Versatile Route to *trans*-Platinum(II) Complexes via Manipulation of a Coordinated 3-(Pyridin-3-yl)propanoic Acid Ligand

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ABSTRACT: We describe the direct coupling of alcohols and amines to a 3-(pyridin-3-yl)propanoic acid ligand coordinated to a Pt(II) to afford ester and amide derivatives. Using this approach, a family of *trans*-Pt(II) compounds with amine ligands bearing long perfluorinated chains was prepared, as these chains potentially endow the complexes with thermoactivatable proper- ties. Related compounds with alkyl chains in place of the perfluorinated chains were also prepared as controls using the same direct coupling method. The stability of the complexes in solution, their reactivity with DNA and proteins, and their antiproliferative activity evaluated in tumorigenic (A2780 and A2780cisR) and nontumorigenic (HEK293) cells at 37 °C and following exposure to elevated temperatures (that mimic the temperatures employed in thermotherapy) were also studied to assess their utility as putative (thermoactivated) anticancer agents.

1. INTRODUCTION

The limitations associated with traditional platinum agents, combined with improved knowledge of tumor biology, and the fact that cisplatin still is the most used metallodrug in the clinic, supports the further development of platinum drugs. Nonconventional platinum anticancer agents are particularly promising due to their selective activity against specific cancer cells types.[1]

DNA is the main pharmacological target for platinum drugs, although many studies have shown that proteins are also involved in their therapeutic effect, such as proteins that remove Pt-DNA adducts.[2,3] However, specific interactions with proteins are difficult to control due to the relative structural simplicity of square-planar platinum(II) compounds. Since DNA is the main target of platinum drugs and many additional nonselective interactions with proteins take place, these compounds have high general toxicities resulting in severe side effects. In order to improve the efficacy of platinum drugs and reduce their side effects, they tend to be applied in combination therapies, either with other drugs or with other types of therapies, e.g., radiotherapy, thermotherapy, etc.[4,5]

In general, clinically approved platinum drugs are used in different combinations without adapting their structure to improve their performance in the given combination. For example, carboplatin is extensively used in the treatment of retinoblastoma in combination with thermotherapy.[6] Here, following administration of carboplatin, the tumor is selectively heated with a laser through the pupil of the eye, and the combination is considerably more effective than the two monotherapies alone. However, carboplatin was not designed for application in combination with heat, and a heat-activated platinum drug could further enhance the efficacy of the combination.

It has previously been shown that the action of nonconventional platinum drugs with aliphatic amines in the trans configuration can be modulated by replacing one of the trans aliphatic amines. As examples, a steroid-functionalized amine increases the selectivity of the complex,[7,8] phosphines modulate drug uptake,[9,10] and functionalizing the ligand with a DNA intercalator affords a dual-action platinum complex.[11,12] In the present study, we developed an alternative synthetic methodology that affords perfluorinated chain-modified platinum(II) complexes by directly coupling the perfluorinated chain to a 3-(pyridin-3-yl)propanoic acid already coordinated to a Pt(II) center, which to the best of our knowledge is the first time such an approach has been reported for platinum(II) complexes. Perfluorinated chains are known to endow compounds with thermoresponsive properties,[13,14] and related complexes with long hydrocarbon chains were also prepared for comparison purposes. The ability of the complexes to bind to DNA and proteins was evaluated at 37 °C and following a hyperthermia treatment. Their cytotoxicity

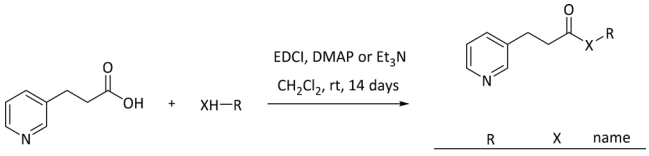
also evaluated in vitro under both normal conditions and hyperthermia.

2. RESULTS AND DISCUSSION

2.1. Synthesis and Characterization of the Compounds.

The synthesis of the platinum compounds with ligands modified with fluorinated and/or aliphatic chains is illustrated in Scheme 1 (ligands are named L1, L2, and L3).

Scheme 1. Synthesis of the Ligands L1, L2, and L3[a]



R	X	name
$-(CH_2)_{17}CH_3$	O	L1
$-(CH_2)_2(CF_2)_5CF_3$	O	L2
$-CH_2(CF_2)_6CF_3$	NH	L3

[a]EDCI (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride) and DMAP (4-(dimethylaminopiridine).

The method comprises a coupling reaction using 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide (EDCI) to activate the carboxylic acid group and dimethylaminopyridine (DMAP) in catalytic amounts. The synthesis of L3 involves the formation of an amide bond from the *O*-acyl-isourea intermediate and perfluorinated fragment.

The traditional method used to prepare *trans*-platinum complexes involves the direct reaction of *cis*-[PtCl (amine)]

compounds with the desired ligand.[15,16] 2 2

However, applying this method for the synthesis of *trans*-platinum complexes containing ligands L1, L2, and L3 resulted in complicated mixtures due to the harsh conditions required. Consequently, an alternative synthetic route was required in order to obtain the target complexes in good yield. First, we prepared a *trans*-platinum complex which incorporates a picolinic-type ligand giving a general formula *trans*-[$PtCl_2$(amine)(3-(pyridin-3-yl)propanoic acid)] (amine = dimethylamine, dma and isopropylamine, ipa). These starting complexes are named Pdma and Pipa, respectively, and are depicted in Scheme 2. Second, the *trans*-Pt(II) compounds bearing the perfluorinated chains were obtained from a coupling reaction between the 3-(pyridin-3-yl)propanoic acid ligand coordinated to the Pt(II) center with the appropriate perfluorinated precursors shown in Scheme 1, employing the same coupling conditions used in the synthesis of ligands L1, L2, and L3 (see Scheme 1). The desired complexes, 1−6, were obtained as pure compounds after flash chromatography.

Using this approach, we prepared a family of *trans*-Pt(II) compounds with ligands L1, L2, and L3. The synthesis of *trans*-[$PtCl_2$(ipa)(L3)] 6, was more complicated than expected. The established route (Scheme 2) led to the formation of the complex together with other byproducts. Moreover, after several attempts to purify 6 by flash chromatography, the resulting yield was low. Therefore, we decided to activate the acid using $SOCl_2$, which in spite of generating SO_2, does not afford any platinated byproducts, allowing the successful synthesis and isolation of complex 6 (see Scheme 3).[17]

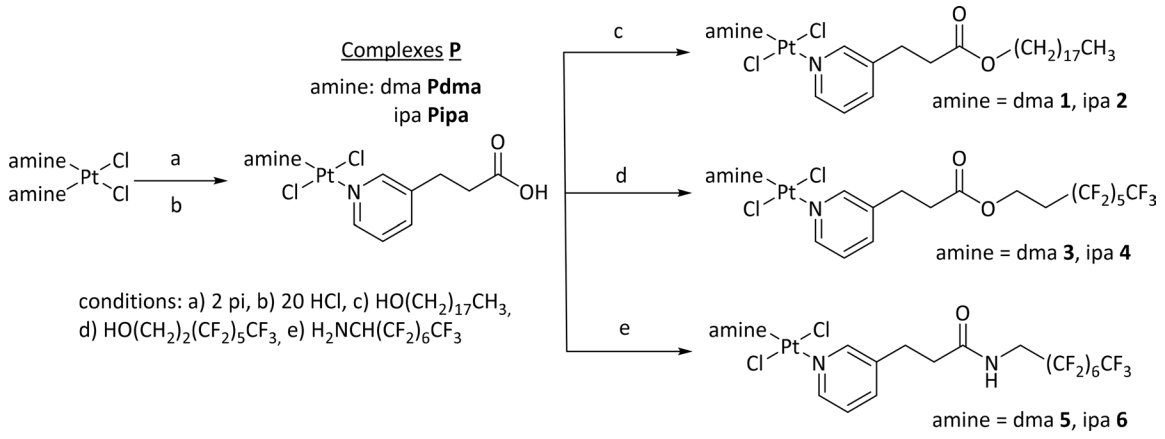
Complexes 1−6 were characterized by ^{1}H, ^{13}C, and ^{195}Pt nuclear magnetic resonance (NMR) spectroscopy and elemental analysis (see Experimental Section for full details). The NMR spectra confirm the expected structures and are essentially as expected following coordination of the ligand to the Pt(II) center. In brief, the ^{195}Pt NMR spectra of each trans complex contains only one signal between −2100 and −2000 ppm, which is consistent with a *trans*-PtN_2Cl_2 unit.[18,19,11] The ^{1}H NMR confirms the presence of dma and ipa and ligands L1 to L3 in 1−6. Following coordination, the ^{1}H NMR signals corresponding to the pyridine moiety move to a lower frequency relative to the free ligands ($\Delta\delta H_\alpha$ = 0.3 to $\Delta\delta H_\beta$ = 0.1), in agreement with the coordination of the N of the pyridine to the platinum center. The signals in the ^{13}C NMR

