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Exploring the Scope of $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ as a Precursor for New Organometallic Platinum(II) and Platinum(IV) Antitumor Agents

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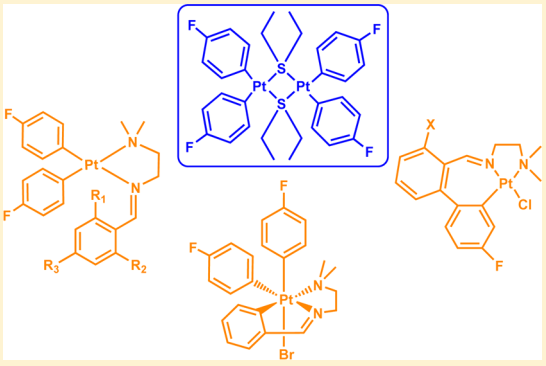
Supporting Information

ABSTRACT: The new compound $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**) was prepared and fully characterized. The reactions of compound **A** with ligands $ArCH{=}NCH_2CH_2NMe_2$ (Ar = 2-BrC_6H_4, **1a**; 2,6-$Cl_2C_6H_3$, **1b**; 4-ClC_6H_4, **1c**; 2-Cl,6-FC_6H_3, **1d**) were studied under different conditions and produced platinum(II) compounds $[Pt(4\text{-}FC_6H_4)_2(ArCH{=}NCH_2CH_2NMe_2)]$ (**2b−2d**), containing a bidentate [N,N′] ligand, as well as cyclometalated platinum(IV) or platinum(II) compounds such as $[PtBr(4\text{-}FC_6H_4)_2(C_6H_4CH{=}NCH_2CH_2NMe_2)]$ (**4a**) or $[PtCl\{(3\text{-}FC_6H_3)(2\text{-}XC_6H_3)CH{=}NCH_2CH_2NMe_2\}]$ (**5b**: X = Cl; **5d**: X = F), containing a tridentate [C,N,N′] ligand and either a five (**4a**) or a seven (**5b**, **5d**) membered metallacycle. These compounds exhibit a great antiproliferative activity against non-small lung cancer cells (A549), and the best result was obtained for compound **2c** (IC_{50} = 0.3 ± 0.1 μM). While compounds **5** alter the mobility of plasmid DNA in a similar way to cisplatin, compound **4** was less efficient in removing the supercoils from DNA. In spite of the very low IC_{50} value obtained for compound **2c**, this compound does not interact with DNA, and it is neither an intercalator nor a topoisomerase I inhibitor.



INTRODUCTION

Nowadays platinum(II) complexes (cis-, carbo-, and oxaliplatin) dominate the field of metal-based chemotherapy in worldwide cancer treatment protocols.[1] However, major limitations of these drugs are (i) dose-limiting severe toxicities, (ii) poor bioavailability, and (iii) intrinsic or acquired resistance.[2,3] As a consequence, different approaches have emerged to improve the cytotoxic profile of these anticancer platinum compounds.[4] Relevant strategies are focused on (i) the stabilization of the Pt(II) ion in the complexes, (ii) the design of Pt(IV) complexes as prodrugs, and (iii) the exploitation of the promising properties associated with organometallic compounds based not only on platinum but also on other metal ions such as palladium, ruthenium, gold, copper, or iron. Cyclometalated platinum(II) complexes containing either bidentate [C,N][5] or terdentate [C,N,N′][6] ligands have been recently screened against tumor cells with very promising outcomes. In these compounds, the presence of a σ(Pt−C) bond increases the stability of the complexes, thus allowing them to reach the cell unaltered. Furthermore, the aromatic groups in the cyclometalated ligand might favor intercalative binding to DNA through π−π stacking,[7] while the labile positions in the coordination sphere of the platinum atom favor covalent coordination to DNA as for cisplatin. Therefore a high cytotoxic activity may result from the combined effect of intercalation and platination operating for cyclometalated platinum compounds.[5,6a] On the other hand, platinum(IV) complexes, able to produce Pt(II) species by reductive elimination[8] or photoactivation,[9] offer several potential advantages. They are stable enough to be administered orally, their stability should result in diminished side effects, and they

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are amenable to structural modifications via the axial ligands, which can be used to improve their pharmacological properties.

In recent years we have been involved in the use of diarylplatinum(II) complexes as precursors in the synthesis of [C,N,N′] cyclometalated platinum(II) and platinum(IV) compounds[10,11] and the study of the mechanisms involved in these processes.[12,13] In particular, along these studies, a novel class of seven-membered platinacycles has been obtained in a reaction involving formation of a C_{aryl}–C_{aryl} bond, and these compounds were shown to display a remarkable antiproliferative activity, even greater than cisplatin, in several human cancer cell lines.[6a] In order to further explore this area, we undertook a project aimed at the preparation of a new precursor, $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**), analogous to compound $[Pt_2(4\text{-}MeC_6H_4)_4(\mu\text{-}SEt_2)_2]$ previously used as metalating agent.[11] Binuclear compound **A**, upon reaction with adequate dinitrogen ligands, should produce new series of organometallic platinum complexes potentially useful as antitumor agents. In addition, the presence of a fluoro substituent in the aryl ligands of compound **A** should allow an analysis on the importance of the electronic effects in the subsequent reactions. Interestingly, several examples of biologically active platinum fluoroaryl compounds have been previously reported.[5l-o,14] Moreover, the presence of the NMR-active ^{19}F nucleus will provide an additional spectroscopic handle to characterize the obtained compounds.[15,16] An additional interest in this system relies on the fact that fluoro substituents may enhance the binding efficacy and selectivity in pharmaceuticals.[17]

The results presented here include the synthesis of the new dimer **A**, which was found to be an adequate precursor for the synthesis of several organoplatinum compounds such as diarylplatinum(II) compounds containing a bidentate [N,N′] ligand (**2b**–**2d**) and cyclometalated platinum(II) (**5b**, **5d**) and platinum(IV) (**4a**) compounds containing a terdentate [C,N,N′] ligand. Their antiproliferative activity against the A549 human lung cancer cell line has been investigated by means of the MTT colorimetric assay. Additionally, electrophoretic DNA migration studies, in the absence and in the presence of topoisomerase I, have been performed, in order to get further insights into the biological behavior of the synthesized compounds.

RESULTS AND DISCUSSION

Synthesis and Characterization of Platinum Compounds. Although the synthesis of compound *cis*-$[Pt(4\text{-}FC_6H_4)_2(SMe_2)_2]$ has been recently reported,[18] our target compound **A** has not been described so far. The dinuclear compound $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**) was obtained from the reaction of *cis*-$[PtCl_2(SEt_2)_2]$ with 4-fluorophenyllithium, prepared *in situ*, and was fully characterized including single-crystal X-ray diffraction analysis (Figure 1). Suitable crystals of compound **A** were grown from dichloromethane–methanol at room temperature as the solvate $\mathbf{A}\cdot CH_2Cl_2$. The crystal structure is composed of discrete molecules held together by van der Waals interactions. Both platinum atoms display square-planar coordination geometry, while the sulfur atoms are tetrahedrically bonded to both platinum centers and two ethyl groups. Bond parameters are similar to those found for the analogous compound $[Pt_2(4\text{-}MeC_6H_4)_4(\mu\text{-}SEt_2)_2]$, whose structure has been previously reported.[19] In particular, the Pt(1)···Pt(2) separation is 3.605 Å. The mean planes of the

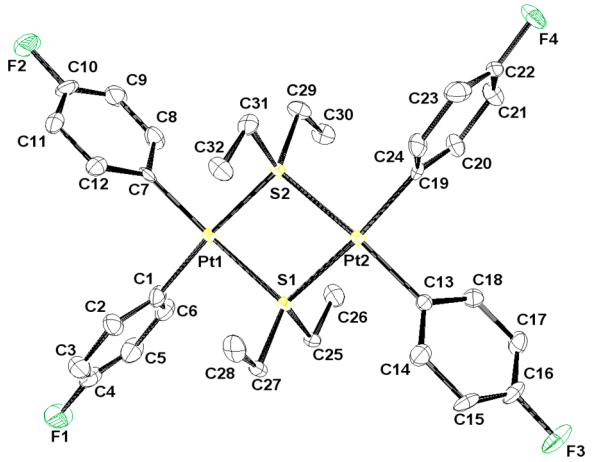

Figure 1. Molecular structure of compound **A**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (deg) with estimated standard deviations: Pt(1)–C(7): 2.006(8); Pt(1)–C(1): 2.015(7); Pt(1)–S(2): 2.3504(16); Pt(1)–S(1): 2,3581(19); Pt(2)–C(19): 2,029(6); Pt(2)–C(13): 2,039(7); Pt(2)–S(2): 2.3545(18); Pt(2)–S(1): 2.3705(16); C(7)–Pt(1)–C(1): 89.8(3); C(7)–Pt(1)–S(2): 96.11(11); C(1)–Pt(1)–S(1): 93.6(2); S(2)–Pt(1)–S(1): 80.45(6); C(19)–Pt(2)–C(13): 91.2(3); C(19)–Pt(2)–S(2): 94.1(2); C(13)–Pt(2)–S(1): 94.53(18); S(2)–Pt(2)–S(1): 80.11(6).

para-fluorophenyl groups form dihedral angles of 85.9(3)°, 78.6(3)°, 66.3(3)°. and 84.9(3)° with the Pt_2S_2 plane.

