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Kinetics and thermodynamics of irreversible inhibition of matrix metalloproteinase 2 by a Co(III) Schiff base complex

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Abstract

Cobalt(III) Schiff base complexes have been used as potent inhibitors of protein function through the coordination to histidine residues essential for activity. The kinetics and thermodynamics of the binding mechanism of Co(acacen)(NH₃)₂Cl [Co(acacen); where H₂acacen is bis(acetylaceton)ethylenediimine] enzyme inhibition has been examined through the inactivation of matrix metalloproteinase 2 (MMP-2) protease activity. Co(acacen) is an irreversible inhibitor that exhibits time- and concentration-dependent inactivation of MMP-2. Co(acacen) inhibition of MMP-2 is temperature-dependent, with the inactivation increasing with temperature. Examination of the formation of the transition state for the MMP-2/Co(acacen) complex was determined to have a positive entropy component indicative of greater disorder in the MMP-2/Co(acacen) complex than in the reactants. With further insight into the mechanism of Co(acacen) complexes, Co(III) Schiff base complex protein inactivators can be designed to include features regulating activity and protein specificity. This approach is widely applicable to protein targets that have been identified to have clinical significance, including matrix metalloproteinases. The mechanistic information elucidated here further emphasizes the versatility and utility of Co(III) Schiff base complexes as customizable protein inhibitors.

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Keywords

Protein inhibition; Histidine; Matrix metalloproteinase; Cobalt

Introduction

The high affinity of transition metals for biological macromolecules and the unique properties of the metal ions have led to the exploitation of transition metal coordination complexes for therapeutic use in medicine. The reactivity of transition metal ions can be modulated because metals can adopt numerous geometries, coordination numbers, and redox states as demonstrated endogenously in biological systems. These unique properties of transition metals allow the optimization of thermodynamic and kinetic properties for enhanced drug specificity and activity [1]. Co(III) Schiff base complexes have been used to develop irreversible inhibitors targeting zinc finger transcription factors and enzymes [2-8]. Previous studies have shown that Co(III) Schiff base complexes with labile axial ligands, such as Co(acacen)(NH₃)₂Cl [Co(acacen); where H₂acacen is bis(acetylacetonate)ethylenediimine], bind to the nitrogen of the imidazole ring of a histidine residue of proteins and model peptides [2-5]. Histidine residues are essential for the catalytic activity of many enzymes and for proper folding of zinc finger transcription factors; thus, the observed inhibition is due to the Co(III) Schiff base complex interrupting the role of histidine [2, 4, 6]. Specificity for histidine has been further demonstrated as lysine, arginine, and cysteine residues are not involved in the binding of Co(III) Schiff base complexes to peptides [2].

Co(III) Schiff base complex inhibitors have been targeted to a specific enzyme or transcription factor of interest through the conjugation of a peptide or oligonucleotide [4, 6, 9]. As a result, not only can specificity be incurred, but the affinity of the Co(III) Schiff base complex for the target protein increases by more than 100-fold [4, 6]. In Co(III)-peptide or Co(III)-DNA conjugates, the targeting ligand is a reversible inhibitor that targets the entire conjugate to the target protein, allowing the Co(III) to bind to a histidine residue for irreversible inhibition of protein function. In this two-step reaction, the irreversible inhibition cannot be readily distinguished from the reversible reaction during enzyme inhibition. Previous studies on enzyme and protein inactivation have provided an understanding of the efficacy of targeting moieties or the effect of variable axial ligands on protein inhibition [4, 9], but the Co(III)-protein interaction has not been examined in as much detail.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endoproteases that alter the extracellular matrix during tissue remodeling and play an important role in embryogenesis, including skeletal formation and angiogenesis [10, 11]. Proteases are required in these processes to degrade components of the extracellular matrix as well as to activate or deactivate protein substrates. The aberrant activity of MMPs has been implicated in diseases including neuroinflammation, arthritis, cardiovascular disease, and cancer metastasis [12-15]. MMPs contain three histidine residues that coordinate one Zn(II) ion in a structural site and three histidine residues that coordinate one Zn(II) ion in the catalytic site [16, 17]. Owing the critical role of MMPs in development and disease progression, and the requirement for histidine residues in catalytic activity, MMP-2 has been used as the protein target for examining the mechanism of enzyme inactivation by a Co(III) Schiff base complex.

