En Route to Osmium Analogues of KP1019: Synthesis, Structure, Spectroscopic Properties and Antiproliferative Activity of trans-[OsIVCl4(Hazole)2]

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ABSTRACT:

By controlled Anderson type rearrangement reactions complexes of the general formula trans-[OsIVCl4(Hazole)2], where Hazole = 1H-pyrazole, 2H-indazole, 1H-imidazole, and 1H-benzimidazole, have been synthesized. Note that 2H-indazole tautomer stabilization in trans-[OsIVCl4(2H-indazole)2] is unprecedented in coordination chemistry of indazole. The metal ion in these compounds possesses the same coordination environment as ruthenium(III) in (H2 ind)[RuIIICl4(Hind)2], where Hind = 1H-indazole, (KP1019), an investigational anticancer drug in phase I clinical trials. These osmium(IV) complexes are appropriate precursors for the synthesis of osmium(III) analogues of KP1019. In addition the formation of an adduct of trans-[OsIVCl4(Hpz)2] with cucurbit[7]uril is described. The compounds have been comprehensively characterized by elemental analysis, EI and ESI mass spectrometry, spectroscopy (IR, UV—vis, 1D and 2D NMR), cyclic voltammetry, and X-ray crystallography. Their antiproliferative activity in the human cancer cell lines CH1 (ovarian carcinoma), A549 (nonsmall cell lung carcinoma), and SW480 (colon carcinoma) is reported.

INTRODUCTION

In the past six years several research groups reported on the synthesis and screening for antiproliferative activity in vitro of osmium coordination compounds and osmium(II)-arene species, as well as on studies of their reactivity with DNA model compounds. These investigations showed that osmium is another metal that deserves attention for the development of effective inorganic antitumor drugs. Osmium analogues that are equipotent to their ruthenium congeners have the advantage of being more inert under conditions relevant for drug formulation. Of particular academic interest are also the observed or established differences between ruthenium and osmium compounds in stabilization of higher oxidation states, magnitude of spin—orbit coupling, metal—ligand exchange reactions, and so forth.

In pursuing the goal of establishing structure—cytotoxicity relationships for osmium-azole-chlorido series and creating active metal-based antitumor drugs, we already reported the synthesis of trans-[OsIIICl2(Hazole)4]Cl, cis-[OsIIICl2(Him)4]Cl, and mer-[OsIIICl3(Hazole)4]. Compounds of the last type were insoluble in water, rendering tests for antiproliferative activity in vitro impossible, while trans-[OsIIICl2(Hazole)4]Cl showed noteworthy antiproliferative effects in the cell lines A549 (nonsmall cell lung cancer), CH1 (ovarian carcinoma), and SW480 (colon carcinoma), with IC50 values in the 10−6 to 10−7 M concentration range. trans-[OsIIICl2(Hpz)4]Cl was found to be more active than the ruthenium counterpart, whereas the osmium(III) imidazole complex was less cytotoxic than the ruthenium analogue in all three cell lines.

Quite recently, Anderson type rearrangement reactions of (H2 azole)2[OsIVCl6] in alcohols in the presence of tetrabutylammonium chloride enabled the synthesis of the next family of compounds, namely, (n-Bu4N)[OsIVCl4(Hazole)], where Hazole = 1H-pyrazole, 1H-indazole, 1H-imidazole, 1H-benzimidazole, or 1H2,4-triazole. The (n-Bu4N)+ was also substituted...
Chart 1. Compounds Reported in This Worka

| L         | A               |
|-----------|-----------------|
| Hpz       | (H2azole)2[OsCl6] |
| Hind      | (H2indazole)2[OsCl6] |
| Him       | (H2imidazole)2[OsCl6] |
| Hbzim     | (H2benzimidazole)2[OsCl6] |

*aAll complexes have been characterized by X-ray crystallography; atom labeling was introduced for assignments of resonances in NMR spectra.

by biologically relevant or nontoxic cations such as Na+ and (H2azole)4. Comparison of these osmium complexes with related ruthenium analogues, such as (H2azole)2[RuCl4(Hazole)]12–15 and (H2azole)[RuCl4(Hazole)]16 showed a clear preference for higher oxidation states of the heavier congener. The efficacy of the observed transformation is dependent on the electron-donating potency of the azole heterocycle and the solvent used. A deeper substitution at osmium(IV) was first observed in the case of imidazole and benzimidazole ligands with highest basicity and further investigated for other azole heterocycles.

