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Cytotoxicities of Polysubstituted Chlorodicarbonyl(cyclopentadienyl) and (Indenyl)ruthenium Complexes

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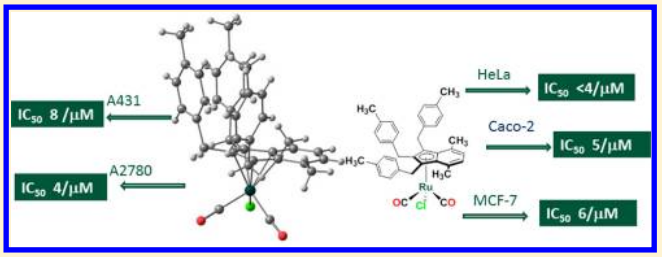
Supporting Information

ABSTRACT: Polysubstituted cyclopentadienyl and indenyl complexes of ruthenium were synthesized and investigated to elucidate their potential cytotoxic activities. In particular, substituted (indenyl)ruthenium complexes inhibited the proliferation of a panel of human adherent cancer cells with comparable activity to reference agent cisplatin. One of the active compounds exerted a concentration dependent inhibition of cell cycle at G1−S transiton as evidenced by flow cytometry.



INTRODUCTION

Cisplatin (**1**) (Figure 1), along with other Pt derivatives including carboplatin and oxaliplatin, belongs to the most well-known drugs

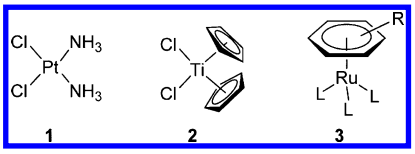


Figure 1. Examples of organometallic compounds with anticancer activity.

containing transition metals.[1] Because of the generally high toxicities and adverse side effects of platinum compounds in therapeutic applications, and also to the potentially occurring and developing drug resistance in rapidly mutating cancer cells, alternative approaches toward other metal-based anticancer agents have been under active investigation. For example, titanocene dichloride (**2**) has been extensively tested as an anticancer drug although its low hydrolytic stability sets limits for in vivo utilization and, so far, this compound has not been approved for clinical use.[2] Ruthenium complexes in turn have gained significant attention due to their lower toxicities toward normal cells and different spectrum of activity compared to platinum compounds.[3] Earlier research on π-complexes of ruthenium has focused mainly on η^6-arene complexes such as **3**,[4] whereas cytotoxicity studies on η^5-(cyclopentadienyl)ruthenium complexes have remained relatively scarce.[5] In this context, the potential cytotoxicities of chlorodicarbonyl ligated (cyclopentadienyl) ruthenium complexes have remained virtually unexplored.

In recent work, we have prepared and investigated, as part of structure optimization work, several cyclopentadienyl and indenyl chlorodicarbonyl complexes of ruthenium for use as racemization catalysts in chemoenzymatic dynamic kinetic resolution of *sec*-alcohols (Figure 2).[6,7] As a spin-off from this work, and with the

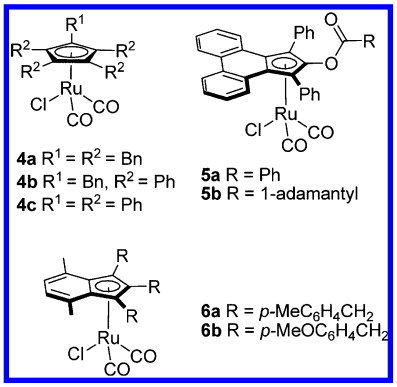


Figure 2. Structures of chlorodicarbonyl(cyclopentadienyl) and (indenyl) complexes **4**−**6** investigated in the present study.

limited information available on the potential cytotoxicities of such compounds in mind, we became interested in their potential use as cytotoxic agents. In this paper, initial insights into their pharmaceutical potential is presented and briefly discussed.

RESULTS AND DISCUSSION

The synthesis of **4a**,[7b] **4b**,[7b] **5a**,[7a] and **5b**[7a] has been described earlier by us and that of **4c** by Bäckvall and co-workers.[8]

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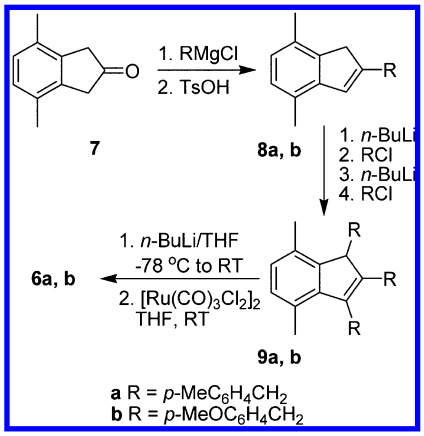
The new polysubstituted indenyl complexes **6a** and **6b** were prepared from the corresponding tribenzylindenes by a method similar to that utilized earlier for **5a** and **5b** (Scheme 1).[7a] All

Scheme 1. Preparation of Indenyl Complexes 6a and 6b



new compounds were characterized by ^{1}H and ^{13}C NMR and HRMS and in case of **6a** also by X-ray crystallography. The X-ray structures of **4a**,[7b] **4b**,[7b] and **4c**[7c] have been described earlier, while this is the first report on the X-ray structure of **5a**.

