ORGANOMETALLICS

Article

pubs.acs.org/Organometallics

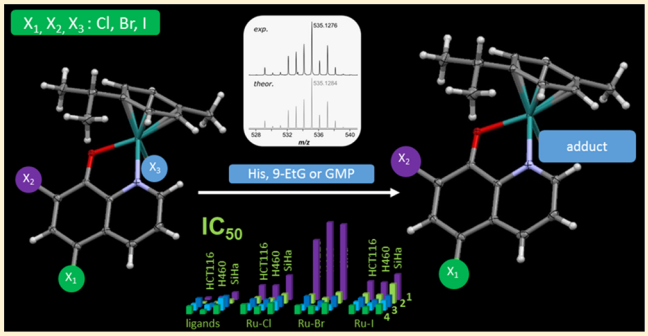
Impact of the Halogen Substitution Pattern on the Biological Activity of Organoruthenium 8-Hydroxyquinoline Anticancer Agents

Mario Kubanik,[†] Hannah Holtkamp,[†] Tilo Söhnel,[†] Stephen M. F. Jamieson,[‡] and Christian G. Hartinger*[,†]

[†]School of Chemical Sciences and [‡]Auckland Cancer Society Research Centre, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

Supporting Information

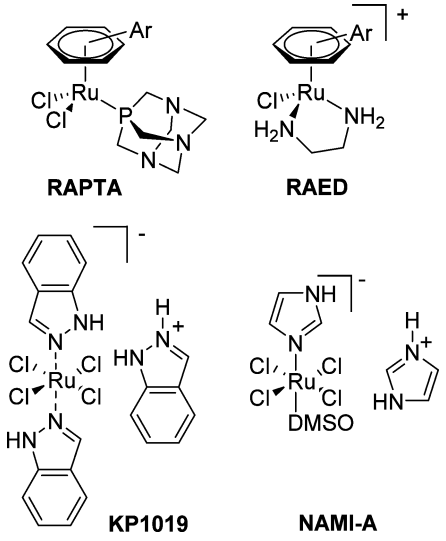
ABSTRACT: 8-Hydroxyquinoline and its derivatives have a broad variety of pharmacological properties, which make them an ideal bioactive building block in the development of metal-based anticancer drugs. In this account we aimed to rationalize the antiproliferative efficacy of organoruthenium compounds featuring 8-hydroxyquinoline-derived ligands and to elucidate structural determinants by using biological assays and bioanalytical methods. By systematically varying the halide substitution pattern at the 5- and 7-positions of the 8-hydroxyquinoline ligand, as well as the halido leaving group, a series of 5,7-dihalido-8-hydroxyquinoline $Ru^{II}(\eta^6\text{-}p\text{-cymene})$ complexes were obtained. Studies on their cytotoxic activity revealed the minor impact of the substitution pattern (with the exception of complexes of 8-hydroxyquinoline) on their activity. Notably, the cellular accumulation showed no correlation with the cytotoxic activity, while the nature of the halido leaving group only had a significant influence in the case of the 8-hydroxyquinoline organoruthenium compounds. However, the compounds were shown to be very stable under a wide variety of pH conditions, making them possible candidates for further development as orally active anticancer agents.



INTRODUCTION

The severe side effects and intrinsic and acquired resistance of platinum-based cancer chemotherapeutics are the driving force for the development of novel coordination compounds with different modes of action and toxicity profiles.[1−3] Ruthenium is considered as a promising metal center for new anticancer agents, with NAMI-A and KP1019 (Chart 1) as the most promising ruthenium complexes reaching clinical trials;[4,5] however, more recently organometallic Ru(arene) complexes have attracted increasing attention.[5−8] RAPTA and RAED compounds (see Chart 1 for general structures) are the most intensively investigated organoruthenium complexes and have shown promise in drug development.[9−11] Notably, the choice of the ligands is of utmost importance for the mode of action of such compounds and of metal-based anticancer drugs in general.[12,13] It affects the ligand exchange kinetics and the 3D shape of the complex and thereby influences the biological activity and also the route of administration.[14,15] Slow ligand exchange may make the metal center act purely as a scaffold that organizes the ligands and makes the compound suitable for oral administration, if insensitive to low pH.[16] In contrast, labile ligands may be replaced with donor atoms of biological target molecules to form covalent biomolecule adducts that affect their functions.[17] The arene ligand in these complexes stabilizes the oxidation state and also influences the lipophilicity and interaction with biomolecules.[5,18,19]

Chart 1. Structures of Anticancer Ru Complexes



A contemporary approach in anticancer metallodrug design is the use of nature-inspired, bioactive ligands such as

Received: October 15, 2015

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

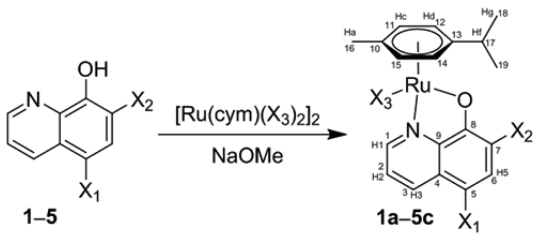
flavones[20−22] and quinones[23] or ligands to target proteins such as maleimide,[24,25] biotin,[26] and ethacrynic acid.[27,28] Another approach is the use of so-called privileged structures. The definition of the concept underwent some refinements since the introduction by Evans in the late 1980s and refers nowadays to substructures or scaffolds which appear frequently in drugs, natural compounds, or bioactive molecules.[29,30] One of those privileged frameworks is the nitrogen-containing heterocycle quinoline. Quinolines and especially 8-hydroxyquinoline (8-HQ) derivatives are used as drugs for a broad range of applications and exhibit a wide range of bioactive properties, such as anticancer, anti-HIV, antifungal, antileishmanial, antischistosomal, antioxidant, antibacterial, and neuroprotective activity. Furthermore, they are inhibitors of iron-dependent enzymes and act as chelators for metal ions in a biological environment.[31,32] Due to the use of 8-hydroxyquinoline and its 5,7-dihalido derivatives in veterinary and human applications for decades, those compounds have been studied extensively.[33] Especially, clioquinol (5-chloro-7-iodoquinolin-8-ol) has shown promising results in Parkinson's and Alzheimer's disease studies.[34,35] Notably, 8-HQ has also been used as a ligand in the orally available gallium(III) complex KP46, currently undergoing clinical trials.[15,36]

Several Ru complexes of 8-hydroxyquinoline (8-HQ) derivatives have been reported.[37−41] However, there is a gap in terms of a systematic study on the effect of the variation of the halide substituents at the 5- and 7-positions of the 8-HQ ligand as well as the leaving halido ligand on the anticancer activity of the complex type. Therefore, we have expanded in this paper on the available structures and bioanalytical characterization.

RESULTS AND DISCUSSION

In order to study rationally the anticancer activity of [Ru(cym)(8-HQ)X] (cym = η^6-*p*-cymene) complexes (Scheme 1), **1a−c** to **5a−c** were prepared using ligands **1−5** (1 equiv), the dimeric ruthenium precursors bis[dihalogenido(η^6-*p*-cymene)ruthenium(II)] with varying halogenido ligands (halogenido = Cl, Br, I; 0.45 equiv), and sodium methoxide (1.1 equiv). Either the solution was then refluxed under nitrogen for 1.5−4 h in methanol (method A) or the ligand was dissolved in a small amount of a chloroform/methanol mixture at room temperature and addition of dimer caused formation and precipitation of the desired products (method B). After an additional 1 h of stirring at room temperature the solvent was evaporated or removed by filtration, the residue was dissolved in dichloromethane, the solution was filtered, and the complex was precipitated with *n*-hexane. The yields of the complexes were in the range of 58% (**4c**) to 83% (**4b**). Compounds **1a**,[37] **2a**,[39] and **5a**[41] were reported earlier, however, by using different methods with two-step procedures or different bases.

Scheme 1. Synthesis of [Ru(cym)(8-HQ)X] Complexes 1a−c to 5a−c and ^{1}H and ^{13}C{^{1}H} NMR Numbering Scheme



	a (X_3 = Cl)	b (X_3 = Br)	c (X_3 = I)
1	X_1, X_2 = H [37]	X_1, X_2 = H	X_1, X_2 = H
2	X_1, X_2 = Cl [39]	X_1, X_2 = Cl	X_1, X_2 = Cl
3	X_1, X_2 = Br	X_1, X_2 = Br	X_1, X_2 = Br
4	X_1, X_2 = I	X_1, X_2 = I	X_1, X_2 = I
5	X_1 = Cl, X_2 = I [41]	X_1 = Cl, X_2 = I	X_1 = Cl, X_2 = I

All complexes were characterized by ^{1}H and ^{13}C{^{1}H} NMR spectroscopy, mass spectrometry, elemental analysis, and melting point. For selected compounds, 2D NMR spectroscopy was performed to unambiguously assign the peaks. Furthermore, the molecular structures of **1a**,**b**, **3a**, and **5b** were determined by single-crystal X-ray diffraction analysis (see Figure 1 for the structures of **1b**, **3a**, and **5b**).

The ^{1}H NMR spectra of the complexes show the typical signals for the isopropyl group protons of *p*-cymene at around 1.2 ppm, of the CH_3 protons at 2.4 ppm, of H_f at 2.8 ppm, and of the aromatic protons in the range of 5.3−5.6 ppm. The aromatic protons of the 8-HQ ligands were found in the area of 6.8−10 ppm. Low-field shifts of H_f, H_g and H_a can be observed by changing X_3 from a chlorido to bromido and iodido leaving groups. The influence of the substitution pattern at X_1 and X_2 is most pronounced at H5, with a downfield shift of almost 1 ppm when substituting the dichloro with the diiodo derivative. This substitution also affects the chemical shifts of C5 and C7 in the ^{13}C{^{1}H} NMR spectra.