Ligands $ArCH{=}NCH_2CH_2NMe_2$ (Ar = 2-BrC_6H_4, **1a**; 2,6-$Cl_2C_6H_3$, **1b**; 4-ClC_6H_4, **1c**; 2-Cl,6-FC_6H_3, **1d**) were selected for this study since the presence of two nitrogen atoms allows formation of a [N,N′]-chelate upon coordination to platinum and further reaction should produce cyclometalated platinum(II) or platinum(IV) compounds in agreement with previous studies for similar systems.[20] The presence of different substituents in the *ortho* positions of the aryl ring (Br, Cl, or H) is determinant in the reaction pathway and the nature of the final products of such reactions.

The reactions of compound **A** with ligands **1a**–**1d** are summarized in Scheme 1. The reaction of $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**) with imines **1b**–**1d** carried out in toluene at room temperature produced compounds of general formula $[Pt(4\text{-}FC_6H_4)_2(ArCH{=}NCH_2CH_2NMe_2)]$ (**2b**–**2d**). For **1a**, the C–Br bond in the *ortho* position is easily activated at room temperature to produce cyclometalated platinum(IV) compound $[PtBr(4\text{-}FC_6H_4)_2(C_6H_4CH{=}NCH_2CH_2NMe_2)]$ (**4a**). For this reason, compound **2a** was not isolated, although the reaction is expected to proceed through this species in agreement with previous mechanistic studies for related systems.[13] Compounds **2b**–**2d** were characterized by usual techniques, and in addition, **2d** was also characterized crystallographically. The ^1H NMR spectra of compounds **2b** and **2c** display only one group of signals, which was assigned, in agreement with the observed $^3J(H{-}Pt)$ values for the imine hydrogen (ca. 50 Hz), to the *E* isomer. In contrast, compound **2d** was obtained as a mixture of *Z* and *E* isomers, as readily deduced from the ^1H and ^{19}F NMR spectra. This result prompted us to monitor by ^1H NMR spectroscopy the stability of compounds **2b** and **2c** in $CDCl_3$ solution. While compound **2c** was stable in solution as the *E* isomer, compound **2b** gave after several hours at room temperature a mixture of three compounds, as evidenced by the three resonances observed in

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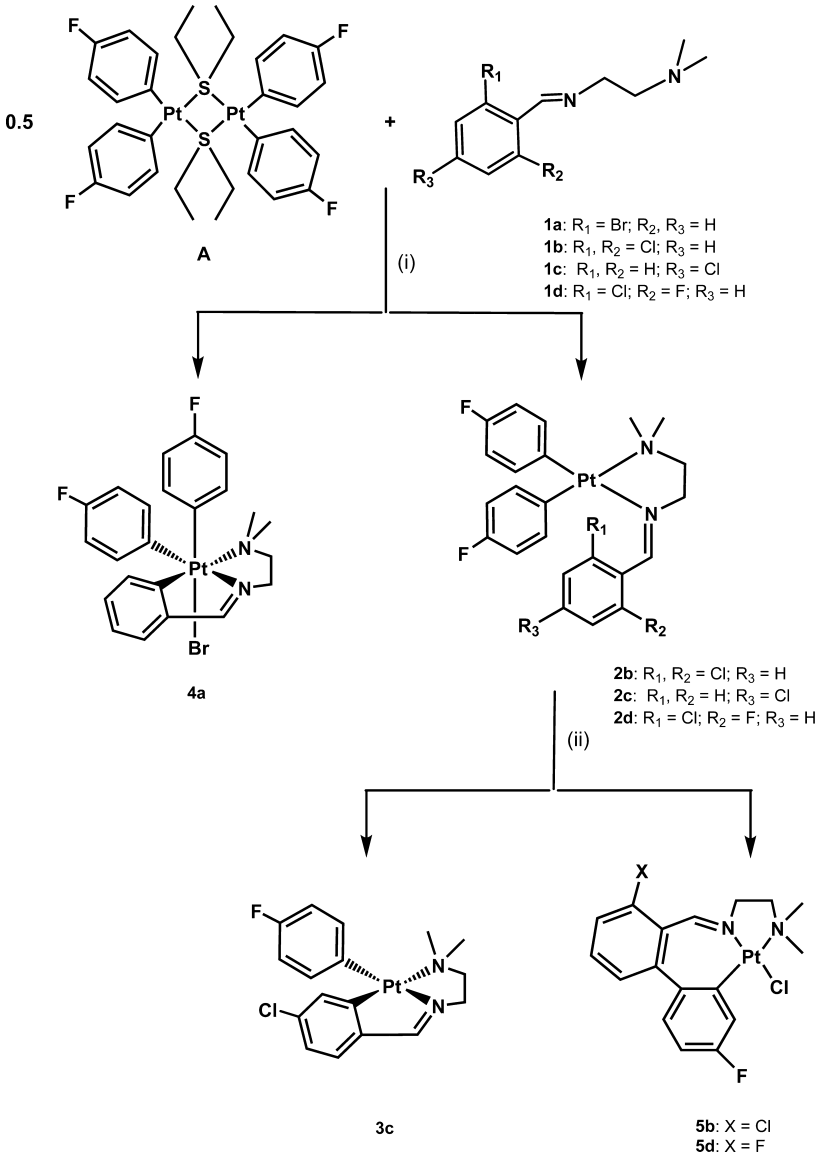
Scheme 1. Synthesis of Coordination and Cyclometalated Platinum Compounds from Precursor A[a]



[a]Conditions: (i) toluene, room temperature, 4 h; (ii) toluene, reflux, 6 h.

the imine region. These were assigned, based on the chemical shifts and the ^3J(H−Pt) values, to isomer *E* of compound **2b** initially present, to the *Z* isomer (δ = 8.17 ppm, ^3J(H−Pt) = 28 Hz), and to the platinum(IV) species **4b** (δ = 9.29 ppm, ^3J(H−Pt) = 48 Hz) arising from intramolecular C−Cl bond activation. After several days at room temperature, **4b** was the major component, the ratio *E*:*Z* was 1:2.2, and a new peak corresponding to **5b** was also observed. Formation of the *Z* isomer releases the steric congestion by placing the aryl group away from the platinum atom; however, formation of compound **4b** takes place from the *E* isomer. These results support the reaction sequence shown in Scheme 2, in agreement with previously reported mechanisms for analogous systems.[12,13]

Suitable crystals of compound **2d** were grown in dichloromethane−methanol at room temperature. The crystal structure is composed of discrete molecules held together by van der Waals forces. The molecular structure (Figure 2) consists of the *E* isomer, and bond distances and angles are similar to those reported for analogous compounds. In particular, the angles CPtC and CPtN are close to 90°, while the bite angle NPtN is 80.62°. The chelate ring is nearly coplanar with the coordination plane (dihedral angle 10.5(2)°), and the *para*-fluorophenyl groups *trans* to the imine and to the amine are tilted 89.6(3)° and 64.4(3)°, respectively, to that plane. This arrangement allows for an intramolecular $\pi-\pi$ stacking[21] between the C_7-C_{12} and the $C_{16}-C_{21}$ rings, the distance being 3.663(4) Å.

