Dirhodium(II,II) Complexes: Molecular Characteristics that Affect in Vitro Activity

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In the series $Rh_2(O_2CR)_4$ (R = CH_3, **1**; R = CF_3, **2**), $[Rh_2(O_2CR)_2(phen)_2]^{2+}$ (R = CH_3, **3**; R = CF_3, **4**), and $[Rh_2(O_2CR)_2(dppz)_2]^{2+}$ (R = CH_3, **5**; R = CF_3, **6**), **2**, **4**, and **6** are twice as cytotoxic as **1**, **3**, and **5**, respectively. The substitution reactions of **2** with 9-ethylguanine at various temperatures take place at faster rates than those of **1**, and the activation energy $E_a(\mathbf{1}) = 69 \pm 4$ kJ/mol is twice $E_a(\mathbf{2}) = 35 \pm 2$ kJ/mol. The higher cytotoxicities of $[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)L(MeOH)]^+$ (L = dppz, **7**; L = dppn, **8**) relative to $[Rh_2(\mu\text{-}O_2CCH_3)_2(bpy)L]^{2+}$ (L = dppz, **10**; L = dppn, **11**) are attributed to the labile equatorial groups in **7** and **8** not present in **10** and **11**. The toxicities of complexes **1**−**8** are not related to their charge or the ease by which they transverse the cellular membrane but to the lability of the ligands on the dirhodium core.

Introduction

Because of the exceptional effectiveness of cisplatin (*cis*-$Pt(NH_3)_2Cl_2$), it is one of the most widely used antitumor agents, particularly for the treatment of testicular, ovarian, bladder, and head/neck tumors.[1,2] Nuclear DNA has been established as the primary target of cisplatin, which covalently binds to adjacent guanine bases resulting in a cascade of events that ultimately lead to cell death. The success of cisplatin and its analogues notwithstanding, their relatively high toxicity and the resistance of tumor cells (inherent and acquired) have prompted the search for new metal complexes with improved properties.[1,3] One such class is represented by the structurally unique polynuclear platinum compounds, as exemplified by the trinuclear BBR3464,[4] where the results of phase II clinical trials showed partial responses in cisplatin relapsed ovarian cancer.[4]

Among the promising non-platinum anticancer agents are metal−metal bonded rhodium carboxylate complexes,[5] which have received considerable attention because of their notable antitumor activity and their limited side effects. Pioneering studies that emanated in the 1970s showed that dirhodium carboxylate compounds $Rh_2(O_2CR)_4$ (R = Me, Et, Pr) exhibit significant in vivo antitumor activity against L1210 tumors,[6,7] Ehrlich ascites,[8−10] and sarcoma 180 and P388 tumor lines.[11] It has been demonstrated that this class of compounds inhibits DNA, RNA, and protein synthesis in a manner akin to cisplatin.[12−15] Moreover, cationic compounds of general formulae $[Rh_2(O_2CCH_3)_2(N{-}N)_2(H_2O)_2]^{2+}$, N−N = bpy or phen, exhibit anticancer activity against human oral carcinoma KB cell lines comparable to $Rh_2(O_2CCH_3)_4$.[16] Despite the obvious importance of the medicinal chemistry of dirhodium complexes, the precise mechanism of their action has not yet been elucidated. Recent findings from our laboratories, however, have provided valuable insight into their plausible biological activity. The interactions of dirhodium compounds with nucleobases,[17−20] dinucleotides,[21−23] oligonucleotides,[24] and single-[25,26] and double-stranded (ds)[27] DNA were investigated, and it has been demonstrated that dirhodium complexes bind covalently to DNA.[28]

We have recently been investigating dirhodium tetraacetate derivatives that contain the intercalating ligand dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) coordinating to the dirhodium core.[29−31] These compounds exhibit increased toxicity upon irradiation with low-energy light and thus are quite promising as photosensitizers for photodynamic therapy (PDT).[29−31] These complexes have the ability to cleave DNA upon irradiation with visible light, both in the presence and in the absence of oxygen.[30,31] Since many cancer cells are hypoxic, these complexes provide a potential advantage over currently used sensitizers because their action does not require O_2.

Recent studies of various dirhodium compounds have indicated that there is a correlation between their molecular characteristics and their cytotoxic behavior. Two important factors that likely affect the cytotoxicity of these compounds are the ease of their transport through cellular membranes and their ability to react with the cellular target(s). Their membrane permeability can be assessed by measuring the lipophilicity of the compounds, whereas their relative reactivity can be estimated by determining the kinetic parameters of binding to biomolecules. The importance of the lipophilicity in the observed bioactivity has been demonstrated in the class of dirhodium carboxylate paddlewheel compounds $Rh_2(O_2CR)_4$ (R = CH_3, C_2H_5, C_3H_7). In this series, the activity against Ehrlich ascites tumor, leukemia L1210, and sarcoma 180 cell lines increases as the hydrophobicity of the R group increases;[32] further lengthening of the carboxylate group beyond the pentanoate, however, reduces their efficacy and indicates that other factors are also responsible for the activity of the compounds. These factors may include the lability of the groups bound to the metal,[33] the presence of open coordination sites, the overall charge on the complex, and the hydrophobicity of the carrier ligands. Reactions of dirhodium with single-stranded oligonucleotides containing dipurine sites exhibit the following order of reactivity, $Rh_2(O_2CCH_3)_4 \ll$ *cis*-$[Rh_2(O_2CCH_3)_2(CH_3CN)_6](BF_4)_2 < Rh_2(O_2CCF_3)_4$, which correlates with the lability of the equatorial groups.[26] Furthermore, a recent study showed that

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[a] Abbreviations: BBR3464, $[\{trans\text{-}PtCl(NH_3)_2\}_2\text{-}\mu\text{-}\{trans\text{-}Pt(NH_3)_2(NH_2(CH_2)_6NH_2)_2\}](NO_3)_4$; bpy, 2,2′-bipyridine; cisplatin, *cis*-$Pt(NH_3)_2Cl_2$; dppn, benzodipyrido[3,2-*a*:2′,3′-*c*]phenazine; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine; 9-EtGuaH, 9-ethylguanine; Hs-27, human skin fibroblasts; phen, 1,10-phenanthroline; PDT, photodynamic therapy.

