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A novel dizinc bridged hydroxamate model for hydroxamate inhibited zinc hydrolases†

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Reaction of $Zn(OAc)_2{\cdot}2H_2O$ with tmen leads to the formation of $[Zn(tmen)(OAc)_2]$ (I) which reacts with benzohydroxamic acid to form $Zn(BA)_2{\cdot}H_2O$ (II) and the novel dizinc hydroxamate bridged complex $[Zn_2(\mu\text{-}OAc)_2(OAc)(\mu\text{-}BA)(tmen)]$ (III), which may also be prepared by self-assembly and whose structure closely mimics that of the native hydroxamate inhibited *Aeromonas proteolytica* aminopeptidase.

Dinuclear metal hydrolases are an important group of metalloenzymes which catalyse the hydrolysis of a range of peptide and phosphate ester bonds and include the amidohydrolases, amidinohydrolases and peptide hydrolases.[1] A common structural feature of these metallohydrolases is a dinuclear metal active site featuring Zn(II), Ni(II), Co(II) and Mn(II) and carboxylate bridges which occur respectively in leucine aminopeptidase,[2] urease,[3] methionineaminopeptidase[4] and arginase.[5] Hydroxamic acids are ubiquitous bioligands which inhibit many enzymes including one-zinc endopeptidases such as thermolysin[6] and colleganases[7] in which the catalytic zinc centre is pentacoordinated by three protein ligands and chelated by the hydroxy and carbonyl oxygen atoms of the inhibiting hydroxamate group, –CONHOH. In contrast, the acetohydroxamate-inhibited C319A variant of *Klebsiella aerogenes* urease shows the deprotonated hydroxy oxygen of the hydroxamic acid bridging the two nickel centres with the carbonyl oxygen bonding one nickel centre only.[8] A similar structure occurs in the dizinc enzyme, *Aeromonas proteolytica* aminopeptidase (Zn_2AAP) inhibited by *p*-iodo-D-phenylalanine hydroxamic acid.[9] We have modelled the hydroxamate inhibition of urease by the reaction of aceto- and benzo-hydroxamic acids (AHA, BHA) with the model dinickel complexes, $[Ni_2(\mu\text{-}OAc)_2(OAc)_2(\mu\text{-}H_2O)(tmen)_2]$ and $[Ni_2(\mu\text{-}OAc)_3(urea)(tmen)_2][OTf]$ which gave respectively the hydroxamate bridged complexes, $[Ni_2(\mu\text{-}OAc)(\mu\text{-}AA)_2(tmen)_2][OAc]$ and $[Ni_2(\mu\text{-}OAc)_2(\mu\text{-}AA)(urea)(tmen)_2][OTf]$ with structures very close to the above inhibited C319A variant.[10] Similar complexes are formed by Co(II)[11] and Mn(II).[12] In this communication, the isolation and structural characterisation of the novel dizinc bridged hydroxamate complex, $[Zn_2(\mu\text{-}OAc)_2(OAc)(\mu\text{-}BA)(tmen)]$ (**III**), which closely mimics a number of features of the above hydroxamate inhibited Zn_2AAP, are described.

In view of the relative ease of formation of $[M_2(\mu\text{-}OAc)_2(OAc)_2(\mu\text{-}H_2O)(tmen)_2]$, M = Ni, Co, Mn, and their reactions with hydroxamic acids,[10–12]attempts were made to synthesise the analogous dinuclear zinc complex by the reaction of a 1:1 molar ratio of $Zn(OAc)_2{\cdot}2H_2O$ and tmen in methanol at room temperature, but resulted in the formation of the mononuclear complex, $[Zn(tmen)(OAc)_2]$ (**I**) only. **I** is octahedral with some distortion of the metal ligand bond angles from 90° (Fig. 1) and some slight asymmetry in the chelating acetates with Zn1–O1 = 2.052(4) and Zn1–O2 = 2.353(4) Å.

† Abbreviations: OAc = $CH_3CO_2^-$, BHA = benzohydroxamic acid, BA = deprotonated hydroxamic acid, tmen = tetramethylethylenediamine.

