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Mechanistic insight into hydroxamate transfer reaction mimicking the inhibition of zinc-containing enzymes†

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A hydroxamate transfer reaction between metal complexes has been investigated by a combination of experimental and theoretical studies. A hydroxamate-bound cobalt(II) complex bearing a tetradentate macrocyclic ligand, $[\text{Co}^{\text{II}}(\text{TBDAP})(\text{CH}_3\text{C}(\text{=NO})\text{O})]^+$ (**1**), is prepared by the reduction of a hydroximatocobalt(III) complex with a biological reductant. Alternatively, **1** is accessible via a synthetic route for the reaction between the cobalt(II) complex and acetohydroxamic acid in the presence of a base. **1** was isolated and characterized by various physicochemical methods, including UV-vis, IR, ESI-MS, and X-ray crystallography. The hydroxamate transfer reactivity of **1** was examined with a zinc complex, which was followed by UV-vis and ESI-MS. Kinetic and activation parameter data suggest that the hydroxamate transfer reaction occurs via a bimolecular mechanism, which is also supported by DFT calculations. Moreover, **1** is able to inhibit the activity against a zinc enzyme, *i.e.*, matrix metalloproteinase-9. Our overall investigations of the hydroxamate transfer using the synthetic model system provide considerable insight into the final step involved in the inhibition of zinc-containing enzymes.

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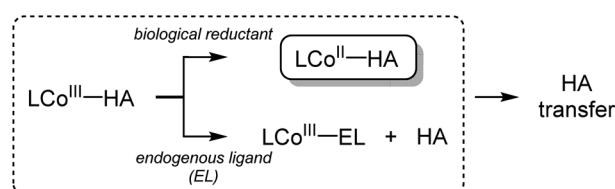
Introduction

Hydroxamic acids are important pharmacophores in diverse biological functions such as antimicrobial activity and metal detoxification.^{1–5} The derivatives of hydroxamic acids are well known as zinc-binding groups; thus, they can inhibit the activity of zinc-containing enzymes, including carbonic anhydrase, histone deacetylase and carboxypeptidase.^{6–11} So far, hydroxamic acids have also been considered as highly promising anti-cancer reagents: they can serve as inhibitors of matrix metalloproteinases (MMPs) that are overexpressed in cancer cells and cause tumor invasion and metastasis.^{12–17} The use of hydroxamate functional groups as traditional anticancer reagents, however, is rather restricted within tumor cells because the environment disrupts drug supply.^{18–21}

Recently, numerous hydroxamate-bound metal complexes have been explored as prodrugs targeting cancer cells. For example, cobalt(III) complexes of hydroxamic acids are reported

to be potential prodrugs for hypoxia-selective anticancer agents.^{22–25} As shown in Scheme 1, Hambley and co-workers have provided experimental support for two possible pathways of releasing the hydroxamate group (*i.e.*, bio-reduction and endogenous ligand exchange pathways).^{22,26} Recent studies on the characterization and reactivity of cobalt(III)-hydroxamate and -hydroxamato complexes bearing TPA ligands revealed their redox behaviors and ligand exchange reactions.^{27,28} Little is known about the molecular-level mechanism of the hydroxamate transfer, which is the final step of inhibiting the activity of zinc-containing enzymes by hydroxamate, however.

Herein, we report a novel approach to investigate the hydroxamate transfer activity of a hydroxamatocobalt(II) complex, $[\text{Co}^{\text{II}}(\text{TBDAP})(\text{CH}_3\text{C}(\text{=NO})\text{O})]^+$ (**1**; TBDAP = *N,N*-di-*tert*-butyl-2,11-diaza[3.3](2,6)-pyridinophane), which is derived from the reduction of $[\text{Co}^{\text{III}}(\text{TBDAP})(\text{CH}_3\text{C}(\text{=NO})\text{O})]^+$ (**2**).²⁹ **1** was characterized by X-ray crystallography and multiple spectroscopic methods. To the best of our knowledge, **1** represents



Scheme 1 Proposed pathways for the release of hydroxamate (HA).

