CH_2Cl_2)/nm 228, 281 and 356 (ε/dm^3 mol^{-1} cm^{-1} 289 600, 138 520 and 18 800). Fluorescence: λ_{exc} = 281 nm, λ_{em} = 375 nm, Φ = 0.9% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3053 (w), 2958 (w), 2921 (w), 2848 (w), 1515 (w), 1483 (s), 1459 (s), 1436 (s), 1408 (s), 1133 (w), 1101 (s), 1031 (w), 997 (w), 958 (w), 910 (w), 787 (m), 751 (s), 721 (m), 692 (w). δ_H (600.13 MHz, $CDCl_3$) 4.05 (3 H, s, 11-H), 7.29 (1 H, d, $^3J_{HH}$ 8, 9-H), 7.39–7.44 (5 H, m, 14-H, 6-H, 7-H, 8-H), 7.49–7.52 (3 H, m, 14-H, 4-H), 7.60 (1 H, t, $^3J_{HH}$ 8, 3-H), 7.72 (2 H, t, $^3J_{HH}$ 8, 15-H), 7.77 (2 H, dd, $^3J_{HH}$ 8, $^3J_{PH}$ 13, 13-H), 7.82 (2 H, dd, $^3J_{HH}$ 8, $^3J_{PH}$ 11, 13-H), 9.42 (1 H, d, $^3J_{HH}$ 8.4, 2-H). δ_P (242.94 MHz, $CDCl_3$) 42.6. δ_C (150.90 MHz, $CDCl_3$) 48.3 (11-C), 128.2 (d, $^3J_{PC}$ 12.5, 4a-C), 128.3 (9-C), 128.4 (d, $^3J_{PC}$ 8.6, 3-C), 128.5 (7-C), 128.8 (6-C, 8-C), 128.9 (5a-C), 129.2 (d, $^3J_{PC}$ 12.4, 14-C), 129.4 (d, $^3J_{PC}$ 12.1, 14-C), 131.7 (15-C), 131.9 (d, $^1J_{PC}$ 2.6, 1-C), 132.0 (15-C), 132.6 (d, $^1J_{PC}$ 2.3, 12-C), 132.7 (d, $^1J_{PC}$ 3.2, 12-C), 133.4 (d, $^2J_{PC}$ 9.7, 4-C, 2-C), 133.8 (d, $^2J_{PC}$ 11.3, 13-C), 134.1 (d, $^2J_{PC}$ 11.5, 13-C), 143.1 (9a-C), 151.4 (d, $^2J_{PC}$ 16.8, 10a-C).

[$PtCl_2$(10-Me-1-PPh_2-Ptz)] (8) and (9). 49 mg [$PtCl_2$(cod)] (0.13 mmol), 52 mg **1** (0.13 mmol). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3055 (w), 3004 (w), 2955 (w), 2870 (w), 1471 (m), 1431 (s), 1402 (s), 1333 (w), 1183 (w), 1134 (w), 1097 (s), 1029 (w), 1002 (w), 904 (w), 867 (w), 750 (s), 694 (s). δ_H (600.13 MHz, $CDCl_3$) 3.32 (6 H, s, CH_3 **9**), 4.27 (3 H, s, CH_3 **8**), 6.28 (2 H, d, $^3J_{HH}$ 8, 9-H **9**), 6.81–6.83 (3 H, m, 9-H **8**; 7-H **9**), 6.92 (1 H, t, $^3J_{HH}$ 8, 7-H **8**), 6.95–6.99 (5 H, m, 6-H (or 8-H) **8**; 6-H, 8-H **9**), 7.06–7.10 (4 H, m, 3-H, 4-H **9**), 7.13–7.17 (7 H in total, m; 8-H (or 6-H) **8**, 3-H, 4-H **8** (3 H); Ph **9** (4 H)), 7.22–7.25 (4 H, m, 14-H **8**), 7.35–7.50 (15 H, m, Ph **9**), 7.57 (2 H, t, $^3J_{HH}$ 7, 2-H **9**), 7.67 (1 H, d, $^3J_{HH}$ 8, **9**), 7.77 (3 H, dd, $^3J_{PH}$ 13, $^3J_{HH}$ 7, Ph **8**), 7.81 (3 H, dd, $^3J_{PH}$ 13, $^3J_{HH}$ 7, Ph **8**), 9.51 (1 H, d, $^3J_{HH}$ 8, 2-H **8**). δ_P (242.94 MHz, $CDCl_3$) 14.8, $^1J_{PtP}$ = 3808 Hz for **8** and 14.9, $^1J_{PtP}$ = 2714 Hz for **9**.