spectra corresponding to the C atoms adjacent to the donor N atom are also shifted to lower values. This kind of shielding produced by the platination has been reported previously with other complex examples.[7,12,20−22] The ^{19}F NMR spectra contain resonances that are readily assigned to the perfluorinated chains, and since they are far from the site of coordination, do not show significant differences in chemical shifts compared to the free ligands.

2.2. Stability of the *trans*-Pt(II) Complexes in DMSO and Aqueous (1% DMSO) Solution.

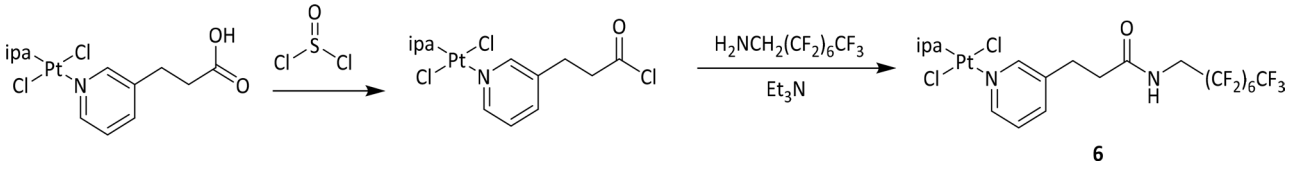
The stability of Pdma, Pipa, and 1−6 was studied in DMSO using ^{1}H NMR spectroscopy (spectra were recorded after 0, 2, 6, and 24 h on samples maintained at 37 °C in a thermoshaker in the dark).

Scheme 2. Route Used to Obtain the *trans*-[$PtCl_2$(amine)L] Complexes 1−6[a]



[a]L = L1, L2, or L3.

Scheme 3. Synthesis of *trans*-[$PtCl_2$(ipa)(L3)] 6 Using $SOCl_2$ and Et_3N



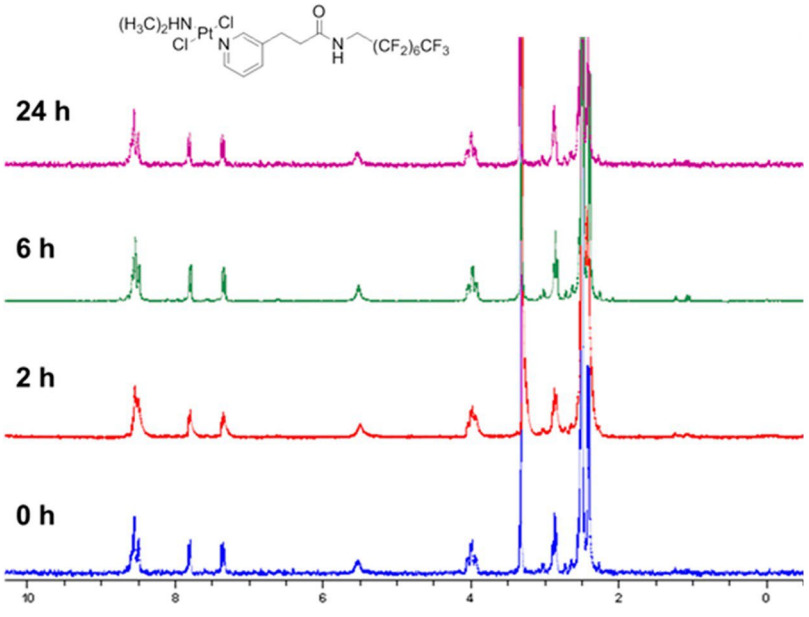


Figure 1. ^{1}H NMR spectra of 3 in DMSO as a function of time.

The spectra remain unchanged demonstrating the stability of the compounds (see Figure 1 for the ^{1}H NMR spectra of 3, other spectra are provided in the Supporting Information). Note that DMSO was selected as the solvent for the stability studies as the compounds are predissolved in DMSO prior to dilution with water for the cytotoxicity studies.

In order to confirm that no other species with similar chemical shifts (possible speciation with DMSO) are present in solution, we performed stabilities studies of the complexes in DMSO solution by reverse-phase HPLC using mixtures of water/acetonitrile as the mobile phase. The stability of the complexes was also monitored by HPLC as a function of time (see Supporting Information), which confirms their high stability in DMSO solution, except for 1, which precipitates after 6 h under the experimental conditions.

The stability of 1−6 in aqueous solution (containing 1% DMSO) was also evaluated using UV−vis spectroscopy over 24 h, as it has previously been shown that water can induce reactions of complexes with DMSO (see in Supporting Information).[23] The stability constants (k_{obs}, Table 1) were estimated by assuming a pseudo-first-order reaction, expressing the absorption as a function of time. Applying the equation $Abs = Abs_0 \cdot e^{-kt}$, we obtained the k_{obs} values collected in Table 1. The nonfluorinated complex 1 (k_{obs}: 7.53 × 10^{-5} s^{-1}) is less stable in aqueous solution than its ipa analogue 2 (k_{obs}: 4.04 × 10^{-5} s^{-1}), which exhibits a stability similar to cisplatin.[24] Complexes with perfluorinated ligands (3−6) display variable stability, with 3 being less stable than its ipa counterpart 4. In general, the complexes with perfluorinated ligands (3−6) are more stable in aqueous solution than the complexes with the alkyl ligands (1 and 2).

Table 1. k_{obs} (× 10^{-5} s^{-1}) Values of 1−6 and Cisplatin

complex	k_{obs} (10^{-5} s^{-1})
trans-[$PtCl_2$(dma)(L1)], 1	11.6[a]
trans-[$PtCl_2$(ipa)(L1)], 2	2.92
trans-[$PtCl_2$(dma)(L2)], 3	7.04
trans-[$PtCl_2$(ipa)L2)], 4	4.04
trans-[$PtCl_2$(dma)(L3)], 5	9.41
trans-[$PtCl_2$(ipa)(L3)], 6	8.28
cisplatin	4.45

[a]Calculated value after 6 h in aqueous solution containing 1% DMSO.

2.3. Interaction of the *trans*-Pt(II) Complexes with Supercoiled Plasmid DNA. Supercoiled pBR322 plasmid DNA is a broadly used model for evaluating the interaction of platinum compounds with a secondary structure DNA model.[11,25] Figure 2 shows the agarose gels of the DNA incubated with 1−6 at 37 and 42 °C (the latter temperature representing hyperthermia). For comparison, the Pdma and Pipa starting materials were included in the study. Pdma interacts with the ccc form of the supercoiled, migrating along with the oc form at r_i of 0.1, and Pipa shows the same pattern but at a higher r (0.2). The elevated temperature leads to only

a slight enhancement in the interaction of these complexes with DNA.

