ORGANOMETALLICS

ARTICLE

pubs.acs.org/Organometallics

Mono- and 1,1′-Disubstituted Organoruthenium Cyclopentadiene Complexes: Synthesis, Structural Characterization, and Antitumoral Evaluation

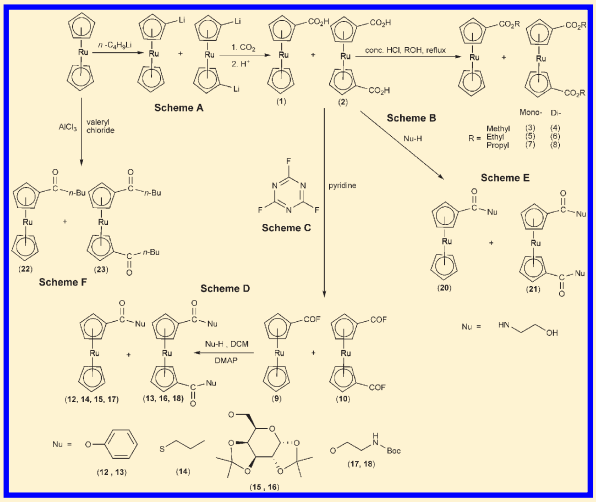
Leanne S. Micallef,[†] Bradley T. Loughrey,[†] Peter C. Healy,[†] Peter G. Parsons,[‡] and Michael L. Williams*[,†]

[†]Eskitis Institute for Cell and Molecular Therapies, Griffith University, Brisbane, Australia

[‡]Drug Discovery Group, Queensland Institute of Medical Research, Brisbane, Australia

Supporting Information

ABSTRACT: This article outlines the synthesis and characterization of a structurally diverse range of mono- and 1,1′-disubstituted ruthenocenyl complexes. Compounds were prepared through organic manipulation of ruthenocenefluorocarbonyl and carboxylic acid functional groups and via the Friedel−Crafts acylation of ruthenocene. A dimetalated acid anhydride of the formula $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\text{-}CO)]_2O$ was also prepared, and the X-ray structure of this molecule is reported. Complexes were evaluated for their antiproliferative properties against a range of tumorigenic cell lines and a control human fibroblast, with results indicating these organoruthenium metallocenes to possess moderate to weak cytotoxicity toward cancerous cells.



INTRODUCTION

Biological inorganic chemistry is a rapidly developing multidisciplinary field that encompasses the preparation and investigation of inorganic complexes of significant biological importance.[1−5] Metals and metal-based complexes have to date played key roles in the development of modern pharmacology, and future generations of these molecules hold the potential to aid in the treatment and diagnoses of disease states that are currently intractable. The most commonly accepted pharmacological role for metal-based complexes is as chemotherapeutic agents, where the square-planar platinum compound cisplatin and its second-generation analogues are the most extensively used complexes in clinical therapy.[6] Considerable research has been carried out investigating the anticancer properties of inorganic complexes comprising an array of different transition metals, and such elements as titanium, gallium, gold, and ruthenium have all produced promising libraries of novel anticancer complexes.[1] Out of the assortment of metallo-drugs that contain metals other than platinum, ruthenium compounds have proven to be the most promising,[7] with two of these coordination complexes, KP1019 and NAMI-A (Figure 1), currently progressing through clinical trials.[8]

While studies into the biological properties of inorganic coordination complexes have been highly topical over the past decade, organometallic complexes have only been sparingly investigated, with recent results suggesting that these systems hold the potential to find use as therapeutic agents.[9] The most widely studied organoruthenium compounds are the ruthenium(II) arene compounds (also referred to as piano-stool complexes) pioneered by Sadler and Dyson.[10−12] Complexes of the type [(R-Ph)Ru(Y−Z)L] (R-Ph = substituted arene, Y-Z = bidentate ligand, and L = monodentate anion) produced by Sadler's laboratory have proven to be potent cytotoxic agents against a range of tumor cell lines both *in vitro* and *in vivo*.[10,12]

The RAPTA series of compounds from Dyson's laboratory [(R-Ph)Ru(YZ)PTA], which incorporate a 1,3,5-triaza-7-phospha-adamantane (PTA) ligand and two monodentate ligands (YZ), have proven to be effective antimetastatic agents with comparable *in vivo* biological activity to NAMI-A.[11,12] The promising biological effects displayed by both of these classes of ruthenium(II) arene half-sandwich complexes prompted our research group to synthesize and biologically evaluate two distinct series of organoruthenium full-sandwich complexes of the type $[(R_5\text{-}Cp)RuCp]$, where R_5 represented a series of pentasubstituted ester functional groups,[13] and $[(R\text{-}Ph)RuCp^*]^+BPh_4^-$, where R represents an array of monosubstituted functional groups including esters, ketones, carbamates,

Received: September 28, 2010
Published: March 01, 2011

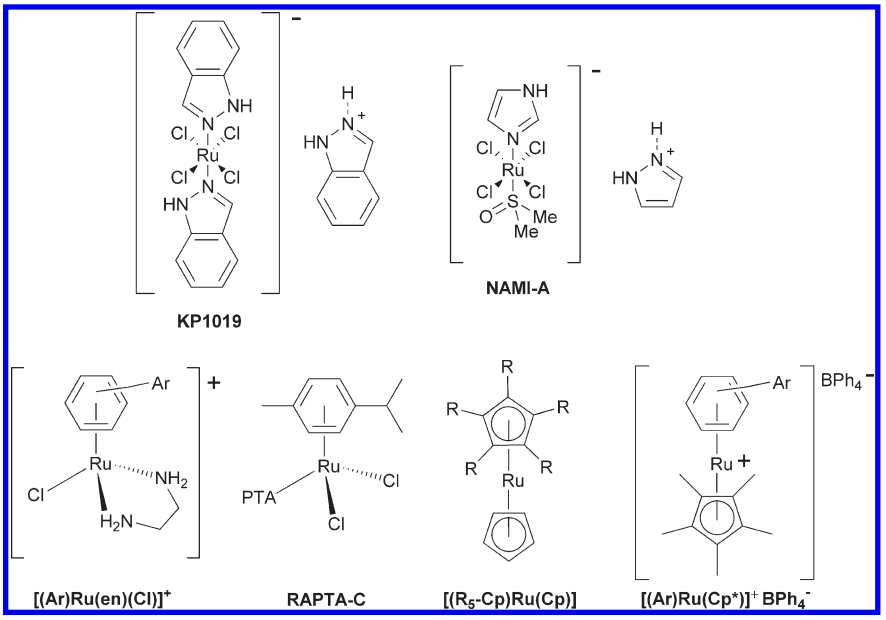


Figure 1. Ruthenium(III) coordination compounds currently progressing through clinical trials (KP1019 and NAMI-A) and four organoruthenium(II) anticancer complexes.

alkyl groups, amines, and sulfonamides.[14−16] The results from these studies demonstrated the cationic organoruthenium complexes to possess potent and selective antiproliferative activity toward a range of cancerous cell lines *in vitro*, with the degree of growth inhibition dependent on the lipophilicity of the arene ligand.[14−16] Of particular interest, however, was the relative inactivity of the neutral ruthenocenyl molecules in comparison to their corresponding cationic derivatives. These neutral ruthenocenyl molecules were on average over 2 orders of magnitude less active than the cationic complexes, highlighting the relationship between a delocalized cationic charge and biological activity. This result was in accordance with those achieved during the cytotoxic evaluation of a myriad of ferrocenyl complexes, where it had been shown previously that ferrocenium $[Fe(III)Cp_2]^+$ salts (delocalized cationic derivatives of ferrocene) were drastically more active both *in vitro* and *in vivo* compared to their neutral counterparts.[17] This increased biological activity is generally regarded within the literature to be a consequence of the increased aqueous solubility imparted through the addition of the positive charge.[17] It therefore could be envisioned that the increased cytotoxicity observed when transitioning from the $[(R_5\text{-Cp})RuCp]$ complexes to the $[(R\text{-Ph})RuCp^*]^+$ salts may principally be due to an overall increase in the hydrophilicity of the organoruthenium moiety. If this were true, then it is highly likely that neutral ruthenocenyl complexes incorporating highly hydrophilic functional groups would display comparative antiproliferative activity to both the $[Fe(III)Cp_2]^+$ and $[(R\text{-}Ph)RuCp^*]^+$ complexes, respectively. To test this hypothesis, we prepared, characterized, and biologically evaluated a series of neutral, mono- and 1,1′-disubstituted ruthenocenyl complexes incorporating functional groups with a varied hydrophilic/hydrophobic balance such as carboxylic acids, acid fluorides, esters, thioesters, ketones, alcohols, amides, and glycoconjugates. Preparation of these complexes afforded us the opportunity to further evaluate how the nature of both charge and aqueous solubility impact the overall antiproliferative activity of organoruthenium metallocenes.