Single crystals suitable for X-ray diffraction analysis were obtained for complexes **1a**,**b**, **3a**, and **5b** (Figure 1 and the Supporting Information). The molecular structures of all complexes featured enantiomeric mixtures with the ruthenium complexes showing piano-stool configuration where the quinoline ligand and X_3 act as the legs and the π-bound *p*-cymene ring forms the seat of the stool, through coordination in an η^6 fashion to the Ru center. Compounds **1a**,**b** (from chloroform/*n*-hexane) and **5b** (from DMSO) crystallized in the monoclinic space group $P2_1/n$, whereas compound **3a** crystallized from DMSO in the orthorhombic space group *Pbca*. Quinoline acts as a chelating ligand and binds via the nitrogen and oxygen atoms to the ruthenium metal center, forming a five-membered ring. The Ru−N distance is slightly longer than the Ru−O bond in all complexes, except in **3a**. The Ru−Br bonds in **1b** and **5b** are approximately 0.1 Å longer than the Ru−Cl bonds of **1a** and **3a** (Table 1). The molecular structures of all compounds featured two enantiomers. However, neither hydrogen bonds nor π stacking was observed between the planar 8-HQ ligands, which is a common feature for aromatic, planar ligands in such complexes.[25]

Stability in DMSO and Aqueous Solutions. Many metal complexes show low aqueous solubility, which limits their development as anticancer agents. Therefore, the complexes are commonly dissolved in DMSO and these DMSO stock solutions are used for biological assays. For many transition-metal complexes aquation, i.e., a halogenido/aqua ligand exchange reaction, is an important step in their modes of action, facilitating the formation of covalent bonds with the donor atoms of their biomolecular targets.[42,43] However, DMSO may coordinate to the metal center, which changes the structure of the pharmacophore or even causes decomposition of the complex.[44]

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

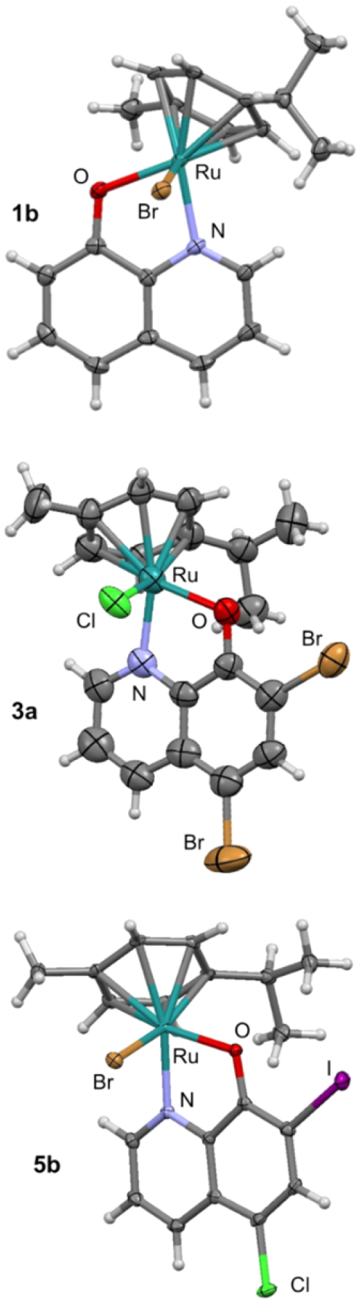


Figure 1. Molecular structures of one of the enantiomers of **1b**, **3a**, and **5b** drawn at the 50% probability level. Solvent molecules were removed for clarity.

The halido/aqua ligand exchange reaction of **1a**−**5a** in aqueous media occurs immediately to yield $\mathbf{1a}^{H_2O}$−$\mathbf{5a}^{H_2O}$ and was too fast to follow by ^{1}H NMR or UV/vis spectroscopy (Figure 2). To confirm the immediate formation of the aqua species, **1a** was incubated with 1 equiv of $AgNO_3$ (Supporting Information). No change in the chemical shifts of the ^{1}H NMR spectra was observed, which indicates that **1a** was already present as $\mathbf{1a}^{H_2O}$. The stability of the formed aqua species in H_2O was determined by ^{1}H NMR spectroscopy, and no decomposition was detectable over a period of more than 50 days at room temperature and in the presence of light. Furthermore, the complexes are stable in DMSO, given that the single crystals for **3a** and **5b** were obtained from a saturated DMSO solution (Figure 2). The stability of **1a** was also

Table 1. Selected Bond Lengths (Å) and Angles (deg) for Complexes 1b, 3a, and 5b in Comparison to Those for 1a[37]

	1a[a]	1b	3a	5b
	Bond Lengths (Å)			
Ru−O	2.073(2)	2.074(3)	2.11(1)	2.084(2)
Ru−N	2.094(2)	2.096(3)	2.08(1)	2.100(3)
Ru−X_3	2.4219(7)	2.5525(5)	2.429(3)	2.5552(4)
C5−X_1			1.90(2)	1.740(3)
C7−X_2			1.90(2)	2.094(3)
Ru−centroid$_{arene}$	1.662	1.667	1.670	1.667
	Bond Angles (deg)			
O−Ru−N	78.80(7)	79.0(1)	78.6(4)	78.65(9)
O−Ru−X_3	86.47(5)	86.48(8)	85.8(3)	84.94(6)
N−Ru−X_3	84.25(5)	84.13(9)	84.3(3)	82.90(7)

[a]Taken from ref 37.

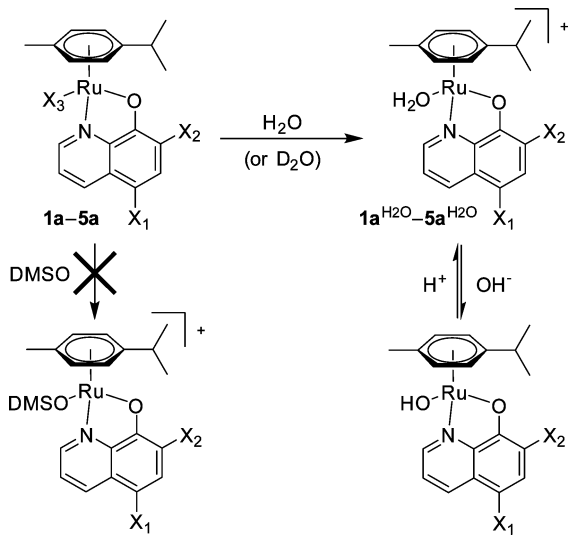


Figure 2. Halogenido/aqua (or D_2O) ligand exchange reaction occurring upon dissolution of the 8-HQ complexes in aqueous solution and observation of pH-dependent protonation/deprotonation of the complexes.

measured in mixtures of 10% d_6-DMSO and D_2O, which again demonstrated the stability of the compound in solution.

pH Titration. The pH-dependent conversion of **1a** to the aqua/hydroxido analogue was studied by ^{1}H NMR spectroscopy in 10% DMSO/D_2O (Figure 3). After dissolution of **1a**, a pD of 7.00 was recorded. The pD of the solution was cycled in both directions to more basic (up to pD 12.34) and acidic pD values and back, using diluted DCl and NaOD. Addition of NaOD resulted in a high-field shift of the cymene ring protons in the ^{1}H NMR spectrum and initial broadening of the peaks, which resolved into four peaks at around pD 11.10. These signals were assigned to a hydroxido species which remained stable upon acidification of the solution with DCl until pH 3.90. Further addition of DCl led to a ^{1}H NMR spectrum with a second set of signals appearing with the same chemical shifts observed as in the spectrum measured after dissolution of **1a** in D_2O. The ratio between this main product and the signals assigned to the hydroxido complex was around 1:0.6. Increasing the pD again showed the reversibility of the process and also proved that complex **1a** is stable under conditions comparable to the acidic environment of the stomach, which would allow oral administration of the drug.

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

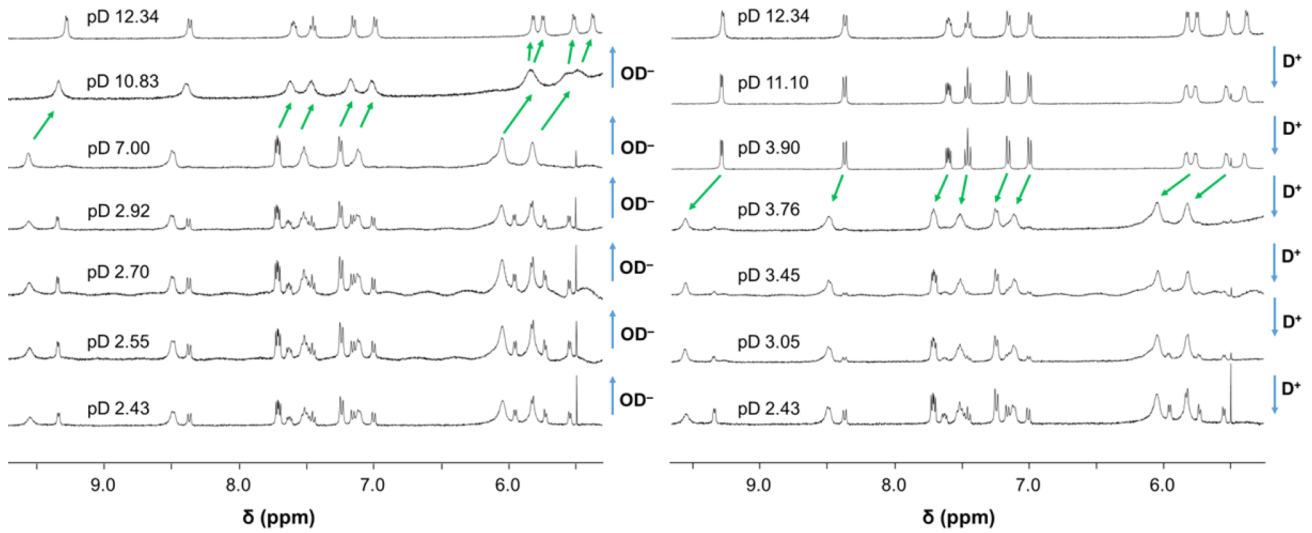


Figure 3. ^{1}H NMR spectroscopic study on the reversible protonation of $\mathbf{1a}^{H_2O}$ using pH titration in the pD range 2.4−12.3.