The crystal structures of **5a** and **6a** are presented in Figures 3 and 4, respectively, with the selected bond distances reported in

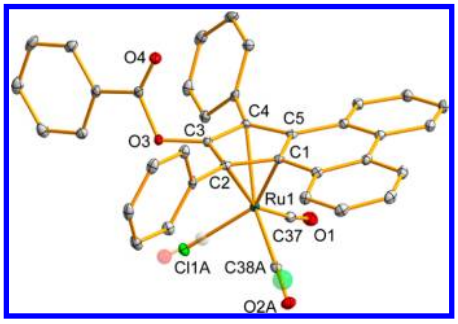


Figure 3. The molecular structure of **5a**. Thermal ellipsoids have been drawn at 30% probability level, and hydrogen atoms are omitted for clarity.

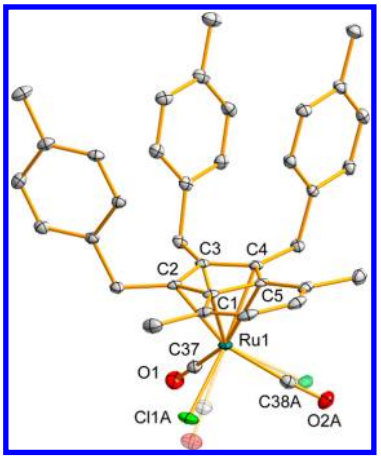


Figure 4. The molecular structure of **6a**. Thermal ellipsoids have been drawn at 30% probability level, and hydrogen atoms are omitted for clarity.

Table 1. In both complexes, the modified cyclopentadienyl (Cp) ring is η^5-coordinated to the ruthenium metal (similar

Table 1. Selected Bonding Distances in the Complexes 5a and 6a

distances (Å)	5a	6a
Ru−C1	2.244(2)	2.322(2)
Ru−C2	2.238(2)	2.202(2)
Ru−C3	2.241(2)	2.237(2)
Ru−C4	2.240(2)	2.218(2)
Ru−C5	2.238(2)	2.323(2)
Ru−C37	1.933(3)	1.874(2)
Ru−C38A[a]	1.879(4)	1.93(1)
Ru−C38B[a]	1.80(3)	1.90(1)
Ru−Cl1A[a]	2.3194(9)	2.398(2)
Ru−Cl1B[a]	2.38(1)	2.387(2)
Ru−Ct[b]	1.875	1.899

[a]The molecular structure shows a disorder in which the Cl ligand and one of the two CO ligands have two orientations (rotamers) in about 9:1 and 1:1 ratio for **5a** and **6a**, respectively. [b]Ct is the C_5 ring centroid.

Ru−C bond distances are observed for all carbon atoms of the five-membered ring). In complex **6a**, however, the Cp fragment is slightly tilted producing marginally longer M−C1 and M−C5 bonds (2.322 and 2.323 Å, respectively) compared to the other M−C(Cp) distances (ranging from 2.218 to 2.237 Å), even though it is still best described as being η^5-coordinated. The observed distribution of M−C distances (three shorter and two longer) is typical of the η^5 coordination mode of indenyl, often described as $\eta^3 + \eta^2$, even in the absence of steric constraints. The fused ring systems around Cp rings of **5a** and **6a** are nearly planar, while the other substituents have been rotated away from the molecular plane. In **5a**, the phenyl substituents are canted about 56−72° from the cyclopentadienyl level while in **6a** the benzyl substituents are arranged at the top of the ring pointing at the opposite direction from the ruthenium cation. The five M−C distances are very similar in this C5 ring, reflecting the different nature of its π orbitals. The remaining coordination sites of Ru are occupied by two carbonyl groups and a chloride ion, producing a typical pseudo-octahedral three-legged piano stool conformation. The carbonyl and chloride ligands of both complexes are disordered in two orientations (rotamers) with the main component 0.89 for **5a** and a 1:1 ratio in **6a**, respectively (the minor or other component is presented partly transparent in Figures 3 and 4). The formal oxidation state of ruthenium in **5a** and **6a** can be assigned as +2, being further supported by the relatively short Ru−carbonyl and Ru−C_5 centroid distances (Table 1). All bond lengths around ruthenium are in good accordance with previous studies.[7a,b,8]

The cytotoxic activity of these ruthenium complexes was assayed against HeLa (cervical carcinoma), MCF-7 (breast carcinoma), Caco-2 (colorectal adenocarcinoma), A2780 (ovarian cancer), and A431 (skin epidermoid carcinoma) cell lines. The cells were exposed to each of the compounds for a total of 48 or 72 h. The IC_{50} values (final concentration ≤0.5% DMSO) were calculated from dose−response curves obtained by nonlinear regression analysis from the colorimetric mitochondrial function-based MTT viability assay. IC_{50} values are concentrations of drug required to inhibit tumor cell proliferation by 50%. The results are summarized in Table 2.