As stated above, the reaction of precursor **A** with ligand **1a** produced a cyclometalated platinum(IV) compound containing a terdentate [C,N,N′] cyclometalated ligand. Compound **4a** was fully characterized including X-ray molecular structure determination of the crystals grown in dichloromethane−methanol. Multinuclear (^{1}H, ^{19}F, ^{13}C, and ^{195}Pt) NMR spectra indicated formation of a single isomer, and bidimensional ^{1}H−^{1}H COSY and NOESY experiments allowed the assignment of all signals observed in the ^{1}H NMR spectrum. The presence of eight cross-peaks in the aromatic region of the

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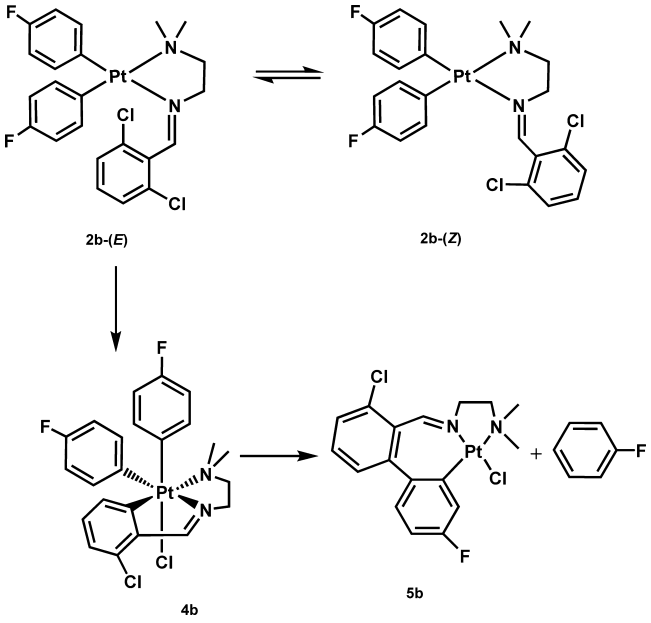
Scheme 2. Isomerization and Reactivity of Compound 2b



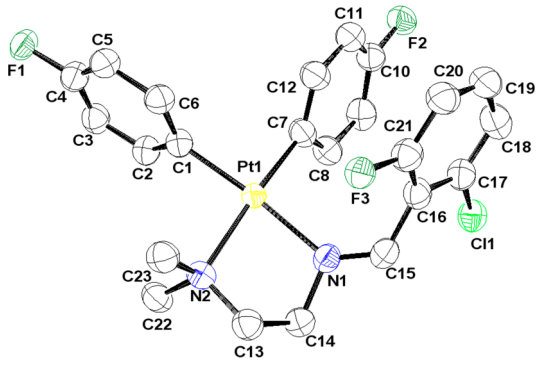


Figure 2. Molecular structure of compound **2d**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (deg) with estimated standard deviations: Pt−C(7): 2.007(6); Pt−C(1): 2.025(6); Pt−N(1): 2.096(4); Pt−N(2): 2,214(5); N(1)−C(15): 1.260(7); N(1)−C(14): 1.501(8); N(2)−C(13): 1.464(8); C(13)−C(14): 1.525(9); C(7)−Pt−C(1): 88.4(2); C(7)−Pt−N(1): 96.0(2); C(1)−Pt−N(2): 94.9(2); N(1)−Pt−N(2): 80.62(18).

^{1}H−^{13}C HSQC is also consistent with the proposed structure. All observed coupling constants are in the range expected for monofluorinated aromatic compounds[22] and in good agreement with the values reported for cyclometalated compounds.[23] In addition, the chemical shift of the observed signal in the ^{195}Pt NMR spectra (δ = −1929.6 ppm) is consistent with the presence of a platinum(IV) compound.[24]

The crystal structure is composed of discrete molecules held together by van der Waals interactions. The molecular structure (Figure 3) confirmed the proposed structure in which the platinum atom displays an octahedral coordination with the three Pt−C bonds in a *fac* arrangement. The Pt−C and Pt−N distances are in the expected range, and the coordination angles involving the *mer*-[C,N,N′] are smaller than 90° (C(1)−Pt−N(1) = 81.1(2)° and N(1)−Pt−N(2) = 78.6(2)°).

As indicated above, the reactions of ligands **1b**−**1d** with precursor **A** in toluene at room temperature gave compounds **2b**−**2d**, in which the ligands act as bidentate [N,N′] ligands; further reaction of these compounds was tested in refluxing

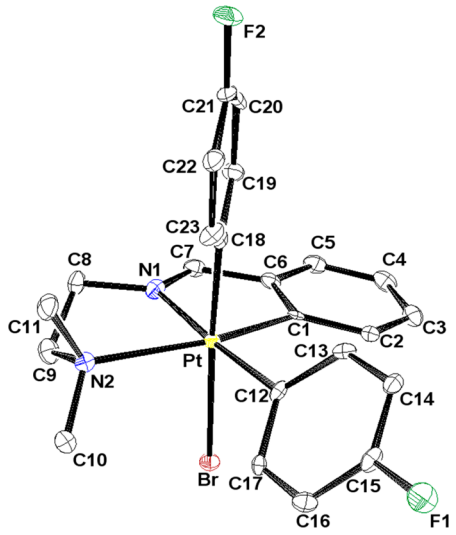


Figure 3. Molecular structure of compound **4a**. Hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (deg) with estimated standard deviations: Pt−C(1): 2.001(6); Pt−C(12): 2.051(6); Pt−C(18): 2.060(7); Pt−Br: 2.5919(6); Pt−N(1): 2.082(5); Pt−N(2): 2,337(5); N(1)−C(7): 1.272(8); N(1)−C(8): 1.455(8); N(2)−C(9): 1.506(8); C(8)−C(9): 1.515(9); C(1)−Pt−C(12): 95.6(2); C(1)−Pt−N(1): 81.1(2); C(12)−Pt−N(2): 104.9(2); N(1)−Pt−N(2): 78.6(2); C(1)−Pt−C(18):92.9(2); C(12)−Pt−C(18): 87.3(2); C(18)−Pt−N(1): 90.7(2); C(18)−Pt−N(2): 93.0(2); C(1)−Pt−Br: 84.76(17); C(12)−Pt−Br: 91.54(17); N(1)−Pt−Br: 90.29(15); N(2)−Pt−Br: 89.66(13).

toluene. As reported for analogous systems,[10,11] compound **2c** is expected to produce under these conditions a five-membered cyclometalated platinum(II) compound, **3c**. In the present case, this compound could only be characterized in solution by ^{1}H and ^{19}F NMR spectra, but could not be isolated in a pure form. Residual amounts of the coordination compound **2c** were present after a reaction time of six hours under reflux. Attempts to achieve full conversion of **2c** into **3c** using longer reaction times as well as attempts to purify compound **3c** were unsuccessful and result in decomposition with formation of metallic platinum. Formation of compounds analogous to **3c** has been reported, along with reductive elimination of either benzene or toluene, from complexes such as *cis*-$[PtPh_2(SMe_2)_2]$[10,25] or $[Pt_2(4\text{-}MeC_6H_4)_4(\mu\text{-}SEt_2)_2]$.[11] In addition several compounds of general formula $[PtAr_2L_2]$ (L = dmso or SMe_2) have been used as metalating agents in the synthesis of cycloplatinated compounds.[26] The failure to obtain pure **3c** could be related to the low nucleophilic character of **A** that renders the intramolecular C−H bond activation more difficult than for the previously reported precursors. In this sense, it has been previously reported that only intramolecular C−Br bond activation, and not C−Cl or C−H bond activation, took place when *cis*-$[Pt(C_6F_5)_2(SMe_2)_2]$ was used as starting material in analogous reactions.[27]

When toluene solutions of **2b** or **2d** were heated at reflux temperature for six hours, compounds **5b** and **5d**, depicted in Scheme 1, were obtained as pure solids. These [C,N,N′] cyclometalated platinum(II) compounds containing a seven-membered metallacycle are formed from the corresponding compounds **2** in a process involving intramolecular C−Cl bond activation to produce a platinum(IV) cyclometalated compound, which further reacts to produce compound **5** and eliminates fluorobenzene, as depicted in Scheme 2 for **2b**.

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Alternatively, compounds **5** could also be obtained in a one-pot procedure after stirring for four hours a toluene mixture of compound **A** and the corresponding ligand. Compounds **5b** and **5d** were characterized by NMR spectra (^{1}H, ^{19}F, and ^{195}Pt). The $\delta(^{195}\text{Pt})$ values are in the range expected for platinum(II) compounds,[24] and in the ^{1}H NMR spectra the nonequivalence of the protons in both the methyl and the methylene groups indicates that the molecule deviates from planarity. In addition, two-dimensional ^{1}H−^{1}H COSY and NOESY were also carried out for **5d** in order to achieve a complete assignment. Moreover, a ^{1}H−^{13}C HSQC hetero-correlation evidenced the presence of six cross-peak signals in the aromatic region for **5d**.

Crystals of **5d** were grown in dichloromethane−methanol at room temperature. In spite of the disorder problems encountered, the obtained molecular structure (Figure 4) confirms the geometry predicted by NMR spectroscopy. The platinum atom displays an approximately square-planar coordination with a terdentate [C,N,N′] and a chloro ligand. As expected, the seven-membered platinacycle includes a biaryl fragment, and the position of the fluoro substituent (F1) in *para* position to the newly formed C_{aryl}−C_{aryl} bond and *meta* to the platinum atom supports the mechanism previously suggested for analogous reactions.[13] The process shown in Scheme 3 takes place through reductive coupling of one *para*-fluorobenzene ligand and the aryl ring of the imine ligand to produce a biaryl moiety, which is consequently cyclometalated with elimination of fluorobenzene.