Speciation upon Interaction with Biomolecules. After intravenous administration, metallodrugs are exposed to a variety of biomolecules as possible binding partners in the blood. Interactions in the blood plasma can lead either to deactivation of the pharmacophore, for example by release of essential ligands, or to protein-mediated transport to their target tissue.[17,24,45−47] In order to compare the impact of the halido leaving group on the adduct formation, the binding behavior of the chlorido (**1a**) and iodido (**1c**) complexes to the amino acids L-cysteine (Cys), L-methionine (Met), and L-histidine (His) was investigated by ^{1}H NMR spectroscopy in 10% d_6-DMSO/D_2O solution. The reaction of **1a** with His resulted in the replacement of the chlorido leaving group with His, which was coordinated to the ruthenium center in a monodentate fashion (Figure 4A). The result was confirmed by electrospray ionization mass spectrometry (ESI-MS; Figure 4B), which allowed unambiguous identification of the His conjugate as $[\mathbf{1a} + \text{His} - \text{Cl}]^+$ with high mass accuracy although at low relative abundance in comparison to the $[\mathbf{1a} - \text{Cl}]^+$ base peak. This may be explained by the lability of such complexes during the electrospray process, observed for similar compound types.[17] The reaction of the iodido derivative **1c** with His occurs at a rate slightly faster than that observed for the chlorido congener **1a**. Within 1 h, 80% of **1c** and 70% of **1a** were converted to the His conjugate.

Many Ru(arene) complexes undergo reactions with Met, while incubation with Cys causes decomposition of such complexes.[48,49] Surprisingly, neither **1a** nor **1c** underwent halido/Met ligand exchange reactions, as shown by ^{1}H NMR spectroscopy and ESI-MS. In contrast, Cys incubation with both **1a** and **1c** led to an instant release of the arene moiety and degradation of the complex.

DNA is widely accepted as the primary target for platinum anticancer agents, and many Ru complexes form conjugates as well, as was recently demonstrated for the ethylenediamine RAED complexes.[50] In order to estimate the potential of forming DNA adducts, **1a** was incubated with the model compounds guanosine 5′-monophosphate (GMP) and the less bulky 9-ethylguanine (EtG) in 10% d_6-DMSO/D_2O and studied by ^{1}H NMR spectroscopy. The reaction occurred instantly and was too fast to follow by NMR spectroscopy. By titrating **1a** with EtG, we confirmed the formation of a EtG

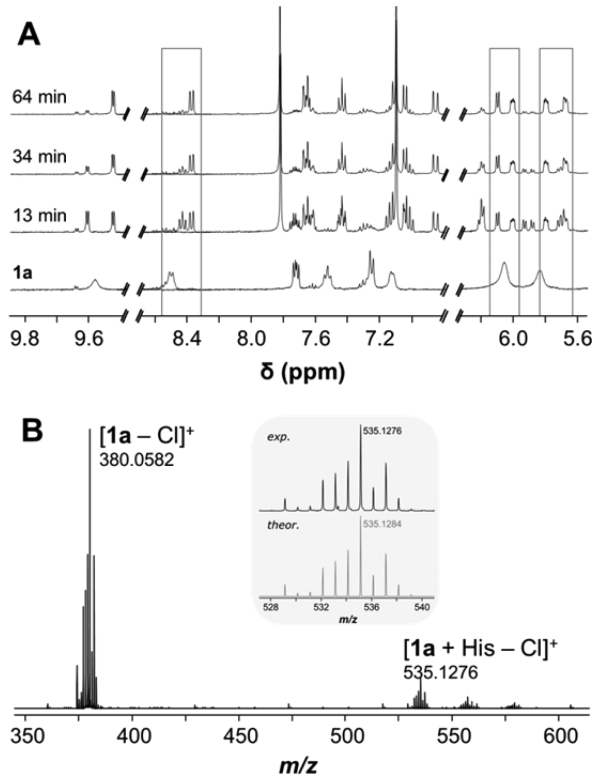


Figure 4. (A) Time-dependent reaction of **1a** with L-histidine studied by ^{1}H NMR spectroscopy in 10% d_6-DMSO/D_2O. (B) ESI-mass spectrum of the reaction mixture after 24 h in 10% DMSO/H_2O.

conjugate (Figure 5). Notably the chemical shift of the H8 of EtG was found at only slightly higher field ($\Delta\delta$ = 0.02 ppm) upon coordination to Ru. ESI-MS confirmed the substitution of the chlorido leaving group with both GMP and EtG. NMR studies showed that the nucleotide complex is stable in aqueous media for at least 36 h and does not undergo any changes during this period.

These experiments show that the compounds form stable complexes with biological nitrogen donors from DNA and protein building blocks while they show no reactivity with Met and decomposition with Cys. This may provide an opportunity

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

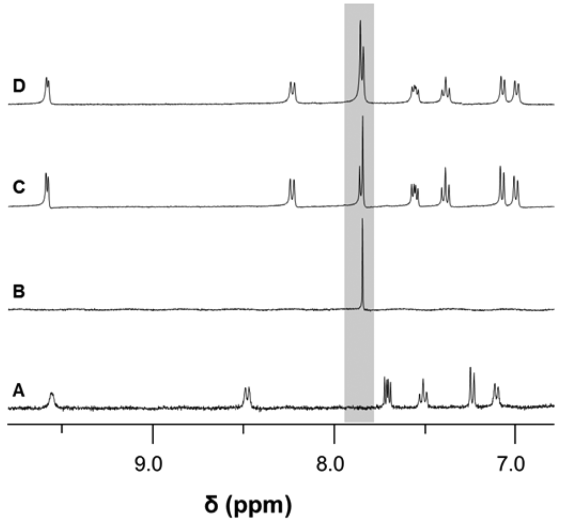


Figure 5. ^{1}H NMR spectroscopic study on the reaction of **1a** with 9-ethylguanine, revealing a minor high-field shift of the EtG H8 signal upon coordination to the Ru center: (A) **1a**; (B) EtG; (C) **1a** + EtG (1 equiv); (D) **1a** + EtG (2 equiv).

for selective ruthenation of His-containing proteins in future work.

Biological Studies in Human Cancer Cells. The in vitro antiproliferative activity of complexes **1a−5a** to **1c−5c** and the respective ligands **1−5** was determined in HCT116 human colorectal, NCI-H460 non-small cell lung, and SiHa cervical carcinoma cells (Table 2). All Ru(cym) compounds show excellent activity, with IC_{50} values in the low micromolar range which is clearly associated with the cytotoxic activity of the 8-HQ ligands. The IC_{50} values of the ligands were lower than or at least similar to those of the respective complexes.

Table 2. Cytotoxic Activity of Complexes 1a−c to 5a−c in the Human Cancer Cell Lines HCT116, NCI-H460, and SiHa in Comparison to That of Their 8-HQ Ligands (1−5) after 72 h Incubation

	IC_{50} value (μM)		
	HCT116	NCI-H460	SiHa
1	1.98 ± 0.81	2.41 ± 0.42	5.96 ± 0.74
1a	11.5 ± 1.4	11.4 ± 2.0	19.3 ± 2.0
1b	47.1 ± 1.2	61.0 ± 6.0	59.4 ± 4.8
1c	14.6 ± 2.8	13.7 ± 2.9	20.2 ± 5.7
2	1.15 ± 0.35	1.11 ± 0.15	3.07 ± 0.13
2a	5.01 ± 0.70	3.95 ± 0.71	7.61 ± 1.27
2b	7.03 ± 1.20	4.38 ± 0.46	8.67 ± 1.01
2c	7.77 ± 2.29	6.83 ± 1.35	15.4 ± 0.4
3	6.91 ± 0.68	2.72 ± 0.47	8.72 ± 0.93
3a	6.27 ± 0.93	5.76 ± 0.55	8.28 ± 0.92
3b	8.55 ± 1.83	4.54 ± 0.53	8.28 ± 0.70
3c	7.26 ± 0.44	5.48 ± 0.18	8.89 ± 1.44
4	5.40 ± 1.04	4.57 ± 0.78	9.70 ± 1.34
4a	5.15 ± 1.91	4.60 ± 1.29	7.30 ± 0.87
4b	4.44 ± 0.85	2.92 ± 0.53	4.38 ± 0.98
4c	7.04 ± 0.39	5.88 ± 0.77	7.96 ± 1.21
5	6.26 ± 1.75	3.28 ± 0.35	6.79 ± 0.27
5a	7.65 ± 0.64	5.63 ± 0.28	8.54 ± 0.37
5b	6.78 ± 1.20	6.43 ± 1.03	8.54 ± 1.41
5c	6.85 ± 0.61	5.60 ± 0.93	7.57 ± 0.22

Interestingly, the complexes of **1**, which was the most active 8-HQ derivative, were the least active in the cytotoxicity assays.

With the aim of explaining the low antiproliferative activity of the complexes of **1** over those of ligands **2−5**, the cellular accumulation of **1a−5a** and, for comparison, **1c** was determined through the Ru levels in HCT116 cells after incubations of 2, 4, and 8 h. The different incubation times had only a minor impact on the uptake of **1a−4a** and **1c** into HCT116 cells (Figure 6). On average, the largest amount of Ru

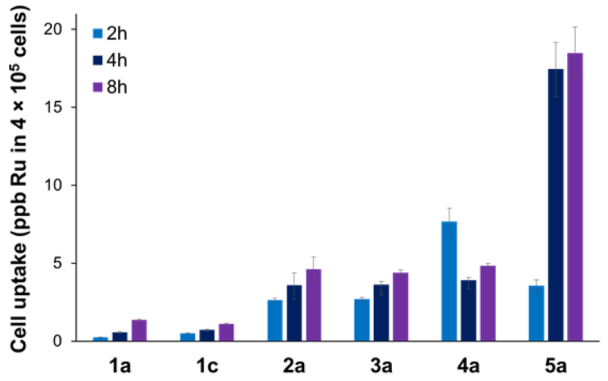


Figure 6. Cellular uptake of compounds **1a,c** and **2a−5a** after incubation for 2, 4, or 8 h with HCT116 cells.

was detected for **5a**, while **1a,c** showed the lowest cell uptake. For **5a** significantly higher Ru contents were observed after 4 and 8 h in comparison to 2 h incubations. However, this is not reflected by the IC_{50} values obtained from the in vitro studies, where **2a−5a** have similar IC_{50} values. The low amount of cellular uptake for **1a,c**, however, correlates with their low cytotoxic activity, where a small amount of uptake translates to a lower efficacy of the compounds in vitro.

