Published in final edited form as:

Dalton Trans. 2013 March 21; 42(11): 4002–4012. doi:10.1039/c2dt32565a.

Rational Design of $[\text{Co}(\text{acacen})\text{L}_2]^+$ Inhibitors of Protein Function

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Abstract

Cobalt(III) Schiff base complexes, such as $[\text{Co}(\text{acacen})\text{L}_2]^+$, inhibit the function of Zn(II)-dependent proteins through dissociative exchange of the axial ligands with key histidine residues of the target protein. Consequently the efficacy of these compounds depends strongly on the lability of the axial ligands. A series of $[\text{Co}(\text{acacen})\text{L}_2]^+$ complexes with various axial ligands was investigated using DFT to determine the kinetics and thermodynamics of ligand exchange and hydrolysis. Results showed excellent agreement with experimental data, indicating that axial ligand lability is determined by several factors: pK_a of the axial ligand, the kinetic barrier to ligand dissociation, and the relative thermodynamic stability of the complexes before and after exchange. Hammett plots were constructed to determine if the kinetics and thermodynamics of exchange can be modulated by the addition of an electron-withdrawing group (EWG) to either the axial ligand itself or to the equatorial acacen ligand. Results predict that addition of an EWG to the axial ligand will shift the kinetics and thermodynamics so as to promote axial ligand exchange, while addition of an EWG to acacen will decrease axial ligand lability. These investigations will aid in the design of the next generation of $[\text{Co}(\text{acacen})\text{L}_2]^{2+}$, allowing researchers to develop new, more effective inhibitors.

Introduction

The field of medicinal inorganic chemistry has grown in recent years as metal-based therapeutics have begun to transition from the laboratory to the clinic.^{1–6} The clinical success of DNA-binding derivatives of cisplatin and other metal complexes has facilitated

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greater understanding of the interaction between biomolecules and inorganic coordination complexes.^{7–10} This in turn has led to the development of transition metal complexes targeted to particular metalloproteins that have been shown to disrupt the coordination environment of the native metal thereby inhibiting specific biological activity.^{3, 11–16}

Cobalt(III) Schiff base complexes such as $[\text{Co}(\text{acacen})\text{L}_2]^+$ are known to inhibit a wide variety of zinc-dependent proteins.^{14–20} Recently, we have shown that they selectively inhibit the function of several transcription factors implicated in both normal development and cancer progression.^{14–16} Evidence suggests that the metal center of $[\text{Co}(\text{acacen})\text{L}_2]^+$ binds to key histidine residues of the target protein by a dissociative exchange mechanism with the labile axial ligands (L) (Figure 1).¹⁸

Experiments show that the inhibitory activity of $[\text{Co}(\text{acacen})\text{L}_2]^+$ depends strongly on the nature of the axial ligands.²¹ Enzyme inhibition is observed when the axial positions are occupied by either sterically-hindered 2-methylimidazole (2-MeIm) or labile ammine (NH_3) ligands. Complexes in which L = imidazole (Im) are comparatively poor enzyme inhibitors.¹⁷ The degree of enzyme inhibition can be further modulated by modification of the acacen backbone. Inhibition is significantly reduced by the addition of a chloride at position B and eliminated entirely by the addition of a trifluoromethyl group at position A (Figure 1).¹⁷

The structural dependence of inhibitory activity can be exploited to modulate the efficacy of $[\text{Co}(\text{acacen})\text{L}_2]^+$ complexes. Consequently, the kinetics and thermodynamics of axial ligand exchange and pH stability as well as the steric and electronic factors that affect the ligand exchange process must be understood to fully realize the potential of these complexes.

Experimental studies have employed ^1H NMR, x-ray crystallography, and mass spectrometry to understand the pH stability of several $[\text{Co}(\text{acacen})\text{L}_2]^+$ derivatives (where L = *N*-methylimidazole (*N*-MeIm), 2-methylimidazole (2-MeIm), 4-methylimidazole (4-MeIm), pyridine (py), Im, and NH_3) and to examine the lability of these complexes with respect to ligand exchange with 4-MeIm (a model axial ligand for histidine residues present in target biomolecules) (Figure 2).²¹