The objective of this study is to provide insight into the kinetics and thermodynamics of the irreversible inhibition of MMP-2 protease activity by the Co(III) Schiff base complex

Co(acacen) (Fig. 1). The kinetic studies of MMP-2 inactivation by Co(acacen) allowed the determination of the rate constants and dissociation constants of the irreversible inactivation of MMP-2. The rate of Co(acacen) binding to MMP-2 was determined to be temperature-dependent. Transition state analysis revealed that the Gibbs free energy of activation decreases as temperature increases. This trend is a result of the positive entropy from the binding reaction, which is likely a result of a decrease in protein structure at the Co(acacen) binding site. These data provide an insight into the mechanisms of the reaction of Co(acacen) for the inactivation of the histidine-containing enzyme MMP-2 to develop a new class of irreversible inhibitors of MMPs with a versatile targeting strategy.

Materials and methods

Materials

The purified human recombinant catalytic domain of MMP-2 was obtained from Enzo Life Sciences (Plymouth Meeting, PA, USA). The MMP-2 fluorescence resonance energy transfer (FRET) peptide substrate MCA-Pro-Leu-Gly~Leu-Dpa(Dnp)-Ala-Arg-NH₂, where MCA is (7-methoxycoumarin-4-yl)acetyl, Dpa is N-3-(2,4-dinitro-phenyl)-L-2,3-diaminopropionyl, and Dnp is dinitrophenyl, was purchased from Bachem (Torrance, CA, USA). Co(acacen) was synthesized as previously described [18]. Materials and solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Deionized water was obtained from a Millipore Q-Gard system equipped with a Quantum EX cartridge. Cobalt(II) acetate tetrahydrate was purchased from EM.

Co(acacen) stock solution preparation

Co(acacen) was dissolved in water and the Co(III) concentration was determined by inductively coupled plasma mass spectrometry using an XSERIES 2 inductively coupled plasma mass spectrometer (Thermo Fisher). Co(acacen) was further diluted in MMP-2 assay buffer—50 mM tris(hydroxymethyl)aminomethane, 150 mM NaCl, 10 mM CaCl₂, and 0.02 % NaN₃, pH 7.5—to achieve a set of Co(acacen) serial dilutions.

Irreversible inhibition of MMP-2 by Co(acacen)

MMP-2 (80 IM) was incubated with an equal volume of MMP-2 assay buffer or 0.8 mM Co(acacen) for 2, 6, or 21 h at 10, 20, or 37 °C. Remaining MMP-2 enzyme activity was determined in duplicate by diluting samples ten times in MMP-2 assay buffer with 1.5 IM MMP-2 FRET peptide substrate and incubating samples at 37 °C for 60 min. MCA fluorescence (recorded at 393 nm) from the cleaved FRET peptide from each condition was measured in duplicate in a 96-well plate using a BioTek Synergy 4 fluorescence microplate reader with MCA excitation at 325 nm using a xenon flash bulb. The percentage of remaining activity (A) for each inhibitor concentration at each minute was calculated relative to the activity of the untreated enzyme (A₀). The remainder of each sample was subjected to dilution and centrifugal filtration five times using a filter with a 3,000 molecular weight cutoff to remove any Co(acacen) that was not irreversibly bound to the protein (Table S1). The remaining MMP-2 enzyme activity of each sample of treated and untreated Co(acacen) was determined in duplicate.

Time- and concentration-dependent inactivation of MMP-2 by Co(acacen)

MMP-2 activity was determined using an MCA fluorophore with a Dnp quencher FRET peptide—MCA-Pro-Leu-Gly~Leu-Dpa(Dnp)-Ala-Arg-NH₂. MMP-2 was diluted in the MMP-2 assay buffer and preincubated with a range of concentrations of Co(acacen), from 1 to 32 IM, in MMP-2 assay buffer for 0, 10, 20, 30, 40, or 50 min. Time- and concentration-dependent inactivation of MMP-2 was determined at five temperatures (32.0, 33.5, 35.0,

36.5, and 38.0 °C) for analysis of the temperature dependence of inactivation. Each sample was diluted ten times in MMP-2 assay buffer to quench the reaction, and MMP-2 fluorimetric substrate FRET peptide was added to measure the remaining enzyme activity. The final concentration of MMP-2 was 1 nM and the Co(acacen) concentration ranged from 0.1 to 3.2 μ M. MMP-2 enzyme function was quenched after 60 min incubation at 37 °C using 10 nM EDTA with 0.002 % NaN₃. MCA fluorescence (recorded at 393 nm) from the cleaved FRET peptide from each condition was measured in duplicate in a 96-well plate using a BioTek Synergy 4 fluorescence microplate reader with MCA excitation at 325 nm using a xenon flash bulb.