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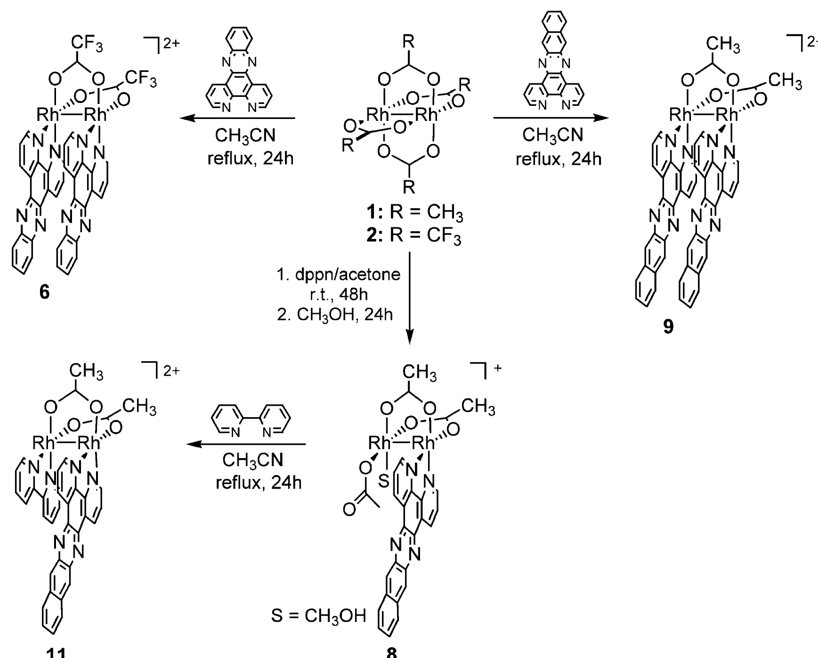

Figure 1. Synthetic routes for the new dirhodium(II,II) complexes **6**, **8**, **9**, and **11**.

the extent of interstrand cross-link formation of a DNA fragment containing 123 base pairs also correlates with the lability of the leaving groups.[27] Herein, we have undertaken the evaluation of the type and lability of the equatorially bound groups to the dirhodium core, as well as the effect of the overall charge and lipophilicity on the cytotoxicity of the complexes toward human skin cells. The determination of some general correlations between the molecular characteristics of these compounds and their cytotoxic behavior has been established. These findings will aid in the design of more effective dirhodium compounds as potential drugs and PDT agents.

Results and Discussion

I. Syntheses. The cytotoxicities and hydrophobicities of the dirhodium compounds **1**−**11** have been studied with respect to their structural features. The compounds $Rh_2(O_2CCH_3)_4$ (**1**),[34] $Rh_2(O_2CCF_3)_4$ (**2**),[35] $[Rh_2(O_2CCH_3)_2(phen)_2]^{2+}$ (**3**),[36] $[Rh_2(O_2CCF_3)_2(phen)_2]^{2+}$ (**4**),[36] $[Rh_2(O_2CCH_3)_2(dppz)_2]^{2+}$ (**5**),[30] $[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(dppz)(MeOH)]^+$ (**7**),[29,30] and $[Rh_2(O_2CCH_3)_2(dppz)(bpy)]^{2+}$ (**10**)[31] have been previously reported. In Figure 1, the synthetic routes for the newly synthesized dirhodium(II,II) complexes **6**, **8**, **9**, and **11** are outlined.

The ligand dppn was introduced into compounds **8**, **9**, and **11** because of its higher lipophilicity as compared to dppz. Heating **1** with 2 equiv of benzodipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppn) in refluxing CH_3CN yields **9** (87%) as a red solid. The reaction temperature and time are critical for the formation of **9**. If the reaction is performed at lower temperatures or for shorter times, a mixture of the mono- and bis-substituted dirhodium complexes is obtained and starting material is still present in the reaction mixture. Compound **8** is synthesized at a 40% yield by the reaction of **1** with 1 equiv of dppn. The solvent used is important, and the reaction is most successful in acetone at room temperature. The reaction can be performed in CH_2Cl_2 at 45 °C, but the temperature is critical in this solvent. The intermediate product $Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^2\text{-}O_2CCH_3)(\eta^1\text{-}O_2CCH_3)$(dppn) in the reaction has a chelating acetate ion, which occupies equatorial and axial positions of one rhodium atom.[37] This intermediate exhibits low solubility in both polar and nonpolar solvents; it is converted, however, into the more soluble

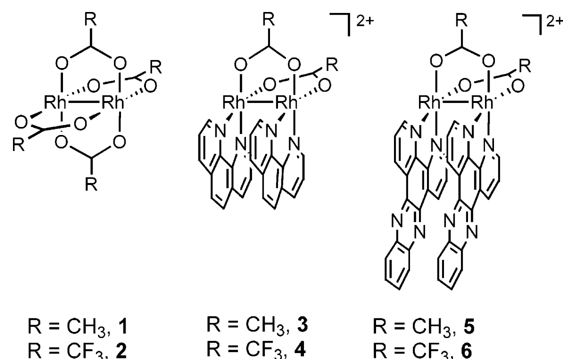

Figure 2. Structural representation of compounds **1**−**6**.

product **8** by stirring a slurry of the insoluble compound in methanol until a clear green solution is obtained. In the last step, the monodentate acetate group is displaced by methanol. Compound **8** is reacted with 1 equiv of bpy in acetonitrile to produce **11** at 42% yield. In compounds **10** and **11**, a chelating bpy group occupies two equivalent sites of one rhodium atom, which decreases the accessibility of these sites to other nucleophiles. In compounds **7** and **8** these sites are occupied by labile η^1-acetate or solvent molecules.