Complex 1 with the aliphatic branched chain (L1) ligand and dma does not interact with the plasmid even at 42 °C, presumably due to its poor solubility (see above). Complex 2, *trans*-[$PtCl_2$(ipa)(L1)], on the other hand, shows a similar interaction profile to its precursor *trans*-[$PtCl_2$(ipa)(3-(pyridin-3-yl)propanoic acid)] Pipa. Complexes 1 and 2 and their

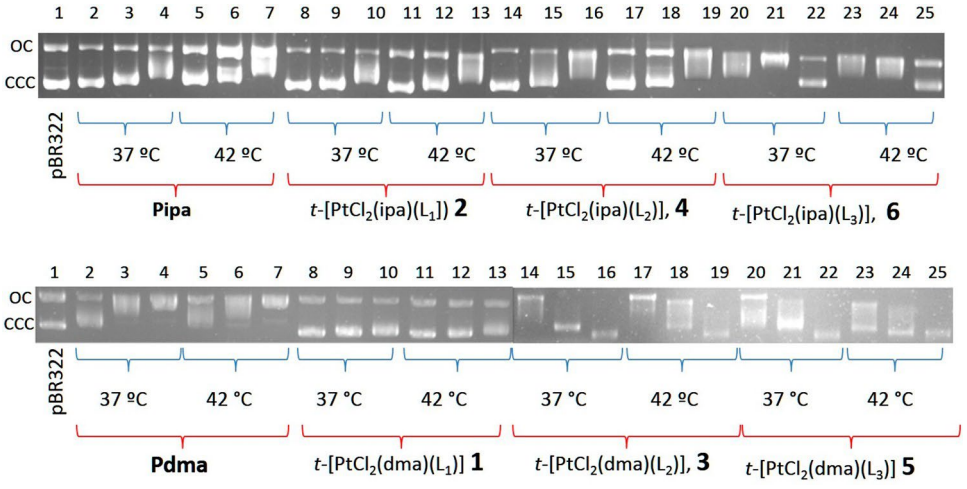


Figure 2. Agarose gels of pBR322 plamid DNA incubated with *trans*-[$PtCl_2$(ipa)(L)] (top) and *trans*-[$PtCl_2$(dma)(L)] (bottom) at 37 and 42 °C for 24 h at r_i values of 0.05, 0.1, and 0.2. r_i = [complex]/[no. nucleotide].

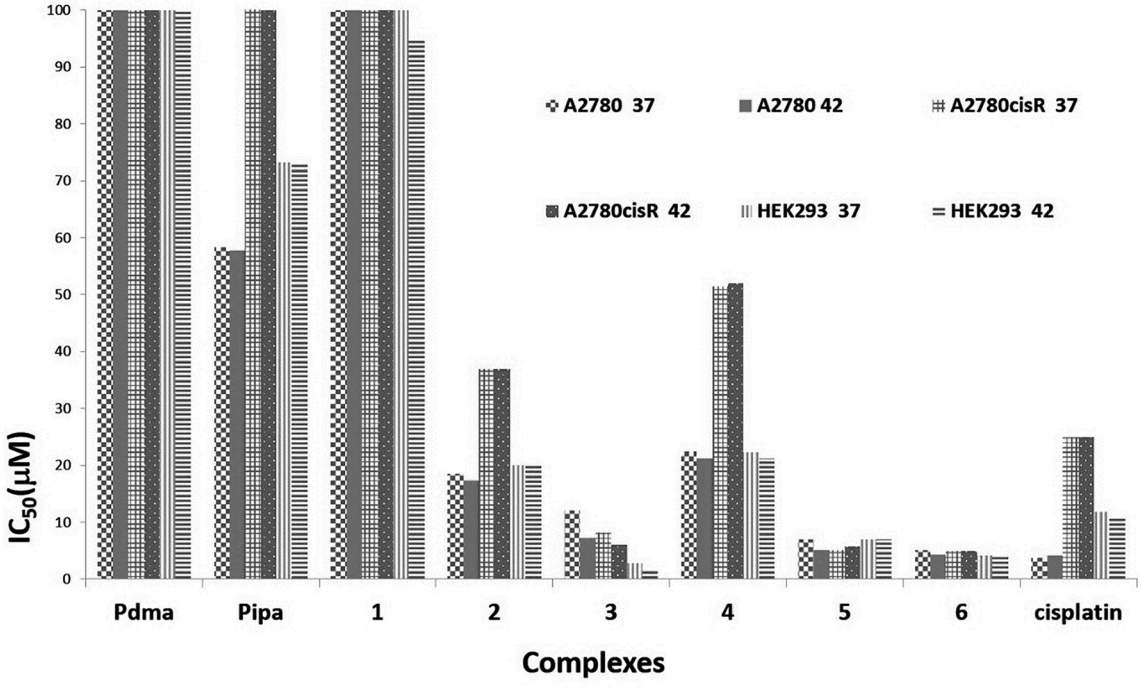


Figure 3. Cytotoxic activity of the *trans*-Pt(II) complexes in A2780, A2780cisR, and HEK293 cell lines at 37 °C and with a 2 h period at 42 °C (total incubation time = 72 h).

precursors do not contain a perfluorinated chain, so the low impact in their reactivity with plasmid at higher temperatures is as expected.

Notably, the *trans*-Pt(II) complexes with perfluorinated chains (3−6) show a different and interesting interaction with DNA compared to the aliphatic counterparts (1−2) and the precursor compounds (Pdma and Pipa). Complex 3 exhibits greater interactions with DNA than its counterpart, 4, which seems to be in agreement with the stability data obtained in solution in which 3 is less stable than 4 and therefore more reactive with the DNA. Notably, complexes 5 and 6 with the longest perfluorinated chains show more extensive reactivity with the plasmid DNA at 42 °C relative to that observed at 37 °C. They reach comigration at even lower r_i values, indicating that they should be more cytotoxic than the other complexes of the series, which is indeed the case (see below). The data are also in agreement with the k_{obs} values obtained in solution (see Table 1).

2.4. Cytotoxicity of the *trans*-Pt(II) Complexes. The cytotoxicity of Pdma, Pipa, and complexes 1−6 was determined at 37 °C over 72 h and additionally at 37 °C over 72 h with a 2 h period at 42 °C at the start of the incubation period (Figure 3). The study was carried out in two cancer cell lines, i.e., A2780 (human ovarian carcinoma) and A2780cisR (Human ovarian carcinoma with acquired resistance to cisplatin) and also in the nontumorigenic HEK293 (human embryonic kidney) cell line. The two precursors (Pdma and Pipa) do not show significant cytotoxic activity or are inactive (at values up to 100 μM). Regarding the complexes with alkyl ligands, complex 1 is also inactive (at

values up to 100 μM), whereas 2 is more cytotoxic but to a lesser extent than cisplatin.

In general, the inclusion of hyperthermia did not lead to large changes in the cytotoxicity of the complexes, although slight increases in the cytotoxicity of the fluorinated complexes at higher temperatures was observed. Moreover, the most active complexes, 3, 5, and 6, contain ligands with perfluorinated chains, but they are not endowed with cancer cell selectivity.

2.5. Interaction Studies with Model Proteins. Although DNA is probably the main target for these *trans*-Pt(II) complexes, with more extensive binding observed at the higher temperature, the interaction of the complexes with proteins cannot be discarded. For this reason, we studied the interaction of the complexes with a model protein at 37 and 42 °C, employing bovine pancreatic ribonuclease A (RNase A), often used as a model to study metallodrug binding interactions.[26−30] The rate constants ($k_{obs}R$) of the complexes were calculated by assuming a pseudo-first-order reaction, and the values obtained at 37 and 42 °C are listed in Table 2.