[MCl_2\{(10-methyl-3-diphenylphosphino-phenothiazine)-κ*P*\}$_2$] (alkyl = methyl, ethyl; M = Pd, Pt).

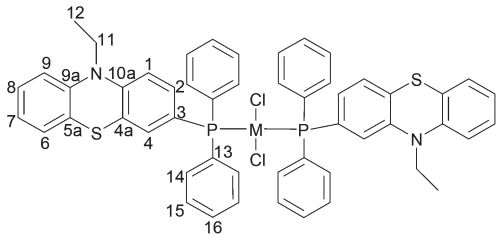


[$PdCl_2$(10-Me-3-PPh_2-Ptz)$_2$] (10). 37 mg [$PdCl_2$(cod)] (0.13 mmol), 103 mg **2** (0.26 mmol). **10** (115 mg, 91%). mp > 236 °C (decomp.). Found: C 61.9, H 4.1, N 2.8, S 6.7. Calc. for $C_{50}H_{40}Cl_2N_2P_2PdS_2$: C 61.8, H 4.15, N 2.9, S 6.6%. UV-vis: λ_{max} (CH_2Cl_2)/nm 227, 263, 335 and 400 (ε/dm^3 mol^{-1} cm^{-1} 58 690, 70 600, 22 360 and 9530). Fluorescence: λ_{exc} = 263 nm, λ_{em} = 375 nm, Φ = 4.8% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 2960 (w), 2871 (w), 1566 (w), 1462 (s), 1430 (m), 1386 (m), 1331 (m), 1253 (m), 1155 (w), 1104 (m), 998 (w), 929 (w), 879 (w), 810 (w), 745 (s), 698 (m). δ_H (600.13 MHz, $CDCl_3$) 3.38 (6 H, s, 11-H), 6.80–6.83 (4 H, m, 1-H, 9-H), 6.94–6.97 (2 H, m, 7-H), 7.10 (2 H, br s, 4-H), 7.17–7.20 (2 H, m, 2-H), 7.36–7.42 (12 H, m, 6-H, 8-H, 14-H), 7.48 (4 H, br s, 15-H), 7.67–7.72 (8 H, m, 13-H). δ_P (242.94 MHz, $CDCl_3$) 22.3. δ_C (150.90 MHz, $CDCl_3$) 35. 7 (11-C), 114.5 (2-C), 114.8 (1-C, 9-C), 123.1 (4a-C), 123.4 (7-C), 128.2 (t, $^3J_{PC}$ 5.1, 14-C), 128.6 (d, $^1J_{PC}$ 12.1, 12-C), 129.9 (5a-C), 130.6 (4-C), 131.1 (d, $^1J_{PC}$ 11.6, 3-C), 131.8 (15-C), 134.8 (6-C, 8-C), 135.1 (t, $^2J_{PC}$ 6.2, 13-C), 147.9 (10a-C), 148.8 (9a-C).

[$PtCl_2$(10-Me-3-PPh_2-Ptz)$_2$] (11). 49 mg $PtCl_2$(cod) (0.13 mmol), 103 mg **2** (0.26 mmol). **11** (115 mg, 83%). mp = 255–256 °C. Found: C 56.5, H 3.9, N 2.8, S 5.9. Calc. for $C_{50}H_{40}Cl_2N_2P_2PtS_2$: C 56.6, H 3.8, N 2.6, S 6.0%. UV-vis: λ_{max}