II. Effect of Substituent on Bridging Ligands. To explore the effect of the bridging ligand on the cytotoxicity of the dirhodium complexes, compounds **2**, **4**, and **6**, which represent the trifluoroacetate analogues of **1**, **3**, and **5**, respectively, were synthesized (Figure 2). Compounds **1**−**6** (Figure 2) were evaluated for their capability to inhibit cell growth in vitro using human skin fibroblasts (Hs-27), and the LC_{50} values (concentration required to reduce cell survival by 50%) are listed in Table 1. The LC_{50} values of the acetate derivatives **1**, **3**, and **5** were found to be 15 ± 2, 290 ± 15, and 135 ± 8, respectively, and those for the trifluoroacetate analogues **2**, **4**, and **6** were 7.7 ± 0.5, 152 ± 7, and 58 ± 3, respectively. The LC_{50} value for each complex with acetate bridging ligands is in each case twice the magnitude of that of its trifluoroacetate counterpart. Since the charge and the lipophilicity of the other ligands within the subsets of dirhodium complexes **1**/**2**, **3**/**4**, and **5**/**6** are the same, the observed differences in the cytotoxicities were investigated

Table 1. Cytotoxicities (LC_{50}) and log P Values for Complexes **1**−**6**

complex		LC_{50} (μM)[a]	log P [b]
$Rh_2(O_2CCH_3)_4$	**1**	15 ± 2	−0.10 ± 0.02
$Rh_2(O_2CCF_3)_4$	**2**	7.7 ± 0.5	0.83 ± 0.01
$[Rh_2(O_2CCH_3)_2(phen)_2]^{2+}$	**3**	290 ± 15	−1.90 ± 0.01
$[Rh_2(O_2CCF_3)_2(phen)_2]^{2+}$	**4**	152 ± 7	−0.85 ± 0.02
$[Rh_2(O_2CCH_3)_2(dppz)_2]^{2+}$	**5**	135 ± 8	0.60 ± 0.03
$[Rh_2(O_2CCF_3)_2(dppz)_2]^{2+}$	**6**	58 ± 3	1.55 ± 0.01

[a] Hs-27 human skin cells exposed to each compound for 30 min in the dark. [b] Partition coefficient $P = C_o/C_w$ (C_o and C_w are the complex concentrations in n-octanol and water, respectively).

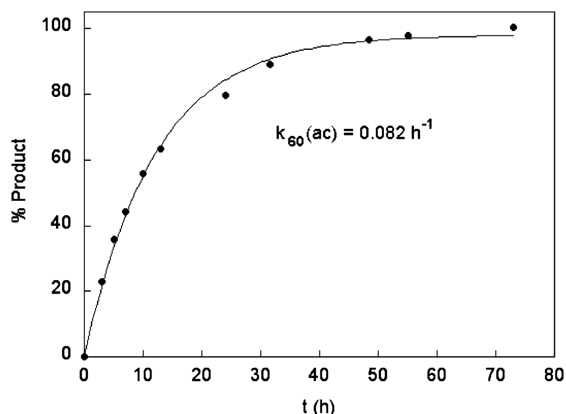

Figure 3. Plot of the % product from the reaction of dirhodium tetraacetate with 9-EtGuaH as a function of time t at 60 °C (k_{60}(ac) = 0.082 h^{-1}).

with respect to the relative labilities of the acetate vs the trifluoroacetate leaving groups. Differences in the reactivity of complexes **1** and **2** with short oligonucleotides[26] and double-stranded DNA[27] have already been reported and correlated with the different labilities of the two leaving groups, $CH_3CO_2^-$ as compared to $CF_3CO_2^-$.

III. Ligand Exchange Kinetics. The substitution reactions of **1** and **2** with 9-EtGuaH were performed at various temperatures between 40 and 75 °C in order to gain insight into the relative lability of acetate and trifluoroacetate bridging ligands in dirhodium complexes. At each temperature, the product concentration follows pseudo-first-order kinetics according to the equation

$$[C] = [C_0](1 - e^{-k_T t}) \qquad (1)$$

where [C] is the product concentration, $[C_0]$ is the initial concentration of the reagent, k_T is the rate of the reaction at a certain temperature T, and t represents reaction time. When the experimental data points for **1** and **2** are fitted at each temperature T, the rate k_T for each reaction at a certain temperature T is derived. For example, at 60 °C, the product formed as a function of time and the corresponding fits to eq 1 for the reactions of **1** and **2** with 9-EtGuaH are shown in Figures 3 and 4, respectively.