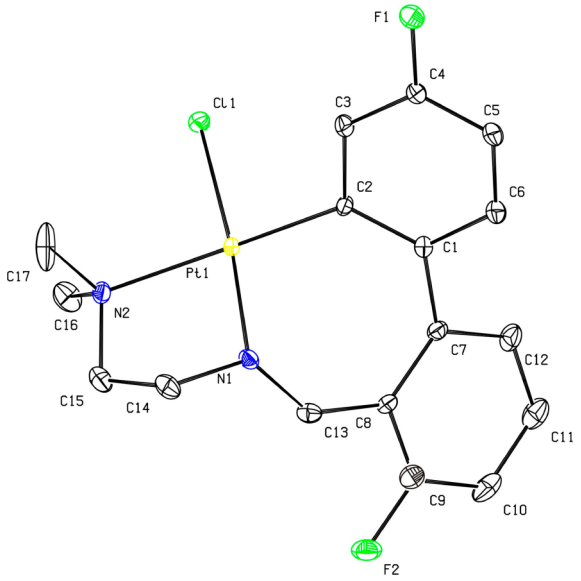
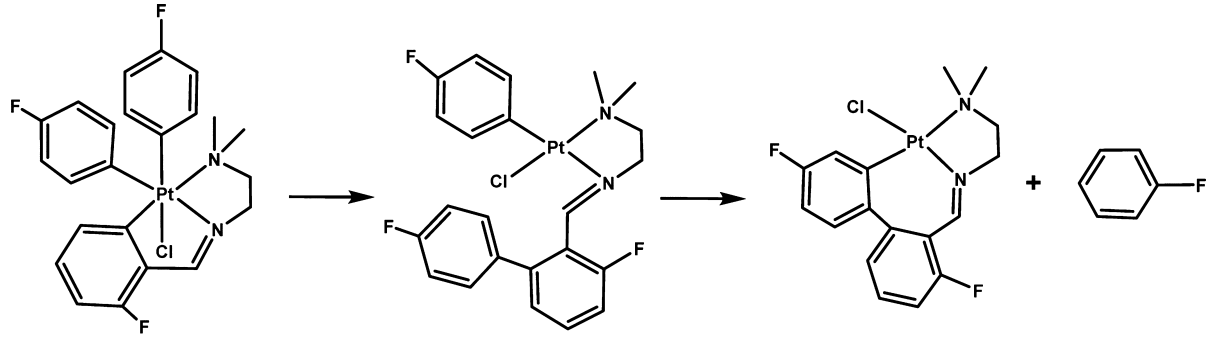


Figure 4. Molecular structure of compound **5d**. The disordered moiety and the hydrogen atoms have been omitted for clarity. Selected bond lengths (Å) and angles (deg) with estimated standard deviations: Pt(1)−C(2): 1.986(7); Pt(1)−N(1): 1.992(7); Pt(1)−N(2): 2.183(6); Pt(1)−Cl(1): 2.293(2); C(1)−C(2): 1.407(8); C(1)−C(7):1.513(8); N(1)−C(13): 1.272(11); N(1)−C(14): 1.483(11); N(2)−C(15): 1.505(13); C(7)−C(8): 1.406(11); C(8)−C(13): 1.463(11); C(14)−C(15): 1.505(14); C(2)−Pt(1)−N(1): 92.1(3); N(1)−Pt(1)−N(2): 82.9(3); C(2)−Pt(1)−Cl(1): 93.1(2); N(2)−Pt(1)−Cl(1): 92.0(2).

Biological Studies. In this work, a set of compounds with different properties (**2b**, **2c**, **2d**, **4a**, **5b**, and **5d**) and the corresponding free ligands (**1a**−**1d**) were evaluated *in vitro* to assess their activity on the inhibition of A549 human lung cancer cell proliferation, using cisplatin as positive control. Compounds **2b**−**2d** are organometallic platinum(II) compounds with one labile position (the dimethylamino fragment), compounds **5b** and **5d** are cyclometalated platinum(II) compounds containing two labile positions (both the chloro ligand and the dimethylamino fragment), and compound **4a** is a cyclometalated platinum(IV) compound with a *fac*-PtC_3 arrangement and a meridional [C,N,N′] terdentate ligand, thus leaving one bromide and one aryl ring as axial ligands. Their effect on the growth of the selected cell line was evaluated after 72 h, and the results are displayed in Figure 5. The obtained IC_{50} values resulting from an average of two experiments are shown in Table 1.

It can be seen from Table 1 that compounds **5b** and **5d** exhibit a great antiproliferative activity and lower IC_{50} values than cisplatin itself. These compounds show little difference in their cytotoxic effectiveness among them and when compared with similar seven-membered platinacycles previously described.[6a] Although the presence of fluorine substituents could favor DNA binding, no increase in potency is observed for compounds **5b** and **5d**, containing fluoro substituents. As previously reported,[6a] the seven-membered metallacycles are not planar, the tilt angle between both aryl rings contained in the seven-membered ring is in the range 50.6−54.2°, and consequently, intercalative binding to DNA is not expected. Compounds **2b** and **2d** show a notable antiproliferative activity with lower IC_{50} values than that of cisplatin. With regard to the same standard reference, compound **2c** exhibited a ca. 50-fold increase in potency. Interestingly compounds **2b**−**2d** have a very similar structure and only differ in the substitution pattern

Scheme 3. Formation of Compound 5d



5d

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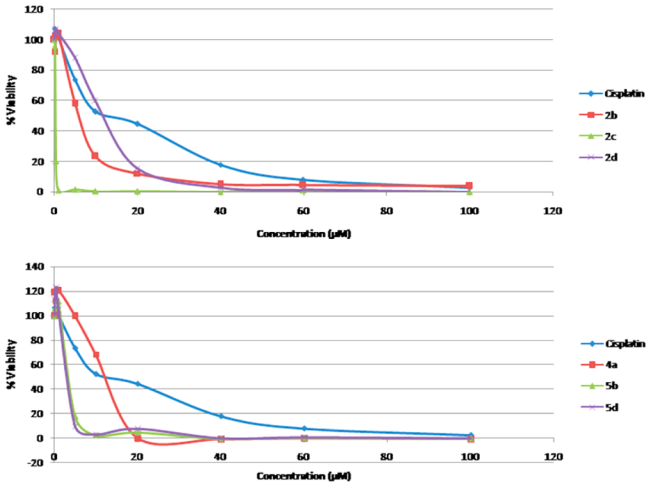

Figure 5. Inhibition of cell growth proliferation in the A549 human lung cancer cell line, after 72 h of exposure to coordination compounds (**2b**–**2d**) (top), cyclometalated compounds (**4a**, **5b**, **5d**) (bottom), and cisplatin.

Table 1. Cytotoxic activities on the A549 Lung Human Cancer Cell Line for Studied Compounds and Cisplatin

compound	IC_{50} (μM)[a]
1a	>100
1b	>100
1c	>100
1d	>100
2b	6.5 ± 2.0
2c	0.3 ± 0.1
2d	12.1 ± 0.8
4a	11.6 ± 2.2
5b	2.8 ± 0.5
5d	2.5 ± 0.1
cisplatin	14.1 ± 1.3

[a]Data are shown as the mean values of two experiments performed in triplicate with the corresponding standard deviation (SD).

in the imine aryl group. However, the presence of two substituents in the *ortho* positions for **2b** and **2d** could favor *E*–*Z* isomerization around the imine bond or even formation of a platinum(IV) compound as depicted in Scheme 2 for **2b**. Complex **2c** (without an *ortho* substituent) turned out to be ca. 20-fold more active than **2b** and ca. 40-fold more active than **2d**.

Most evidence to date indicates that platinum(IV) complexes exhibiting symmetrical axial ligands (Cl, OH, and OAc) are reduced under physiological conditions by biologically relevant reducing agents (ascorbic acid, glutathione, metallothionein) to release two axial ligands and yield the cytotoxic platinum(II) species.[8a] Investigations with a series of three model Pt(IV) complexes with axial chloro, acetato, and hydroxo ligands revealed that they have reduction potentials such that the ease of reduction follows the trend Cl > OAc > OH.[8j] In addition, the difficulty of reduction of Pt(IV) analogues exhibiting OH^- ligands has been correlated with good *in vivo* biological activity.[8k] However it was found recently that redox potential does not always correlates with the rate of reduction of the platinum(IV) complexes, and also the precise mechanisms of reduction are not always fully understood.[8e,f]

On the other hand, there are no structure–activity rules for platinum(IV) complexes *per se*, except that the platinum(II) congeners used for constructing a platinum(IV) complex must be active.[8a] Furthermore, to the best of our knowledge, no cytotoxicity has been evaluated so far for [C,N,N′]-cyclometalated Pt(IV) complex. Therefore we intended to determine within this project if it is possible that unsymmetrical monomeric Pt(IV) complexes, featuring halide and 4-fluorophenyl as axial ligands, exhibit cytotoxicity versus the cell line selected (A549 human lung cancer). Interestingly the Pt(IV) complex **4a** synthesized in this study exhibited IC_{50} values in non-small lung cancer cells (A549) very close to that of the standard reference cisplatin.

