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Synthesis, Crystal Structure, Studies in Solution and Cytotoxicity of Two New Fluorescent Platinum(II) Compounds Containing Anthracene Derivatives as a Carrier Ligand

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Two new cytotoxic fluorescent platinum(II) compounds, *cis*-[Pt(A9opy)Cl_2] (**1**) and *cis*-[Pt(A9pyp)(DMSO)Cl_2] (**2**), have been designed, synthesized, and characterized by IR, ^{1}H NMR, and ^{195}Pt NMR spectroscopy; electrospray ionization mass spectrometry (ESI-MS); and single-crystal X-ray diffraction. The carrier ligands selected for the synthesis of these fluorescent platinum(II) compounds are *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] (abbreviated as A9opy) and *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone (abbreviated as A9pyp). The compound *cis*-[Pt(A9opy)Cl_2] (**1**) comprises a peculiar *cis*-platinum(II) organometallic compound, in which the platinum(II) ion is bound to the photoisomerizable carbon−carbon double bond of the carrier ligand. The effects of the metal-ion coordination on the photoisomerization of the carbon−carbon double bond of the ligand have been studied. In contrast, the carrier ligand A9pyp used for the synthesis of the *cis*-[Pt(A9pyp)(DMSO)Cl_2] compound (**2**) does not undergo such an isomerization process and remains in the E conformation, while coordinated to the platinum(II) ion through the nitrogen of the pyridine ring. In addition to the synthesis and characterization, solution studies of both compounds have also been performed in detail, including NMR and ESI-MS spectroscopy. Moreover, a high degree of cytotoxic activity of compound **1** was found, as compared to cisplatin and its corresponding platinum-free molecule, in a series of human tumor cell lines. Compound **2** was also found to be highly active against these cell lines but appeared less active compared to the platinum-free molecule.

Introduction

The clinical success of cisplatin has triggered considerable interest in the search of improved metal-based drugs for their use as chemotherapeutic agents. Several approaches have been taken over the past three decades, which include the synthesis of cisplatin analogues (e.g., carboplatin and oxaliplatin), variation of the metal ion, and preparation of platinum compounds that are not a structural analogue to cisplatin.[1,2] The goal behind the design of new platinum compounds is the synthesis of compounds that remain active against resistant cell lines, with a wider spectrum of antitumor activity, and with a lower toxicity than cisplatin.

Many strategies have been explored to understand the mechanism of action of cisplatin and second-generation platinum(II) compounds. Although these studies have been successful, helping to develop and improve this field widely, still several questions remain unanswered.[3−6] Since optical measurements can also be used in molecular biology studies,

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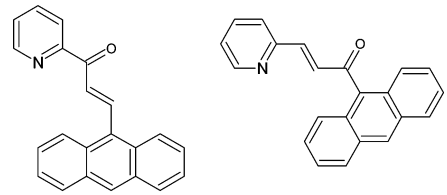

Figure 1. Chemical structure of carrier ligands *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] (abbreviated as A9opy) and *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone (abbreviated as A9pyp).

it has been shown that digital fluorescence microscopy is an adequate technique to obtain images of specific structures in living cells.[7,8] Unfortunately, most of the known platinum(II) anticancer compounds are not intrinsically fluorescent; so they need to be modified with a fluorescent tag in order to be visualized within the cell. This approach has been recently introduced,[9] and this technique has been found to be useful in studying the cellular responses of several fluorescent platinum(II) compounds.[7,9−13] Our current work is focused on the synthesis of new fluorescent platinum(II) compounds coordinated to organic emissive molecules. The strategy reported herein is to link the platinum and anthracene functionalities in such a way that the fluorescent property of the ligand can help to localize the platinum drug within the tumor cell, providing new insights into platinum(II) complex cellular processing. In addition, the coordination sphere of the platinum(II) ion can be modified by the substitution in the periphery of the complex. The heterocyclic ligands may be introduced to achieve additional interaction, such as intercalation into the DNA base pairs, and thus reinforce the interaction and decrease, or even circumvent, repair mechanisms present in the resistant cell lines.[14] The design and synthesis of two new fluorescent platinum(II) compounds presented herein are based on these two latter ideas. Two organic molecules of the family of 9-anthryl pyridyl enones,[15,16] *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] (abbreviated as A9opy) and *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone (abbreviated as A9pyp), were chosen as carrier ligands (Figure 1) for the synthesis of two platinum(II) compounds. First the organometallic compound *cis*-[Pt(A9opy)Cl_2] (**1**), containing the carrier ligand *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] coordinated through the nitrogen of the pyridine, is presented. The compound yields a five-membered chelating ring formed by coordination of the platinum(II) ion with the isomerizable carbon−carbon double bond, which stays in the E conformation, according to spectroscopic data and as subsequently confirmed by X-ray diffraction. The chemical structure and its high cytotoxicity in a panel of human tumor cell lines prompted the synthesis of a derivate platinum(II) compound. For this reason, the reverse enone of the A9opy ligand, the *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone ligand (abbreviated as A9pyp), was chosen (see Figure 1) to synthesize a neutral platinum(II) compound, *cis*-[Pt(A9pyp)(DMSO)Cl_2] (**2**). The structure of compound **2** shows as a common feature with compound **1**, the nitrogen of the pyridine ring; it being the anchor to the ligand A9pyp. The coordination sphere of compound **2** is completed with two chloride leaving groups and one DMSO molecule.

Because the carrier ligand A9opy is coordinated to the platinum(II) ion through the isomerizable carbon−carbon double bond in compound **1**, the study of such a compound in solution, and the influence of the light in its stability toward isomerization of the coordinated carbon−carbon double bond, was undertaken and performed by NMR spectroscopy. In addition, the stability of these new platinum compounds in solution was explored, since many differences in the action of cisplatin and second-generation compounds have been found, depending on the test conditions, such as light and solvent.[17−27] The present contribution describes the synthesis, crystal structure, and solution behavior of these two new fluorescent platinum(II) compounds in DMSO. In addition, the cytotoxic activity of both platinum(II) compounds and their corresponding carrier ligands was measured in human tumor cell lines, including human ovarian carcinoma cell line A2780 and its cisplatin-resistant counterpart A2780R.