By applying fits for eq 1 as described above, the rate constant k_T was determined for each reaction of **1** and **2** conducted at a given temperature T. For each complex, the plots of k_T vs $1/T$ resulted in a straight line and were subsequently fit to eq 2, with a slope equal to $-E_a/R$:

$$\ln k_T = \ln A - \frac{E_a(\mathbf{n})}{RT} \qquad (2)$$

where $E_a(\mathbf{n})$ is the activation energy of complex **n** (**n** = **1**,**2**) and A represents the pre-exponential factor of the reaction. The

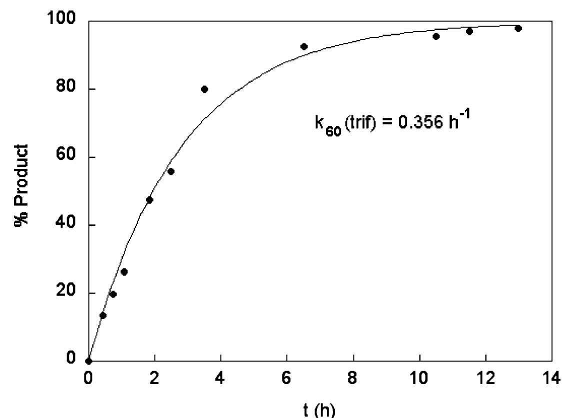

Figure 4. Plot of the % product from the reaction of dirhodium trifluoroacetate with 9-EtGuaH as a function of time t at 60 °C (k_{60}(trif) = 0.356 h^{-1}).

least-squares best fits to eq 2 for the reactions of **1** and **2** with 9-EtGuaH afford activation energy values of $E_a(\mathbf{1}) = 69 \pm 4$ kJ/mol and $E_a(\mathbf{2}) = 35 \pm 2$ kJ/mol, respectively.

The 2:1 ratio of the acetate vs the trifluoroacetate activation energies (for the systems under investigation) is in accord with the greater lability of the trifluoroacetate vs the acetate group because of the lower basicity of $CF_3CO_2^-$ (pK_b = 13.5) as compared to $CH_3CO_2^-$ (pK_b = 9.2).[38] This 2:1 ratio of the activation energies for acetate/trifluoroacetate compounds **1** and **2** correlates well with the difference in the LC_{50} values of the acetate derivatives **1**, **3**, and **5** being half as cytotoxic as the trifluoroacetate derivatives **2**, **4**, and **6**, respectively (Table 1). Since the trifluoroacetate groups are substituted by the same nucleophile more easily than the acetate, the dirhodium compounds **2**, **4**, and **6** react faster than the acetate analogs **1**, **3**, and **5** with biomolecules within cells, and thus the lability of the leaving groups accounts for the higher toxicity of the trifluoroacetate derivatives.

It is notable that a relationship exists between the ligand exchange kinetics and the in vitro toxicity for other classes of compounds, which bind covalently to DNA.[39] For example, the response toxic level for cisplatin and carboplatin against sarcoma 180 cells is 9 and 150 mg/kg, respectively, and correlates well with the greater lability of Cl^- vs the chelating $C_6H_6O_4^{2-}$ group, making cisplatin more toxic than carboplatin.[40] Likewise, in a series of diamine platinum(II) compounds, the complexes with chloride or oxalate leaving groups are cytotoxic against ovarian cancer cell lines at much lower concentrations than their congeners with 1,1′-cyclobutanedicarboxylato leaving groups (for the same carrier ligand).[41] The importance of the lability of the ligands has also been demonstrated in a series of platinum−amine complexes, in which the aqua derivatives of the series are the most toxic as compared to the tetramino complexes lacking labile ligands, which are the least toxic.[42] Similarly, in the series cis-$Pt(NH_3)_2X_2$ with various groups X, it is shown that the Pt−X bond strength has a substantial influence on the intrinsic reactivity of the compounds (e.g., complexes with the strongly bound isocyanate (−SCN^-) and nitrite (−NO_2) ligands show no antitumor potential).[43] Additionally, in the series of compounds [PtCI(R′R″SO)(diam)]-NO_3 (diam = bidentate amine such as 1,2-diaminocyclohexane (dach) or 1,1-bis(aminomethyl)cyclohexane (damch) and R′R″SO substituted sulfoxides such as dimethyl (Me_2SO), methyl phenyl (MePhSO), methyl benzyl (MeBzSO), diphenyl (Ph_2SO), and dibenzyl sulfoxide (Bz_2SO)), the presence of the more labile Ph_2SO sulfoxide group renders the platinum compounds more toxic than the other sulfoxide groups do.[44] Likewise, in the series

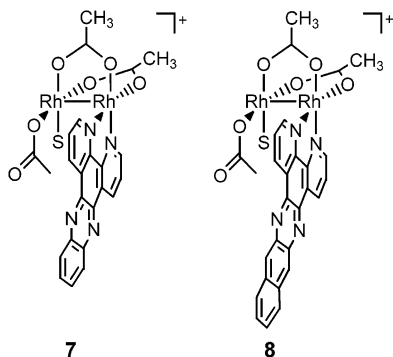

Figure 5. Structural representations of compounds **7** and **8**.

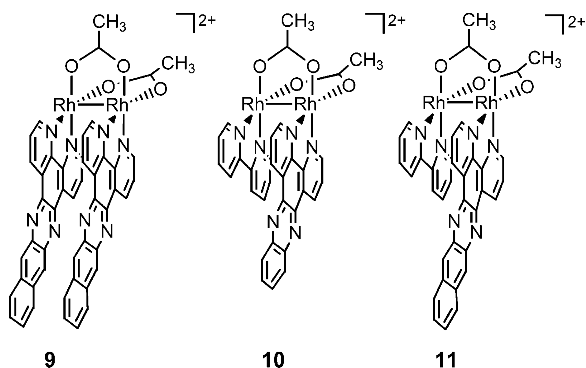

Figure 6. Structural representations of compounds **9**−**11**.