In this paper, we report on the synthesis, X-ray diffraction studies, and spectroscopic characterization of osmium(IV) complexes, namely, trans-[OsH2Cl4(Hazole)2], where Hazole = 1H-pyrazole, 2H-indazole, 1H-imidazole, and 1H-benzimidazole. Of these compounds the osmium imidazole complex is the first example in which the ortho-quinoid tautomer is stabilized upon coordination to the transition metal ion. The cytotoxic activity of all complexes has been assessed in three human cancer cell lines originating from different solid malignancies, namely, CH1 (ovarian carcinoma), A549 (nonsmall cell lung carcinoma), and SW480 (colon carcinoma). In addition, attempts to encapsulate the complexes prepared into the macrocyclic host molecule cucurbit[7]uril are also described (Chart 1).

**EXPERIMENTAL SECTION**

**Materials.** The starting compounds [(DMSO)2H]+[OsCl4] and (H2azole)2[OsCl4] (Hazole = Hpz, Him, Hind, Hbzim) were synthesized as previously reported in the literature.16,17 OsO4 (99.8%) and N2H4·2HCl were purchased from Johnson Matthey and Fluka, respectively. 1H-Pyrazole, 1H-indazole, 1H-imidazole, 1H-benzimidazole, and 1-hexanol were from Aldrich and Fluka. All these chemicals were used without further purification. trans-[OsCl4(Hazole)2] (Hazole = Hpz, Him, Hbzim) complexes were prepared under argon atmosphere using standard Schlenk techniques. The synthesis of trans-[OsCl4(2H-ind)2] was performed in the solid state in an evacuated glass oven. Cucurbit[7]uril was generously offered by Marcel Wieland, Institute of Organic Chemistry, University of Vienna.

trans-[OsCl4(Hpz)2]2 (1). A suspension of (H2pz)2[OsCl4] (100 mg, 0.18 mmol) in 1-hexanol (2 mL) was stirred at 160 °C for 2 h. The red precipitate formed was filtered off, washed with ethanol and dried in vacuo. Yield: 40 mg, 47%. Anal. Calc for C6H8Cl4N4Os (M = 547.40 g/mol): C, 15.70; H, 2.13; N, 9.86. Found: C, 15.80; H, 1.86; N, 9.25. ESI-MS in MeOH (positive): m/z 567 [OsCl4(Hpz)2]+, 467 [OsCl4(Hpz)2−]+. ESI-MS in MeOH (negative): m/z 332 [OsCl4−], 467 [OsCl4(Hpz)2−]+, ESI-MS in MeOH (positive): m/z 491 [OsCl4(Hpz)2]+, 956 [OsCl4(Hpz)2+2Na]+. ESI-MS (negative): m/z 192 [Os]+, 329 [OsCl4(Hpz)+], 346 [OsCl4(Hpz)+], 432 [OsCl4(Hpz)+], 467 [OsCl4(Hpz)+].

trans-[OsCl4(Him)2]2 (2). A suspension of (H2im)2[OsCl4] (100 mg, 0.25 mmol) was heated in the solid state at 150 °C in a glass oven for 45 h. The black product was obtained with methanol in a Soxhlet extractor for 24 h. The dark blue residue was precipitated as a solid from the extraction solvent used. A deeper substitution at osmium(IV) was first observed in the case of imidazole and benzimidazole ligands with highest basicity and further investigated for other azole heterocycles.

In this paper, we report on the synthesis, X-ray diffraction studies, and spectroscopic characterization of osmium(IV) complexes, namely, trans-[OsH2Cl4(Hazole)2], where Hazole = 1H-pyrazole, 2H-indazole, 1H-imidazole, and 1H-benzimidazole. Of these compounds the osmium imidazole complex is the first example in which the ortho-quinoid tautomer is stabilized upon coordination to the transition metal ion. The cytotoxic activity of all complexes has been assessed in three human cancer cell lines originating from different solid malignancies, namely, CH1 (ovarian carcinoma), A549 (nonsmall cell lung carcinoma), and SW480 (colon carcinoma). In addition, attempts to encapsulate the complexes prepared into the macrocyclic host molecule cucurbit[7]uril are also described (Chart 1).
Table 1. Crystal Data and Details of Data Collection for 1, 2, 3·2DMSO, 4, and 5·11.25H2O