RESULTS AND DISCUSSION

Synthesis and Characterization. Interest in the functionalization of metallocenes began shortly after the discovery of ferrocene in 1951,[18] with a slew of strategies for the derivatization of this molecule emerging shortly after Pauson's serendipitous find. Numerous studies were undertaken that highlighted the aromatic reactivity of the ferrocene molecule, particularly demonstrating its ability to act as an electrophile in a plethora of substitution reactions.

These include, but are not limited too, Friedel−Crafts acylation[19] and alkylation,[20] formylation,[21] sulfonation,[22] metalation (with *n*-butyllithium,[23,24] phenylsodium,[25] and mercuric acetate[23]), arylation with diazonium salts,[23,26] and N-terminal amidation upon treatment with isocyanates.[27,28] Derivatization of ruthenocene has been less extensively studied; however comparison of the aromatic reactivity of group 8 metallocenes in 1960 by Rausch et al. indicated that both ruthenocene and osmocene, like ferrocene, exhibit substitution reactions characteristic of those observed for generic aromatic systems.[29] This publication reported the Friedel−Crafts acylation and arylation of ruthenocene, its N-terminal amidation upon treatment with isocyanates, and also the novel preparation of both mono- (**1**) and 1,1′-disubstituted (**2**) ruthenocenecarboxylic acids.[29] These carboxylic acids of ruthenocene were of particular interest to this study due to the potential they provide as intermediates for the synthesis of a library of mono- and 1,1′-disubstituted ruthenocenes through modification using simple organic procedures. As mentioned, complexes **1** and **2** were originally prepared in 1960 by Rausch et al., who synthesized these molecules via a two-step procedure (Figure 2, Scheme A) beginning with the lithiation of ruthenocene to yield a mixture of both the mono- and 1,1′-dimetalated ruthenocenyl derivatives. Carbonation and acid-catalyzed hydrolysis of the reaction mixture then prompted formation of complexes **1** and **2** in identical yields of 24%, respectively.[29] This literature method proved to be an effective route for preparation of the required quantities of complexes **1**

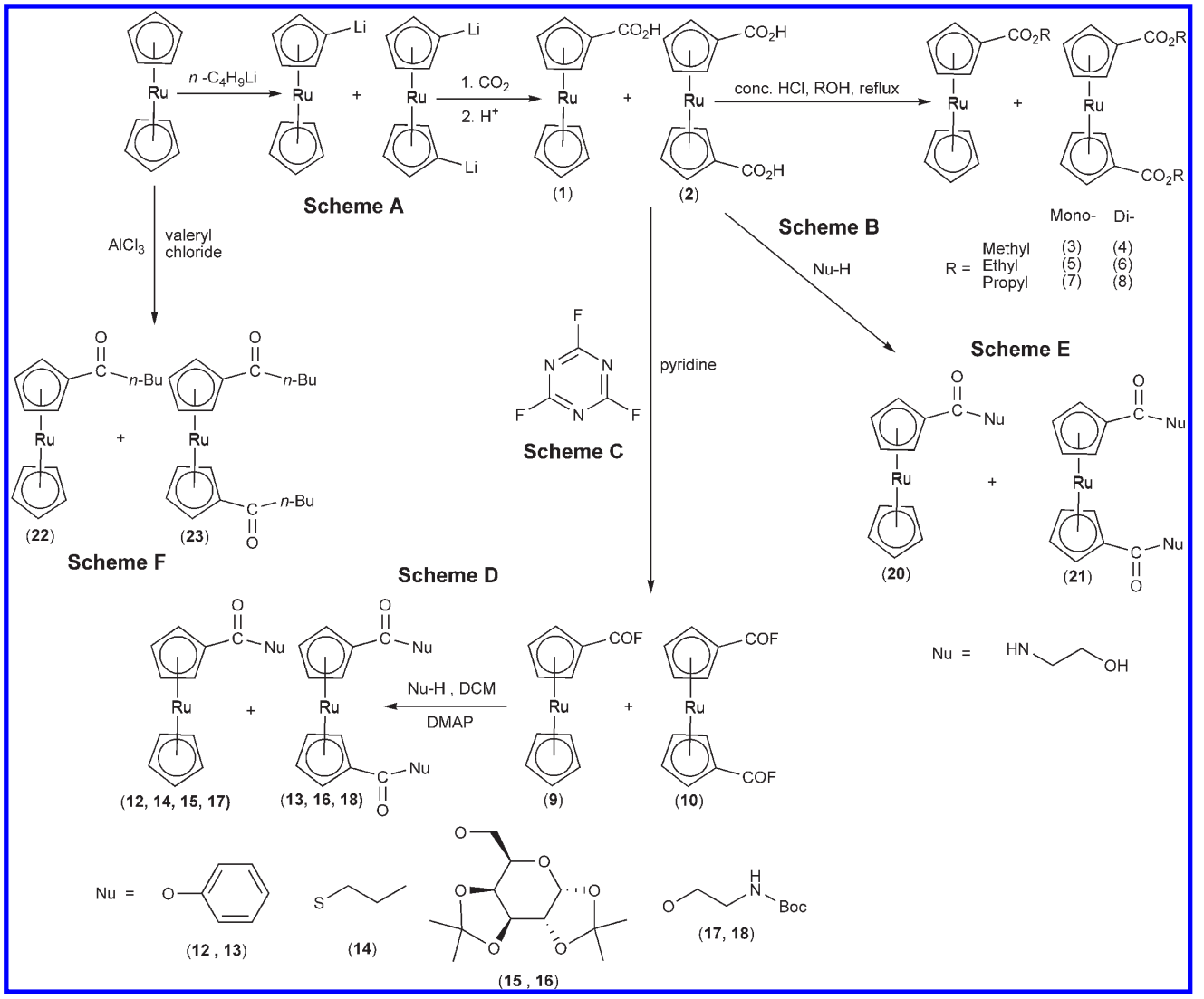


Figure 2. Preparatory methods incorporated for the synthesis of a structurally diverse library of mono- and 1,1′-disubstituted ruthenocenyl complexes.

and **2**; however, due to the relative insolubility of these organoruthenium carboxylic acids in common organic solvents, their separation by chromatography or recrystallization was found to be laborious. It was therefore found to be more efficient to convert complexes **1** and **2** to other desired reaction intermediates/products as a mixture prior to separation of the mono- and 1,1′-disubstituted products via silica column chromatography.

The organoruthenium carboxylic acids (**1** and **2**) were found to be viable intermediates for the preparation of a range of ruthenocenyl alkyl esters. Complexes **1** and **2** readily esterify in the presence of an alcohol solvent and a catalytic volume of concentrated hydrochloric acid under reflux conditions (Figure 2, Scheme B). This method afforded the preparation of a series of mono- and 1,1′-disubstituted methyl (**3**, **4**), ethyl (**5**, **6**), and propyl (**7**, **8**) esters, respectively, with complexes obtained in yields ranging between 39% and 84%. As observed during our previous studies into the preparation and reactivity of pentasubstituted ruthenocenyl ester complexes,[13] attempts to perform further substitution reactions with complexes **1** and **2** using poor nucelophiles prompted minimal to no conversion of the organoruthenium carboxylic acids to the target complexes. It

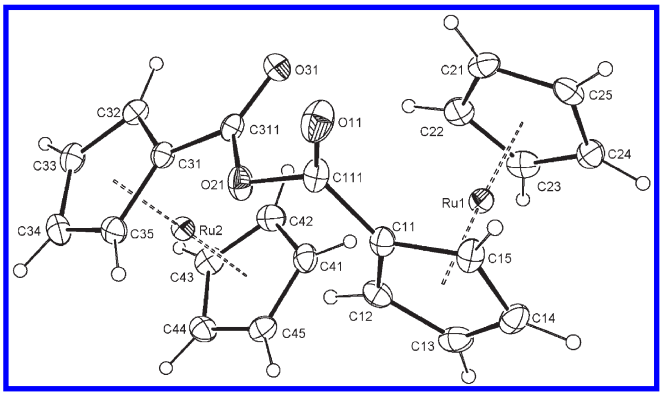


Figure 3. Molecular projection of ruthenocenecarboxylic anhydride; $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO)]_2O$ (**11**).

was therefore necessary to convert complexes **1** and **2** to more electrophilic intermediates such as mono- and 1,1′-disubstituted alkyl halides as a means of facilitating the preparation of a wider array of ruthenocenyl complexes.

dx.doi.org/10.1021/om100928s |*Organometallics* 2011, 30, 1395–1403

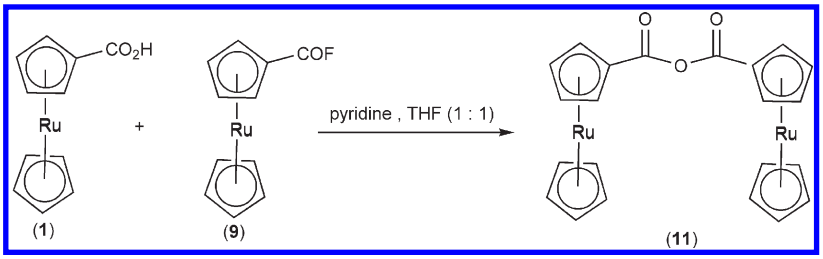


Figure 4. Synthetic scheme for the preparation of $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO)]_2O$ (**11**).