Table 2. Calculated $k_{obs}R$ Values for the Interaction of 1−6 and Cisplatin with Rnase at 37 and 42 °C

	K (× 10^{-5} s^{-1})	
complex	$k_{obs}R$ (37 °C)	$k_{obs}R$ (42 °C)
1	11.5	10.2
2	17.5	26.6[a]
3	9.23	13.2
4	7.79	0.03
5	7.93	7.00
6	8.07	44.4[a]
cisplatin	18.8	

[a]Calculated values after 6 h in solution.

Caution should be applied when comparing the values at the two different temperatures, as the structure of the protein probably differs as hyperthermia is known to lead to protein denaturation.[31,32]

The $k_{obs}R$ values of the nonfluorinated complexes 1 and 2 at 37 °C indicate a higher affinity of these complexes for the protein than the other complexes in the series, exhibiting similar protein affinities to cisplatin.[33] Notably, 1 and 2 are the least reactive with DNA. The long aliphatic chain in 1 and 2 presumably leads to interactions with the lipophilic residues of RNase. However, this effect is not as strong with the complexes bearing perfluorinated ligands, although the values are indicative of interactions.

At the elevated temperature of 42 °C, the trend is less clear, reflecting the lower stability and increased dynamic properties of the complexes at this temperature. Moreover, at 42 °C the *k* observed values have very short detection times, making it increasingly difficult to obtain good-quality absorptions, as the sample shows little differences with the control. Complexes 2 and 6 have very high $K_{obs}R$ values at the elevated temperature, leading to precipitation after 6 h. In contrast, the $K_{obs}R$ value of 4 is very low indicating that it interacts differently with the model protein.

synthetic route used allows alcohols and amines to be directly coupled to a platinum-coordinated 3-(pyridin-3-yl)propanoic acid (pi) ligand, which in principle allows a wide range of groups to be added to the Pt(II) scaffold. Such a synthetic approach opens the way to rapidly generate small libraries of Pt(II) complexes for cancer cell screening and other applications. Concerning the specific compounds prepared here, complexes 5 and 6, with perfluorinated ligands, are able to overcome cisplatin resistance in the A2780cisR cell line, but they are not endowed with cancer cell selectivity, and their thermoresponsive behavior is rather limited. Nonetheless, their cytotoxicity correlates reasonably well to the observed complex−DNA binding interactions.

4. EXPERIMENTAL SECTION

4.1. General Methods. NMR spectra were acquired on a Bruker 300 spectrometer, running at 300, 75, and 64.5 MHz for ^{1}H, ^{13}C, and ^{195}Pt, respectively. Chemical shifts (δ) are reported in ppm relative to residual solvent signals ($CDCl_3$: 7.26 ppm for ^{1}H NMR, 77.0 ppm for ^{13}C NMR; DMSO-d_6: 2.50 ppm for ^{1}H NMR, 39.52 for ^{13}C NMR; acetone-d_6: 2.05 ppm for ^{1}H NMR, 29.84 for ^{13}C NMR). ^{13}C NMR spectra were acquired on a broad band decoupled mode. ^{195}Pt NMR spectra were obtained with chemical shifts reported in ppm downfield relative to the external reference 1.0 M Na_2PtCl_6 in D_2O. Elemental analyses were performed on a PerkinElmer 2400 Series II micro-analyzer. UV−vis in a Thermo Fisher Scientific Evolution 260 Bio spectrophotometer. Analytical thin layer chromatography (TLC) was performed using precoated aluminum-backed plates (Merck Kieselgel 60-F254) and visualized by ultraviolet irradiation or iodine dip. All reagents and materials were purchased from commercial sources and used without further purification. Cytotoxicity studies were performed as described previously.[34]

4.2. Synthesis of the Starting Materials. *4.2.1. Ligands. Method (a): Synthesis of Ligands L1 and L2.* To a suspension of picolinic acid (py) (300 mg, 1 equiv) in 10 mL of CH_2Cl_2, 1 equiv of 1-octadecanol or 1*H*,1*H*,2*H*,2*H*-perfluorooctanol (for L1 or L2 ligand, respectively), 1 equiv of EDCI and DMAP (10 mol %) were added, and the mixture was stirred at rt for 14 days. The mixture was washed with a saturated solution of $NaHCO_3$ (3 × 10 mL), brine (3 × 10 mL), and then the organic phase was dried over anhydrous $MgSO_4$. Finally, the organic solvent was eliminated under reduced pressure. Final purification by flash column chromatography using silica gel and eluting with hexane and ethyl acetate (5:1) afforded the desired compounds.

L1: Yield: 64%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.43 (s, 1H, Ar_{Py}), 8.41 (d, 1H, J = 4.8 Hz, Ar_{Py}), 7.47 (d, 1H, J = 7.1 Hz, Ar_{Py}), 7.14 (dd, 1H, J = 7.1, 4.8 Hz, Ar_{Py}), 4.00 (t, 2H, J = 6.4 Hz, O−CH_2), 2.90 (t, 2H, J = 6.4 Hz, Py-$\underline{CH_2}$), 2.58 (t, 2H, J = 6.4 Hz, CH_2−CO), 1.55−1.51 (m, 2H, O- CH_2−$\underline{CH_2}$), 1.21 (s, 30H, CH_2), 0.83 (t, 3H, J = 6.4 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 172.3 ($\underline{C}$OO−), 149.9 (N_{py}-CH-C), 147.7 (N_{py}-CH), 135.8 (N_{py}-CH-$\underline{C}$), 135.7 (N_{py}-CH-C-$\underline{C}$H), 123.3 (N_{py}-CH-$\underline{C}$H), 64.8 (O−CH_2), 35.4 ($\underline{C}H_2$−COO−), 31.9 ($\underline{C}H_2$−CH_2−CO−), 29.7 (11C, $(CH_2)_n$-), 29.3 (1C, (CH_2)−), 28.6 (1C, (CH_2)-), 28.1 (1C, $(CH_2)_n$-), 25.8 (1C, (CH_2)−), 22.7 (-$\underline{C}H_2$− CH_3), 14.1 (CH_2−$\underline{C}H_3$). Anal. Calcd for $C_{26}H_{45}NO_2$: C, 77.37%; H, 11.24%; N, 3.47%. Found: C, 77.70%; H, 11.48%; N, 3.26%.

L2: Yield: 62%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.44 (s, 1H, Ar_{Py}), 8.42 (d, 1H, J = 4.9 Hz, Ar_{Py}), 7.48 (d, 1H, J = 7.6 Hz, Ar_{Py}), 7.16 (dd, 1H, J = 7.6, 4.9 Hz, Ar_{Py}), 4.33 (t, 2H, J = 6.4 Hz, O−CH_2), 2.92 (t, 2H, J = 6.4 Hz, Py-$\underline{CH_2}$), 2.64 (t, 2H, J = 6.4 Hz, CH_2−CO), 2.49−2.37 (m, 2H, CH_2−CF_2). ^{13}C NMR ($CDCl_3$): δ 171.9 ($\underline{C}$OO−),

3. CONCLUSIONS

We have synthesized a new family of *trans*-Pt(II) complexes with long-chain aliphatic or perfluorinated ligands. The

149.8 (N_{py}-$\underline{C}H-C$), 147.9 (N_{py}-CH), 135.7 (N_{py}-CH-C-$\underline{C}H$), 135.6 (N_{py}-CH-$\underline{C}$-CH), 123.3 (N_{py}-CH-$\underline{C}H$), 105.8−121.5 (6 C−F), 56.4 (O−$\underline{C}H_2$), 35.0 ($\underline{C}H_2$−COO−), 30.6 ($\underline{C}H_2$−CH_2−O−), 28.1 ($\underline{C}H_2$−CH_2−CO−). Anal. Calcd for $C_{16}H_{12}F_{13}NO_2$: C, 38.65%; H, 2.43%; N, 2.82%. Found C, 39.01%; H, 2.58%; N, 2.77%.