Overall, of the compounds studied here, the polysubstituted cyclopentadienyl complexes **4a**, **4b**, and **4c** and the 2-adamantanoyloxy substituted cyclopenta[*l*]phenanthrenyl complex **5b** proved ineffective, failing to show 50% inhibition of cancer cell

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Table 2. Calculated IC_{50} Values for Selected Ruthenium Complexes against HeLa, Caco-2, MCF7, A2780, and A431 Cell Lines

	IC_{50} (μM)[a]					
complex	HeLa[b]	Caco-2[b]	HeLa	MCF7	A431	A2780
4a	46.0		>30	>30	>30	>30
4b	>95		>30	>30	>30	>30
4c	>250		>30	>30	>30	>30
5a	17.6	81.7	14.3	23.0	>30	19.1
5b	45.0					
6a	5.9	5.2	4.7	6.3	8.0	3.9
6b	4.7	2.4	7.8	5.1	5.6	4.3
cisplatin (1)			5.7	8.0	8.8	0.9

[a]Main value from two independent determinations, standard deviation less than 15%. [b]Results from experiments with 48 h of incubation. All other data represent 72 h.

proliferation in up to 30 μM final concentrations. The larger range of concentrations tested for these four complexes with 48 h incubation showed high IC_{50} for **4a** (46.0 μM) and **5b** (45.0 μM), and even higher for **4b** and **4c**. Owing to this low activity (IC_{50} > 95 or 250 μM), it was not considered relevant to determine a precise value for these two complexes. Treatment with the 2-benzoyloxy substituted cyclopenta[*l*]phenanthrenyl complex **5a**, however, resulted in moderate inhibition of the cell growth, whereas the activities of the two 1,2,3-tri(benzyl)substituted indenyl complexes **6a** and **6b** were the most remarkable in all cell lines tested and comparable with that of the clinically used reference agent cisplatin **1**.

To obtain information on the cell cycle distribution as a function of the treatment of cancer cells with a test substance, flow cytometric analysis was used. In short, diploid cells (cells in "basic state") contain a unit amount of DNA (population G1). As the cell prepares for division, its DNA content gradually increases, characterizing the synthetic (S) phase. Upon completion of the synthesis, the G2 (G2/M) phase, with a DNA content double from that of cells in the G1 phase, is reached. Next, a G2 cell undergoes division to produce two G1 cells. Cells containing less-than-G1 amount of DNA (subG1 population) are considered resulting from the organized self-decomposition (apoptosis), typically elicited by harmful stimuli.

In the flow cytometric experiment, A2780 cells were incubated for 24 h with 1 and 3 μM concentrations of complex **6b** prior to analysis. Treatment with the tested complex resulted in a substantially increased population of G1 cells. A significant and concentration-dependent decrease in the ratio of cells from S and G2/M phases was also observed (Figure 5). On the basis of these experimental results, a cell cycle blockade at G1−S transition could be suggested as a mechanism of action of the detected antiproliferative property. Interestingly, the subdiploid (subG1) cell population, which is generally accepted as an apoptotic marker, exhibited no characteristic change under the applied conditions.[9]

The binding of complexes **5a**, **6a**, and **6b** to DNA was also studied by electronic absorption spectroscopy.[10] The absorption pattern of each complex was followed upon adding increasing amounts of ct DNA (200 μM bp^{-1}). The absorption maxima at 268 nm (**5a**), 281 nm (**6a**), and 290 nm (**6b**) were shifted to higher wavelengths while the intensity decreased, suggesting that the complexes may be acting as intercalators. The intrinsic binding constants (K_b) of each compound were calculated as 1.05×10^5 (**5a**), 7.12×10^4 (**6a**), and 3.26×10^4 (**6b**) M^{-1}. These values

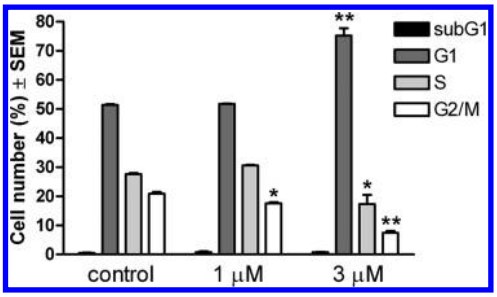

Figure 5. Effect of complex **6b** on A2780 cell cycle distribution after incubation for 24 h. * and ** indicate $p < 0.05$ and $p < 0.01$, respectively, as compared with the control cells.

compare very well with the binding constants of known intercalators,[11] such as doxorubicin and ethidium bromide, determined under the same conditions to be 1.52×10^5 and 2.78×10^5 M^{-1}, respectively. The plot in Figure 6 depicts the

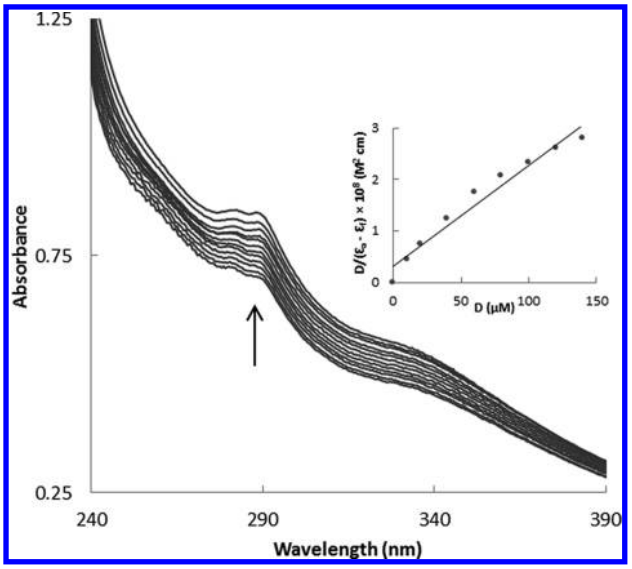

Figure 6. UV−vis absorption spectra of **6b** (20 μM) in Tris buffer in the presence of increasing amounts of ct DNA (0−200 μM). The inset plot represents [DNA]/($\varepsilon_a - \varepsilon_f$) ($M^2$ cm) vs [DNA] (μM) for the titration. The arrow indicates the absorbance changes monitored at 281 nm upon increasing DNA concentration.