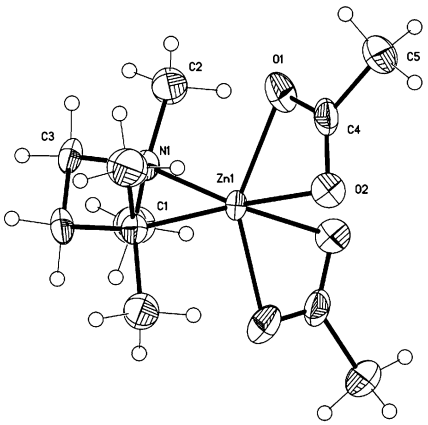
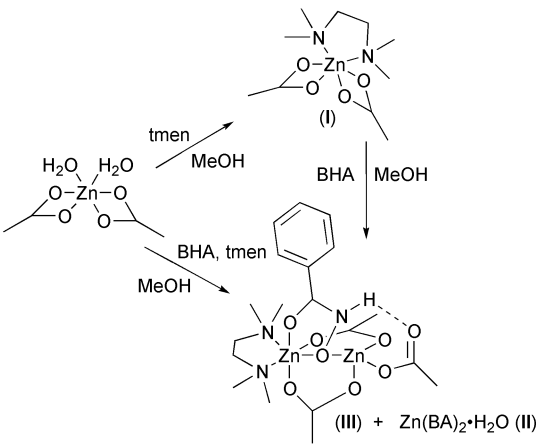


Fig. 1 Molecular structure of complex **I**. Selected bond distances (Å) and angles (°): Zn1–O1 2.052(4), Zn1–O2 2.353(4), Zn1–N1 2.140(4), O1–C4 1.250(6), O2–C4 1.243(6), O1–Zn1–O2 58.76(14), N1–Zn1–N1#1 85.3(2), O2–Zn1–O2#1 97.78(19).

Although there was no evidence for formation of a dimeric species in the above reaction, further reaction of **I** with BHA in a 1:1 molar ratio in methanol for 3 h at room temperature gave a mixture of bisbenzohydroxamatozinc(II), $Zn(BA)_2{\cdot}H_2O$ (**II**) and the dizinc hydroxamate **III**, which were separated by filtration and work up.‡ The same products were obtained by self-assembly from the reaction of $Zn(OAc)_2{\cdot}2H_2O$, tmen and BHA in molar ratio 1:1:1 in methanol for 3 h at room temperature and identical work up (Scheme 1). The formation of **II** was confirmed from previously reported data.[13]

The structure of **III** (Fig. 2) shows the presence of a bridging benzohydroxamate with the same type of bonding previously found in all model dinuclear bridged hydroxamates[10–12] and in the hydroxamate-inhibited urease[8] and Zn_2AAP,[9] namely bridging of the two metal centres by the deprotonated



Scheme 1

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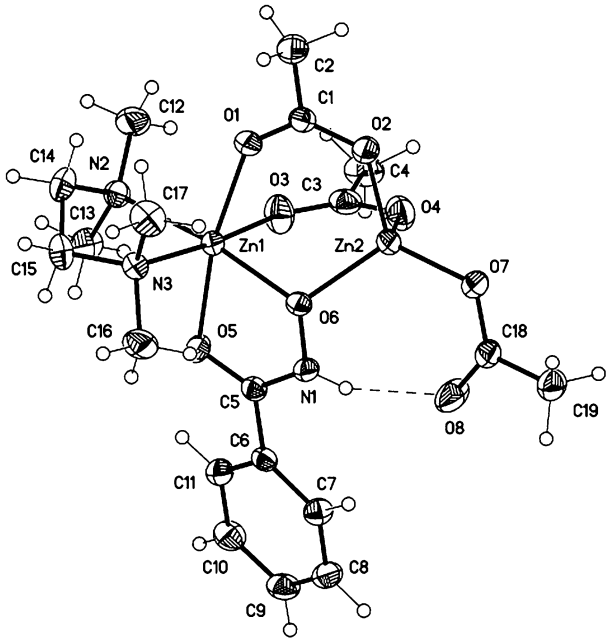

Fig. 2 Molecular structure of complex **III**. Selected bond distances (Å) and angles (°): Zn1–O6 2.0760(16), Zn2–O6 1.9729(16), Zn1–O5 2.1160(16), Zn1–O1 2.1140(16), Zn2–O2 1.9614(16), Zn1–N2 2.1469(16), Zn2–O7 1.9320(17), Zn2–O8 2.9922(19), N1–H···O8 2.7138(28), Zn1–Zn2 3.2368(5); O6–Zn1–O1 92.74(6), O6–Zn1–O5 78.13(6), O1–Zn1–N2 94.36(7), O7–Zn1–O2 106.63(7), O4–Zn2–O2 105.66(8), O4–Zn2–O6 109.00(7).

