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Structural comparison of suberanilohydroxamic acid (SAHA) and other zinc-enzyme inhibitors bound to a monomeric zinc species

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ABSTRACT

Histone Deacetylase (*HDAC*) and similar proteins contain an active Zn–OH group and have been targets for inhibitors. Known inhibitors such as suberanilohydroxamic acid (SAHA), suberohydroxamic acid (SBHA), valproic acid, and 8-hydroxyquinoline (8-HQ) derivatives act on these enzymes by binding to the zinc center. To examine how these drugs interacted with zinc centers and to examine their structural differences, a homogeneous zinc complex was synthesized and reacted with SAHA, SBHA, valproic acid, and 8-HQ to form stable adducts, which were characterized by spectroscopy and X-ray crystallography. A comparison of their structures with known complexes and enzyme active sites bound to drugs was performed in order to understand how these drugs interact with their targets.

1. Introduction

One of the approaches for treating disease states such as cancer is to target zinc-dependent enzymes such as *Histone Deactylase* (*HDAC*) or *Matrix Metalloprotease* (*MMP*). The role of zinc-based enzymes in disease has made them promising targets for inhibition with a variety of zinc-binding groups (ZBGs) [1]. An example of a successful ZBG-based drug is suberanilohydroxamic acid (SAHA, Vorinostat), which has been shown to be a *HDAC* inhibitor and has been crystallized bound to a *HDAC* protein [2,3]. Vorinostat has been approved by the FDA for the treatment of certain cases of cutaneous T-cell lymphoma [4]. Hydroxamic acids such as SAHA exhibit a variety of interesting coordination chemistry and biological activities [5,6]. The structures of many hydroxamic acids have been determined bound to a variety of metal complexes [7], and the structure of free SAHA has been determined [8,9]. In addition, Griffith and co-workers synthesized and characterized a zinc complex of SAHA, $[Zn(SAHA)_2(H_2O)]\cdot CH_3OH$ [8]. However, to our knowledge, there are no crystal structures of small molecule zinc complexes bound to SAHA. Therefore, we set out to synthesize and structurally characterize a monomeric, stable zinc species coordinated to SAHA and other ZBG-containing species in order to examine their structures.

Working with model complexes often affords a number of advantages when compared to studying native protein systems. Model complexes are easier to synthesize and prepare than whole proteins and can be systematically modified. A variety of zinc enzyme model complexes have been synthesized and examined [10–12]. Notably, structural data for zinc complexes with hydroxamic acids has shown that they tend to be clusters of zinc species or have more than one hydroxamic acid bound to the metal center [13–16]. These cases are not appropriate mimics of the crystal structure of SAHA bound to *HDAC* with its monomeric zinc center. Although both classes of enzymes belong to the broader zinc hydrolase family [17], the active site of *HDAC* differs from that of *MMP* in that the zinc center is coordinated by two aspartate residues and a histidine residue in *HDAC*, but is coordinated by three histidine residues in *MMP*. We were inspired by the success of Vahrenkamp [18,19] and, more recently, Cohen and co-workers using the hydrotris(3-phenyl-5-methylpyrazol-1-yl) ($Tp^{Ph,Me}$) ligand to bind a variety of ZBGs, including acetohydroxamic acid, a small hydroxamic acid [20–23]. These complexes were also attractive because they were soluble in organic solvents and amenable to study using solution methods. The coordination geometry of the $Tp^{Ph,Me}$ ligand also mimics *MMP* and similar enzymes with three histidines bound to zinc. We selected SAHA as well as three other zinc-binding compounds for examination (Fig. 1). Our progress using the ($Tp^{Ph,Me}$)Zn system to study SAHA and other compounds bound to zinc is reported below.

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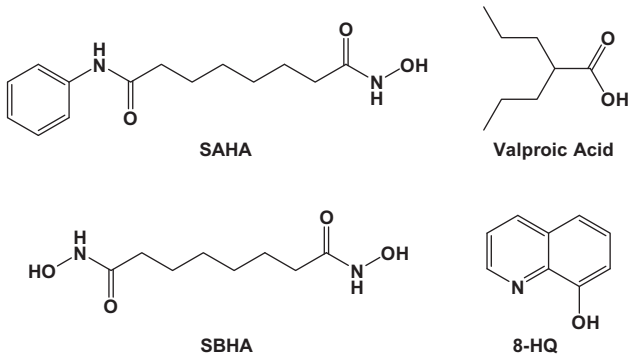

Fig. 1. Zinc-binding compounds examined in this study.