UV−vis absorption spectra of complex **6b** (20 μM) in Tris buffer in the presence of increasing amounts of ct DNA (0−200 μM). The arrow indicates the absorbance changes monitored at 281 nm upon increasing DNA concentration. The inset plot represents [DNA]/($\varepsilon_a - \varepsilon_f$) ($M^2$ cm) as a function of [DNA] (μM) for the titration and allows the determination of K_b.

SUMMARY AND CONCLUSIONS

In this work, two new indenyl chlorodicarbonyl complexes of ruthenium(II) were synthesized and characterized and together with three earlier prepared chlorodicarbonyl ruthenium cyclopentadienyl and two cyclopenta[*l*]phenanthrenyl complexes screened against several cancer cell lines for investigation of their potential cytotoxic activities. New crystal structures of two of the complexes were determined, displaying the expected piano stool coordination environment of the metal. In both complexes, the five-membered rings of the ligands are η^5-coordinated to ruthenium, although in the indenyl complex the

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two Ru−C bonds to the hinge carbon atoms are longer than the other three.

Testing of the antiproliferative activity of these seven complexes revealed five complexes as ineffective, inhibiting less than 50% of cancer cell proliferation in up to 30 μM final concentration. Two η^5-indenyl ligated ruthenium complexes containing 1,2,3-tribenzyl substituents in the five-membered rings of the indenyl moieties displayed activities similar to that of the reference metal complex cisplatin. Treatment with a selected test agent resulted in a profound cell cycle disturbance which could be the consequence of intercalation into the DNA. Titration experiments showed that the binding constants to DNA of the more active complexes were comparable to those of known intercalators (doxorubicin and ethidium bromide). On the basis of the results obtained in this study, it can be concluded that chlorodicarbonyl indenyl complexes of ruthenium are viable candidates for further search and optimization of new lead compounds for anticancer therapies with promising efficacies.

EXPERIMENTAL SECTION

General Considerations. All glassware utilized for organometallic reactions was oven-dried at 150 °C overnight and cooled down in a desiccator over phosphorus pentoxide. Solvents were dried according to standard procedures. Starting material for the indenyl ligand precursors, 4,7-dimethyl-2-indanone (**7**), was prepared according to a literature procedure.[12] The RPMI 1640 cell culture medium, fetal bovine serum, tripsin, glutamine, and pen-step were purchased from LONZA Co. MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide), doxorubicin, and ethidium bromide were purchased from Sigma Aldrich. A stock solution of calf thymus DNA (ct DNA) from Sigma Aldrich was prepared by diluting in a buffer solution (50 mM NaCl/5 mM Tris-HCl, pH 7.2) and stirring at 4 °C for two days. The solution was stored at 4 °C. The ratio of UV absorbance at 260 and 280 nm (A_{260}/A_{280}) of 1.83−1.86 of this ct DNA stock solution indicated that the DNA was sufficiently free of protein contamination. The DNA concentration was determined by the UV absorbance at 260 nm after 1:10 dilution using ε = 6600 M^{-1} cm^{-1}.[13] MTT was dissolved in RPMI 1640 medium (0.5 mg/mL). Ruthenium complexes **4a**,[7b] **4b**,[7b] **4c**,[8] **5a**,[7a] and **5b**[7a] were prepared as described previously. For X-ray structure determination, **5a** was recrystallized from a DCM−heptane mixture. NMR spectra were recorded using a a 600 MHz NMR spectrometer equipped with a BBI-5 mm-Zgrad-ATM probe or BBO-5 mm-Zgrad probe at 298 K operating at 600.13 MHz for ^{1}H and 150.92 MHz for ^{13}C. Numbering of the indene moiety, in accordance with the IUPAC rules, used for assignment of the NMR spectra but deviating from the crystallographic numbering used in Figures 3 and 4 and Table 1 is shown in Figure 7.

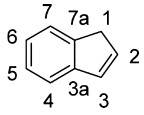

Figure 7. Numbering of the indene moiety in NMR spectra.