hydroxamate hydroxy group and bonding of only one metal centre by the hydroxamate carbonyl oxygen. In addition, **III** shows a number of structural features very close to that of the native Zn_2AAP inhibited by *p*-iodo-D-phenylalanine hydroxamic acid.[9] Firstly, in both cases the zinc atoms in **III** and in inhibited Zn_2AAP have different coordination numbers (Zn1, 6 and Zn2, 4 in **III** and Zn1, 5 and Zn2, 4 in the inhibited enzyme). Secondly, the bridge bonds in **III** are unequal, Zn1–O6 = 2.0760(16) and Zn2–O6 = 1.9729(16) Å, as are those in the inhibited enzyme with values of 2.4 and 1.8 Å, respectively, consistent with the different coordination numbers of the two zinc centres in both cases. Thirdly, the capping acetate in **III** is clearly η^1 with Zn2–O7 = 1.9320(17) and Zn2–O8 = 2.9922(19) Å due to a hydrogen bond between the N1H of the hydroxamate and the free acetate oxygen, O8 with an N1–H···O8 distance of 2.7138(28) Å. This behaviour in **III** mimics the hydrogen bonding occurring between an oxygen of the carbonyl group of Glu151 and the hydroxylamino nitrogen (3.3 Å) in inhibited Zn_2AAP.[9]

In conclusion, the very similar structures of **III** and hydroxamate inhibited Zn_2AAP illustrate the ability of hydroxamic acids to induce dimerisation of mononuclear species and to then mimic closely the inhibition of related dinuclear metalloenzymes. Extension to other hydroxamic acids, including the secondary series, is in progress to establish criteria for their possible pharmaceutical efficacy in enzyme inhibition.

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Notes and references

‡ Satisfactory microanalyses were obtained for compounds **I**–**III**. *Preparation* of **I**: to a solution of $Zn(OAc)_2{\cdot}2H_2O$ (20 mmol) in methanol was added dropwise 20 mmol of tmen in methanol followed by stirring for 1 h. After work-up, recrystallisation from acetone gave colourless crystals of **I** suitable for X-ray crystallography.

Preparation of **II** and **III** by self-assembly: to a solution of $Zn(OAc)_2{\cdot}2H_2O$ (20 mmol) and tmen (20 mmol) in methanol was added dropwise BHA (20 mmol) in methanol followed by stirring for 3 h. **II** was filtered off leaving on concentration a pale yellow oil which on stirring in diethyl ether for 1 h gave a white solid which on recrystallisation from acetone gave colourless crystals of **III** suitable for X-ray crystallography.

Preparation of **II** and **III** from **I**: to a solution of **I** (20 mmol) in methanol was added dropwise a solution of BHA (20 mmol) in methanol followed by stirring for 3 h. Identical work-up as in the preceding method gave **II** and **III** as above.

Crystallography: crystal data: for **I**: $C_{10}H_{22}N_2O_4Zn$, M = 299.67, monoclinic, space group $C2/c$, a = 14.9905(19), b = 7.3672(14), c = 12.5105(17) Å, β = 97.015(3)°, U = 1371.3(4) Å^3, Z = 4, λ = 0.71073 Å, μ = 1.796 mm^{-1}, 1327 independent reflections were measured. Final $R1$ = 0.0661 and $wR2$ = 0.1666.

For **III**: $C_{19}H_{31}N_3O_8Zn_2$, M = 560.21, orthorhombic, space group *Pbca*, a = 8.9976(11), b = 15.9235(19), c = 33.533(2) Å, U = 4804.4(9) Å^3, Z = 8, λ = 0.71073 Å, μ = 2.044 mm^{-1}, 6118 independent reflections were measured. Final $R1$ = 0.0355 and $wR2$ = 0.0663.

Data were collected using a Siemens SMART CCD area-detector diffractometer. Refinement was by full-matrix least squares on F^2 for all data using SHELXL-97.[14] Hydrogen atoms were added at calculated positions and refined using a riding model.

CCDC reference numbers 182248 and 182249. See http://www.rsc.org/suppdata/cc/b2/b202612k/ for crystallographic data in CIF or other electronic format.

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