ABSTRACT: The development of photoactivatable metal complexes with potential anticancer properties is a topical area of current investigation. Photoactivated chemotherapy using coordination compounds is typically based on photochemical processes occurring at the metal center. In the present study, an innovative approach is applied that takes advantage of the remarkable photochemical properties of diarylethenes. Following a proof-of-concept study with two complexes, namely, C1 and C2, a series of additional platinum(II) complexes from dithienylcyclopentene-based ligands was designed and prepared. Like C1 and C2, these new coordination compounds exhibit two thermally stable, interconvertible photoisomers that display distinct properties. The photochemical behavior of ligands L3−L7 has been analyzed by 1H NMR and UV−vis spectroscopies. Subsequently, the corresponding platinum(II) complexes C3−C7 were synthesized and fully characterized, including by single-crystal X-ray diffraction for some of them. Next, the interaction of each photoisomer (i.e., containing the open or closed ligand) of the metal complexes with DNA was examined thoroughly using various techniques, revealing their distinct DNA-binding modes and affinities, as observed for the earlier compounds C1 and C2. The antiproliferative activity of the two forms of the complexes was then assessed with five cancer cell lines and compared with that of C1 and C2, which supported the use of such diarylethene-based systems for the generation of a new class of potential photochemotherapeutic metallodrugs.

INTRODUCTION
An elegant drug-design approach aimed at enhancing tumor selectivity while reducing systemic toxicity is the use of light activation. Recent advances in laser and fiber-optic technologies have driven the development of various medical applications based on light, including the design of metal-containing prodrugs that can be activated through irradiation. Photoactivated chemotherapy (PACT) is a prominent field of investigation, which provides both temporal and spatial control over drug activity, and offers tremendous potential for the treatment of different types of cancer. Photoactivatable prodrugs based on coordination compounds typically involve activation at the metal center, with concomitant generation of pharmacologically active species. Metal-mediated PACT can be achieved via distinct mechanisms of action, namely, photodissociation and photosensitization, which are often associated with the redox properties of the metal center, and photothermal reaction. A number of research groups worldwide...
are developing elegant systems that are photoactivatable through the action of light on the metal.9−16

The activation of a metallodrug through the photomodification of a coordinated ligand has not been investigated extensively. Different types of organic molecular photoswitches may be used for this purpose, like azobenzenes, spiropyans, or diarylethenes.17 Azobenzenes and spiropyans are thermally unstable and gradually revert back to their initial state in the absence of light.18 In contrast, diarylethenes are more suitable for such biological application, as they exhibit negligible thermal relaxation; as a matter of fact, these unique properties have converted them as one of the most interesting classes of photoresponsive molecular devices, as reflected by their numerous applications.19

In the present study, the potential use of diarylethene moieties to generate potential light-activatable metallodrugs has been investigated further.20 Indeed, it has recently been shown that the two thermally stable states of two molecular switches (complexes C1 and C2 in Figure 1) exhibit distinct biological activities, one of the two photoisomers being cytotoxic, whereas the other one is not.20 Hence, on the basis of these results, a series of new simple diarylethene-based ligands were designed and prepared, and the corresponding platinum(II) complexes were synthesized.21 Subsequently, the DNA-interacting properties of their open and closed forms were examined using various techniques, and their cytotoxic behavior against various cancer cell lines was then evaluated and compared with those of C1 and C2. Promising results were achieved, further indicating that this innovative mechanism of photochemical control may be applied to produce a new class of photoactivatable metallodrugs. It can indeed be stressed that the photoactivation of the cytotoxic properties of the platinum(II) compounds reported herein is based on the photomodification of the coordinated ligand and not on the metal center (as commonly described in the literature).

■ EXPERIMENTAL DETAILS

Materials and Methods. All reagents and high-performance liquid chromatography (HPLC)-grade solvents for the synthesis of the ligands and complexes were purchased from commercial sources and used as received. Ethidium bromide, sodium cacodylate, tris acetate-ethyl-
enediaminetetraacetic acid (TAE), and calf thymus DNA (ct-DNA) were purchased from Sigma-Aldrich. Plasmid pBR322 DNA was purchased from Roche. All reagents used for the in vitro DNA-interaction studies were obtained from Sigma-Aldrich and Invitrogen. Anhydrous solvents were distilled under an inert atmosphere using a PureSolv solvent purification system from Innovative Technologies.

When required, the reactions were performed under an atmosphere of dinitrogen using standard procedures. Column chromatography purifications were performed in air, using ultrapure silica gel (60–200 μm, 60 Å) from Acrhos Organics, and monitored by analytical thin-layer chromatography (TLC) using precoated aluminum plates.

Nuclear magnetic resonance (NMR) spectra were recorded at 298 K on a Varian Mercury 400 MHz spectrometer. Chemical shifts (δ) are reported in parts per million, and coupling constants (J) are given in hertz. Proton chemical shifts are referenced to the corresponding nondeuterated solvent peak (CHCl3: 7.26 ppm; dimethyl sulfoxide (DMSO): 2.50 ppm); fluorine chemical shifts are referenced to trifluoroacetic acid (−76.55 ppm). Signal multiplicities are defined as s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), br (broad signal), and m (multiplet).

Electrospray ionization (ESI) mass spectrometry was performed using an LC/MSD-TOF spectrometer from Agilent Technologies equipped with an ESI source, at the Centres Científics i Tecnològics de la Universitat de Barcelona. Samples were eluted with a H2O/CH3CN 1:1 mixture and measured in the positive mode. C, H, N, and S elemental analyses were performed at the Centres Científics i Tecnològics de la Universitat de Barcelona, using a Thermo EA 1108 CHNS/O analyzer from Carlo Erba Instruments.

Spectroscopic measurements in buffered aqueous media were performed in a Cary 300 spectrophotometer (Varian) in 1 cm path length quartz cuvettes, using a deionized water reference.

The stock solutions for the DNA-interaction studies were prepared as follows: the commercial plasmid pBR322 stock solution (250 μg mL⁻¹, ca. 385 μM) was used as received. A 150 μM stock solution of ct-DNA was prepared by dissolving the highly polymerized sodium salt of the biomolecule in cacodylate buffer, and the exact concentration was determined by absorbance at 260 nm (ε₂₆₀ = 13 200 M⁻¹ cm⁻¹).

**Synthesis.** 1,5-Bis(5-chloro-2-methyl-3-thienyl)pentane-1,5-dione (H)² Aluminum(III) trichloride (29.0 g, 171 mmol) was slowly added to a mixture of 2-chloro-5-methylthiophene (25.0 g, 189 mmol) and glutaryl dichloride (12.0 mL, 94 mmol) dissolved in 200 mL of ice-cold nitrogen. After the addition of AlCl₃, the resulting dark red solution was stirred for 3 h at room temperature, and 150 mL of ice-cold water was subsequently added in small portions. The reaction mixture was then placed in an ice bath and vigorously stirred for 1 h, until an abundant precipitate formed. This precipitate was then poured over a glass filter, washed with cold n-pentane, and finally dried under reduced pressure to yield the crude final product 1 as a pale brown solid (27.6 g, 81%).