4,7-Dimethyl-2-(4-methylbenzyl)-1*H*-indene (8a). A solution of 4,7-dimethyl-2-indanone (**7**) (3.6 g, 23 mmol) in THF was slowly added to 50 mL of a 0.5 M solution of 4-methylbenzylmagnesium chloride in THF over 4 h. The reaction mixture was stirred at room temperature overnight. The reaction was quenched by slowly adding 20 mL of a saturated solution of NH_4Cl upon which a white precipitate formed. Next, 15 mL of water were added to dissolve the precipitate. The organic layer was separated and dried over Na_2SO_4 and concentrated, providing 4.91 g of the crude product. Part of this product (2 g, approximately 7.5 mmol) was dissolved in 80 mL of toluene, and 130 mg of *p*-toluenesulfonic acid was added. The reaction mixture was refluxed for 2 h and then cooled down to room temperature and washed with 2 × 100 mL of a saturated solution of $NaHCO_3$ and 100 mL of brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by filtration through silica using hexane as an eluent. Yield: 1.36 g (24% based on **7**). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.12 (d, 2 H, $^3J_{\text{2-Bn-}o\text{-H, 2-Bn-}m\text{-H}}$ = 8.2 Hz, 2-Bn-*o*-H), 7.10 (m, 2 H, 2-Bn-*m*-H), 6.94 (d, 1 H, ^3J = 7.6 Hz, H-6), 6.81 (d, 1 H, ^3J = 7.6 Hz, H-5), 6.62 (m, 1 H, H-3), 3.78 (s, 2 H, 2-CH_2Ph), 3.12 (s, 2 H, H-1), 2.35 (s, 3 H, 7-Me), 1.32 (s, 3 H, 2-*p*-*Me*Bn). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 148.6 (C-2), 143.8 (C-4), 142.0 (C-3′), 137.3 (2-Bn-*i*-*C*), 135.7 (2-Bn-*p*-*C*), 130.0 (C-7′), 129.3 (2-Bn-*m*-*C*), 128.8 (2-Bn-*o*-*C*), 127.8 (C-6), 127.1 (C-7), 126.4 (C-3), 125.3 (C-5), 40.0 (C-1), 37.8 (2-*C*H_2Ph), 21.2 (2-*p*-*Me*Bn), 18.5 (4-Me), 14.4 (7-Me)

4,7-Dimethyl-2-(4-methoxybenzyl)-1*H*-indene (8b). Magnesium turnings (7.1 g, 0.3 mol) and 100 mL of dry THF were placed into a round-bottom flask under argon atmosphere, and then a single crystal of iodine was added. Next, 4-methoxybenzyl chloride (12 mL, 0.09 mmol) was added slowly over 1.5 h. To the resulting mixture containing the Grignard reagent, 4,7-dimethyl-2-indanone (**7**) (5 g, 32 mmol) was added slowly and the reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with saturated NH_4Cl and filtered, and the residue was washed with TBME. Solvent was removed in vacuo, providing 5 g (approximately 55%) of oil crystallizing upon overnight storage at RT. This product was dissolved in 100 mL of toluene, and 200 mg of *p*-toluenesulfonic acid were added. The reaction mixture was refluxed for 1.5 h and then cooled down to room temperature and washed with 2 × 100 mL of a saturated solution of $NaHCO_3$ and 100 mL of brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by filtration through silica (hexane → hexane:DCM 10:1 → hexane:DCM 4:1). Yield: 3.9 g (47% based on **7**). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.16 (d, 2 H, $^3J_{\text{2-Bn-}o\text{-H, 2-Bn-}m\text{-H}}$ = 8.7 Hz, 2-Bn-*o*-H), 6.95 (d, 2 H, ^3J = 7.7 Hz, H-6), 6.85 (m, 2 H, 2-Bn-*m*-H), 6.83 (d, 1 H, ^3J = 7.7 Hz, H-5), 6.61 (m, 1 H, H-3), 3.80 (s, 3 H, 2-*p*-*Me*OBn), 3.77 (s, 2 H, 2-CH_2Ph), 3.14 (s, 2 H, H-1), 2.36 (s, 3 H, 7-Me), 2.24 (s, 3 H, 4-Me). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 158.2 (2-Bn-*p*-*C*), 148.9 (C-2), 143.8 (C-7′), 142.0 (C-3′), 132.5 (2-Bn-*i*-*C*), 130.1 (C-4), 129.9 (2-Bn-*o*-*C*), 127.8 (C-6), 127.1 (C-7), 126.2 (C-3), 125.3 (C-5), 114.0 (2-Bn-*m*-*C*), 55.4 (2-*p*-*Me*Bn), 40.0 (C-1), 37.4 (2-*C*H_2Ph), 18.5 (4-Me), 18.4 (7-Me).

4,7-Dimethyl-1,2,3-tris-(4-methylbenzyl)-1*H*-indene (9a). 4,7-Dimethyl-2-(4-methylbenzyl)-indene (1.3 g, 5.2 mmol) was dissolved in 100 mL of dry THF under argon atmosphere, and the mixture was cooled down to −50 °C. Then a 1.6 M solution of *n*-BuLi in hexane (3.6 mL, 5.8 mmol) was added slowly, keeping the temperature below −50 °C. The reaction mixture was allowed to warm up to 0 °C and then cooled down to −70 °C. Next, 4-methylbenzyl chloride (0.76 mL, 5.8 mmol) was added slowly, keeping the temperature at −70 °C. The reaction mixture was stirred at −70 °C for 10 min and then allowed to warm up to room temperature. The reaction mixture was then cooled down to −50 °C again, and another 3.6 mL (5.8 mmol) of a 1.6 M solution of *n*-BuLi in hexane were added slowly and the reaction mixture was allowed to warm up to 0 °C and then again cooled down to −70 °C. Another 0.76 mL (5.8 mmol) of 4-methylbenzyl chloride was added slowly, and then the reaction mixture was stirred at room temperature overnight. The reaction was quenched by carefully adding 10 mL of a saturated solution of NH_4Cl. The reaction mixture was then extracted with 3 × 100 mL EtOAc, washed with 100 mL brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography using hexane:DCM 5:1 as an eluent. Yield: 1.14 g (48%). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.07 (m, 2 H, 2-Bn-*m*-H), 7.03 (d, 2 H, ^3J = 7.9 Hz, 2-Bn-*o*-H), 6.89 (m, 2 H, 1-Bn-*m*-H), 6.85,(d, 1 H, ^3J = 8.0 Hz, H-6), 6.84 (m, 2 H, 3-Bn-*m*-H), 6.83 (d, 1 H, ^3J = 8.0 Hz, H-5), 6.67 (m, 2 H, 2-Bn-*m*-H), 6.40 (d, 2 H, ^3J = 7.9 Hz, 3-Bn-*o*-H), 4.02 (d, 1 H, ^2J = 17.1 Hz, 3-CH_2Ph), 3.92 (d, 1 H, ^2J = −17.1 Hz, 3-CH_2Ph), 3.86 (d, 1 H, ^2J = −15.6 Hz, 2-CH_2Ph), 3.56 (d, 1 H, ^2J = −15.6 Hz, 2-CH_2Ph), 3.62 (dd, 1 H, ^3J = 4.7 Hz, ^3J = 4.6 Hz, H-1), 3.46 (dd, 1 H, ^2J = −14.1 Hz, ^3J = 4.6, 1-CH_2Ph), 3.16 (dd, 1 H, ^2J = −14.1 Hz, ^3J = 4.7 1-CH_2Ph), 2.47 (s, 3 H, 4-Me), 2.32 (s, 3 H, *p*-*Me*Bn),