The organoruthenium carboxylic acids were converted to highly stable, versatile acid fluoride intermediates using a modified preparative scheme originally incorporated by our group as a route to pentafluorocarbonyl ruthenocene.[13] Synthesis of the mono- (**9**) and 1,1′-disubstituted (**10**) fluorocarbonyl ruthenocenyl derivatives was achieved in yields of 82% and 73%, respectively, through the reaction between a mixture of both carboxylic acids **1** and **2**, cyanuric fluoride, and pyridine in anhydrous dichloromethane (Figure 2, Scheme C). Complexes **9** and **10** could be isolated as pure, yellow, microcrystalline solids via silica column chromatography using a solution of 1:5 ethyl acetate/hexane as the eluent. Unlike pentafluorocarbonyl ruthenocene, which displayed limited stability in the presence of atmosphere,[13] both mono- (**9**) and 1,1′-disubstituted (**10**) fluorocarbonyl ruthenocene appear to possess relatively high levels of stability under standard atmospheric conditions. Despite this apparent stability however, fresh samples of complexes **9** and **10** were always prepared prior to use.

Column chromatography of freshly prepared samples of complexes **9** and **10** often resulted in the isolation of small quantities (∼2% yield) of a third unique product as a pale yellow powder. Recrystallization of this compound from ethyl acetate yielded crystals suitable for X-ray diffraction. Structure determination indicated the molecule to be the diruthenocenyl anhydride complex of the structure $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO)]_2O$ (**11**, Figure 3). Preparation of **11** as the major product was achieved through the reaction between the ruthenocenecarboxylic acid (**1**) and an equimolar quantity of monofluorocarbonyl ruthenocene (**9**) in a mixture of pyridine and THF at room temperature (Figure 4). Complex **11** is highly stable under standard atmospheric conditions and soluble in organic solvents and has a melting point of 150−152 °C. The stability of this organoruthenium molecule is consistent with the high stability reported for the analogous ferrocenecarboxylic anhydride prepared and studied by Wang et al.[30]

Following the synthesis and isolation of the mono- (**9**) and 1,1′-disubstituted (**10**) fluorocarbonyl ruthenocene complexes, it was of interest to investigate the versatility of these molecules as starting materials for a range of substitution reactions using such poor nucleophiles as phenol, 1-propanethiol, and 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (a glycoconjugate). These organoruthenium acid fluorides were also incorporated for the attempted C-terminus coupling of *N*-Boc-ethanolamine to the ruthenocene moiety. Each nucleophile was reacted with the respective organoruthenium acid halide in an anhydrous DCM solution in the presence of the nucleophilic acylation catalyst 4-dimethylaminopyridine (DMAP) (Figure 2, Scheme D). This strategy proved to be an effective synthetic route to the target molecules, with the mono- (**12**, **14**, **16**, **17**) and 1,1′-disubstituted (**13**, **15**, **18**) ruthenocenyl complexes forming from these nucleophiles in yields ranging from 31% to 68%. Compound **12** was isolated as a pale yellow, waxy solid, while the remaining complexes (**13**−**18**) were isolated as pale yellow or white, microcrystalline solids, respectively. Complex **17** was successfully deprotected via a 2 M solution of HCl in ethyl acetate at 0 °C, yielding complex **19**, $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO_2\text{-}(CH_2)_2NH_3)]Cl$, as a white powder in a yield of 95%.

Ethanolamine was also found to be eligible for coupling to the ruthenocene moiety through the N-terminus via the reaction between an excess of the unprotected, bifunctional ethanolamine and the mono- (**1**) and 1,1′-disubstituted (**2**) carboxylic acids of ruthenocene (Figure 2, Scheme E). These reactions yielded the amide products, complexes **20** and **21**, respectively, with no evidence of esterification present in either reaction mixture.

Ruthenocenyl ketones $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO(CH_2)_3CH_3)]$ (**22**) and $[Ru(\eta^5\text{-}C_5H_4CO(CH_2)_3CH_3)_2]$ (**23**) were prepared through the Friedel−Crafts acylation of ruthenocene in an anhydrous DCM solvent (Figure 2, Scheme F). The bright yellow acyl cation was first formed *in situ* through the reaction between aluminum trichloride and valeryl chloride, prior to the dropwise addition of an appropriate strength DCM solution of ruthenocene. Upon reaction completion, complexes **22** and **23** were isolated as bright yellow, crystalline solids using silica column chromatography (1:4 ethyl acetate/hexane) in yields of 13% and 27%, respectively.

In summary, a structurally diverse range of mono- and 1,1′-disubstituted ruthenocenyl complexes was successfully prepared through organic manipulation of ruthenocenefluorocarbonyl and carboxylic acid functional groups and via the Friedel−Crafts acylation of ruthenocene. A dimetalated acid anhydride of ruthenocene was also prepared, and the X-ray structure of this molecule reported. This series of pure, mono- and 1,1′-disubstituted ruthenocenyl complexes afforded us the opportunity to study these molecules *in vitro* and ascertain their biological activity. Where possible (stability pending), all prepared complexes (**1**−**23**) were characterized using Fourier transform infrared and NMR spectroscopy, electrospray mass spectrometry, melting point, and microanalysis (C, H %) prior to biological evaluation.

Cell Survival Studies. The antiproliferative properties of the mono- and 1,1′-disubstituted ruthenocenyl complexes were established by monitoring their ability to inhibit the growth of both cancerous and normal cells over a six-day period using the SRB (sulforhodamine B) colorimetric assay.[31] Cell lines chosen for this investigation included MCF7 (hormone-dependent breast cancer), DU145 (prostate cancer grade II), CI80-13S (ovarian cancer), two individual phenotypes of human melanoma (MM96L and MM418c5). and a control human fibroblast (NFF, neonatal foreskin fibroblasts). The carcinoma cell lines MCF7, CI80-13S, and MM96L have previously demonstrated susceptibility to inhibition by organoruthenium complexes,[13−16] while DU145 and MM418c5 provided two additional tumor models that are both susceptible to a variety of applied

Table 1. Inhibitory Concentration That Limits Cellular Proliferation by 50% (IC_{50}) for the Mono- [$Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4\text{-}R)$] and 1,1′-Disubstituted [$Ru(\eta^5\text{-}C_5H_4\text{-}R)_2$] Ruthenocenyl Complexes

			IC_{50} values (μM)[a]					
complex	substitution	R	NFF	MCF7	DU145	CI80−13S	MM96L	MM418c5
1	mono	CO_2H	>1000	>1000	>1000	254	>1000	>1000
2	1,1′-di	CO_2H	>1000	>1000	>1000	689	>1000	>1000
3	mono	CO_2Me	>1000	>1000	>1000	622	>1000	>1000
4	1,1′-di	CO_2Me	>1000	821	>1000	570	>1000	>1000
5	mono	CO_2Et	>1000	577	>1000	198	890	742
6	1,1′-di	CO_2Et	>1000	582	655	582	>1000	218
7	mono	CO_2Pr	394	79.0	221	66.0	189	46.0
8	1,1′−di	CO_2Pr	242	166	149	12.0	50.0	40.0
9	mono	COF	>1000	830	>1000	>1000	812	>1000
10	1,1′-di	CO_2F	>1000	>1000	>1000	>1000	>1000	>1000
12	mono	CO_2Ph	925	216	398	23.0	171	85.0
13	1,1′-di	CO_2Ph	>1000	>1000	>1000	170	>1000	>1000
14	mono	CO_2Glyco	676	531	>1000	676	>1000	>1000
15	1,1′-di	CO_2Glyco	398	821	883	547	821	826
16	mono	COSPr	255	120	225	30.0	150	24.0
17	mono	$CO_2(CH_2)_2NHBoc$	657	179	299	131	180	454
18	1,1′-di	$CO_2(CH_2)_2NHBoc$	>1000	355	165	206	429	182
19	mono	$CO_2(CH_2)_2NH_3^+\ Cl^-$	113	70.0	42.0	36.0	92.0	101
20	mono	$CONH(CH_2)_2OH$	251	>1000	>1000	>1000	>1000	770
21	1,1′-di	$CONH(CH_2)_2OH$	>1000	>1000	>1000	>1000	>1000	840
22	mono	COBu	222	238	>1000	349	317	>1000
23	1,1′-di	COBu	751	250	476	163	175	526
cisplatin			3.30	1.80	1.78	3.20	1.70	0.80

[a] Errors are within the range of ±5−10% of the reported value. Results are the average of three separate experiments. Ruthenocene has been previously screened using this assay technique against the same six cell lines and was found to exhibit no growth inhibitory effect at maximal concentration (IC_{50} > 1000).[13]

chemotherapeutics and also display different mechanisms of resistance to chemotherapeutic treatment.