²H NMR (400 MHz, CDCl₃): δ = 2.06 (m, 2H, J = 6.8 Hz, CH₂), 2.66 (s, 6H, Me), 2.86 (t, 4H, J = 6.8 Hz, COCH₂), 7.18 (s, 2H, thiophene). Mass spectrometry (MS) [ESI]: m/z = 361 [M⁺] (expected: 360.98). Anal. Calc. for C₁₅H₁₄Cl₂O₂S₂ (%): C 49.86, H 3.91, S 17.75; found: C 50.19, H 3.94, S 16.56.

1,2-Bis(5-chloro-2-methyl-3-thienyl)cyclopentene (2). Titanium(IV) tetrachloride (4.4 mL, 40 mmol) was carefully added under a nitrogen atmosphere using a syringe to an ice-cooled suspension of zinc dust (5.2 g, 80 mmol) in 150 mL of anhydrous tetrahydrofuran (THF). The resulting gray-blue reaction mixture was allowed to cool down to 50 °C and stirred for 2 h, after which pyridine (3.2 mL, 40 mmol) was added dropwise, producing a brown solution. After 10 min, 1 (7.2 g, 20 mmol) was added, and the resulting mixture was stirred in the dark overnight. Next, the solution was cooled in an ice bath and quenched with a 20% aqueous K₂CO₃ solution (25 mL), yielding an abundant black precipitate. After the precipitate was vigorously stirred with diethyl ether (40 mL), the remaining solid was isolated by filtration over a glass filter and washed with additional diethyl ether (2 × 25 mL). The combined organic phase was placed in an ice bath for 30 min and filtered again to eliminate the resulting undesired white precipitate. The solution was washed with acetic acid (2 × 20 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give an orange oil. This oil was purified by column chromatography on silica gel with cyclohexane as the eluent, yielding an oil that slowly solidified to colorless product 2 (4.75 g, 72%).

³H NMR (400 MHz, CDCl₃): δ = 1.88 (s, 6H, Me), 2.02 (m, 2H, J = 7.6 Hz, cyclopentene), 2.71 (t, 4H, J = 7.6 Hz, cyclopentene), 6.57 (s, 2H, thiophene). MS (ESI): m/z = 329 [M⁺] (expected: 328.99). Anal. Calc. for C₁₅H₁₄Cl₂S₂ (%): C 54.71, H 4.29, S 19.47; found: C 54.89, H 3.94, S 16.56.

4-Bromo-5-methylthiophene-2-boronic acid (3). n-Butyllithium (10 mL of a 1 M solution in hexane, 16.0 mmol) was added dropwise to a solution of 3,5-dibromo-2-methylthiophene (2.0 mL, 15.6 mmol) in anhydrous diethyl ether (25 mL) at −78 °C, under a dinitrogen atmosphere, resulting in a color change from colorless to bright orange. The reaction mixture was stirred for 30 min, and then tributyl borate (8.0 mL, 29.6 mmol) was added. After the resulting solution was stirred at room temperature overnight, 5% HCl aqueous solution (20 mL) was added to redissolve the pale yellow precipitate formed. The organic phase was then separated and extracted with an aqueous NaOH solution (1 M, 2 × 20 mL); the combined aqueous phase was acidified (HCl, 37%) under vigorous stirring until the appearance of an off-white precipitate. The product 3 was then filtered, washed with acified water, and dried under reduced pressure (3.3 g, 96%).

²H NMR (400 MHz, DMSO-δ₆): δ = 2.36 (s, 3H, Me), 7.49 (s, 1H, thiophene), 8.32 (s, 2H, B(OH)₂). MS (ESI): m/z = 220.0 [M⁺] (expected: 220.94).

4-Bromo-5-methyl-2-(4-pyridyl)thiophene (4). 4-Bromopyridine hydrochloride (1.3 g, 6.7 mmol) and 3 (1.0 g, 4.5 mmol) were subsequently added under a nitrogen atmosphere to a solution of [Pd(PPh₃)₄] (260 mg, 5% mol) in anhydrous THF (20 mL) and 20% aqueous K₂CO₃ (20 mL). The resulting two-phase system was heated to 50 °C, stirred in the dark overnight, and then cooled to room temperature before dichloromethane (25 mL) and water (15 mL) were added. The organic layer was separated, washed with brine (25 mL), dried over Na₂SO₄, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel with dichloromethane as the eluent, yielding the final product 4 as an off-white solid (0.94 g, 82%).

²H NMR (400 MHz, CDCl₃): δ = 2.45 (s, 3H, Me), 7.32 (s, 1H, thiophene), 7.38 (m, 2H, J = 6.0 Hz, J₂ = 2.4 Hz, pyridine), 8.59 (m, 2H, J₁ = 6.0 Hz, J₂ = 2.4 Hz, pyridine). MS (ESI): m/z = 254.0 [M⁺] (expected: 253.96).

4-Bromo-5-methyl-2-(3-quinolyl)thiophene (5). Compound 5 was prepared following the procedure described for compound 4 but using 3-bromoquinoline (0.9 mL, 6 mmol) instead of 4-bromopyridine. The crude product was purified by column chromatography on silica gel with dichloromethane as the eluent, yielding the final product 5 as an off-white solid (0.81 g, 59%).

²H NMR (400 MHz, CDCl₃): δ = 2.48 (s, 3H, Me), 7.31 (s, 1H, thiophene), 7.58 (m, 2H, quinoline), 7.71 (m, 2H, quinoline), 7.84 (d, 2H, J = 8.8 Hz, quinoline), 8.12 (d, 2H, J = 8.8 Hz, quinoline), 8.21 (d, 2H, J = 2.4 Hz, quinoline), 9.11 (d, 2H, J = 2.4 Hz, quinoline). MS (ESI): m/z = 306.0 [M + H⁺] (expected: 305.97).