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2.31 (s, 3 H, *p*-*Me*Bn), 2.26 (s, 3 H, *p*-*Me*Bn), 2.11 (s, 3 H, 7-Me). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 130.0 (C-5), 129.1 (2-Bn-*o*-*C*), 129.0 (1-Bn-*o*-C), 128.9 (3-Bn-*m*-*C*), 128.6 (1-Bn-*m*-*C*), 128.5 (2-Bn-*m*-*C*), 127.8 (3-Bn-*o*-*C*), 126.2 (C-6), 49.3 (C-1), 33.3 (1-CH_2Ph), 32.3 (2-CH_2Ph), 31.9 (3-CH_2Ph), 21.0 and 21.1 (1-*p*-*Me*Bn, 2-*p*-*Me*Bn, 3-*p*-*Me*Bn), 19.6 (4-Me), 19.0 (7-Me). The quaternary carbons were omitted due to overlapping and complex coupling patterns caused by the highly conjugated system.

4,7-Dimethyl-1,2,3-tris-(4-methoxybenzyl)-1*H*-indene (9b). Compound **8a** (3.9 g, 15 mmol) was dissolved in 100 mL of dry THF under argon atmosphere, and the mixture was cooled down to −50 °C. Then a 1.6 M solution of *n*-BuLi in hexane (11 mL, 18 mmol) was added slowly, keeping the temperature below −50 °C. The reaction mixture was allowed to warm up to 0 °C and then cooled down to −70 °C. Next, 4-methoxybenzyl chloride (2.2 mL, 16 mmol) was added slowly, keeping the temperature at −70 °C. The reaction mixture was stirred at −70 °C for 10 min and then allowed to warm up to room temperature. The reaction mixture was then cooled down to −50 °C again, and another 11 mL (16 mmol) of a 1.6 M solution of *n*-BuLi in hexane wasadded slowly and the reaction mixture was allowed to warm up to 0 °C and then again cooled down to −70 °C. Another 2.2 mL (16 mmol) of 4-methoxybenzyl chloride was added slowly, and then the reaction mixture was stirred at room temperature overnight. The reaction was quenched by careful addition of 10 mL of a saturated solution of NH_4Cl. The reaction mixture was then extracted with 3 × 100 mL EtOAc, washed with 100 mL of brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography using hexane:DCM 2:1 as an eluent. Yield: 4.33 g (58%). The product was still unpure after the purification, and was used as such in the following reactions.

Chlorodicarbonyl(η^5-4,7-dimethyl-1,2,3-tris-(4-methylbenzyl)-indenyl)ruthenium(II) (6a). Compound **9a** (540 mg, 1.18 mmol) was dissolved in 50 mL of dry THF under argon atmosphere, and the reaction mixture was cooled down to −60 °C. Then a 1.6 M solution of *n*-BuLi in hexane (0.81 mL, 1.30 mmol) was added slowly, keeping the temperature at −60 °C. The reaction mixture was allowed to return to room temperature and stirred for 30 min, after which 302 mg of $[Ru(CO)_3Cl_2]_2$ dissolved in 5 mL of dry THF were added. The reaction mixture was stirred at room temperature for 70 h. The reaction was quenched by adding a few drops of H_2O, and then the solvent was evaporated. The crude product was purified by column chromatography (hexane:DCM 5:1 → hexane:DCM 2:1 → DCM). Yield: 310 mg (40%). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.00 (s, 2 H, H-5, H-6), 6.91 (m, 4 H, 1-Bn-*m*-H, 3-Bn-*m*-H), 6.71 (d, 4 H, ^3J = 8.0 Hz, 1-Bn-*o*-H, 3-Bn-*o*-H), 6.41 (m, 2 H, 2-Bn-*m*-H), 4.55 (d, 2 H, ^2J = −17.6 Hz, 1-CH_2Ph, 3-CH_2Ph), 4.02 (d, 2 H, ^2J = −17.6 Hz, 1-CH_2Ph, 3-CH_2Ph), 3.78 (s, 2 H, 2-CH_2Ph), 2.43 (s, 6 H, 4-Me, 7-Me), 2.27 (s, 6 H, 1-*p*-*Me*Bn, 3-*p*-*Me*Bn), 2.01 (s, 3 H, 2-*p*-*Me*Bn). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 197.3 (Ru-CO), 136.0 (2-Bn-*p*-*C*), 135.8 (1-Bn-*i*-*C*, 3-Bn-*i*-*C*). 135.7 (1-Bn-*p*-*C*, 3-Bn-*p*-*C*), 133.6 (2-Bn-*i*-*C*), 132.9 (4-C, 7-C), 131.1 (5-C, 6-C), 129.1 (1-Bn-*m*-*C*, 3-Bn-*m*-*C*), 128.7 (2-Bn-*m*-*C*), 128.4 (2-Bn-*o*-*C*), 127.1 (1-Bn-*o*-*C*, 3-Bn-*o*-*C*), 116.3 (2-C), 110.3 (3′-C, 7′-C), 89.3 (1-C, 3-C), 32.1 (1-CH_2Ph, 3-CH_2Ph), 31.8 (2-CH_2Ph), 21.0 (4-Me, 7-Me), 20.7 (1-*p*-*Me*Bn, 2-*p*-*Me*Bn, 3-*p*-*Me*Bn). MS: exact mass calcd for $C_{37}H_{35}{}^{102}RuO_2$ (M^+-Cl) 613.1681, found 613.1681; 585.16 (M^+-Cl-CO), 557.16 (M^+-Cl-2CO). Anal. Calcd for $C_{37}H_{35}ClO_2Ru$: C, 68.56; H, 5.44. Found: C, 68.38; H, 5.32 (average of two runs).