The effect of binding of the compounds investigated in this study on supercoiled DNA was determined by their ability to alter the electrophoretic mobility of pBluescript plasmid DNA: supercoiled closed circular (ccc) and open circular (oc) forms. Figure 6 shows the electrophoretic mobility of native

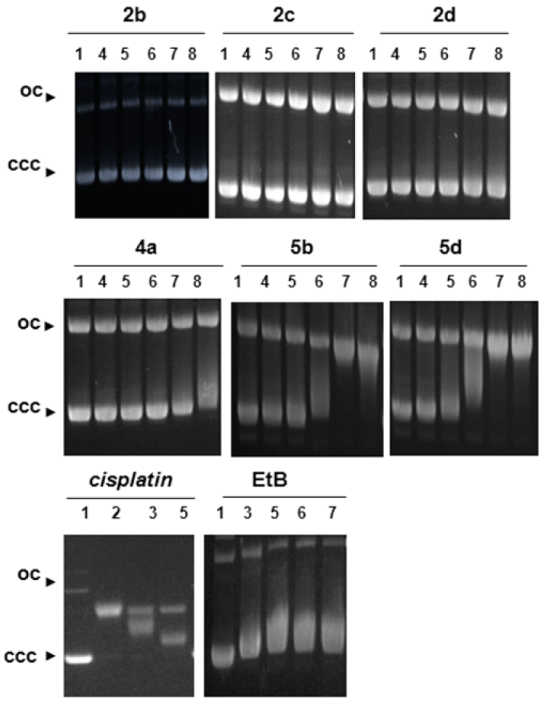

Figure 6. Interaction of pBluescript SK+ plasmid DNA (0.8 μg) with increasing concentrations of compounds **2**, **4**, and **5**, cisplatin, and ethidium bromide. Lane 1: DNA only. Lane 2: 2.5 μM. Lane 3: 5 μM. Lane 4: 10 μM. Lane 5: 25 μM. Lane 6: 50 μM. Lane 7: 100 μM. Lane 8: 200 μM. ccc = supercoiled closed circular DNA; oc = open circular DNA.

pBluescript DNA incubated with the synthesized compounds (**2b**, **2c**, **2d**, **4a**, **5b**, and **5d**) at increasing amounts ranging from 0 to 200 μM. To provide a basis for comparison, incubation of DNA with cisplatin and ethidium bromide (EtB) was also performed. As expected, cisplatin greatly altered the electrophoretic mobility of pBluescript DNA at all concentrations tested. At concentrations up to 50 μM none of the assayed compounds produced a significant effect on the electrophoretic mobility of native pBluescript DNA. Compounds **5b** and **5d** greatly alter the mobility of plasmid DNA at 50 μM, and at 100 μM concentration, the rate of migration of the supercoiled band (ccc) decreases even more and tends to approach that of the nicked relaxed band (oc). Platinum(IV) compound **4a**

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displayed a much lower effect on plasmid DNA mobility, while compounds **2b**−**2d** did not modify the DNA migration in spite of their low IC_{50} values. These results indicated that compounds **5** alter the electrophoretic mobility of pBluescript plasmid DNA and hence interact with DNA like the standard reference, cisplatin. However, compounds **2** and **4** showed a weak effect on DNA electrophoresis, pointing out another mechanism of action or another biomolecular target.

Since compound **2c** was found to be very active (IC_{50} = 0.3 μM) against A549 lung cancer cells, and $\pi-\pi$ stacking interactions are plausible for these types of compounds, as observed in the crystal structure of compound **2d**, we hypothesized that compounds **2** might behave as intercalating agents. Although intercalation has been traditionally associated with molecules containing fused bi- or tricyclic ring structures, atypical intercalators might be more prevalent than originally thought.[28] In order to ascertain whether compound **2c** could be a DNA intercalator, a topoisomerase-based gel assay was performed upon this compound.[29,30] Figure 7 shows the

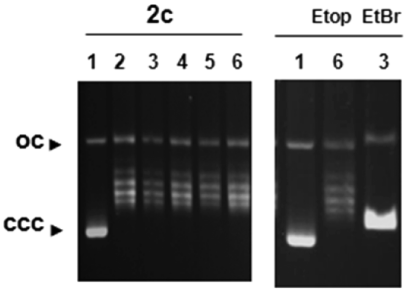

Figure 7. Analysis of **2c** as a putative DNA intercalator or topoisomerase I inhibitor. Conversion of supercoiled pBluescript plasmid DNA (0.8 μg) to relaxed DNA by the action of topoisomerase I (3 units) in the absence or in the presence of increasing amounts of compound **2c** was analyzed by agarose gel stained with ethidium bromide (EtBr). Also shown are the negative and positive intercalator controls, etoposide (Etop, 100 μM) and ethidium bromide (EtBr, 10 μM). Lanes 1, DNA only, lane 2, 0 μM compound; lanes 3, 10 μM; lane 4, 25 μM, lane 5, 50 μM; lanes 6, 100 μM. Except for lane 1, all the lanes included topoisomerase I. ccc = supercoiled closed circular DNA form; oc = open circular DNA form.

electrophoretic mobility of supercoiled DNA treated with topoisomerase I in the presence of compound **2c** at increasing amounts ranging from 10 to 100 μM. To provide a basis for comparison, unwinding assays with etoposide (100 μM) and ethidium bromide (10 μM) as examples of nonintercalative and intercalative drugs, respectively, were also performed. Results presented in Figure 7 showed that **2c** does not prevent unwinding of DNA by the action of topoisomerase I, indicating that this compound is not an intercalator nor an inhibitor of topoisomerase I.

CONCLUSION

The new compound $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**) appears to be a suitable precursor for the synthesis of several types of organometallic species such as diarylplatinum(II) compounds containing a bidentate [N,N′] ligand (**2b**−**2d**) and cyclometalated platinum compounds containing a terdentate [C,N,N′] ligand with either a five-membered platinum(IV) (**4a**) or a seven-membered platinum(II) (**5b**−**5d**) metallacycle. The fluoro substituent in the aryl group remains *para* to the platinum in coordination compounds **2** and in the cyclometalated platinum(IV) compound **4a**. However, for compounds **5** the fluoro group is now *para* to the formed $C_{aryl}-C_{aryl}$ bond and *meta* to the platinum center. This result is consistent with a process involving $C_{aryl}-C_{aryl}$ coupling between the carbon atoms bound to platinum of the metalated aryl ring and the *para*-fluoroaryl ligand, as expected for a biaryl reductive elimination from a platinum(IV) compound. On the other hand, the failure to obtain pure **3c** could be related to the presence of electron-withdrawing fluorosubstituents that reduce the nucleophilic character of compound **A** compared to previously reported precursors.

The new compounds exhibited notable to great antiproliferative activities against the A549 human lung cancer cell line. The behavior of compounds **5** is very similar to that obtained for analogous seven-membered platinacycles, which suggests that the presence of fluoro substituents is not relevant to their biological properties. In spite of the very high potency of compound **2c** (ca. 50-fold greater than the standard reference cisplatin), electrophoretic studies carried out for this compound do not show any evidence of either covalent binding or intercalation with DNA, nor a topoisomerase I inhibitor behavior. The results presented here constitute the first step of current work centered on (i) mechanistic studies for elucidating the mode of action of compounds **2b**−**2d** and **4a** in terms of cell cycle arrest, induction of apoptosis, etc., and (ii) structure−activity relationship analysis upon platinum(IV) complex **4a** and structurally related analogues, previously synthesized in our group, in order to elucidate the structural requirements for activity. These studies may provide valuable information for the design of new organometallic compounds with improved potency and pharmacokinetic properties.

EXPERIMENTAL SECTION

General Procedures. Microanalyses were performed at the Centres Científics i Tecnològics (Universitat de Barcelona). Mass spectra were performed at the Unitat d'Espectrometria de Masses (Universitat de Barcelona) in an LC/MSD-TOF spectrometer using H_2O-CH_3CN (1:1) to introduce the sample. NMR spectra were performed at the Unitat de RMN d'Alt Camp de la Universitat de Barcelona using a Mercury-400 (^{1}H, 400 MHz; ^{1}H−^{1}H COSY; ^{1}H−^{1}H NOESY; ^{1}H−^{13}C HSQC; ^{13}C, 100.6 MHz; ^{19}F, 376.5 MHz) or a Varian VNMRS-400 (^{195}Pt, 85.68 MHz) spectrometer and referenced to $SiMe_4$ (^{1}H, ^{13}C), $CFCl_3$ (^{19}F), or H_2PtCl_6 in D_2O (^{195}Pt). δ values are given in ppm, and J values in Hz. Abbreviations used: s = singlet; d = doublet; t = triplet; m = multiplet; br = broad.

