ORGANOMETALLICS

Article

pubs.acs.org/Organometallics

Cytotoxicity of Ruthenium–Arene Complexes Containing β-Ketoamine Ligands

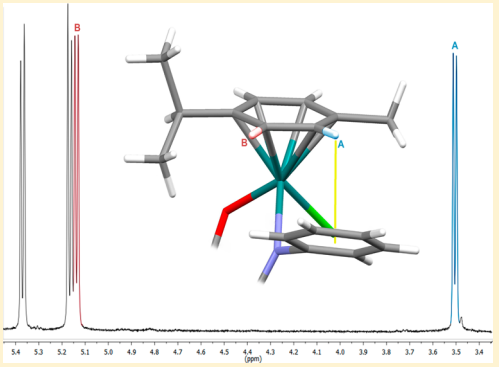
Riccardo Pettinari,*[,†] Claudio Pettinari,[†] Fabio Marchetti,[‡] Catherine M. Clavel,[§] Rosario Scopelliti,[§] and Paul J. Dyson*[,§]

[†]School of Pharmacy and [‡]School of Science and Technology, University of Camerino, via S. Agostino 1, 62032 Camerino MC, Italy

[§]Institute of Chemical Sciences and Engineering, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland

Supporting Information

ABSTRACT: New ruthenium(II) arene derivatives (arene = *p*-cymene, benzene, hexamethylbenzene) containing β-ketoamine ligands L′ (HL′ in general; in detail, $HL^{ph,ph}$ = (4*Z*)-3-methyl-4-((phenylamino)(phenyl)-methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one, $HL^{naph,ph}$ = (4*Z*)-3-methyl-4-((phenyamino)(naphthalen-2-yl)methylene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one, $HL^{et,ph}$ = (4*Z*)-3-methyl-4-(1-(phenylamino)propylidene)-1-phenyl-1*H*-pyrazol-5(4*H*)-one) have been synthesized and characterized by spectroscopy (IR, ESI-MS, ^1H and ^{13}C NMR) and elemental analysis. The ligands in the anionic form coordinate ruthenium in a chelating κ^2N,O-bidentate fashion, affording 1:1 derivatives of the formula [Ru(arene)(L′)Cl]. Further reaction of [Ru(*p*-cymene)(L′)Cl] with $AgPF_6$ or PTA (PTA = 1,3,5-triaza-7-phosphaadamantane) in methanol affords [Ru(*p*-cymene)(L′)(CH_3OH)]-[PF_6] and [Ru(*p*-cymene)(L′)(PTA)]Cl, respectively. The solid-state structures of the ligand $HL^{et,ph}$ and complexes [Ru(*p*-cymene)($L^{ph,ph}$)Cl] (**1**), [Ru(p-cymene)($L^{naph,ph}$)Cl] (**4**), and [Ru(*p*-cymene)($L^{et,ph}$)Cl] (**7**) have been determined by single-crystal X-ray diffraction. The antitumor activity of both the ligands and complexes has been evaluated against the human ovarian carcinoma cell line A2780 and its cisplatin-resistant equivalent A2780R, some of the complexes showing significant cytotoxicity toward the cisplatin-resistant cell line.



INTRODUCTION

Organometallic compounds play a unique role in medicinal chemistry because of their physicochemical properties that include chemical stability and structural diversity combined with relevant photo- and electrochemical properties.[1] Among the various classes of metal complexes developed as anticancer agents, ruthenium arene based organometallics have recently attracted considerable interest, especially the RAPTA family[2] and ethylene-1,2-diamine complexes,[3] which have undergone extensive in vivo evaluation. It is interesting to note that RAPTA complexes have a low cytotoxicity in vitro but a high antimetastatic activity in vivo as well as an intrinsic antiangiogenic activity and the ability to reduce growth of certain primary tumors.[4] In contrast, ruthenium arene complexes containing ethylenediamine chelating ligands show very high cytotoxicities and in vivo reduce tumor growth.[5] In addition to these families of compounds a number of different types of auxiliary supporting ligands have been used in conjugation with the ruthenium arene fragment, including acetylacetones,[6] pyridines,[7] bipyridines,[8] and N,O-chelating ligands such as glycine, alanines, and phenylalanines.[9]

β-Ketoamine ligands are an interesting class of N,O-bidentate ligands that can be readily modified to fine tune the steric and electronic environment around a ruthenium ion. Interestingly, the two donor atoms of these β-ketoamine ligands, i.e. the N and O atoms, exhibit two opposing features: the oxygen atom is a hard donor able to stabilize a higher oxidation state of the ruthenium atom, whereas the nitrogen atom is less hard and, accordingly, is suitable to better stabilize the lower oxidation state of the ruthenium atom. On coordination, both atoms could help a metal to form stable direct (coordination) bonds with potential biomolecular targets. We have previously reported an extensive study[10] on the coordination chemistry of ruthenium arene fragments with 4-acyl-5-pyrazolone ligands, unsymmetrical β-diketones containing a pyrazole ring fused to the chelating moiety, and in this paper, we report the preparation and characterization of some ruthenium arene complexes containing some β-ketoamine bases derived from the same 4-acyl-5-pyrazolones. The effect on cytotoxicity of β-ketoamine, arene, and ancillary ligands coordinated to ruthenium has been widely investigated against human ovarian carcinoma cells sensitive to (A2780) and resistant to (A2780R) cisplatin.

EXPERIMENTAL SECTION

Materials and Methods. The dimers $[Ru(\eta^6\text{-arene})Cl_2]_2$ (arene = *p*-cymene, benzene, hexamethylbenzene) were purchased from Aldrich and TCI Europe and were used as received. The acylpyrazolone ligands HQ^{ph} (3-methyl-1-phenyl-4-benzoyl-5-pyrazolone), HQ^{naph} (3-

Received: November 20, 2012
Published: December 18, 2012

methyl-1-phenyl-4-(1-naphthoyl)-5-pyrazolone), and HQ^{et} (3-methyl-1-phenyl-4-propionyl-5-pyrazolone) were synthesized using literature methods.[11] All other materials (products) were obtained from commercial sources and were used as received. IR spectra were recorded from 4000 to 600 cm^{-1} on a Perkin-Elmer Spectrum 100 FT-IR instrument. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded on a 400 Mercury Plus Varian instrument operating at room temperature (400 MHz for ^1H, 100 MHz for ^{13}C, and 161 MHz for ^{31}P). Referencing is relative to TMS (^1H and ^{13}C) and 85% H_3PO_4 (^{31}P). Positive and negative ion electrospray mass spectra were obtained with an HP Series 1100 MSI detector spectrometer, using an acetonitrile mobile phase. Solutions (3 mg/mL) for electrospray ionization mass spectrometry (ESI-MS) were prepared using reagent-grade acetonitrile. Mass and intensities were compared to those calculated using IsoPro Isotopic Abundance Simulator, version 2.1.28. Melting points are uncorrected and were taken on an STMP3 Stuart scientific instrument and on a capillary apparatus. Samples for microanalysis were dried in vacuo to constant weight (20 °C, ca. 0.1 Torr), and analyses were performed on a Fisons Instruments 1108 CHNS-O elemental analyzer. Electrical conductivity measurements (Λ_M, reported as S cm^2 mol^{-1}) of acetonitrile and dichloromethane solutions of the complexes were recorded using a Crison CDTM 522 conductimeter at room temperature.

Synthesis of the Proligands HL′. *HL^{ph,ph}*. To a solution of HQ^{ph} (1-phenyl-3-methyl-4-benzoyl-5-pyrazolone, 5.00 g, 18 mmol) in ethanol (75 mL) was added dropwise a solution of aniline (1.70 g, 18 mmol). The solution was stirred under reflux for 24 h. The solvent was removed under reduced pressure, and dichloromethane (10 mL) was added. The mixture was filtered, and *n*-hexane (20 mL) was added to the solution to form a diphase that was stored at 4 °C. Yellow crystals were obtained and collected (4.70 g, 13 mmol, yield 94%). The compound is soluble in diethyl ether, alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents. Anal. Calcd for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 78.07; H, 5.42; N, 11.70. IR (cm^{-1}): 3350−3070 br ν(N−H···O), 1609 s ν(C═O), 1583 s, 1574 s, 1534 w, 1499 s ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): δ 1.61 (s, 3H, C3-CH_3), 6.82 (d, 2H), 7.04−7.52 (m, 11H), 8.02 (s, 2H), 13.03 (sbr, 1H, −NH). ^{13}C NMR ($CDCl_3$, 298 K): δ 16.3 (s, C3-CH_3), 101.6 (s, *C*4), 119.5, 124.0, 126.2, 128.7, 129.23, 130.7, 131.7, 137.7, 139.1, 148.3, 162.4, 165.9 (s, ligand). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 354 [100] [$H_2L^{ph,ph}$]$^+$. ESI-MS (−) CH_3OH (*m*/*z*, relative intensity %): 352 [100] [$L^{ph,ph}$]$^-$.