Experimental Section

Materials and Reagents. The compound K_2PtCl_4 was obtained on a loan scheme from Johnson and Matthey (Reading, U.K). The compound *cis*-Pt(DMSO)$_2Cl_2$ was synthesized as previously reported.[28] All other chemicals and solvents were reagent-grade commercial materials and were used as received. The ligand *E*-2-

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[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] and its reverse enone *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone were prepared according to the published methods.[15,16]

Synthesis of the Compounds. Synthesis of the Carrier Ligands. Both ligands were synthesized with a method based on an earlier reported synthetic route.[15,16]

***E*-2-[1-(9-Anthryl)-3-oxo-3-prop-2-enylpyridine] (A9opy).** A solution of 1.5 M NaOH (20 mL, 30 mmol) was added to a solution containing 2-acetylpyridine (2.4 g, 20 mmol) and 9-anthralaldehyde (4.12 g, 20 mmol) in 40 mL of ethanol at room temperature. This reaction mixture was stirred at room temperature for 4 h. The yellow-orange solid was obtained in a yield of 89%, after recrystallization from methanol. ^{1}H NMR ($CDCl_3$): δ 8.9 (d, 1H), 8.68 (1H, d), 8.48 (1H, s), 8.34 (2H, m), 8.29 (1H, d; 1H, s), 8.03 (2H, m), 7.93 (2H, td), 7.5 (4H, m). Anal. found (calcd) for $C_{22}H_{15}NO$: C, 85.2 (85.4); H, 5.2 (4.9); N, 4.6 (4.5)%.

***E*-1-(9-Anthryl)-3-(2-pyridyl)-2-propenone (A9pyp).** A warm solution of 9-acetylanthracene (2 g, 9.08 mmol) in 40 mL of ethanol was added to a stirred solution of 2-pyridinecarboxaldehyde (0.9 mL, 9 mmol) and 1.5 M NaOH (8 mL, 12 mmol) in 50 mL of ethanol. The reaction mixture was stirred for 4 h at room temperature. The orange precipitate was collected and recrystallized in ethanol. An orange-yellow solid was obtained at a yield of 85%. ^{1}H NMR ($CDCl_3$): δ 8.6 (1H, d), 8.55 (1H, s), 8.06 (2H, m), 7.89 (2H, m), 7.69 (1H, d; 1H, td), 7.47 (4H, m), 7.34 (1H, d), 7.23 (1H, d), 7.21 (1H, d). Anal. found (calcd) for $C_{22}H_{15}NO$: C, 85.2 (85.4); H, 5.1 (4.9); N, 4.6 (4.5)%.

Synthesis of the Platinum(II) Compounds. *cis*-[Pt(A9opy)Cl$_2$] (1). To a stirred solution of *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] (38 mg, 0.12 mmol) in 2.5 mL of DMF was added a filtered aqueous solution of K_2PtCl_4 (50 mg, 0.12 mmol). The reaction mixture was stirred in the dark at 70 °C for about 1 h to afford an orange solution. The mixture was stirred overnight in the dark at 35 °C. The resulting orange precipitate was washed with water, ethanol, and diethyl ether. The orange solid was dried in vacuo. Yield: 40% (28 mg). ^{1}H NMR (DMF-d_7): δ 9.59 (2H, dd), 8.86 (1H, s), 8.63 (1H, t), 8.45 (1H, d), 8.37 (1H, d), 8.20 (3H, m) 7.75 (2H, m), 7.64 (1H, d), 7.54 (2H, m), 6.23 (1H, d). ^{195}Pt NMR (DMF-d_7): δ −2391. Anal. found (calcd) for $C_{22}H_{15}NOPtCl_2$: C, 45.9 (45.9); H, 2.7 (2.6); N, 2.7 (2.4)%.

***cis*-[Pt(A9pyp)(DMSO)Cl$_2$] (2).** A DMF solution of *cis*-$Pt(DMSO)_2Cl_2$ (50 mg, 0.12 mmol) was mixed with a solution of 38 mg of *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone (0.12 mmol) in 2 mL of DMF. The yellowish reaction mixture was stirred at room temperature for 72 h. The resulting orange solution was evaporated under reduced pressure. The orange oily product was washed with hexane and diethyl ether and dried in vacuo, to obtain an orange solid. Yield: 48% (37 mg). ^{1}H NMR ($(CD_3)_2CO$): δ 8.79 (1H, s), 8.75 (1H, d), 8.55 (1H, d), 8.33 (1H, d), 8.21 (3H, m), 7.95 (2H, m), 7.59 (6H, m). ^{195}Pt NMR ($(CD_3)_2CO$): δ −3010. Anal. found (calcd) for $C_{24}H_{21}NO_2SPtCl_2$: C, 44.4 (44.1); H, 3.7 (3.3); N, 2.1 (2.1)%.

Physical Measurements. C, H, and N analyses were performed with a Perkin-Elmer 2400 series II analyzer. Infrared spectra (4000−300 cm^{-1}) were recorded on a Perkin-Elmer Paragon 1000 FTIR spectrometer equipped with a Golden Gate ATR device, using the reflectance technique (resolution 4 cm^{-1}). Electrospray ionization mass spectra (ESI-MS) in DMSO or DMF were recorded on a Thermo Finnigan AQA apparatus. All NMR spectra were recorded with a 300 MHz Bruker DPX300 spectrometer with a 5-mm multinucleus probe. The temperature was kept constant at 20 °C by using a variable-temperature unit. ^{1}H and ^{195}Pt chemical shifts were referenced to TSP and $Na_2[PtCl_6]$ (δ = 0 ppm), respectively.