(CH_2Cl_2)/nm 234, 268 and 318 (ε/dm^3 mol^{-1} cm^{-1} 48 100, 57 750 and 10 440). Fluorescence: λ_{exc} = 268 nm, λ_{em} = 371 nm, Φ = 3.5% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3051 (w), 2964 (w), 1594 (m), 1464 (s), 1434 (m), 1389 (m), 1360 (m), 1258 (m), 1162 (w), 1105 (s), 1021 (m), 998 (w), 891 (w), 804 (m), 748 (m), 694 (m). δ_H (600.13 MHz, $CDCl_3$) 3.28 (6 H, s, 11-H), 6.44 (2 H, d, $^3J_{HH}$ 8, 1-H), 6.78 (2 H, d, $^3J_{HH}$ 8, 9-H), 6.91 (4 H, m, 4-H, 7-H), 7.00 (2 H, d, $^3J_{HH}$ 8, 2-H), 7.16 (4 H, t, $^3J_{HH}$ 8, 6-H, 8-H), 7.22 (8 H, t, $^3J_{HH}$ 7, 14-H), 7.35 (4 H, t, $^3J_{HH}$ 7, 15-H), 7.56 (8 H, t, $^3J_{PH}$ 2, $^3J_{HH}$ 8, 13-H). δ_P (242.94 MHz, $CDCl_3$) 13.2, $^1J_{PtP}$ 3699. δ_C (150.90 MHz, $CDCl_3$) 35.5 (11-C), 100.3 (1-C), 113.2 (2-C), 114.6 (9-C), 122.7 (4a-C), 123.1 (3-C), 123.3 (7-C), 127.3, 127.8 (6-C, 8-C), 128.0 (t, $^3J_{PC}$ 5.5, 14-C), 130.0 (5a-C), 131 (15-C), 132.5 (4-C), 134.6 (12-C), 134.9 (t, $^2J_{PC}$ 5.1, 13-C), 144.7 (10a-C), 147.9 (9a-C).

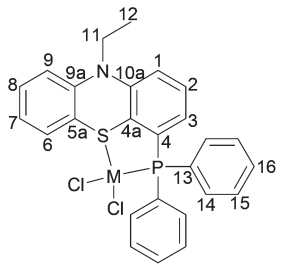
[MCl_2{(10-ethyl-3-diphenylphosphino-phenothiazine)-κ*P*}$_2$] (alkyl = methyl, ethyl; M = Pd, Pt).



[$PdCl_2$(10-Et-3-PPh_2-Ptz)$_2$] (12). 17 mg [$PdCl_2$(cod)] (0.06 mmol), 49 mg **3** (0.12 mmol). **12** (54 mg, 90%). mp > 260 °C (decomp.). Found: C 62.3, H 4.3, N 2.7, S 6.55. Calc. for $C_{52}H_{44}Cl_2N_2P_2PdS_2$: C 62.4, H 4.4, N 2.8, S 6.4%. UV-vis: λ_{max} (CH_2Cl_2)/nm 229, 263, 336 and 404 (ε/dm^3 mol^{-1} cm^{-1} 51 850, 72 530, 23 890 and 9630). Fluorescence: λ_{exc} = 263 nm, λ_{em} = 368 nm, Φ = 2.8% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 2872 (w), 2816 (w), 1644 (w), 1566 (m), 1463 (s), 1429 (m), 1387 (m), 1371 (m), 1331 (m), 1253 (m), 1156 (w), 1104 (m), 878 (w), 808 (w), 746 (m), 698 (m). δ_H (600.13 MHz, $CDCl_3$) 1.42 (6 H, br s, 12-H), 3.93 (4 H, br s, 11-H), 6.87–6.93 (6 H, m, 1-H, 9-H, 7-H), 7.07 (2 H, br s, 4-H), 7.15–7.19 (4 H, m, 2-H, 6-H), 7.37–7.48 (10 H, m, 8-H, 15-H), 7.48 (2 H, br s, 16-H), 7.58 (2 H, br s, 16-H), 7.68–7.71 (8 H, m, 14-H). δ_P (242.94 MHz, $CDCl_3$) 22.1. δ_C (150.90 MHz, $CDCl_3$) 13.0 (12-C), 42.3 (11-C), 114.5 (2-C), 115.4 (1-C), 115.6 (9-C), 122.9 (7-C), 123.3 (4a-C), 128.2 (t, $^3J_{PC}$ 5.4, 15-C), 128.6 (d, $^1J_{PC}$ 11.9, 13-C), 130.0 (5a-C), 130.6 (4-C), 131.0 (d, $^1J_{PC}$ 11.9, 3-C), 131.8 (16-C), 134.7, 134.8 (6-C, 8-C), 135.0 (t, $^2J_{PC}$ 6.3, 14-C), 147.2 (10a-C), 148.2 (9a-C).