These studies revealed a direct correlation between pH stability, propensity for hydrolysis, and substitutional reactivity. Each of these properties was found to be axial ligand dependent and the experimental observations can be summarized as follows: (1) The trend in pH stability of the axial ligands is not simply a function of ligand pK_a . (2) When 2 equivalents of 4-MeIm are added to complexes in which L = 2-MeIm or NH_3 , complete conversion to the *bis*-4-MeIm adduct is observed. (3) When 2 equivalents of 4-MeIm are added to complexes in which L = py, Im or 4-MeIm, a mixture of products is formed consisting of starting material with both initial axial ligands (*bis*-L), a mixed product containing one initial axial ligand and one 4-MeIm (*mixed*-4-MeIm/L), and the product in which both initial ligands have been exchanged for 4-MeIm (*bis*-4-MeIm) (Figure 3). The proportion of products within the mixture is dependent on the starting complex, with the *bis*-py complex converting most readily to the *bis*-4-MeIm derivative.²¹

The goal of this work is to employ computational methods to i.) explain the experimental results in terms of the underlying reactivity of $[\text{Co}(\text{acacen})\text{L}_2]^+$ complexes and ii.) predict how modifications to these complexes will affect inhibitory activity. In order to validate the predictive ability of the computational model we employed, computational results were compared to experimental data. The model was extended to a variety of modified Co(III) complexes to determine how specific structural modifications to either the axial ligand or acacen backbone affect axial ligand lability.

All intermediate structures corresponding to a dissociative axial ligand exchange with either water or 4-MeIm were geometry optimized. The kinetic and thermodynamic parameters associated with both processes were calculated to i.) validate the computational methods employed by comparing the results to experimental information and ii.) provide further insight into the observations described.²¹

Both the geometries of the calculated structures and the trends in exchange dynamics predicted by the calculated kinetic and thermodynamic parameters are in excellent agreement with experimental data.²¹ Moreover, these calculations reveal that the axial ligand lability is determined by an interplay between multiple equilibria. Specifically, lability is a function of ligand pK_a , the activation barrier for ligand dissociation, and the relative thermodynamic stability of the complexes. This information was used to predict how further chemical modifications to the complex affect ligand lability. Hammett studies indicate that the addition of electron withdrawing groups (EWGs) to either the axial ligand or the acacen backbone affect the kinetics and thermodynamics of both hydrolysis and ligand exchange.

Computational Methods

Calculations were performed using Q-Chem 3.1 and graphics were generated using Visual Molecular Dynamics (VMD).^{22, 23} All possible conformations of each structure were fully optimized using the B3LYP density functional in conjunction with the 6-31G(d) Pople basis set for all atoms.^{24, 25} This functional has been used for several studies of similar systems and shown to provide accurate results.^{26–30} Optimization and frequency calculations of the lowest energy conformation of each structure were repeated using the same functional in conjunction with a correlation consistent, double zeta basis set (cc-pVDZ) to further validate results. Both basis sets produced structures that are in excellent agreement with x-ray crystal data, with Co-N and Co-O bond distances differing by less than 2 or 3% for calculations performed with 6-31G(d) and cc-pVDZ, respectively. The trends in kinetic and thermodynamic data were consistent between both basis sets. The data reported here is from calculations performed with 6-31G(d); data obtained using the cc-pVDZ basis set can be found in the supporting information (Figures SI-5 through SI-9).

Frequency analyses were performed on all optimized structures to confirm the absence of imaginary frequencies and obtain thermal corrections to the electronic energy. For all calculations, a charge of 3^+ and a low-spin d^6 configuration were assumed for the cobalt metal, in accordance with the NMR data obtained in reference [21]. To examine the specific stereoelectronic effects of various axial ligands, Natural Bond Orbital analysis was performed using the NBO 5.0 program³¹ embedded within Q-Chem 3.1 (results in Supporting Information). The potential energy surfaces (PES) for the approach of 2-MeIm and py were generated by fixing the approaching ligand 2.75 Å away from the metal center and rotating the ligand about the Co-N_L bond in 15-degree increments. Single point energy calculations were performed at each point, and the surface generated using the E_{SCF} values.

Attempts to identify a 7-coordinate intermediate consistent with an associative ligand exchange mechanism were unsuccessful, in agreement with previous studies that suggest both hydrolysis and ligand exchange of Co(III) complexes occur through a dissociative^{32–35} or dissociative interchange^{36–39} (I_D) mechanism. An I_D exchange mechanism implies that while dissociation of the outgoing ligand is rate-limiting, the incoming ligand is associated with the complex in such a way that the exchange occurs too rapidly for experimental detection of a 5-coordinate intermediate. The dissociative character of the mechanism increases for complexes with more sterically hindered metal centers.^{40, 41} The tetradentate acacen ligand can be considered sterically demanding on the metal center, thus all kinetic

and thermodynamic parameters reported herein were calculated under the assumption of a dissociative mechanism.