Lipophilicity. Lipophilicity is an important factor for the cellular accumulation and oral bioavailability of drugs. It is often expressed as the *n*-octanol/water partition coefficient (log *P*), which is also a central parameter in many in silico medicinal chemistry approaches, such as the determination of the drug likeliness of a new drug.[16,51] However, high or low aqueous solubilities do not necessarily imply that compounds are hydrophilic or lipophilic, respectively (Table 3).

Table 3. Aqueous Solubility (mM), *n*-Octanol/Water Partition Coeffients (log *P*), and QED_w^{mo} Values of Complexes 1a−5a

complex	solubility (mM)	log *P*	QED_w^{mo}
1a	0.458	0.46 ± 0.01	0.61
2a	0.450	0.43 ± 0.07	0.50
3a	0.222	0.61 ± 0.02	0.42
4a	0.026	0.85 ± 0.09	0.38
5a	0.028	0.24 ± 0.002	0.41

To compare the lipophilic properties of the Ru(cym)(8-HQ) compounds **1a−5a**, the calculated octanol/water partition coefficients (*c* log *P*) of the ligands were determined using ChemDraw 12.0, Molinspiration (www.molinspiration.com), and ALOGPS 2.1 (Supporting Information).[52] As the Ru(cym)Cl moiety is contained in all of these organoruthenium compounds, the relative *c* log *P* values should depend on the 8-HQ derivatives only. Indeed, although the values differ from

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

program to program, the trend was the same: **1** < **2** < **3** ≈ **5** < **4**. Notably, the ligands' antiproliferative activity seems to be independent of their lipophilicity, taking the *c* log *P* values as a measure.

As **1a**−**5a** undergo rapid Cl/H_2O exchange in aqueous solution, we determined the log *P* values for the aqua complexes $\mathbf{1a}^{H_2O}$−$\mathbf{5a}^{H_2O}$ of **1a**−**5a** (vide infra) using the shake flask method (Table 3). In general, the log *P* values are in the range of 0−1. The relatively low log *P* values may be explained by the ligand exchange reaction of the complexes quickly yielding the aqua derivatives. The log *P* values for the complexes are also significantly lower than the *c* log *P* values for the ligands (Supporting Information). The experimentally determined values show overall a trend similar to that found for the 8-HQ ligands for **1a**−**4a**, and this resembles to some extent what would be expected from the cellular accumulation determined for the compounds. The value for **5a** was significantly lower than expected, as we would anticipate it to be between those for the dichloro (**3a**) and diiodo (**4a**) derivatives and there is no correlation between the lipophilicity and the cytotoxic activity in the three cell lines studied. However, we observed an effect of the nature of the leaving group on the cytotoxic activity which may be surprising, given the formation of the same aqua products. Nevertheless, the reactions with amino acids also demonstrated an effect on the reaction rates which may explain the different behavior in cell culture.

Quantitative Estimate of Druglikeness of the Complexes. Since the lipophilicity does not appear to be a good predictor for the anticancer activity of this class of compounds, we determined the weighted quantitative estimate of druglikeness of the compounds on the basis of the highest information content (QED_w^{mo}) for **1a**−**5a** and the respective 8-HQ ligands (Table 3 and Supporting Information).[16,51] The ligands **1**−**5** gave in general higher QED_w^{mo} values than the respective complexes **1a**−**5a** (Supporting Information). Interestingly, the calculation of the QED_w^{mo} values is fairly independent of the data set used for the lipophilicity, i.e., log *P* vs *c* log $P_{ALOGPS\ 2.1}$ (with the latter being the average obtained from ALOGPS 2.1). Despite these values being very different in absolute terms, the QED_w^{mo} values vary only by ±2% and both follow the order of the lipophilicity according to the *c* log *P* values for the ligands.

The overall highest QED_w^{mo} values were found for cliquinol **5** and the dibromo derivative **3** with a value of 0.76. Surprisingly, their complexes together with **4a** are the least druglike, while the 8-hydroxyquinoline complex **1a** was the most druglike compound studied. This is an interesting result, given the fact that the complexes of **1** are in all cell lines the least active, while all other complexes have fairly similar QED_w^{mo} and IC_{50} values in all cell lines. Notably, the QED_w^{mo} values were higher than those observed for pyridinecarbothioamide complexes developed as orally active anticancer agents.[16]

CONCLUSIONS

To systematically study the influence of the 5,7-dihalogen substitution pattern and the leaving halido group on the cytotoxicity of 8-hydroxyquinoline-derived $Ru^{II}(\eta^6$-*p*-cymene) complexes, a series of derivatives was synthesized featuring different halido ligands as leaving groups. For 8-HQ derivatives, we also included 8-hydroxyquinoline, currently being clinically investigated as an anticancer agent in the form of a Ga(III) complex, and clioquinol, a potential remedy against Alzheimer's and Parkinson's diseases. Spectroscopic techniques and elemental analysis were used to determine the nature and purity of the complexes prior to biological studies. The behavior of representative complexes in aqueous media, lipophilicity, quantitative estimated druglikeness, and interactions with biological molecules were studied to rationalize the in vitro efficacy in human cancer cell lines. In vitro anticancer activity studies of the compounds revealed IC_{50} values in the low micromolar range driven by the cytotoxicity of the 8-HQ-derived ligands. The leaving groups appear to influence the cytotoxic activity of the Ru complexes which may be explained by different ligand exchange reaction rates. Surprisingly, the correlation between the cytotoxicity and cellular uptake data was poor for **5a**, since usually a higher accumulation in the cell results in higher anticancer activity. However, most of the complexes showed similar activity levels, with the exception of the unsubstituted 8-HQ compounds. Reactivity studies with amino acids showed that the complexes undergo halido/L-histidine ligand exchange reactions, whereas L-cysteine causes cleavage of the arene ring, while L-methionine does not react with the metal complexes at all. Similar to the studies with L-histidine, the DNA model compounds 9-ethylguanine and guanosine 5′-monophosphate coordinate to the Ru center by replacing the leaving halido ligand. These observations make it likely that the metal-based pharmacophore acts as a prodrug. The high stability of the compounds in an acidic environment and their high QED_w^{mo} values make them potential candidates for further development for oral application.

EXPERIMENTAL SECTION

All reactions were carried out under standard Schlenk conditions in dry solvents unless otherwise stated. Chemicals obtained from commercial suppliers were used without prior purification. Solvents were dried according to literature procedures.[53] Ruthenium(III) chloride hydrate (99%) was purchased from Precious Metals Online, 5,7-dibromo-8-hydroxyquinoline (98%) and 8-hydroxyquinoline (99%) were obtained from AK Scientific, and α-terpinene, *n*-octanol, and 5,7-diiodo-8-hydroxyquinoline (97%) were purchased from Sigma-Aldrich. 5-Chloro-7-iodo-8-hydroxyquinoline (ultrapure) was purchased from OFC Inc., 5,7-dichloro-8-hydroxyquinoline (99%) from Acros, and sodium methoxide from Fluka. Potassium bromide was purchased from May and Baker (Sanofi-Aventis) and potassium iodide from Ajax Finechem. Bis[dichlorido(η^6-*p*-cymene)ruthenium-(II)] was prepared as described in the literature[54] and converted into bis[dibromido(η^6-*p*-cymene)ruthenium(II)] and bis[diiodo(η^6-*p*-cymene)ruthenium(II)].[55]

Elemental analyses for the synthesized compounds were performed at the Campbell Microanalytical Laboratory, The University of Otago.

NMR spectra were recorded on Bruker Avance AV 300 MHz, AVIII 400 MHz, and AVIII-HD 500 MHz NMR spectrometers at ambient temperature at 400.13 MHz (^{1}H) or 75.48, 100.61, or 75.48 MHz (^{13}C{^{1}H}). Chemical shifts are reported versus $SiMe_4$ and were determined by reference to the residual ^{1}H and ^{13}C{^{1}H} solvent peaks. For an unambiguous assignment of the characteristic resonances, multinuclear 2D (^{1}H−^{1}H COSY, ^{1}H−^{13}C HSQC, and HMBC) NMR spectroscopic experiments were conducted.

Melting points were measured in capillary tubes using a SMP30 Stuart Scientific melting point apparatus. High-resolution mass spectra were recorded on a Bruker microOTOF-Q II mass spectrometer in positive ion electrospray ionization (ESI) mode.

X-ray diffraction measurements of single crystals of **1a**,**b**, **3a**, and **5b** were carried out on a Siemens/Bruker SMART APEX II single-crystal diffractometer with a CCD area detector using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The data were processed using the SHELX2013 software packages.[56] All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were inserted at

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

calculated positions and refined with a riding model or without restrictions. Molecular structures were visualized using Mercury 3.5.1.

General Procedures for the Synthesis of Ru Complexes. The complexes were prepared using one of the following methods:

Method A. The ruthenium dimer $[Ru(\eta^6\text{-}p\text{-cymene})X_2]_2$ (with X = Cl, Br, I (0.45 equiv)) was added to a stirred solution of sodium methoxide (1.1 equiv) and ligand (1 equiv) in methanol. The solution was stirred under reflux for 1.5−4 h under a nitrogen atmosphere. Afterward the solvent was evaporated, the residue was dissolved in dichloromethane, the solution was filtered, and the complex was precipitated with *n*-hexane.

Method B. The ligand (1 equiv) and sodium methoxide (1.1 equiv) were dissolved in a mixture of chloroform (8 mL) and methanol (15 mL). Upon addition of the ruthenium dimer $[Ru(\eta^6\text{-}p\text{-cymene})X_2]_2$ (with X = Cl, Br, I (0.45 equiv)) to the stirred solution, the complex precipitated. After an additional 1 h of stirring at room temperature under a nitrogen atmosphere, the precipitate was collected by filtration, washed with *n*-hexane, and dried under vacuum.