1-(2-Methyl-5-chloro-3-thienyl)-2-(2-methyl-5-phenyl-3-thienyl)cyclopentene (6). 2 (10 g, 30 mmol) was dissolved in anhydrous THF (10 mL) under a nitrogen atmosphere and treated with n-butyllithium (2.0 mL of a 1.6 M solution in hexane, 3.2 mmol) at room temperature. After it was stirred in the dark for ~45 min, tributyl borate (1.2 mL, 4.5 mmol) was added, and the resulting bright orange solution was then
Hence, in a separate flask, [Pd(PPh3)4] (180 mg, 5% mol) and bromobenzene (0.4 mL, 3.75 mmol) were dissolved in a solvent mixture containing anhydrous THF (20 mL) and 20% aqueous K2CO3 solution (20 mL) under a dinitrogen atmosphere. This two-phase system was stirred at 50 °C for 15 min, and the previous freshly prepared boronic derivative solution was added dropwise with a syringe. The resulting reaction mixture was stirred in the dark overnight, after which it was cooled to room temperature; dichloromethane (25 mL) and water (15 mL) were subsequently added. The organic layer was separated, washed with brine (25 mL), dried over Na2SO4, and concentrated under reduced pressure. The crude product was purified by a column chromatography on silica gel with a 19:1 mixture of cyclohexane and ethyl acetate as the eluent, yielding 6 as a slightly colored oil (0.74 g, 66%).

1H NMR (400 MHz, CDCl3): δ = 1.88 (s, 3H, Me), 2.00 (s, 3H, Me), 2.05 (m, 2H, 7.6 Hz, cyclopentene), 2.75 (m, 2H, Japp = 7.6 Hz, cyclopentene), 2.81 (m, 2H, Japp = 7.6 Hz, cyclopentene), 6.62 (s, 1H, thiophene), 6.99 (s, 1H, thiophene), 7.23 (m, 1H, Japp = 7.2 Hz, phenyl), 7.34 (m, 2H, Japp = 7.2 Hz, phenyl), 7.49 (m, 2H, Japp = 7.2 Hz, phenyl). MS (ESI): m/z = 371.1 [M+H]+ (expected: 371.07).

1,2-Bis(2-methyl-5-(4-pyridyl)-3-thienyl)cyclopentene (L1). Ligand L1, whose synthetic pathway was described earlier, was prepared as described below. This procedure was applied for all ligands including a central cyclopentene ring (ligands L3, L5, L6, and L7).

2.05 (m, 2H, 7.6 Hz, cyclopentene), 2.75 (m, 2H, Japp = 7.6 Hz, cyclopentene), 2.81 (m, 2H, Japp = 7.6 Hz, cyclopentene), 6.62 (s, 1H, thiophene), 6.99 (s, 1H, thiophene), 7.23 (m, 1H, Japp = 7.2 Hz, phenyl), 7.34 (m, 2H, Japp = 7.2 Hz, phenyl), 7.49 (m, 2H, Japp = 7.2 Hz, phenyl). MS (ESI): m/z = 371.1 [M+H]+ (expected: 371.07).

1,2-Bis(2-methyl-5-(3-quinolyl)-3-thienyl)cyclopentene (L2). Ligand L2, whose synthetic pathway was described earlier, was prepared as described below. This procedure was applied for the other ligand containing a central perfluorocyclopentene ring, namely, L4.

1H NMR (400 MHz, CDCl3): δ = 2.03 (s, 6H, Me), 2.11 (m, 2H, J = 7.6 Hz, cyclopentene), 2.85 (s, 4H, J = 7.6 Hz, cyclopentene), 7.22 (s, 2H, thiophene), 7.35 (m, 4H, Japp = 6.4 Hz, pyridine), 8.53 (m, 4H, Japp = 6.4 Hz, pyridine). 13C NMR (100 MHz, CDCl3): δ = 14.8, 23.1, 38.6, 119.4, 126.4, 134.9, 136.8, 137.2, 137.4, 141.4, 150.4. MS (ESI): m/z = 623.1 [M+H]+ (expected: 623.1). Anal. Calc for C33H26N2S2 (%): C 63.59, H 3.70, N 4.47, S 9.76.

1,2-Bis(2-methyl-5-(5-methyl-3-pyridyl)-3-thienyl)cyclopentene (L5). L5 was prepared following the procedure described for L1 but using 3-bromo-5-methylpyridine (0.85 mL, 7.5 mmol) as the bromide derivative in the Suzuki cross-coupling reaction. The crude product was then purified by column chromatography on silica gel with a 4:1 mixture of dichloromethane and ethyl acetate as the eluent, producing L5 as a bright white solid (0.87 g, 56%).

1H NMR (400 MHz, CDCl3): δ = 2.03 (s, 6H, Me), 2.11 (m, 2H, J = 7.6 Hz, cyclopentene), 2.85 (s, 4H, J = 7.6 Hz, cyclopentene), 7.22 (s, 2H, thiophene), 7.35 (m, 4H, Japp = 6.4 Hz, pyridine), 8.53 (m, 4H, Japp = 6.4 Hz, pyridine). 13C NMR (100 MHz, CDCl3): δ = 14.8, 23.1, 38.6, 119.4, 126.4, 134.9, 136.8, 137.2, 137.4, 141.4, 150.4. MS (ESI): m/z = 623.1 [M+H]+ (expected: 623.1). Anal. Calc for C33H26N2S2 (%): C 63.59, H 3.70, N 4.47, S 9.76.

1,2-Bis(2-methyl-5-(1-methyl-1H-imidazol-2-yl)-3-thienyl)cyclopentene (L6). L6 was prepared following the procedure described for L1 but using 2-bromo-1-methyl-1H-imidazole (1.0 g, 6.2 mmol) as the bromide derivative in the Suzuki cross-coupling reaction. The crude product was then purified by column chromatography on silica gel with a 4:1 mixture of dichloromethane and ethyl acetate as the eluent, producing L6 as a bright white solid (0.87 g, 52%).
the bromide derivative in the Suzuki cross-coupling reaction. The crude product was then purified by column chromatography on silica gel with ethyl acetate (containing 1% NH₃) as the eluent, to yield L6 as a slightly pink solid (0.47 g, 37%).

1H NMR (400 MHz, CDCl₃): δ = 2.08 (m, 2H, J = 7.6 Hz, cyclopentene), 2.10 (s, 6H, Me), 2.83 (t, 4H, J = 6.7 Hz, cyclopentene), 3.65 (s, 6H, N-Me), 6.84 (d, 2H, J = 1.2 Hz, imidazole), 6.96 (s, 2H, thiophene), 7.01 (d, 2H, J = 1.2 Hz, imidazole).

13C NMR (100 MHz, CDCl₃): δ = 14.4, 23.2, 34.6, 38.2, 122.4, 126.9, 128.5, 129.8, 133.0, 136.2, 136.2, 142.4. MS (ESI): m/z = 422.1 [M⁺] (expected: 422.16).

Analy. Calc. for C₁₃H₁₄Cl₂N₂O₂Pt₂ (326.98): C 37.51, H 3.08, N 2.48, S 11.36; found: C 37.51, H 3.08, N 2.48, S 11.36.

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(400 MHz, CDCl₃): δ = 2.08 (m, 2H, J = 7.6 Hz, cyclopentene), 2.10 (s, 6H, Me), 2.83 (t, 4H, J = 6.7 Hz, cyclopentene), 3.65 (s, 6H, N-Me), 6.84 (d, 2H, J = 1.2 Hz, imidazole), 6.96 (s, 2H, thiophene), 7.01 (d, 2H, J = 1.2 Hz, imidazole).