The synthesis was performed as for HL^{naph,ph} using 1-phenyl-3-methyl-4-naphthoyl-5-pyrazolone (5.90 g, 18 mmol) and aniline (1.70 g, 18 mmol). The compound is soluble in diethyl ether, alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents. Mp: 162−163 °C. Anal. Calcd for $C_{27}H_{21}N_3O$: C, 80.37; H, 5.25; N, 10.41. Found: C, 79.97; H, 5.25; N, 10.37. IR (cm^{-1}): 3400−3050 br ν(N−H···O), 1614 s ν(C═O), 1589 sh, 1578 s, 1539 s ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): δ 1.57 (s, 3H, C3-CH_3), 6.85 (d, 2H), 7.04−7.91 (m, 13H), 8.03 (d, 2H), 13.19 (sbr, 1H, -NH). ^{13}C NMR ($CDCl_3$, 298 K): δ 16.4 (s, C3-CH_3), 101.9 (s, *C*4), 119.5, 123.9, 124.7, 125.2, 126.1, 127.5, 128.0, 128.3, 128.6, 128.9, 129.0, 129.2, 132.7, 133.9, 137.7, 139.1, 148.3, 162.2, 165.9 (s, ligand). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 829 [100] [$(HL^{naph,ph})_2Na$]$^+$; 404 [50] [$H_2L^{naph,ph}$]$^+$. ESI-MS (−) CH_3OH (*m*/*z*, relative intensity %): 827 [100] [$(L^{naph,ph})(HL^{naph,ph})Na$]$^-$; 402 [50] [$L^{naph,ph}$]$^-$.

HL^{et,ph}. The synthesis was performed as for HL^{ph,ph} using 1-phenyl-3-methyl-4-propionyl-5-pyrazolone (4.14 g, 18 mmol) and aniline (1.70 g, 18 mmol). The compound is soluble in diethyl ether, alcohols, acetone, acetonitrile, DMSO and chlorinated solvents. Anal. Calcd for $C_{19}H_{19}N_3O$: C, 74.73; H, 6.26; N, 13.76. Found: C, 74.36; H, 6,10; N, 13.53. IR (cm^{-1}): 3400−3050br ν(N−H···O), 1616 ν(C═O), 1580 s, 1537 s, 1500 w ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): 1.20 (t, 3H, CH_2CH_3), 2.46 (s, 3H, C3-CH_3), 2.71 (q, 2H, CH_2CH_3), 7.14−7.46 (m, 8H), 8.03 (d, 2H), 13.06 (sbr, 1H, −NH). ^{13}C NMR ($CDCl_3$, 298 K): δ 13.8 (s, CH_2CH_3), 17.1 (s, C3-CH_3), 22.4 (s, CH_2CH_3), 99.1 (s, *C*4), 119.5 s, 124.6 s, 126.4 s, 128.1 s, 128.9 s, 129.8, 136.9, 139.2, 146.9, 166.4, 169.5 (s, ligand). ESI-MS (+) CH_3CN (*m*/*z*, relative intensity %): 305 [100] [$H_2L^{et,ph}$]$^+$. ESI-MS (−) CH_3CN (*m*/*z*, relative intensity %): 305 [100] [$L^{et,ph}$]$^-$.

Synthesis of the Ruthenium Complexes. *[Ru(η^6-cymene)-(L^{ph,ph})Cl] (**1**).* To the proligand HL^{ph,ph} (230.8 mg, 0.653 mmol) dissolved in methanol (20 mL) was added KOH (36.6 mg, 0.653 mmol). The mixture was stirred for 1 h at room temperature, and then [Ru(η^6-*p*-cymene)Cl_2]$_2$ (200.0 mg, 0.326 mmol) was added. The resulting solution was refluxed with stirring for 24 h. The solvent was removed under reduced pressure, and dichloromethane (10 mL) was added. The mixture was filtered to remove sodium chloride. The solution was concentrated to ca. 2 mL and stored at 4 °C. The red crystals obtained and collected (366.3 mg, 0.587 mmol, yield 91%) were soluble in diethyl ether, alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 248−250 °C. Anal. Calcd for $C_{33}H_{32}N_3RuClO$: C, 63.61; H, 5.18; N, 6.74. Found: C, 63.51; H, 5.19; N, 6.66. IR (cm^{-1}): 3050 w, 3123 w, 1589 s, 1570 s, 1525 m, 1499 s ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): δ 1.19 (s, 3H, C3-CH_3), 1.23 (d, 3H, ^3J = 6.9 Hz, $CH_3C_6H_4CH(CH_3)_2$), 1.30 (d, 3H, ^3J = 6.9 Hz, $CH_3C_6H_4CH(CH_3)_2$), 2.06 (s, 3H, $CH_3C_6H_4CH(CH_3)_2$), 2.74 (sept, 1H, ^3J = 6.9 Hz, $CH_3C_6H_4CH(CH_3)_2$), 3.50 (d, 1H, ^3J = 5.6 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.14 (d, 1H, ^3J = 5.6 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.16 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.36 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.79−7.97 (m, 15H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.5 (s, C3-CH_3), 18.6 (s, $CH_3C_6H_4CH(CH_3)_2$), 21.1 and 23.7 (s, $CH_3C_6H_4CH(CH_3)_2$), 30.8 (s, $CH_3C_6H_4CH(CH_3)_2$), 80.2, 83.6, 84.2, 86.8, 96.5, 101.4 (s, $CH_3C_6H_4CH(CH_3)_2$), 102.3 (s, *C*4), 120.6, 124.7, 124.9, 125.1, 127.3, 127.4, 128.1, 128.3 128.4, 128.6, 129.0, 136.0, 139.5, 149.4, 156.1, 160.3, 168.5 (s, ligand L^{ph,ph}). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 588 [100] [Ru(η^6-cym)(L^{ph,ph})]$^+$. ESI-MS (−) CH_3OH (*m*/*z*, relative intensity %): 659 [100] [Ru(η^6-cym)(L^{ph,ph})Cl_2]$^-$.

*[Ru(η^6-benzene)(L^{ph,ph})Cl] (**2**).* The synthesis was performed as for **1** using [Ru(η^6-benzene)Cl_2]$_2$ (163.3 mg, 0.326 mmol). **2** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 278−280 °C. Anal. Calcd for $C_{29}H_{24}N_3ORuCl$: C, 60.53; H, 4.36; N, 7.57. Found: C, 60.40; H,4.26; N, 7.44. IR (cm^{-1}): 3058 w, 1602 s, 1591 s, 1567 s, 1531 s ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): δ 1.20 (s, 3H, C3-CH_3), 5.20 (s, 6H, C_6H_5), 6.80−7.94 (m, 15H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.4 (s, C3-CH_3), 84.7s (s, C_6H_6), 102.8 (s, *C*4), 120.8, 125.0, 125.1, 125.3, 127.4, 127.5, 127.6, 128.2, 128.3, 128.8, 129.1, 135.8, 139.4, 149.5, 156.3, 160.5s, 168.7 (s, ligand L^{ph,ph}). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 532 [100] [Ru(η^6-benz)(L^{ph,ph})]$^+$.

*[Ru(η^6-hexamethylbenzene)(L^{ph,ph})Cl] (**3**).* The synthesis was performed as for **1** using [Ru(η^6-hexamethylbenzene)Cl_2]$_2$ (218.3 mg, 0.326 mmol). **3** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 131−132 °C. Anal. Calcd for $C_{35}H_{36}N_3ClORu$: C, 64.55; H, 5.57; N, 6.64. Found: C, 64.2; H, 5.46; N, 6.41. IR (cm^{-1}): 3062 w, 1602 s, 1588 s, 1572 s, 1523 m, 1500 w ν(C═C; C═N). ^1H NMR ($CDCl_3$, 298 K): δ 1.15 (s, 3H, C3-CH_3), 1.77 (s, 18H, $C_6(CH_3)_6$), 6.67−8.03 (m, 15H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.2 (s, $C_6(CH_3)_6$), 15.8 (s, C3-CH_3), 92.0 (s, $C_6(CH_3)_6$), 102.2 (s, *C*4), 119.5, 122.0, 124.7, 124.8, 124.9, 126.9, 127.8, 128.3, 128.4, 128.7, 129.0, 129.1, 129.2, 137.1, 139.4, 149.5, 154.5, 160.3, 169.3 (s, ligand H^{ph,ph}). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 616 [100][Ru(η^6-hmb)(L^{ph,ph})]$^+$.