Table 1. Crystal Data and Structure Refinement Details for *cis*-Pt(A9opy)Cl_2 (**1**) and *cis*-Pt(A9pyp)(DMSO)Cl_2 (**2**)

	1	**2**
chemical formula	$PtCl_2C_{22}H_{15}NO$	$PtCl_2C_{24}H_{21}NO_2S$
fw [g/mol]	575.33	653.47
cryst syst	triclinic	monoclinic
space group	$P\bar{1}$	P 2_1/c
a [Å]	8.3704(10)	17.0002(10)
b [Å]	9.356(2)	15.2874(9)
c [Å]	12.525(3)	8.7615(6)
α [deg]	100.730(16)	90.00
β [deg]	108.535(16)	93.1930(10)
γ [deg]	91.917(18)	90.00
V [Å^3]	909.2(3)	2273.5(2)
Z	2	4
D_{calcd} [g/cm^3]	2.1016(7)	1.909
μ [mm^{-1}]	8.023	9.684
cryst size [mm^3]	0.10 × 0.10 × 0.05	0.05 × 0.02 × 0.02
Nref	4129	5577
Npar	250	282
R ($I/\sigma(I) > 2$)	0.0226	0.0441
wR2 (all data)	0.0450	0.0981
S	1.038	0.92
residual density [e/Å^3]	−1.13, 0.92	3.31

X-Ray Crystallographic Analysis and Data Collection. Crystal data, data collection parameters, and structure refinement details are given in Table 1. X-ray data for compound **1** were collected at 150 K for an oil-covered crystal mounted on a Lindemann capillary with a Nonius Kappa CCD diffractometer (rotating anode, Mo Kα radiation, graphite monochromator, λ = 0.71073 Å). The intensity data were corrected for absorption with PLATON/MULABS.[29,30] The programs COLLECT,[31] DIRDIF,[32] and SHELXL-97[33] were used for data reduction, structure solution, and structure refinement, respectively. Data collection on an orange small parallelepiped crystal of compound **2** was performed on station 16.2SMX of the Synchrotron Radiation Source at CCLRC Daresbury Laboratory, at 0.7848 Å, from a silicon 111 monochromator and using a Bruker APEX II CCD diffractometer. Absorption corrections were done with SADABS.[34] The structure was solved by direct methods, and the refinement and all further calculations were carried out using the SHELX-TL suite.[35] All non-hydrogen atoms were refined aniostropically. The methyl hydrogens were found in the difference map, while others were placed geometrically; all were refined using a riding model. The high residual largest peak and hole (3.314 and −2.206 e Å^3) remained in the difference map at the end of the refinement, which are located next to the Pt atom. Crystal data and selected bond distances and angles are given for both compounds in Tables 1−3.

Cell Lines, Culture Conditions, and Cytotoxicity Assays. The human ovarian carcinoma cell line A2780 and its cisplatin-resistant counterpart A2780R were grown as monolayers at 37 °C in a 7% CO_2 atmosphere and were maintained in a continuous logarithmic culture in Dulbecco's modified eagle's medium (Gibco BRL, Invitrogen Corporation, The Netherlands) supplemented with 10% heat-inactivated fetal calf serum (Hyclone, Perbio Science, The

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Table 2. Selected Bond Lengths [Å] and Angles [deg] for *cis*-Pt(A9opy)Cl_2 (**1**)

bond lengths		bond angles	
Pt(1)−Cl(1)	2.3125(10)	Cl(1)−Pt(1)−Cl(2)	89.86(4)
Pt(1)−Cl(2)	2.2934(10)	Cl(1)−Pt(1)−N(1)	93.15(7)
Pt(1)−N(1)	2.036(3)	Cl(1)−Pt(1)−C(8)	167.13(10)
Pt(1)−C(8)	2.103(4)	Cl(1)−Pt(1)−C(9)	153.64(10)
Pt(1)−C(9)	2.175(3)	Cl(2)−Pt(1)−N(1)	173.61(8)
C(8)−C(9)	1.414(5)	Cl(2)−Pt(1)−C(8)	93.21(11)
		Cl(2)−Pt(1)−C(9)	93.82(9)
		N(1)−Pt(1)−C(8)	82.64(13)
		N(1)−Pt(1)−C(9)	85.98(11)

Table 3. Selected Bond Lengths [Å] and Angles [deg] for *cis*-Pt(A9pyp)(DMSO)Cl_2 (**2**)

bond lengths		bond angles	
Pt(1)−Cl(1)	2.3068(18)	Cl(1)−Pt(1)−Cl(2)	91.43(7)
Pt(1)−Cl(2)	2.3181(17)	Cl(1)−Pt(1)−S(1)	90.10(6)
Pt(1)−S(1)	2.2110(16)	Cl(1)−Pt(1)−N(1)	176.94(13)
Pt(1)−N(1)	2.050(6)	Cl(2)−Pt(1)−S(1)	175.88(6)
		Cl(2)−Pt(1)−N(1)	86.87(14)
		S(1)−Pt(1)−N(1)	91.77(14)

Netherlands), penicillinG Sodium (100 units/mL: Dufecha, Biochemie BV, The Netherlands), streptomycin (100 μg/mL: Dufecha, Biochemie BV, The Netherlands), and Glutammax 100x (Gibco BRL, The Netherlands). The trypsinized cells (2000−2500 cells/well) were plated in 96-well flat-bottom microtiter plates. The plates were preincubated for 48 h at 37 °C in 7% CO_2 to allow the cells to adhere. On day 2, a 3-fold dilution sequence of 10 steps was made in full medium, starting with the stock solution of the compounds in the corresponding solvent, and from a saline solution of cisplatin used as a reference compound. Every dilution was used in triplicate by adding 50 μL to a column of the wells. On day 4, the incubation was terminated by adding 50 μL of an MTT solution (5 mg/mL in PBS buffer)[36] in each well, incubating at 37 °C for 3 h. The solution was carefully removed, and the amount of blue formazan crystals formed via mitochondrial reduction were dissolved in 100 μL of DMSO, and the absorbance was read at 550 nm using an automatic microplate reader (labsystems Multiskan MS). The IC_{50} values (i.e., the concentration of the compound that restricts cell growth to 50% of that of the control) were calculated from curves constructed by plotting cell survival (%) versus compound concentration (μM), and these are summarized in Table 3. All experiments were carried out in triplicate, and the IC_{50} values (drug concentration that inhibits cell growth for 50% with respect to control) were determined graphically, using Graphpad Prism analysis software. The resistance factor was calculated by dividing IC_{50} in the resistant variant by the IC_{50} in the respective sensitive cell line.

In addition, cytotoxicity of these new platinum(II) compounds, and their corresponding free carrier ligands, were studied by means of a colorimetric microculture assay (SRB assay)[37] in seven well-characterized human tumor cell lines containing examples of breast (MCF7 and EVSA-T), colon (WIDR), ovarian (IGROV), melanoma (M19 MEL), renal (A498), and non-small-cell lung cancer (H226), yielding IC_{50} values in the micromolar range (Table 4). Cell lines WIDR, M19, A498, IGROV, and H226 belong to the currently used anticancer screening panel of the National Cancer Institute (U.S.A.). The MCF7 cell line is estrogen (ER)+/progesterone (pgR)+, and the cell line EVSA-T is (ER)−/(pgR).