All intermediate structures corresponding to axial ligand exchange with either water or 4-MeIm were located (Scheme 1). Unfortunately, attempts to locate transition structures for the dissociation or addition of axial ligands were unsuccessful. Consequently, all structures discussed in this work are ground state minima and therefore the kinetic parameters discussed herein are not activation barriers. However, the 5-coordinate intermediate resulting from ligand dissociation is significantly higher in energy than the initial octahedral complex. A dissociative exchange must proceed through this intermediate; consequently, we have assumed that the difference in energy between the initial octahedral complex and the 5-coordinate intermediate can be taken as a lower bound for the activation barrier (Scheme 1). The numbers obtained are in good agreement (within ~ 4 kcal/mol) with experimentally determined activation enthalpies for a similar system, providing validation for our assumption.⁴²

Result and Discussion

Geometric Analysis

Comparison to crystal structures—The optimized structures are in excellent agreement with experimental observations.²¹ For all structures in which the axial positions are occupied by aromatic rings, the two rings are orthogonal to one another: one is oriented parallel to the C_2 axis of acacen (defined in Figure 4) and the other perpendicular to it. This orientation of the axial ligands is apparent in the experimentally-determined crystal structures (Figure 4). By defining an average plane (N_2O_2) that contains the chelating atoms of acacen (O_{eq} and N_{eq}), it is obvious that all structures show a significant deformation of the ethylenediimine backbone with one carbon lying above the plane and the other approximately in the plane, further coinciding with crystal structure data (Figure 4).²¹

Though both basis sets overestimate the Co-N bond lengths (by ~0.02 and 0.03 Å for 6-31G(d) and cc-pVDZ, respectively), 6-31G(d) produces geometries more consistent with crystal structure data, with all calculated bond distances lying within 2% of the experimental value and all bond angles within 3% (Table SI-1). Both basis sets capture qualitative trends within the data: the slightly elongated bond between Co(III) and the nitrogen of the axial ligand ($Co-N_L$) for the *bis*-py and *bis*-2-MeIm complexes, slight compression of the O_{eq} -Co- O_{eq} and N_{eq} -Co- N_{eq} angles, very minor bending of the axial ligands toward the oxygen atoms of the acacen ligand, and significant bending of the acacen backbone in the *bis*-2-MeIm complex (Figure 5).

Geometric changes during hydrolysis and ligand exchange reactions—The high-energy, five-coordinate intermediate (or *mono*-L complex, Scheme 1) formed by dissociation of an axial ligand from $[Co(acacen)L_2]^+$ adopts a square pyramidal geometry for all ligands examined in this study. As a result of ligand dissociation, several noticeable changes to the geometry of the complexes about the metal center occur. The Co- O_{eq} and Co- N_{eq} bond distances are reduced by ~0.03 Å, and the Co- N_L distance is reduced by ~0.1 Å (Figure SI-1A, 1B, and 1C). In the initial *bis*-L complexes, the Co(III) ion is located in the plane of the chelating atoms of acacen (the N_2O_2 plane shown in Figure 5); in the *mono*-L complexes, the metal is displaced from the plane, toward the axial ligand by ~0.2 Å (Figure SI-1E).

Coordination of a 4-MeIm ligand to the *mono*-L complex results in the formation of the *mixed*-4-MeIm/L complex, which exhibits a geometry similar to the initial *bis*-L complexes. The Co- O_{eq} , Co- N_{eq} , and Co- N_L distances are all relatively unaffected by the exchange, and

Co(III) remains within the N_2O_2 plane for both types of complexes. Water coordination to the *mono*-L complex results in the formation of the octahedral, *mixed*-H₂O/L complex (Figure 4A), which displays geometric features that are intermediate between the original *bis*-L complex and the 5-coordinate *mono*-L complex. While the Co-O_{eq} and Co-N_{eq} bond distances of the *mixed*-H₂O/L species are only slightly shorter than those of the *bis*-L species, the Co-N_L distances are shortened by ~ 0.04 Å. Additionally, the Co(III) is slightly displaced from the N_2O_2 plane, deviating ~ 0.1 Å in the direction of L.

Dissociation of the second axial ligand from the *mixed*-H₂O/L causes significant distortions in geometry. Unlike the *mono*-L species, the 5-coordinate, $[Co(acacen)H_2O]^+$ complex adopts a geometry midway between a square pyramid and trigonal bipyramid, with a significant buckling of the acacen ligand, and Co-O_{eq} and Co-N_{eq} distances ~ 0.03 Å shorter than the initial *bis*-L structure. Coordination of a second water molecule to $[Co(acacen)H_2O]^+$ results in the expected octahedral structure, which displays a Co(III)-water (Co-O_w) distance that is ~ 0.03 Å shorter than the Co-O_w distance calculated for the *mixed*-H₂O/L species.