Method (b): Synthesis of Ligand L3. To a suspension of picolinic acid (py) (300 mg, 1 equiv) in 10 mL of CH_2Cl_2, 1 equiv of 1*H*,1*H*-

perfluorooctilamine, 1 equiv of EDCI, and 1 equiv of Et_3N were added, and the mixture stirred at rt for 14 days. The mixture was washed with a saturated solution of $NaHCO_3$ (3 × 10 mL) and brine (3 × 10 mL), and then the organic phase was dried over anhydrous $MgSO_4$. Finally, the organic solvent was eliminated under reduced pressure. Final purification by flash column chromatography afforded the desired compounds. L3: Yield: 52%. ^{1}H NMR (acetone-d_6, 300 MHz): δ 8.46 (s, 1H, Ar_{Py}), 8.39 (d, 1H, J = 4.4 Hz, Ar_{Py}), 7.74 (s, 1H, NH), 7.62 (d, 1H, J = 7.8 Hz, Ar_{Py}), 7.24 (dd, 1H, J = 7.8, 4.4 Hz, Ar_{Py}), 4.15−4.02 (m, 2H, Py-$\underline{CH_2}$), 2.95 (td, 2H, $J_{H,H}$ = 6.2 Hz, $J_{F,H}$ = 16.0 Hz, NH-$\underline{CH_2}$), 2.63 (t, 2H, J = 6.4 Hz, CH_2−CO). ^{13}C NMR ($CDCl_3$): δ 172.7 ($\underline{C}$ON-), 150.7 (N_{py}-$\underline{C}$H−C), 148.3 (N_{py}-CH), 137.4 (N_{py}-CH-C-$\underline{C}$H), 136.5 (N_{py}-CH-$\underline{C}$), 124.0 (N_{py}-CH-$\underline{C}$H), 118.0−109.2 (7 C−F), 56.5 (CON-$\underline{C}$H$_2$), 39.5 ($\underline{C}$H$_2$−CON), 29.8 ($\underline{C}$H$_2$−CH$_2$−NH−). Anal. Calcd for $C_{16}H_{11}F_{15}N_2O$: C, 36.11%; H, 2.08%; N, 5.26%. Found: C, 36.39%; H, 2.36%; N, 5.12%.

4.2.2. Starting cis-Platinum Complexes. cis-[PtCl$_2$(amine)$_2$] Complexes. The synthesis of the cis compounds was performed following the protocol published by our group.[35,36] *cis*-[$PtCl_2(dma)_2$] was synthesized by treating K_2PtCl_4 (500 mg; 1.2 mmol) with 4 equiv of dimethylamine (dma) or isopropylamine (ipa) (4.8 mmol) in 1 mL of H_2O. The reaction was stirred at room temperature in dark conditions for 24 h. The yellow residue paste was filtered off and washed with water, acetone, and chloroform. Finally the solid was air-dried. Characterization data agree with the data reported.

cis-[PtCl$_2$(ipa)$_2$]. Yield: 82%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.74 (s, 2H, NH_2), 3.09 (sept, J = 6.3 Hz, 1H, CH); 1.20 (d, J = 6.5 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 47.6 (CH), 23.5 (2C, CH_3). Anal. Calcd for $PtC_6H_{18}Cl_2N_2$: C, 18.75%; H, 4.72%; N, 7.29%. Found $PtC_6H_{18}N_2Cl_2$: C, 18.10%; H, 4.56%; N, 7.30%.

cis-[PtCl$_2$(dma)$_2$. Yield: 60%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.69 (s, 1H, NH), 2.52 (s, 6H, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 43.6. Anal. Calcd for $PtC_4H_{14}N_2Cl_2$: C, 13.48%; H, 3.96%; N, 7.86%. Found $PtC_4H_{14}Cl_2N_2$: C, 13.66%, H, 3.93%, N, 7.90%.

trans-[PtCl$_2$(amine)(L)] Complexes (P). To a suspension of the corresponding *cis*-[$PtCl_2$(amine)$_2$] (500 mg, 1 equiv) in 5 mL of H_2O, 2.5 equiv of 3-(pyridin-3-yl)propanoic acid was added, and the mixture was stirred at 80 °C for 24 h. The colorless solution was cooled and then concentrated slowly until the minimum solvent. To this solution, 20 equiv of HCl (36% aqueous solution) was added, and the mixture was maintained at the reflux temperature for 24 h. The final yellow solid was isolated, washed with water, and dried under a vacuum.

trans-[$PtCl_2$(ipa)(3-(pyridin-3-yl)propanoic acid)], Pipa Yield: 71%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 12.23 (s, 1H, COOH), 8.55 (s, 1H, Ar_{Py}), 8.53 (d, 1H, J = 6.5 Hz, Ar_{Py}), 7.83 (d, 1H, J = 6.5 Hz, Ar_{Py}), 7.36 (t, 1H, J = 6.7 Hz, Ar_{Py}), 4.60 (s, 2H, NH_2), 3.10 (m, 1H, $\underline{CH}$-(CH_3)$_2$), 2.86 (t, 2H, J = 6.4 Hz, Py-$\underline{CH_2}$), 2.55 (t, 2H, J = 6.4 Hz, CH_2−CO), 1.22 (d, 6H, J = 6.4 Hz, CH-($\underline{CH_3}$)$_2$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.3 (COOH), 154.0 (N_{py}-$\underline{C}$H−C), 151.9 (N_{py}-CH), 139.5 (N_{py}-CH-C-$\underline{C}$H), 139.3 (N_{py}-CH-$\underline{C}$), 125.7 (N_{py}-CH-$\underline{C}$H), 49.2 ($\underline{C}$H(CH_3)$_2$), 34.8 (Py-$\underline{CH_2}$), 28.2 (Py-CH_2−$\underline{CH_2}$), 24.5 (CH($\underline{C}$H$_3$)$_2$). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2066. Anal. Calcd for $C_{11}H_{18}Cl_2N_2O_2Pt$: C, 27.74%; H, 3.81%; N, 5.88%. Found: C, 27.97%; H, 3.62%; N, 5.62%.

trans-[$PtCl_2$(dma)(3-(pyridin-3-yl)propanoic acid)], Pdma Yield: 81%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 11.78 (s, 1H, COOH), 8.11 (s, 1H, Ar_{Py}), 8.06 (d, 1H, J = 6.4 Hz, Ar_{Py}), 7.38 (d, 1H, J = 6.4 Hz, Ar_{Py}), 6.91 (t, 1H, J = 6.8 Hz, Ar_{Py}), 5.06 (bs, 1H, NH), 2.39 (t, 2H, J = 6.4 Hz, Py-CH_2), 2.15 (t, 2H, J = 6.4 Hz, CH_2−CO), 1.96 (d, 6H, J = 6.4 Hz, CH$_3$). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.2 (COOH), 152.7 (N_{py}-$\underline{C}$H−C), 150.9 (N_{py}-CH), 138.6 (N_{py}-CH-C-$\underline{C}$H), 138.4 (N_{py}-CH-$\underline{C}$), 125.0 (N_{py}-CH-$\underline{C}$H), 43.2 (2C, NH($\underline{C}$H$_3$)$_2$), 34.1 (Py-$\underline{C}$H$_2$), 26.9 (Py-CH_2−$\underline{C}$H$_2$). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2044. Anal. Calcd for $C_{10}H_{16}Cl_2N_2O_2Pt$: C, 25.98%; H, 3.49%; N, 6.06%. Found: C, 26.43%; H, 3.40%; N, 6.05%.