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a rare example of a structurally characterized cobalt(II) complex bearing an acetohydroxamate ligand that has been prepared by the reaction of a hydroximatocobalt(III) complex with a biological reductant. In this work, we have examined the mechanism of the hydroxamate transfer from **1** towards a zinc complex which is a model of zinc-containing active sites in enzymes.³⁰ Kinetic studies and density functional theory (DFT) calculations support the notion that the hydroxamate transfer occurs through a bimolecular mechanism. Moreover, **1** performs the better inhibitory activity against matrix metalloproteinase-9 (MMP-9), compared to **2**.

Results and discussion

The hydroximatocobalt(II) complex, **1**, was synthesized by reacting 1 equiv. of acetohydroxamic acid in CH₃CN with a starting Co^{II} complex, [Co^{II}(TBDAP)(NO₃)(H₂O)]⁺, in the presence of 2 equiv. of triethylamine (TEA) under ambient conditions, where the solution color changed from pink to orange. The UV-vis spectrum of **1** in CH₃CN at 25 °C revealed two characteristic absorption bands at $\lambda_{\text{max}} = 361$ ($\epsilon = 1900 \text{ M}^{-1} \text{ cm}^{-1}$) and 468 nm ($\epsilon = 100 \text{ M}^{-1} \text{ cm}^{-1}$). The electrospray ionization mass spectrometry (ESI-MS) spectrum of **1** showed a single signal at mass-to-charge (m/z) ratio of 485.3 (calcd m/z 485.2); the mass and isotope distribution pattern correspond to [Co(TBDAP)(CH₃C(-NHO)O)]⁺ (Fig. S1†). The FT-IR spectrum of **1** revealed the existence of N-H vibrational frequency at 3206 cm⁻¹, which also corroborates the hypothesis that acetohydroxamic acid is bound in the form of singly deprotonated monoanionic hydroxamate rather than doubly deprotonated dianionic hydroxamate (Fig. S2†).³¹ The effective magnetic moment of **1** ($\mu_{\text{eff}} = 4.41 \text{ B.M.}$) was determined using the ¹H NMR spectroscopy method of Evans in CD₃CN at 25 °C,³² suggesting the high spin state ($S = 3/2$) of the Co^{II} ion (see ESI†). **1** has a slightly higher effective magnetic moment due to spin-orbit coupling.³³ Thus, **1** is characterized as the cobalt(II) complex with the singly deprotonated hydroxamate ligand.

The X-ray crystal structure of **1** reveals a mononuclear acetohydroxamate cobalt complex in a distorted octahedral

geometry, in which the hydroxamate group coordinated in a bidentate mode (Fig. 1). The average Co–O bond length (2.034 Å) in **1** is similar to that of the hydroximatocobalt(II) complex with 6-(Me₂Ph)₂TPA ligand (2.038 Å)³⁴ but is longer than that in **2** (1.856 Å) and other hydroximatocobalt(III) complexes.^{24,25,27–29,35} **1** is the rare example of a structurally characterized hydroximatocobalt(II) complex, which would be a reactive species towards inhibition of metalloenzymes.

It has been proposed that the hydroximatocobalt(II) species is a key intermediate in the inhibition against MMP.^{22,23} We investigated the intermolecular transfer of the hydroxamate group from **1** to a zinc complex, [Zn^{II}(Me₃-TACN)(NO₃)]⁺ (**3**), which is a model of the active site of MMP (Scheme S1†).³⁰ Upon addition of **3** to **1**, the characteristic absorption band of **1** disappeared (Fig. 2a). The hydroxamate transfer from **1** to **3** was confirmed by ESI-MS analysis in the course of the reaction, where the mass peak at m/z 485.3 corresponding to **1** vanished with a concomitant appearance of the mass peak at m/z 309.2 corresponding to [Zn^{II}(Me₃-TACN)(CH₃C(-NHO)O)]⁺ (**4**) (Fig. 2b). Many attempts to isolate the product as single crystals have been unsuccessful. The structural information was obtained from an alternative synthetic route: the complex **4** was crystallized from the solution of the reaction mixture of **3** and excess acetohydroxamic acid in the presence of TEA (see ESI and Fig. S3†). Although the equilibrium constant ($K_{\text{eq}} = 5.9 \times 10^{-2}$) of the transfer reaction determined by optical titrations is small (Fig. S4†), the reaction readily occurs upon the addition of an excess amount of **3**.