[$PtCl_2$(10-Et-3-PPh_2-Ptz)$_2$] (13). 22 mg [$PtCl_2$(cod)] (0.06 mmol), 49 mg **3** (0.12 mmol). **13** (56 mg, 86%). mp > 284 °C (decomp.). Found: C 57.5, H 4.0, N 2.7, S 5.8. Calc. for $C_{52}H_{44}Cl_2N_2P_2PtS_2$ C 57.35, H 4.1, N 2.6, S 5.9%. UV-vis: λ_{max} (CH_2Cl_2)/nm 227, 267 and 318 (ε/dm^3 mol^{-1} cm^{-1} 53 390, 82 570 and 16 490). Fluorescence: λ_{exc} = 267 nm, λ_{em} = 366 nm, Φ = 2.9% in CH_2Cl_2, $c = 10^{-10}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 2963 (w), 2872 (w), 2817 (w), 1596 (w), 1565 (m), 1462 (s), 1383 (w), 1332 (m), 1253 (m), 1155 (w), 1105 (m), 928 (w), 878 (w), 808 (m), 745 (m), 699 (m). δ_H (600.13 MHz, $CDCl_3$) 1.36 (6 H, t, $^3J_{HH}$ 7, 12-H), 3.84 (4 H, q, $^3J_{HH}$ 9, 11-H), 6.48 (2 H, d, $^3J_{HH}$ 8, 1-H), 6.84 (2 H, d, $^3J_{HH}$ 8, 9-H), 6.91 (2 H, t, $^3J_{HH}$ 8, 7-H), 6.94 (2 H, dd, $^3J_{HH}$ 11, $^4J_{PH}$ 2, 4-H), 6.98 (2 H, dd, $^3J_{HH}$ 8, $^3J_{PH}$ 2, 2-H), 7.13–7.17 (4 H, m, 6-H, 8-H), 7.22 (8 H, t, $^3J_{HH}$ 7, 15-H), 7.34 (4 H, t, $^3J_{HH}$ 7, 16-H), 7.56 (8 H, t, $^3J_{PH}$ 2, $^3J_{HH}$ 8, 14-H). δ_P (242.94 MHz, $CDCl_3$) 13.0, $^1J_{PtP}$ 3672. δ_C (150.90 MHz, $CDCl_3$) 12.9 (12-C), 42.1 (11-C), 100.3 (1-C), 113.9 (2-C), 115.4 (9-C), 123.2 (7-C), 123.5 (4a-C), 123.7 (3-C), 127.6 (6-C, 8-C), 128.0 (t, $^3J_{PC}$ 5.7, 15-C), 130.1 (5a-C), 130.9 (16-C), 132.9 (4-C), 134.7 (13-C), 134.9 (t, $^2J_{PC}$ 5.1, 14-C), 143.7 (10a-C), 147.1 (9a-C).

[MCl_2{(10-ethyl-4-diphenylphosphin-phenothiazinyl)-κ^2*S*,*P*}] (M = Pd, Pt).