13C NMR (100 MHz, CDCl₃): δ = 14.4, 23.2, 34.6, 38.2, 122.4, 126.9, 128.5, 129.8, 133.0, 136.2, 136.2, 142.4. MS (ESI): m/z = 422.1 [M⁺] (expected: 422.16).

Analy. Calc. for C₁₃H₁₄Cl₂N₂O₂Pt₂ (326.98): C 37.51, H 3.08, N 2.48, S 11.36; found: C 37.51, H 3.08, N 2.48, S 11.36.
phenyl), 7.39 (m, 2H), J₁ = 5.6 Hz, J₂ = 1.6 Hz, pyridine), 7.48 (m, 2H, J₁ = 7.6 Hz, J₂ = 1.6 Hz, pyridine). 1°C NMR (100 MHz, CDCl₃): δ = 145.5, 151.1, 23.1, 38.4, 38.8, 44.3, 120.5, 122.8, 125.4, 127.3, 129.0, 129.3, 133.5, 134.0, 134.3, 136.3, 136.5, 138.4, 140.1, 144.8, 151.7. MS (ESI): m/z = 66.0 [M + Na]+ (expected: 66.02). Anal. Calcd for C₂₈H₂₉Cl₂NOPtS₃ (%): C 44.38, H 3.94, N 2.04, S 12.46.

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X-ray Structure Determination. Data for the open and closed forms of complex C3 and the open form of complex C4 were collected on a Bruker PHOTON100 CMOS diffractometer at beamline 11.3.1 of the Advanced Light Source (synchrotron radiation, λ = 0.7749 Å), respectively, on a dark-purple/orange (depending on crystal orientation) plate at 200 K and a colorless plate at 100 K. Data reduction was done with SAINT. 24 The structures were solved by intrinsic phasing using SHELXT25 and refined by full-matrix least-squares on F² with SHELXL-2014.26 Absorption corrections for the structure of C4 were done with SADAB. 24 The crystal of C3 was found to contain both the open and closed forms. It is unclear if this is a consequence of cocrystallization or solid-state transformation upon exposure to light. The structure was refined as a 2-component twin using cells and twin law determined with CELL_NOW. 27 Absorption corrections were done with TWINABS. 27 The heavy disorder of the central part of the ligand associated with the presence of both photoisomers in the same crystal, initially defined from electron-density peaks, was refined with both 1.2- and 1.3- σ distances and displacement parameters restraints. Crystallographic and refinement parameters are summarized in Tables S1 and S3, for complexes C3 and C4, respectively. Additional crystallographic information is available in the Supporting Information.

Agarose Gel Electrophoresis. Ccadylate-buffered solutions (20 μL) containing 15 μM (in base pair) PBR322 plasmid DNA, 0.5–2.0 equiv of the complexes (7.5–30 μM), and 5% DMSO were incubated in the dark for 24 h at 37 °C. Analogue sample solutions of free plasmid DNA and DNA bound to cisplatin (0.5 equiv, without DMSO) were also prepared and used as controls. After the incubation, all samples were treated with SYBR Safe DNA gel stain and subsequently recorded as a 2-component twin using SHELXL-2014.26 Absorption corrections for the structure of C4 were done with SADAB. 24 The crystal of C3 was found to contain both the open and closed forms. It is unclear if this is a consequence of cocrystallization or solid-state transformation upon exposure to light. The structure was refined as a 2-component twin using cells and twin law determined with CELL_NOW. 27 Absorption corrections were done with TWINABS. 27 The heavy disorder of the central part of the ligand associated with the presence of both photoisomers in the same crystal, initially defined from electron-density peaks, was refined with both 1.2- and 1.3- σ distances and displacement parameters restraints. Crystallographic and refinement parameters are summarized in Tables S1 and S3, for complexes C3 and C4, respectively. Additional crystallographic information is available in the Supporting Information.

Ethyridium Bromide Displacement Assays. Samples containing 15 μM (in base pair) ct-DNA and 75 μM ethidium bromide in cadylate buffer were incubated for 1 h at 37 °C, after which they were treated with increasing amounts of the complex stock solutions (see Materials and Methods). The ratio of DNA/ethidium bromide (EB) of 1:5 (15 μM cp DNA, 75 μM EB) was determined experimentally, by fluorescence spectroscopy. It corresponds to the saturation of the emission signal (i.e., when a plateau is reached), which indicates that EB occupies all possible intercalation sites. The resulting samples, containing 1–25 μM of the studied complexes and up to 5% DMSO in a final volume of 3 mL, were then incubated in the dark for 24 h at 37 °C. Following the incubation, the fluorescence emission spectra of all samples were recorded at room temperature in the range of 350–800 nm, using a Horiba Jovin Yvon SPEX Fluorolog 3–22 spectrophuometer and applying an excitation wavelength of 335 nm. Ccadylate-buffered solutions of ct-DNA/EB (i.e., without complex) were used as control references.

Circular Dichroism Spectroscopy. Samples containing 50 μM ct-DNA (in base pair), 5–25 μM of the studied complexes, and up to 5% DMSO in a final volume of 3 mL of cadylate-buffered solution were incubated for 24 h at 37 °C. Following the incubation, the circular dichroism spectra of all samples were recorded at room temperature using a Jasco J-815 CD spectropolarimeter. A wavelength range of 235–351 nm was used to minimize DMSO interference, using a bandwidth of 2 nm, a data pitch of 0.5 nm, scanning speed of 50 nm min⁻¹, response time of 1 s, and 4 accumulations. Ccadylate-buffered solutions of ct-DNA were used as control references.

Cell Lines and Culture. Human lung adenocarcinoma (A549), melanoma (A375), breast adenocarcinoma (MCF7), colorectal adenocarcinoma (SW620), and ovarian adenocarcinoma (SKOV3) cell lines used in this study were purchased from the American Type Culture Collection (ATCC). All cell lines were tested and authenticated by ATCC using short tandem repeat analysis and were cultured (passage number 10–25) following ATCC recommended media. A549, A375, SW620, and SKOV3 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 unit/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine. MCF7 cells were cultured in DMEM-F12 (Ham) media (1:1) supplemented with 5% horse serum (v/v), 100 μM sodium pyruvate, 10 μg/mL insulin, 100 unit/mL penicillin, 100 μg/mL streptomycin, and 2 mM l-glutamine. All cell lines were grown at 37 °C under a 5% CO₂ atmosphere. The cell lines were routinely tested using a specific standard PCR to control mycoplasma contamination.