All cell lines were maintained in a continuous logarithmic culture in the RPMI 1640 medium with hepes and phenol red. The medium was supplemented with 10% FCS, 100 units/mL penicillin, and 100 μg/mL streptomycin. The cells were mildly trypsinized for passage and for use in the experiments.

On the first day, 150 μL of trypsinized tumor cells (1500−2000 cells/well) were plated in 96-well flat-bottom microtiter plates. The plates were preincubated for 48 h at 37 °C in 8.5% CO_2 to allow the cells to adhere. On the second day, a 3-fold dilution sequence of 10 steps was made in full medium, starting with the 1 mg compound/200 μL stock solution. Every dilution was used in quadruplicate by adding 50 μL to a column off the wells. On day 7, the incubation was terminated by washing the plate twice with PBS. Cytotoxicity was estimated by the microculture sulforhodamineB (SRB) test.[37] The absorbance was read at 540 nm using an automatic microplate reader (Labsystems Multiskan MS). Data were used for the construction of concentration response curves and determination of the IC_{50} value by use of the Deltasoft 3 software. The IC_{50} values were defined as the drug concentrations (μM) that reduced the absorbance by 50% compared to the drug-free control.

Results and Discussion

General Characterization. The reaction between K_2PtCl_4 and the ligand *E*-2-[1-(9-anthryl)-3-oxo-3-prop-2-enylpyridine] (abbreviated as A9opy) results in an organometallic *cis*-platinum(II) compound (**1**). This ligand allows the formation of a chelating ring with coordination through the nitrogen of the pyridine ring and the carbon−carbon double bond to the platinum(II) ion (the molecular structure, as determined from XRD, is given in Figure 2). The reaction between *cis*-Pt(DMSO)$_2$$Cl_2$ and ligand *E*-1-(9-anthryl)-3-(2-pyridyl)-2-propenone (abbreviated as A9pyp) gives a *cis*-platinum(II) compound (**2**) with the platinum(II) ion, where one DMSO molecule from the platinum precursor is replaced by the ligand, coordinated through the nitrogen of the pyridine ring.

Both compounds were characterized by several physical methods including infrared spectra. As expected for *cis*-[PtL_2Cl_2] compounds, two bands are observed at 327 and 312 cm^{-1} for the ν(Pt−Cl) in the cis configuration for compound **1** and at 339 and 317 cm^{-1} for compound **2**. A new band is also observed at 1608 cm^{-1}, which can be related with the coordinated C═C double bond in compound **1**.[38] In the infrared spectrum of compound **2**, a characteristic band is observed at 440 cm^{-1} corresponding to the ν(Pt−S) vibration, which is in agreement with other Pt(DMSO)LCl_2 compounds.[39] Fresh solutions of these two compounds were used to characterize and confirm the chemical structures using ^{1}H and ^{195}Pt NMR spectroscopy. The ^{1}H NMR of ligand A9opy was recorded in DMF-d_7 as a fresh solution in the dark, to prevent any *E*−*Z* photoisomerization,[16] and compared to a fresh solution of compound **1** in DMF-d_7. Significant changes in the ^{1}H NMR are observed for the proton peaks upon coordination of the ligand to the platinum(II) ion. The proton (H_6), in the ortho position to the coordinated nitrogen of the pyridine ring, is shifted compared

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Table 4. IC_{50} Values in Micromoles after 5 Days Incubation with Compounds **1** and **2** and the Corresponding Free Ligands (A9opy and A9pyp) Compared to Cisplatin on Human Tumor Cell Lines[a]

test compound	A498	EVSA-T	H226	IGROV	M19	MCF-7	WIDR
A9opy	36.7	16.7	12.3	33.4	14.3	10.8	23.5
cis-[Pt(A9opy)Cl_2] (**1**)	9.9	6	10.1	5.7	8.2	5.1	6.8
A9pyp	1.7	1	0.8	2.6	2.5	1.1	1.3
cis-[Pt(A9pyp)(DMSO)Cl_2)] (**2**)	3.1	1.6	1.5	2.9	4.5	1.6	2.0
cisplatin	7.5	1.4	10.9	0.6	1.9	2.3	3.2

[a] A498, renal cancer; EVSA-T, breast cancer; H226, non-small lung cancer; IGROV, ovarian cancer; M19, melanoma; MCF-7, breast cancer; WIDR, colon cancer.

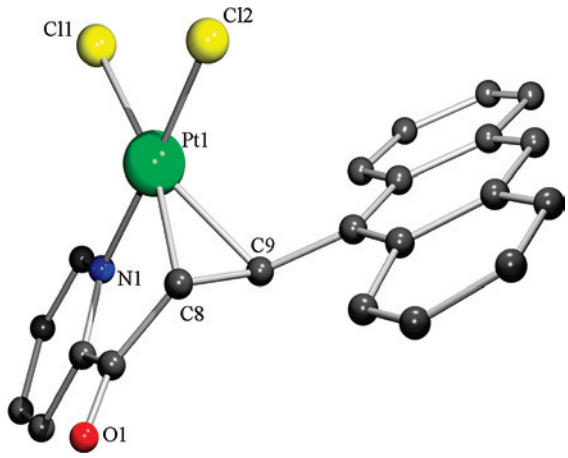

Figure 2. PLATON/POVRAY view of *cis*-[Pt(A9opy)Cl_2] (**1**). Hydrogen atoms have been omitted for clarity.

to the free ligand, from 8.78 in the free ligand to 9.58 ppm in compound **1**. The most important feature from compound **1** is the chemical shift change of the protons in the carbon−carbon double bond. The doublets corresponding to H_8 and H_9 appear shifted as compared to the free ligand, from 8.87 to 7.64 ppm for H_8 and from 8.27 to 6.23 ppm for H_9. It was found that the coupling constant between these protons decreases from 16 to 14 Hz with the formation of the organometallic bond.