[$PdCl_2${(10-Et-4-PPh_2-Ptz)-κ*S*,*P*}] (14). 37 mg [$PdCl_2$(cod)] (0.13 mmol), 54 mg **4** (0.13 mmol). **14** (54 mg, 71%). mp > 310 °C (decomp.). Found C 52.85, H 3.8, N 2.5, S 5.5. Calc. for $C_{26}H_{22}Cl_2NPSPd$: C 53.0, H 3.8, N 2.4, S 5.45%. UV-vis: λ_{max} (CH_2Cl_2)/nm 228, 287 and 417 (ε/dm^3 mol^{-1} cm^{-1} 26 450, 9565 and 1490). Fluorescence: λ_{exc} = 287 nm, λ_{em} = 370 nm, Φ = 0.3% in CH_2Cl_2, $c = 10^{-8}$ M (standard: naphthalene solution in cyclohexane). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3058 (m), 2981 (w), 2933 (w), 1593 (m), 1564 (m), 1478 (s), 1449 (m), 1413 (m), 1364 (m), 1253 (s), 1165 (m), 1101 (w), 997 (w), 853 (s), 813 (m), 753 (m), 695 (m). δ_H (600.13 MHz, $CDCl_3$) 1.46 (3 H, t, $^3J_{HH}$ 7, 12-H), 4.12 (2 H, t, $^3J_{HH}$ 7, 11-H), 7.12–7.15 (2 H, m, 1-H, 9-H), 7.23 (1 H, t, $^3J_{HH}$ 8, 7-H), 7.27 (1 H, d, $^3J_{HH}$ 8, 6-H), 7.37–7.41 (3 H, m, 8-H, 16-H), 7.48–7.51 (4 H, m, 15-H), 7.58 (1 H, br s, 2-H), 7.70 (2 H, br s, 14-H), 7.85 (2 H, br s, 14-H), 8.76 (1 H, d, $^3J_{HH}$ 7, 3-H). δ_P (242.94 MHz, $CDCl_3$) 59.5. δ_C (150.90 MHz, $CDCl_3$) 13.4 (12-C), 42.2 (11-C), 116.4 (1-C), 118.3 (2-C), 119.1 (9-C), 125.3 (7-C), 127.6 (16-C), 128.3 (4-C), 129.2 (d, $^3J_{PC}$ 11.1, 15-C), 130.2 (8-C), 130.7 (6-C), 131.9 (d, $^2J_{PC}$ 8.4, 3-C), 132.6 (13-C), 134.2 (d, $^2J_{PC}$ 11.3, 14-C), 141.8 (10a-C). Because of low solubility, quaternary carbon atoms 4a-C, 5a-C and 9a-C could not be assigned.

[$PtCl_2${(10-Et-4-PPh_2-Ptz)-κ*S*,*P*}] (15). 50 mg [$PtCl_2$(cod)] (0.14 mmol), 56 mg **4** (0.14 mmol). **15** (80 mg, 87%). mp > 339 °C (decomp.). Found: C 46.2, H 3.4, N 2.3, S 4.9. Calc. for $C_{26}H_{22}Cl_2NPSPt$: C 46.1, H 3.3, N 2.1, S 4.7%. UV-vis: λ_{max} (CH_2Cl_2)/nm 229 and 327 (ε/dm^3 mol^{-1} cm^{-1} 33 610 and 3375). IR (KBr, $\tilde{\nu}_{max}$/cm^{-1}) 3056 (m), 2981 (w), 1593 (m), 1563 (m), 1479 (s), 1449 (m), 1413 (m), 1364 (m), 1253 (s), 1164 (m), 1098 (m), 996 (w), 810 (m), 754 (s), 694 (m). δ_H (600.13 MHz, $CDCl_3$) 1.48 (3 H, t, $^3J_{HH}$ 7, 12-H), 4.12 (2 H, dd, $^3J_{HH}$ 7, 11-H), 7.13 (1 H, d, $^3J_{HH}$ 8, 1-H), 7.22–7.25 (3 H, m, 9-H, 6-H, 7-H),

7.34–7.40 (3 H, m, 8-H, 16-H), 7.46–7.49 (4 H, m, 15-H), 7.55 (1 H, t, $^3J_{HH}$ 8, 2-H), 7.69 (2 H, dd, $^3J_{HH}$ 8, $^3J_{PH}$ 13, 14-H), 7.83 (2 H, dd, $^3J_{HH}$ 8, $^3J_{PH}$ 13, 14-H), 8.63 (1 H, d, $^2J_{PH}$ 8, 3-H). δ_P (242.94 MHz, $CDCl_3$) 36.6, $^1J_{PtP}$ 3490. δ_C (150.90 MHz, $CDCl_3$) 13.4 (12-C), 42.0 (11-C), 116.4 (1-C), 117.7 (2-C), 119.2 (9-C), 125.1 (7-C), 127.2 (3-C), 127.7 (4a-C), 128.2 (5a-C), 128.9 (15-C), 129.0 (15-C), 129.1 (15-C), 129.9 (4-C), 130.1 (16-C), 130.2 (16-C), 131.9 (d, $^1J_{PC}$ 8.9, 13-C), 132.3, 132.4 (6-C, 8-C), 134.1 (t, $^3J_{PC}$ 11.8, 14-C), 141.9 (10a-C), 145.6 (9a-C).