Cell Viability Assays. Cell proliferation was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Cells were plated in 96-well sterile plates at a density of 1 × 10⁵ cells per mL (100 μL) and allowed to grow for 24 h. After attachment to the surface, the cells were then incubated with various concentrations of the studied complexes (10 and 50 μM for single-point experiments and within the range of 0.5–5 μM for dose–response curves), freshly dissolved in DMSO and diluted in the corresponding culture medium (DMSO final concentration = 1%), for 48 h at 37 °C. Control cells were cultured in the corresponding culture medium plus the carrier (DMSO final concentration = 1%). Following the treatment, 10 μM MTT was added to each well for an additional 4 h. Afterward, the medium was aspirated, the purple formazan precipitate was dissolved in 100 μL of DMSO, and the absorbance at 570 nm was measured in a multiwell plate reader Multiskan FC (Thermo Scientific). The cell viability was expressed as percentage values with respect to control cells, and the data are shown as the mean value ± standard deviation (SD) of three independent experiments. Dose–response curves and the corresponding IC₅₀ values were obtained by means of nonlinear regression (curve fit), calculated with the GraphPad Prism 5.0 software. For comparison purposes, the cytotoxic effect of cisplatin was also evaluated under identical experimental conditions.

RESULTS AND DISCUSSION

Preparation of the Ligands and Their Respective Platinum(II) Complexes. The diarylethene-based ligands were prepared using common synthetic procedures (Figure S1).26,27 Ligands L1, L3, and L5–L7 were obtained in moderate-to-good yields, ranging from 37% to 72%, by Suzuki cross-coupling reaction between the in situ-generated bisboronic ester derivative of 1,2-bis(5-chloro-2-methyl-3-thienyl)-cyclopentane and the corresponding bromo(hetero)arene (see Experimental Section). 29 Ligands L2 and L4, containing a perfluorinated cyclopentene ring, were synthesized in low yields (26% and 14%, respectively), by reaction of octafluorocyclopentene with the corresponding bromo heteroarylthiophene (see Experimental Details).

The photoswitching abilities of the ligands can be assessed by UV–vis and ¹H NMR spectroscopies. For example, the colorless, open form of L1 exhibits intense absorption bands in the UV region of the spectrum (Figures S2). The absorption observed at λ = 322 nm corresponds to the highest occupied molecular orbital (HOMO) → lowest unoccupied molecular orbital (LUMO) transition, which, according to the Woodward–Hoffmann rules, is required for the photocyclization process to occur. 30 UV irradiation of open L1 gives rise to the development of an intense new absorption band in the visible region, that is, at λ = 544 nm (Figures S2), as reflected by the deep purple color of the solution. It can be pointed out here that a photostationary state (PSS) is reached, which corresponds to a final equilibrium situation of the photocyclization reaction; although complete photoconversion is rarely achieved, the PSS is assumed to
represent the closed form of the molecule.\textsuperscript{31} Similar features were observed for all other ligands, except for ligand L6, which cannot undergo photocyclization; this distinctive behavior was investigated theoretically and was ascribed to the presence of a non-photoreactive HOMO$\rightarrow$LUMO transition with a higher computed oscillator strength value than that of the HOMO$\rightarrow$LUMO one, therefore impeding the photocyclization process.\textsuperscript{32} The photochemical transformation of all ligands, except L6, can also be followed by $^1$H NMR. For instance, NMR spectra of L2 after different photoirradiation times are illustrated in Figure S3. These spectra are representative of the other compounds inspected in the present study (excluding L6, which does not cyclize). Upon UV exposure, a gradual disappearance of the original NMR signals (open form of L2) is observed, which is accompanied by the concomitant, progressive apparition of a new set of peaks ascribed to closed L2 (see blue asterisks in Figure S3). The upfield shift of the proton at the 4-position of the thiophene, from 7.47 to 6.85 ppm, corroborates the occurrence of the expected electrocyclic reaction, generating a new intramolecular bond at the proximal 2-position. For irradiation times longer than 5 min, no further variation of the peaks was noticed, therefore indicating that the PPS was reached. At this equilibrium situation, a photoconversion ratio of 0.86 could be determined from the relative intensity of the two sets of signals (i.e., from the open and closed forms of L2).

After full characterization, platination of the ligands was performed, generating complexes C1$-$C7 with good yields, from 42% to 95% (Figure 1). Hence, reaction of 2 equiv of cis-dichlorobis(dimethyl sulfoxide)platinum(II) (cis-[PtCl$_2$(DMSO)$_2$]) with 1 equiv of L1$-$L6 produced the corresponding complexes C1$-$C6, while the reaction between 1 equiv of the platinum(II) salt and 1 equiv of L7 yielded C7.

**Table 1. Calculated Stern–Volmer Quenching Constants for the Titration of ct-DNA/EB ([DNA]$_{bp}$ = 15 $\mu$M, [EB] = 75 $\mu$M) with the Different Complexes (open and closed forms), at $\lambda_{exc}$ = 514 nm and $\lambda_{em}$ = 610 nm**

| complex | $K_{SV}$ (1 × 10$^3$ M$^{-1}$), open form | $K_{SV}$ (1 × 10$^3$ M$^{-1}$), closed form |
|---------|---------------------------------|-------------------------------|
| C1      | 6.6 ± 0.8                       | 42.5 ± 0.6                    |
| C2      | 4.8 ± 0.9                       | 26.8 ± 0.9                    |
| C3      | 13.7 ± 0.7                      | 58.0 ± 3.0                    |
| C4      | 18.4 ± 0.7                      | 45.0 ± 3.0                    |
| C5      | 9.9 ± 0.4                       | 37.4 ± 0.8                    |
| C6$^a$  | 17.1 ± 0.9                      |                               |
| C7      | 1.4 ± 0.3                       | 21.7 ± 0.6                    |

$^a$This complex does not undergo photocyclization.

**Figure 3.** Fluorescent emission spectra of ct-DNA/EB samples ([DNA]$_{bp}$ = 15 $\mu$M, [EB] = 75 $\mu$M) treated with increasing quantities (1$-$25 $\mu$M) of open C1 (top) and closed C1 (bottom), registered after incubation for 24 h at 37 °C.
Single crystals, suitable for X-ray diffraction studies, could be obtained for C2, C3, C4, and C6. The crystal structures of C2 and C6 were described earlier. The X-ray structures of C3 and C4 are reported herein. Crystallographic and refinement parameters are summarized in Tables S1 and S3 for C3 and C4, respectively, and selected coordination bond lengths and angles for C3 are listed in Table S2, whereas those for C4 are shown in Table S4. The molecular structures of C3 and C4 (Figures S4 and S5) are related to those of C2 and C6. In all cases, the platinum atom is in a square-planar environment defined by two trans chlorido ligands, an S-coordinated DMSO solvent molecule, and a nitrogen atom from the heteroaromatic moiety of the ligand. It can be pointed out here that the solid-state structure of C3 contains both the open and closed forms of the metal complex, in a 31.6/68.4 (Figure S4) ratio, which is not commonly observed with such photoswitching molecules.