The Co-O_{eq} and Co-N_{eq} distances are nearly independent of the identity of the axial ligand within each class of complexes (*bis*-L, *mono*-L, *mixed*-H₂O/L and *mixed*-4-MeIm/L: see Figure SI 1A and 1B). The Co-N_L distance shows only slightly more ligand dependence than the Co-acacen distances, with elongated bond lengths for the py and 2-MeIm complexes.

Factors affecting axial ligand lability

pH Stability to Hydrolysis—Acidification of an aqueous solution of $[Co(acacen)L_2]^+$ initiates hydrolysis as the axial ligands are replaced with water molecules (Scheme 1A). Multiple equilibria are involved in this process, including the protonation of the free ligand (Scheme 2). In an aqueous solution the axial ligands are in a dynamic equilibrium with the solvent molecules. Ligands with higher pK_a values will be more prone to protonation in an acidic environment. Protonation of the ligand hinders its re-coordination to the metal, promoting the hydrolysis reaction. Thus, it is reasonable to assume that the pK_a of L will play a substantial role in determining the pH stability of $[Co(acacen)L_2]^+$.

Experimental evidence indicates that other factors play a role in determining the pH stability of these complexes.²¹ The pH stability can be quantified by determining the pH at which 50% of the initial *bis*-L complex has been hydrolyzed. If ligand pK_a were the sole factor in determining stability to hydrolysis, the complexes containing axial ligands with lower pK_a values would be expected to maintain 50% stability at lower pH levels. However, a plot of pH stability vs. ligand pK_a shows that both the py and 2-MeIm complexes show unexpectedly low stability, while the *N*-MeIm complex exhibits greater stability than expected (Figure SI-2).²¹

To explore other factors that may influence the pH stability of these complexes, the kinetic and thermodynamic parameters for the hydrolysis reaction were calculated (see Scheme 1A). Results indicate that the complexes that exhibit unusually low stability (i.e., L = py and 2-MeIm) have the lowest kinetic barrier for axial ligand dissociation. Meanwhile, the complex in which L = *N*-MeIm (exhibiting unusually high stability) has the largest barrier (Figure 5). Interestingly, the barrier for dissociation of the second axial ligand from the *mixed*-H₂O/L complex (k_2) is significantly higher than the barrier for the initial dissociation (k_1) for all complexes with the exception of L = NH₃. This is consistent with experimental NMR data, which indicates that the ammine complex proceeds all the way to the *bis*-aqua product, $[Co(acacen)(H_2O)_2]^+$, rather than reaching equilibrium at the *mixed*-H₂O/L product as is the case for the pyridine, 4-MeIm, and Im complexes.²¹

For all complexes investigated, hydrolysis is an endothermic process (as indicated by the positive values for T_1 , T_2 , and T_{tot} ; parameters defined in Scheme 1A). However, the thermodynamic destabilization of the hydrated complexes (*mixed*-H₂O/L and *bis*-aqua) relative to the initial *bis*-L complexes is greater for the more pH stable complexes (*N*-MeIm and 4-MeIm) and smaller for the least stable (py and 2-MeIm).

Therefore each of the equilibria shown in Scheme 2 plays a role in pH stability. When combined with knowledge of ligand pK_a values, the kinetic and thermodynamic parameters governing axial ligand dissociation and subsequent water coordination can be used to understand the pH-dependent hydrolysis of these complexes.

Ligand Exchange with 4-MeIm—Both hydrolysis and ligand exchange of $[\text{Co}(\text{acacen})\text{L}_2]^+$ with 4-MeIm are related to the lability of the axial ligands. Consequently, we applied the same methodology described above to understand the experimentally observed trends in the ligand exchange reaction, calculating the kinetic and thermodynamic parameters associated with 4-MeIm exchange (Figure 6; parameters defined in Scheme 1B).