4.3. Synthesis of the *trans*-Platinum Complexes 1−6. *Method (a): Synthesis of complexes trans-[PtCl$_2$(amine)(L1)] (1− 2),* acid)] complex (250 mg, 1 equiv) in 8.25 mL of CH_2Cl_2, 1 equiv of 1-octadecanol or 1*H*,1*H*,2*H*,2*H*-perfluorooctanol (for the formation of *trans*-[$PtCl_2$(amine)(L1)] or *trans*-[$PtCl_2$(amine)(L2)], respectively), 1 equiv of EDCI and DMAP (10 mol %) were added, and the mixture was stirred at rt for 14 days. The mixture was washed with a saturated solution of $NaHCO_3$ (3 × 10 mL) and brine (3 × 10 mL), and then the organic phase was dried over anhydrous $MgSO_4$. Finally, the organic solvent was eliminated under reduced pressure. Final purification by flash column chromatography afforded the desired compounds, using 90 active neutral alumina Merck 0.063−0.200 mm (70−230 mesh ASTM), dichloromethane/ethyl acetate (10:1 for complex 1 and 2), and dichloromethane/EtOH (8:1 for complexes 3−6) as eluent.

trans-[PtCl$_2$(dma)(L1)], 1. Yield: 45%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.56 (s, 1H, Ar_{Py}), 8.51 (d, 1H, J = 6.0 Hz, Ar_{Py}), 7.84 (d, 1H, J = 7.8 Hz, Ar_{Py}), 7.36 (dd, 1H, J = 7.7, 6.0 Hz, Ar_{Py}), 5.50 (bs, 1H, NH), 4.06 (t, 2H, J = 6.7 Hz, CH_2−O), 2.88 (t, 2H, J = 7.6 Hz, Py-$\underline{CH_2}$), 2.62 (t, 2H, J = 7.4 Hz, CH_2−CO), 2,68 (6 H, d, J 5.7, NH-$\underline{C}$H$_3$), 2.42 (d, 2H, J = 6.4 Hz), 1.52 (m, 2H), 1.23 (bs, 34H), 0.85 (t, 3H, J = 6.9 Hz, CH_3). ^{13}C NMR ($CDCl_3$): δ 171.9 ($\underline{C}$OO−), 153.4 (N_{py}-$\underline{C}$H−C), 151.5 (N_{py}-CH), 138.3 (N_{py}-CH-C-$\underline{C}$H), 129.9 (N_{py}-CH-$\underline{C}$-CH), 124.8 (N_{py}-CH-$\underline{C}$H), 65.1 (O−CH_2), 43.4 (($\underline{C}$H$_3$)$_2$NH), 34.7 ($\underline{C}$H$_2$−COO−), 31.9 ($\underline{C}$H$_2$−CH_2−COO−), 29.7 (1C, $(CH_2)_n$-), 29.5 (1C, $(CH_2)_n$-), 29.3 (9C, $(CH_2)_n$-), 28.6 (1C, (CH_2)−), 27.7 (1C, $(CH_2)_n$-), 25.9 (1C, (CH_2)−), 22.7 ($\underline{C}$H$_2$−CH_3), 14.1 (CH_2−$\underline{C}$H$_3$). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2046. MS (ESI) *m/z*: 737.29 [M + Na]$^+$. Anal. Calcd for $C_{28}H_{52}Cl_2N_2O_2Pt[(CH_3)_2CO]_{0,5}$: C, 47.75%; H, 7.48%; N, 3.74%. Found: C, 48.05%; H, 7.39%; N, 3.73%.

trans-[PtCl$_2$(ipa)(L1)], 2. Yield: 31%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.69 (s, 1H, Ar_{Py}), 8.67 (d, 1H, J = 6.7 Hz, Ar_{Py}), 7.62 (d, 1H, J = 6.7 Hz, Ar_{Py}), 7.17 (t, 1H, J = 6.9 Hz, Ar_{Py}), 4.05 (t, 2H, J = 6.4 Hz, CH_2−O), 3.46 (bs, 2H, NH_2), 3.40 (m, 1H, $\underline{CH}$-(CH_3)$_2$), 2.94 (t, 2H, J = 6.4 Hz, Py-$\underline{CH_2}$), 2.62 (t, 2H, J = 6.4 Hz, CH_2−CO), 1.38 (d, 6H, J = 6.4 Hz, CH-($\underline{CH_3}$)$_2$), 1.21 (s, 32H, $\underline{CH_2}$), 0.86 (t, 3H, J = 6.4 Hz, CH_2-CH_3). ^{13}C NMR ($CDCl_3$): δ 171.9 ($\underline{C}$OO−), 153.2 (N_{py}-$\underline{C}$H−C), 151.3 (N_{py}-CH), 138.3 (N_{py}-CH-C-$\underline{C}$H), 129.5 (N_{py}-CH-$\underline{C}$-CH), 124.7 (N_{py}-CH-$\underline{C}$H), 65.1 (O−CH_2), 49.1 ((CH_3)$_2$$\underline{C}HNH_2$), 34.8 ($\underline{C}H_2$−COO−), 29.7 ($\underline{C}H_2$−$CH_2$−COO−), 29.6 (1C, $(CH_2)_n$-), 29.2 (9C, $(CH_2)_n$-), 28.6 (1C, (CH_2)−), 27.7 (1C, $(CH_2)_n$-), 25.9 (($\underline{C}$H$_3$)$_2$CHNH$_2$), 24.0 (1C, $(CH_2)_n$-), 22.7 ($\underline{C}$H$_2$−CH_3), 14.1 (CH_2−$\underline{C}$H$_3$). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2071. MS (ESI) *m/z*: 751.4 [M + Na]$^+$. Anal. Calcd for $C_{29}H_{54}Cl_2N_2O_2Pt$: C, 47.80%; H, 7.47%; N, 3.84%. Found C, 48.12%; H, 7.69%; N, 3.56%.

trans-[PtCl$_2$(dma)(L2)], 3. Yield: 52%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.57 (s, 1H, Ar_{Py}), 8.51 (d, 1H, J = 5.7 Hz, Ar_{Py}), 7.85 (d, 1H, J = 7.9 Hz, Ar_{Py}), 7.37 (dd, 1H, J = 5.7, 4.9 Hz, Ar_{Py}), 5.50 (bs, 1H, NH), 4.31 (t, 2H, J = 6.0 Hz, CH_2−O), 2.95 (t, 2H, J = 7.4 Hz, Py-$\underline{CH_2}$), 2.72−2.62 (m, 4H, CH_2−CO and CH_2−CF_2), 2.40 (d, 2H, J = 5.7 Hz). ^{13}C NMR ($CDCl_3$) δ 171.3 ($\underline{C}$OO−), 153.3 (N_{py}-$\underline{C}$H− C), 151.6 (N_{py}-CH), 138.3 (N_{py}-CH-C-$\underline{C}$H), 137.7 (N_{py}-CH-$\underline{C}$-CH), 136.5 (N_{py}-CH-$\underline{C}$H), 124.8−108.1 (6 C−F), 56.1 (O−CH_2), 43.4 ($\underline{C}$H$_3$)$_2$NH), 34.4 ($\underline{C}$H$_2$−COO−), 30.4 ($\underline{C}$H$_2$−CH_2−O), 27.5 ($\underline{C}$H$_2$−CH_2−COO). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2046. MS (ESI) *m/z*: 831.0 [M + Na]$^+$. ^{19}F-NMR (acetone-d_6, 282.40 MHz): δ −82.80 (t, 3F, $J_{F,F}$ = 8.5 Hz), −115.04 (m, 2F), −123.50 (m, 2F), −124.51 (m, 2F), −125.20 (m, 2F), −127.86 (m, 2F). Anal. Calcd for $C_{18}H_{19}Cl_2F_{13}N_2O_2Pt$: C, 26.75%; H, 2.37%; N, 3.47%. Found C, 26.82%; H, 2.58%; N, 3.45%.