It can also be stressed that all complexes contain a DMSO, which is trans to the N-coordinated ligand (strong trans effect of S-coordinated DMSO); in principle, this solvent molecule may be used to further functionalize the platinum compounds, hence increasing the versatility of the system (since numerous monodentate ligands may be used to replace this DMSO). Moreover, one may expect that the high chloride concentration in blood (95−105 mM) would convert the compounds into more hydrophilic "[PtLCl3]−" species (for instance, a complex of the type [PtCl2(DMSO)L], where L is phosphane ligand, was reported that could convert into [PtCl3L]− in the presence of NBu4Cl3); this species, which can be hydrolyzed inside cells (with subsequent DNA binding).

**Agarose Gel Electrophoresis.** After full characterization of complexes C1−C7, the interaction of their open and closed photoisomers with pBR322 DNA was investigated. The corresponding gel images are depicted in Figure 2. It can be mentioned here that one may expect that complexes C1−C7 will exhibit a mechanism of action related to that of cisplatin. Hence, hydrolysis of the chlorides of C1−C7 should lead to the formation of aquated species, which will bind to DNA by substitution of the water molecules for purine bases. Because of the trans disposition of the chlorides, it is expected that each platinum ion will mostly produce 3-intrastand cross-links and...
monofunctional adducts, which will undergo conversion to interstrand cross-links;\(^40\) the diplatinum complexes should therefore show strong DNA interactions. It can be pointed out that polynuclear platinum complexes (PPCs) have shown remarkable biological activities.\(^41\) Furthermore, one may anticipate that the photoinduced closing/opening of the ligand will alter the DNA-binding properties of the corresponding complex isomers. Indeed, the electronic changes taking place upon ring closure will affect the hydrolysis of the chlorido ligands (and therefore their DNA-binding aptitude), and the change of flexibility between the closed and open species will modify their DNA binding; for instance, interstrand binding may be less favorable for the closed, more rigid complexes.

As a matter of fact, for complexes C1−C5, a clearly distinct DNA-interacting behavior of the two isomeric forms is observed. At low concentration, open C1 appears to produce a decrease of the electrophoretic mobility of supercoiled DNA (lane 3), which may be explained by the unwinding of the double helix. However, this effect becomes less pronounced with the increase of the concentration of open C1 (lanes 4 to 6). In contrast to lane 3, DNA form I is detected in lanes 4−6, and the diminution of its mobility with the increase of [open C1] resembles that of supercoiled DNA treated with cisplatin (lane 2).\(^{42,43}\) This

Figure 7. CD spectra of ct-DNA samples ([DNA]bp = 50 μM) treated with increasing concentrations (5−25 μM) of open complexes C1−C7, registered after incubation for 24 h at 37 °C.
concentration-dependent interaction of open C1 with DNA suggests that it exhibits different binding modes, most likely arising from cooperative effects between the platinum centers and the organic ligand. It seems that with only 0.5 equiv of open C1, noncovalent interactions play a role (lane 3), whereas for [open C1] ≥ 1 equiv, typical platination effects are taking place (lanes 4 to 6).

On the contrary, closed C1 behaves as a strong DNA intercalator (lanes 7 to 10), probably as the result of its higher planarity and electronic conjugation. It is well-known that DNA intercalation induces a stronger local unwinding of the double helix than covalent binding; this may result in the relaxation of negatively supercoiled DNA sequences until reaching an open circular conformation, whereas further addition of the intercalating agent will lead to positive supercoiling. Such phenomenon is clearly occurring with closed C1 (lanes 7 to 10). At lane 7, an initial decrease of the mobility is observed, as the result of a partial twist reduction of the biomolecule. When the amount of closed C1 is increased, this effect is enhanced, so that all initial DNA form I is relaxed and comigrates with DNA form II (lane 8). Upon further increase of [closed C1], the DNA mobility is improved (lanes 9 and 10), which is likely due to a subsequent induction of positive supercoiling to the previously relaxed structure (lane 8). Finally, note that a vanishing of the band intensities takes place when the amount of closed C1 is increased (lanes 8 to 10). This feature points to the precipitation of highly platinated DNA species.

Replacement of the cyclopentene ring by a perfluorocyclopentene one (from C1 to C2) clearly modifies the DNA-interacting properties of the resulting complex. Like open C1, open C2 also induces the unwinding of DNA, the total conversion to form II now being observed for the highest complex concentration (lane 6). This feature may be explained by single-strand cleavage of the plasmid upon platinum binding, producing the complete relaxation of supercoiled DNA fragments through free rotation around the nicked strand.

However, closed C2 exhibits intercalating properties as closed C1, but higher concentrations are required to give the same effect. Indeed, 1.0 equiv of closed C1 is sufficient to provoke complete relaxation of supercoiled circular DNA (lane 8), whereas 2.0 equiv are required with closed C2 (lane 10). This weaker intercalating behavior exhibited by closed C2 may be due to repulsive interactions between the fluorine atoms of ligand L2 and the phosphate backbone.

For complexes C3 and C4, minor differences were observed between their open and closed forms (Figure 2). In all cases, the treatment of plasmid DNA with increasing quantities of the two photoisomers of each compound gives rise to a noticeable decrease of the electrophoretic mobility of supercoiled DNA; the open and closed forms of C3 are even capable of inducing the comigration of supercoiled and open circular DNA (lanes 4–6 and lanes 8–10). The analogous activities of both forms of the complexes may be attributed to the larger, planar quinoline moieties of ligands L3 and L4, compared to the pyridine rings in L1 and L2, which apparently confers a comparable intercalating behavior to the photoisomers. It can also be noticed that C4 is less active than C3, confirming the negative influence of the fluorinated backbone of ligand L4 on the DNA interaction.

Open C5 follows a dose-dependent pattern that is like that of open C1 (lanes 3–6). 0.5 equiv of open C5 produces the complete unwinding of supercoiled circular DNA, generating its form II (lane 3). Higher amounts of open C5 induce effects that are comparable to those observed with cisplatin, therefore suggesting metal binding to DNA.

Closed C5 exhibits the same behavior as its open form (see lanes 3–6 and lanes 7–10 in Figure 2). Thus, closed C5 does not seem to show the intercalative properties observed with the closed isomers of the previous complexes (see above). This feature may arise from (i) steric hindrance caused by the methyl substituents of the pyridine ring of ligand L5 and/or (ii) the relative disposition of the metal centers (meta position instead of para position for the previous complexes). This drastic alteration of the DNA-interacting properties of closed C5 (compared, for instance, to those of closed C1 and C2), triggered by a subtle modification of the metal-coordinating unit, again indicates that the binding activity of the two photoisomers proceeds via two different mechanisms.