*[Ru(η^6-cymene)(L^{naph,ph})Cl] (**4**).* The synthesis was performed as for **1** using HL^{naph,ph} (263.3 mg, 0.653 mmol). **4** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 260−262 °C. Anal. Calcd for $C_{37}H_{34}ClN_3ORu$: C, 66.01; H, 5.09; N, 6.24. Found: C, 65.96; H, 5.13; N, 6.16. IR (cm^{-1}): 3048 w, 1589 s, 1570 s, 1530 s ν(C═C; C═N). ^1H NMR (DMSO, 273 K): δ 0.9 (s, 6H, C3-CH_3), 1.25t and 1.34 (dd, 12H, $CH_3C_6H_4CH(CH_3)_2$), 1.95 (d, 6H, $CH_3C_6H_4CH(CH_3)_2$), 2.68 (m, 2H, $CH_3C_6H_4CH(CH_3)_2$), 3.60 (d, 1H, ^3J = 6.3 Hz, $CH_3C_6H_4CH(CH_3)_2$), 3.65 (d, 1H, ^3J = 6.3 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.26 (d, 1H, ^3J = 6.3 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.31 (d, 1H, ^3J = 6.3 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.40 (d, 1H, ^3J = 5.8 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.43 (d, 1H, ^3J = 5.8 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.55 (d, 2H,

dx.doi.org/10.1021/om301115e | *Organometallics* 2013, 32, 309−316

^3J = 6.3 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.80−7.90 (m, 30H), 7.98 (d, 4H). ^{1}H NMR (DMSO, 363 K): δ 0.9 (s, 3H, C3-CH_3), 1.21 and 1.29 (sbr, 6H, $CH_3C_6H_4CH(CH_3)_2$), 1.99 (s, 3H, $CH_3C_6H_4CH(CH_3)_2$), 2.72 (m, 1H, $CH_3C_6H_4CH(CH_3)_2$), 3.90 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 5.19 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 5.37 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 5.47 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 6.80−7.86 (m, 15H), 7.99 (d, 2H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.6 (s, C3-CH_3), 15.8 (s, C3-CH_3), 18.7 (s, $CH_3C_6H_4CH(CH_3)_2$), 21.2, 21.3, 23.8 (s, $CH_3C_6H_4CH(CH_3)_2$), 30.8s ($CH_3C_6H_4CH(CH_3)_2$), 80.2, 80.3, 83.8, 84.3, 84.4, 86.9, 96.5, 101.5, 101.6 ($CH_3C_6H_4CH(CH_3)_2$), 102.5, 102.7 (s, *C*4), 120.7, 120.8, 124.5, 124.8, 125.1, 125.3, 126.1, 126.3, 126.6, 126.7, 126.7, 126.8, 127.1, 127.2, 127.3, 127.4, 127.5, 127.7, 127.8, 120.0, 128.1, 128.3, 128.6, 128.7, 132.2, 132.4, 132.6, 132.7, 133.5, 133.6, 139.5, 149.4, 149.5, 156.1, 156.2, 160.3, 160.5, 168.3, 168.6 (s, ligand $L^{naph,ph}$). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 638 [100] [Ru(η^6-cym)($L^{naph,ph}$)]$^+$. ESI-MS (−) CH_3OH (*m*/*z*, relative intensity %): 710 [100] [Ru(η^6-cym)($L^{naph,ph}$)Cl_2]$^-$.

*[Ru(η^6-benzene)($L^{naph,ph}$)Cl] (**5**).* The synthesis was performed as for **1** using $HL^{naph,ph}$ (263.3 mg, 0.653 mmol) and [Ru(η^6-benzene)Cl_2]$_2$ (163.3 mg, 0.326 mmol). **5** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 350 °C dec. Anal. Calcd for $C_{33}H_{26}ClN_3ORu$: C, 64.23; H, 4.25; N, 6.81. Found: C, 64.56; H, 4,13; N, 6.56. IR (cm^{-1}): 3053 w, 1589 s, 1568 s, 1523 s ν(C═C; C═N). ^{1}H NMR ($CDCl_3$, 298 K): δ 1.08 (s, 3H, C3-CH_3), 5.22 (d, 6H, C_6H_6), 6.80−7.80 (m, 15H), 7.94 (d, 2H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.6 (s, C3-CH_3), 84.8s (s, C_6H_6), 102.9 (s, *C*4), 120.6, 124.8, 125.0, 125.4, 127.6, 127.7, 127.8, 128.4, 128.5, 128.9, 129.1, 135.7, 139.6, 149.6, 156.5, 160.7, 168.9 (s, ligand $L^{naph,ph}$). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 582 [100] [Ru(η^6-benz)($L^{naph,ph}$)]$^+$.

*[Ru(η^6-hexamethylbenzene)($L^{naph,ph}$)Cl] (**6**).* The synthesis was performed as for **1** using $HL^{naph,ph}$ (263.3 mg, 0.653 mmol) and [Ru(η^6-hexamethylbenzene)Cl_2]$_2$ (218.3 mg, 0.326 mmol). **6** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 233−235 °C. Anal. Calcd for $C_{39}H_{38}ClN_3ORu$: C, 66.80; H, 5.46; N, 5.99. Found: C, 66.56; H, 5,33; N, 5.76. IR (cm^{-1}): 3047 w, 1601 s, 1589 s, 1567 s, 1528 s ν(C═C; C═N). ^{1}H NMR ($CDCl_3$, 298 K): δ 1.03 (d, 3H, C3-CH_3), 1.78 (s, 18H, $C_6(CH_3)_6$), 6.67−8.00 (m, 17H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.2 (s, $C_6(CH_3)_6$), 16.1, 16.2 (s, C3-CH_3), 89.8, 92.0 (s, $C_6(CH_3)_6$), 101.94 (s, *C*4), 119.6, 122.0, 124.0, 124.7, 125.0, 126.2, 126.4, 126.5, 127.8, 127.9, 128.2, 128.3, 128.6, 129.1, 131.9, 132.7, 132.8, 133.9, 134.5, 134.7, 139.1, 139.3, 148.3, 149.4, 149.5, 154.4, 154.6, 162.3, 169.3, 169.7 (s, ligand $L^{naph,ph}$). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 666 [100] [Ru(η^6-hmb)($L^{naph,ph}$)]$^+$.

*[Ru(η^6-cymene)($L^{et,ph}$)Cl] (**7**).* The synthesis was performed as for **1** using $HL^{et,ph}$ (199.3 mg, 0.653 mmol). **7** is soluble in alcohols, acetone, acetonitrile, DMSO, and chlorinated solvents and sparingly soluble in water. Mp: 221−222 °C dec. Anal. Calcd for $C_{29}H_{32}ClN_3ORu$: C, 60.56; H, 5.61; N, 7.31. Found: C, 60.37; H, 5.49; N, 7.16. IR (cm^{-1}): 3061 w, 1603 s, 1592 s, 1577 s, 1519 w ν(C═C; C═N). ^{1}H NMR ($CDCl_3$, 298 K): 0.99 (t, 3H, CH_2CH_3), 1.15 (d, 3H, ^3J = 6.8 Hz, $CH_3C_6H_4CH(CH_3)_2$), 1.19 (d, 3H, ^3J = 6.9 Hz, $CH_3C_6H_4CH(CH_3)_2$), 1.99 (s, 3H, $CH_3C_6H_4CH(CH_3)_2$), 2.34 (s, 3H, C3-CH_3), 2.45 (q, 2H, CH_2CH_3), 2.66 (sept, 1H, ^3J = 6.8 Hz, $CH_3C_6H_4CH(CH_3)_2$), 3.43 (d, 1H, ^3J = 5.6 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.02 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.12 (d, 1H, ^3J = 5.6 Hz, $CH_3C_6H_4CH(CH_3)_2$), 5.30 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 7.13−7.46 (m, 7H), 7.73 (d, 1H), 7.96 (d, 2H). ^{13}C NMR ($CDCl_3$, 298 K): δ 14.0 (s, C3-CH_3), 17.6 (s, CH_2CH_3), 18.5 (s, $CH_3C_6H_4CH(CH_3)_2$), 21.1 and 23.6 (s, $CH_3C_6H_4CH(CH_3)_2$), 24.9 (s, CH_2CH_3), 30.7 (s, $CH_3C_6H_4CH(CH_3)_2$), 79.6, 83.9, 84.8, 86.5, 95.9, 101.56 (s, $CH_3C_6H_4CH(CH_3)_2$), 100.6 (s, *C*4), 119.5, 120.7, 123.2, 124.6, 124.7, 125.9, 126.2, 126.4, 128.0, 128.6, 128.9, 129.7, 129.8, 139.6, 147.3, 156.0, 160.9, 172.0 (s, ligand $L^{et,ph}$). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 540 [100] [Ru(η^6-cym)($L^{et,ph}$)]$^+$.