The irreversible inhibition of MMP-2 by the irreversible inhibitor Co(acacen) can be modeled by the following equation [19]:

where E is enzyme and I is inhibitor.

In this model K_I is the dissociation constant in the first step for the formation of the reversible enzyme-inhibitor (EI) complex. Once the EI complex has formed, the first-order rate constant, k_i , is for the formation of an irreversible bond between the inhibitor and the enzyme resulting in EI^* . From the experimental MMP-2 activity determined by peptide proteolysis, the percentage of remaining activity (A) for each inhibitor concentration at each time point was calculated relative to the original enzyme activity (A_0). These values were converted to the natural log percentage and plotted against the total preincubation time (t) to determine the mechanism of inhibition. For irreversible inhibitors, a saturable pseudo-first-order rate constant (k_{obs}) is observed for MMP-2 inactivation from the linear portion of the plot of the natural log of the percentage of remaining MMP-2 enzyme activity and time. A double-reciprocal plot of k_{obs} and the concentration of inhibitor was generated, from which K_I , k_i , and the second-order rate constant were determined [19]. The positive y-intercept indicates that the inhibitor can reversibly saturate the enzyme prior to inactivation.

$$1/k_{obs} = 1/k_i + K_I/k_i [I] \quad (2)$$

As the concentrations of Co(acacen) inhibitor were typically well below K_I , the second-order rate constant, k , was derived from Eq. 3:

$$k = k_i/K_I. \quad (3)$$

Multisite binding analysis was performed by plotting the percentage of remaining enzyme activity versus the concentration of Co(acacen). Linearity of this plot suggests single-site modification of the enzyme, whereas a curved dependence implies that inhibition occurs at multiple sites, or that the inhibitor is depleted, as some inhibitors are also substrates [20].

Temperature-dependent inactivation of MMP-2 by Co(acacen)

The activation energy for Co(acacen) binding to MMP-2 is described by the Arrhenius equation (Eq. 4):

$$\ln k = -E_a/RT + \ln A. \quad (4)$$

A plot of $\ln k$, the second order rate constant for the inactivation of MMP-2 by Co(acacen), against T^{-1} (K^{-1}) gave a straight line; the activation energy (E_a) is calculated from the slope of the line [21]. Transition state analysis was performed using the Eyring plot. The enthalpy and entropy of inactivation ($\Delta H^\ddagger$ and $\Delta S^\ddagger$, respectively) were determined from Eq. 5:

$$\ln(k/T) - \ln(k_B/h) = -\Delta H^\ddagger/RT + \Delta S^\ddagger/R, \quad (5)$$

where k_B is the Boltzmann constant, h is the Planck constant, and R is the gas constant [22].

The Gibbs free energy of activation ($\Delta G^\ddagger$) can be calculated for individual temperatures from Eq. 6:

$$\Delta G^\ddagger = -RT [\ln(hk/k_B T)]. \quad (6)$$

Results

Co(acacen) irreversibly inhibits MMP-2 activity

Evidence for the formation of the irreversibly bound EI complex was obtained from a dilution and filtration assay. Tight binding inhibitors often display many of the characteristics of irreversible inhibitors; however, they do not chemically modify the enzyme and extended dialysis or dilution and filtration are often used to differentiate between tight binding inhibitors and irreversible inhibitors. These methods cause tight binding inhibitors to dissociate, resulting in the return of the activity of the enzyme to that of the untreated enzyme. After a preincubation of MMP-2 with Co(acacen) for 2, 6, or 21 h at 10, 20, or 37 °C, MMP-2 enzyme activity was reduced compared with the activity of untreated control MMP-2 (Fig. S1). Subsequently, the EI complex was diluted and washed five times in cold MMP-2 assay buffer using a centrifugation filter to remove any unbound Co(acacen) (Fig. S1). For a reversible binding mechanism, the dilution and subsequent washing would remove any reversibly bound inhibitor (Table S1) and no changes in MMP-2 activity would be expected relative to the untreated control MMP-2 sample.