Chlorido(8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (1a). The synthesis was performed according to method A using **1** (200 mg, 1.377 mmol) and bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] (380 mg, 0.62 mmol) to afford a brown solid (393 mg, 76%). Mp: 222−223 °C dec. Anal. Calcd for $C_{19}H_{20}NOClRu\cdot0.25H_2O$: C, 54.41; H, 4.93; N, 3.34. Found: C, 54.32; H, 4.76; N, 3.66. MS (ESI+): m/z 380.0587 $[M - Cl]^+$ (m_{theor} = 380.0588). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.14−1.18 (m, 6H, H_g), 2.31 (s, 3H, H_a), 2.76−2.83 (m, 1H, H_f), 5.31 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.42 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.48 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 5.60 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 6.82 (d, $^3J_{H5,H6}$ = 8 Hz, 1H, H6), 7.02 (d, $^3J_{H_4,H_5}$ = 8 Hz, 1H, H4), 7.30−7.34 (m, 2H, H2/H5), 8.06 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.90 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 18.1 (C16), 21.7 (C18), 21.9 (C19), 30.4 (C17), 80.0 (C11), 80.5 (C15), 81.7 (C12), 82.4 (C14), 97.9 (C10) 99.9 (C13), 109.0 (C5), 113.0 (C7), 122.3 (C2), 129.4 (C6), 129.7 (C4), 137.0 (C3), 144.0 (C9), 149.8 (C1), 168.8 (C8) ppm.

Bromido(8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (1b). The synthesis was performed according to method B using **1** (82 mg, 0.562 mmol) and bis[dibromido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.253 mmol) to afford a red solid (166 mg, 71%). Mp: 221−222 °C dec. Anal. Calcd for $C_{19}H_{20}NOBrRu$: C, 49.68; H, 4.39; N, 3.05. Found: C, 49.41; H, 4.34; N, 3.04. MS (ESI+): m/z 380.0589 $[M - Br]^+$ (m_{theor} = 380.0588). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.16−1.21 (m, 6H, H_g), 2.35 (s, 3H, H_a), 2.83−2.90 (m, 1H, H_f), 5.33 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.42 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.48 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 5.59 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 6.82 (d, $^3J_{H_5,H_6}$ = 8 Hz, 1H, H6), 7.02 (d, $^3J_{H_4,H_5}$ = 8 Hz, 1H, H4), 7.29−7.34 (m, 2H, H2/H5), 8.05 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.87 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 18.5 (C16), 21.7 (C18), 21.8 (C19), 30.5 (C17), 80.4 (C11), 88.2 (C15), 81.5 (C12), 81.9 (C14), 97.2 (C10), 100.8 (C13), 109.0 (C5), 113.1 (C7), 122.4 (C2), 129.3 (C6), 129.6 (C4), 137.0 (C3), 144.2 (C9), 150.2 (C1), 168.7 (C8) ppm.

Iodido(8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (1c). The synthesis was performed according to method B using **1** (66 mg, 0.454 mmol) and bis[diiodido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.204 mmol) to afford a brown solid (166 mg, 64%). Mp: 200−201 °C dec. Anal. Calcd for $C_{19}H_{20}NOIRu$: C, 45.07; H, 3.98; N, 2.77. Found: C, 44.84; H, 3.88; N, 2.97. MS (ESI+): m/z 380.0588 $[M - I]^+$ (m_{theor} = 380.0588). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.24 (m, 6H, H_g), 2.40 (s, 3H, H_a), 2.92−2.99 (m, 1H, H_f), 5.34 (d, $^3J_{H_c,H_d}$ = 5 Hz, 1H, H_c), 5.45 (d, $^3J_{H_c,H_d}$ = 5 Hz, 1H, H_c), 5.51 (d, $^3J_{H_d,H_c}$ = 7 Hz, 2H, H_d), 6.81 (d, $^3J_{H_5,H_6}$ = 8 Hz, 1H, H6), 6.99 (d, $^3J_{H_4,H_5}$ = 8 Hz, 1H, H4), 7.28−7.34 (m, 2H, H2/H5), 8.03 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.83 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 19.2 (C16), 21.9 (C18), 21.8 (C19), 308 (C17), 81.0 (C11), 81.4 (C15), 81.5 (C12), 82.1 (C14), 96.6 (C10), 102.4 (C13), 109.0 (C5), 113.2 (C7), 122.5 (C2), 129.3 (C6), 129.6 (C4), 136.9 (C3), 144.5 (C9), 151.0 (C1), 167.6 (C8) ppm.

Chlorido(5,7-dichloro-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (2a). The synthesis was performed according to method A using **2** (200 mg, 0.930 mmol) and bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] (258 mg, 0.420 mmol) to afford an ocher solid (288 mg, 71%). Mp: 242−246 °C dec. Anal. Calcd for $C_{19}H_{18}NOCl_3Ru$: C, 47.17; H, 3.75; N, 2.90%. Found: C, 47.08; H, 3.76; N, 2.97. MS (ESI+): m/z 447.9819 $[M - Cl]^+$ (m_{theor} = 447.9809). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.16−1.25 (m, 6H, H_g), 2.33 (s, 3H, H_a), 2.80−2.87 (m, 1H, H_f), 5.36 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.47 (t, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.69 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.44 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.51 (s, 1H, H4), 8.38 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.94 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 18.0 (C16), 21.6 (C18), 21.9 (C19), 30.4 (C17), 80.0 (C11), 81.2 (C15), 81.9 (C12), 82.4 (C14), 98.2 (C10), 100.5 (C13), 109.8 (C5), 116.0 (C7), 123.7 (C2), 125.5 (C4), 128.8 (C6), 133.9 (C3), 144.2 (C9), 151.8 (C1) 163.2 (C8) ppm.

Bromido(5,7-dichloro-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (2b). The synthesis was performed according to method B using **2** (120 mg, 0.4562 mmol) and bis[dibromido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.253 mmol) to afford an orange solid (212 mg, 79%). Mp: 249−252 °C dec. Anal. Calcd for $C_{19}H_{18}NOBrCl_2Ru$: C, 43.20; H, 3.43; N, 2.65. Found: C, 43.13; H, 3.54; N, 2.69. MS (ESI+): m/z 447.9790 $[M - Br]^+$ (m_{theor} = 447.9809). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.18−1.26 (m, 6H, H_g), 2.38 (s, 3H, H_a), 2.86−2.92 (m, 1H, H_f), 5.36 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.49 (d, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.63 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.43 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.50 (s, 1H, H4), 8.36 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.92 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 18.9 (C16), 22.1 (C18), 22.3 (C19), 31.0 (C17), 81.0 (C11), 82.1 (C15), 82.3 (C12), 82.4 (C14), 97.8 (C10), 102.0 (C13), 110.3 (C5), 116.6 (C7), 124.2 (C2), 125.9 (C4), 129.2 (C6), 134.4 (C3), 144.9 (C9), 152.7 (C1), 163.6 (C8) ppm.

Iodido(5,7-dichloro-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (2c). The synthesis was performed according to method A using **2** (97 mg, 0.454 mmol) and bis[diiodido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.204 mmol) to afford a orange-brown solid (161 mg, 68%). Mp: 230−231 °C dec. Anal. Calcd for $C_{19}H_{18}NOICl_2Ru$: C, 39.67; H, 3.15; N, 2.43. Found: C, 39.74; H, 3.40; N, 2.45. MS (ESI+): m/z 447.9799 $[M - I]^+$ (m_{theor} = 447.9809). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.29 (m, 6H, H_g), 2.43 (s, 3H, H_a), 2.94−3.00 (m, 1H, H_f), 5.35 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.55 (m, 3H, H_c/H_d), 7.40 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.49 (s, 1H, H4), 8.34 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.88 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 19.7 (C16), 22.1 (C18), 22.3 (C19), 31.3 (C17), 81.7 (C11), 81.7 (C15), 82.1 (C12), 83.4 (C14), 97.0 (C10), 103.7 (C13), 110.1 (C5), 116.8 (C7), 124.2 (C2), 125.9 (C4), 129.6 (C6), 134.3 (C3), 145.1 (C9), 153.4 (C1), 163.4 (C8) ppm.

Chlorido(5,7-dibromo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (3a). The synthesis was performed according to method B using **3** (220 mg, 0.726 mmol) and bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.326 mmol) to afford an ochre solid (287 mg, 76%). Mp: 258−259 °C dec. Anal. Calcd for $C_{19}H_{18}NOBr_2ClRu\cdot0.125C_6H_{14}$: C, 40.66; H, 3.41; N, 2.40%. Found: C, 40.85; H, 3.65; N, 2.34. MS (ESI+): m/z 537.8773 $[M - Cl]^+$ (m_{theor} = 537.8831). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.16−1.27 (m, 6H, H_g), 2.33 (s, 3H, H_a), 2.81−2.88 (m, 1H, H_f), 5.37 (d, $^3J_{H_c,H_d}$ = 5 Hz, 1H, H_c), 5.46 (t, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.68 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.45 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.80 (s, 1H, H5), 8.34 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.92 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 18.0 (C16), 21.7 (C18), 21.9 (C19), 30.5 (C17), 80.1 (C11), 81.5 (C15), 81.8 (C14), 82.3 (C12), 98.0 (C10), 98.7 (C5), 100.6 (C13), 106.1 (C7),

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

124.1 (C2), 127.3 (C4), 134.1 (C6), 136.4 (C3), 144.0 (C9), 151.7 (C1), 164.8 (C8) ppm.

Bromido(5,7-dibromo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (3b). The synthesis was performed according to method A using **3** (170 mg, 0.560 mmol) and bis[dibromido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.253 mmol) to afford a brown solid (185 mg, 59%). Mp: 239−242 °C dec. Anal. Calcd for $C_{19}H_{18}NOBr_3Ru{\cdot}0.5H_2O$: C, 36.44; H, 3.06; N, 2.24. Found: C, 36.42; H, 2.98; N, 2.31. MS (ESI+): *m*/*z* 537.8779 $[M - Br]^+$ (m_{theor} = 537.8831). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.19−1.30 (m, 6H, H_g), 2.37 (s, 3H, H_a), 2.87−2.94 (m, 1H, H_f), 5.37 (d, $^3J_{H_c,H_d}$ = 5 Hz, 1H, H_c), 5.48 (d, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.62 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.44 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.80 (s, 1H, H4), 8.32 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.89 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 18.9 (C16), 22.1 (C18), 22.3 (C19), 31.0 (C17), 81.1 (C11), 82.0 (C15), 82.2 (C14), 82.8 (C12), 97.6 (C10), 99.2 (C5), 102.1 (C13), 106.7 (C7), 124.6 (C2), 127.7 (C4), 134.5 (C6), 136.8 (C3), 144.6 (C9), 152.7 (C1), 165.2 (C8) ppm.