Interactions with DNA

For the DNA electrophoresis tests, different volumes of stock solutions of compounds **1** ($c = 10^{-3}$ M), **3** ($c = 10^{-3}$ M) and **4** ($c = 5 \times 10^{-4}$ M) and of the transition metal complexes **14** ($c = 2 \times 10^{-4}$ M) and **15** ($c = 4 \times 10^{-4}$ M) prepared in DMSO were mixed with 200 ng of plasmid DNA (pTZ57R, 2836 bp) at different ratios. The plasmid DNA was purified from an overnight culture of *Escherichia coli* cells using the Gene JET Plasmid Miniprep Kit (Thermo Scientific). The mixtures were incubated for 30 min at room temperature and loaded into an agarose gel 1% in TAE (tris-acetate ethylenediaminetetraacetic acid) buffer (40 mM tris-acetate, 1 mM EDTA (ethylenediaminetetraacetic acid), pH 8.0) without ethidium bromide. After migration, gels were stained for 30 min in a TAE buffer containing ethidium bromide (0.5 μg ml^{-1}), according to standard procedures.[50]

Electronic absorption titration. The absorption titration of complexes **14** and **15** in DMSO was performed at a fixed concentration (**14**: 0.025 mM; **15**: 0.040 mM) with increments of 10 μL CT-DNA (stock solution 1.054 mg ml^{-1}). The experiment was followed by UV-Vis with a Perkin Elmer Lambda 35 spectrophotometer.

Ethidium bromide fluorescence quenching. Competitive binding studies were carried out with a Perkin Elmer LS 55 spectrophotometer. The emission spectra of a DMSO solution of free ethidium bromide (0.25 × 10–5 M) and ethidium bromide bound-DNA (Tris buffer stock solution 1.054 mg ml^{-1}) were recorded. Constant increments (100 μL) of complex **15** were added to a mixture of 400 μL ethidium bromide and 100 μL DNA. The samples were irradiated at 535 nm and the emission maxima (650 nm) corresponding to ethidium bromide were recorded.

Thermal denaturation. Thermal denaturation studies were carried out with a CECIL CE7450 spectrophotometer equipped with a Peltier temperature-controlling programmer. The thermal denaturation of CT-DNA was performed with complexes **14** and **15** in DMSO. CT-DNA (30 μM) was treated with complexes at 1.5 : 1 ratio (DNA–complex). The samples were heated from 40 to 90 °C at a rate of 1 °C min^{-1} measuring the absorbance at 260 nm. The melting temperatures (T_m) for the CT-DNA were determined as the temperature at which half of the dsDNA becomes ssDNA. Data are presented as $(A - A_o)/(A_f - A_o)$ *versus* temperature, where A is the initial, $A_o$is the observed and A_f is the final absorbance at 260 nm.

Cytotoxic activity

Cell lines, culture. Three tumour cell lines were used to evaluate the cytotoxic effect of the compounds: MCF7 (human breast adenocarcinoma) and HepG2 (hepatocyte carcinoma) cultured in Eagle's MEM (minimum essential medium), and DLD1 (colorectal adenocarcinoma) grown in RPMI 1640; all media were supplemented with 10% foetal bovine serum, 1% glutamine and antibiotics. All media were purchased from Sigma. Cells seeded on 96-well plates were treated with different concentrations of the compound for 24 h (each concentration in at least three wells). The concentrations were as follows: 162.5; 81.25; 40.62; 16.25; 6.5; 3.25; 1.63 μg ml^{-1}. At least three wells were left untreated (control).

Determination of cell survival. The cytotoxic effect of the treatments was assessed using the MTT assay. This method is based on the reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] to a coloured compound (formazan) only by living cells with active mitochondrial hydrogenase, which represents a part of the respiratory chain. 24 h after treatments, 100 μl of MTT (Sigma) was added and cells were incubated for 1 h. After that interval, formazan crystals were dissolved by adding 150 μl of DMSO. The absorbance was measured with a Biotek Synergy 2 microplate reader at 570 nm. Statistical analysis was done and graphs were plotted using Graph Pad Prism 5 software.

Acknowledgements

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