The inhibition of MMP-2 by Co(acacen) is both time- and temperature-dependent (Figs. 2, S1). After 2 h of incubation followed by dilution and washing, 42.0 ± 2.7 , 26.0 ± 4.5 , and 18.3 ± 5.8 % of MMP-2 activity remained after incubation at 10.0, 20.0, and 37.0 °C, respectively (Fig. 2), demonstrating that inhibition is temperature-dependent. These data further validate that MMP-2 is irreversibly inhibited by Co(acacen) even at 10.0 °C, although to a much lesser extent than at 37.0 °C. The inhibition increases with time, as 28.1 ± 0.2 % of MMP-2 activity remains after incubation with Co(acacen) at 10.0 °C, which is reduced to 1.9 ± 1.3 % at 20.0 °C, and enzyme activity becomes undetectable at 37.0 °C. These data indicate that an irreversible EI complex of MMP-2 and Co(acacen) forms during the preincubation period.

Multisite binding analysis

The inactivation of MMP-2 protease activity has been used to study the rate of irreversible Co(acacen) binding to a protein. MMP-2 contains histidine residues that are critical for maintaining protein structure and enzyme activity. A Zn(II) ion coordinated by three histidine ions is required for structural stability. Additionally, the catalytic active site of MMP-2 contains three histidine residues that coordinate one Zn(II) which is required for enzyme activity [23, 24]. Since there are three catalytic histidine residues in the active site of MMP-2, Co(acacen) can bind to at least one of three histidine residues and inhibit enzyme activity, but has the ability to bind at both the structural and the catalytic Zn(II)-containing

sites. Analysis of the plot of the percentage of remaining activity versus inhibitor concentration shows multiple site inhibition of MMP-2 by Co(acacen) as evidenced by the nonlinearity of the line, or depletion of Co(acacen) during the reaction (Fig. 3).

Kinetics of MMP-2 inactivation by Co(acacen)

Inhibition of MMP-2 by Co(acacen) conforms to the criteria established for irreversible inhibition. Time- and concentration-dependent loss of activity of MMP-2, which are hallmarks of an irreversible chemical reaction between Co(acacen) and the protease, are observed (Figs. 4, 5). The semilog plots of the percentage of remaining activity versus time demonstrate that the kinetics of inactivation display pseudo-first-order reaction rates between 32 and 38 °C (Figs. 4, 5), with clear saturation kinetics at the highest concentrations of Co(acacen) at 36.5 and 38.0 °C (Fig. 5). This plateau indicates the formation of an EI complex during the inactivation process, as seen for substrate binding in Michaelis-Menten enzyme kinetics. The linear portions of these semilog plots were used to determine the pseudo-first-order rate constants (k_{obs}) (Tables S2, S3, S4, S5, S6).

The double-reciprocal plots of the pseudo-first-order rate constants (k_{obs}) and inhibitor concentration are linear with positive y-intercepts at all temperatures examined (Figs. 6, 7). The positive y-intercept further indicates the formation of a reversible EI complex that is saturated before the irreversible reaction occurs. From the double-reciprocal plots, K_I and k_i were calculated (Tables S7, S8). Both the dissociation constant and the inactivation rate constant showed dependence on temperature. As the temperature increases from 32.0 to 38.0 °C, the dissociation constant K_I decreases from 13.2 to 4.0 μM . The rate constant of inactivation k_i increases with increasing temperature from $8.9 \times 10^{-4} \text{ s}^{-1}$ at 32.0 °C to $1.7 \times 10^{-3} \text{ s}^{-1}$ at 38.0 °C. From these data, the second-order rate constant for inactivation (k_i/K_I) was calculated for each temperature studied. The second-order rate constants (k) for 32.0 and 38.0 °C are 67.6 and 435.3 $\text{M}^{-1} \text{ s}^{-1}$ respectively.