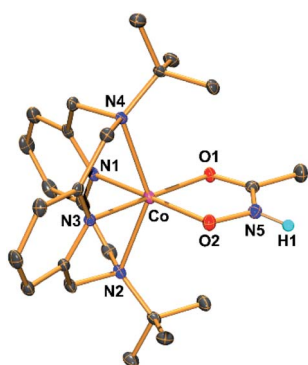


Fig. 1 ORTEP diagram of the hydroximatocobalt(II) complex, [Co^{II}(TBDAP)(CH₃C(-NHO)O)]⁺ (**1**), with thermal ellipsoids drawn at the 30% probability level. All hydrogen atoms except H1 are omitted for clarity. H1 was found in the Fourier difference map.

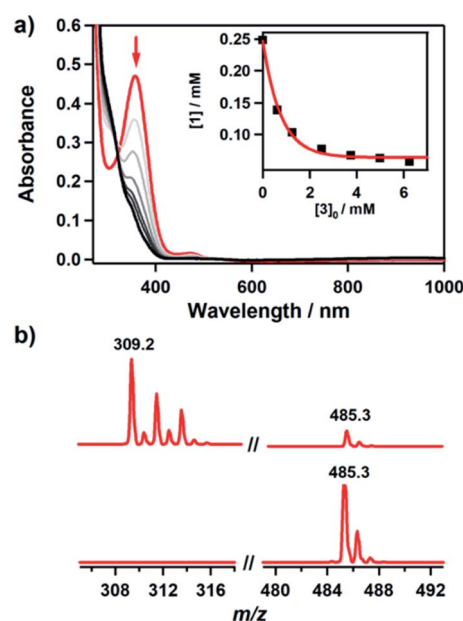


Fig. 2 Reaction of **1** with **3**. (a) UV-vis spectral change during the reaction of **1** (0.25 mM) with **3** (10 mM) in CH₃CN/H₂O (99 : 1) at -10 °C. Inset shows the decrease of concentration of **1** with increasing concentration of **3**. (b) ESI-MS spectra obtained for the reaction of **1** (0.25 mM) with **3** (25 mM) before (lower) and after (upper) the reaction. The peaks at m/z 309.2 and 485.3 are assigned to [Zn^{II}(Me₃-TACN)(CH₃C(-NHO)O)]⁺ (calcd m/z 309.1) and [Co^{II}(TBDAP)(CH₃C(-NHO)O)]⁺ (calcd m/z 485.2), respectively.



Kinetic studies of the hydroxamate group transfer reaction from **1** to **3** were carried out in a mixture of CH₃CN and H₂O (99 : 1). Upon addition of **3** (2.5 mM) to the solution of **1** (0.25 mM) at −10 °C, the characteristic absorption bands of **1** decayed (Fig. 2). The rate constant increased with the concentration of **3**, providing a second-order rate constant (k_2) of 4.0(2) M^{−1} s^{−1} (Fig. 3a). The temperature dependence of the k_2 value was investigated in the range of 253–283 K, from which a linear Eyring plot was obtained with activation parameters of $\Delta H^\ddagger = 59(3)$ kJ mol^{−1} and $\Delta S^\ddagger = -5(1)$ J mol^{−1} K^{−1} (Fig. 3b). The observed data suggest that the hydroxamate transfer reaction occurs through a bimolecular mechanism, in which the formation of an undetected [(TBDAP)Co-(CH₃C(-NHO)O)-Zn(Me₃-TACN)]³⁺ species is the rate-determining step.

DFT calculations were performed for the hydroxamate transfer reaction (see ESI†). According to the DFT results (Fig. 4), the oxygen atom in the NHO[−] moiety of hydroxamate in the Co(II) complex is first coordinated by the Zn(II) complex at **Int1**. Subsequently, the carbonyl oxygen of hydroxamate is transferred from the Co(II) center to the Zn(II) center *via* a transition state (**TS1**) to form a product complex (**Int3**). The free energy profile also suggests that the reaction is not a very favorable process thermodynamically, which is reasonably consistent with the small equilibrium constant (*vide supra*).