Chlorodicarbonyl(η^5-4,7-dimethyl-1,2,3-tris-(4-methoxybenzyl)-indenyl)ruthenium(II) (6b). Compound **9b** (395 mg, 0.78 mmol) was dissolved in 50 mL of dry THF under argon atmosphere, and the reaction mixture was cooled down to −60 °C. Then 0.54 mL (0.86 mmol) of a 1.6 M solution of *n*-BuLi in hexane was added slowly, keeping the temperature at −60 °C. The reaction mixture was allowed to warm up to room temperature and stirred for 30 min, after which 200 mg of $[Ru(CO)_3Cl_2]_2$ dissolved in 5 mL of dry THF were added. The reaction mixture was stirred at room temperature for 70 h. The reaction was quenched by adding a few drops of H_2O, and then the solvent was evaporated. The crude product was purified by column chromatography (hexane:DCM 2:1 → DCM). The obtained product was recrystallized from 10 mL of heptane:DCM (4:1). Yield: 62 mg (11%). ^{1}H NMR (600.13 MHz, $CDCl_3$): δ 7.01 (s, 2 H, H-5, H-6), 6.75 (d, 4 H, ^3J = 8.6 Hz, 1-Bn-*o*-H, 3-Bn-*o*-H), 6.66 (m, 4 H, 1-Bn-*m*-H, 3-Bn-*m*-H), 6.34 (d, 2 H, ^3J = 8.7 Hz, 2-Bn-*o*-H), 6.16 (m, 2 H, 2-Bn-*m*-H), 4.54 (d, 2 H, ^2J = −17.5 Hz, 1-CH_2Ph, 3-CH_2Ph), 4.02 (d, 2 H, ^2J = −17.5 Hz, 1-CH_2Ph, 3-CH_2Ph), 3.77 (s, 2 H, 2-CH_2Ph), 3.74 (s, 6 H, 1-*p*-*Me*OBn, 3-*p*-*Me*OBn), 3.54 (s, 3 H, 2-*p*-*Me*OBn), 2.44 (s, 6 H, 4-Me, 7-Me). ^{13}C NMR (150.90 MHz, $CDCl_3$): δ 197.2 (Ru-CO), 158.1 (1-Bn-*p*-*C*, 2-Bn-*p*-*C*, 3-Bn-*p*-*C*), 132.9 (C-4, C-7), 131.1 (C-5, C-6), 131.0 (1-Bn-*i*-*C*, 3-Bn-*i*-*C*), 129.9 (2-Bn-*o*-*C*), 128.7 (2-Bn-*i*-*C*), 128.2 (1-Bn-*o*-*C*, 3-Bn-*o*-*C*), 116.2 (C-2), 113.9 (1-Bn-*m*-*C*, 3-Bn-*m*-*C*), 113.2 (2-Bn-*o*-*C*), 110.4 (C-3′, C-7′), 89.1 (C-1, C-3), 55.2 (1-*p*-*Me*OBn, 3-*p*-*Me*OBn), 55.0 (2-*p*-*Me*OBn), 31.7 (1-CH_2Ph, 3-CH_2Ph), 31.3 (1-CH_2Ph), 20.6 (4-Me, 7-Me). MS: exact mass calcd for $C_{37}H_{35}{}^{102}RuO_5$ (M^+-Cl) 661.1528, found 661.1528; 633.14 (M^+-Cl-CO); 605.15 (M^+-Cl-2CO). Anal. Calcd for $C_{37}H_{35}ClO_5Ru$: C, 63.56; H, 5.34. Found: C, 63.00; H, 5.06. Because of the small amount of sample (>2 mg) remaining for elemental analysis, also contributing to the accuracy of analysis, this run could not be duplicated. While the carbon analysis for this compound is slightly greater than the 0.4% range viewed as establishing analytical purity, it is provided to illustrate the best value obtained. For spectroscopic analytical data (NMR, MS), see Supporting Information.