As reported earlier, complex C6 does not display photo-switching properties. Consequently, only the DNA interaction of open C6 could be investigated. Hence open C6 behaves as open C1; namely, 0.5 equiv of the complex leads to the formation of DNA form II (lane 3). Higher [open C6] result in effects comparable with those of the interaction of cisplatin with DNA, that is, metal binding to the biomolecule. Actually, this covalent binding of open C6 appears to be particularly strong, since a vanishing of the bands is observed with the increase of its concentration (lanes 5–6), which is indicative of the precipitation of the resulting complex–DNA adducts.

The mononuclear complex C7 has completely distinct behavior. No photoisomeric differentiation is observed; in both cases, a comparable retardation of the DNA mobility takes place (lanes 3–6 and lanes 7–10). Surprisingly, closed C7 does not exhibit better DNA-intercalating properties than open C7, even though one may have expected that substitution of one of the metal-binding units by a phenyl ring (ligand L1 to ligand L7) would have somewhat favored the insertion of closed C7 between DNA base pairs. Consequently, it can be inferred that the strong DNA-unwinding ability displayed by most closed complexes of the present study is due to not only their insertion between DNA base pairs but also to the subsequent binding of the two platinum atoms.

Fluorescent Intercalator Displacement Assays. Competitive binding studies were performed subsequently, using EB bound to ct-DNA and the open and closed forms of the complexes, as fluorescence quenchers (through the release of EB). Fluorescence spectra were therefore recorded at constant concentrations of ct-DNA and EB, respectively, 15 and 75 μM, by adding increasing amounts of both photoisomers of the platinum(II) complexes, specifically, in the concentration range of 1–25 μM. Representative fluorescence spectra for open and closed C1 are shown in Figure 3. In all cases, a decrease of the fluorescent emission of the DNA/EB adduct45 is observed, which is indicative of the displacement of the dye resulting from the interaction of the complex with the double helix.

It can be noticed that the quenching effect is clearly more pronounced for closed C1 than for open C1 (Figure 3), hence suggesting that the closed photoisomer has a higher capacity to expel DNA-bound EB, thus confirming its better intercalating properties (see Gel Electrophoresis results).46 To properly compare the DNA affinity of all studied species, the fluorescence emission data obtained were used to determine the corresponding Stern–Volmer quenching constants (KSV) for intermolecular deactivations, by applying the Stern–Volmer eq 1:47
In this expression, $I_0$ is the initial fluorescence intensity of the DNA/EB system, $I$ represents the fluorescence emission intensity after the addition of a quencher, and $Q$ is the quenching molecule, which is the metal complex in the present study. A plot of $I_0/I$ versus [complex] at the maximum emission wavelength (i.e., $\lambda_{\text{max}} = 610$ nm) gives a straight line, whose slope is equal to $K_{SV}$. Applying this procedure, Stern–Volmer constants of $K_{SV} = 6.6 \pm 0.8 \times 10^3$ M$^{-1}$ and $K_{SV} = 42.5 \pm 0.6 \times 10^3$ M$^{-1}$ were determined for open and closed C1, respectively (Figure 4).

Similar trends were observed for the open and closed photoisomers of all compounds investigated, whose respective Stern–Volmer constants are listed in Table 1.

In all cases, a significantly higher $K_{SV}$ value was obtained for the closed isomer, hence corroborating their higher intercalative behavior. Thus, one may expect that the closed forms of the platinum(II) complexes will induce higher growth inhibitory effects than their open counterparts. It can also be noticed that lower quenching constants were systematically achieved for the fluorinated closed-ring species, compared with their corresponding nonhalogenated analogues (see C1 and C2, and C3 and C4 in Table 1). These features agree with the data achieved by gel electrophoresis (see above) and confirm the negative effect of the fluorinated cyclopentane ring on the intercalation of the corresponding coordination compounds. These differences may result from a slight destabilization of the DNA/complex adducts through electrostatic repulsion between the fluorine atoms and the negatively charged phosphate backbone of the double helix.48

A noticeably reduced dye-displacement ability is exhibited by the mononuclear complex C7 (in both its forms; see Table 1). For instance, closed C7 shows a $K_{SV}$ value that is twice lower than that of closed C1. These results are in line with the earlier data obtained with plasmid DNA and confirm that the binding of dinuclear species like C1 to neighboring nucleobases (after the insertion of the compound between base pairs) is crucial regarding the stabilization of the resulting DNA/complex adducts.

This new set of data again shows that the closed form of the diplatinum complexes are inducing stronger alterations of the double-helix conformation than the open one, apparently through a combination of intercalative and covalent interactions. It may therefore be expected that such closed species can be more efficient in inhibiting gene expression and DNA-replication processes, which may be reflected by a higher antiproliferative efficiency.

The results achieved with photoinert C6 requires some attention. As indicated by its $K_{SV}$ value (Table 1), this imidazolic species exhibits a far superior degree of interaction with DNA than open C1 and C2 (Table 1). Its dye-displacement activity is comparable to or even greater than that of quinoline-containing complexes C3 and C4. Contrary to these two compounds, C6 does not have potentially intercalating structural motifs; hence, C6 can expel ethidium bromide through a different mode of action. Most likely, the binding of C6 strongly alters the conformation of the double helix. Local modifications of the secondary or tertiary structure of DNA, like those produced by 1,3-interstrand cross-links, may indeed force the dye to leave the nearby interbase positions as the result of the destabilization of the π-stacking interactions. This phenomenon may also explain the apparent higher degree of platination and consequent band fading observed by gel electrophoresis for this complex (Figure 2).

**Cell Viability Assays.** The potential application of such metalloswitches as a novel type of photochemotherapeutic agents was examined through the evaluation of the effect of the open and closed isomers of C1–C7 (only the open form in the case of C6) on cancer cell proliferation and survivability. Five cancer cell lines were selected for these studies, namely, lung adenocarcinoma (A549), melanoma (A375), breast carcinoma (MCF7), colorectal adenocarcinoma (SW620), and ovarian adenocarcinoma (SKOV3).

In a first instance, single-point screening assays at constant complex concentrations, that is, 10 and 50 μM, were performed to obtain a general comparative estimation of the cytotoxic behavior of both photoisomers of each complex. The results achieved after an incubation time of 48 h are depicted in Figure 5 for complex C2, Figure 6 for C6, and Figure S6 for C1, C3–C5, and C7. Surprisingly (considering the DNA-binding studies), most of the screened platinum(II) complexes exhibit very poor cytotoxic properties; moreover, there is no discrimination between the activity of their open and closed isomers. Hence, treatment of the cells with open/closed C1, C3, C4, and C5 gives rise to above 75% survival of the cell populations (Figure S6). Mononuclear C7 shows slightly better results, especially for the SKOV3 cell line, for which the closed form is more active than the open one (Figure S6). At $[C7] = 10$ μM, closed C7 can induce 50% growth inhibition, whereas cell viability of ~95% is observed with the open form. Much more favorable results were achieved with compound C2. Indeed, whereas the open form of this complex is not active in all tested cell lines, its closed photoisomer presents a remarkably high cytotoxic activity against most of them (Figure 5). For instance, incubation of the cells with 50 μM C2 led to survival levels below 35% in all cases, except for the ovarian SKOV3 line. Even more interesting is the fact that comparable cancer-cell death ratios were also obtained with the same cell lines using a lower amount of the complex, namely, with a concentration of 10 μM (Figure 5).