On the other hand, upon addition of **3** to a solution of **2** (0.5 mM), **2** remained intact without showing any absorption spectral change (Fig. S5a†), indicating that **2** is not able to conduct the hydroxamate group transfer reaction. The ESI-MS spectrum of the reaction solutions confirmed that no transferred product was formed (Fig. S5b†). Notably, the transfer reaction occurs by

adding a biological reductant such as ascorbic acid, which is a 2H⁺/2e[−] donor. Addition of 0.5 equiv. of ascorbic acid to a reaction mixture of **2** and **3** resulted in the conversion of **2** to **1**, and then the hydroxamate in **1** was transferred to **3** (Fig. S6a†). After the reaction had been completed, **4** was produced, which was confirmed by ESI-MS (Fig. S6b†).

The cyclic voltammograms of **1** and **2** in CH₃CN exhibit a reversible couple between the Co(II) and Co(III) complexes (Fig. S7†). From the $E_{1/2}$ values, the one-electron redox potentials of **1** and **2** were determined to be 0.28 and −0.47 V (*versus* SCE), respectively. The redox potential of **2** is more negative than that of ascorbic acid.³⁶ In earlier studies, however, the protonation of hydroximatocobalt(III) complexes resulted in more positive potential affording facile reduction.²⁷ The proton-assisted reduction process was confirmed by the cyclic voltammetry (CV) experiments, where the redox signal of **2** disappeared with the concomitant generation of the redox signal of **1** upon addition of proton (Fig. S8†). Alternatively, the hydroxamatocobalt(III) complex, [Co^{III}(TBDAP)(CH₃C(-NHO)O)]²⁺ (**5**), which is not only a protonated form of **2** but also an one electron oxidized species of **1**, was prepared by the reaction of **2** with 1 equiv. of HClO₄ (Fig. S11†). The formation of **5** was confirmed by UV-vis and ESI-MS (Fig. S12†). **2** and **5** are interconvertible through the acid–base chemistry.

In order to verify the influence of **1**, relative to acetohydroxamic acid or **2**, on a zinc-containing enzyme, their inhibitory activity against MMP-9 was evaluated. After incubation of activated MMP-9 with a peptide as a substrate in the presence of **1**, **2**, or acetohydroxamic acid, the amount of the substrate that was not cleaved by the enzyme was analyzed. Inhibition against MMP-9 by **1** and **2**, relative to that by acetohydroxamic acid, was 77(2)% and 24(3)%, respectively. The more noticeable inhibitory activity of **1** than **2** against MMP-9 was expected from our reactivity and mechanistic studies (*vide supra*).

To visualize possible interactions between **1** and MMP-9 at the molecular level, docking studies were carried out employing the catalytic domain of MMP-9 (PDB 4H3X).³⁷ As illustrated in Fig. 5, **1** could access to the catalytic cleft of MMP-9 where three histidine residues (*i.e.*, H226, H230, and H236) are coordinated to the active Zn(II) center. According to the docked structures, **1** may have multiple contacts with the catalytic domain of MMP-9: (i) hydrogen bonding {[C–H from **1** and oxygen (O) donor atoms from A189, A191, E227, and D235; a nitrogen (N) donor atom from H236] and [C–H from H236 and an O donor atom from **1**]} and (ii) C–H⋯ π interaction (C–H from the side chain of L187 and H236 and pyridine groups from **1**). In addition, the representative conformations exhibited the possibility of the hydroxamate moiety onto **1** to be located close to the Zn(II) center in the catalytic domain of MMP-9. Collectively, **1** may interact with the catalytic site of MMP-9.

To explore how the substituent onto the ligand affects the hydroxamate transfer reactivity towards **3** and the inhibitory activity of Co(II) complexes against MMP-9, a benzohydroxamatocobalt(II) complex, [Co^{II}(TBDAP)(C₆H₅C(-NHO)O)]⁺ (**6**), was prepared (see ESI†). Kinetic studies for the reaction of **6** with **3** were carried out, affording k_2 of 4.9(5) × 10^{−1} M^{−1} s^{−1} at −10 °C (Fig. S16 and S17†). The equilibrium constant for the