The ^{1}H NMR spectrum of compound **2** in acetone-d_6 was recorded and compared with a solution of its free ligand A9pyp. The ligand A9pyp is stable in acetone-d_6 solution, and the *E* isomer was characterized with a coupling constant of 16 Hz between H_8 and H_9. Several shifts in the proton signals can be observed in ^{1}H NMR after coordination; for instance, the proton H_6, in the ortho position to the nitrogen of the pyridine ring, appears shifted from 8.62 to 8.74 ppm. In addition, the DMSO ligand influences the chemical shifts of the protons in the carbon−carbon double bond, where the H_9 and H_8 are shifted as compared to the free ligand, from 7.73 to 8.52 ppm and from 7.21 to around 7.68 ppm, respectively.

The ^{195}Pt NMR spectrum of compound **1** was measured in DMF-d_7, giving a single peak at −2391 ppm corresponding to a $NCCl_2$ environment.[39] The ^{195}Pt NMR of compound **2** was recorded in acetone-d_6, yielding a single peak at −3010 ppm, which corresponds to NCl_2S around the platinum;[40] however, some compounds of the type *cis*-[Pt(DMSO)LCl_2] are known in the literature within a large range of chemical shifts, such as −2867 to −3528 ppm.[39]

Description of Molecular and Crystal Structures. Single crystals of *cis*-Pt(A9opy)Cl_2 (**1**) were obtained by the slow diffusion of its DMF solution and diethyl ether. Single crystals of *cis*-Pt(A9pyp)(DMSO)Cl_2 (**2**) were obtained by the vapor diffusion of hexane into a saturated solution of the compound in CH_2Cl_2 and hexane. Details of the structure determination are given in the Experimental Section.

Structure of *cis*-[Pt(A9opy)Cl_2] (1). The reaction between K_2PtCl_4 and the ligand A9opy results in a *cis*-platinum(II) organometallic compound that crystallizes in the triclinic space group $P\bar{1}$. A PLATON/POVRAY view of compound **1** is shown in Figure 2 (vide supra). Selected bond lengths and angles are given in Table 2.

In this compound, the platinum(II) ion is coordinated in a cis fashion to two chloride ions and chelating to the A9opy ligand. A9opy Pt binding includes the organometallic bond interaction with the carbon−carbon double bond. The carbonyl oxygen of the A9opy does not interact with the platinum(II) ion, and it does not form any significant hydrogen bond in the lattice. This oxygen is located out of the platinum(II) coordination and pyridine ring plane by an angle of 38° and 12°, respectively; this conformation may result from minimized repulsions in the crystal lattice. In addition, the conjugation of the double bonds, present in the ligand A9opy, might contribute to a more constrained configuration in the platinum(II) compound.

The carbon−carbon double bond is almost perpendicular to the platinum(II) plane, with a distance of 2.019(4) Å for Pt to the center of the C═C bond and a carbon−carbon double-bond length of 1.414(5) Å. The organometallic bond contributes to a distortion in the square-planar configuration. The largest deviations from 90° were found in Cl(1)−Pt−N(1), Cl(2)−Pt(1)−C(8), and Cl(2)−Pt(1)−C(9), with values of 93.15(7), 93.21(11), and 93.82(9)°, respectively. In contrast to these data, the Cl(1)−Pt−Cl(2) angle is 89.86(4)°, which shows almost no deviation. These values are consistent with the values reported for other Pt(II)−C_2H_4 compounds, which have been taken as examples found in the literature for platinum(II) compounds coordinated to a C═C bond of an olefin.[39] To the best of our knowledge, no X-ray data are present in the CSD of a carbon−carbon double bond conjugated to a pyridine ring on its ortho position and chelated, at the same time, to a platinum(II) ion. Only a few metal compounds of 2-allylpyridine are known, including platinum(II) compounds, where such rearrangement has been suggested by IR spectroscopy only.[38,41] Also, a few examples of transition-metal compounds coordinated to 2-vinylpyridine

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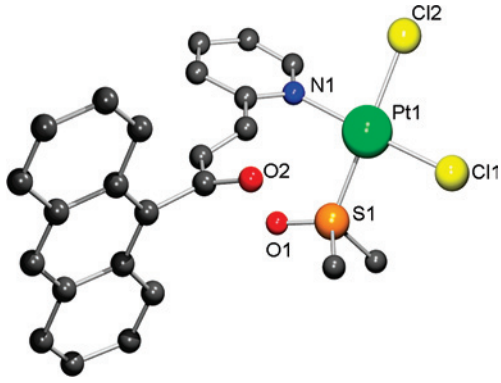

Figure 3. PLATON/POVRAY view of *cis*-[Pt(A9pyp)(DMSO)Cl_2] (**2**). Hydrogen atoms have been omitted for clarity.

through the nitrogen of the pyridine ring and the carbon−carbon double bond of the vinyl moiety have been reported.[42−44]

It is of interest to note that the trans effect is clearly observed from the Pt−Cl(1) and Pt−Cl(2) bond lengths. The Pt−Cl(1) distance is larger than the one trans to the N of the pyridine ring Cl(2), which agrees with a labilizing effect of the carbon−carbon double bond.

The angle between the plane of the pyridine ring and the coordination plane (containing the Pt atom and the two chloride ligands) is approximately 28°, which shows clearly that the pyridine ring is distorted from coplanarity. The Pt−N(1) bond length is 2.036(3) Å, which is found to be consistent with other platinum(II) compounds containing pyridine ligands.[45−47] The platinum(II) ion in compound **1** is not located on the axis of symmetry of the pyridine ligand, which is indicative of constrained coordination. Platinum(II) compounds containing pyridine ligands tend to achieve as much coplanarity as possible, if there is no hindrance of the ortho hydrogen atoms from other ligands or apparent stacking present.[47] In the case of compound **1**, the ortho position of the pyridine ring is linked to the carbon−carbon double bond that coordinates to the metal center. Moreover, the packing shows no peculiar intermolecular contacts or relevant $\pi-\pi$ stacking interactions. This suggests that the coplanarity cannot be achieved because of steric hindrance related with the organometallic bond, which forces the pyridine ring to be closer to the platinum(II) coordination plane. The anthracene ring conjugated to the coordinated carbon−carbon double bond forms an angle of 61° to the platinum(II) coordination plane.

Structure of *cis*-[Pt(A9pyp)(DMSO)Cl_2] (2). The molecular structure of *cis*-[Pt(A9pyp)(DMSO)Cl_2] is shown in Figure 3. This compound crystallizes in the monoclinic space group $P2_1/c$. Relevant bond lengths and angles are listed in Table 3.