Results obtained during this study are listed in Table 1 and demonstrate these mono- and 1,1′-disubstituted ruthenocenyl derivates to be, on average, rather ineffectual growth inhibitors of each cell line compared to a known chemotherapeutic agent such as cisplatin. The results achieved during this study are in accordance with those obtained for the prior evaluated penta-substituted molecules of ruthenocene.[13] The degree of ruthenocene substitution appears to play little, if any, role in imparting these complexes with biological activity, with the average growth inhibitory effect of these ruthenocenyl compounds appearing similar between the mono-, 1,1′-di-, and pentasubstituted series of molecules.[13] Cytotoxicity appears predominantly governed via choice of the substituted functional group, with the average antiproliferative effect of the monosubstituted complexes following the sequence **19** > **16** ≈ **7** > **12** ≈ **17** > **22** > **5** > **14** ≈ **1** ≈ **20** ≈ **3** > **9** and the 1,1′-disubstituted complexes following the sequence **8** > **18** ≈ **23** > **6** ≈ **15** > **13** ≈ **4** > **2** ≈ **21** > **10**, respectively.

As mentioned previously, it is generally regarded that increasing the aqueous solubility of ferrocenyl complexes is a key strategy for imparting the resulting compounds with increased antiproliferative activity both *in vitro* and *in vivo*.[17]

Our results appear to suggest the opposite is true for ruthenocenyl molecules, particularly *in vitro*, where the neutral hydrophilic carboxylic acid (**1**, **2**), fluorocarbonyl (**9**, **10**), glycoconjugate (**14**, **15**), and ethanolamide (**20**, **21**) derivatives of ruthenocene achieve an average IC_{50} value greater than 880 μM against tumorigenic cells. The most cytotoxic neutral ruthenocenyl derivatives assayed during this study were found to be the lipophilic monosubstituted propyl (**7**), phenyl (**12**), and thiopropyl (**16**) esters in addition to the 1,1′-disubstituted propyl ester (**8**), respectively. These hydrophobic complexes of ruthenocene achieved an average IC_{50} value of 123 μM against cancerous cells, with the 1,1′-disubstituted propyl ester (**8**) in particular achieving low micromolar IC_{50} values against the CI80-13S (12.0 μM), MM418c5 (40.0 μM), and MM96L (50.0 μM) tumorigenic cell lines. Cellular specificity of complex **8** is also high, with this molecule demonstrating, on average, 10-fold greater growth inhibition of these cancerous cells versus control human fibroblasts (NFF).

Of particular interest, however, are the results achieved by the cationic monosubstituted ruthenocenyl derivative **19**, of the structure $[Ru(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5H_4CO_2(CH_2)_2NH_3)]^+$ (assayed as the chloride salt). This molecule prompted the highest levels of growth inhibition observed during this study, achieving an average IC_{50} value of 68.2 μM against cancerous cell lines. Compared to its neutral counterpart (complex **17**), protonation of this molecule results in a 4-fold increase in cytotoxicity, indicating that the presence of the cationic charge drastically increases the biological activity of complex **19**. Results achieved during this study suggest that the increased cytotoxicity observed here cannot merely be attributed to increased aqueous solubility, as the neutral hydrophilic ruthenocenyl molecules (**1**, **2**, **9**, **10**, **14**, **15**, **20**, **21**) are around 7-fold less active than the lipophilic ruthenocenyl derivatives (**7**, **8**, **12**, **16**), respectively. Comparison of the cumulative results obtained over the course

of our studies on both neutral[13] and cationic organoruthenium full-sandwich complexes[14−16] indicates that the biological activity of these metallocenes appears to hinge on a balance between hydrophilicity and lipophilicity, with optimized complexes generally being polar (due to the presence of a delocalized positive charge) and hydrophobic (due to substitution of a lipophilic functional group). Aqueous solubility imparted through the presence of the delocalized positive charge is likely to be a benefit for complex delivery, while substituted hydrophobic functional groups maintain the lipid solubility required for the traversal of cellular membranes. It also appears likely that the presence of a positive charge delocalized over the structure of these metallocenes contributes to the biological activity of the molecules in additional ways other than simply improving aqueous solubility. Previous studies on a variety of both organic and inorganic delocalized lipophilic cations have demonstrated that the positive charge is crucial for directing these molecules toward appropriate drug targets within cancerous cells, thus significantly increasing their antiproliferative activity.[32−34] It is unclear as yet how the presence of the cationic charge directly influences the biological behavior of both iron- and ruthenium-based metallocenes; however it is likely that the presence of this positive charge prompts these organoiron and organoruthenium complexes to operate in a similar fashion within cells to other delocalized lipophilic cations previously studied within the literature.

EXPERIMENTAL SECTION

General Procedures. All reactions were carried out in an atmosphere of dry nitrogen or argon unless stated otherwise. Tetrahydrofuran (THF), diethyl ether, and dichloromethane (DCM) were supplied by Sigma-Aldrich and distilled under a nitrogen atmosphere prior to use. All other solvents were supplied by Sigma-Aldrich and used as received. Ruthenocene was purchased from Strem Chemicals Inc., cyanuric fluoride from Lancaster Chemicals, silica gel (for column chromatography) from Merck Chemicals, and silica gel preparative plates from Alltech Associates Australia Pty Ltd., respectively. All other chemical reagents were commercial products purchased from Sigma-Aldrich and were used as received. All deuterated solvents were supplied by Cambridge Isotope Laboratories and were used as received.

Melting points were obtained on a Gallenkamp variable temperature apparatus. Fourier transform infrared spectroscopy was conducted on a Thermo Nicolet-Nexus FT-IR spectrometer with all samples made up as KBr discs. The following abbreviations apply to the intensity of peaks found within the spectra: vs, very strong; s, strong; m, medium; w, weak. Electrospray mass spectrometry experiments were conducted on a direct injection Waters ZQ 4000 mass spectrometer utilizing electrospray ionization. All data were processed using Mass Linx version IV (IBM) software. ^{1}H and ^{13}C NMR spectra were obtained on a 400 MHz Varian Gemini spectrometer with samples of complexes **3**, **5**−**14**, **16**−**18**, and **21**−**23** being prepared in solutions of $CDCl_3$. Samples of complexes **1**, **2**, **4**, **15**, **19**, and **20** were characterized in d_6-DMSO solutions due to insolubility in $CDCl_3$. Peaks obtained for the deuterated solvent were used as the internal reference points for the spectra (reference peak: $CDCl_3$, ^{1}H, δ 7.26 ppm, ^{13}C, δ 77.0 ppm; d_6-DMSO, ^{1}H, δ 2.49 ppm, ^{13}C, δ 39.5 ppm). All signals have been recorded using their appropriate chemical shift (δ in ppm), multiplicity, integral ratio, and coupling constants (Hz). The following abbreviations apply to the signal multiplicity of peaks within spectra: s = singlet, d = doublet, t = triplet, m = multiplet. Microanalyses were performed by Mr. George Blazak at the Microanalytical Unit of the University of Queensland.

The chemical identity of complexes **1**−**6** was confirmed through comparison of experimental results achieved during characterization of these compounds with current literature data previously reported for these molecules.[29,30]

Synthesis and Characterization. *[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2H$)] (**1**).* Compound **1** was prepared and purified using literature procedures.[29] Yield: 42%. Mp: 185 °C (dec). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[2\,M + Na]^+$ 573.6, found 574.3, −ve ion, calcd *m*/*z* for $[M - H]^-$ 274.3, found 275.4. NMR: ^{1}H (d_6-DMSO), δ 4.61 (s, 5H, C_5H_5), 4.74 (m, 2H, C_5H_4 *meta*), 5.01 (m, 2H, C_5H_4 *ortho*).

