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Inorganica Chimica Acta 360 (2007) 264–272
Inorganica Chimica Acta
www.elsevier.com/locate/ica

Characterization and evaluation of pyrone and tropolone chelators for use in metalloprotein inhibitors

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Received 17 June 2006; accepted 7 July 2006
Available online 27 July 2006

Inorganic Chemistry – The Next Generation.

Abstract

The tetrahedral zinc and cobalt complexes [($Tp^{Ph,Me}$)ZnOH] ($Tp^{Ph,Me}$ = hydrotris(3,5-phenylmethylpyrazolyl)borate) and [($Tp^{Ph,Me}$)-CoCl] were combined with 3-hydroxy-2*H*-pyran-2-one (3,2-pyrone), 3-hydroxy-4*H*-pyran-4-one (3,4-pyrone), and tropolone to form the corresponding [($Tp^{Ph,Me}$)M(**L**)] complexes (**L** = bidentate ligand, M = Zn^{2+}, Co^{2+}). X-ray crystal structures of these complexes were obtained to determine the mode of binding for each chelator and the coordination geometry of each complex. The complexes [($Tp^{Ph,Me}$)-M(3,2-pyrone)] (M = Zn^{2+}, Co^{2+}) are the first structurally characterized metal complexes with this chelator. These complexes with the various chelators show that the cobalt(II) complexes are generally isostructural with their zinc(II) counterparts. In addition to structural characterization, inhibition data for each ligand against two different zinc(II) metalloproteins, matrix metalloproteinase-3 (MMP-3) and anthrax lethal factor (LF), were obtained. Examination of these chelators in the MMP-3 active site demonstrates the possible mode of inhibition.

Keywords: Inhibitors; Metalloproteinases; Pyrone; Tropolone; Zinc

1. Introduction

Matrix metalloproteinases (MMPs) are a zinc(II)-dependent class of hydrolytic enzymes required for the breakdown of connective tissue [1–4]. MMPs can function on a range of substrates in the extracellular matrix including collagen, elastin, and lamin. The hydrolytic activity of these metalloproteins is performed by a zinc(II) ion bound by three nitrogen ligands in a highly conserved tris(histidine) motif. The remaining sites in the zinc(II) ion are open for coordinating substrate and aquo/hydroxo nucleophiles. The aquo/hydroxo nucleophiles are known to perform amide bond cleavage [5,6]. MMPs are required for a variety of normal biological functions such as growth and wound healing; however, unregulated MMP activity has been associated with a number of pathologies including cancer, arthritis, heart, and inflammatory diseases. The importance of MMPs in these disease states has prompted the development of a large number of MMP inhibitors (MMPi) [1,7,8]. MMPi are generally comprised of two major components; a metal binding group (ligand) that forms a coordinate bond(s) to the catalytic zinc(II) ion, and a peptidomimetic backbone for generating non-covalent interactions within the active site pocket. A variety of ligands has been used for the metal-binding group, including carboxylates, phosphinates, thiolates, and hydroxamates [1,7,8]. However, the overwhelming majority of MMPi use hydroxamic acids as the metal binding ligand, which are implicated in unwanted side-effects and exhibit poor oral availability [9,10]. The

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0020-1693/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ica.2006.07.044

limitations of hydroxamates have prompted the search for other functional groups that can be used as the zinc-binding group (ZBG).

Model complexes of metalloenzyme active sites based on the use of tris(pyrazolyl)borate ligands and their derivatives are well established [11–16]. The coordination mode for a prospective ligand can be rapidly obtained from high-resolution structural data. The complex [($Tp^{Ph,Me}$)ZnOH] has been shown to be a useful model for determining how a ligand will bind to the zinc(II) ion in the active site of matrix metalloproteinases [17–20]. In addition, recent investigations have examined [($Tp^{Ph,Me}$)Co(**L**)] as a spectroscopic model for the inhibited protein active site [21]. Differences in the electronic absorption and EPR spectra may be useful in elucidating the mode of binding of a particular ligand to the active site of a zinc(II) enzyme that has been reconstituted with cobalt(II) [21–23].