The coordination polyhedron around the platinum(II) ion corresponds to a square-planar geometry with only small angular distortions. The platinum(II) ion is coordinated by the pyridine ring of the ligand A9pyp, two chloride ions, and a sulfur of a dimethyl sulfoxide molecule. According to the literature, the average value for the distance of the Pt−S bond in Pt−DMSO compounds is approximately 2.21 Å,[39,48,49] corresponding nicely to the value found in compound **2**. The sulfur atom is in an approximate tetrahedral environment. As observed for other Pt−DMSO compounds, the angle Pt−S−O (115.8°) is larger than the two Pt−S−C angles (111.6 and 110.1 °C from the methyl groups) and the two O−S−C angles, correspondingly (108.1 and 109°).[39,45,48−52] The S−O and the two S−C bond lengths are normal in this type of coordination, being 1.439, 1.779, and 1.774 Å, respectively. The angles O−S−C and C−S−C are close to the ones found for a free DMSO, 107 and 97.4°, respectively.[49] In addition, the S═O bond length is 1.439 Å, which is consistent with those in other dimethyl sulfoxide platinum(II) compounds.[39,45,48−52]

The bond lengths found in compound **2** for the Pt−Cl(1) and Pt−Cl(2) are uneventful, being 2.3068 and 2.3181 Å, respectively, and the one located trans to the pyridine ring is the shortest (Pt−Cl(1)). This difference is also in perfect agreement with the larger trans effect of the DMSO. Consequently, it may be suggested that the trans effects of the DMSO in compound **2** and that of the carbon−carbon double bond for compound **1** are almost of the same strength, that is, labilizing both the Pt−Cl bonds in the trans position.

Finally, the platinum completes its coordination sphere by a nitrogen of the pyridine ring giving a Pt−N(1) bond length of 2.051 Å, typical for Pt−N(py) bonds trans to chloride ligands.[45−47] The platinum(II) ion of compound **2** is nearly located on the axis of symmetry of the pyridine ligand. As compared with compound **1**, the coordination geometry of compound **2** is almost unstrained, and there is no additional coordination of the platinum(II) ion to the ligand. In addition, the dihedral angle between the platinum(II) coordination plane and the pyridine plane is 65°, which is much larger than the one found in compound **1**. This value may indicate that the pyridine ring forms such a dihedral angle with the platinum(II) coordination plane to minimize steric hindrance. The anthracene ring is planar and almost perpendicular to the plane of the pyridine ring. The angle to the platinum(II) coordination plane is 67°, similar to the one found for compound **1**. In contrast to compound **1**, intermolecular contacts in the form of weak $\pi-\pi$ stacking interactions are observed in the crystal packing of compound **2**, with a

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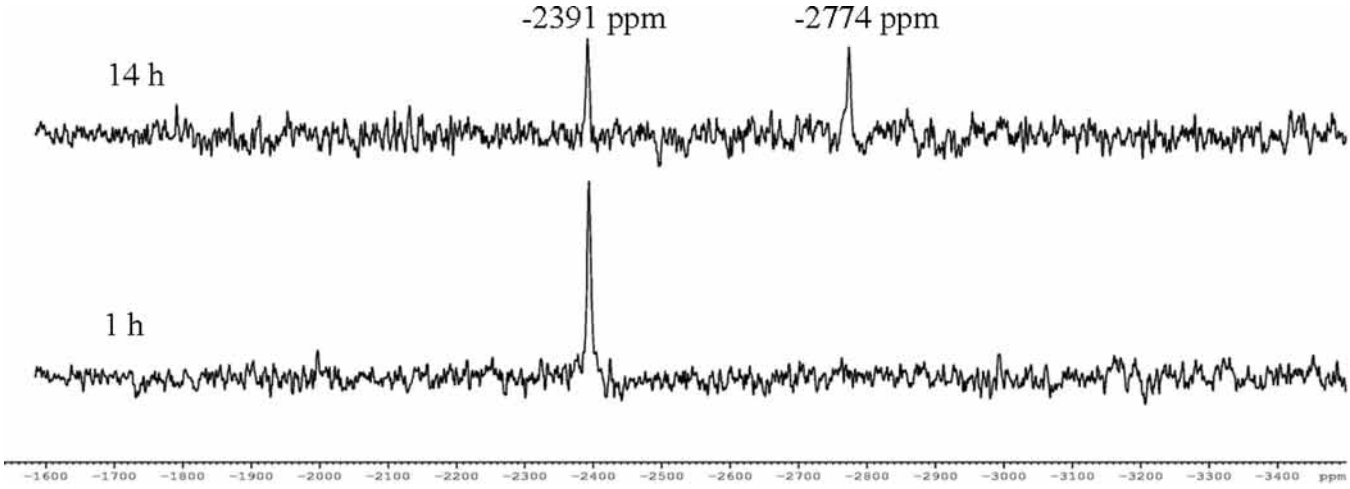

Figure 4. Time-dependent ^{195}Pt NMR spectra of compound **1** in DMF-d_7 in the presence of a 10-fold DMSO-d_6.

distance between two anthracene rings of approximately 3.66 Å. These two rings are not completely parallel, but slightly shifted.

Studies of Solvolysis in Solution by NMR. Dimethyl sulfoxide is commonly used in studies of the biochemical reactivity of platinum compounds; however, platinum(II) has a well-known affinity to the sulfur donor ligand;[53] therefore, spectral changes may be expected from the substitution of chloride ion by DMSO molecules in the platinum(II) compounds. Since the biological assays performed with compounds **1** and **2**, both of which are not markedly water-soluble, were studied in DMSO and DMF solutions, it was felt to be necessary to investigate the behavior of these active platinum(II) compounds in the presence of DMSO. So, the solvolysis in DMSO solution has been studied by NMR and ESI-MS spectroscopy.

In contrast to the absence of solvolysis of compound **1** in DMF-d_7, as clearly seen by its ^{1}H NMR and ^{195}Pt spectroscopy, compound **1** in DMSO-d_6 shows changing resonance patterns in time-dependent studies. The changing signal intensities versus time prove solvolysis reactions in DMSO, generating new platinum species. The signals corresponding to authentic compound **1** decrease during the first 48 h, while new signals appear that are assigned to the free ligand. The release of all ligands was reached after 72 h, as confirmed from ^{13}C NMR and by comparisom to the solution of free ligand in DMSO-d_6.