*[Ru(η^5-$C_5H_4CO_2H$)$_2$] (**2**).* Compound **2** was prepared and purified using literature procedures.[29] Yield: 21%. Mp: 270 °C [325 °C]. ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 342.3, found 343.0, calcd *m*/*z* for $[2\,M + K]^+$ 661.6, found 662.2, −ve ion, calcd *m*/*z* for $[M - H]^-$ 318.3, found 319.6. NMR: ^{1}H (d_6-DMSO), δ 4.78 (m, 4H, C_5H_4 *meta*), 5.02 (m, 4H, C_5H_4 *ortho*).

*[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2R$)] (**3**, **5**, **7**).* Compound **1** (0.20 g, 7.72 × 10^{-4} mol) and a catalytic quantity of concentrated hydrochloric acid (20 μL) were added to neat alcohol (ROH, 50 mL), and the mixture was heated under reflux conditions for 48 h. The solvent was concentrated *in vacuo*, and complexes were isolated using a TLC preparative plate (8:7 EtOAc/Hex).

*[Ru(η^5-$C_5H_4CO_2R$)$_2$] (**4**, **6**, **8**).* Compound **2** (0.20 g, 7.72 × 10^{-4} mol) and a catalytic quantity of concentrated hydrochloric acid (20 μL) were added to neat alcohol (ROH, 50 mL), and the mixture was heated under reflux conditions for 48 h. The solvent was concentrated *in vacuo*, and complexes were isolated using a TLC preparative plate (8:7 EtOAc/Hex).

*[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2CH_3$)] (**3**).* Yield: 0.16 g, 73%. Mp: 105−106 °C. ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + H]^+$ 290.3, found 290.0. NMR: ^{1}H ($CDCl_3$), δ 3.69 (s, 3H, CH_3), 4.56 (s, 5H, C_5H_5), 4.67 (m, 2H, C_5H_4 *meta*), 5.19 (m, 2H, C_5H_4 *ortho*).

*[Ru(η^5-$C_5H_4CO_2CH_3$)$_2$] (**4**).* Yield: 0.16 g, 69%. Mp: 135−136 °C. NMR: ^{1}H ($CDCl_3$), δ 3.75 (s, 6H, CH_3), 4.74 (m, 4H, C_5H_4 *meta*), 5.18 (m, 4H, C_5H_4 *ortho*).

*[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2CH_2CH_3$)] (**5**).* Yield: 0.20 g, 84%. Mp: 76−77 °C. NMR: ^{1}H ($CDCl_3$), δ 1.29 (t, *J* = 7.4 Hz, 3H, CH_3), 4.21 (q, *J* = 7.4 Hz, 2H, OCH_2), 4.60 (s, 5H, C_5H_5), 4.71 (m, 2H, C_5H_4 *meta*), 5.15 (m, 2H, C_5H_4 *ortho*).

*[Ru(η^5-$C_5H_4CO_2CH_2CH_3$)$_2$] (**6**).* Yield: 0.19 g, 83%. Mp: 85−86 °C. NMR: ^{1}H ($CDCl_3$), δ 1.31 (t, *J* = 7.4 Hz, 6H, CH_3), 4.22 (q, *J* = 7.4 Hz, 4H, OCH_2), 4.74 (m, 4H, C_5H_4 *meta*), 5.17 (m, 2H, C_5H_4 *ortho*).

*[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2CH_2CH_2CH_3$)] (**7**).* Yield: 0.10 g, 41%. Mp: 49−51 °C. IR (cm^{-1}): 1710 (s, C═O), 1271 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 340.4, found 341.0. NMR: ^{1}H ($CDCl_3$), δ 0.97 (t, *J* = 7.4 Hz, 3H, CH_3), 1.68 (m, 2H, CH_2CH_3), 4.10 (t, *J* = 7.4 Hz, 2H, OCH_2), 4.58 (s, 5H, C_5H_5), 4.69 (m, 2H, C_5H_4 *meta*), 5.14 (m, 2H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 10.70 (s, CH_3), 22.35 (s, CH_2CH_3), 65.87 (s, OCH_2), 71.85, 71.95, 72.94 (s, C_5H_4, C_5H_5), 76.12 (s, $C(CO_2(CH_2)_2CH_3)$), 170.50 (s, CO_2). Anal. Calcd for $C_{14}H_{16}O_2Ru$: C 53.0, H 5.08. Found: C 52.9, H 5.10.

*[Ru(η^5-$C_5H_4CO_2CH_2CH_2CH_3$)$_2$] (**8**).* Yield: 0.10 g, 39%. Mp: 65−67 °C. IR (cm^{-1}): 1708 (s, C═O), 1280 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 426.5, found 427.0, calcd *m*/*z* for $[2\,M + Na]^+$ 829.9, found 829.6. NMR: ^{1}H ($CDCl_3$), δ 0.98 (t, *J* = 7.4 Hz, 6H, CH_3), 1.69 (m, 4H, CH_2CH_3), 4.11 (t, *J* = 7.4 Hz, 4H, OCH_2), 4.73 (m, 4H, C_5H_4 *meta*), 5.17 (m, 4H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 10.70 (s, CH_3), 22.35 (s, CH_2CH_3), 65.13 (s, OCH_2), 73.34, 74.50 (s, C_5H_4), 76.15 (s, $C(CO_2(CH_2)_2CH_3)$), 169.38 (s, CO_2). Anal. Calcd for $C_{18}H_{22}O_4Ru$: C 53.6, H 5.50. Found: C 52.9, H 5.49.

*Ru(η^5-C_5H_5)(η^5-C_5H_4COF)] (**9**).* A suspension of **1** (0.63 g, 2.15 × 10^{-3} mol) and pyridine (0.35 mL, 4.30 × 10^{-3} mol) in DCM (25 mL) was cooled to 0 °C. Cyanuric fluoride (0.37 mL, 4.30 × 10^{-3} mol) was added, and the reaction mixture stirred at 0 °C for two hours.

The mixture was poured into a solution of ice-cold H_2O (approximately 30 mL) and filtered, and the organic layer was collected. The solution was concentrated *in vacuo*, and the product **9** isolated as a yellow, crystalline material using silica column chromatography (1:5 ethyl acetate/hexane). Yield: 0.49 g, 82%. Mp: 81−82 °C. IR (cm^{-1}): 1805 (s, C═O), 1266, 1069 (s, C−F). NMR: ^1H ($CDCl_3$), δ 4.69 (s, 5H, C_5H_5), 4.85 (t, J = 1.6 Hz, 2H, C_5H_4 *meta*), 5.17 (t, J = 1.6 Hz, 2H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 70.23 (s, *C*(COF)), 72.64 (s, C_5H_4 *meta*), 72.82 (s, C_5H_5), 74.60 (s, C_5H_4 *ortho*), 159.91 (s, COF). Anal. Calcd for $C_{11}H_9FORu$: C 47.7, H 3.27. Found: C 47.7, H 2.86.

[Ru(η^5-$C_5H_4COF)_2$] (***10***). Compound **10** was prepared by a similar method to that described for **9**, using **2** (0.38 g, 1.08×10^{-3} mol) as a starting material. Compound **10** was isolated as a yellow, crystalline material using silica column chromatography (1:5 ethyl acetate/hexane). Yield: 0.25 g, 73%. Mp: 196−197 °C. IR (cm^{-1}): 1806 (s, C═O), 1268, 1069 (s, C−F). NMR: ^1H (d_6-DMSO), δ 5.26 (t, J = 2.0 Hz, 4H, C_5H_4 *meta*), 5.40 (t, J = 2.0 Hz, 4H, C_5H_4 *ortho*); ^{13}C (d_6-DMSO), δ 72.56 (s, *C*(COF)), 74.71 (s, C_5H_4 *meta*), 76.89 (s, C_5H_4 *ortho*), 160.85 (s, COF). Anal. Calcd for $C_{12}H_8F_2O_2Ru$: C 44.6, H 2.50. Found: C 46.9, H 2.86.