Experimentally, when two equivalents of 4-MeIm are added to aqueous solutions of $[\text{Co}(\text{acacen})\text{L}_2]^+$, the complexes in which $\text{L} = \text{NH}_3$ and 2-MeIm exchange with 4-MeIm to form the *bis*-4-MeIm product.²¹ Conversely, complexes in which $\text{L} = \text{Im}$, *N*-MeIm, or py exchange more slowly and do not reach completion (i.e., full conversion to the final *bis*-4-MeIm product is not observed).²¹ Instead, the final product is an equilibrium mixture of the initial *bis*-L complex, the *mixed*-4-MeIm/L complex, and the *bis*-4-MeIm complex. It should be noted, that the *bis*-py complex does exchange to a greater extent than either the Im or *N*-MeIm complex, particularly at lower pH values.²¹

As with $[\text{Co}(\text{acacen})\text{L}_2]^+$ hydrolysis, we propose that the axial ligand exchange of $[\text{Co}(\text{acacen})\text{L}_2]^+$ with 4-MeIm can be explained by considering the pK_a of L in combination with the kinetics and thermodynamics of exchange. The pK_a of NH_3 (10.25) is significantly higher than any of the other ligands considered in this study, including 4-MeIm (7.51).⁴³ Thus, during the course of dynamic equilibrium with solvent molecules, NH_3 is readily protonated upon dissociation (even at neutral pH), preventing its re-coordination and promoting ligand exchange with 4-MeIm, which has a significantly lower pK_a (Scheme 3).

There is little variation within the pK_a values of various imidazole derivatives. However, as with the hydrolysis reaction, the kinetic barrier for ligand dissociation is significantly lower for the complex in which $\text{L} = 2\text{-MeIm}$ as compared to $\text{L} = \text{Im}$ or *N*-MeIm, promoting more rapid and complete ligand exchange. The kinetic barrier is similarly low for the $\text{L} = \text{py}$ complex; however, pyridine has a pK_a of 5.23, making re-coordination of pyridine more competitive than re-coordination of 2-MeIm (Figure 6).

Re-coordination of 2-MeIm is further hindered by the steric bulk of the methyl group near the coordinating nitrogen. A scan of the potential energy surface (PES) about the Co-N_L bond for the $\text{L} = \text{py}$ and $\text{L} = 2\text{-MeIm}$ complexes indicates that there is a stronger angular dependence for the attack of 2-MeIm on the 5-coordinate species (Figure 7). While the PES for the pyridine complex has two discernable maxima at 90° and -90° , the surface is more flat than that of the 2-MeIm complex, which shows steeper, more sustained maxima. Consequently, the probability of free 2-MeIm associating with the 5-coordinate intermediate with an energetically favorable approach trajectory (and thus moving the reaction toward the starting material) is significantly lower than for pyridine.

The high barriers for ligand dissociation from the $\text{L} = \text{Im}$ and *N*-MeIm complexes, combined with their intermediate (between pyridine and ammine) pK_a , are consistent with the slow,

incomplete exchange with 4-MeIm that was observed for these complexes.²¹ With the exception of the L = *N*-MeIm complex, the exchange of axial ligands for 4-MeIm is thermodynamically favorable for all [Co(acacen)L₂]⁺ species (Figure 6). The exchange is most favorable for complexes in which L = 2-MeIm, NH₃, and py. This is consistent with the experimental observation that complexes in which L = 2-MeIm and NH₃ undergo complete conversion to the *bis*-4-MeIm species.²¹ Although the L = py complex does not undergo complete conversion, it does exchange with 4-MeIm more readily than the L = Im or L = *N*-MeIm species, particularly at lower pH values where the low pK_a of pyridine would be expected to play a smaller role in the exchange process.

Modifications to the axial ligand

The ability to modulate the lability of the axial ligands (either to tune pH stability or to enhance the exchange of axial ligands for the histidine residues present in target proteins) would be useful in optimizing the inhibitory efficacy of these complexes. Previous work has indicated that the addition of an electron-withdrawing group (EWG) to the axial ligand has a significant effect on the redox properties of Schiff base complexes.⁴⁴ Our results suggest that the axial ligand lability of [Co(acacen)L₂]⁺ complexes is affected by the pK_a of L as well as the kinetics and thermodynamics of the system. For this reason, the effect of axial ligand modification on the kinetics and thermodynamics of both hydrolysis and ligand exchange were examined.

A Hammett study was performed to investigate how the addition of an EWG to the 4-position of an axial imidazole ligand affects the kinetics and thermodynamics of exchange with both water and 4-MeIm. The axial ligands investigated (Figure 8) include 4-fluoroimidazole (4-F-Im, $\sigma_m = 0.34$), 4-cyanoimidazole (4-CN-Im, $\sigma_m = 0.56$), and 4-nitroimidazole (4-nitro-Im, $\sigma_m = 0.71$).⁴⁵ The kinetic and thermodynamic parameters for imidazole ($\sigma_m = 0$) and 4-MeIm ($\sigma_m = -0.07$) (which are calculated and discussed in the previous section) were incorporated in the construction of Hammett plots.⁴⁵ Sigma-meta (σ_m) constants were used as a measure of electron-withdrawing (EW) capability.⁴⁶

Results suggest a correlation between the EW capacity of axial ligand substituents and the kinetics and thermodynamics of hydrolysis and ligand exchange (Figure 9). As the strength of the EWG increases, the kinetic barrier for ligand dissociation decreases (see Figure 9A and SI-3). The thermodynamics of exchange show a similar correlation with EW capacity. As the strength of the EWG increases, the thermodynamic drive to exchange the initial ligand for 4-MeIm increases (Figure 9B), and the thermodynamic destabilization resulting from hydrolysis decreases (see Figure SI-3).