X-ray Diffraction. Suitable crystals were grown in dichloromethane−methanol at room temperature. For **2d**, X-ray diffraction data were collected on a Mar 345 diffractometer with image plate detector at 293 K, and the structure was solved by direct methods using the SHELX97 software package and refined by the full-matrix least-squares method with the SHELX97 software package.[31] For **A**, **4a**, and **5d** X-ray diffraction data were collected on a D8 VENTURE system equipped with a multilayer monochromator and a Mo high brilliance Incoatec Microfocus Source (λ = 0.71073 Å) at 100 K (**A** and **5d**) or 90 K (**4a**), and the structures were solved and refined using the Bruker SHELXTL software package.[31] For **5d**, the compound displays molecular disorder for all atoms except for Pt, C1, and C17, which lie on a mirror plane. CIFs for all four structures and a table of crystallographic data are included in the Supporting Information.

Preparation of the Complexes. Ligands **1a**−**1d**[19,32] and compound *cis*-$[PtCl_2(SEt_2)_2]$[33] were prepared as reported elsewhere.

Compound $[Pt_2(4\text{-}FC_6H_4)_4(\mu\text{-}SEt_2)_2]$ (**A**) was prepared using the following procedure: 3.5 mL (37.15 mmol) of *n*-butyllithium in hexane was added under N_2 to 30 mL of diethyl ether, and the solution was cooled to 0 °C. 4-Fluoroiodobenzene (1.225 g; 5.52 mmol) was slowly added, and the mixture was stirred for 30 min at 0 °C After this time,

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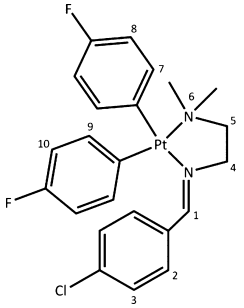
$[PtCl_2(SEt_2)_2]$ (0.502 g; 1.23 mmol) was added, and the mixture was stirred for 2 h at room temperature. After cooling to 0 °C, water (5 mL) was added, the aqueous layer was extracted with dichloromethane (3 × 15 mL), and the combined organic layers were dried over magnesium sulfate, filtered, and evaporated to give an oily residue. The solid obtained upon addition of hexane was filtered and dried under vacuum. Yield: 314 mg (53.8%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (dd, $^3J_{H-H}$ = 8.8, $^4J_{H-F}$ = 6.4, 8H, H^{ortho}), 6.72 (dd, $^3J_{H-H}$ = 8.8, $^3J_{H-F}$ = 9.2, 8H, H^{meta}), 2.51 (q, $^3J_{H-H}$ = 7.2, 8H, CH_2), 1.82 (t, $^3J_{H-H}$ = 7.2, 12H, CH_3). ^{19}F NMR (376.5 MHz, $CDCl_3$): δ −121.6 (tt, $^4J_{F-H}$ = 6.4, $^3J_{F-H}$ = 9.4). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 968.1805 (calcd for $C_{32}H_{40}F_4NPt_2S_2$ 968.1828) $[M + NH_4]^+$. Anal. Found (calcd) for $C_{32}H_{36}F_4Pt_2S_2$: C: 40.3 (40.4); H: 3.9 (3.8); S: 6.9 (6.7).

Compound $[Pt(4\text{-}FC_6H_4)_2\{Me_2NCH_2CH_2N{=}CH(2,6\text{-}Cl_2C_6H_3)\}]$ (**2b**) was obtained after stirring for 4 h a mixture containing 0.100 g (0.105 mmol) of *cis*-$[Pt(4\text{-}FC_6H_4)_2(\mu\text{-}SEt_2)]_2$ and 0.055 g (0.224 mmol) of ligand **1b** in toluene at room temperature. The solvent was evaporated, and the residue was treated with diethyl ether. The yellow solid was filtered and dried under vacuum. Yield: 106 mg (80.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.71 (s, $^3J_{H-Pt}$ = 54.4, 1H, CHN), 7.26 (m, 2H, H^{meta}), 6.97 (br s, 3H, H^{Ar}), 6.77 (m, 2H, H^{meta}), 6.62 (m, 2H, H^{ortho}), 6.14 (m, 2H, H^{ortho}), 4.18 (t, $^3J_{H-H}$ = 5.6, 2H, CH_2), 2.81 (t, $^3J_{H-H}$ = 5.6, 2H, CH_2), 2.60 (s, $^3J_{H-Pt}$ = 18.8, 6H, NMe_2). ^{19}F NMR (376.5 MHz, $CDCl_3$): δ −126.7 (m, 1F), −124.9 (m, 1F). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 534.0475 (calcd for $C_{17}H_{18}Cl_2FN_2Pt$ 535.0473) $[M - C_6H_4F]^+$; 652.0697 (calcd for $C_{23}H_{22}Cl_2F_2N_2NaPt$ 652,0668) $[M + Na]^+$; 1276.1858 (calcd for $C_{46}H_{48}Cl_4F_4N_5Pt_2$ 1276.1890) $[2M + NH_4]^+$. Anal. Found (calcd for $C_{23}H_{22}Cl_2F_2N_2Pt$): C: 44.0 (43.8); H: 3.6 (3.5); N: 4.2 (4.4).

Compound $[Pt(4\text{-}FC_6H_4)_2\{Me_2NCH_2CH_2N{=}CH(4\text{-}ClC_6H_4)\}]$ (**2c**) was obtained using the same procedure from **1c**. Yield: 108 mg (86.4%).

NMR labeling for **2c**:

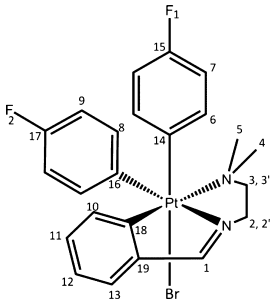


^{1}H NMR (400 MHz, CD_3COCD_3): δ 9.11 (s, $^3J_{H-Pt}$ = 47.2, 1H, H^1), 8.20 (d, $^3J_{H-H}$ = 8.8, 2H, H^2), 7.38 (dd, $^3J_{H-H}$ = 8.4, $^4J_{H-F}$ = 6.8, 2H, H^7), 7.09 (d, $^3J_{H-H}$ = 8.4, 2H, H^3), 6.84 (dd, $^3J_{H-H}$ = 8.8, $^4J_{H-F}$ = 6.8, 2H, H^9), 6.67 (dd, $^3J_{H-H}$ = 8.8, $^4J_{H-F}$ = 10, 2H, H^8), 6.13 (dd, $^3J_{H-H}$ = 8.8, $^3J_{H-F}$ = 10, 2H, H^{10}), 4.36 (t, $^3J_{H-H}$ = 5.6, 2H, H^4), 2.96 (t, $^3J_{H-H}$ = 5.6, 2H, H^5), 2.67 (s, $^3J_{H-Pt}$ = 21.2, 6H, H^6). ^{19}F NMR (376.5 MHz, CD_3COCD_3): δ −126.9 (tt, $^3J_{F-H}$ = 10.2, $^4J_{F-H}$ = 6.8, 1F), −126.1 (tt, $^3J_{F-H}$ = 10.2, $^4J_{F-H}$ = 6.8, 1F). ^{195}Pt NMR (85.68 MHz, $CDCl_3$): δ −3388.6 (s). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 404.0473 (calcd for $C_{11}H_{14}ClN_2Pt$ 404.0487) $[M - 2FC_6H_4 - H]^+$; 613.1484 (calcd for $C_{23}H_{27}ClF_2N_3Pt$ 613.1503) $[M + NH_4]^+$; 1208.2616 (calcd for $C_{46}H_{50}Cl_2F_4N_5Pt_2$ 1208.2669) $[2M + NH_4]^+$. Anal. Found (calcd for $C_{23}H_{23}ClF_2N_2Pt{\cdot}C_4H_{10}O$): C: 48.5 (48.4); H: 4.6 (5.0); N: 4.3 (4.2).