Transition state analysis of Co(acacen) inactivation of MMP-2

The temperature dependence for the inactivation of MMP-2 by Co(acacen) was studied over a temperature range from 32.0 to 38.0 °C. From the linear plot of $\ln k$ against T^{-1} the activation energy, E_a , of the reaction was determined to be $241.3 \pm 30.2 \text{ kJ mol}^{-1}$ from the Arrhenius equation (Fig. 8, Table S9). The linearity of the Arrhenius plot in Fig. 8 indicates that inactivation by Co(acacen) involves the same rate-limiting step over the temperature range studied. From the Eyring plots in Fig. 9, the enthalpy of activation ($\Delta H^\ddagger$) and the entropy of activation ($\Delta S^\ddagger$) for the inactivation of MMP-2 by Co(acacen) were determined to be $238.7 \pm 30.2 \text{ kJ mol}^{-1}$ and $570.1 \pm 97.9 \text{ J mol}^{-1} \text{ K}^{-1}$, respectively (Table S9). The positive entropy of activation indicates increased disorder in the protein active site, which is consistent with Co(acacen) deforming the protein structure upon binding [5]. Additionally, the ligand-exchange mechanism, where the dissociation of the labile axial ligands during protein binding creates more products than reactants, could be a contributing factor to the positive entropy of activation.

The Gibbs free energy of activation ($\Delta G^\ddagger$) at physiological temperature (37 °C) was calculated to be $61.9 \pm 30.2 \text{ kJ mol}^{-1}$. Although the entropy of activation is relatively small, the entropy contribution, $-T\Delta S^\ddagger$, increases at higher temperatures, rendering the reaction more favorable at higher temperatures (Table S8). The enthalpy of activation ($\Delta H^\ddagger$) has a positive contribution, possibly due to the energy requirement for ligand dissociation of Co(acacen) (Fig. 9).

Discussion

Through this work examining the mechanism of Co(acacen) enzyme inhibition, we have found that MMP-2 inhibition by Co(acacen) exhibits slow binding kinetics consistent with irreversible inhibition. Additionally, we have shown that Co(acacen) inhibition is temperature-dependent, revealing features of Co(III) Schiff base complexes that render them promising inhibitors of MMPs.

MMP-2 contains three histidine residues that coordinate Zn(II) in the enzyme active site, and the same histidine coordination of Zn(II) is present in a site required for structural support. Multisite binding analysis of Co(acacen) with MMP-2 demonstrates that Co(acacen) can bind to multiple sites or that the inhibitor is consumed during the inhibition reaction. Since the concentration of inhibitor is in far excess of that of MMP-2, it is likely that Co(acacen) is able to bind to multiple histidine residues in both the structural and the catalytic Zn(II) sites. Since Co(acacen) is not targeted to the catalytic site or the structural site, this is a likely possibility. Targeting of Co(acacen) derivatives to the active sites of proteases has been accomplished by the conjugation of a short peptide containing the protease cleavage site [4, 9]. This approach results in active-site targeting and increases the efficacy of inhibition as 100-fold less targeted complex is required to achieve the same degree of inhibition as with the untargeted complex. Similar Co(III) Schiff base complexes could be readily applied to targeting MMP family members [9].

A significant focus in the development of MMP inhibitors is to utilize zinc binding groups that bind to the Zn(II) in the active site to block MMP protease activity. This approach has been effective in animal models, ultimately resulting in many clinical trials [25, 26]. The enzyme active site is most similar among MMPs, rendering it difficult to selectively target individual MMPs, and as a result many of the MMP inhibitors in clinical trials broadly inhibit several MMPs [26]. As these challenges persist in clinical trials, alternative strategies for targeting and inhibition have been employed, including peptidomimetics that target the binding pocket, deforming the protein structure, and a mechanism-based inhibitor [27-29]. Inactivation of MMP-2 by Co(acacen) demonstrates that Co(acacen) is an effective and irreversible inhibitor. The ability to use Co(III) Schiff base complexes targeted to the histidine residues in MMPs presents a class of inhibitor with alternative strategies for inhibition. Co(acacen) has been shown to deform the secondary structure of the protein [5], which is further supported in this study. Therefore, targeting the structural site might prove to be an alternative to targeting the active site, which could be effective owing to the ability of Co(acacen) to deform the protein structure.

Inactivation of MMP-2 by Co(acacen) is dependent on concentration, time, and temperature. The effectiveness of an inhibitor is measured by k_i/K_i . The effectiveness of inhibition of MMP-2 by Co(acacen) increases with temperature as k_i/K_i was determined to be $67.6 \text{ M}^{-1} \text{ s}^{-1}$ at 32.0°C and $435.3 \text{ M}^{-1} \text{ s}^{-1}$ at 38.0°C . Therefore, Co(acacen) is more than six times more effective in inhibiting MMP-2 activity at 38.0°C than at 32.0°C . The mechanism of this dramatic temperature dependence was further investigated by transition state analysis.