|         | 1              | 2              | 3·2DMSO         | 4              | 5·11.25H2O      |
|---------|----------------|----------------|----------------|----------------|-----------------|
| empirical formula | C26H23ClN4O3  | C26H23ClN4O3  | C26H23ClN4O3  | C26H23ClN4O3  | C26H23ClN4O3  |
| fw      | 568.28         | 568.28         | 624.42         | 568.28         | 1833.88         |
| space group | P21/c         | P21/c         | P21/c         | Pna21         | Pna21         |
| a [Å]   | 6.5110(4)      | 9.925(3)      | 9.2546(3)     | 9.8508(3)     | 31.5673(9)     |
| b [Å]   | 7.1695(5)      | 12.022(3)     | 14.6087(5)    | 12.1309(4)    | 16.9392(6)     |
| c [Å]   | 7.4983(5)      | 6.9802(15)    | 7.6323(3)     | 6.9496(2)     | 14.3896(5)     |
| α [deg] | 115.562(4)     |                |               |               |                 |
| β [deg] | 110.932(4)     | 108.128(11)   | 99.294(2)     | 108.036(2)    |                 |
| γ [deg] | 92.719(4)      |                |               |               |                 |
| V [Å³]  | 286.36(3)      | 791.5(3)      | 1018.32(6)    | 789.66(4)     | 7694.5(4)      |
| Z       | 1              | 2              | 2              | 2              | 4               |
| λ [Å]   | 0.70170        | 0.71073        | 0.70173        | 0.71073        | 0.71073         |
| ρcalo [g cm⁻³] | 2.715        | 2.384          | 2.036          | 2.390          | 1.583           |
| crystal size [mm³] | 0.20 ± 0.06 × 0.04 | 0.20 ± 0.06 × 0.01 | 0.50 ± 0.25 × 0.03 | 0.44 ± 0.20 × 0.02 | 0.20 ± 0.18 × 0.04 |
| T [K]   | 100            | 100            | 100            | 100            | 100             |
| µ [mm⁻¹] | 12.035         | 8.732          | 7.001          | 8.753          | 1.890           |
| R₁       | 0.0237         | 0.0313         | 0.0205         | 0.0184         | 0.0486          |
| wR₂     | 0.0552         | 0.0729         | 0.0462         | 0.0450         | 0.1310          |
| GOF      | 1.071          | 0.949          | 1.067          | 1.090          | 1.050           |

a R₁ = Σ||F₁| - |F₂||/Σ|F₁||, \( wR₂ = \left( \sum w(F_{₁}^2 - F_{₂}^2)^2/\sum w(F_{₂}^2)^2 \right)^{1/2} \cdot \text{GOF} = \left( \sum (w(F_{₁}^2 - F_{₂}^2))/(n - p) \right)^{1/2} \), where n is the number of reflections and p is the total number of parameters refined.

Found: C, 29.64; H, 1.90; N, 9.68. ESI-MS in MeOH (negative): m/z 331 [OsCl(C₅Hⱸ)₄], 448 [OsCl(Hbzim) – H]⁻, 567 [OsCl₂(Hbzim)₂ – H]⁻. MIR, cm⁻¹: 591, 658, 689, 733, 795, 988, 1127, 1246, 1420, 1945, 2018, 2150, 2189, 2235, 2364, and 3338. FIR, cm⁻¹: 161, 320, 349, 643. UV–vis (DMSO), λmax nm (ε M⁻¹ cm⁻¹): 354 (10 000), 373 (6176), 390 (5310), 418 (4333), 437 (6176), 454 (3362), 513 (4075). ¹H NMR (DMSO-d₆, 500.32 MHz): δ = 5.58 (s, 1 H, H₅), 5.48 (d, 1 H, J = 8.17 Hz, H₆), 7.61 (d, 1 H, J = 8.18 Hz, H₆ ar), 6.71 (t, 1 H, J = 7.26 Hz, H₅ ar), 9.00 (s, 1 H, H₂) ppm. ¹³C [¹H] NMR (DMSO-d₆, 125.81 MHz): δ = 114.64 (C₆ ar), 122.64 (67.1) (C₆ ar), 127.45 (67.4) (C₆ ar), 133.73 (45.8) (C₅ ar), 135.48 (5.55) (C₅ ar), 159.43 (C₁ ar), 200.46 (C₂ ar) ppm. Suitable crystals for X-ray diffraction study were grown from a solution of 4 in DMSO.