The ^{195}Pt NMR spectrum of compound **1** dissolved in absolute DMSO-d_6 shows two peaks at −2767 and −3442 ppm in time-dependent studies. The latter peak appears with increasing intensity, while the intensity of the peak at −2767 ppm decreases. The solvolysis reaction reaches completion after 72 h, just as observed for the ^{1}H NMR time-dependent spectra, where a single peak at −3442 ppm appears as the remaining platinum species in DMSO-d_6 solution. This peak can be assigned to the formation of [Pt(DMSO)$_2$Cl$_2$] in the DMSO-d_6 solution of compound **1**, in agreement with literature values.[54] The formation of [Pt(DMSO)$_2$Cl$_2$] in a DMSO solution of platinum(II) compounds has also been reported in an oxadiazoline platinum(II) compound, where the complete conversion into Pt(DMSO)$_2$Cl$_2$ takes place even after 2 h.[55] In addition, the same solvolysis has been observed for a platinum(II) compound coordinated by the 3,3′-disubstituted-2,2′-bipyridine ligand, where the DMSO molecules displaced the ligand without invoking the dissociation of the coordinated chloride ions.[54] However, in compound **1**, the predissociation of the chloride ligands can be followed in some detail when compound **1** is dissolved in DMF-d_7 and treated with only a 10-fold molar excess of DMSO-d_6. Two peaks are observed after 7 h of data collection, one at −2391 ppm corresponding to authentic compound **1** and one at −2774 ppm, which has been assigned to the *cis*-[Pt(A9opy)(DMSO)Cl]$^+$ species (see Figure 4).

Similar solvolysis studies were performed for compound **2** by use of NMR spectroscopy. Changes in the ^{1}H NMR of a DMSO-d_6 solution of compound **2** after 1 h can be observed. Similar behavior as compared to that of compound **1** was found for compound **2** in a DMSO-d_6 solution, where a release of the free ligand is observed. However, in the case of compound **2**, this release is faster than for compound **1**. The ^{195}Pt NMR of compound **2** in DMSO-d_6 over time gives a constant peak at −3442 ppm corresponding to [Pt(DMSO)$_2$Cl$_2$] formation. The ^{195}Pt NMR spectra over time of compound **2** in acetone-d_6 treated with a 10-fold molar excess of DMSO-d_6 shows a gradual decrease of compound **2** as seen from the peak at −3010 ppm, with the increase of a new one at −3441 ppm (see Figure S1, Supporting Information). In this case, there is no evidence of a predissociation of a chloride ligand by a DMSO molecule in the ^{195}Pt NMR time-dependent spectra. In addition, it is noteworthy to mention that cis−trans isomerization upon substituting chloride ligands by DMSO was not observed with ^{1}H NMR during solvolysis studies of compound **2**.[40]

The kinetic differences found in the solvolysis for compounds **1** and **2** can be explained if the trans effect of the

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DMSO ligand is considered. It is generally accepted that DMSO has a high trans influence, as mentioned above. In compound **2**, the DMSO ligand affects the Pt−Cl(2) bond, which is labilized and made more reactive for substitution by a DMSO molecule in solution. A strong cis-labilization effect of DMSO, however, could conceivably result in a loss of coordination to the nitrogen of the pyridine ligand, as was observed for both compounds. Subsequently, the ligand is released faster in a DMSO-d_6 solution as compared to compound **1**. Moreover, the hydrophobic substituents in the ortho position in the pyridine ring might change the solvation sphere around the platinum(II) ion, preventing the approach of the molecules of solvent. In both compounds, the chloride substitution is possible; although compound **1** is remarkably more sterically crowded around the platinum(II) ion, therefore, preventing solvolysis, or as observed in this case, making the chloride ion substitution less kinetically favorable, as compared to compound **2**.

ESI-MS studies. In addition to the NMR spectroscopic studies, the solution behavior in DMSO of compounds **1** and **2** was investigated by ESI-MS. A fresh solution of 1 mg of the compound in 1 mL of DMSO was prepared in the dark and measured after 10 min in solution.

The ESI-MS spectrum of both compounds shows ionic species containing the DMSO molecule. In particular, an ionic species at *m*/*z* 617 corresponding to the $[Pt(A9opy)(DMSO)Cl]^+$ ion is observed for both complex solutions (see Figure S2, Supporting Information). In addition, it is noteworthy to mention the *m*/*z* 675 peak corresponding to the $[Pt(A9pyp)(DMSO)_2(OH)]^+$ species, which has been observed in the spectrum of compound **1**. This particular *m*/*z* peak suggests the coordination of a second DMSO molecule to the platinum(II) ion, substituting the Pt−C═C bond.

A second molecule of DMSO coordinated to the platinum(II) ion in the spectrum of compound **2** can be assigned to *m*/*z* 695, yielding the $[Pt(A9pyp)(DMSO)_2Cl]^+$ species (see Figure S3a and b, Supporting Information). In addition, a peak at *m*/*z* 464 corresponding to the platinum species $[Pt(DMSO)_3Cl]^+$ can be observed in the same spectrum; however, this peak is not visible in the case of compound **1**.

Finally, the ESI-MS was recorded from the same solution after being kept for 72 h in the dark. The spectrum of compound **1** shows a peak at *m*/*z* 617, which corresponds to the remaining $[Pt(A9opy)(DMSO)Cl]^+$ molecular ion of compound **1** with one chloride ligand substituted by a DMSO molecule. No ligand-containing platinum species were found in the case of compound **2**.

Photoinduced Isomerization in Solution. The coordination of the platinum(II) ion to the carrier ligand A9opy through the isomerizable carbon−carbon double bond might affect its isomerization, when irradiated with light. The poor solubility of compound **1** in common solvents required performance of these studies in DMF. In order to establish a comparison between the free carrier ligand A9opy and its corresponding platinum(II) compound in photochemical reactions, both were studied under the same conditions in this solvent. Spectral changes due to irradiation at 366 nm under the UV lamp were followed with NMR spectroscopy.

Table 5. IC_{50} Values in μM after 2 Days Incubation with Compounds **1** and **2** and the Corresponding Free Ligands (A9opy and A9pyp), Compared to Cisplatin in Human Ovarian Carcinoma Cell Lines A2780 and A2780R

test compound	A2780	A2780R	RF[a]
A9opy	29	39	1.3
cis-$[Pt(A9opy)Cl_2]$ (**1**)	1.8	2.3	1.3
A9pyp	1.1	1.3	1.2
cis-$[Pt(A9pyp)(DMSO)Cl_2]$ (**2**)	9.1	12.4	1.4
cisplatin	2.4	14.7	6.1

[a] RF = IC_{50} (A2780R)/ IC_{50} (A2780).