On the basis of the transition state analysis of Co(acacen) binding to MMP-2, the Gibbs free energy of activation decreases with increasing temperature owing to the favorable entropy of the transition state over the reactants. This result is consistent with previous data that proposed the deformation of the protein structure upon Co(acacen) binding [2, 5]. Additionally, the positive entropy could be due to the ligand-exchange mechanism, where the release of axial ligands when Co(acacen) binds MMP-2 results in more products than reactants. The positive enthalpy of activation is postulated to be due to the energy

requirement for the release of the first axial ligand to generate an open binding site for histidine.

With this expanded knowledge of the mechanism of protein inhibition by Co(III) Schiff base complexes, we can better design inhibitors of proteins, including MMPs, by taking advantage of all of the properties of these inhibitors. Most significantly, we have determined the strong temperature dependence of inhibition by Co(III) Schiff base complexes. Temperature-dependent inhibition of zinc finger transcription factors by Co(III) Schiff base complexes has been demonstrated in vivo [7]. In *Xenopus laevis*, a targeted Co(III) Schiff base complex has been used to inhibit cell migration during embryonic development by inhibiting Snail family transcription factors. Inhibition of cell migration was seen when embryos were reared at 28 °C, whereas no effect was observed in embryos reared at 14 °C [7], lending support to the temperature-dependent inhibition of histidine-containing proteins by Co(III) Schiff base complexes. Thus, temperature may be used to modulate the inhibitory activity or the timing of Co(acacen) inhibition in model organisms and may have clinical applications with the advancing use of hyperthermia therapies [30]. Temperature regulation presents an additional method for selective targeting and regulation of Co(III) Schiff base complexes in addition to the use of targeting moieties and variable axial ligands that have been previously described [2, 4, 6, 7, 9]. Our examination of the mechanism of Co(III) Schiff base complexes as irreversible protein inhibitors underscores the remarkable reactivity and versatility of Co(III) Schiff base complexes and their derivatives as protein inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Bruijninx PC, Sadler PJ. New trends for metal complexes with anticancer activity. *Curr Opin Chem Biol.* 2008; 12(2):197–206. [PubMed: 18155674]
2. Louie AY, Meade TJ. A cobalt complex that selectively disrupts the structure and function of zinc fingers. *Proc Natl Acad Sci USA.* 1998; 95(12):6663–6668. [PubMed: 9618469]
3. Boettcher A, Takeuchi T, Hardcastle K, Meade TJ, Gray HB. Spectroscopy and electrochemistry of Cobalt(III) Schiff base complexes. *Inorg Chem.* 1997; 36:2498–2504.
4. Takeuchi T, Boettcher A, Quezada CM, Meade TJ, Gray HB. Inhibition of thermolysin and human alpha-thrombin by cobalt(III) Schiff base complexes. *Bioorg Med Chem.* 1999; 7(5):815–819. [PubMed: 10400334]
5. Blum O, Haiek A, Cwikel D, Dori Z, Meade TJ, Gray HB. Isolation of a myoglobin molten globule by selective Cobalt(III)-induced unfolding. *Proc Natl Acad Sci USA.* 1998; 95(12):6659–6662. [PubMed: 9618468]
6. Harney AS, Lee J, Manus LM, Wang P, Ballweg DM, LaBonne C, Meade TJ. Targeted inhibition of Snail family zinc finger transcription factors by oligonucleotide-Co(III) Schiff base conjugate. *Proc Nat Acad Sci USA.* 2009; 106(33):13667–13672. [PubMed: 19666616]
7. Harney AS, Meade TJ, Labonne C. Targeted inactivation of Snail family EMT regulatory factors by a Co(III)-Ebox conjugate. *PLoS ONE.* 2012; 7(2):e32318. [PubMed: 22393397]