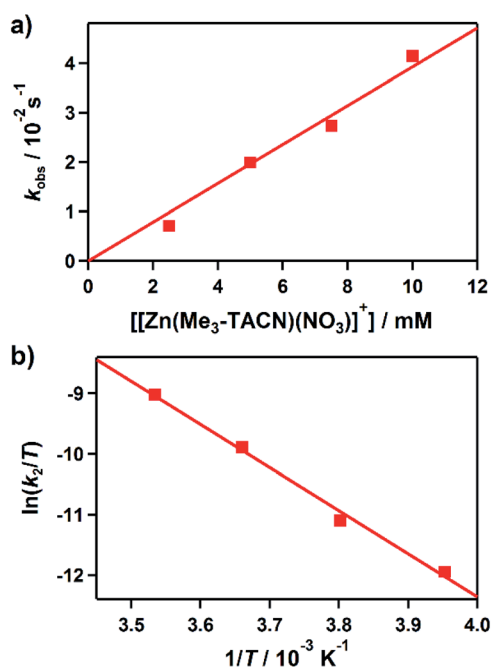


Fig. 3 Rate and activation parameters for the reaction of **1** with **3** in CH₃CN/H₂O (99 : 1). (a) Plot of k_{obs} against the concentration of **3** at −10 °C to determine a second-order rate constant (k_2). (b) Eyring plot of $\ln(k_2/T)$ against $1/T$ to obtain the activation parameters.



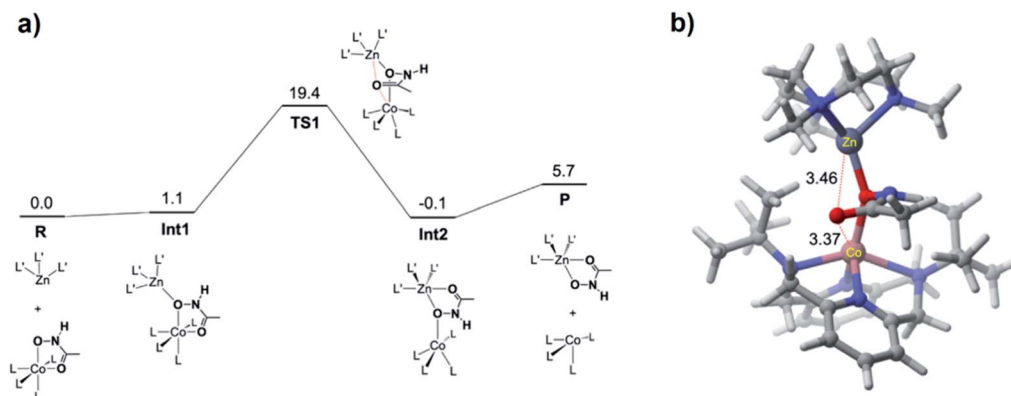


Fig. 4 (a) DFT-calculated free energy profile (in kcal mol⁻¹) for the hydroxamate transfer reaction. L and L' denote the ligands for the Co and Zn complexes, respectively. (b) Optimized geometry of TS1, with key distances shown in Å.

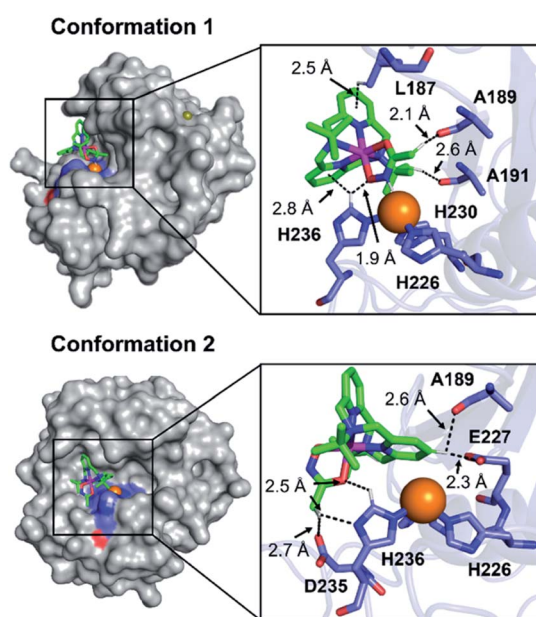


Fig. 5 Two possible representative conformations of **1** docked with the catalytic domain of MMP-9 (PDB 4H3X) by AutoDock Vina [surface (left) and cartoon (right) versions]. These conformations exhibited the calculated binding energies in a range from -5.5 to -3.5 kcal mol⁻¹. Hydrogen bonding and C-H... π interactions within 3.0 Å are indicated with dashed lines. The Zn(II) center in the catalytic domain of MMP-9 is highlighted as an orange sphere.