*[Ru(η^6-cymene)($L^{ph,ph}$)(CH_3OH)][PF_6] (**8**).* Silver hexafluorophosphate (81.1 mg, 0.320 mmol) was added to a solution of [Ru(η^6-cymene)($L^{ph,ph}$)Cl] (**1**; 200.0 mg, 0.320 mmol) in methanol. The reaction mixture was refluxed for 24 h and then cooled to room temperature. The solvent was removed under reduced pressure, and chloroform (15 mL) was added. The mixture was filtered to remove AgCl, the solution was dried under vacuum, and the red residue was identified as **8** (159.6 mg, 208.6 mmol, 65% yield), which is soluble in alcohols, acetone, acetonitrile, and DMSO and sparingly soluble in water and chlorinated solvents. Mp: 143−145 °C. Anal. Calcd for $C_{34}H_{36}F_6N_3O_2PRu$: C, 53.40; H, 4.75; N, 5.49. Found: C, 53.03; H, 4.43; N, 5.44. Λ_m (MeCN, 298 K, 10^{-4} mol/L): 136 S cm^2 mol^{-1}. IR (cm^{-1}): 3500 br ν(H_2O), 3060 w, 1589 s, 1567 s, 1524 s ν(C═C; C═N), 827 s ν(PF_6). ^{1}H NMR (($CD_3)_2CO$, 298 K): δ 1.19 (s, 3H, C3-CH_3), 1.22 (d, 6H, $CH_3C_6H_4CH(CH_3)_2$), 2.04 (s, 3H, $CH_3C_6H_4CH(CH_3)_2$), 2.70 (sept, 1H, $CH_3C_6H_4CH(CH_3)_2$), 3.10 (s, 3H, CH_3OH), 5.25 and 5.64 (sbr, 4H, $CH_3C_6H_4CH(CH_3)_2$), 7.06−7.59 (m, 13H), 8.19 (d, 2H). ^{13}C NMR (($CD_3)_2CO$, 298 K): δ 15.3 (s, C3-CH_3), 18.4 (s, $CH_3C_6H_4CH(CH_3)_2$), 22.5 (s, $CH_3C_6H_4CH(CH_3)_2$), 32.3 (s, $CH_3C_6H_4CH(CH_3)_2$), 85.5, 98.0 (s, $CH_3C_6H_4CH(CH_3)_2$), 102.6 (s, *C*4), 121.8, 122.1, 127.1, 129.3, 129.5, 129.9, 130.1, 130.3, 136.1, 137.8, 138.5, 140.2, 142.0, 156.5, 158.5, 160.1 (s, ligand $L^{ph,ph}$). ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 588 [100][Ru(η^6-cym)($L^{ph,ph}$)]$^+$.

*[Ru(η^6-cymene)($L^{ph,ph}$)(PTA)]Cl (**9**).* PTA (PTA = 1,3,5-triaza-7-phosphaadamantane, 50.4 mg, 0.320 mmol) was added to a solution of [Ru(η^6-cymene)($L^{ph,ph}$)Cl] (**1**; 200.0 mg, 0.320 mmol) in methanol. The reaction mixture was refluxed for 2 h and then cooled to room temperature. The solution was concentrated to ca. 2 mL, and diethyl ether (25 mL) was added. The mixture was left at 277 K until an orange precipitate formed. The orange crystalline powder that was recovered by filtration and air-dried (162 mg, 0.208 mmol, 65%) was identified as **9**, which is soluble in alcohols, acetonitrile, DMSO,and acetone, sparingly soluble in water, and poorly soluble in chlorinated solvents. Mp: 170−172 °C. Anal. Calcd for $C_{39}H_{44}N_6ClOPRu$: C, 62.89; H, 5.95; N, 11.28. Found: C, 62.95; H, 6.07; N, 11.11. Λ_m (MeCN, 298 K, 10^{-4} mol/L): 145 S cm^2 mol^{-1}. IR (cm^{-1}): 3159 br, 3050 w, 1560 s, 1521 s ν(C═C; C═N). ^{1}H NMR ($CDCl_3$, 298 K): δ 1.12 (d, 3H, ^3J = 7.2 Hz, $CH_3C_6H_4CH(CH_3)_2$), 1.14 (d, 3H, ^3J = 7.2 Hz, $CH_3C_6H_4CH(CH_3)_2$), 1.25 (s, 3H, C3-CH_3), 1.59 (s, 3H, C$H_3C_6H_4CH(CH_3)_2$), 2.37 (sept, 1H, ^3J = 6.9 Hz, $CH_3C_6H_4CH(CH_3)_2$), 4.04 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 4.54 and 4.62 (d, 6H, J_{AB} = 13 Hz, PCH^AH^BN, PTA), 4.78 and 4.90 (d, 6H, J_{AB} = 14 Hz, NCH^AH^BN, PTA), 5.27 (d, 1H, ^3J = 6.0 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.12 (d, 1H, ^3J = 6.0 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.69 (d, 1H, ^3J = 7.6 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.93−7.83 (m, 15H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.6 (s, C3-CH_3), 17.8 (s, $CH_3C_6H_4CH(CH_3)_2$), 20.5 and 23.0 (s, $CH_3C_6H_4CH(CH_3)_2$), 30.9 (s, $CH_3C_6H_4CH(CH_3)_2$), 52.4 (d, PCH_2N, PTA), 73.1 (d, NCH_2N, PTA), 82.2, 86.8, 86.9, 90.1, 95.0, 96.9 (s, $CH_3C_6H_4CH(CH_3)_2$), 104.5 (s, *C*4), 120.2, 121.7, 124.2, 126.0, 126.3, 127.8, 128.2, 128.8, 129.2, 129.4, 135.2, 138.4, 149.6, 155.6, 159.4, 170.8 (s, ligand $L^{ph,ph}$). ^{31}P NMR ($CDCl_3$, 298 K): δ −29.31. ESI-MS (+) CH_3OH (*m*/*z*, relative intensity %): 745 [100][Ru(η^6-cym)($L^{ph,ph}$)(PTA)]$^+$, 588 [60][Ru(η^6-cym)($L^{ph,ph}$)]$^+$.

*[Ru(η^6-cymene)($L^{naph,ph}$)(CH_3OH)][PF_6] (**10**).* The synthesis was performed as for **8** using **4** (216.0 mg, 0.320 mmol). **10** is soluble in alcohols, acetone, acetonitrile, and DMSO and sparingly soluble in water and chlorinated solvents. Mp: 142−144 °C. Anal. Calcd for $C_{38}H_{38}F_6N_3O_2PRu$: C, 56.02; H, 4.70; N, 5.16. Found: C, 56.17; H, 4.54; N, 5.22. Λ_m (MeCN, 298 K, 10^{-4} mol/L): 122 S cm^2 mol^{-1}. IR (cm^{-1}): 3554 br ν(H_2O), 3060 w, 1591 s, 1571 s, 1525 s ν(C═C; C═N), 831 ν(PF_6). ^{1}H NMR (($CD_3)_2CO$, 298 K): δ 1.05 (3H, C3-CH_3), 1.26 (d, 6H, $CH_3C_6H_4CH(CH_3)_2$), 2.05 (s, 3H, C$H_3C_6H_4CH(CH_3)_2$), 2.77 (m, 1H, $CH_3C_6H_4CH(CH_3)_2$), 3.24 (s, 3H, CH_3OH), 5.18 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 5.27 (sbr, 1H, $CH_3C_6H_4CH(CH_3)_2$), 5.81 (sbr, 2H, $CH_3C_6H_4CH(CH_3)_2$), 6.98−7.85 (m, 13H), 8.19 (d, 2H). ^{13}C NMR (($CD_3)_2CO$, 298 K): δ 15.6 (s, C3-CH_3), 18.3 (s, $CH_3C_6H_4CH(CH_3)_2$), 22.3, 22.4 (s, $CH_3C_6H_4CH(CH_3)_2$), 31.9 (s, $CH_3C_6H_4CH(CH_3)_2$), 83.8, 84.2, 85.1, 85.3, 97.5, 100.2 ($CH_3C_6H_4CH(CH_3)_2$), 102.4 (s, *C*4), 119.1, 120.3, 125.9, 126.7, 128.6, 129.0, 129.4, 129.8, 133.3, 133.6, 133.8, 140.1, 149.5, 156.4,

160.5, 170.5. ESI-MS (+) CH_3OH (m/z, relative intensity %): 638 [100] [Ru(η^6-cym)Ru($L^{naph,ph}$)]$^+$.

*[Ru(η^6-cymene)($L^{naph,ph}$)(PTA)]Cl (**11**).* The synthesis was performed as for **9** using **4** (216.0 mg, 0.320 mmol). **11** is soluble in alcohols, acetonitrile, DMSO, and acetone, sparingly soluble in water, and poorly soluble in chlorinated solvents. Mp: 154−156 °C. Anal. Calcd for $C_{43}H_{46}ClN_6OPRu$: C, 62.20; H, 5.58; N, 10.12. Found: C, 62.04; H, 5.38; N, 9.96. Λ_m (MeCN, 298 K, 10^{-4} mol/L): 129 S cm^2 mol^{-1}. IR (cm^{-1}): 1590 s, 1571 s, 1524 s ν(C═C; C═N). ^{1}H NMR ($CDCl_3$, 298 K): δ 1.13−1.21 (m, 3H, C3-CH_3 and 6H, $CH_3C_6H_4CH(CH_3)_2$), 1.61 (s, 3H, $CH_3C_6H_4CH(CH_3)_2$), 2.40 (m, 1H, $CH_3C_6H_4CH(CH_3)_2$), 4.02 (d, 1H, ^3J = 6.0 Hz, $CH_3C_6H_4CH(CH_3)_2$), 4.05 (d, 1H, ^3J = 6.0 Hz, $CH_3C_6H_4CH(CH_3)_2$), 4.56 and 4.63 (d, 6H, J_{AB} = 13 Hz, PCH^{A}H^{B}N, PTA), 4.83 and 4.90 (d, 6H, J_{AB} = 14 Hz, NCH^{A}H^{B}N, PTA), 5.29 (d, 1H, ^3J = 6.4 Hz, $CH_3C_6H_4CH(CH_3)_2$), 6.13 (m, 1H, $CH_3C_6H_4CH(CH_3)_2$), 6.80−7.80 (m, 17H). ^{13}C NMR ($CDCl_3$, 298 K): δ 15.6, 15.9 (s, C3-CH_3), 17.9 (s, CH_3−C_6H_4−$CH(CH_3)_2$), 20.5, 23.0, 24.3 (s, CH_3−C_6H_4−$CH(CH_3)_2$), 30.9 (s, CH_3−C_6H_4-$CH(CH_3)_2$), 52.6 (d, PCH_2N, PTA), 73.2 (d, NCH_2N, PTA), 82.2, 86.9, 87.1, 90.1, 95.1, 97.1 (s, $CH_3C_6H_4CH(CH_3)_2$), 104.6, 104.9 (s, C4), 120.2, 120.6, 121.7, 121.9, 122.3, 124.6, 126.0, 126.3, 127.2, 127.6, 127.9, 128.1, 128.6. 128.9, 129.1, 129.4, 132.2, 132.5, 132.7, 132.8, 138.4, 149.5, 149.6, 155.6, 155.7, 159.5, 159.6, 170.6, 170.7 (s, ligand $L^{naph,ph}$). ^{31}P NMR ($CDCl_3$, 298 K): δ −31.35, −31.30. ESI-MS (+) CH_3OH (m/z, relative intensity %): 638 [60] [Ru(η^6-cym)($L^{naph,ph}$)]$^+$, 795 [100] [Ru(η^6-cym)(PTA)($L^{naph,ph}$)]$^+$.