trans-[OsCl₂(Hpz)₂-cucurbit[7]uril]·2DMSO (5·11.25H₂O). A suspension of 1 (4 mg, 0.009 mmol) and cucurbit[7]uril (10 mg, 0.009 mmol) in water (3 mL) was heated at 100 °C for 2.5 h in a closed Schlenk vial. The hot solution was filtered. Suitable crystals of 5·11.25H₂O for X-ray diffraction study were grown from the filtrate at room temperature over 24 h.

Physical Measurements. Elemental analyses were performed by the Microanalytical Laboratory of the Faculty of Chemistry of the University of Vienna. MIR spectra were obtained by an ATR unit with a Perkin-Elmer 370 FTIR 2000 instrument (4000–400 cm⁻¹). FTR spectra were recorded on the same instrument in transmission mode using CsI pellets. UV–vis spectra were recorded on a Perkin-Elmer Lambda 20 UV–vis spectrophotometer using samples dissolved in DMSO, DMF, methanol, or water containing 1% DMSO. Electrospary ionization mass spectrometry was carried out with a Bruker Esquire 3000 instrument (Bruker Daltonics, Bremen, Germany) by using methanol as solvent. Electron impact mass spectrometry was carried out on a Finnigan MAT95 mass spectrometer. Expected and measured isotope distributions were compared. Cyclic voltammograms were measured in a three-electrode cell using a 2 mm diameter glassy carbon disk working electrode, a platinum auxiliary electrode, and an Ag/AgCl reference electrode containing 0.1 M AgNO₃. Measurements were performed at room temperature using a EG&G PARC potentiostat/galvanostat model 273A. Deaeration of solutions was accomplished by passing a stream of argon through the solution for 5 min prior to the measurement and then maintaining a blanket atmosphere of argon over the solution during the measurement. The potentials were measured in 0.2 M (n-Bu₄N)[BF₄]₀/DMSO using [Fe(n-C₅H₇)₂]⁺ (E₁/₂ = +0.68 V vs NHE) as internal standard and are quoted relative to NHE. Thermogravimetry was performed under nitrogen atmosphere on a Mettler Toledo TGA/SDTA851 instrument. The ¹H, ¹³C and ¹⁵N NMR spectra were recorded at 500.32, 125.81, and 150.91 MHz on a Bruker DPX500 (UltraShield Magnet) in DMSO-d₆, D₂O, and D₂O/DMSO. UV, VIS spectrophotometry was performed on a Perkin-Elmer 370 FTIR 2000 instrument in transmission mode.

Cell Lines and Culture Conditions. CH1 (ovarian carcinoma, human) cells were a gift from Lloyd R. Kelland (CRC Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, U.K.). A549 (non-small cell lung cancer, human) and SW480 (colon carcinoma, human) cells were kindly provided by Brigitte Marian (Institute of Cancer Research, Department of Medicine I, Medical University of Vienna, Austria). Cells were grown in 75 cm² culture flasks (Iwaki/Asahi Technoglass) as adherent monolayer cultures in Minimal Essential Media 199 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S).
Cytotoxicity in Cancer Cell Lines. Cytotoxicity in the cell lines mentioned above was determined by the colorimetric MTT assay (MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, purchased from Fluka). For this purpose, cells were harvested from culture flasks by trypsinization and seeded in 100 μL/well aliquots in MEM supplemented with 10% heat-inactivated fetal bovine serum, 1 mM sodium pyruvate, 4 mM l-glutamine, and 1% nonessential amino acids (100 × stock solution) into 96-well microculture plates (Iwaki/Asahi Technoglass) in the following densities to ensure exponential growth of untreated controls throughout the experiment: 1.5 × 10^4 (CH1), 4.0 × 10^3 (AS49), and 2.5 × 10^3 (SW480) viable cells per well. Cells were allowed to settle and resume proliferation for 24 h and were then exposed to the test compounds by addition of 100 μL/well aliquots of appropriate dilutions in the same medium. For this purpose, compounds were dissolved in DMSO first and diluted in medium such that the effective DMSO content did not exceed 0.5% whenever necessary. After exposure for 96 h, the medium was replaced by 100 μL/well RPMI 1640 medium (supplemented with 10% heat-inactivated fetal bovine serum and 4 mM l-glutamine) plus 20 μL/well solution of MTT in phosphate-buffered saline (5 mg/mL) (all purchased from Sigma-Aldrich). After incubation for 4 h, medium/MTT mixtures were removed, and the formazan precipitate formed by viable cells was dissolved in DMSO (150 μL/well). Optical densities at 550 nm (corrected for unspecific absorbance at 690 nm) were measured with a microplate reader (Tecan Spectra Classic) to yield relative quantities of viable cells as percentage of untreated controls, and 50% inhibitory concentrations (IC50) were calculated by interpolation. Evaluation is based on at least two (in case of inactivity) or three independent experiments, each comprising triplicate samples.