Compound $[Pt(4\text{-}FC_6H_4)_2\{Me_2NCH_2CH_2N{=}CH(2\text{-}Cl\text{-}6\text{-}F\text{-}C_6H_3)\}]$ (**2d**) was obtained using the same procedure from **1d**. Yield: 107 mg (82.9%). ^{1}H NMR (400 MHz, CD_3COCD_3): δ 8.74 (s, $^3J_{H-Pt}$ = 55.6, 1H, CHN, *E* isomer), 8.27 (s, $^3J_{H-Pt}$ = 26.0, 1H, CHN, *Z* isomer), 7.43 (m, 2H), 7.34 (t, $^3J_{H-H}$ = 8.0, 4H), 7.08−7.02 (m, 2H), 6.87 (m, 2H), 6.78−6.71 (m, 10H), 6.14 (dd, $^3J_{H-F}$ = 12.0, $^3J_{H-H}$ = 8.0, 2H), 4.20 (m, 4H, CH_2), 2.81 (t, $^3J_{H-H}$ = 8.0, 2H, CH_2), 2.78 (t, $^3J_{H-H}$ = 8.0, 2H, CH_2), 2.62 (s, 6H, NMe_2), 2.61 (s, 6H, NMe_2). ^{19}F NMR (376.5 MHz, CD_3COCD_3): δ −126.5 (tt, $^3J_{H-F}$ = 10.2, $^4J_{H-F}$ = 7.2, 1F), −124.8 (tt, $^3J_{H-F}$ = 10.2, $^4J_{H-F}$ = 6.8, 1F), −124.5 (tt, $^3J_{H-F}$ = 10.2, $^4J_{H-F}$ = 7.2, 1F), −124.2 (tt, $^3J_{H-F}$ = 10.2, $^4J_{H-F}$ = 6.8, 1F), −108.5 (dd, $^3J_{H-F}$ = 9.0, $^4J_{H-F}$ = 6.0, 1F), −107.5 (dd, $^3J_{H-F}$ = 8.6, $^4J_{H-F}$ = 6.0 Hz, 1F). ^{195}Pt NMR (85.68 MHz, $CDCl_3$): δ (ppm) −3359.0 (s). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 518.0774 (calcd for $C_{17}H_{18}ClF_2N_2Pt$ 518.0768) $[M - FC_6H_4]^+$; 614.1133 (calcd for $C_{23}H_{23}ClF_3N_2Pt$ 614.1146) $[M + H]^+$. Anal. Found (calcd for $C_{23}H_{22}ClF_3N_2Pt$): C: 45.3 (45.0); H: 4.0 (3.6); N: 4.3 (4.6).

Compound $[PtBr(4\text{-}FC_6H_4)_2\{Me_2NCH_2CH_2N{=}CHC_6H_4\}]$ (**4a**) was obtained as a white solid following the same procedure as for compounds **2b**−**2d** from 0.100 g (0.105 mmol) of *cis*-$[Pt(4\text{-}FC_6H_4)_2(\mu\text{-}SEt_2)]_2$ and 0.054 g (0.212 mmol) of ligand **1a** for 4 h. Yield: 77.5 mg (57.8%).

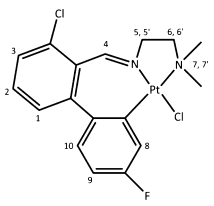
NMR labeling for **4a**:



^{1}H NMR (400 MHz, CD_3COCD_3): δ 8.95 (s, $^3J_{H-Pt}$= 47.6, 1H, H^1), 7.69 (t, $^3J_{H-Pt}$ = 32.0, $^3J_{H-H}$ = $^4J_{H-F}$ = 6.8, 2H, H^8), 7.51 (d, $^3J_{H-H}$ = 6.8, 1H, H^{13}), 7.21 (d, $^3J_{H-H}$ = 7.6, 1H, H^{10}), 7.15 (t, $^3J_{H-H}$ = 6.8, 1H, H^{11}), 7.05 (t, $^3J_{H-H}$ = 6.8, 1H, H^{12}), 6.88 (dd, $^3J_{H-Pt}$= 52.0, $^3J_{H-H}$ = 8.0, $^4J_{H-F}$ = 6.0, 2H, H^6), 6.88 (t, $^3J_{H-F}$ = $^3J_{H-H}$ = 8.7, 2H, H^7), 6.62 (m, 2H, H^9), 4.70 (m, 1H, H^2), 4.45 (m, 2H, $H^{3'}$, $H^{2'}$), 3.08 (m, 1H, H^3), 2.93 (s, $^3J_{H-Pt}$ = 11.2, 3H, H^4), 2.64 (s, $^3J_{H-Pt}$ = 15.2, 3H, H^5). ^{19}F NMR (376.5 MHz, CD_3COCD_3): δ −123.2 (tt, $^5J_{F-Pt}$ = 12.8, $^3J_{F-H}$ = 9.0, $^4J_{F-H}$ = 6.0, 1F, F^2), −122.4 (tt, $^5J_{F-Pt}$ = 18.8, $^3J_{F-H}$ = 9.4, $^4J_{F-H}$ = 6.0, 1F, F^1). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 171.9 (C^1), 161.7, 147.3, 139.0 (d, $^4J_{H-F}$ = 5.0, C^8), 135.5 (d, $^3J_{C-F}$ = 6.4, C^6), 134.86, 131.75, 131.6 (C^{10}), 131.5 (C^{11}), 129.9 (C^{13}), 128.8, 128.1, 124.1 (C^{12}), 113.1 (d, $^2J_{C-F}$ = 19.6, C^7), 112.9 (d, $^2J_{C-F}$ = 19.1, C^9), 66.0 (C^2), 52.6 (C^3), 50.0 (C^4), 47.9 (C^5). ^{195}Pt NMR (85.68 MHz, $CDCl_3$): δ −1929.6 (s). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 464.1092 (calcd for $C_{17}H_{18}FN_2Pt$ 464.1096) $[M - FC_6H_4 - H - Br]^+$; 544.0354 (calcd for $C_{17}H_{19}BrFN_2Pt$ 544.0357) $[M - FC_6H_4]^+$; 560.1469 (calcd for $C_{23}H_{23}F_2N_2Pt$ 560.1471) $[M - Br]^+$; 657.1006 (calcd for $C_{23}H_{27}BrF_2N_3Pt$ 657.0998) $[M + NH_4]^+$; 1199.2099 (calcd for $C_{46}H_{46}BrF_4N_4Pt_2$ 1199.2132) $[2M - Br]^+$; 1279.1362 (calcd for $C_{46}H_{47}Br_2F_4N_4Pt_2$ 1279.1393) $[2M + H]^+$. Anal. Found (calcd for $C_{23}H_{23}BrF_2N_2Pt$): C: 43.2 (43.2); H: 3.8 (3.6); N: 4.4 (4.4).

Compound $[PtCl\{(3\text{-}FC_6H_3)(2\text{-}ClC_6H_3)CH{=}NCH_2CH_2NMe_2\}]$ (**5b**) was obtained after stirring under reflux for 6 h a solution containing 0.075 g (0.119 mmol) of compound **2b**. The solvent was evaporated, and the residue was treated with diethyl ether. The yellow solid was filtered and dried under vacuum. Yield: 29 mg (45.9%).

NMR labeling for **5b**:



^{1}H NMR (400 MHz, $CDCl_3$): δ 9.24 (s, $^3J_{H-Pt}$ = 144.0, 1H, H^4), 7.51 (t, $^3J_{H-H}$ = 7.6, 1H, H^2), 7.38 (m, 1H, H^8), 7.31 (d, $^3J_{H-H}$ = 7.6, 2H, $H^{1,3}$), 6.92 (m, 1H, H^9), 6.70 (m, 1H, H^{10}), 4.52 (m, 1H, H^5), 3.98 (d, $^2J_{H-H}$ = 10.8, 1H, $H^{5'}$), 3.01 (s, 3H, H^7), 2.73 (m, 4H, $H^{7',6'}$), 2,61 (m, 1H, H^6). ^{19}F NMR (376.5 MHz, $CDCl_3$): δ (ppm) −116.6

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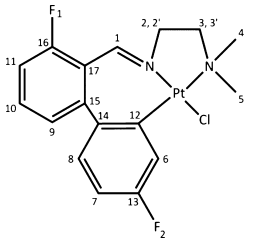
(ddd, $^3J_{F-H}$ = 10.5, $^3J_{F-H}$ = 7.9, $^4J_{F-H}$ = 6.0). ^{195}Pt NMR (85.68 MHz, $CDCl_3$): δ −3075.1 (s). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 534.0466 (calcd for $C_{17}H_{18}Cl_2FN_2Pt$ 534.0473) $[M + H]^+$; 551.0734 (calcd for $C_{17}H_{21}Cl_2FN_3Pt$ 551.0738) $[M + NH_4]^+$; 1084.1108 (calcd for $C_{34}H_{38}Cl_4F_2N_5Pt_2$ 1084.1139) $[2M + NH_4]^+$. Anal. Found (calcd for $C_{17}H_{17}Cl_2FN_2Pt$): C: 37.9 (38.2); H: 3.2 (3.2); N: 4.8 (5.2).