Research efforts from our laboratory have evaluated several alternative chelators to hydroxamic acid, including hydroxypyridinones and pyrones, and found them to be potent ZBGs [24,25]. As an extension of these studies, this report details three O,O donor ligands, 3-hydroxy-2*H*-pyran-2-one (3,2-pyrone), 3-hydroxy-4*H*-pyran-4-one (3,4-pyrone), and tropolone (Chart 1), in the same general class of chelators. The cyclic nature of these ZBGs should rigidify these ligands, increasing their affinity for metal binding relative to hydroxamic acids, and thereby lead to more potent enzyme inhibition. To demonstrate their potency in zinc metalloproteins their inhibition is reported against MMP-3 and anthrax lethal factor (LF). All of these ZBGs show greater potency at inhibiting MMP-3 and LF as compared to acetohydroxamic acid (AHA), which is used as a comparative benchmark of the hydroxamate ligand. Furthermore, model compounds have been used to help determine the binding orientation of these chelators in the MMP active site. The crystal structures of these model complexes, formed with [($Tp^{Ph,Me}$)ZnOH] and [($Tp^{Ph,Me}$)CoCl], demonstrate that all of the ligands in Chart 1 act as bidentate chelators. Notably, the [($Tp^{Ph,Me}$)M(3,2-pyrone)] ($M = Zn^{2+}$, Co^{2+}) complexes described here are the first structurally characterized metal complexes with this pyrone-based chelator. Finally, the electronic absorption spectra of the cobalt(II) complexes imply, that while 3,2-pyrone is bidentate in the solid state, in solution there appear to be d–d transitions suggesting a fluctuation between monodentate and bidentate. This may explain why 3,2-pyrone is a weak inhibitor of zinc(II)-dependent metalloproteinases.

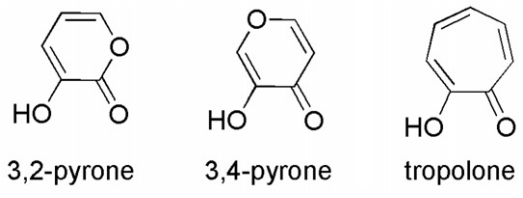

Chart 1. Compounds examined in this study for use as chelators in metalloprotein inhibitors.

2. Experimental

2.1. General

Unless otherwise noted, starting materials were obtained from commercial suppliers (Aldrich) and used without further purification. [($Tp^{Ph,Me}$)ZnOH], [($Tp^{Ph,Me}$)Co(Cl)], and 3,4-pyrone (3-hydroxy-4*H*-pyran-4-one, pyromeconic acid) were synthesized as previously described [17,26–28]. $^{1}H/^{13}C$ NMR spectra were recorded on a Varian FT NMR spectrometer running at 300 or 400 MHz located in the Department of Chemistry and Biochemistry, University of California, San Diego. Elemental analysis was performed at NuMega Laboratories, San Diego, California. UV–Visible spectra were collected on a Perkin–Elmer Lambda 25 spectrophotometer. Absorbance maxima are given as λ_{max}/nm ($\varepsilon/M^{-1}\,cm^{-1}$). Infrared spectra were collected on a Nicolet AVATAR 360 FT-IR instrument at the Department of Chemistry and Biochemistry, University of California, San Diego. *Caution*! Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of these materials should be prepared and they should be handled with great care.

2.2. 3-Hydroxy-2H-pyran-2-one (3,2-pyrone)

Mucic acid (100 g, 0.49 mol) and potassium hydrogen sulfate (100 g, 0.73 mol, 1.51 equiv.) were combined in a 400 mL beaker and mixed thoroughly. A 1 L Pyrex round bottom flask was preheated for about 30 min in a heating mantle with sand to a temperature of ~350 °C with the top half of the flask covered in aluminum foil. The powder mixture of mucic acid and potassium hydrogen sulfate was added quickly to the hot round bottom flask. After adding the powder, a distillation apparatus was immediately attached to the reaction flask. Within seconds, yellow oil began to distill over into the collection flask. The head temperature during the distillation ranged from 100 to 170 °C over ~3.5 h. After the reaction was complete, the yellow oil was extracted into diethyl ether using a continuous liquid/liquid extraction apparatus (Kontes Article No. 584000). The extraction ran overnight under a dinitrogen atmosphere. The ether fraction was concentrated to dryness on a rotary evaporator to obtain a yellow microcrystalline material. The yellow solid was sonicated with 2×100 mL hexanes for about 15 min. The product was isolated by vacuum filtration and dried in vacuo for about 3 h. Yield: 16%. ^{1}H NMR (d_6-DMSO, 300 MHz, 25 °C): δ 6.22 (t, $J = 6.0$ Hz, 1H, Ar–H), 6.61 (d, $J = 6.9$ Hz, 1H, Ar-H), 7.31 (d, $J = 4.5$ Hz, 1H, Ar–H), 9.97 (s, 1H, –OH). ^{13}C NMR (d_6-DMSO, 100 MHz, 25 °C): δ 106.7 (Ar–C), 115.7 (Ar–C), 142.6 (Ar–C), 143.0 (C–OH), 159.8 (C=O). APCI-MS(-): m/z 111.01 $[M-H]^-$.