The cell-viability results achieved with photochemically inert C6 were also noteworthy. In accordance with the DNA-interaction studies (see above), open C6 is highly cytotoxic, as reflected by the cell-death ratios of over 85% observed in all cell lines with a complex concentration of 50 μM (Figure 6). Related high antiproliferative levels were even achieved using a complex concentration of 10 μM, thus highlighting the different mode of action of this imidazole-based compound compared with the open forms of the pyridine- and -quinoline-containing ones (which are not cytotoxic).

Subsequently, half-maximal inhibitory concentrations ($IC_{50}$) were determined for the two most interesting platinum(II) compounds, namely, C2 and C6 (Figure S7), using the two cell lines A375 and SW620, carefully selected from the single-point results (see Figures 5 and 6) and specific characteristics. The melanoma A375 cells constitute the most suitable target for PACT drug candidates such as those herein reported, owing to the inherent accessibility to the treatment area.49 The choice of the colorectal cancer line SW620 arises from its bad prognosis and current shortfall of efficient therapies.50 The $IC_{50}$ for cisplatin with these two cell lines was also determined, for comparison purposes.

The $IC_{50}$ data listed in Table 2 corroborate the single-point cell viability assays (see above) and the cytotoxic potential of the selected compounds, which are more efficient than cisplatin for the two cell lines. The $IC_{50}$ values for closed C2 and open C6 are
in the low micromolar range, the compounds being up to 8 times more active than cisplatin (Table 2).

**Circular Dichroism Measurements.** The distinct results achieved with open C6, compared with those of all other open complexes, were analyzed further by circular dichroism (CD) spectroscopy. Thus, increasing quantities of the open form of C1−C7 were added to solutions of ct-DNA, and the corresponding CD spectra were recorded in the near-UV region, specifically, between 235 and 315 nm, after incubation for 24 h at 37 °C (see Experimental Section for details). The spectra for C6 and open C1−C5 and C7 are shown in Figure 7.

Direct comparison of the CD spectra of C1 and C6 interacting with B-DNA reveals noticeable differences. The addition of increasing amounts of C1 to ct-DNA results in a minor bathochromic shift of both the characteristic positive and negative bands, and a progressive slight increase of ellipticity of the positive signal, up to saturation. Such CD features are indicative of the monofunctional covalent binding of platinum(II) drugs to DNA, and therefore agree with the previously proposed metal-mediated activity of the open photoisomers.51

Furthermore, these spectroscopic data corroborate the minor impact of the complex binding on the helical conformation nor on the base-pair stacking of the biomolecule. Apart from C6, similar spectra were obtained for all other open complexes (see Figure 7); the spectral changes induced upon treatment of ct-DNA with the highest concentration of each complex are listed in Table S5.

Open C6 induces a drastically distinct alteration of the conformation of the double helix (Figure 7 and Table S5). The progressive binding of this imidazolic species leads to a severe decrease of the whole CD signal of B-DNA, particularly for the negative band. These spectral changes suggest that C6 has a distinct effect on the secondary structure of the biomolecule, most likely generating local disruptions of the helical turn, and destabilization of the alignment of adjacent base pairs. This effect is consistent with the efficient expulsion of ethidium bromide noted by fluorescence assays (see above).

Since C6 is the sole metal complex of the series exhibiting this behavior, it may be attributed to the methylimidazole units; however, the exact mechanism through which C6 induces such a distinct structural alteration of B-DNA remains to be elucidated. Probably, the specific electronic structure of ligand H632 has a direct effect on the ligand-exchange kinetics and/or the binding affinity of the platinum(II) centers, which may thus become more prone to mediate DNA cross-links. These different DNA-interacting properties may explain the higher cytotoxicity of C6, compared with that of the other open complexes (Table 2 and Figures 5, 6, and S7).

Finally, the CD spectra for the closed form of complexes C1−C5 and C7 were recorded (Figure S6); the spectroscopic data obtained are comparable to those of the corresponding open complexes (see Figures 7 and S6), indicating that the closed compounds are also not capable of significantly altering the secondary structure of DNA.

**CONCLUSIONS**

Following earlier promising results,20 a series of photoswitchable platinum(II) complexes based on photoresponsive dithienycyclopentene moieties were prepared and fully characterized. In particular, the connectivity of the metal ions was clearly established by single-crystal X-ray diffraction, which revealed a trans disposition of the chlorido ligands and the S-coordination of DMSO. The photochemical properties of the ligands and metal complexes prepared were investigated, which showed that the ligand L6 and the corresponding complex C6 were photoinactive.32

DNA-interaction studies performed with all complexes revealed that the activity of these photoisomerisable agents can be efficiently modified through their light-mediated conversion. Clearly distinct DNA-interacting properties for the open and closed forms of the coordination compounds were observed. For instance, different metal covalent binding and ligand intercalative association were noticed for the two forms. These remarkable results suggest that the activity of each photoisomer may be tailored through appropriate structural modifications, thus opening design possibilities for the future development of more effective DTC-based metallo drugs (DTC stands for dithienycyclopentene).

The antiproliferative activity of the open and closed forms of the complexes were examined, which showed that most of the compounds surprisingly were not cytotoxic; actually, the distinct DNA-interacting ability of each isomer did not translate into interesting results with cells. Remarkable data were though obtained with complex C2, whose closed form is highly cytotoxic against melanoma and colorectal cancer cells, whereas its open form is nontoxic. These drastically distinct behaviors of open/closed C2 further strengthen the innovative approach proposed in a previous proof of concept study,32 consisting in using DTC photoswitches to develop novel PACT agents.

**ASSOCIATED CONTENT**

*Supporting Information*

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.inorgchem.8b00146.

The schematic representations of the structures of L1−L7; UV−vis spectra of open/closed C1; 1H NMR spectra of L2 upon UV irradiation; crystal data and structure refinement for C3 and C4; representations of the crystal structures of C3 and C4; selected bond lengths and angles for C3 and C4; CD data of the open complexes; CD spectra of the closed complexes; cell viability assays and dose-response curves (PDF)

**Accession Codes**

CCDC 1565000−1565001 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

Financial support from the Spanish Ministerio de Economía y Competitividad/FEDER (Project Nos. CTQ2015−70371−REDT, CTQ2014−55293−P, and CTQ2017−88446−R) is ac-
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