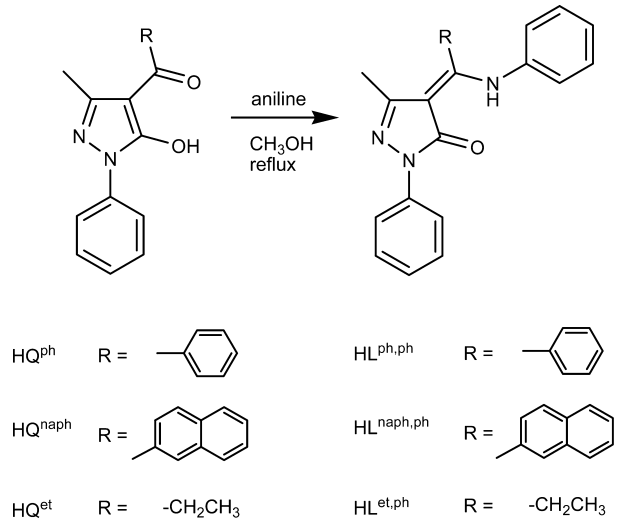
Single Crystal X-ray Diffraction Analysis. The diffraction data of the proligand $HL^{et,ph}$ and of complexes **1** and **7** were measured at low temperature (100(2) or 170(2) K) using Mo Kα radiation on a Bruker APEX II CCD diffractometer equipped with a κ geometry goniometer. The data sets were reduced by EvalCCD[12] and then corrected for absorption.[13] The data collection of compound **4** was carried out at room temperature using Mo Kα radiation on an Agilent Technologies SuperNova dual system in combination with an Atlas CCD detector. The data reduction was performed using Crysalis PRO,[14] and the solutions and refinements were performed with SHELX.[15] The crystal structures were refined using full-matrix least squares based on F^2 with all non-hydrogen atoms anisotropically defined. Hydrogen atoms were placed in calculated positions by means of the "riding" model.

Cell Culture and Evaluation of the Anticancer Activity. The human A2780 and A2780R ovarian carcinoma cells were obtained from the European Collection of Cell Cultures (Salisbury, U.K.). Cells were grown routinely in RPMI-1640 medium with 10% fetal calf serum (FCS) and antibiotics at 37 °C and 5% CO_2. Cytotoxicity was determined using the MTT assay (MTT = 3(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide). Cells were seeded in 96-well plates as monolayers with 100 μL of cell solution (approximately 20000 cells) per well and preincubated for 24 h in medium supplemented with 10% FCS. Compounds were prepared as DMSO solutions and then dissolved in the culture medium and serially diluted to the appropriate concentration, to give a final DMSO concentration of 0.5%. A 100 μL portion of the drug solution was added to each well, and the plates were incubated for another 72 h. Subsequently, MTT (5 mg/mL solution) was added to the cells and the plates were incubated for a further 2 h. The culture medium was aspirated, and the purple formazan crystals formed by the mitochondrial dehydrogenase activity of vital cells were dissolved in DMSO. The optical density, directly proportional to the number of surviving cells, was quantified at 590 nm using a multiwell plate reader, and the fraction of surviving cells was calculated from the absorbance of untreated control cells. Evaluation is based on means from two independent experiments, each comprising three microcultures per concentration level.

RESULTS AND DISCUSSION

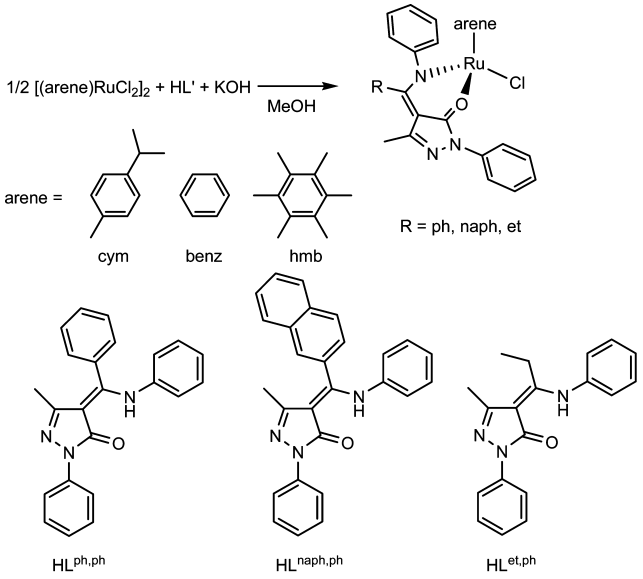
The proligands (HL′) were obtained in high yields via the condensation of 4-acyl-5-pyrazolones (HQ′) with aniline (Chart 1).

Chart 1



The novel ruthenium arene complexes **1**−**7** were prepared in high yield from the reaction of the appropriate dimer, [Ru(η^6-arene)Cl_2]$_2$, with the appropriate proligand and KOH in methanol (Chart 2). The complexes are air stable in solution

Chart 2



Complex	Arene	N-O ligand
1	cym	$L^{ph,ph}$
2	benz	$L^{ph,ph}$
3	hmb	$L^{ph,ph}$
4	cym	$L^{naph,ph}$
5	benz	$L^{naph,ph}$
6	hmb	$L^{naph,ph}$
7	cym	$L^{et,ph}$

and in the solid state and are highly soluble in most organic solvents and sparingly soluble in water. Conductivity measurements indicate a slight dissociation of the chloride in DMSO at room temperature; however, ionization increases with temperature and at 353 K it is almost complete (see the Supporting Information).

dx.doi.org/10.1021/om301115e | *Organometallics* 2013, 32, 309−316

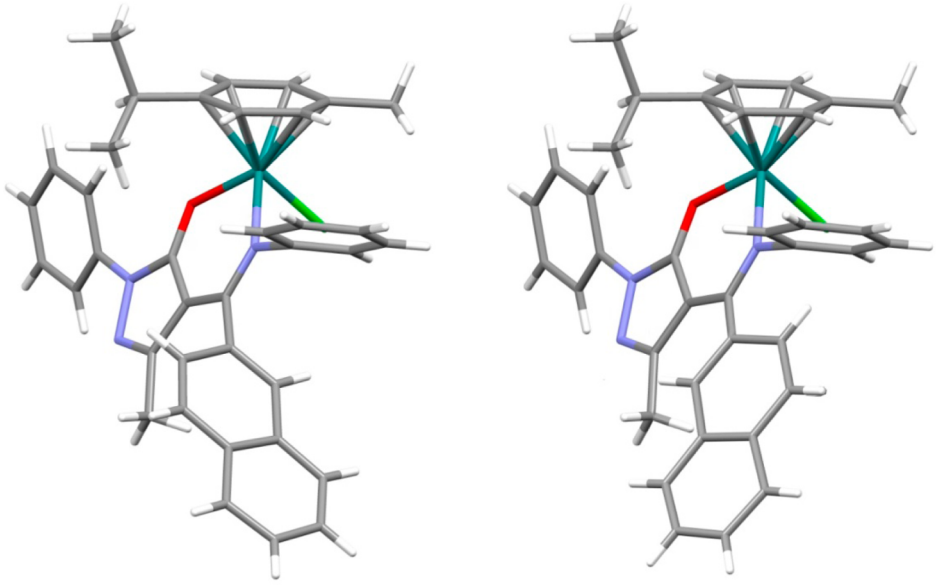


Figure 1. Proposed conformers of **4**, which differ in the orientation of the naphthyl group.