2. Experimental

2.1. General considerations

All reactions and processes were carried out on the benchtop in the presence of air unless otherwise noted. Potassium hydrotris(3-phenyl-5-methylpyrazol-1-yl)borate ($Tp^{Ph,Me}K$) and zinc tetrafluoroborate hydrate were purchased from Strem, Inc. All other reagents and solvents were purchased from commercial suppliers and used as supplied. All NMR spectra were collected using a Bruker Avance 300 MHz NMR spectrometer. ^1H NMR spectra were referenced against residual solvent peaks and reported downfield of tetramethylsilane ($\delta = 0$ ppm). Acetonitrile, dichloromethane, and pentane were purified using an Innovative Technology Pure-Solv system under N_2. Tetrabutylammonium hexafluorophosphate was recrystallized from HPLC-grade methanol and dried prior to use. UV–Visible (UV–Vis) spectra were obtained with a Varian Cary 100 UV–Vis spectrometer. Infrared (IR) spectra were collected on an ABB FTLA2000 IR spectrometer equipped with a PIKE Technologies MIRacle diamond ATR (attenuated total reflectance) anvil. Elemental analyses were performed by Midwest Microlab, LLC in Indianapolis, Indiana. X-ray diffraction analyses were performed at the North Carolina State University Department of Chemistry X-ray Structural Facility by R. D.S.

2.2. Synthesis of $(Tp^{Ph,Me})ZnOH$

$Tp^{Ph,Me}K$ (234.7 mg, 0.45 mmol) was dissolved in CH_2Cl_2 (20 mL) and the resulting solution was added to a stirring solution of $Zn(BF_4)_2{\cdot}6H_2O$ (114.3 mg, 0.48 mmol) in MeOH (5 mL). After the mixture was allowed to stir for 2 h at room temperature under a nitrogen atmosphere, KOH (28.6 mg, 0.51 mmol) was added. The solution was stirred at room temperature overnight under a nitrogen atmosphere. The resulting solid was filtered through a glass frit and 30 mL of methanol was added to the filtrate. The filtrate was concentrated on a rotary evaporator until 5 mL of solution remained. The remaining solution was allowed to stand at room temperature overnight producing the desired compound as translucent white crystals (199.0 mg). Yield: 78.3%. Spectroscopic data matched literature values [20].

2.3. Synthesis of $(Tp^{Ph,Me})Zn(SAHA)$ (**1**)

$(Tp^{Ph,Me})ZnOH$ (56.7 mg, 0.10 mmol) was dissolved in 15 mL of CH_2Cl_2. To this solution, suberanilohydroxamic acid (28.8 mg, 0.11 mmol) dissolved in 10 mL of MeOH was added. The reaction was allowed to stir at room temperature overnight under a nitrogen atmosphere. The solution was then evaporated to dryness on a rotary evaporator. The solid was dissolved in a small amount of toluene (5 mL) and the material was recrystallized by vapor diffusion of pentane into the toluene solution. Clear crystals were produced (28.6 mg, yield: 35.1%). ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 8.18$ (s, 1H, NH), 7.69 (d, 6H, $J_{H-H} = 6.9$ Hz, ArH), 7.06–7.36 (m, 9 H, ArH), 6.18 (s, 3H, pyrazole C–H), 2.51 (s, 9H, CH_3), 2.20 (t, 2H, $J_{H-H} = 7.5$ Hz, CH_2), 1.65 (s, 1H, NH). 1.52 (m, 2H, CH_2), 1.02 (m, 4H, CH_2), 0.79 (m, 2H, CH_2), 0.54 (m, 2H, CH_2). IR (solid ATR): 2543, 1683, 1603 cm^{-1}. *Anal.* Calc. for $C_{44}H_{47}O_3N_8BZn$: C, 65.07; H, 5.83; N, 13.86. Found: C, 65.41; H, 6.03; N, 13.69%.