transfer reaction from **6** was also determined as 4.4×10^{-2} (Fig. S18†). These results are similar to those of the hydroxamate transfer reaction of **1**. Under our experimental conditions, the inhibitory activity of **6** towards MMP-9 was same as that of **1** (data not shown). Thus, replacing a structural moiety of the ligand from acetohydroxamate to benzohydroxamate may not significantly change the hydroxamate transfer reactivity towards **3** and the inhibitory activity of its corresponding Co(II) complex against MMP-9.

Based on the experimental and computational studies, it is suggested that the hydroxamate transfer can be accelerated in the presence of a weak acid, which reduces the coordination

ability of the hydroxamate group in the hydroxamatocobalt(II) complex. Thus, we further investigated the equilibrium between **1** and **4** under acidic conditions. By adding acetic acid to the solution of **1** and **3**, the absorption band of **1** exponentially decreased with increasing concentration of the acetic acid (Fig. S19†). This result indicates that, in the presence of acetic acid, the equilibrium is shifted in favor of **4**, which was also confirmed by ESI-MS (Fig. S20†). Taken together, the hydroxamate transfer reaction effectively occurs in relatively acidic conditions.

Conclusions

We have succeeded in the isolation and structural characterization of a hydroxamatocobalt(II) complex bearing macrocyclic tetradentate N4 ligand, [Co^{II}(TBDAP)(CH₃C(=NHO)O)]⁺ (**1**), in which the acetohydroxamate ligand is bound in a bidentate mode. The intermediate is further characterized by various physicochemical methods such as FT-IR, UV-vis, and ESI-MS. **1** exhibited hydroxamate group transfer reactivity towards a zinc(II) complex. The observation of the hydroxamate transfer between metal complexes is unprecedented. In addition, the proton-assisted reduction mechanism was examined by the CV measurements. Kinetic studies suggest that the transfer reaction proceeds by a bimolecular mechanism, which is supported by DFT calculations. Moreover, the inhibitory activity of **1** towards a zinc-containing enzyme, MMP-9, was confirmed. The interaction and accessibility of **1** to MMP-9 as well as the substitution effect onto the hydroxamate ligand were also examined.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- 1 C. Apfel, D. W. Banner, D. Bur, M. Dietz, T. Hirata, C. Hubschwerlen, H. Locher, M. G. P. Page, W. Pirson, G. Rosse and J.-L. Specklin, *J. Med. Chem.*, 2000, **43**, 2324–2331.
- 2 M. J. Miller, *Chem. Rev.*, 1989, **89**, 1563–1579.
- 3 E. M. Muri, M. J. Nieto, R. D. Sindelar and J. S. Williamson, *Curr. Med. Chem.*, 2002, **9**, 1631–1653.
- 4 C. J. Marmion, D. Griffith and K. B. Nolan, *Eur. J. Inorg. Chem.*, 2004, **2004**, 3003–3016.
- 5 S. Parvathy, I. Hussain, E. H. Karran, A. J. Turner and N. M. Hooper, *Biochemistry*, 1998, **37**, 1680–1685.
- 6 V. Alterio, A. Di Fiore, K. D'Ambrosio, C. T. Supuran and G. De Simone, *Chem. Rev.*, 2012, **112**, 4421–4468.
- 7 C. T. Supuran, A. Scozzafava and A. Casini, *Med. Res. Rev.*, 2003, **23**, 146–189.