RESULTS AND DISCUSSION

Synthesis. By exploring further the Anderson type rearrangement reactions, which already enabled the synthesis of (azole)penta-chloridoosmate(IV) complexes, we obtained trans-bis(azole)tetrachloridoosmium(IV) complexes following the reaction scheme:

$$\text{(H}_2\text{azole})_2\text{[OsIVCl}_6\text{]} \rightarrow \text{(H}_2\text{azole})\text{[OsIVCl}_5\text{(Hazole)}\text{]} \rightarrow \text{[OsIVCl}_4\text{(Hazole)}_2\text{]}$$

The reactions were performed in suspensions of the starting compounds (H2azole)2[OsIVCl6], where Hazole = 1H-pyrazole, 1H-imidazole, and 1H-benzimidazole, in 1-hexanol at 160 °C to yield 1, 3, and 4. The indazole product 2 was obtained by heating the indazolium hexachloridoosmate(IV) in the solid state at 150 °C. The liberation of two molecules of hydrochloric acid was followed by thermogravimetric analysis and the formation of bis-azole complexes by NMR spectroscopy. The trans-[OsIVCl4-H(Hazole)2] complexes can also be prepared starting from (H2azole)[OsIVCl5(Hazole)] providing further evidence for Anderson type rearrangement reaction in the solid state (Supporting Information, Figure S1). The complexes 1–4 were synthesized in yields ranging from 19 to 82%. It is worth noting that in the case of indazole the only product isolated was trans-[OsIVCl4(2H-ind)2]2. All four compounds are non-electrolytes and hence show poor solubility in aqueous media. Therefore, attempts to encapsulate them in a cucurbit[7]uril host molecule to improve the aqueous solubility and use the latter as a vehicle for drug delivery to the cell, as reported for platinum24,25 and metallocene26 anticancer agents, have been undertaken. However, instead of inclusion complexes adduct 5 resulted from reaction of 1 with cucurbit[7]uril, as described for other metal chlorido complexes.27–30

Crystal Structures. The results of X-ray diffraction studies of complexes 1, 2, 3·2DMSO, 4, and 5·11.25H2O are shown in Figures 1 and 2, respectively. Selected bond lengths and angles are quoted in Table 2. To our knowledge 1–4 are the first bis(azole)tetrachloridoosmium(IV) complexes characterized by X-ray crystallography as individual compounds.

Complex 1 crystallized in the triclinic centrosymmetric space group P1, while compounds 2, 3·2DMSO, and 4 crystallized in the monoclinic space group P21/c. There is one molecule of 1
and two molecules of 2–4 in the corresponding unit cells. In addition, complex 3 contains one molecule of cocristallized DMSO per asymmetric unit. The osmium ion in all four complexes lies on a center of symmetry, and the centrosymmetric moieties are of point group symmetry C3. The osmium atom displays distorted octahedral coordination geometry with four chlorido ligands in the equatorial positions and two azole ligands bound axially. Comparison of the bond lengths in the coordination environment of osmium in 1–4 shows (Table 2) that the longer the axial bonds, the shorter are the equatorial ones.

Both trans-azole ligands in 1–4 lie in one plane in contrast to what was found in (Ph2P)[(trans-RuIIICl4 (1H-ind)2]], where the 1H-indazole ligands are significantly twisted.31 The plane through both azole ligands crosses the equatorial plane between chloride ligands, the torsion angles ΘC1–Os–N1–N2l, ΘCl2–Os–N2–N1l, ΘCl2–Os–N1–Cl2, and ΘCl1–Os–N1–Cl1 being −30.4(4), −33.2(6), −31.6(2), and −47.8(2)°, respectively.