The IR spectra of **1**−**7** show the typical shift of the ν(C═O) vibrations to lower frequency upon coordination of the β-ketoamine proligands to the metal ion.[16] In the positive ESI mass spectra of **1**−**7** peaks due to the cationic fragment [Ru(arene)(L′)]$^+$, generated from loss of Cl$^-$, are observed as the predominant species.

The ^{1}H NMR spectra of **1**−**7** display a distinct shift of resonances of the β-ketoamine protons in comparison with the equivalent protons in the free proligands. The asymmetry (C_1) of the complexes induces significant modifications to the ^{1}H and ^{13}C NMR signals of the *p*-cymene moiety in **1**, **4**, and **7**; the ^{1}H NMR spectra of **1** and **7** in $CDCl_3$ show a doublet for each of the four *p*-cymene ring protons and two doublets for the methyl groups of the isopropyl moiety. Surprisingly, one of the four proton resonances attributable to the *p*-cymene ring is strongly shifted to higher fields, in the range 3.43−3.50 ppm, whereas the other three doublets are in the range 5.02−5.36 ppm, which is typical of ruthenium arene systems.[6]

The aforementioned high-field shift of one of the aromatic protons is likely due to the close vicinity of the phenyl group in the ammine moiety of the coordinated ligand, as confirmed by X-ray diffraction studies (see below). Also in the ^{13}C NMR spectra of **1** and **7** four different *p*-cymene ring carbons are observed in the range 79.6−86.5 ppm, together with two different methyl groups of the isopropyl moiety in the range 20.5−23.7 ppm.

A similar pattern has been observed in the ^{1}H and ^{13}C NMR of **4**, where, however, two sets of signals are observed. They are likely due to the presence in solution of two conformers differing in the orientation of the naphthyl group in the ligand (Figure 1).[17]

The presence of two conformers (1:1 ratio) has been observed also in the NMR spectra of the other complexes containing the L^{naph,ph} ligand (**5** and **6**). A variable-temperature ^{1}H NMR study of **4** in the range 273−323 K, carried out in $CDCl_3$, reveals unchanged spectra. In contrast, the same study performed in DMSO-d_6 shows the coalescence of the two sets of resonances at higher temperatures (Figure 2). In detail, at 273 K the aromatic protons of *p*-cymene show six separate doublets (integrating to 1H each) and a doublet (integration 2H), while the methyl groups of the isopropyl moiety give rise to two partially superimposed doublets. However, at higher temperature (343 K) all the peaks of the *p*-cymene ring protons broaden and at 363 K they coalesce into four broad resonances, due to rapid isomerization between the two forms on the NMR time scale. The ^{13}C NMR spectra of **4**−**6** further confirm the presence of two conformational isomers in solution—eight different *p*-cymene ring carbons being observed in the range 80.2−86.9 ppm (four for each isomer), together with the presence of four different resonances in the range 20.5−23.7 ppm for the methyl groups of the isopropyl moiety.

The chloride ligand in **1** and **4** can be easily removed by reaction with $AgPF_6$ in methanol, to afford [Ru(η^6-cymene)(L^{ph,ph})(CH_3OH)][PF_6] (**8**) and [Ru(η^6-cymene)(L^{naph,ph})(CH_3OH)][PF_6] (**10**), in which one methanol molecule takes the place of the chloride in the Ru coordination sphere. The chloride of **1** and **4** can also be replaced by the water-soluble phosphine 1,3,5-triaza-7-phosphaadamantane (PTA), affording the complexes [Ru(η^6-cymene)(L^{ph,ph})(PTA)]Cl (**9**) and [Ru(η^6-cymene)(L^{naph,ph})(PTA)]Cl (**11**), as depicted in Scheme 1.

Complexes **8**−**11** are soluble in alcohols, acetonitrile, DMSO, and acetone, sparingly soluble in water, and poorly soluble in chlorinated solvents. In acetonitrile **8**−**11** display conductivity values within the range typical of 1:1 electrolytes.[18] In the IR spectra of **8** and **10** a strong sharp absorption is observed at 827 and 831 cm^{-1} due to the PF_6^- counteranion.[19]

The ^{1}H NMR spectra of **9** and **11** show all the expected signals due to the coordinated *p*-cymene ring, β-ketoamine, and PTA. A doublet for each of the four *p*-cymene ring protons and two doublets for the methyl groups of the isopropyl moiety are observed. Moreover, the four doublets are shifted to lower field with respect to those observed in the spectra of **1** and **4**, in accordance with a stronger donor interaction between the arene moiety and the cationic ruthenium(II) center. The ^{1}H NMR spectra of **9** and **11** show two types of methylene protons in the coordinated PTA ligand, both displaying AB spin systems centered respectively at 4.58 (J_{AB} = 13 Hz) and 4.84 ppm (J_{AB} = 14 Hz).[20] The ^{31}P NMR spectrum of **9** contains a singlet at

dx.doi.org/10.1021/om301115e | *Organometallics* 2013, 32, 309−316

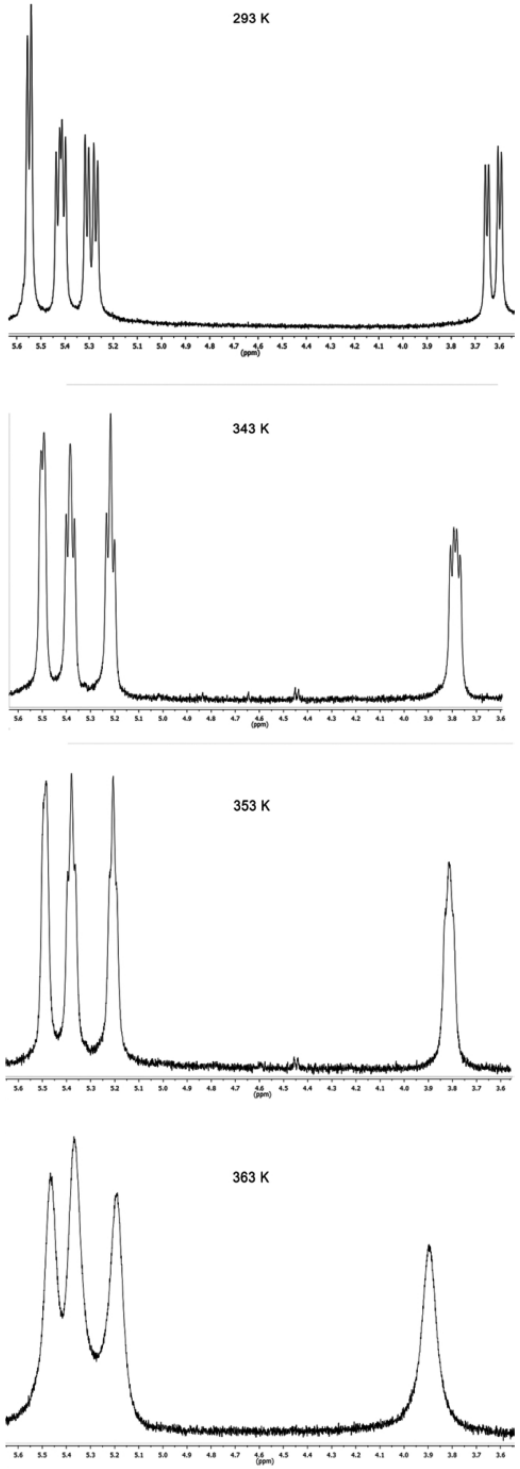
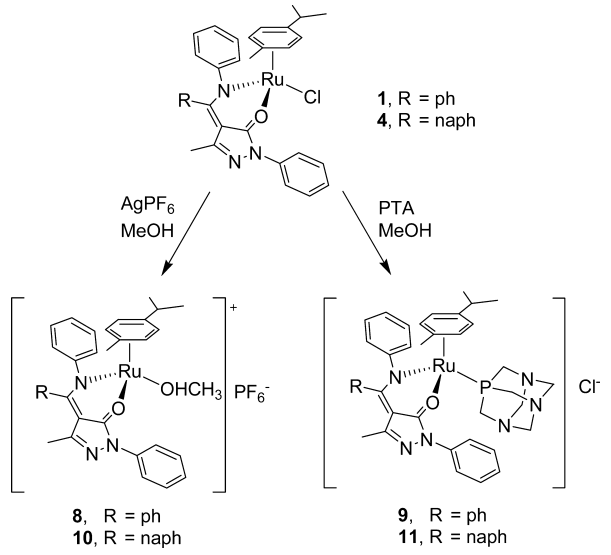


Figure 2. ^{1}H NMR spectra of **4** in DMSO-d_6 in the range 3.5−5.5 ppm at different temperatures.

−29.31 ppm, which is in the region typical for related complexes, in accordance with the existence of only one species in solution.[21] However, in the ^{31}P NMR spectrum of **11** a doublet centered at δ −32.3 ppm is observed, confirming the presence of two different conformers in solution.

The ESI mass spectra of **9** and **11** show the presence of two peaks, the most intense corresponding to [Ru(η^6-cymene)(L′)(PTA)]$^+$ species and the less intense peak assignable to the fragment [Ru(η^6-cymene)(L′)]$^+$, due to dissociation of the PTA ligand.