[Ru(η^5-C_5H_5)(η^5-C_5H_4CO]$_2O$ (***11***). Compound **1** (0.03 g, 1.08×10^{-4} mol) and compound **9** (0.03 g, 1.08×10^{-4} mol) were stirred in a solution of pyridine (5 mL) and THF (5 mL) for a period of 30 min. The solution was cooled to 0 °C in an ice bath prior to the dropwise addition of concentrated HCl (0.5 mL). The product was extracted from solution with diethyl ether (10 mL), and the organic phase was washed three times with water (10 mL) and once with a saturated solution of sodium bicarbonate (10 mL). The organic phase was retained, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo* to yield a pale yellow solid. Compound **11** was then isolated as a yellow powder using silica column chromatography (1:5 ethyl acetate/hexane). Yield: 0.03 g, 22%. Mp: 150−152 °C (dec). IR (cm^{-1}): 1713 (s, C═O), 1243 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 555.5, found 556.4, calcd *m*/*z* for $[2\ M + Na]^+$ 1088.1, found 1088.5. NMR: ^1H ($CDCl_3$), δ 4.70 (s, 10H, C_5H_5), 4.83 (m, 4H, C_5H_4 *meta*), 5.18 (m, 4H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 70.28 (s, *C*(CO_2)), 72.60 (s, C_5H_4-*meta*), 74.15 (s, C_5H_4-*ortho*), 74.24 (s, C_5H_5), 165.78 (s, CO_2). Anal. Calcd for $C_{22}H_{18}O_3Ru_2$: C 49.6, H 3.41. Found: C 50.6, H 3.71.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2C_6H_5$)] (***12***). Compound **9** (0.07 g, 2.60×10^{-4} mol), phenol (0.05 g, 5.20×10^{-4} mol), and DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (3:7 ethyl acetate/hexane) to afford compound **12** as a yellow solid. Yield: 0.05 g, 55%. Mp: 96−97 °C. IR (cm^{-1}): 1735 (s, C═O), 1269 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 374.4, found 375.3. NMR: ^1H ($CDCl_3$), δ 4.69 (s, 5H, C_5H_5), 4.81 (m, 2H, C_5H_4 *meta*), 5.30 (m, 2H, C_5H_4 *ortho*), 6.99−7.49 (m, 5H, C_6H_5); ^{13}C ($CDCl_3$), δ 70.24, 72.21, 73.59, 74.94 (s, C_5H_4, C_5H_5), 121.74, 125.71, 129.53, 151.02 (s, C_6H_5), 168.97 (s, CO_2). Anal. Calcd for $C_{17}H_{14}O_2Ru$: C 58.1, H 4.02. Found: C 59.2, H 4.27.

[Ru(η^5-$C_5H_4CO_2C_6H_5)_2$] (***13***). Compound **10** (0.07 g, 2.23×10^{-4} mol), phenol (0.04 g, 4.46×10^{-4} mol), and DMAP (0.05 g, 4.46×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (3:7 ethyl acetate/hexane) to afford compound **13** as a yellow, crystalline material. Yield: 0.03 g, 31%. Mp: 154−156 °C. IR (cm^{-1}): 1729 (s, C═O), 1270 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 494.5, found 494.3. NMR: ^1H ($CDCl_3$), δ 4.91 (m, 4H, C_5H_4 *meta*), 5.41 (m, 4H, C_5H_4 *ortho*), 6.99−7.39 (m, 10H, C_6H_5); ^{13}C ($CDCl_3$), δ 74.18, 75.05 (s, C_5H_4), 76.91 (s, *C*($CO_2C_6H_5$)), 121.92, 125.82, 129.46, 150.77 (s, C_6H_5), 167.85 (s, CO_2). Anal. Calcd for $C_{24}H_{18}O_4Ru$: C 61.1, H 3.85. Found: C 61.4, H 4.58.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2CH_2C_5H_5O_5C_2(CH_3)_4$)] (***14***). Compound **9** (0.07 g, 2.53×10^{-4} mol), 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (0.05 g, 5.20×10^{-4} mol), and DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (3:7 ethyl acetate/hexane) to afford compound **17** as a colorless powder. Yield: 0.07 g, 51%. Mp: 112−114 °C. IR (cm^{-1}): 1721 (s, C═O), 1291, 1222 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 540.6, found 541.0; NMR: ^1H ($CDCl_3$), δ 1.35, 1.36, 1.48, 1.55 (s, 12H, CH3), 3.99−4.69 (m, 6H, CH, CH2), 4.63 (s, 5H, C5H5), 4.71 (m, 2H, C5H4 *meta*), 5.16 (m, 2H, C5H4 *ortho*), 5.57 (m, 1H, OCHO); ^{13}C ($CDCl_3$), δ 24.79, 25.23, 26.25, 26.39 (s, CH_3), 63.21, 66.16, 70.68, 70.93, 71.30, 75.38, 96.54, 108.92, 109.76 (s, $O_2C(CH_3)_2$, $C(CO_2CH_2C_5O_5C_2(CH_3)_4)$, CH_2, CH), 71.88, 73.05 (s, C_5H_4), 72.10 (s, C_5H_5), 170.32 (s, CO_2). Anal. Calcd for $C_{23}H_{28}O_7Ru$: C 53.4, H 5.46. Found: C 54.8, H 5.81.

[Ru(η^5-$C_5H_4CO_2CH_2C_5H_5O_5C_2(CH_3)_4)_2$] (***15***). Compound **10** (0.07 g, 2.17×10^{-4} mol), 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (0.05 g, 5.20×10^{-4} mol), and DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (3:7 ethyl acetate/hexane) to afford compound **18** as a colorless powder. Yield: 0.08 g, 45%. Mp: 159−160 °C. IR (cm^{-1}): 1721 (s, C═O), 1292, 1272 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 826.9, found 826.8. NMR: ^1H ($CDCl_3$), δ 1.35, 1.36, 1.48, 1.56 (s, 24H, CH_3), 3.99−4.69 (m, 12H, CH, CH_2), 4.79 (m, 4H, C_5H_4 *meta*), 5.21 (m, 2H, C_5H_4 *ortho*), 5.57 (m, 2H, OCHO); ^{13}C ($CDCl_3$), δ 24.79, 25.27, 26.25, 26.39 (s, CH_3), 63.43, 66.13, 70.68, 70.90, 71.23, 76.87, 96.50, 108.92, 109.76 (s, $O_2C(CH_3)_2$, $C(CO_2CH_2C_5O_5C_2(CH_3)_4)$, CH_2, CH), 73.34, 75.09 (s, C_5H_4), 169.34 (s, CO_2). Anal. Calcd for $C_{36}H_{46}O_{14}Ru$: C 53.8, H 5.77. Found: C 54.0, H 5.86.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4COS(CH_2)_2CH_3$)] (***16***). Compound **9** (0.07 g, 2.53×10^{-4} mol), *n*-thiopropanol (0.04 mL, 5.20×10^{-4} mol), and DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (3:7 ethyl acetate/hexane) to afford compound **19** as a colorless powder. Yield: 0.04 g, 57%. IR (cm^{-1}): 1656 (s, C═O), 1237 (s, C−S). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[2\ M + Li]^+$ 673.8, found 673.9. NMR: ^1H ($CDCl_3$), δ 0.99 (t, J = 7.4 Hz, 3H, CH_3), 1.63 (m, 2H, CH_2CH_3), 2.92 (t, J = 7.4 Hz, 2H, SCH_2), 4.59 (s, 5H, C_5H_5), 4.75 (m, 2H, C_5H_4 *meta*), 5.20 (m, 2H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 13.61 (s, CH_3), 23.52 (s, CH_2CH_3), 30.55 (s, SCH_2), 70.46, 72.83, 73.16 (s, C_5H_4, C_5H_5), 84.63 (s, *C*($COS(CH_2)_2CH_3$)), 192.65 (s, COS). Anal. Calcd for $C_{14}H_{16}OSRu$: C 50.4, H 4.85. Found: C 51.4, H 5.08.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2(CH_2)_2NHBoc$)] (***17***). Compound **9** (0.07 g, 2.60×10^{-4} mol), *N*-Boc-ethanolamine (0.08 mL, 4.96×10^{-4} mol), and DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (1:1 ethyl acetate/hexane) to afford compound **14** as a colorless powder. Yield: 0.07 g, 68%. Mp: 176−177 °C. IR (cm^{-1}): 3351 (m, N−H stretch), 1700, 1712 (s, C═O), 1536 (m, N−H bend), 1291, 1260 (m, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 441.5, found 441.4. NMR: ^1H ($CDCl_3$), δ 1.66 (s, 9H, CH_3), 3.43 (m, 2H, CH_2NHBoc), 4.21 (t, J = 5.0 Hz, 2H, OCH_2), 4.60 (s, 5H, C_5H_5), 4.73 (m, 2H, C_5H_4 *meta*), 5.15 (m, 2H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 28.65 (s, CH_3), 40.12 (s, CH_2NH), 63.32 (s, OCH_2), 71.88, 72.06, 73.19, (s, C_5H_4, C_5H_5), 79.89 (s, *C*($CO_2(CH_2)_2NHBoc$)), 155.86 (s, $NHCO_2$), 170.50 (s, $C_5H_4CO_2$). Anal. Calcd for $C_{18}H_{23}NO_4Ru$: C 51.7, H 5.54, N 3.35. Found: C 51.9, H 5.75, N 3.27.