2.4. Synthesis of $[(Tp^{Ph,Me})Zn]_2$(suberohydroxamic acid) (**2**)

$(Tp^{Ph,Me})ZnOH$ (36.0 mg, 0.06 mmol) was dissolved in 15 mL of CH_2Cl_2. To this solution, suberohydroxamic acid (6.6 mg, 0.03 mmol) dissolved in 10 mL of MeOH was added. The reaction was allowed to stir at room temperature overnight under a nitrogen atmosphere, and was then evaporated to dryness on a rotary evaporator. The resulting solid was dissolved in a small amount of toluene (5 mL) and the material was purified by vapor diffusion of hexane into the toluene solution. Clear crystals were produced (16.8 mg, Yield 43.2%). Crystals suitable for X-ray diffraction were grown by slow evaporation of an acetonitrile solution. ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 8.10$ (s, 2H, NH), 7.68 (d,12H, $J_{H-H} = 7.1$ Hz, ArH), 7.15–7.30 (m, 18H, ArH), 6.18 (s, 6H, pyrazole C–H), 2.51 (s, 18H, CH_3), 0.90 (t, 4H, $J_{H-H} = 7.65$ Hz, CH_2), 0.28–0.47 (br m, 8H, CH_2). IR (solid ATR): 2525, 1600 cm^{-1}. *Anal.* Calc. for $C_{68}H_{70}O_4N_{14}B_2Zn_2$: C, 62.84; H, 5.43; N, 15.09. Found: C, 62.53; H, 5.49; N, 14.63%.

2.5. Synthesis of $(Tp^{Ph,Me})Zn$(valproate) (**3**)

$(Tp^{Ph,Me})ZnOH$ (18.6 mg, 0.03 mmol) was dissolved in 10 mL of CH_2Cl_2. To this solution, valproic acid (3.8 mg, 0.03 mmol) dissolved in 5 mL of MeOH was added. The reaction was allowed to stir at room temperature overnight under a nitrogen atmosphere. The solution was then evaporated to dryness on a rotary evaporator. The solid was dissolved in heptane and recrystallized by slow evaporation. Clear crystals were produced (16.7 mg, 73.4% yield). X-ray quality crystals were obtained by slow evaporation from acetonitrile. ^1H NMR ($CDCl_3$, 300 MHz): $\delta = 7.58–7.65$ (m, 6H, ArH), 7.27–7.37 (m, 9H, ArH), 6.20 (s, 3H, pyrazole C–H), 2.53 (s, 9H, CH_3), 1.64 (p, 1H, $J_{H-H} = 5.9$ Hz, O_2CCH), 0.93–1.16 m, 8H, CH_2), 0.77 (t, 6H, $J_{H-H} = 6.8$ Hz, CH_3).). IR (solid ATR): 2548, 1545 cm^{-1}. *Anal.* Calc. for $C_{38}H_{43}O_2N_6BZn$: C, 65.95; H, 6.26; N, 12.15. Found: C, 66.86; H, 6.73; N, 11.47%.

2.6. Synthesis of $(Tp^{Ph,Me})Zn(8\text{-}HQ)$ (**4**)

$(Tp^{Ph,Me})ZnOH$ (10.2 mg, 0.018 mmol) was dissolved in 15 mL of CH_2Cl_2. To this solution, 8-hydroxyquinoline (3.0 mg, 0.021 mmol) dissolved in 5 mL of MeOH was added. The combined solution rapidly formed a yellow solution. The reaction was allowed to stir at room temperature overnight under a nitrogen atmosphere. The solution was then evaporated to dryness on a rotary evaporator, and the yellow solid was recrystallized from methanol (10.8 mg, 86.5% yield). X-ray crystals were obtained by dissolving the solid in a small amount of toluene (5 mL) and allowing heptane vapor to diffuse into the toluene solution under a nitrogen atmosphere. ^1H NMR ($CDCl_3$, 300 MHz): 7.47 ppm (m, 6H, ArH), 7.43 (dd, 1H, $J_{H-H} = 8.2$ Hz, $J_{H-H} = 1.5$ Hz, 8HQ), 7.31 (t, 1H, $J_{H-H} = 7.9$ Hz, 8HQ), 7.01 (dd, 1H, $J_{H-H} = 4.5$ Hz, $J_{H-H} = 1.5$ Hz, 8HQ), 6.77–6.79 (m, 8H, ArH), 6.67 (dd, 1H, $J_{H-H} = 8.0$ Hz, $J_{H-H} = 0.9$ Hz, 8HQ), 6.58 (dd, 1H, $J_{H-H} = 8.2$ Hz, $J_{H-H} = 4.4$ Hz, 8HQ), 6.20 (s, 3H, pyrazole C–H), 2.60 (s, 9H, CH_3). UV–Vis (CH_2Cl_2): $\lambda_{max} = 392$ nm.). IR (solid ATR): 2546 cm^{-1}. *Anal.* Calc. for $C_{39}H_{34}ON_7BZn$: C, 67.60; H, 4.95; N, 14.15. Found: C, 67.22; H, 5.05; N, 14.12%.