Iodido(5,7-dibromo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)-ruthenium(II) (3c). The synthesis was performed according to method B using **3** (138 mg, 0.454 mmol) and bis[diiodo(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.204 mmol) to afford a red crystalline product (214 mg, 78%). Mp: 250−253 °C dec. Anal. Calcd for $C_{19}H_{18}NOBr_2IRu{\cdot}0.125C_6H_{14}$: C, 35.14; H, 2.95; N, 2.08. Found: C, 34.94; H, 3.05; N, 2.06. MS (ESI+): *m*/*z* 537.8772 $[M - I]^+$ (m_{theor} = 537.8831). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.21−1.32 (m, 6H, H_g), 2.42 (s, 3H, H_a), 2.95−3.02 (m, 1H, H_f), 5.34 (d, $^3J_{H_c,H_d}$ = 5 Hz, 1H, H_c), 5.54 (m, 2H, H_c/H_d), 5.58 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.41 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.80 (s, 1H, H4), 8.30 (d, $^3J_{H3,H2}$ = 9 Hz, 1H, H3), 8.85 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 19.2 (C16), 21.6 (C18), 21.8 (C19), 30.8 (C17), 81.1 (C11), 81.3 (C15), 81.6 (C14), 83.2 (C12), 96.6 (C10), 98.6 (C5), 103.3 (C13), 106.3 (C7), 124.1 (C2), 127.1 (C4), 133.9 (C6), 136.2(C3), 144.4 (C9), 152.9 (C1), 164.6 (C8) ppm.

Chlorido(5,7-diiodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)-ruthenium(II) (4a). The synthesis was performed according to method B, using **4** (300 mg, 0.760 mmol) and bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] (208 mg, 0.340 mmol) to afford an ocher product (297 mg, 64%). Mp: 246−248 °C dec. Anal. Calcd for $C_{19}H_{18}NOI_2ClRu{\cdot}0.5H_2O$: C, 33.77; H, 2.83; N, 2.07. Found: C, 33.48; H, 2.57; N, 2.21. MS (ESI+): *m*/*z* 631.8524 $[M - Cl]^+$ (m_{theor} = 631.8521). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.18−1.31 (m, 6H, H_g), 2.33 (s, 3H, H_a), 2.84−2.91 (m, 1H, H_f), 5.37 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.43 (d, $^3J_{H_c,H_d}$ = 5 Hz, 2H, H_c/H_d), 5.66 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.43 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 8.16 (s, 1H, H5), 8.19 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.85 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 18.4 (C16), 22.3 (C18), 22.3 (C19), 30.9 (C17), 73.4 (C5), 80.7 (C11), 82.1 (C15), 82.5 (C14), 82.6 (C12), 83.1 (C7), 97.9 (C10), 101.2 (C13), 124.9 (C2), 131.0 (C4), 141.3 (C3), 142.8 (C9), 145.5 (C6), 151.9 (C1), 168.9 (C8) ppm.

Bromido(5,7-diiodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)-ruthenium(II) (4b). The synthesis was performed according to method A and the reaction mixture was refluxed for 1.5 h, using **4** (223 mg, 0.560 mmol) and bis[dibromido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.253 mmol) to afford an ocher product (303 mg, 83%). Mp: 215−216 °C dec. Anal. Calcd for $C_{19}H_{18}NOI_2BrRu{\cdot}H_2O$: C, 31.28; H, 2.76; N, 1.92. Found: C, 31.26; H, 2.53; N, 2.09. MS (ESI+): *m*/*z* 631.8533 $[M - Br]^+$ (m_{theor} = 631.8521). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.32 (m, 6H, H_g), 2.36 (s, 3H, H_a), 2.88−2.95 (m, 1H, H_f), 5.35 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.46 (t, $^3J_{H_c,H_d}$ = 5 Hz, 2H, H_c/H_d), 5.59 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.41 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 8.15 (s, 1H, H5), 8.17 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.83 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 18.9 (C16), 22.3 (C18), 22.3 (C19), 31.0 (C17), 73.4 (C5), 81.3 (C11), 81.8 (C15), 82.1 (C14), 83.2 (C7), 83.3 (C12), 97.1 (C10), 102.2 (C13), 124.9 (C2), 131.0 (C4), 141.3 (C3), 143.0 (C9), 145.5 (C6), 152.5 (C1), 168.8 (C8) ppm.

Iodido(5,7-diiodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)-ruthenium(II) (4c). The synthesis was performed according to method B using **4** (180 mg, 0.450 mmol) and bis[diiodido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.204 mmol) to afford a red product (163 mg, 53%). Mp: 219−220 °C dec. Anal. Calcd for $C_{19}H_{18}NOI_3Ru$: C, 30.10; H, 2.39; N, 1.85. Found: C, 30.21; H, 2.43; N, 1.93. MS (ESI+): *m*/*z* 631.8533 $[M - I]^+$ (m_{theor} = 631.8521). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.23−1.35 (m, 6H, H_g), 2.41 (s, 3H, H_a), 2.97−3.04 (m, 1H, H_f), 5.35 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.53 (t, $^3J_{H_c,H_d}$ = 5 Hz, 2H, H_c/H_d), 5.59 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.39 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 8.15 (s, 1H, H4), 8.16 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.81 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (125.8 MHz, DMSO): δ 19.6 (C16), 22.3 (C18), 22.3 (C19), 31.3 (C17), 73.4 (C5), 81.3 (C11), 82.0 (C15), 82.1 (C14), 83.3 (C7), 84.2 (C12), 96.2 (C10), 103.9 (C13), 124.9 (C2), 131.0 (C4), 141.2 (C3), 143.3 (C9), 145.4 (C6), 153.2 (C1), 168.7 (C8) ppm.

Chlorido(5-chloro-7-iodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (5a). The synthesis was performed according to method A using **5** (300 mg, 0.982 mmol) and bis[dichlorido(η^6-*p*-cymene)ruthenium(II)] (270 mg, 0.442 mmol) to afford an olive green product (374 mg, 73%). Mp: 250−252 °C dec. Anal. Calcd for $C_{19}H_{18}NOCl_2IRu{\cdot}0.25H_2O$: C, 39.36; H, 3.22; N, 2.41. Found: C, 39.24; H, 3.04; N, 2.57. MS (ESI+): *m*/*z* 539.9139 $[M - Cl]^+$ (m_{theor} = 539.9165). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.18−1.32 (m, 6H, H_g), 2.34 (s, 3H, H_a), 2.85−2.92 (m, 1H, H_f), 5.38 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.44 (d, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.66 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.45 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.80 (s, 1H, H4), 8.36 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.90 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 17.9 (C16), 21.8 (C18), 21.8 (C19), 30.4 (C17), 79.8 (C7), 80.2 (C12), 81.6 (C14), 82.0 (C15), 82.1 (C11), 97.4 (C10), 100.7 (C13), 110.5 (C5), 123.9 (C2), 126.5 (C4), 134.0 (C3), 135.7 (C6), 141.4 (C9), 151.5 (C1), 167.2 (C8) ppm.

Bromido(5-chloro-7-iodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (5b). The synthesis was performed according to method B and stirred at room temperature for 1 h using **5** (173 mg, 0.562 mmol) and bis[dibromido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.253 mmol) to afford an orange product (238 mg, 75%). Mp: 253−254 °C dec. Anal. Calcd for $C_{19}H_{18}NOClBrIRu$: C, 36.83; H, 2.93; N, 2.26. Found: C, 37.00; H, 2.85; N, 2.33. MS (ESI+): *m*/*z* 539.9168 $[M - Br]^+$ (m_{theor} = 539.9165). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.20−1.33 (m, 6H, H_g), 2.38 (s, 3H, H_a), 2.91−2.97 (m, 1H, H_f), 5.38 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.47 (t, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.61 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.45 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.80 (s, 1H, H4), 8.35 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.88 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (75.5 MHz, DMSO): δ 18.8 (C16), 22.3 (C18), 22.3 (C19), 31.0 (C17), 80.4 (C7), 81.2 (C12), 81.9 (C14), 82.0 (C15), 83.3 (C11), 97.1 (C10), 102.2 (C13), 111.0 (C5), 124.4 (C2), 126.9 (C4), 134.4 (C3), 136.2 (C6), 142.1 (C9), 152.5 (C1), 167.6 (C8) ppm.

Iodido(5-chloro-7-iodo-8-quinolinolato-$\kappa N^1,\kappa O^8$)(η^6-*p*-cymene)ruthenium(II) (5c). The synthesis was performed according to method B, using **5** (139 mg, 0.454 mmol) and bis[diiodido(η^6-*p*-cymene)ruthenium(II)] (200 mg, 0.204 mmol) to afford a red product (176 mg, 65%). Mp: 225−226 °C dec. Anal. Calcd for $C_{19}H_{18}NOClI_2Ru$: C, 34.23; H, 2.72; N, 2.10. Found: C, 34.40; H, 2.88; N, 2.19. MS (ESI+): *m*/*z* 539.9165 $[M - I]^+$ (m_{theor} = 539.9165). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.23−1.36 (m, 6H, H_g), 2.41 (s, 3H, H_a), 2.97−3.04 (m, 1H, H_f), 5.35 (d, $^3J_{H_c,H_d}$ = 6 Hz, 1H, H_c), 5.53 (t, $^3J_{H_c,H_d}$ = 6 Hz, 2H, H_c/H_d), 5.59 (d, $^3J_{H_d,H_c}$ = 6 Hz, 1H, H_d), 7.41 (dd, $^3J_{H2,H1}$ = 5 Hz, $^3J_{H2,H3}$ = 5 Hz 1H, H2), 7.79 (s, 1H, H4), 8.33 (d, $^3J_{H3,H2}$ = 8 Hz, 1H, H3), 8.85 (d, $^3J_{H1,H2}$ = 5 Hz, 1H, H1) ppm. $^{13}C\{^1H\}$ NMR (100.6 MHz, DMSO): δ 19.1 (C16), 21.8 (C18), 21.8 (C19), 30.8 (C17), 80.0 (C7), 80.8 (C12), 81.5 (C14), 81.5 (C15), 83.7 (C11), 95.7 (C10), 103.4 (C13), 110.4 (C5), 123.9 (C2),

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

126.4 (C4), 133.9 (C3), 135.6 (C6), 141.9 (C9), 152.8 (C1), 167.0 (C8) ppm.