Of particular interest is the structure of indazole derivative 2 (Figure 1), in which the indazole is stabilized in a quinoid form (Supporting Information, Figure S2). The H1 proton in the 1H NMR spectra of trans-[OsIVCl4(Hpz)2] was enabled by a homonuclear decoupling experiment. H4 shows a broad signal at 6.48 ppm. In the 13C NMR spectrum of trans-[OsIVCl4(Hpz)2] through the portal of cucurbit[7]uril could experience strong repulsive forces at distances less than the sums of van der Waals radii (portal diameter 5.4 Å, cavity width 7.3 Å, and Cl2...Cl4 vector in 5 of 4.74 Å). Although the cucurbit[7]uril does form an inclusion complex with a metal halide, namely, cis-[SnCl2(H2O)2], a more sophisticated mechanism of portal passage and formation of the complex was suggested.34 First a tetrahedral SnCl4 molecule enters the host molecule, which then undergoes hydration. Partial inclusion of oxaloplain into cucurbit[7]uril has been also reported.35

**NMR Spectra.** The 1H, 13C, and 15N NMR spectra show signals due to coordinated azole heterocycles (1–4). The assignment of the protons and carbon atoms of the azole heterocycle is in some cases hindered by the temperature-independent paramagnetism of low-spin d3 osmium(IV) which causes some broadening of the signals and large shifts relative to uncoordinated azoles at room temperature. Protons close to the metal center generally show larger shifts to negative values. However, it should be noted that signals are remarkably sharp for the majority of the compounds studied. We succeeded to obtain two-dimensional NMR spectra for the compounds 1, 3, and 4 ([13C,1H HSQC, 15N,1H HSQC (1, 3), 13C,1H HMBC, 1H,1H COSY]).

trans-[OsCl4(Hpz)2]. The H1 proton in 1 at 19.21 ppm was identified from the 15N,1H HSQC plot with a chemical shift for N1 at 115.50 ppm. Because of the C3 local symmetry of the coordinated pyrazole, the H3 and H5 signals do not overlap as is the case for the metal-free pyrazole. The resonance signal H5 is observed as a singlet at −2.97 ppm, while H3 is observed as a doublet at −5.59 ppm. The assignment of the H3 and H5 signals was enabled by a homonuclear decoupling experiment. H4 shows a broad signal at 6.48 ppm. In the 13C [1H] NMR spectra of 1 the carbon resonances of C4, C5, and C3 appear at 73.52, 181.14, and 218.41 ppm, respectively.

trans-[OsCl4(Him)2]. The H1 proton in 3 at 12.29 ppm was identified from the 15N,1H HSQC plot with the chemical shift for N1 at 157.08 ppm. The H3 and H5 resonances do not overlap as is the case for the metal-free imidazole. The singlets at −0.51, 7.29, and 7.86 ppm were attributed to H2, H6, or H3; a more precise assignment failed. The corresponding carbon resonances of C2, C4, and C3 were found at 119.52 (7.29), 114.45 (7.86), and 188.36 (−0.51) ppm. The 13C,1H HMBC plot does not enable the assignment of C2, C4, C5, and H2, H6, H3 because of the same number of cross peaks.

trans-[OsCl4(Hzim)2]. Compound 4 shows two singlets, two doublets, and two triplets in the 1H NMR spectrum. The H3
The proton is found as a singlet at 9.00 ppm, while a strongly shifted singlet at 5.58 ppm was assigned to H₂, which is in close proximity to the coordination site. The two doublets were attributed to H₄ and H₇, whereas the two triplets to H₅ and H₆. In the ¹³C{¹H} NMR spectra the carbon resonances of C₄ and C₇ are seen at 122.64 ± 6.71 and 133.73 ± 4.58 ppm, while those for C₅, C₆ at 127.45 ± 6.74, 135.48 ± 5.55 and C₂ at 200.46 ppm, respectively. There are two signals at 114.64 and 159.43 ppm for the quarternary carbons C₈ and C₉. Neither the ¹³C,¹H HMBC nor the ¹H,¹H COSY allowed to differentiate between H₄/H₇ (C₄/C₇) and H₅/H₆ (C₅/C₆).