X-ray Structure Determination. For crystal data and other experimental details for compounds **5a** and **6a**, see Supporting Information. The data collection was performed with Agilent SuperNova dual wavelength diffractometer equipped with Atlas CCD area detector using Cu Kα radiation and CrysAlisPro program package.[14] The empirical (**5a**) absorption correction with SCALE3 ABSPACK scaling algorithm or analytical numeric one using multifaceted crystal (**6a**) was performed as implemented in CrysAlisPro program.[14] The structures were solved by direct methods by using the SHELXS-97[15] program or the SIR-97[16] program, and the full-matrix least-squares refinements on F^2 were performed using the SHELXL-97[15] program. Figures were drawn with Diamond 3.[17] For all complexes, the heavy atoms were refined anisotropically. The CH hydrogen atoms were included at the calculated distances with fixed displacement parameters from their host atoms (1.2 or 1.5 times of the host atom).

The site occupations of the groups involved in the rotational disorder of compound **5a** were refined to be 0.89 and 0.11. The atoms of the main component could be refined anisotropically (ISOR restraint for atoms O2A, C37, and C38A was used), while the atoms of the minor component had to be refined with isotropic displacement parameters and the C38B−O2B distance had to be fixed to 1.15 Å. For **6a**, the site occupation factors were initially refined, but as they converged to values very close to 0.5, the parameters were fixed (to 0.5 for both parts). Atoms of the both groups were refined anisotropically, but the ISOR restraint for atoms C38A, O1, O2a, and O2b had to be used.

Cytotoxicity Studies. HeLa (cervical adenocarcinoma) and Caco-2 (colorectal adenocarcinoma) cell lines were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 200 U/mL penicillin, 100 μg/mL streptomycin, and 0.3 g/mL L-glutamine. MCF7 (breast adenocarcinoma), A2780 (ovarian cancer), and A431 (skin epidermoid carcinoma) cell lines were maintained in MEM supplemented with 10% fetal bovine serum, 1% nonessential amino acids, and an antibiotic antimycotic mixture (AAM). All cell lines were purchased from the European Collection of Cell Culture (Salisbury, UK) and cultivated in a humidified atmosphere of 95% air/5% CO_2 at 37 °C.

For the first screening of the complexes, exponentially growing cells were seeded at a density of approximately 5000 cells/well, in 96-well flat-bottomed microplates, and were treated with the complexes after 48 h. The complexes were dissolved in DMSO and tested in concentrations ranging from 1 to 200 μM. Each experiment included 10 replicates for each concentration of complexes, and the results usually represent at least two independent experiments. The MTT assay was used to determine the cell viability or the cytotoxicity of test compounds.[18] The optical density was measured at 570 nm using a 96-well multiscanner autoreader. The IC_{50} were calculated by nonlinear regression analysis (GraphPad Prism 4.0, GraphPad Software; San Diego, CA, USA). Selected complexes were reevaluated similarly but

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after 72 h of incubation. Near-confluent cells were seeded in 96-well microplates at a density of 5000 cells/well and allowed to stand overnight, after which the medium containing the tested compound was added. Cisplatin, a clinically used anticancer complex, was used as reference compound.

Flow Cytometric Studies. Flow cytometric analysis was performed in order to characterize the cellular DNA content of treated A2780 cells as described earlier.[19] Briefly, after treatment for 24 h, the cells (200000/condition) were trypsinized (Gibco BRL, Paisley, U.K.), washed with phosphate-buffered saline (PBS), and fixed in 1.0 mL of cold 70% ethanol overnight at −20 °C. After fixation, the cells were collected and resuspended in PBS containing 100 μg/mL propidium iodide, 20 μg/mL RNA-ase, and 0.3% Triton-X-100 and then incubated at room temperature for 1 h. The cells were evaluated by flow cytometry using Partec CyFlow ML instrument (Partec GmbH, Münster, Germany). In each analysis, 20000 events were recorded, and the percentages of the cells in the different cell-cycle phases (subG1, G1, S, and G2/M) were calculated by using ModFit LT (Verity Software House, Topsham, ME, USA).

DNA Binding Studies. Calf thymus DNA (ct DNA) solutions of various concentrations (0−100 μM) were added to 20 μM buffered solutions (5 mM Tris, 50 mM NaCl, pH 7.2) of the metal complexes, and the same amount of ctDNA solution was added to the reference cell. Absorption spectra were recorded after equilibration at 37.0 °C for 10 min. The intrinsic binding constant, K, was determined from a plot of $D/\Delta\varepsilon_{ap}$ vs D according to eq 1.[20] D is the concentration of DNA in base pairs, $\Delta\varepsilon_{ap} = |\varepsilon_A - \varepsilon_F|$, $\varepsilon_A = A_{obs}/[\text{complex}]$, and $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$, with ε_B and ε_F corresponding to the extinction coefficient of the DNA-bound complex and unbound complex, respectively.

$$D/\Delta\varepsilon_{ap} = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \quad (1)$$

■ ASSOCIATED CONTENT

Supporting Information

Copies of ^{1}H and ^{13}C NMR for all new ligands and metal complexes, HRMS for new complexes. X-ray crystallographic data and data for all complexes in CIF format [CDCC 929859 (**5a**) and CCDC 929860 (**6a**)]. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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