The Co-O_{eq} and Co-N_{eq} bond distances are unaffected by substitution of the axial ligand. Effects on the Co-N_L distance are only observed for the *mixed*-L/4-MeIm complexes (see Scheme 1); for these complexes the Co-N_L distance is slightly longer than the Co-N_{4-MeIm} distance. This difference between the Co-N_L and Co-N_{4-MeIm} distance roughly increases with the strength of the EWG. The σ -donor strength of the axial ligands in the mixed-4-MeIm/L complexes reflects this trend. When an EWG is added to L, the Co-4-MeIm interaction becomes slightly stronger than the Co-L interaction. This discrepancy increases with the strength of the EWG (reaching a maximum for the mixed-4-MeIm/4-nitro-Im complex) where the Co-4-MeIm interaction is 10% stronger than the Co-4-nitro-Im interaction, suggesting that the addition of an EWG to the axial ligand weakens the Co-L interaction.

These results indicate that the addition of an EWG to the axial ligand will alter its interaction with [Co(acacen)]⁺, shifting the kinetics and thermodynamics of the system so as to favor both hydrolysis and exchange with 4-MeIm. However, such modifications to the

ligand will likely lower its pK_a (e.g. pK_a of 4-F-Im = 2.44),⁴⁷ which would have the opposite effect on ligand lability as the predicted shifts in kinetics and thermodynamics. This complicates predictions for how axial ligand modifications will affect pH stability and 4-MeIm exchange.

Modifications to acacen

Inspired by previous studies that show that the redox properties of Schiff-base complexes are affected by the addition of EWGs to the equatorial ligand,^{48, 49} the effect of substitution at various positions on the acacen backbone was investigated to determine whether it is possible to manipulate the kinetic and thermodynamic parameters for hydrolysis and ligand exchange without affecting the axial ligand pK_a . This would allow us to predict how such modifications will modulate the axial ligand lability. As mentioned above, a previous study examined the effects of acacen modification on the inhibitory activity of $[Co(acacen)L_2]^+$ -type complexes. This study found that the addition of a Cl group *meta* to the chelating atoms (here, termed position B, refer to Figure 10) caused a reduction in inhibitory activity, while the addition of a CF_3 group *para* to the chelating atoms (position A, see Figure 1) eliminated inhibition entirely.¹⁷

As with our examination of the effect of axial ligand substitution, the EW capacity of the substituent at either position A or position B was systematically altered, and the kinetic and thermodynamic parameters for the hydrolysis and ligand exchange reactions calculated (Figures 11 and 12). Imidazole was used as the axial ligand for all calculations involving acacen modifications. Sigma-*para* (σ_p) constants were used for the construction of Hammett plots for position A, σ_m constants were used to analyze the data for position B.

Our results for the ligand exchange reaction indicate that there is a correlation between the EW capacity of the acacen substituent and the kinetic barrier for the dissociation of the first imidazole ligand (k_1), with the barrier increasing with the strength of the EWG (Figure 13). There is a similar correlation between the barrier for dissociation of the second imidazole to form the *mono*-4-MeIm species. In both cases, the correlation is stronger when the substitution is made at position A rather than position B.

The effect of substitution on the kinetics of hydrolysis appears more complicated. The first steps in the hydrolysis and ligand exchange reactions are identical, thus the correlation between EW capacity of the substituent and k_1 is still present. However, this correlation is significantly reduced for k_2 (the barrier for dissociation of the second imidazole to form the *mono*-aqueous species) if the substitution is made at position A; for substitutions at position B, the correlation disappears entirely (see Figure SI-4).

In contrast to the kinetic data, thermodynamic parameters associated with ligand exchange appear unaffected by substitution of the acacen ligand, showing no correlation between EW capacity of the substituent and the exothermicity of 4-MeIm exchange (Figures 12A and 12B). However, the thermodynamics of hydrolysis do indicate a slight trend (at least for substitutions at position A) where increases in the EW capacity decrease the thermodynamic destabilization resulting from hydrolysis (Figure 12C). Substitutions at position B show a much weaker correlation (Figure 12D).