trans-[PtCl$_2$(ipa)(L2)], 4. Yield: 41%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.76 (m, 2H, Ar), 7.61 (d, 1H, J = 6.8 Hz, Ar), 7.19 (dd,

trans-[PtCl$_2$(amine)(L2)] (3−4) and trans-[PtCl$_2$(dma)(L3)] (5). To a suspension of *trans*-[$PtCl_2$(amine)(3-(pyridin-3-yl)propanoic

1H, J = 6.9, 5.7 Hz, Ar_{Py}), 4.37 (t, 2H, J = 6.4 Hz, CH_2-O), 3.53 (bs, 2H, NH_2), 3.41 (m, 1H, $\underline{CH}$-$(CH_3)_2$), 2.97 (t, 2H, J = 6.4 Hz, Py-$\underline{CH_2}$), 2.66 (t, 2H, J = 6.4 Hz, CH_2-CO), 2.47−2.39 (m, 2H, CH_2-CF_2) 1.30 (d, 6H, J = 6.4 Hz, CH-$\underline{(CH_3)_2}$). ^{13}C NMR ($CDCl_3$): δ 170.4 ($\underline{C}OO-$), 152.2 (N_{py}-$\underline{C}H-C$), 150.5 (N_{py}-CH), 137.2 (N_{py}-CH-C-$\underline{C}H$), 136.8 (N_{py}-CH-$\underline{C}$-CH), 136.2 (N_{py}-CH-$\underline{C}H$), 123.9−105.1 (6 C−F), 55.7 ($O-CH_2$), 48.0 ($(CH_3)_2\underline{C}HNH_2$), 33.5 ($\underline{C}H_2-COO$), 29.5 ($\underline{C}H_2-CH_2-O$), 26.5 ($\underline{C}H_2-CH_2-COO$), 23.0

($\underline{C}H_3)_2CHNH_2$). ^{195}Pt-NMR (DMSO-$d_6$, 64 MHz): δ −2079. ^{19}F- NMR (acetone-d_6, 282.40 MHz): δ −82.80 (t, 3F, $J_{F,F}$ = 8.5 Hz), −115.04 (m, 2F), −123.49 (m, 2F), −124.49 (m, 2F), −125.18 (m, 2F), −127.83 (m, 2F). MS (ESI) *m/z*: 844,0 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{21}Cl_2F_{13}N_2O_2Pt$: C, 27.75%; H, 2.57%; N, 3.41%. Found: C, 28.10%; H, 2.67%; N, 3.52%.

trans-[PtCl$_2$(dma)(L3)], 5. Yield: 40%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.58−8.49 (m, 3H, Ar_{Py} and CO-NH), 7.82 (d, 1H, *J* = 7.9 Hz, Ar_{Py}), 7.35 (dd, 1H, *J* = 7.9, 5.7 Hz, Ar_{Py}), 5.53 (bs, 1H, NH), 4.01−3.92 (td, 2H, $J_{H,H}$ = 6.1 Hz, $J_{F,H}$ = 17.2 Hz, CH_2-CF_2), 2.87 (t, 2H, *J* = 7.6 Hz, Py-$\underline{CH_2}$), 2.58 (m, 2H, CH_2-CO), 2.40 (d, 6H, *J* = 5.6 Hz, CH). ^{13}C NMR (CDCl ,): δ 171.1 (CON-), 153.2 (N - $\underline{C}H-$ $_3$ $_3$ $_{py}$ C), 151.5 (N_{py}-CH), 138.7 (N_{py}-CH-C-$\underline{C}$H), 137.9 (N_{py}-CH-$\underline{C}$-CH), 124.9 (N_{py}-CH-$\underline{C}$H), 118.1−109.0 (7 C−F), 43.4 ($\underline{C}H_2-$CON), 39.3 -(m, CON-$\underline{C}H_2C-$F), 36.7 $(\underline{C}H_3)_2NH$), 27.9 ($\underline{C}H_2-CH_2-CO$). ^{195}Pt-NMR (DMSO-d_6, 64 MHz): δ −2044. ^{19}F-NMR (acetone-d_6, 282.40 MHz): δ −82.78 (t, 3F, $J_{F,F}$ = 11.3 Hz), −119.45 (m, 2F), −123.43 (m, 2F), −123.68 (m, 2F), −124.34 (m, 2F), −125.12 (m, 2F), −127.81 (m, 2F). MS (ESI) *m/z*: 866.0 $[M + Na]^+$. Anal. Calcd for $C_{18}H_{18}Cl_2F_{15}N_3OPt$: C, 25.59%; H, 2.21%; N, 4.95%. Found: C, 25.15%; H, 2.15%; N, 4.98%.

Method (b) trans-[PtCl$_2$(ipa)(L3)], 6. On a flask containing 70 mg (1 equiv) of *trans*-[$PtCl_2$(ipa)(3-(pyridin-3-yl)propanoic acid)], 0.25 mL of $SOCl_2$ was added, and the mixture was stirred at rt for 90 min. The yellow solution was dried under a vacuum, and then a solution containing 1 equiv of 1*H*,1*H*-perfluorooctilamine with 1 equiv of Et_3N in 5 mL of CH_2Cl_2 anhydrous was added, and then stirred at rt overnight. Finally, the yellow solution was dried with $MgSO_4$ and then filtered, and the final solution was dried under reduced pressure.

trans-[PtCl$_2$(ipa)(L3)], 6. Yield: 29%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.72 (s, 1H, Ar_{Py}), 8.67 (d, 1H, *J* = 5.7 Hz, Ar_{Py}), 7.86 (d, 1H, *J* = 8.1 Hz, Ar_{Py}), 7.33 (dd, 1H, *J* = 8.1, 5.7 Hz, Ar_{Py}), 5.95 (bs, 1H, CO-NH), 3.95 (td, 2H, $J_{H,H}$ = 6.3 Hz, $J_{F,H}$ = 15.9 Hz, CH_2-CF_2), 3.57 (bs, 1H, NH), 3.41 (m, 1H, $\underline{CH}$-$(CH_3)_2$), 3.00 (t, 2H, *J* = 6.4 Hz, Py-$\underline{CH_2}$), 2.58 (t, 2H, *J* = 6.4 Hz, CH_2-CO), 1.37 (d, 6H, *J* = 6.4 Hz, CH-$\underline{(CH_3)_2}$). ^{13}C NMR (acetone-d_6) δ 171.4 ($\underline{C}OO-$), 152.9 (N_{py}-$\underline{C}H-C$), 150.8 (N_{py}-CH), 139.5 (N_{py}-CH-C-$\underline{CH}$), 138.4 (N_{py}-CH-$\underline{C}$-CH), 125.0 (N_{py}-CH-$\underline{C}$H), 118.0−109.2 (7 C−F), 48.6 ($(CH_3)_2\underline{C}HNH_2$), 46.1 ($\underline{C}H_2-$CON), 39.7 (m, CON-$\underline{C}H_2C-$F), 27.0 ($\underline{C}H_2-CH_2-CO$), 22.9 ($(\underline{C}H_3)_2CHNH_2$). ^{19}F-NMR (acetone-$d_6$, 282.40 MHz): δ −82.77 (m, 3F), −119.46 (m, 2F), −123.45 (m, 2F), −123.67 (m, 2F), −124.36 (m, 2F), −125.13 (m, 2F), −127.83 (m, 2F). MS (ESI) *m/z*: 880,0 $[M + Na]^+$. Anal. Calcd for $C_{19}H_{20}Cl_2F_{15}N_3OPt$: C, 30.10%; H, 3.06%; N, 4.68%. Found $C_{19}H_{20}Cl_2F_{15}N_3OPt[(CH_3)_2CO]_{0.5}$: C, 29,68%; H, 2,55%; N, 4,61%.