The $E-Z$ isomerization of the platinum-free ligand molecule A9opy occurs after 2 h of irradiation with the UV lamp at 366 nm, in DMF-d_7 (1.0 mg, 0.0032 mmol), which can be observed in the ^{1}H NMR spectra. The same study was performed for compound **1** in a DMF-d_7 solution (1.6 mg, 0.0028 mmol), irradiated with a UV lamp at 366 nm for 4 h. The ^{1}H and ^{195}Pt NMR spectra show no changes as compared to the solution kept in the dark. This suggests that the carbon−carbon double bond coordinated to the platinum(II) ion is stable and prevents isomerization under these conditions.

The photoisomerization studies of compound **2** and its corresponding carrier ligand were undertaken under the same conditions as described for compound **1**; however, no $E-Z$ isomerization was observed as known for the free ligand.[16]

Biological Studies. The DMSO solution of compound **1** is stable only up to 10 h; therefore, handling and storage of the stock solutions should be avoided, and fresh solutions of such compounds are used to perform biological studies. When the stability studies were performed with a DMSO solution of compound **2**, the release of free ligand and formation of $Pt(DMSO)_2Cl_2$ were observed already after the first hour. For this reason, the biological studies of compound **2** were done using freshly prepared DMF stock solutions. It is noteworthy to mention that dilutions of such stocks were done in a complete medium, which contains different substances potentially susceptible to interaction with the platinum(II) compounds before or during the biological studies. Final concentrations of the employed organic solvent were $<1.5\%$ for DMSO and $<0.5\%$ for DMF, which were found not to be cytotoxic in every test.

The platinum(II) compounds **1** and **2**, and cisplatin for comparison, have been tested for their cytotoxic activity against a series of human tumor cell lines. The experimental data are listed in Tables 4 and 5. To determine the extent to which compounds **1** and **2** improve the cytotoxic behavior of their corresponding metal-free molecules A9opy and A9pyp, all were compared with regard to their cytotoxicity against the same human tumor cell lines and under the same conditions.

Interestingly, the metal free molecules A9opy and A9pyp have already a quite good activity in most of the cell lines tested; however, A9opy is less active than its reverse enone, A9pyp. For instance, A9opy is 22 times less active than A9pyp against the A498 renal cancer cell line, although the difference in toxicity becomes less pronounced in the M19

cell line. These metal free molecules are expected to have large intercalative properties to interact with the DNA, because of the presence of the planar conjugated anthracene ring. The differences in the cytotoxic activity of these two free ligands suggest that the cellular processing of such compounds is likely to be different, despite the fact that their interaction with DNA might be very similar on the basis of their chemical structure.

The platinum(II) compounds are compared to their corresponding metal free molecules in cytotoxic activity. As shown in Tables 4 and 5, platinum(II) compound **1** is found to have better activity in all of the cell lines tested than its carrier ligand A9opy. This is different in the case of compound **2**, where the metal free molecule A9pyp, in most of the cases, is 2 times more active than its platinum(II) compound. Even though compound **2** has a high activity against all tested cell lines, when compared to cisplatin, the data suggest that the coordination to the platinum(II) ion results in a lack of improvement in cytotoxic activity as compared to the free carrier ligand. The data obtained suggest that both metal-free molecules interact in a different mechanistic pathway as compared to their corresponding platinum(II) compounds, therefore, having a very different cytotoxic activity.

Moreover, the cytotoxicities of these platinum(II) compounds were tested in human ovarian carcinoma cell line A2780 and its cisplatin-resistant counterpart A2780R (data summarized in Table 5). The RF value between 0.5 and 2 indicates similar cytotoxicity of a compound in both sensitive and resistant cell lines, while compounds having RF > 2 are at least partially cross-resistant to cisplatin. As shown in Table 5, both platinum(II) compounds tested have an RF < 2, overcoming in this way the resistance of the cell line to platinum compounds. With the exception of the A9opy carrier ligand, all of the compounds tested are highly cytotoxic in the human ovarian carcinoma cell lines. Compound **1** has been found to be the most cytotoxic, as compared to its platinum-free molecule and cisplatin.

The differences found in the cytotoxic activity of these compounds may be related to uptake and transport to targets, which might vary for the platinum(II) compounds, in relation to their metal free molecules. More detailed studies, such as interaction with DNA, accumulation within human ovarian carcinoma cell lines, and cellular processing by fluorescence microscopy, of these platinum(II) compounds and the metal free molecules will be reported later elsewhere.

Conclusions

Two new cytotoxic fluorescent platinum(II) compounds have been synthesized and characterized. To the best of our knowledge, these compounds are the first examples of metal compounds coordinated to A9opy and A9pyp ligands. Time-dependent studies in solution reveal the solvolysis of both platinum(II) compounds when dissolved in absolute DMSO, with the release of the carrier ligand and the formation of $Pt(DMSO)_2Cl_2$ as the ultimate result. For compound **1**, the solvolysis is slower than for compound **2**, which can be explained by the different steric hindrances found in both compounds. In addition, the trans effect of the DMSO ligand in compound **2** labilizing the trans chloride appears to be stronger than the carbon−carbon double-bond trans effect in compound **1**. Moreover, the coordination of ligand A9opy to the platinum(II) ion was found to stabilize the carbon−carbon double bond, which inhibits the *E*−*Z* photoisomerization of this olefinic bond.

Cytotoxicity studies of these compounds and their free carrier ligands show a high degree of activity, as compared to cisplatin. Compound **1** is more active against most of the tumor cell lines tested, as compared to its carrier ligand; interestingly, compound **2** has similar cytotoxic activity to its corresponding free ligand, and both have lower IC_{50} values than that of cisplatin. More detailed biological studies of these and other related compounds, such as uptake, binding to intracellular DNA, and fluorescence microscopy, are underway and will be published elsewhere.

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Supporting Information Available: CIF files for compounds **1** and **2** giving full X-ray crystallographic data. ^{195}Pt NMR solvolysis studies compound **2** and ESI-MS spectra for both compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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