These results suggest that the addition of an EWG to the acacen ligand, particularly at a position *ortho/para* to the chelating atoms, may increase both the pH stability of the complex and its resistance to 4-MeIm exchange. This is consistent with experimental results which showed that substitutions at these positions result in reduced enzyme inhibition.¹⁷

Conclusions

In summary, we have constructed a computational model to explain the experimental observations of $[\text{Co}(\text{acacen})\text{L}_2]^+$ inhibitory activity and predict how modifications to these complexes affect their efficacy. These predictions will be used to develop new generations of $[\text{Co}(\text{acacen})\text{L}_2]^+$ complexes in which the inhibitory activity can be tailored to the intended application. We determined that the axial ligand lability of $[\text{Co}(\text{acacen})\text{L}_2]^+$ complexes depends on multiple factors that include the ligand pK_a , the kinetic barrier for ligand dissociation, and the relative thermodynamic stability of the initial and final complexes. The kinetics and thermodynamics of the system can be modulated by substitution of either a.) the axial ligand itself or b.) the acacen ligand (Figure 1). Addition of an EWG to the axial ligand shifts the kinetics and thermodynamics in a direction that favors both exchange and hydrolysis. However, such modifications also affect the pK_a , complicating predictions of axial ligand lability. The addition of an EWG to acacen, particularly *ortho/para* to the chelating atoms, will kinetically hinder axial ligand dissociation, but have little effect on the thermodynamics of exchange with 4-MeIm. The results of this study will guide future modification of $[\text{Co}(\text{acacen})\text{L}_2]^+$, allowing researchers to tune the pH stability of these complexes and enhance the exchange of axial ligands for the histidine residues of target proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors gratefully acknowledge Shane M. Parker for helpful discussions. This work was supported by the National Institutes of Health's Centers of Cancer Nanotechnology Excellence initiative of the National Cancer Institute under award U54CA119341 and the Rosenberg Cancer Foundation. MAR thanks the NSF Chemistry Division for support of this work (Grant Number CHE-1058896). LMM and MCH would like to acknowledge the National Science Foundation Graduate Research Fellowship.

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Figure 1.

Co(III) Schiff base complexes $[\text{Co}(\text{acacen})\text{L}_2]^+$ that inhibit protein function. The degree of inhibition is dependent on the nature of the axial ligand (L) and the substituents located at positions A and B on the acacen ligand.

Figure 2. $[\text{Co}(\text{acacen})]^+$ complexes with axial ligands prepared and characterized in reference [21]. The axial ligands 2-MeIm and NH_3 are significantly more labile to hydrolysis and substitution by 4-MeIm.

Figure 3.

The addition of two equivalents of 4-Melm to $[\text{Co}(\text{acacac})\text{L}_2]^+$ (where $\text{L} = \text{py}$, $N\text{-MeIm}$, or Im) results in a solution containing three species: a) the original complex (*bis-L*) b) a complex in which one of the initial ligands is replaced by 4-Melm (*mixed-4-Melm/L*) and c) a complex in which both axial ligands are replaced by 4-Melm (*bis-4-Melm*).²¹

Figure 4. B3LYP/6-31G(d) optimized structure of the *bis*-2-MeIm complex. Atom labels correspond to those used in Table 1. The carbons of the ethylenediimine backbone and the N₂O₂ plane have been indicated. It is clear that the acacen ligand is significantly distorted out of the plane by the 2-MeIm ligand perpendicular to the C₂ axis. Hydrogens have been omitted for clarity.

Figure 5.

A.) Kinetics and B.) thermodynamics of hydrolysis of $[\text{Co}(\text{acacen})\text{L}_2]^+$. Calculated values are reported as free energy change at 298 K, and the kinetic and thermodynamic parameters are defined in Scheme 1. The complexes with unexpectedly low pH stability ($\text{L} = \text{pyridine}$ and 2-MeIm) have the lowest barriers for ligand dissociation and experience the least thermodynamic destabilization upon hydrolysis. Similarly, the complex exhibiting greater than expected pH stability ($\text{L} = \text{N-MeIm}$) has a very high barrier for ligand dissociation and is destabilized by hydrolysis.

Figure 6.

A.) Kinetics and B.) thermodynamics of axial ligand exchange of $[\text{Co}(\text{acacen})\text{L}_2]^+$ with 4-MeIm. The kinetic barrier for ligand dissociation is much lower for complexes in which $\text{L} = \text{py}$, 2-MeIm. These complexes show the largest thermodynamic stabilization upon exchange with 4-MeIm.