Table 2. Cytotoxicities (LC_{50}) and log *P* Values for Complexes **7**−**11**

complex		LC_{50} (μM)[a]	log *P* [b]
$[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(dppz)(MeOH)]^+$	**7**	27 ± 2	−0.30 ± 0.02
$[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(dppn)(MeOH)]^+$	**8**	51 ± 5	0.91 ± 0.01
$[Rh_2(O_2CCH_3)_2(dppn)_2]^{2+}$	**9**	384 ± 24	1.02 ± 0.03
$[Rh_2(O_2CCH_3)_2(dppz)(bpy)]^{2+}$	**10**	208 ± 10	−0.75 ± 0.01
$[Rh_2(O_2CCH_3)_2(dppn)(bpy)]^{2+}$	**11**	200 ± 20	0.32 ± 0.02

[a] Hs-27 human skin cells exposed to each compound for 30 min in the dark. [b] Partition coefficient $P = C_o/C_w$ (C_o and C_w are the complex concentrations in *n*-octanol and water, respectively).

of anticancer organometallic "piano-stool" ruthenium(II) complexes of the type $[(\eta^6\text{-arene})Ru(ethylenediamine)(X)]^{n+}$ (X is the leaving group, e.g., Cl), only the ones that readily hydrolyze exhibit high carcinostatic activity, whereas those that do not hydrolyze are inactive or weakly active.[45]

IV. Effect of Accessibility of Coordination Sites. The role of the lability of the equatorial ligands is also nicely demonstrated by comparing the cytotoxicities of compounds **7** and **8** (Figure 5) with those of **10** and **11**, as well as **5** and **9** (Figure 6), respectively (Tables 1 and 2). Complexes **7** and **8** are 8 and 4 times more cytotoxic than **10** and **11**, respectively, and their toxicities are 5 and 7 times greater than those measured for **5** and **9**, respectively. The greater toxicities of **7** and **8** relative to the other complexes can be attributed to the presence of solvent molecules and monodentate acetate groups in the equatorial positions of one rhodium atom. These ligands are more labile than the diimine, bpy, dppz, and dppn ligands occupying the same equatorial positions in **5**, **9**, **10**, and **11**. The labile solvent molecules and the monodentate acetate groups in **7** and **8** provide potential "open sites" accessible for nucleophilic substitution, as opposed to these sites being occupied by the chelating N−N diimine ligands in **5** and **9**−**11**, which reduce the reactivity of the latter.[37] This is supported by the fact that the dirhodium compound *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(bpy)(CH_3OH)]^+$, with a structure analogous to the structures of **7** and **8**, has been shown to react with biologically relevant molecules such as 9-EtGuaH.[46] Similarly, the higher toxicity of **1** as compared to the toxicities of **3** and **5** may be attributed to the greater lability of the carboxylate groups as compared to the chelating N−N diimine groups (phen and dppz for **3** and **5**, respectively). A 2- to 3-fold decrease in the cytotoxicity of $Rh_2(O_2CCH_3)_4$ toward human oral carcinoma KB cell lines has also been observed upon substituting two acetate groups with bpy and phen.[16]

V. Partition Coefficients and Toxicity of the Compounds. The log of the partition coefficient *P*, log *P*, has been previously related to the ability of a compound to transverse cellular membranes[47] and thus represents a simple method that can be used to estimate the potential membrane permeability of a molecule.[48−50] The values of log *P* between the aqueous and organic phases for compounds **1**−**6** are listed in Table 1, all of which are in the range from −1.90 ± 0.01 to 1.55 ± 0.01. The method used herein for the determination of the partition coefficient has been shown to work well for molecules with log *P* values that range from −2 (most hydrophilic) to +4 (most hydrophobic).[51] Compounds containing the CF_3 group (**2**, **4**, and **6**) are more lipophilic than their counterparts with CH_3 (**1**, **3**, and **5**, respectively).[52] The difference between the log *P* values for the acetate/trifluoroacetate pairs **1**/**2**, **3**/**4**, and **5**/**6** is on average 0.98, which is in accordance with the additivity of the lipophilic substituent constant (π).[53] The +2 charge for **3** and its trifluoroacetate analog **4** decreases their hydrophilicity relative to the uncharged complexes **1** and **2**, respectively. In **3** and **4**, the hydrophobic character of the two planar aromatic phen ligands is largely compensated by the positive charge of these compounds. If the aromatic system is further extended, however, as in **5** and its trifluoroacetate counterpart **6**, the increase in hydrophilicity due to the charge is overcome by the increase of hydrophobicity of the chelating groups, resulting in positive log *P* values. Thus, for a given carboxylate leaving group, the hydrophobicity of the compounds increases with the hydrophobicity of the nonleaving group.[54] It is noted that molecules that are both hydrophilic and lipophilic are able to penetrate membranes efficiently, since they do not require the formation of pores or channels at the membrane for entry.[55]

The effect of the charge on the partition coefficients of the compounds is demonstrated in the series of compounds **7**−**11**, since the singly charged monosubstituted compounds **7** and **8** have a hydrophobicity that is between the hydrophobicity of the bis-substituted compounds **5** and **9** and the hydrophobicity of the doubly charged compounds **10** and **11**, respectively (Tables 1 and 2). Although there does not seem to be a correlation between the cytotoxicities and the charge of the complexes (e.g., compounds **5** and **10** have the same +2 charge, and yet **5** is approximately twice as toxic as **10**), the +2 charge of complexes **3**−**6** and **9**−**11** facilitates their solubility in water.