- 8 A. Scozzafava and C. T. Supuran, *J. Med. Chem.*, 2000, **43**, 3677–3687.
- 9 T. A. Miller, D. J. Witter and S. Belvedere, *J. Med. Chem.*, 2003, **46**, 5097–5116.
- 10 M. S. Finnin, J. R. Donigian, A. Cohen, V. M. Richon, R. A. Rifkind, P. A. Marks, R. Breslow and N. P. Pavletich, *Nature*, 1999, **401**, 188–193.
- 11 W. L. Mock and H. Cheng, *Biochemistry*, 2000, **39**, 13945–13952.
- 12 M. Whittaker, C. D. Floyd, P. Brown and A. J. H. Gearing, *Chem. Rev.*, 1999, **99**, 2735–2776.
- 13 R. P. Verma, *Matrix Metalloproteinase Inhibitors: Specificity of Binding and Structure-Activity Relationships*, ed. S. P. Gupta, Springer, Basel, 2012, pp. 137–176.
- 14 S. D. Shapiro, *Curr. Opin. Cell Biol.*, 1998, **10**, 602–608.
- 15 N. Johansson, M. Ahonen and V.-M. Kähäri, *Cell. Mol. Life Sci.*, 2000, **57**, 5–15.
- 16 E. Kerkelä and U. Saarialho-Kere, *Exp. Dermatol.*, 2003, **12**, 109–125.
- 17 M. A. Rudek, J. Venitz and W. D. Figg, *Pharmacotherapy*, 2002, **22**, 705–720.
- 18 V. P. Chauhan, T. Stylianopoulos, Y. Boucher and R. K. Jain, *Annu. Rev. Chem. Biomol. Eng.*, 2011, **2**, 281–298.
- 19 Y. Boucher, L. T. Baxter and R. K. Jain, *Cancer Res.*, 1990, **50**, 4478–4484.
- 20 A. I. Minchinton and I. F. Tannock, *Nat. Rev. Cancer*, 2006, **6**, 583–592.
- 21 R. E. Vandenbroucke and C. Libert, *Nat. Rev. Drug Discovery*, 2014, **13**, 904–927.
- 22 T. W. Hambley, *Science*, 2007, **318**, 1392–1393.
- 23 C. R. Munteanu and K. Suntharalingam, *Dalton Trans.*, 2015, **44**, 13796–13808.
- 24 T. W. Failes, C. Cullinane, C. I. Diakos, N. Yamamoto, J. G. Lyons and T. W. Hambley, *Chem.-Eur. J.*, 2007, **13**, 2974–2982.
- 25 T. W. Failes and T. W. Hambley, *Dalton Trans.*, 2006, **6**, 1895–1901.
- 26 N. Yamamoto, S. Danos, P. D. Bonnitcha, T. W. Failes, E. J. New and T. W. Hambley, *J. Biol. Inorg. Chem.*, 2008, **13**, 861–871.
- 27 P. D. Bonnitcha, B. J. Kim, R. K. Hocking, J. K. Clegg, P. Turner, S. M. Neville and T. W. Hambley, *Dalton Trans.*, 2012, **41**, 11293–11304.
- 28 M. Alimi, A. Allam, M. Selkti, A. Tomas, P. Roussel, E. Galardon and I. Artaud, *Inorg. Chem.*, 2012, **51**, 9350–9356.
- 29 H. Noh, D. Jeong, T. Ohta, T. Ogura, J. S. Valentine and J. Cho, *J. Am. Chem. Soc.*, 2017, **139**, 10960–10963.
- 30 F. E. Jacobsen, J. A. Lewis and S. M. Cohen, *J. Am. Chem. Soc.*, 2006, **128**, 3156–3157.
- 31 B. J. Brennan, J. Chen, B. Rudshiteyn, S. Chaudhuri, B. Q. Mercado, V. S. Batista, R. H. Crabtree and G. W. Brudvig, *Chem. Commun.*, 2016, **52**, 2972–2975.
- 32 D. F. Evans and D. A. Jakubovic, *J. Chem. Soc., Dalton Trans.*, 1988, 2927–2933.
- 33 F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, Wiley, New York, 1988.
- 34 M. M. Makowska-Grzyska, E. Szajna, C. Shipley, A. M. Arif, M. H. Mitchell, J. A. Halfen and L. M. Berreau, *Inorg. Chem.*, 2003, **42**, 7472–7488.
- 35 R. Codd, *Coord. Chem. Rev.*, 2008, **252**, 1387–1408.
- 36 T. Matsui, Y. Kitagawa, M. Okumura and Y. Shigeta, *J. Phys. Chem. A*, 2015, **119**, 369–376.
- 37 C. Antoni, L. Vera, L. Devel, M. P. Catalani, B. Czarny, E. Cassar-Lajeunesse, E. Nuti, A. Rossello, V. Dive and E. A. Stura, *J. Struct. Biol.*, 2013, **182**, 246–254.