[Ru(η^5-$C_5H_4CO_2(CH_2)_2NHBoc)_2$] (***18***). Compound **10** (0.09 g, 2.69×10^{-4} mol), *N*-Boc-ethanolamine (0.08 mL, 4.96×10^{-4} mol), and

DMAP (0.06 g, 5.20×10^{-4} mol) were dissolved in DCM (10 mL) and stirred for 16 h at room temperature. The solvent was removed *in vacuo* to yield an oily, yellow residue, which was purified using silica column chromatography (1:1 ethyl acetate/hexane) to afford compound **15** as a colorless powder. Yield: 0.09 g, 55%. Mp: 140−141 °C (dec). IR (cm^{-1}): 3369 (m, N−H stretch), 1719, 1698 (s, C═O), 1535 (m, N−H), 1287, 1256 (m, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 628.7; found 628.4. NMR: ^1H ($CDCl_3$), δ 1.47 (s, 18H, CH_3), 3.44 (m, 4H, CH_2NHBoc), 4.62 (t, J = 5.2 Hz, 4H, OCH_2), 4.78 (m, 4H, C_5H_4 *meta*), 5.20 (m, 4H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 28.73 (s, CH_3), 40.09 (s, CH_2NH), 63.65 (s, OCH_2), 73.78, 74.50, (s, C_5H_4), 79.68 (s, $C(CO_2(CH_2)_2NHBoc)$), 156.08 (s, $NHCO_2$), 169.34 (s, $C_5H_4CO_2$). Anal. Calcd for $C_{26}H_{36}N_2O_8Ru$: C 51.6, H 5.99, N 4.63. Found: C 51.5, H 6.05, N 4.41.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO_2(CH_2)_2NH_3$)]Cl (***19***). Compound **14** (0.07 g, 1.63×10^{-4} mol) was dissolved in a 2 M solution of HCl in ethyl acetate (20 mL) and stirred at 0 °C for 16 h. The solvent was removed *in vacuo*, and the product extracted into H_2O (20 mL). Evaporation of the aqueous solution yielded compound **16** as a colorless, hydroscopic powder. Yield: 0.07 g, 95%. Mp: 218−220 °C (dec). IR (cm^{-1}): 1701 (s, C═O), 1561 (m, NH_3), 1281 (s, C−O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M]^+$ 320.4, found 319.0. NMR: ^1H (d_6-DMSO), δ 3.09 (m, 2H, CH_2NH_3Cl), 4.24 (t, J = 5.0 Hz, 2H, OCH_2), 4.65 (s, 5H, C_5H_5), 4.81 (m, 2H, C_5H_4 *meta*), 5.22 (m, 2H, C_5H_4 *ortho*), 8.18 (br m, 3H, NH_3); ^{13}C (d_6-DMSO), δ 37.80 (s, CH_2NH_3), 60.38 (s, OCH_2), 71.64, 71.76, 73.03, 74.42, (s, C_5H_4, C_5H_5), 168.89 (s, CO_2). Anal. Calcd for $C_{18}H_{23}NO_4Ru$: C 51.7, H 5.54, N 3.35. Found: C 51.9, H 5.75, N 3.27.

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CONH(CH_2)_2OH$)] (***20***). Compound **1** (0.02 g, 7.27×10^{-5} mol) and ethanolamine (10 mL) were stirred at room temperature for 2 or 3 days until the acid had dissolved. Excess ethanolamine was removed via freeze-drying, yielding compound **20** as a yellow, crystalline solid. Yield: 0.02 g, 86%. Mp: 124−126 °C. IR (cm^{-1}): 3349 (w, O−H), 1650 (s, C═O), 1534 (m, C−N bend). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 341.4, found 341.5. NMR: ^1H (d_6-DMSO), δ 3.15 (m, 2H, CH_2OH), 3.40 (m, 2H, NCH_2), 4.54 (s, 5H, C_5H_5), 4.65 (m, 2H, C_5H_4 *meta*), 5.11 (m, 2H, C_5H_4 *ortho*), 7.58 (m, 1H, NH); ^{13}C (d_6-DMSO), δ 41.30 (s, NCH_2), 59.64 (s, CH_2OH), 69.84, 71.49 (s, C_5H_4), 71.17 (s, C_5H_5), 80.69 (s, $C(CONH(CH_2)_2OH)$), 167.32 (s, CON).

[Ru(η^5-$C_5H_4CONH(CH_2)_2OH)_2$] (***21***). Compound **2** (0.02 g, 6.26×10^{-5} mol) and ethanolamine (10 mL) were stirred at room temperature for 2 or 3 days until the acid had dissolved. Excess ethanolamine was removed via freeze-drying, yielding compound **21** as a yellow, crystalline solid. Yield: 0.02 g, 79%. Mp: 109−112 °C. IR (cm^{-1}): 3350 (w, O−H), 1649 (s, C═O), 1539 (m, C−N bend). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + Na]^+$ 428.4, found 428.4. NMR: ^1H (d_6-DMSO), δ 3.18 (m, 4H, NCH_2), 3.44 (m, 4H, CH_2OH), 4.67 (m, 4H, C_5H_4 *meta*), 5.08 (m, 4H, C_5H_4 *ortho*), 7.62 (m, 2H, NH); ^{13}C (d_6-DMSO):, δ 41.72 (s, NCH_2), 59.74 (s, CH_2OH), 71.54, 73.04 (s, C_5H_4), 82.10 (s, $C(CONH(CH_2)_2OH)$), 167.04 (s, CON).

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO(CH_2)_3CH_3$)] (***22***) *and [Ru(η^5-$C_5H_4CO(CH_2)_3CH_3)_2$]* (***23***). Aluminum chloride (1.50 g, 1.12×10^{-2} mol) and valeryl chloride (1.08 mL, 8.91×10^{-3} mol) were dissolved in DCM (10 mL) to yield a bright yellow mixture. A solution of ruthenocene (0.69 g, 3.00×10^{-3} mol) in DCM was then added dropwise over a period of one hour, and the reaction heated under reflux conditions for 16 h. The solution was allowed to cool to room temperature, after which 10 mL of H_2O was added, the phases were separated, and the organic layer was dried over Na_2SO_4. The solvent was removed *in vacuo* to yield a green, oily residue. Compounds **22** and **23** and unreacted ruthenocene were separated from this mixture using silica column chromatography (1:4 ethyl acetate/hexane).

[Ru(η^5-C_5H_5)(η^5-$C_5H_4CO(CH_2)_3CH_3$)] (***22***). Yield: 0.13 g, 13%. Mp: 45−47 °C. IR (cm^{-1}): 1676 (s, C═O). ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + H]^+$ 316.4, found 317.0, calcd *m*/*z* for $[M + Li]^+$ 322.3, found 322.0. NMR: ^1H ($CDCl_3$), δ 0.94 (t, J = 7.2 Hz, 3H, CH_3), 1.36 (m, 2H, CH_2), 1.65 (m, 2H, CH_2), 2.59 (t, J = 7.4 Hz, 2H, $COCH_2$), 4.59 (s, 5H, C_5H_5), 4.78 (m, 2H, C_5H_4 *meta*), 5.11 (m, 2H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 14.23 (s, CH_3), 22.87, 27.53 (s, CH_2), 39.04 (s, OCH_2), 71.01 (s, C_5H_4 *meta*), 72.14 (s, C_5H_5), 73.67 (s, C_5H_4 *ortho*), 84.30 (s, $C(CO(CH_2)_3CH_3)$), 202.96 (s, CO).

[Ru(η^5-$C_5H_4CO(CH_2)_3CH_3)_2$] (***23***). Yield: 0.33 g, 27%. Mp: 57−58 °C. IR (cm^{-1}): 1679 (s, C═O); ESMS (*m*/*z*): +ve ion, calcd *m*/*z* for $[M + H]^+$ 400.5, found 401.0, calcd *m*/*z* for $[M + Li]^+$ 406.5, found 406.0, calcd *m*/*z* for $[2\ M + Li]^+$ 806.0, found 806.0. NMR: ^1H ($CDCl_3$), δ 0.95 (t, J = 6.6 Hz, 6H, CH_3), 1.37 (m, 4H, CH_2), 1.63 (m, 4H, CH_2), 2.53 (t, J = 7.4 Hz, 4H, $COCH_2$), 4.78 (m, 4H, C_5H_4 *meta*), 5.11 (m, 4H, C_5H_4 *ortho*); ^{13}C ($CDCl_3$), δ 14.19 (s, CH_3), 22.75, 26.90 (s, CH_2), 39.10 (s, OCH_2), 72.50 (s, C_5H_4 *meta*), 75.09 (s, C_5H_4 *ortho*), 85.58 (s, $C(CO(CH_2)_3CH_3)$), 201.75 (s, CO).