By comparison of the partition coefficients of compounds **3**, **5**, and **9** (Tables 1 and 2), it is evident that the log *P* values become more positive as the π-system of the ligands in the complexes is extended within a given series (phen, dppz, dppn),[52] but the LC_{50} values of complexes **3**, **5**, and **9** do not change regularly with increasing hydrophobicity. In the series of dirhodium carboxylate paddlewheel compounds $Rh_2(O_2CR)_4$ (R = CH_3, C_2H_5, C_3H_7), the antitumor activity increases as the hydrophobicity of the R group increases, but further lengthening of the carboxylate group beyond the pentanoate reduces the efficacy of the compounds, a fact that implies that other factors are also responsible for the compound activity.[32] Indeed, the results reported herein indicate that the LC_{50} values of compounds **1**−**11** do not correlate with the partition coefficient

values, which provide an estimate of how well a complex can transverse the cell membrane. For example, compounds **2** and **9** have similar log P values, but **2** is 50 times more cytotoxic than **9**. Likewise, **8** and **9** have similar partition coefficients, and yet **9** is 7 times more toxic than **8**. In the same vein, compounds **10** and **11** have similar cytotoxicity values but markedly different partition coefficients.

Conclusions

The results of the present study imply that the toxicity of the complexes is not related to the ease of the complexes transversing the cellular membrane; the lability of the leaving groups on the dirhodium core, however, is a significant factor that affects the toxicity of the complexes. The kinetic results for the activation energies for acetate/trifluoroacetate compounds **1** and **2** (2:1 ratio) correlate well with the LC_{50} values of the acetate derivatives **1**, **3**, and **5** being half as cytotoxic as the trifluoroacetate derivatives **2**, **4**, and **6** and underscore the importance of the ligand lability/in vitro toxicity relationship. Further evidence of the effect of the ligand lability and the accessibility of the equatorial sites of the dirhodium core is provided by comparing the toxicities of compounds **7** and **8** with those of **10** and **11**, respectively. The labile solvent molecules and the monodentate acetate groups in **7** and **8** easily provide potential "open sites" accessible for nucleophilic substitution, as opposed to these sites being occupied by nonlabile groups, which reduce the reactivity of the complexes. Thus, the labile equatorial groups play a critical role in the toxicity of these complexes in vitro, which may be related to the antitumor activity of similar dirhodium compounds. Tailoring appropriately the groups surrounding the dirhodium core in these complexes may lead to more effective and less toxic drugs.

Experimental Section

Materials. The ligands dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz)[56] and benzodipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppn)[57] were synthesized according to reported procedures. The dirhodium complexes $Rh_2(O_2CCH_3)_4$ (**1**),[34] $Rh_2(O_2CCF_3)_4$ (**2**),[35] $[Rh_2(O_2CCH_3)_2(phen)_2]^{2+}$ (**3**),[36] $[Rh_2(O_2CCF_3)_2(phen)_2]^{2+}$ (**4**),[36] $[Rh_2(O_2CCH_3)_2(dppz)_2]^{2+}$ (**5**),[30] $[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(dppz)(MeOH)]^+$ (**7**),[29,30] and $[Rh_2(O_2CCH_3)_2(dppz)(bpy)]^{2+}$ (**10**)[31] were synthesized according to reported procedures. The starting material $RhCl_3 \cdot nH_2O$ was purchased from Pressure Chemicals and used as received. The compounds 2,2′-bipyridine (bpy), 1,10-phenanthroline (phen), and 2,3-diaminonaphthalene were purchased from Acros. The compounds 9-ethylguanine (9-EtGuaH), HPLC grade *n*-octanol, sodium phosphate (dibasic, anhydrous), and NaCl were purchased from Sigma. The deuterated solvents D_2O, CD_3COCD_3, CD_3OD, and C_6D_6 were purchased from Aldrich.

Instrumentation. The ^{1}H NMR spectra of the synthesized complexes were recorded on a Varian spectrometer at 300 MHz. The ^{1}H NMR spectra for the kinetics studies were recorded at 20 °C on a 500 MHz Varian Inova spectrometer with a 5 mm switchable probe head. The ^{1}H NMR spectra for the dirhodium tetraacetate and trifluoroacetate reactions with 9-EtGuaH were referenced to the residual proton impurities of the deuterated solvents D_2O and $(CD_3)_2CO$, respectively. The ^{1}H NMR spectra for the compounds **6**, **8**, **9**, and **11** were referenced to the residual proton impurities of the deuterated solvents $CD_3OD/CDCl_3$ and CD_3OD, respectively. Absorption spectra were recorded in a UV-1601PC Shimadzu spectrophotometer. Mass spectra were acquired on a PE SCIEX QSTAR Pulsar electrospray ionization mass spectrometer at Texas A & M University. Elemental analyses were performed by Atlantic Microlab Inc., P.O. Box 2288, Norcross, GA 30091.

Syntheses. *cis*-$[Rh_2(\mu\text{-}O_2CCF_3)_2(dppz)_2](O_2CCF_3)_2$ (6). A solution of $Rh_2(\mu\text{-}O_2CCF_3)_4$ (126.3 mg, 0.19 mmol) in CH_3CN (16 mL) was treated with solid dppz (108.2 mg, 0.38 mmol), and the suspension was heated to reflux for 16 h. After this time period, the red mixture was cooled to room temperature, filtered, and washed with CH_3CN to afford a red solid (89% yield). ESI-MS: m/z 499.2 ($[Rh_2(\mu\text{-}O_2CCF_3)_2(dppz)_2]^{2+}$). UV−vis (MeOH) λ, nm (ϵ, M^{-1} cm^{-1}): 530 (455), 378 (sh, 2710), 360 (16 400), 279 (56 300). ^{1}H NMR ($CD_3OD/CDCl_3$) δ (ppm): 9.35 (d, 4H, dppz), 8.95 (d, 4H, dppz), 8.45 (m, 4H, dppz), 8.25 (m, 4H, dppz), 8.10 (m, 4H, dppz). Anal. Calcd for $C_{44}H_{20}N_8O_8F_{12}Rh_2 \cdot H_2O$: C, 42.60; H, 1.79; N, 9.03. Found: C, 42.64; H, 1.75; N, 8.98.

***cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(\eta^1\text{-}O_2CCH_3)(dppn)(CH_3OH)](O_2CCH_3)$ (8).** A suspension of dppn (90 mg, 0.39 mmol) and $Rh_2(\mu\text{-}O_2CCH_3)_4(CH_3OH)_2$ (171 mg, 0.39 mmol) in acetone (15 mL) was stirred at room temperature under N_2 for 48 h. The resulting green precipitate was filtered and washed with acetone (3 × 5 mL). The solid was suspended in CH_3OH (50 mL) and stirred at room temperature for 24 h. The resulting green solution was filtered and concentrated under reduced pressure to 2 mL, and the product was precipitated by addition of Et_2O. The green solid was filtered, washed with Et_2O, and dried under vacuum (93 mg, 40%). ESI-MS: m/z 715 (100%, $[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)(O_2CCH_3)]^+$). UV−vis (MeOH) λ, nm, (ϵ, M^{-1} cm^{-1}): 595 (760), 440 (sh, 770), 403 (11 900), 321 (89 800), 256 (49 100). ^{1}H NMR ($CD_3OD/CDCl_3$, 1:1 v:v) δ (ppm): 9.77 (d, 1H, J = 9.3 Hz), 9.12 (s, 1H), 8.86 (dd, 1H, J = 9.6, 5.3 Hz), 8.35 (d, 1H, J = 7.7 Hz), 8.15 (dd, 1H, J = 9.3, 5.7 Hz), 7.76 (dd, 1H, J = 6.4, 3.2 Hz), 2.49 (s, 3H, CH_3CO_2), 2.45 (s, 3H, CH_3CO_2), 2.02 (s, 3H, CH_3CO_2), 1.23 (s, 3H, CH_3CO_2). Anal. Calcd for $C_{31}H_{32}N_4O_{11}Rh_2 \cdot 3H_2O$: C, 41.51; H, 4.27; N, 6.25. Found: C, 41.58; H, 4.09; N, 6.30.

***cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)_2](O_2CCH_3)_2$ (9).** A mixture of dppn (100 mg, 0.30 mmol) and $Rh_2(\mu\text{-}O_2CCH_3)_4(CH_3OH)_2$ (76.2 mg, 0.15 mmol) in CH_3CN (10 mL) was heated under nitrogen for 24 h. After the mixture was cooled, the precipitate was filtered, washed with CH_3CN (3 × 5 mL), and dried overnight under vacuum to provide a reddish solid (144 mg, 87%). ESI-MS: m/z 494 (100%, $[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)_2]^{2+}$). UV−vis (MeOH) λ, nm (ϵ, M^{-1} cm^{-1}): 418 (16 380), 397 (16 570), 320 (94 180), 259 (71 380). ^{1}H NMR (CD_3OD) δ (ppm): 9.03 (d, 1H, J = 7.1 Hz), 8.62 (d, 1H, J = 5.5 Hz), 8.08 (s, 1H), 7.73 (t, 1H, J = 7.9 Hz), 7.60 (d, 1H, J = 7.4 Hz), 7.41 (dd, 1H, J = 6.8, 2.5 Hz), 2.60 (s, 6H, CH_3CO_2), 1.73 (s, 6H, CH_3CO_2). Anal. Calcd for $C_{52}H_{36}N_8O_8Rh_2 \cdot 4H_2O \cdot 0.25CH_3CN$: C, 53.03; H, 3.79; N, 9.72. Found: C, 53.07; H, 3.42; N, 10.16.

***cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)(bpy)](O_2CCH_3)_2$ (11).** Following the procedure described for *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)_2](O_2CCH_3)_2$, a mixture of **8** (270 mg, 0.35 mmol) and bpy (55 mg, 0.35 mmol) in CH_3CN (10 mL) was heated under N_2 for 24 h to provide a dark-red solid (173 mg, 42%). ESI-MS: m/z 406 (100%, $[Rh_2(\mu\text{-}O_2CCH_3)_2(dppn)(bpy)]^{2+}$). UV−vis (MeOH) λ, nm (ϵ, M^{-1} cm^{-1}): 398 (11 000), 318 (73 160), 260 (61 600). ^{1}H NMR (CD_3OD) δ (ppm): 9.42 (d, 1H, J = 7.5 Hz), 9.05 (s, 1H), 8.61 (d, 1H, J = 5.0 Hz), 8.32 (d, 1H, J = 5.0 Hz), 8.27 (dd, 1H, J = 5.0, 2.5 Hz), 7.78−7.68 (m, 3H), 7.53 (t, 1H, J = 7.4 Hz), 7.31 (t, 1H, J = 7.5 Hz), 2.57 (s, 6H, CH_3CO_2), 1.75 (s, 6H, CH_3CO_2). Anal. Calcd for $C_{52}H_{36}N_8O_8Rh_2 \cdot 4H_2O \cdot 0.25CH_3CN$: C, 48.02; H, 4.06; N, 8.64. Found: C, 47.96; H, 3.54; N, 8.69.

Ligand Exchange Kinetics. Reaction of $Rh_2(O_2CCH_3)_4(H_2O)_2$ with 9-EtGuaH.[17,18,21] In a typical reaction, a slurry of 9-EtGuaH (25 mg, 0.14 mmol) in H_2O (5 mL) was added to a solution of $Rh_2(O_2CCH_3)_4(H_2O)_2$ (30 mg, 0.063 mmol) in 5 mL of H_2O. The reaction solution was heated at a constant temperature (±3 °C) for a few days, during which time its color gradually changed from aqua to emerald-green. Small aliquots (200 μL) were removed from the reaction solution at various time points (depending on the rate of the reaction at the particular temperature), lyophilized a few times, redissolved in D_2O and monitored by ^{1}H NMR spectroscopy to determine the progress of the reaction. The areas of the H8 protons of the reaction product $Rh_2(O_2CCH_3)_2(9\text{-EtGua})_2$ (head-to-head and head-to-tail isomers) in each sample were integrated against the area of the H8 proton of the unreacted 9-EtGuaH.