**Electrochemical Behavior.** The cyclic voltammograms (CVs) of the complexes 1–4 in DMSO (0.2 M (n-Bu₄N)[BF₄]/DMSO) at a carbon disk working electrode, recorded with a scan rate of 0.2 V/s, display a reversible one-electron wave attributed to the Os⁴⁺ → Os³⁺ process with potential values ranging from 0.31 to 0.50 V and an irreversible single electron reduction wave (f⁺⁻) attributed to the Os³⁺ → Os²⁺ process with Eₚ potential values between −1.17 and −1.55 V versus NHE (Figure 3, Table 3). The redox waves Os⁴⁺/Os³⁺ are characterized by a peak-to-peak separation (ΔEₚ) of 68−71 mV and an anodic peak current (iₚᵃ) that is almost equal to the cathodic peak current (iₚᶜ), as expected for reversible electron transfer processes. The one-electron nature of these processes was verified by comparing the peak current height (iₚ) with that of standard ferrocene/ferrocenium couples under identical experimental conditions. The reduction potentials Os⁴⁺/Os³⁺ are in the following order: E₁/₂(1) > E₁/₂(2) > E₁/₂(4) > E₁/₂(3), which agrees quite well with the relative electron-donating

![Figure 3. Cyclic voltammogram of 0.2 M solution of 3 in DMSO at a carbon disk working electrode at a scan rate of 0.2 V/s, starting the scan in cathodic direction.](image)

![Figure 4. UV–vis spectra of an aqueous solution (containing 1% DMSO) of 4, measured immediately after dissolution and 24 h thereafter.](image)

![Figure 5. Concentration–effect curves of compounds 1–4 in (A) A549, (B) CH1, and (C) SW480 cells, obtained by the MTT assay (96 h exposure).](image)
The values of $E_{\text{calc}}$ for Os(IV)/Os(III) are obtained by using this formula for comparison with the $E_0$ values measured for Os(IV)/Os(III) in Table 3.

**Aqueous Solubility and Resistance to Hydrolysis.** The solubility of the complexes 1–4 in water containing 1% DMSO at 298 K varies from 1.9 (1 and 3) to 2.1 mM (4). The complexes remain intact in this medium for at least 24 h as is shown for complex 4 in Figure 4 by UV–vis spectroscopy. The optical spectra of 1–4 in DMSO are shown in Supporting Information, Figure S7.

**Cytotoxicity in Cancer Cell Lines.** The cytotoxicity of compounds 1–4 was assessed by means of the MTT assay in three human cell lines originating from different malignant tumors. A 96 h exposure yielded the concentration IC50, μM a necessary for at least three independent experiments, as obtained by the MTT assay (exposure time: 96 h). 8 C. Bartel, M. A. Jakupe, unpublished results.

**Final Remarks.** Anderson type transformation of (H2azole)2[OsIVCl6] in 1-hexanol and in the solid state resulted in complexes with the general formula trans-[OsIVCl6(Hazole)2], where Hazole = 1H-pyrazole, 1H-imidazole, 2H-indazole, or 1H-benzimidazole. Comparison of the described complexes with their ruthenium analogues (H2azole)[RuIVCl4(Hazole)] provides further evidence for the higher oxidation state preference of the heavier congener. Despite the nonelectrolyte character of the prepared compounds, the solubility in water containing 1% DMSO was borderline to allow the assessment of their antiproliferative activity in vitro against human cancer cell lines. An attempt to improve the solubility of the complexes by encapsulation in cucurbit[7]uril was described. The complexes trans-[OsIVCl6(Hazole)2] are characterized for the first time as individual compounds by X-ray crystallography. A remarkable finding is the stabilization of the quinoid-type 2H-indazole in 2 coordinated to metal via N1. Further attempts to prepare osmium(IV) complexes with 1H-indazole tautomer and inclusion complexes with cucurbit[8]uril and cucurbit[10]uril are underway in our laboratory.

**Supporting Information.** Thermogravimetric data for (H2ind)2[OsCl6] and (H2ind)[OsCl5(1H-ind)] (Figure S1), the unit cells for 1 and 2 (Figures S2, S3) as well as hydrogen bonding interactions in the crystal structures of 2, 3, 2DMSO, and 4 (Figures S4–S6), UV–vis spectra of 1–4 in DMSO (Figure S7). Crystallographic data for 1, 2, 3, 2DMSO, 4, and $\Sigma$ 11.25H2O in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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