4.4. Stability Studies of the *trans*-Pt(II) Complexes in DMSO by ^{1}H NMR. We dissolved 1 mg of each compound in DMSO-d_6 (0.5 mL), placed it into an NMR tube, and analyzed it several times from 0 to 24 h by ^{1}H NMR using a Bruker AMX-300 (300 MHz) spectrometer at room temperature (25 °C).

4.5. Stability Studies of the *trans*-Pt(II) Complexes in DMSO by HPLC. For the stability studies of the *trans*-Pt(II) complexes by HPLC, stock solutions were prepared fresh in DMSO (5 mM). Then, 1 mL of a 100 μM solution in DMSO of the test compound was incubated at 37 °C, and aliquots of 20 μL were taken at various time points (0, 1, 2, 5, and 24 h) and analyzed by RP-HPLC on an Agilent 1200 system using a Zorbax Eclipse Plus C18 column (4.6 × 100 mm, 3.5 μm): flow rate, 1 mL/min; detection, UV 254 nm; gradient solvent system A/B (acetonitrile/water): for complexes *trans*-[$PtCl_2$(dma)(3-(pyridin-3-yl)propanoic acid)], *trans*-[$PtCl_2$(ipa)(3-(pyridin-3-yl)propanoic acid)], *trans*-[$PtCl_2$(dma)(L2)], *trans*-[$PtCl_2$(ipa)(L2)], and *trans*-[$PtCl_2$(dma)(L3)], initial 30% A + 70% B; 10 min linear gradient to 100% A; for complexes *trans*-[$PtCl_2$(dma)(L1)], *trans*-[$PtCl_2$(ipa)(L1)] and *trans*-[$PtCl_2$(ipa)- (L3),] initial 50% A + 50% B; 30 min linear gradient to 100% A. The disappearance of the compound over time was expressed as the remaining percentage in comparison to the initial amount. The different time point alicuots were analyzed by RP_HPLC monitored

4.6. Sample Preparation and UV−vis Kinetic Experiments of the Complex Solutions. The desired concentration of complexes was achieved by dilution of the stock DMSO solution with aqueous tris buffer. All the solutions and buffers were previously tempered to 37 °C for the stability in solution and at 37 °C. The freshly prepared complex solutions were mixed in a thermoshaker at the desired temperature with Milli-Q water (for stability *k*).The electronic spectra of *trans*-Pt complexes were recorded from fresh to 24 h in DMSO/buffer solution monitoring the changes in the absorbance of one characteristic charge transfer band. The absorbance was then represented versus time for a typical kinetic run, and the data were fit to an exponential decay function: $A = A_0 \cdot e^{-kt}$.

4.7. Interaction Studies of the *trans* Pt(II) versus pBR322 using a Detector photodiode array.

Plasmid DNA. The plasmid pBR322 DNA stock was purchased from Gencust at a concentration of 0.5 μg/μL in phosphate buffer 50 mM (pH 7.4). Stock solutions of the complexes were prepared in DMSO at 5 mM and then diluted with water to the desired concentration. The DNA interaction studies were performed with a total volume of 20 μL. A 20 μL portion containing 0.125 μg/μL of DNA in 10 mM Tris-HCl (pH 7.6) and 1 mM EDTA was incubated with the platinum compounds at r_i values ranging from 0.05 to 0.2 (r_i is defined r_i = [complex]/[no. nucleotide]). The samples were incubated at 37 °C for 24 h, after which 2 μL of a loading dye buffer containing 50% glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol was added. The total amount of the sample (20 μL) was loaded in the agarose gel (1.2% w/v), and electrophoresis was carried out for a period of 150 min at approximately 70 V in TAE 1× (Tris-acetate/EDTA) buffer. After electrophoresis, the gel was immersed in 200 mL of Millipore water containing 10 μL from a 10 mg/mL stock solution of ethidium bromide for 30 min to stain the DNA. Finally, the stained gel was analyzed with a UVITEC Cambridge UVIDOC HD2 instrument.

4.8. Cell Culture and Cytotoxicity Measurements. Human A2780 and A2780cisR ovarian carcinoma cells were obtained from the European Centre of Cell Cultures (ECACC, Salisbury, UK). Nontumorigenic HEK-293 cells were obtained from ATCC (Sigma, Switzerland). A2780 and A2780cisR cells were routinely grown in RPMI 1640 medium with GlutaMAX containing 5% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37 °C and 5% CO_2. HEK-293 cells were grown in DMEM medium containing 5% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin) at 37 °C and 5% CO_2. In order to keep the A2780cisR cells resistant to cisplatin, the cells were monthly treated with 2 μM cisplatin for one passage. Cytotoxicity was determined using the MTT assay (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl-2*H*-tetrazolium bromide). Briefly, the cells were seeded in 96-well plates (10 000 cells per well) and grown for 24 h in complete medium. For each testing, compounds were freshly prepared as DMSO stock solution and then dissolved in the culture medium and immediately serially diluted to the appropriate concentration, to give a final DMSO concentration of 0.5% v/v. Drug solution (100 μL) was added to each well, and the plates were incubated at 37 °C for 72 or 2 h at 41.5 °C followed by a 70 h incubation at 37 °C. Following drug exposure, 20 μL of MTT (5 mg/mL solution in PBS) was added to the cells and incubated for 4 h, then the culture medium was aspirated, and the violet formazan crystals were dissolved in DMSO (100 μL). The optical density of each well (96 well plates) was quantified at 590 nm using a multiwell plate reader (Molecular Devices, UK), and the percentage of surviving cells was calculated from the ratio of absorbance of treated to untreated cells. The IC_{50} values for the inhibition of cell growth were determined by fitting the plot of the logarithmic percentage of surviving cells against the logarithm of the drug concentration using a linear regression function. Mean values and standard deviations computed from two independent experiments, each comprising four microcultures per concentration level, are reported.

4.9. Sample Preparation for RNaseA Interaction Studies by UV−vis Spectroscopy. *trans*-Pt complexes were initially dissolved in DMSO to a concentration of 5 mM. The desired concentration of complexes was achieved by dilution of the stock DMSO solution with

aqueous tris buffer. Cisplatin stock solutions were prepared in 5 mM $NaClO_4$ and diluted in Milli-Q water. All the solutions and buffers were previously tempered to 37 °C for the stability in solution, and at 37 and 42 °C for the interaction with Rnase. Afterward, the freshly prepared complex solutions were mixed in a thermoshaker at the desired temperature aqueous buffer tris with the RNase solutions. The final concentration used for these studies never exceeded 1% DMSO (v/v) in the final solution. Control experiments with DMSO were performed, and no changes in the spectra of the model protein RNase were observed.

4.9.1. UV−vis Kinetics Experiments with Proteins. The electronic spectra of the model protein RNase A (bovine pancreatic ribonuclease A) were recorded at 10^{-5} M, monitoring the changes in the absorbance at λ_{max} = 280 nm, and after the addition of the Pt complexes (*trans*-Pt(II) complexes and cisplatin) from fresh to 24 h both at 37 and 42 °C. The assays were performed first using pseudo-first-order conditions (ratios of 10:1 metal to protein) obtaining poor absorbance signals (values range from 0.10 to 0.05 au); then to increase the absorbance, we repeated the experiment using 3:1 ratios. The experimental time dependence spectra profiles (1:10 and 1:3 ratios) were analyzed as a pseudo-first-order reaction by plotting the variation of the absorbance as a function of time obtaining the same results. The data were fit to a exponential decay function: $A = A_0 \cdot e^{-kt}$ expected for a pseudo-first-order reaction.

ASSOCIATED CONTENT

Supporting Information

HPLC spectra gradient conditions and ^{1}H NMR spectra for all the complexes. UV−vis spectra for all the complexes (PDF)

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Notes

The authors declare no competing financial interest.

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