Figure 7.

Potential energy surface for rotation about the Co-N_L bond for *mixed-4-MeIm/L* complexes where L = py, 2-MeIm. The distance between Co(III) and the coordinating nitrogen was fixed at 2.75 Å to model the approach of an incoming ligand to the 5-coordinate species. The incoming ligand was rotated about the Co-N_L bond. Single point energy calculations were performed in 15° increments. The PES for L = 2-MeIm shows much steeper and more sustained maxima than the PES for L = py, indicating that the probability of approaching the 5-coordinate intermediate from a favorable angle is lower for 2-MeIm than for py.

X =

Me, $\sigma_m = -0.07$

H, $\sigma_m = 0.00$

F, $\sigma_m = 0.34$

CN, $\sigma_m = 0.56$

NO₂, $\sigma_m = 0.71$

Figure 8.

Imidazole derivatives (and their associated Hammett constants)⁴⁵ used to investigate how the addition of an EWG to the axial ligand affects the kinetics and thermodynamics of hydrolysis and ligand exchange.

Figure 9.

Kinetics (A) and thermodynamics (B) of ligand exchange. There is a substantial correlation between the electron withdrawing capability of the substituent on the axial ligand and the kinetic barrier for ligand dissociation. As the strength of the EWG is increased, the activation barrier decreases. Further, the thermodynamic drive to exchange the axial ligands for 4-Melm increases with increasing strength of the EWG.

A, B =	
Me:	$\sigma_p = -0.17$, $\sigma_m = -0.07$
H:	$\sigma_p = -0.00$, $\sigma_m = -0.00$
Cl:	$\sigma_p = 0.23$, $\sigma_m = 0.37$
CF ₃ :	$\sigma_p = 0.54$, $\sigma_m = 0.43$
CN:	$\sigma_p = 0.66$, $\sigma_m = 0.56$

Figure 10.

Structures (and associated Hammett constants)⁴⁵ studied to determine the effect of acacen substitution on kinetics and thermodynamics of hydrolysis and 4-MeIm exchange. As position A is *ortho/para* to the chelating atoms of acacen while position B is *meta* to these atoms, σ_p and σ_m constants were used respectively when fitting the Hammett plots discussed below.

Figure 11.

Effect of acacen substitution on the kinetics of 4-MeIm exchange. The addition of an EWG at position A increases the kinetic barrier for dissociation of the first imidazole ligand for both hydrolysis and 4MeIm exchange, though the correlation is stronger for position A than position B. For the 4-MeIm exchange reaction, the barrier for the second dissociation follows the same trend.

Figure 12.

Effect of acen substitution on the thermodynamics of hydrolysis and 4-MeIm exchange. The addition of an EWG to acen appears to have no correlation with the thermodynamics of 4-MeIm exchange; however, the thermodynamic destabilization resulting from hydrolysis appears to increase with increasing EW capability of substituents at position A. This correlation is much weaker for substitutions made at position B.

Scheme 1.

Kinetic and thermodynamic parameters associated with (A) hydrolysis and (B) axial ligand exchange of $[\text{Co}(\text{acacen})\text{L}_2]^+$, where $\text{L} = \text{NH}_3, \text{Im}, 2\text{-MeIm}, N\text{-MeIm}, \text{py}, \text{and } 4\text{-MeIm}$ (hydrolysis). Thermodynamic parameters for the hydrolysis reaction are reported as positive values, indicating that the reaction is endothermic. In contrast, the negative values for the thermodynamic parameters associated with exchange with 4-MeIm indicate that the exchange is exothermic.

Scheme 2.

Equilibria involved in hydrolysis of $[\text{Co}(\text{acacen})\text{L}_2]^+$. In aqueous solution, the axial ligands are in dynamic equilibrium with solvent molecules, though the equilibrium lies heavily to the side of ligand coordination. In an acidic environment, the ligand may become protonated during the course of this equilibrium reaction. A ligand with a higher pK_a is more likely to become protonated, preventing the re-coordination of the ligand to $\text{Co}(\text{III})$ and promoting the hydrolysis reaction.

Scheme 3.

Equilibria involved in ligand exchange with 4-MeIm. Even though the *bis*-2-MeIm and *bis*-py complexes have similar barriers for ligand dissociation, the lower pK_a of pyridine favors the deprotonated form of the free ligand, increasing the likelihood of the reverse reaction (i.e., re-coordination of pyridine). The higher pK_a of 2-MeIm reduces the amount of deprotonated free ligand available for re-coordination, driving the reaction forward toward the *bis*-4-MeIm species.