8. Hurtado RR, Harney AS, Heffern MC, Holbrook RJ, Holmgren RA, Meade TJ. Specific inhibition of the transcription factor Ci by a cobalt(III) Schiff base-DNA conjugate. *Mol Pharm.* 2012; 9(2): 325–333. [PubMed: 22214326]
9. Takeuchi T, Boettcher A, Quezada CM, Simon MI, Meade TJ, Gray HB. Selective inhibition of human α -thrombin by Cobalt(III) Schiff base complexes. *J Am Chem Soc.* 1998; 120(33):8555–8556.
10. Ortega N, Wang K, Ferrara N, Werb Z, Vu TH. Complementary interplay between matrix metalloproteinase-9, vascular endothelial growth factor and osteoclast function drives endochondral bone formation. *Dis Model Mech.* 2010; 3(3-4):224–235. [PubMed: 20142327]
11. Kiran MS, Viji RI, Kumar SV, Prabhakaran AA, Sudhakaran PR. Changes in expression of VE-cadherin and MMPs in endothelial cells: Implications for angiogenesis. *Vasc Cell.* 2011; 3(1):6. [PubMed: 21349163]
12. Morancho A, Rosell A, Garcia-Bonilla L, Montaner J. Metalloproteinase and stroke infarct size: role for anti-inflammatory treatment? *Ann N Y Acad Sci.* 2010; 1207:123–133. [PubMed: 20955435]
13. Hansson J, Vasan RS, Arnlov J, Ingelsson E, Lind L, Larsson A, Michaelsson K, Sundstrom J. Biomarkers of extracellular matrix metabolism (MMP-9 and TIMP-1) and risk of stroke, myocardial infarction, and cause-specific mortality: cohort study. *PLoS ONE.* 2011; 6(1):e16185. [PubMed: 21283828]
14. Wernicke D, Seyfert C, Gromnica-Ihle E, Stiehl P. The expression of collagenase 3 (MMP-13) mRNA in the synovial tissue is associated with histopathologic type II synovitis in rheumatoid arthritis. *Autoimmunity.* 2006; 39(4):307–313. [PubMed: 16891219]
15. Jezierska A, Motyl T. Matrix metalloproteinase-2 involvement in breast cancer progression: a mini-review. *Med Sci Monit.* 2009; 15(2):RA32–40. [PubMed: 19182722]
16. Diaz N, Suarez D. Molecular dynamics simulations of matrix metalloproteinase 2: role of the structural metal ions. *Biochemistry.* 2007; 46(31):8943–8952. [PubMed: 17616173]
17. Kleinfeld O, Kotra LP, Gervasi DC, Brown S, Bernardo MM, Fridman R, Mobashery S, Sagi I. X-ray absorption studies of human matrix metalloproteinase-2 (MMP-2) bound to a highly selective mechanism-based inhibitor. *J Biol Chem.* 2001; 276(20):17125–17131. [PubMed: 11278946]
18. Costa G, Mestroni G, Tauzher G, Stefani L. Organometallic derivatives of cobalt chelates of bis(acetylacetone) ethylenediamine. *J Organomet Chem.* 1966; 6(2):181–187.
19. Kitz R, Wilson IB. Esters of methanesulfonic acid as irreversible inhibitors of acetylcholinesterase. *J Biol Chem.* 1962; 237:3245–3249. [PubMed: 14033211]
20. Segel, IH. *Enzyme kinetics: behavior and analysis of rapid equilibrium and steady-state enzyme systems.* Wiley; New York: 1975.
21. Copeland RA. Evaluation of enzyme inhibitors in drug discovery. A guide for medicinal chemists and pharmacologists. *Methods Biochem Anal.* 2005; 46:1–265. [PubMed: 16350889]
22. Laidler, KJ.; Meiser, JH. *Physical chemistry.* Benjamin/ Cummings; Menlo Park: 1982.
23. Bode W, Gomis-Rueth F-X, Stoeckler W. Astacins, serralyins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the ‘metzincins’. *FEBS Lett.* 1993; 331(1-2):134–140. [PubMed: 8405391]
24. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res.* 2003; 92(8):827–839. [PubMed: 12730128]
25. Jacobsen JA, Major Jourden JL, Miller MT, Cohen SM. To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. *Biochim Biophys Acta Mol Cell Res.* 2010; 1803(1):72–94.
26. Overall CM, Kleinfeld O. Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat Rev Cancer.* 2006; 6(3):227–239. [PubMed: 16498445]
27. Brown S, Bernardo MM, Li Z-H, Kotra LP, Tanaka Y, Fridman R, Mobashery S. Potent and selective mechanism-based inhibition of gelatinases. *J Am Chem Soc.* 2000; 122(28):6799–6800.
28. Ikejiri M, Bernardo MM, Bonfil RD, Toth M, Chang M, Fridman R, Mobashery S. Potent mechanism-based inhibitors for matrix metalloproteinases. *J Biol Chem.* 2005; 280(40):33992–34002. [PubMed: 16046398]