Reaction of $Rh_2(O_2CCF_3)_4$ with 9-EtGuaH. In a typical reaction, a slurry of 9-EtGuaH (25 mg, 0.14 mmol) in H_2O (5 mL) was added to a solution of $Rh_2(O_2CCF_3)_4$ (46 mg, 0.070 mmol) in 5 mL of H_2O. The reaction solution was heated at a constant temperature (±3 °C) for several hours, during which time its color gradually changed from blue to emerald-green. Small aliquots (350 μL) were removed from the reaction solution at various time points (depending on the rate of the reaction at the particular temperature), lyophilized a few times, and redissolved in exactly 450 μL of $(CD_3)_2CO$. To each sample, a precise amount of C_6D_6 (1.5 μL) was added, and the sample was monitored by ^{1}H NMR spectroscopy. The area of the H8 protons of $[Rh_2(O_2CCF_3)_2(9\text{-EtGuaH})_2](O_2CCF_3)_2$ in each sample was integrated against the area of the internal C_6D_6. Sampling of the reaction was discontinued when the integration area of the H8 protons (from the product) did not increase with respect to the integration area of the internal C_6D_6. The internal C_6D_6 had to be added to the NMR sample because, although $[Rh_2(O_2CCF_3)_2(9\text{-EtGuaH})_2](O_2CCF_3)_2$ is readily soluble in $(CD_3)_2CO$, 9-EtGuaH is not (to monitor the unreacted amount). The ^{1}H NMR spectra of the reaction aliquots could not be monitored in D_2O because $[Rh_2(O_2CCF_3)_2(9\text{-EtGuaH})_2](O_2CCF_3)_2$ is not completely soluble in D_2O. Efforts to monitor the progress of the reaction by ^{19}F NMR spectroscopy were not successful because the resonances of the free and bound trifluoroacetate ($CF_3CO_2^-$) are very close, making the integration values unreliable. Monitoring the substitution reactions of the acetate and trifluoroacetate bridging groups with other reagents, e.g., HCOOH or Na_2CO_3, was not possible because the reactions with dirhodium trifluoroacetate are complete within minutes and thus not possible to monitor by NMR.

Partition Coefficient Determination. The lipophilicity of the complexes was determined by the "shake flask" method using a pH 7.4 phosphate buffer (0.129 M NaCl) and *n*-octanol as solvents.[58] Each compound was dissolved in the phase in which it is most soluble, resulting in typical concentrations of 50−350 μM, and duplicate determinations using three different solvent ratios were performed for each complex. Following mixing and phase separation according to literature methods,[58] each phase was analyzed for solute content and the concentration was determined using spectrophotometric methods. All the *n*-octanol/water partition coefficients were determined by UV−vis spectroscopy (the wavelengths λ_{max} used for compounds **1**−**11** are listed in the Supporting Information). *n*-Octanol and buffer solutions were presaturated with each other prior to use. Fifty rotations were performed by hand followed by 1 h of settling time. Equilibration and measurements were made at 20 °C using a UV-1601PC Shimadzu spectrophotometer. The stability of the complexes **1**−**11** in the solvents used for the partition coefficient determination was studied by UV−vis spectroscopy; the complexes proved to be stable in both buffer and *n*-octanol solutions for extended periods of time (Supporting Information).

In Vitro Cytotoxicity Measurements. Human skin fibroblasts (Hs-27) were obtained from the American Type Culture Collection, cell line CRL-1634 (Manassas). Cells were cultured in Dulbecco's modified Eagle medium, containing 10% fetal bovine serum (Life Technologies), 50 μg/mL gentamicin, 4.5 mg/mL glucose, and 4 mM L-glutamine (Invitrogen Life Technology). Cell cultures were incubated in a humidified atmosphere containing 5% CO_2 at 37 °C. To assess the cytotoxicity of the compounds under investigation, subconfluent (50−80% confluent) monolayers of Hs-27 in 60 mm culture dishes were used. The monolayers were washed twice with phosphate buffered saline (PBS) to ensure that the culture dishes were free of any culture medium, and fresh Eagle medium containing different concentrations of each compound was added to cover the fibroblasts. Then the cells were removed from the dishes by trypsinization, seeded into 24-well culture dishes, and incubated for 2−4 days or until the untreated control group reached confluence. *N*-Lauroyl sarcosine (200 μL, 40 mM) was then added to each well, and the cells were allowed to lyse for at least 15 min. Quantitative determination of the protein content in each well was undertaken using Peterson's modification of the micro-Lowry method (Sigma reagent kit), where the lysate was treated with 200 μL of Lowry reagent for 20 min and then with 100 μL of Folin−Ciocalteau phenol reagent for 30 min or until color developed. A portion of the contents (200 μL) of each well was transferred to a 96-well plate for absorbance determination using a multiwell plate reader (Dynatech Laboratory). The absorbance at 630 nm was monitored, which is proportional to the total protein content and the number of cells in each well.

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Supporting Information Available: Analytical data for compounds **6**, **8**, **9**, and **11**; ^{1}H NMR spectra of dppz, dppn, and compounds **6**, **8**, **9**, and **11**; wavelengths λ_{max} (nm) used for the determination of the partition coefficients for compounds **1**−**11**; and UV−vis spectra of fresh and 30-day solutions of compounds **1** (*n*-octanol) and **3** (buffer). This material is available free of charge via the Internet at http://pubs.acs.org.

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