Crystal Structure Determinations. A unique data set for compound **11** was measured at 295(2) K within the specified $2\theta_{max}$ limit using a Rigaku AFC 7R four-circle diffractometer [θ−2θ scan mode, monochromatized Mo Kα radiation (λ = 0.71073 Å), from a 12 kW rotating anode source], yielding N independent reflections, N_o with $I > 2.0\sigma(I)$ being considered "observed" and used in the expression of the conventional refinement residual R. The structure was solved by direct methods and refined by full-matrix least-squares using SHELXL97[35] after semiempirical absorption corrections based on ψ-scans. Anisotropic thermal parameters were refined for all non-hydrogen atoms while (x, y, z, $U_{iso})_H$ were included and constrained at estimated values. Neutral atom complex scattering factors were employed, while computation used the TeXsan crystallographic software package of Molecular Structure Corporation,[36] ORTEP-3,[37] and PLATON.[38]

A full .cif deposition resides with the Cambridge Crystallographic Data Centre with CCDC number 794550. Copies of the data may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK, at the following address: www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi.

Crystal data for 11:. $C_{22}H_{18}O_3Ru_2$. M = 532.5, monoclinic, space group $P2_1/n$, a = 17.126(5) Å, b = 13.960(4) Å, c = 7.6170(16) Å, β = 99.069(19)°, U = 1798.3(8) Å^3, Z = 4, D_c = 1.97 g cm^{-3}, μ = 1.7 mm^{-1}, crystal size = 0.30 × 0.30 × 0.20 mm. $T_{min/max}$ = 0.63, 0.73; 4575 reflections collected, 4119 unique (R_{int} = 0.024), R = 0.026 (3660 reflections with $I > 2\sigma(I)$), wRF^2 = 0.071 (all data).

Cell Survival Studies. All cell lines were cultured in heat-inactivated fetal calf serum (10%, CSL, Australia) in RPMI 1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 μg/mL), and HEPES (3 mM) at 5% CO_2, 99% humidity at 37 °C. Primary human fibroblasts were obtained from neonatal foreskin and cultured in the above medium. Culture media was replaced every three days, and cell monolayers were split when 70−80% confluent. Routine mycoplasma tests were performed using Hoescht stain and were always negative.

Stock solutions of test compounds were prepared by dissolving the complexes (∼10 mg) in DMSO (10 μL). These stock solutions were diluted as necessary for testing. Cells were seeded in 96-well microtiter plates at approximately 5000 cells per 100 μL (NFF), 3000 cells per 100 μL (MCF7, DU145, CI80-13S, MM418c5), and 1000 cells per 100 μL (MM96L). Seven dilutions of each drug were added to triplicate wells. The plates were incubated for a period of 6 days prior to incorporation of the SRB staining method.[31] The culture medium was removed from the plates, and each plate was washed with phosphate-buffered saline (PBS). The plates were fixed with methylated spirits for 15 min, then washed with tap water. SRB solution (50 μL, 0.4% sulforhodamine B dye (w/v) in 1% (v/v) acetic acid) was added to each well and left at room temperature for 45 min. The SRB solution was removed, and the plates were washed quickly, once with tap water and twice with 1% (v/v) acetic acid solution. In the case of the NFF cell assay, these plates were washed

three times with 1% (v/v) acetic acid solution. Tris base (100 μL, 10 mM, unbuffered, pH > 9) was added to each well to solubilize the protein-bound dye. Plates were left for 5 min, and then the absorbance was measured on a multiwell plate reader at 564 nm. The percentage of surviving cells was calculated from the absorbance of untreated control cells. The IC_{50} values for the inhibition of cell viability were determined by fitting the plot of the percentage of surviving cells against drug concentration with a sigmoidal function.

■ ASSOCIATED CONTENT

Supporting Information. Crystallographic data (including cif files) for compound **11** (CCDC 794550). This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +61 07 373 57728. E-mail: michael.williams@griffith.edu.au.

■ ACKNOWLEDGMENT

We thank Griffith University, the Queensland Institute of Medical Research, and the Eskitis Institute for Cell and Molecular Therapies for support of this work.

■ REFERENCES

(1) Guo, Z. J.; Sadler, P. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1512.
(2) Jaouen, G. *Bioorganometallics*; Wiley-VCH: Weinheim, 2006.
(3) Storr, T.; Thompson, K. H.; Orvig, C. *Chem. Soc. Rev.* **2006**, *35*, 534.
(4) Hambley, T. W. *Science* **2007**, *318*, 1392.
(5) van Rijt, S. H.; Sadler, P. J. *Drug Discovery Today* **2009**, *14*, 1089.
(6) Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467.
(7) Fricker, S. P. *Dalton Trans.* **2007**, 4903.
(8) Bratsos, I.; Jedner, S.; Gianferrara, T.; Alessio, E. *Chimia* **2007**, *61*, 692.
(9) Hartinger, C. G.; Dyson, P. J. *Chem. Soc. Rev.* **2009**, *38*, 391.
(10) Yan, Y. K.; Melchart, M.; Habtermariam, A.; Sadler, P. J. *Chem. Commun.* **2005**, *38*, 4764.
(11) Dyson, P. J. *Chimia* **2007**, *61*, 698.
(12) Suess-Fink, G. *Dalton Trans.* **2010**, *39*, 1673.
(13) Micallef, L. S.; Loughrey, B. T.; Healy, P. C.; Parsons, P. G.; Williams, M. L. *Organometallics* **2010**, *29*, 6237.
(14) Loughrey, B. T.; Healy, P. C.; Parsons, P. G.; Williams, M. L. *Inorg. Chem.* **2008**, *47*, 8589.
(15) Loughrey, B. T.; Williams, M. L.; Carruthers, T. J.; Parsons, P. G.; Healy, P. C. *Aust. J. Chem.* **2010**, *63*, 245.
(16) Loughrey, B. T.; Williams, M. L.; Healy, P. C.; Innocenti, A.; Vullo, D.; Supuran, C. T.; Parsons, P. G.; Poulsen, S.-A. *J. Biol. Inorg. Chem.* **2009**, *14*, 935.
(17) Fouda, M. F. R.; Abd-Elzaher, M. M.; Abdelsamaia, R. A.; Labib, A. A. *Appl. Organomet. Chem.* **2007**, *21*, 613.
(18) Kealy, J.; Pauson, P. L. *Nature* **1951**, *168*, 1039.
(19) Woodward, R. B.; Rosenblum, M.; Whiting, M. C. *J. Am. Chem. Soc.* **1952**, *74*, 3458.
(20) Nesmeyanov, A. N.; Kochetkova, N. S. *Dokl. Akad. Nauk* **1956**, *109*, 543.
(21) Broadhead, G. D.; Osgerby, J. M.; Pauson, P. L. *Chem. Ind.* **1957**, 209.
(22) Weinmayr, V. *J. Am. Chem. Soc.* **1955**, *77*, 3009.
(23) Nesmeyanov, A. N.; Perevalova, E. G.; Golovnya, R. V.; Nesmeyanova, O. A. *Dokl. Akad. Nauk* **1954**, *97*, 459.
(24) Benkeser, R. A.; Goggin, D.; Schroll, G. *J. Am. Chem. Soc.* **1954**, *76*, 4025.
(25) Nesmeyanov, A. N.; Perevalova, E. G.; Beinoravichute, Z. A. *Dokl. Akad. Nauk* **1957**, *112*, 439.
(26) Broadhead, G. D.; Pauson, P. L. *J. Am. Chem. Soc.* **1955**, 367.
(27) Weliky, N.; Gould, E. S. *J. Am. Chem. Soc.* **1957**, *79*, 2742.
(28) Rausch, M.; Shaw, P.; Mayo, D.; Lovelace, A. M. *J. Org. Chem.* **1958**, *23*, 505.
(29) Rausch, M. D.; Fischer, E. O.; Grubert, H. *J. Am. Chem. Soc.* **1960**, *82*, 76.
(30) Huang, R.; Wang, Q. *J. Organomet. Chem.* **2001**, *637*−*639*, 94–98.
(31) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J.; Bokesch, H.; Kenney, S.; Boyd, M. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
(32) Barnard, P. J.; Berners-Price, S. J. *Coord. Chem. Rev.* **2007**, *251*, 1889.
(33) Berners-Price, S. J.; Filipovska, A. *Aust. J. Chem.* **2008**, *61*, 661.
(34) Pathania, D.; Millard, M.; Neamati, N. *Adv. Drug Delivery Rev.* **2009**, *61*, 1250.
(35) Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112.
(36) *TeXsan for Windows*, Version 1.06, MSC 9009; Molecular Structure Corporation: The Woodlands, TX, USA, 1997−2001.
(37) Farrugia, L. J. *J. Appl. Crystallogr.* **1997**, *30*, 565.
(38) Spek, A. L. *J. Appl. Crystallogr.* **2003**, *36*, 7.