29. Johnson AR, Pavlovsky AG, Ortwine DF, Prior F, Man C-F, Bornemeier DA, Banotai CA, Mueller WT, McConnell P, Yan C, et al. Discovery and characterization of a novel inhibitor of matrix metalloprotease-13 that reduces cartilage damage in vivo without joint fibroplasia side effects. *J Biol Chem.* 2007; 282(38):27781–27791. [PubMed: 17623656]
30. Hurwitz MD. Today's thermal therapy: not your father's hyperthermia: challenges and opportunities in application of hyperthermia for the 21st century cancer patient. *Am J Clin Oncol.* 2010; 33(1):96–100. [PubMed: 19636240]

Fig. 1.
Structure of the Co(III) Schiff base complex with ammine axial ligands, [Co(acacen)]⁺

Fig. 2. Time- and temperature-dependent irreversible inhibition of matrix metalloproteinase 2 (MMP-2) activity by Co(acacen). Remaining MMP-2 activity after incubation with Co(acacen) for 2, 6, or 21 h at 10.0, 20.0, or 37.0 °C. MMP-2 activity was determined after removal of Co(acacen) using centrifugation filters in MMP-2 assay buffer at pH 7.5. The data are presented as means of duplicate samples $\pm$ the standard error of the mean (SEM)

Fig. 3.

Analysis for multisite interaction of MMP-2 with Co(acacen) by plotting MMP-2 activity remaining after 20 min of preincubation with Co(acacen) from 1 to 32 μM at 35.0 ± 0.1 °C in MMP-2 assay buffer at pH 7.5. The data are presented as means of duplicate samples $\pm$ SEM

Fig. 4.

Time- and concentration-dependent decline in MMP-2 activity in the presence of Co(acacen) at a 32.0 ± 0.1 °C and b 33.5 ± 0.1 °C. MMP-2 (10 nM) was preincubated with Co(acacen) (1-32 μ M) for 0-50 min at pH 7.5 in MMP-2 assay buffer. The data are presented as means of duplicate samples $\pm$ SEM

Fig. 5.

Time- and concentration-dependent decline in MMP-2 activity in the presence of Co(acacen) at a 35.0 ± 0.1 °C, MMP-2 assay buffer. The data are presented as means of duplicate b 36.5 ± 0.1 °C, preincubated with Co(acacen) (1-32 μ M) for 0-50 min at pH 7.5 in and c 38.0 ± 0.1 °C. MMP-2 (10 nM) was samples $\pm$ SEM

Fig. 6.

The dependence of k_{obs} on Co(acacen) inhibitor concentration. Double-reciprocal plots of the pseudo-first-order rate constants of inactivation of MMP-2 at a 32.0 °C and b 33.5 °C. From these plots, K_I and k_i were calculated. The data are presented as means of duplicate samples $\pm \text{SEM}$

Fig. 7.

The dependence of k_{obs} on Co(acacen) inhibitor concentration. these plots, K_I and k_i were calculated. The data are presented as means Double-reciprocal plots of the pseudo-first-order rate constants of of duplicate samples $\pm$ SEM inactivation of MMP-2 at a 35.0 °C, b 36.5 °C, and c 38.0 °C. From these plots, K_I and k_i were calculated. The data are presented as means of duplicate samples $\pm$ SEM

Fig. 8.

Arrhenius plot for the dependence of second-order rate constant, k , on temperature. The second-order rate constants were determined from MMP-2 (10 nM) inactivation by Co(acacen) at 32.0, 33.5, 35.0, 36.5, and 38.0 ± 0.1 °C in assay buffer. The activation energy, E_a , for the reaction of Co(acacen) with MMP-2 was determined from the slope of the line. The data are presented as means of duplicate samples $\pm$ SEM

Fig. 9.

Eyring plot for the dependence of the second-order rate constant, k , on temperature. The second-order rate constants were determined from MMP-2 (10 nM) inactivation by Co(acacen) at 32.0, 33.5, 35.0, 36.5, and 38.0 ± 0.1 °C in assay buffer. The enthalpy and entropy of activation ($\Delta H^\ddagger$ and $\Delta S^\ddagger$) were determined from the slope and the y-intercept according to Eq. 5