Scheme 1



The solid state structures of HL^{et,ph}, **1**, **4**, and **7** were established by X-ray crystallography (see Table 1S in the Supporting Information and also see the Experimental Section for details of the crystals, data collection, and structure refinement). The crystal structure of the proligand HL^{et,ph} (Figure 3) shows two independent molecules within the asymmetric unit. The molecules are mostly planar, except for the ethyl and the −NHPh substituents (mostly due to steric hindrance). A strong intramolecular H bond occurs between the −NH and the O of the pyrazolone moiety (N−H···O: 2.706(2), 2.671(2) Å; 143(2), 145(2)°).

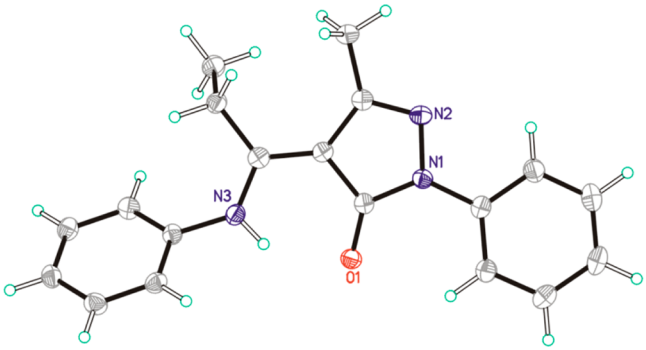


Figure 3. X-ray structure of the proligand HL^{et,ph} Key bond length (Å) for HL^{et,ph}: N2−N3 = 1.408(1) Å.

Complexes **1**, **4**, and **7** (Figure 4) show the usual piano-stool geometry about the ruthenium center. The bond distances around the Ru atom vary over a small range (Ru−O = 2.066(2)−2.098(2) Å; Ru−N = 2.113(4)−2.127(2) Å; Ru−Cl = 2.429(2)−2.440(1) Å) and are comparable to those of similar compounds.[22]

All the compounds were tested using the MTT assay (see Experimental Section for details) for their in vitro anticancer activity against two human ovarian carcinoma cell lines, A2780 and A2780R, the latter having acquired resistance to cisplatin (Table 1).

dx.doi.org/10.1021/om301115e | *Organometallics* 2013, 32, 309−316

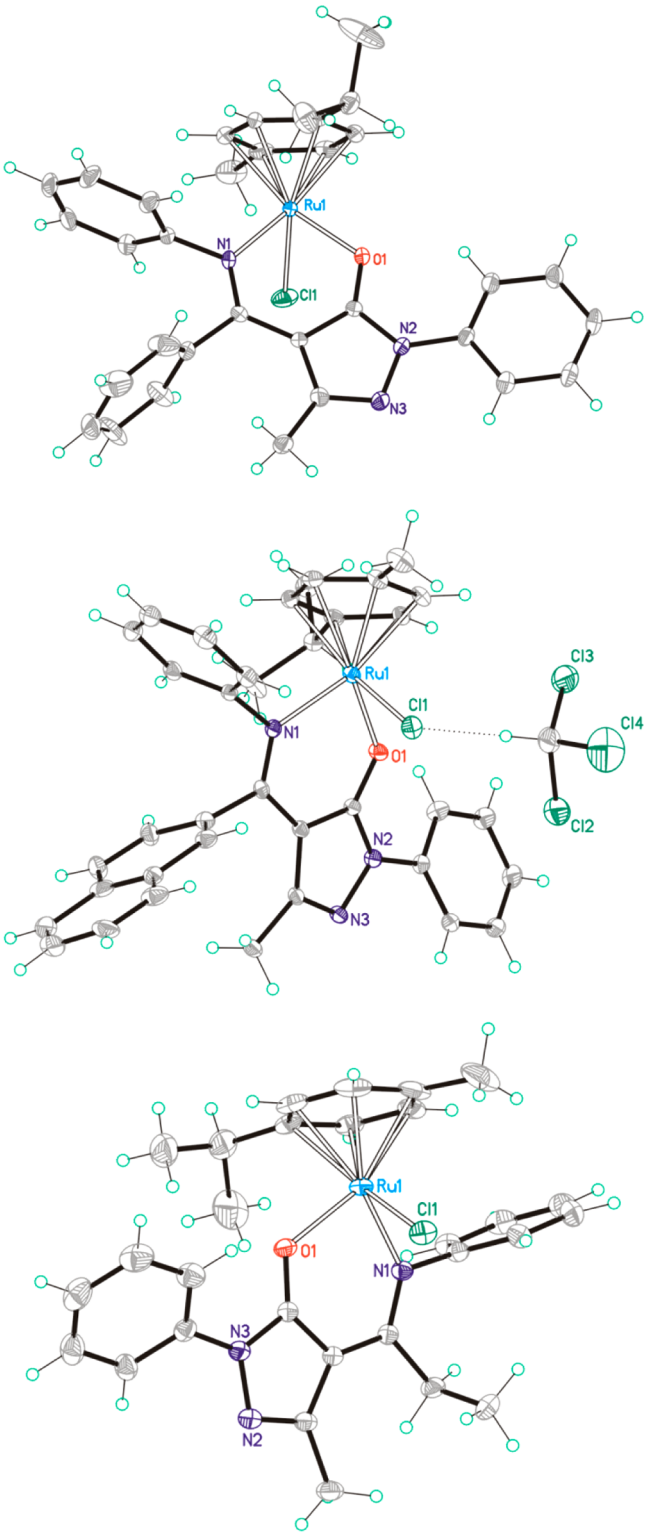


Figure 4. X-ray structures of (top) **1**, (middle) **4**, and (bottom) **7**. Key bond lengths (Å) and angles (deg) for **1**: Ru−O = 2.098(2) Å, Ru−N1 = 2.120(2), Ru−Cl = 2.439(6), N2−N3 = 1.398(4); O−Ru−N1 = 87.60(8), O−Ru−Cl = 84.91(6), N1−Ru−Cl: 83.85(7). Key bond lengths (Å) and angles (deg) for **4**: Ru−O = 2.081(4), Ru−N1 = 2.113(4), Ru−Cl = 2.429(2), N2−N3 = 1.410(7); O−Ru−N1 = 88.84(2), O−Ru−Cl = 85.16(1), N1−Ru−Cl = 84.84(1). Key bond lengths (Å) and angles (deg) for **7**: Ru−O = 2.066(2) Å, Ru−N1 = 2.127(2), Ru−Cl = 2.437(6), N2−N3 = 1.393(3); O−Ru−N1 = 88.46(7), O−Ru−Cl = 83.88(5), N1−Ru−Cl = 82.96(5).

Table 1. IC_{50} Values of $HL^{naph,ph}$, $HL^{ph,ph}$, $HL^{et,ph}$, and Complexes 1−11 in Ovarian Carcinoma Cell Lines A2780 and A2780R (Cisplatin-Resistant)

compd	IC_{50} in A2780 (μM)	IC_{50} in A2780R (μM)
$HL^{naph,ph}$	14.4 ± 1.6	14.0 ± 1.7
$HL^{ph,ph}$	275 ± 76	271 ± 70
$HL^{et,ph}$	116 ± 2.4	204 ± 17
1	7.6 ± 1.1	14.0 ± 1.7
2	10.2 ± 0.7	7.7 ± 1.0
3	>500	51.4 ± 4.5
4	9.1 ± 0.5	10.1 ± 0.2
5	19.1 ± 0.4	21.4 ± 2.0
6	35.6 ± 2.4	80.3 ± 2.9
7	>500	>500
8	9.6 ± 0.7	10.5 ± 0.6
9	18.9 ± 0.8	19.5 ± 0.3
10	5.9 ± 0.2	7.2 ± 0.4
11	6.0 ± 0.5	6.1 ± 0.5
cisplatin[a]	1.0 ± 0.2	25.0 ± 0.2

[a]Cisplatin is used as a reference compound and has been tested before.

The $HL^{naph,ph}$ ligand is quite cytotoxic against the ovarian carcinomas, whereas $HL^{ph,ph}$ and $HL^{et,ph}$ are much less active, $HL^{ph,ph}$ being the least active of the ligands with an IC_{50} value around 270 μM in both cell lines. In comparison, the complexes bearing the $HL^{ph,ph}$ ligand, i.e. **1** and **2**, are more active than their $HL^{naph,ph}$ analogues **4** and **5**. The complexes with the *p*-cymene ligand are the most active of the series, with **1** and **4** followed by the benzene complexes **2** and **5** and finally hexamethylbenzene complexes **3** and **6**. Notably, rather small changes to the β-ketoamine ligand lead to very large differences in cytotoxicity, with **7** being inactive in both cell lines (IC_{50} > 500 μM) in comparison to IC_{50} values of <10 μM for **1** and **2**. Replacing chloride by an aqua ligand in **1** gives **8**, which has a lower activity, whereas the aqua complex **10** is more active than its precursor **4**, with an IC_{50} value in the low micromolar range. Switching to the PTA ligand further decreases the activity of compound **9**, but not that of **11**, which, to within experimental errors, is the most active compound in both cell lines. It is noteworthy that most ruthenium arene complexes with a PTA ligand display limited cytotoxicities unless functionalized with a biologically active group,[2b] indicating the important biological role of the β-ketoamine ligands in these complexes.