Sulforhodamine B Cytotoxicity Assay. HCT116 and NCI-H460 cells were supplied by ATCC, while SiHa cells were from Dr. David Cowan, Ontario Cancer Institute, Canada. The cells were grown in αMEM (Life Technologies) supplemented with 5% fetal calf serum (Moregate Biotech) at 37 °C in a humidified incubator with 5% CO_2. The cells were seeded at 750 (HCT116, NCI-H460) or 4000 (SiHa) cells/well in 96-well plates and left to settle for 24 h. The compounds were added to the plates in a series of 3-fold dilutions, containing a maximum of 0.5% DMSO at the highest concentration. The assay was terminated after 72 h by addition of 10% trichloroacetic acid (Merck Millipore) at 4 °C for 1 h. The cells were stained with 0.4% sulforhodamine B (Sigma-Aldrich) in 1% acetic acid for 30 min in the dark at room temperature and then washed with 1% acetic acid to remove unbound dye. The stain was dissolved in unbuffered Tris base (10 mM; Serva) for 30 min on a plate shaker in the dark and quantified on a BioTek EL808 microplate reader at an absorbance wavelength of 490 nm with 450 nm as the reference wavelength to determine the percentage of cell growth inhibition by determining the absorbance of each sample relative to a negative (no inhibitor) and a no-growth control (day 0). The IC_{50} values were calculated with SigmaPlot 12.5 using a three-parameter logistic sigmoidal dose–response curve between the calculated growth inhibition and the compound concentration. The presented IC_{50} values are the mean of at least 3 independent experiments, where 10 concentrations were tested in duplicate for each compound.

Cellular Accumulation. For the cell uptake studies HCT116 cells (4×10^5/well) were seeded into 6-well plates and allowed to settle for 24 h. The compounds (10 μM, dissolved in DMSO and diluted with media to a concentration of 1% DMSO) were added for 2, 4, and 8 h drug-exposure time at 37 °C and 5% CO_2 before the medium was removed and the wells were washed twice with 1 mL of ice-cold PBS buffer. The cells were lysed with 2 mL of concentrated nitric acid (Suprapure from Merck, containing 0.1 μL of a 1000 ± 3 μg/mL thulium standard as internal standard; CPI International) and digested with an Ethos Up microwave digestion system (Milestone). Then the solutions were diluted with 10 mL of H_2O (18 MΩ, Millipore) and the metal content was determined by ICP-MS. The ICP-MS (Agilent 7700) with an ASX-500 autosampler (CETAC Technologies) in a Serie SuSi laminar flow hood (SPECTEC) was equipped with a MicroMist nebulizer and a Scott double pass spray chamber. The carrier gas flow rate of was 1 mL/min. The instrument was tuned for cerium, cobalt, lithium, magnesium, thallium, and yttrium by using the Tuning Solution for ICP-MS 7500cs (Agilent Technologies). The standards were matched to the samples with regard to HNO_3 concentration and the internal standard. The reported values are the mean of at least 3 independent uptake experiments conducted in duplicates with blank wells for each substance to account for unspecific binding of the lipophilic complexes to the plastic of the well plates.[57]

Lipophilicity. *log P.* The OECD guidelines[58] for the log *P* determination via the shake flask method were slightly modified. A known amount of complex was suspended in water (presaturated with *n*-octanol) and shaken for several days on an orbital shaker. Afterward the solution was centrifuged for 5 min to allow phase separation and the ruthenium content of the saturated aqueous solution was measured by ICP-MS to give the solubility of the compounds in H_2O. To obtain a partition coefficient, different ratios (0.5:1, 1:1, and 2:1) of the saturated solutions were shaken with presaturated *n*-octanol for 20 min on an orbital shaker. After shaking for an additional 5 min by hand and centrifugation for 5 min at 10000 rpm, the aqueous phase was collected with a syringe according to OECD guidelines. For the analysis, the samples were diluted 1:1000 with 5% HNO_3 and 1 ppb of Tb was used as the internal standard (CPI international). The Ru content was determined on an Agilent 7700 ICP-MS equipped with a MicroMist nebulizer, a Scott double pass spray chamber, and an ASX-500 autosampler (CETAC Technologies) in a Serie SuSi laminar flow hood (SPECTEC).

c log P. ChemBioDrawUltra 12.0 and software tools from Molinspiration (http://www.molinspiration.com) and VCCLAB (Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005) were used to estimate the compounds' lipophilicity on the basis of calculated logarithmic octanol–water partition coefficients (*c* log *P*) for the ligands **1**–**5**.[52] The ligands were chosen for the calculations, since the Ru(η^6-*p*-cymene)X moiety is present in all complexes and has therefore no significant influence on the relative values.

Biomolecule Interaction. The biomolecule interactions of the complexes were studied by ^{1}H NMR spectroscopy. The complexes were dissolved in d_6-DMSO and diluted with D_2O to otbain a 10% d_6-DMSO/D_2O solution. Equimolar amounts of the biomolecules L-methionine, L-cysteine, L-histidine, guanosine 5′-monophosphate, and 9-ethylguanine were added to the complexes. NMR spectra were collected over periods of 25–74 h depending on the rate of the reaction.

pH-Dependent Speciation of Hydrolysis Products. The changes in the speciation of the complexes upon dissolution and titration with DCl and NaOD were monitored by ^{1}H NMR spectroscopy. DCl (37 wt % solution in D_2O, 99.5% atom % deuterium) and NaOD (40 wt % solution in D_2O, 99+ atom % deuterium) were diluted 1:4 with D_2O prior to use and the pD value was measured with a freshly calibrated pH meter (Mettler Toledo Seven Multi) at room temperature in the NMR tube. The pH was converted to the pD by adding 0.4 to the reading taken from the glass electrode pH meter.[59] The titration was performed in both directions, from acidic to basic and basic to acidic to ensure reversibility.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.organomet.5b00868.

X-ray diffraction analysis data, NMR and mass spectra for reactions of **1a** with L-cysteine, L-methionine, L-histidine, guanosine 5′-monophosphate, and 9-ethylguananine, *c* log *P* values for ligands **1**–**5**, and calculation of the quantitative estimate of druglikeness (PDF)

Crystallographic data for **1a**,**b**, **3a**, and **5b** (CIF)

■ AUTHOR INFORMATION

Corresponding Author

*C.G.H.: e-mail, c.hartinger@auckland.ac.nz; tel, +64-9-37370599, ext 83220; fax, +64-9-3737 599 ext 87422; web, http://hartinger.wordpress.fos.auckland.ac.nz/.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This paper is dedicated to Prof. Bernhard K. Keppler on the occasion of his 60th birthday. We thank the University of Auckland (Doctoral Scholarship to M.K. and H.H.), the Royal Society of New Zealand, and COST CM1105 for financial support. The authors are grateful to Tanya Groutso for collecting the single-crystal X-ray diffraction data, to Stuart Morrow for support with the ICP-MS experiments, and to Tony Chen for ESI-MS analyses. We thank Auckland Science Analytical Services of the University of Auckland for access to their facilities.

■ REFERENCES

(1) Pasetto, L. M.; D'Andrea, M. R.; Rossi, E.; Monfardini, S. *Crit. Rev. Oncol. Hematol.* **2006**, *59*, 159–168.

(2) Yao, X.; Panichpisal, K.; Kurtzman, N.; Nugent, K. *Am. J. Med. Sci.* **2007**, *334*, 115–24.