Compound [PtCl{(3-FC_6H_3)(2-FC_6H_3)CH=$NCH_2CH_2NMe_2$}] (**5d**) was prepared using the same procedure from **2d**. Alternatively, **5d** was prepared as a yellow solid from 0.150 g (0.158 mmol) of compound *cis*-[Pt(4-FC_6H_4)$_2$(μ-SEt_2)]$_2$ and 0.074 g (0.324 mmol) of ligand **1d** in toluene with continuous stirring at room temperature for 4 h followed by heating under reflux for 6 h. The reaction mixture was evaporated, and the yellow oily residue was treated with dichloromethane−methanol. After cooling the mixture, a solid was produced, filtered, and dried under vacuum. Yield: 124 mg (75.9%).

NMR labeling for **5d**:



^{1}H NMR (400 MHz, CD_3COCD_3): δ 9.38 (s, $^3J_{H-Pt}$ = 144.4, 1H, H^1), 7.69 (td, $^3J_{H-H}$ = 8.0, $^4J_{H-F}$ = 6.0, 1H, H^{10}), 7,27 (dd, $^3J_{H-F}$ = 10.8, $^4J_{H-H}$ = 2.8, 1H, H^6), 7.21 (d, $^3J_{H-H}$ = 8.0, 1H, H^9), 7.20 (ddd, $^3J_{H-F}$ = 12.0, $^3J_{H-H}$ = 8.0, $^4J_{H-H}$ = 0.8, 1H, H^{11}), 6.94 (dd, $^3J_{H-H}$ = 8.4, $^4J_{H-F}$ = 6.0, 1H, H^8), 6.65 (td, $^3J_{H-H}$ = $^3J_{H-F}$ = 8.4, $^4J_{H-H}$ = 2.8, 1H, H^7), 4.56 (dtd, $^2J_{H-H}$ = 12.8, $^3J_{H-H}$= $^3J_{H-H}$ = 4.4, $^4J_{H-H}$ = 1.2, 1H, H^2), 4.31 (ddd, $^2J_{H-H}$ = 11.6, $^3J_{H-H}$ = 3.6, $^3J_{H-H}$ = 0.4, 1H, $H^{2'}$), 2.99 (s, 3H, H^5), 2.85 (m, 2H, H^3, $H^{3'}$), 2.65 (s, 3H, H^4). ^{19}F NMR (376.5 MHz, CD_3COCD_3): δ −120.0 (ddd, $^3J_{F-H}$ = 11.3, $^3J_{F-H}$ = 8.7, $^4J_{F-H}$ = 6.4, 1F, F^2), −118,3 (ddd, $^3J_{F-H}$ = 10.2, $^4J_{F-H}$ = 6.0, $^5J_{F-H}$ = 2.3, 1F, F^1). ^{13}C NMR (100 MHz, CD_3COCD_3): δ 159.8 (C^1), 133.1 (d, $^3J_{C-F}$ = 10.4, C^{10}), 130.1 (d, $^3J_{C-F}$ = 8.2, C^8), 128.8 (d, $^4J_{C-F}$ = 2.8, C^9), 125.8 (d, $^2J_{C-F}$ = 17.5, C^6), 112.9 (d, $^2J_{C-F}$ = 22.2, C^{11}), 109.4 (d, $^2J_{C-F}$ = 23.0, C^7), 67.4 (C^2), 64.8 (C^3), 50.1 (C^4), 47.2 (C^5). ^{195}Pt NMR (85.68 MHz, CD_3COCD_3): δ −3259.0 (s). HRMS-ESI-(+) {H_2O−CH_3CN (1:1)}: m/z 518.0759 (calcd for $C_{17}H_{18}ClF_2N_2Pt$ 518.0768) $[M + H]^+$; 535.1006 (calcd for $C_{17}H_{21}ClF_2N_3Pt$ 535.1034) $[M + NH_4]^+$; 1052.1709 (calcd for $C_{34}H_{38}Cl_2F_4N_5Pt_2$ 1052.1730) $[2M + NH_4]^+$. Anal. Found (calcd for $C_{17}H_{17}ClF_2N_2Pt$): C: 39.7 (39.4); H: 3.4 (3.3); N: 5.3 (5.4).

Compound [Pt{(4-FC_6H_4){$Me_2NCH_2CH_2N$=CH(3-ClC_6H_3)}] (**3c**) was obtained as an impure solid from 0.150 g (0.158 mmol) of compound *cis*-[Pt(4-FC_6H_4)$_2$(μ-SEt_2)]$_2$ and 0.066 g (0.313 mmol) of ligand **1c** in toluene with continuous stirring at room temperature for 4 h followed by heating under reflux for 6 h. The reaction mixture was evaporated, and the yellow oily residue was treated with dichloromethane−methanol. After cooling the mixture, a solid was produced, filtered, and dried under vacuum. Analogous results were obtained when **2c** was refluxed in toluene for 6 h. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.38 (s, $^3J_{H-Pt}$ = 57.2, CHN), 3.97 (t, $^3J_{H-H}$ = 4.0, 2H, CH_2), 3.12 (t, $^3J_{H-H}$ = 4.0, 2H, CH_2), 2.67 (s, $^3J_{H-Pt}$ = 20.0, 6H, NMe_2). ^{19}F NMR (376.5 MHz, $CDCl_3$): δ (ppm) −123.4 (tt, $^3J_{F-H}$ = 10.2, $^4J_{F-H}$ = 6.8, 1F).

Biological Studies. *Cell Culture.* Human lung carcinoma A549 cells were grown as a monolayer culture in minimum essential medium (DMEM with L-glutamine, without glucose and without sodium pyruvate) in the presence of 10% heat-inactivated fetal calf serum, 10 mM D-glucose, and 0.1% streptomycin/penicillin, in standard culture conditions (humidified air with 5% CO_2 at 37 °C).

Cell Viability Assay. For A549 cell viability assays, compounds were suspended in DMSO at 20 mM as stock solution. To obtain final assay concentrations, they were diluted in DMEM (final concentration of DMSO was the same for all final dilutions and always lower than 1%). The assay was performed by a variation of the MTT assay described by Mosmann et al.[34] as specified by Matito and co-workers,[35] which is based on the ability of live cells to cleave the tetrazolium ring of the MTT, thus producing formazan, which absorbs at 550 nm. In brief, 2.5 × 10^3 A549 cells/well were cultured in 96-well plates for 24 h prior to the addition of different compounds at different concentrations, in triplicate. After further incubation for 72 h, the supernatant was aspirated, and 100 μL of filtered MTT (0.5 mg/mL) was added to each well. Following 1 h of incubation with the MTT, the supernatant was removed, and the precipitated formazan was dissolved in 100 μL of DMSO. Relative cell viability, compared to the viability of untreated cells, was measured by absorbance at 550 nm on an ELISA plate reader (Tecan Sunrise MR20-301, TECAN, Salzburg, Austria). Concentrations that inhibited cell growth by 50% (IC_{50}) after 72 h of treatment were subsequently calculated.

DNA Migration Studies. Compounds were dissolved in high-purity DMSO at 10 mM as stock solution. Then, serial dilutions were made in Milli-Q water (1:1). Plasmid pBluescript SK+ (Stratagene) was obtained using QIAGEN plasmid midi kit as described by the manufacturer. Interaction of drugs with pBluescript SK+ plasmid DNA was analyzed by agarose gel electrophoresis following a modification of the method described by Abdullah et al.[36] In brief, plasmid DNA aliquots (40 μg mL^{-1}) were incubated in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5) with different concentrations of the compounds (ranging from 0 to 200 μM) at 37 °C for 24 h. Final DMSO concentration in the reactions was always lower than 1%. For comparison, cisplatin and ethidium bromide were used as controls. Aliquots of 20 μL of compound−DNA complexes containing 0.8 μg of DNA were subjected to 1% agarose gel electrophoresis in TAE buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.0). The gel was stained in the same buffer containing ethidium bromide (0.5 mg mL^{-1}) and visualized and photographed under UV light.

Topoisomerase I-based experiments were performed as described previously.[29] Supercoiled pBluescript DNA, obtained as described above, was treated with topoisomerase I in the absence or presence of increasing concentrations of compound **2c**. Assay mixtures contained supercoiled pBluescript DNA (0.8 μg), calf thymus topoisomerase I (3 units), and complex **2c** (0−100 μM) in 20 μL of Tris-HCl buffer (pH 7.5) containing 175 mM KCl, 5 mM $MgCl_2$, and 0.1 mM EDTA. Ethidium bromide (10 μM) was used as a control of intercalating agents. Reactions were incubated for 30 min at 37 °C and stopped by the addition of 2 μL of agarose gel loading buffer. Samples were then subjected to electrophoresis and DNA bands stained with ethidium bromide as described above.

■ ASSOCIATED CONTENT

Supporting Information

CIFs giving crystallographic data for the four complexes characterized by XRD and a table of crystallographic data are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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