None of the compounds are as cytotoxic as cisplatin in the A2780 cell line, whereas a number of them are substantially more active in A2780R cells, indicating that they operate via a mechanism of action different from that of cisplatin.

CONCLUSIONS

Organometallic compounds are attracting considerable interest in medicinal chemistry—especially in imaging and as putative anticancer compounds.[23] Many different classes of organometallic compounds have been evaluated, from the pioneering studies on titanocene dichloride that underwent extensive testing in the clinic,[24] to rationally designed ferrocifens,[25] to the current situation in which a large range of main-group and transition metals and essentially all the classic ligands encountered in organometallic chemistry have been explored.[1] Of particular current interest are ruthenium arene compounds which have been extensively modified to give compounds with various therapeutic effects.[2,3] Herein we have shown that ruthenium arene compounds with β-ketoamine ligands have

dx.doi.org/10.1021/om301115e | *Organometallics* 2013, 32, 309−316

relevant anticancer properties in vitro: for example, displaying significant cytotoxicity on resistant human ovarian cancer cells. Minor changes to the β-ketoamine ligand, such as changing an ethyl group for a phenyl group, result in considerable changes to their cytotoxicity. Consequently, these ligands represent an interesting class for further study in order to fine tune the anticancer properties of the ruthenium arene unit.

■ ASSOCIATED CONTENT

Supporting Information

Text giving ^{1}H NMR and conductivity data for complex **4** and a table and CIF files giving crystallographic data for $HL^{et,ph}$, **1**, **4**, and **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*R.P.: e-mail, riccardo.pettinari@unicam.it; tel, +39 0737402338; fax, +39 0737 402338.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Swiss National Science Foundation (CMM) and the University of Camerino for financial support.

■ REFERENCES

(1) (a) Noffke, A. L.; Habtemariam, A.; Pizarro, A. M.; Sadler, P. J. *Chem. Commun.* **2012**, *48*, 5219. (b) Patra, M.; Gasser, G. *ChemBioChem* **2012**, *13*, 1232. (c) Hartinger, C. G.; Metzler-Nolte, N.; Dyson, P. J. *Organometallics* **2012**, *31*, 5677. (d) Sava, G.; Bergamo, A.; Dyson, P. J. *Dalton Trans.* **2011**, *40*, 9069. (e) Gasser, G.; Ott, I.; Metzler-Nolte, N. *J. Med. Chem.* **2011**, *54*, 3. (f) Suss-Fink, G. *Dalton Trans.* **2010**, *39*, 1673. (g) Hartinger, C. G.; Dyson, P. *J. Chem. Soc. Rev.* **2009**, *38*, 391.

(2) See for example: (a) Dyson, P. J.; Sava, G. *Dalton Trans.* **2006**, 1929. (b) Ang, W. H.; Casini, A.; Sava, G.; Dyson, P. J. *J. Organomet. Chem.* **2011**, *696*, 989.

(3) See for example: (a) Hoeschele, J. D.; Habtemariam, A.; Muir, J.; Sadler, P. J. *Dalton Trans.* **2007**, 4974. (b) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. *J. Chem. Commun.* **2005**, 4764. (c) Aird, R. E.; Cummings, J.; Ritchie, A. A.; Muir, M.; Morris, R. E.; Chen, H.; Sadler, P. J.; Jodrell, D. I. *Br. J. Cancer* **2002**, *86*, 1652.

(4) (a) Scolaro, C.; Bergamo, A.; Brescacin, L.; Delfino, R.; Cocchietto, M.; Laurenczy, G.; Geldbach, T. J.; Sava, G.; Dyson, P. J. *J. Med. Chem.* **2005**, *48*, 4161. (b) Nowak-Sliwinska, P.; van Beijnum, J. R.; Casini, A.; Nazarov, A. A.; Wagnieres, G.; van den Bergh, H.; Dyson, P. J.; Griffioen, A. W. *J. Med. Chem.* **2011**, *54*, 3895−3902. (c) Chatterjee, S.; Kundu, S.; Bhattacharyya, A.; Hartinger, C. G.; Dyson, P. J. *J. Biol. Inorg. Chem.* **2008**, *13*, 1149.

(5) Bergamo, A.; Masi, A.; Peacock, A. F.; Habtemariam, A.; Sadler, P. J.; Sava, G. *J. Inorg. Biochem.* **2010**, *104*, 79.

(6) (a) Peacock, A. F. A.; Melchart, M.; Deeth, R. J.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem. Eur. J.* **2007**, *13*, 2601. (b) Fernandez, R.; Melchart, M.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem. Eur. J.* **2004**, *10*, 5173.

(7) Betanzos-Lara, S.; Salassa, L.; Habtemariam, A.; Novakova, O.; Pizarro, A. M.; Clarkson, G. J.; Liskova, B.; Brabec, V.; Sadler, P. J. *Organometallics* **2012**, *31*, 3466.

(8) Bugarcic, T.; Habtemariam, A.; Stepankova, J.; Heringova, P.; Kasparkova, J.; Deeth, R. J.; Johnstone, R. D. L.; Prescimone, A.; Parkin, A.; Parsons, S.; Brabec, V.; Sadler, P. J. *Inorg. Chem.* **2008**, *47*, 11470.

(9) Habtemariam, A.; Melchart, M.; Fernández, R.; Parsons, S.; Oswald, I. D. H.; Parkin, A.; Fabbiani, F. P. A.; Davidson, J. E.; Dawson, A.; Aird, R. E.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2006**, *49*, 6858.

(10) Marchetti, F.; Pettinari, C.; Pettinari, R.; Cerquetella, A.; Cingolani, A.; Chan, E. J.; Kozawa, K.; Skelton, B. W.; White, A. H.; Wanke, R.; Kuznetsov, M. L.; Martins, L. M. D. R. S.; Pombeiro, A. J. L. *Inorg. Chem.* **2007**, *46*, 8245.

(11) Marchetti, F.; Pettinari, C.; Pettinari, R. *Coord. Chem. Rev.* **2005**, *249*, 2909.

(12) Duisenberg, A. J. M.; Kroon-Batenburg, L. M. J.; Schreurs, A. M. M. *J. Appl. Crystallogr.* **2003**, *36*, 220.

(13) Blessing, R. H. *Acta Crystallogr., Sect. A* **1995**, *51*, 33.

(14) *Crysalis PRO, release 1.171.35.21*; Agilent Technologies, 2012.

(15) SHELX: Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *64*, 112.

(16) Pettinari, C.; Pettinari, R.; Fianchini, M.; Marchetti, F.; Skelton, B. W.; White, A. H. *Inorg. Chem.* **2005**, *44*, 7933.

(17) Carmona, E.; Cingolani, A.; Marchetti, F.; Pettinari, C.; Pettinari, R.; Skelton, B. W.; White, A. H. *Organometallics* **2003**, *22*, 2820.

(18) Geary, W. J. *Coord. Chem. Rev.* **1971**, *7*, 81.

(19) (a) Kruck, T. *Angew. Chem., Int. Ed.* **1967**, *6*, 53. (b) Collong, W.; Kruck, T. *Chem. Ber.* **1990**, *123*, 1655. (c) Fuss, W.; Ruhe, M. *Z. Naturforsch.* **1992**, *47B*, 1.

(20) Wanke, R.; Smolenski, P.; Guedes da Silva, M. F. C.; Martins, L. M. D. R. S.; Pombeiro, A. J. L. *Inorg. Chem.* **2008**, *47*, 10158.

(21) Kilpin, K. J.; Clavel, C. M.; Edafe, F.; Dyson, P. J. *Organometallics* **2012**, *31*, 7031.

(22) (a) Brunner, H.; Henning, F.; Weber, M.; Zabel, M.; Carmona, D.; Lahoz, F. J. *Synth.* **2003**, 1091. (b) Kumar, K. N.; Venkatachalam, G.; Ramesh, R.; Liu, Y. *Polyhedron* **2008**, *27*, 157. (c) Brunner, H.; Zwack, T.; Zabel, M.; Beck, W.; Bohm, A. *Organometallics* **2003**, *22*, 1741.

(23) See the recent issue of *Organometallics* dedicated to organometallics in biology and medicine: Coogan, M. P.; Dyson, P. J.; Bochmann, M. *Organometallics* **2012**, *31*, 5671.

(24) (a) Köpf, H.; Köpf-Maier, P. *Angew. Chem.* **1979**, *91*, 509. (b) Kröger, N.; Kleeberg, U. R.; Mross, K.; Edler, L.; Hossfeld, D. K. *Onkologie* **2000**, *23*, 60.

(25) (a) Jaouen, G.; Top, S.; Vessieres, A.; Leclercq, G.; McGlinchey, M. J. *Curr. Med. Chem.* **2004**, *11*, 2505−2517. (b) Nguyen, A.; Vessieres, A.; Hillard, E. A.; Top, S.; Pigeon, P.; Jaouen, G. *Chimia* **2007**, *61*, 716−724.