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX–XXX

(3) Martin, L. P.; Hamilton, T. C.; Schilder, R. J. *Clin. Cancer Res.* **2008**, *14*, 1291−1295.
(4) Bergamo, A.; Gaiddon, C.; Schellens, J. H.; Beijnen, J. H.; Sava, G. *J. Inorg. Biochem.* **2012**, *106*, 90−9.
(5) Nazarov, A. A.; Hartinger, C. G.; Dyson, P. J. *J. Organomet. Chem.* **2014**, *751*, 251−260.
(6) Morris, R. E.; Aird, R. E.; del Socorro Murdoch, P.; Chen, H.; Cummings, J.; Hughes, N. D.; Parsons, S.; Parkin, A.; Boyd, G.; Jodrell, D. I.; Sadler, P. J. *J. Med. Chem.* **2001**, *44*, 3616−3621.
(7) Yan, Y. K.; Melchart, M.; Habtemariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, 4764−4776.
(8) 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*, 6858−6868.
(9) Jaouen, G.; Dyson, P. J., Medicinal organometallic chemistry. In *Comprehensive Organometallic Chemistry III-Applications III: Functional Materials, Environmental and Biological Applications*; O'Hare, D., Ed.; Elsevier: Oxford, U.K., 2007; Vol. *12*, pp 445−464.
(10) Noffke, A. L.; Habtemariam, A.; Pizarro, A. M.; Sadler, P. J. *Chem. Commun.* **2012**, *48*, 5219−46.
(11) Murray, B. S.; Babak, M. V.; Hartinger, C. G.; Dyson, P. J. *Coord. Chem. Rev.* **2016**, *306*, 86−114.
(12) Gianferrara, T.; Bratsos, I.; Alessio, E. *Dalton Trans.* **2009**, 7588−7598.
(13) Casini, A.; Hartinger, C.; Nazarov, A.; Dyson, P., Organometallic Antitumour Agents with Alternative Modes of Action. In *Medicinal Organometallic Chemistry*; Jaouen, G., Metzler-Nolte, N., Eds.; Springer: Berlin, Heidelberg, 2010; Vol. *32*, pp 57−80.
(14) Jakupec, M. A.; Keppler, B. K. *Curr. Top. Med. Chem.* **2004**, *4*, 1575−1583.
(15) Enyedy, É.; Dömötör, O.; Bali, K.; Hetényi, A.; Tuccinardi, T.; Keppler, B. *JBIC, J. Biol. Inorg. Chem.* **2015**, *20*, 77−88.
(16) Meier, S. M.; Hanif, M.; Adhireksan, Z.; Pichler, V.; Novak, M.; Jirkovsky, E.; Jakupec, M. A.; Arion, V. B.; Davey, C. A.; Keppler, B. K.; Hartinger, C. G. *Chem. Sci.* **2013**, *4*, 1837−1846.
(17) Meier, S. M.; Hanif, M.; Kandioller, W.; Keppler, B. K.; Hartinger, C. G. *J. Inorg. Biochem.* **2012**, *108*, 91−5.
(18) Kandioller, W.; Hartinger, C. G.; Nazarov, A. A.; Kasser, J.; John, R.; Jakupec, M. A.; Arion, V. B.; Dyson, P. J.; Keppler, B. K. *J. Organomet. Chem.* **2009**, *694*, 922−929.
(19) Hartinger, C. G.; Metzler-Nolte, N.; Dyson, P. J. *Organometallics* **2012**, *31*, 5677−5685.
(20) Kurzwernhart, A.; Kandioller, W.; Bächler, S.; Bartel, C.; Martic, S.; Buczkowska, M.; Mühlgassner, G.; Jakupec, M. A.; Kraatz, H.-B.; Bednarski, P. J.; Arion, V. B.; Marko, D.; Keppler, B. K.; Hartinger, C. G. *J. Med. Chem.* **2012**, *55*, 10512−10522.
(21) Kurzwernhart, A.; Kandioller, W.; Bartel, C.; Bachler, S.; Trondl, R.; Muhlgassner, G.; Jakupec, M. A.; Arion, V. B.; Marko, D.; Keppler, B. K.; Hartinger, C. G. *Chem. Commun.* **2012**, *48*, 4839−4841.
(22) Kurzwernhart, A.; Kandioller, W.; Enyedy, E. A.; Novak, M.; Jakupec, M. A.; Keppler, B. K.; Hartinger, C. G. *Dalton Trans.* **2013**, *42*, 6193−6202.
(23) Kandioller, W.; Balsano, E.; Meier, S. M.; Jungwirth, U.; Goschl, S.; Roller, A.; Jakupec, M. A.; Berger, W.; Keppler, B. K.; Hartinger, C. G. *Chem. Commun.* **2013**, *49*, 3348−3350.
(24) Hanif, M.; Nazarov, A. A.; Legin, A.; Groessl, M.; Arion, V. B.; Jakupec, M. A.; Tsybin, Y. O.; Dyson, P. J.; Keppler, B. K.; Hartinger, C. G. *Chem. Commun.* **2012**, *48*, 1475−1477.
(25) Moon, S.; Hanif, M.; Kubanik, M.; Holtkamp, H.; Söhnel, T.; Jamieson, S. M. F.; Hartinger, C. G. *ChemPlusChem* **2015**, *80*, 231−236.
(26) Babak, M. V.; Plażuk, D.; Meier, S. M.; Arabshahi, H. J.; Reynisson, J.; Rychlik, B.; Błauż, A.; Szulc, K.; Hanif, M.; Strobl, S.; Roller, A.; Keppler, B. K.; Hartinger, C. G. *Chem. - Eur. J.* **2015**, *21*, 5110−5117.
(27) Ang, W. H.; Parker, L. J.; De Luca, A.; Juillerat-Jeanneret, L.; Morton, C. J.; Lo Bello, M.; Parker, M. W.; Dyson, P. J. *Angew. Chem., Int. Ed.* **2009**, *48*, 3854−3857.
(28) Agonigi, G.; Riedel, T.; Zacchini, S.; Păunescu, E.; Pampaloni, G.; Bartalucci, N.; Dyson, P. J.; Marchetti, F. *Inorg. Chem.* **2015**, *54*, 6504−6512.
(29) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S. *J. Med. Chem.* **1988**, *31*, 2235−2246.
(30) Verma, A.; Yadav, M. R.; Giridhar, R.; Prajapati, N.; Tripathi, A. C.; Saraf, S. K. *Comb. Chem. High Throughput Screening* **2013**, *16*, 345−393.
(31) Al-Busafi, S. N.; Suliman, F. E. O.; Al-Alawi, Z. R. *Res. Rev. J. Chem.* **2014**, *3* (1−10), 10.
(32) Song, Y. n.; Xu, H.; Chen, W.; Zhan, P.; Liu, X. *MedChemComm* **2015**, *6*, 61−74.
(33) Mantri, A. P.; Kumar, S.; Sharadamma, K. C.; Kandepu, N.; Radhakrishna, P. M.; Kumara Swamy, B. E. *Int. J. Appl. Chem.* **2012**, *8*, 79−92.
(34) Kaur, D.; Yantiri, F.; Rajagopalan, S.; Kumar, J.; Mo, J. Q.; Boonplueang, R.; Viswanath, V.; Jacobs, R.; Yang, L.; Beal, M. F.; DiMonte, D.; Volitaskis, I.; Ellerby, L.; Cherny, R. A.; Bush, A. I.; Andersen, J. K. *Neuron* **2003**, *37*, 899−909.
(35) Adlard, P. A.; Cherny, R. A.; Finkelstein, D. I.; Gautier, E.; Robb, E.; Cortes, M.; Volitakis, I.; Liu, X.; Smith, J. P.; Perez, K.; Laughton, K.; Li, Q.-X.; Charman, S. A.; Nicolazzo, J. A.; Wilkins, S.; Deleva, K.; Lynch, T.; Kok, G.; Ritchie, C. W.; Tanzi, R. E.; Cappai, R.; Masters, C. L.; Barnham, K. J.; Bush, A. I. *Neuron* **2008**, *59*, 43−55.
(36) Timerbaev, A. R. *Metallomics* **2009**, *1*, 193−198.
(37) Gemel, C.; John, R.; Slugovc, C.; Mereiter, K.; Schmid, R.; Kirchner, K. *J. Chem. Soc., Dalton Trans.* **2000**, 2607−2612.
(38) Schuecker, R.; John, R. O.; Jakupec, M. A.; Arion, V. B.; Keppler, B. K. *Organometallics* **2008**, *27*, 6587−6595.
(39) Thai, T.-T.; Therrien, B.; Süss-Fink, G. *J. Organomet. Chem.* **2009**, *694*, 3973−3981.
(40) Nongbri, S. L.; Therrien, B.; Rao, K. M. *Inorg. Chim. Acta* **2011**, *376*, 428−436.
(41) Gobec, M.; Kljun, J.; Sosic, I.; Mlinaric-Rascan, I.; Ursic, M.; Gobec, S.; Turel, I. *Dalton Trans.* **2014**, *43*, 9045−9051.
(42) Peacock, A. F. A.; Melchart, M.; Deeth, R. J.; Habtemariam, A.; Parsons, S.; Sadler, P. J. *Chem. - Eur. J.* **2007**, *13*, 2601−2613.
(43) Patra, M.; Joshi, T.; Pierroz, V.; Ingram, K.; Kaiser, M.; Ferrari, S.; Spingler, B.; Keiser, J.; Gasser, G. *Chem. - Eur. J.* **2013**, *19*, 14768−14772.
(44) Hall, M. D.; Telma, K. A.; Chang, K. E.; Lee, T. D.; Madigan, J. P.; Lloyd, J. R.; Goldlust, I. S.; Hoeschele, J. D.; Gottesman, M. M. *Cancer Res.* **2014**, *74*, 3913−3922.
(45) Guo, W.; Zheng, W.; Luo, Q.; Li, X.; Zhao, Y.; Xiong, S.; Wang, F. *Inorg. Chem.* **2013**, *52*, 5328−5338.
(46) Renfrew, A. K. *Metallomics* **2014**, *6*, 1324−1335.
(47) Singh, S. K.; Pandey, D. S. *RSC Adv.* **2014**, *4*, 1819−1840.
(48) Kandioller, W.; Hartinger, C. G.; Nazarov, A. A.; Bartel, C.; Skocic, M.; Jakupec, M. A.; Arion, V. B.; Keppler, B. K. *Chem. - Eur. J.* **2009**, *15*, 12283−12291.
(49) Kandioller, W.; Hartinger, C. G.; Nazarov, A. A.; Kuznetsov, M. L.; John, R. O.; Bartel, C.; Jakupec, M. A.; Arion, V. B.; Keppler, B. K. *Organometallics* **2009**, *28*, 4249−4251.
(50) Adhireksan, Z.; Davey, G. E.; Campomanes, P.; Groessl, M.; Clavel, C. M.; Yu, H.; Nazarov, A. A.; Yeo, C. H. F.; Ang, W. H.; Dröge, P.; Rothlisberger, U.; Dyson, P. J.; Davey, C. A. *Nat. Commun.* **2014**, *5*, 3462.
(51) Bickerton, G. R.; Paolini, G. V.; Besnard, J.; Muresan, S.; Hopkins, A. L. *Nat. Chem.* **2012**, *4*, 90−98.
(52) Tetko, I. V.; Gasteiger, J.; Todeschini, R.; Mauri, A.; Livingstone, D.; Ertl, P.; Palyulin, V. A.; Radchenko, E. V.; Zefirov, N. S.; Makarenko, A. S.; Tanchuk, V. Y.; Prokopenko, V. V. *J. Comput.-Aided Mol. Des.* **2005**, *19*, 453−463.
(53) Williams, D. B. G.; Lawton, M. *J. Org. Chem.* **2010**, *75*, 8351−8354.
(54) Bennett, M. A.; Smith, A. K. *J. Chem. Soc., Dalton Trans.* **1974**, 233.

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX

(55) da Silva, A. C.; Piotrowski, H.; Mayer, P.; Polborn, K.; Severin, K. *J. Chem. Soc., Dalton Trans.* **2000**, 2960−2963.
(56) Sheldrick, G. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008**, *64*, 112−122.
(57) Egger, A. E.; Rappel, C.; Jakupec, M. A.; Hartinger, C. G.; Heffeter, P.; Keppler, B. K. *J. Anal. At. Spectrom.* **2009**, *24*, 51−61.
(58) Test No. 107: Partition Coefficient (n-octanol/water): Shake Flask Method. *OECD Guidelines for the Testing of Chemicals, Section 1*; OECD Publishing, 1995.
(59) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.

DOI: 10.1021/acs.organomet.5b00868
Organometallics XXXX, XXX, XXX−XXX