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'3 + 1' mixed-ligand oxorhenium(V) complexes and their inhibition of the cysteine proteases cathepsin B and cathepsin K

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Dedicated to Professor Brian James.

Abstract

The synthesis of several new oxorhenium(V) complexes containing the '3 + 1' mixed-ligand donor set, ReO(SXS)(SR) (where X = S, O, N(R′); R = alkyl, aryl, heterocylce; R′ = H, alkyl, aryl), is described. The X-ray structure for four of these complexes ReO(SN(Ph)S)(SPh) (**6**), ReO(SN($CH_2CH_2NMe_2$)S)(SPhOMe-*p*) (**10**), ReO(SOS)(SPh) (**29**) and ReO(SOS)(SPhNO_2-*p*) (**30**) was determined. The inhibitory activity of all of the oxorhenium(V) complexes reported herein was evaluated against the cysteine proteases cathepsin B and K in vitro. Compound **25**, ReO(SSS)(S-4py) · HCl, was the best inhibitor of the series against cathepsin B with an IC_{50} of 10 nM. Several of the complexes exhibited specificity for cathepsin B over K, suggesting that oxorhenium(V) complexes can be designed to be enzyme specific. The results described in this paper show that the oxorhenium(V) '3 + 1' complexes are potent inhibitors of cathepsin B and K, constituting promising potential for the treatment of cancer and osteoporosis, respectively.

Keywords: Cysteine protease inhibitor; Cathepsin B; Cathepsin K; Oxorhenium(V) compounds; '3 + 1' mixed-ligand complexes

1. Introduction

The lysosomal cathepsins are cysteine proteases that are responsible for normal cellular protein degradation. An imbalance in the regulation of protease activity can contribute to a number of disease processes. The cysteine proteases have been implicated in the pathophysiology of diseases including inflammatory airway diseases, bone and joint disorders, parasitic diseases, and cancer [1–5]. Cathepsins B and K are of particular interest in our laboratory for their respective roles in cancer and osteoporosis.

Cathepsin K is a cysteine protease involved in the degradation of human type I collagen and plays a primary role in bone resorption [6]. Thus it is an attractive target for the development of new agents for treating disorders of increased bone resorption such as osteoporosis where the rate of osteoclastic bone resorption outpaces the laying down of new bone by osteoblasts. Recent work has focused on possible strategies for improved drug design and delivery as reviewed in the cited references [7–9].

Cathepsin B has been implicated in cancer and may provide a prognostic marker for the disease [10,11]. Increased expression and secretion of cathepsin B has been shown to be associated with numerous human and experimental tumors [12–15]. The exact role for cathepsin B in solid tumors has yet to be defined, but it has been proposed to be involved in metastasis, angiogenesis and tumor progression [16,17]. Carcinoma cell invasion and metastasis can be inhibited by the non-specific, irreversible, cysteine protease inhibitor E-64 [18–20]. Cathepsin B therefore presents itself as a possible therapeutic target for the control of tumor progression.

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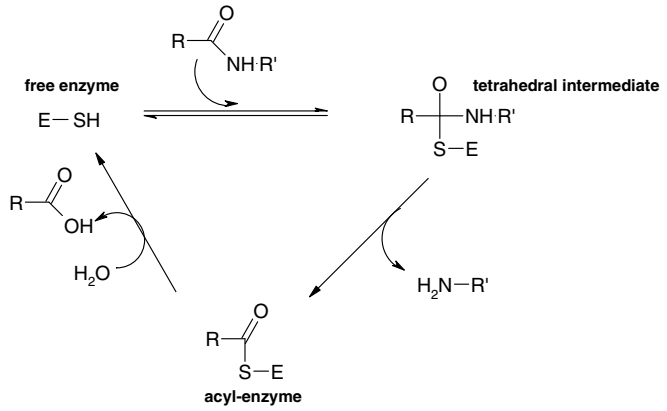
Cathepsin B is unique among the papain family of cysteine proteases in that it has both endopeptidase activity (cleaves proteins in the middle of the molecule) and has dipeptidyl exopeptidase activity (cleaves off two amino acid units form the C-terminus end of proteins). This activity is due to a unique structural feature known as the occluding loop, which partially blocks the active site. The basic residues His110 and His111 are located in this loop in such a way as to be well placed to interact with the free C-terminal carboxylate of a polypeptide chain, directing it into position for hydrolysis of the C-terminal dipeptide. The occluding loop provides structural constraints for the design of an inhibitor, which will be able to functionally access the active site cysteine. On the other hand, the presence of the occluding loop may provide a structural feature, which we can exploit to design cathepsin B specific inhibitors.

The cysteine protease active site is composed of cysteine, histidine, and asparagine residues in a catalytic triad. The cysteine and histidine form a stable thiolate-imidazolium ion pair, which is essential for enzyme activity. Scheme 1 illustrates the nucleophilic attack of the thiolate cysteine on the carbonyl carbon of the substrate, resulting in a tetrahedral intermediate. A covalent-bound acyl enzyme is formed upon cleavage of the peptide bond. Hydrolysis of the acyl-enzyme allows the regeneration of the free enzyme. The majority of cysteine protease inhibitors form either a reversible or irreversible covalent bond with the reactive cysteine in the active site, thereby blocking the proteolytic activity of the enzyme [21]. Several such inhibitors have been synthesized and mechanistic studies have ensued.

To our knowledge the use of metal-based compounds for the inhibition of cathepsin K activity has not been previously reported. As for cathepsin B, only a handful of examples are known where a metal complex was evaluated as a potential inhibitor of the cysteine protease (e.g. organotellurium(IV) [22], gold(I) [23], Pt(II) [24]). Co-ordination compounds of rhenium have not been reported to target cysteine proteases, but their technetium-99m analogues, have attracted much attention over the past several years due to their radionuclide-based application in radiopharmaceuticals. Several labs have been working on oxorhenium(V) complexes using the '3 + 1' approach to give mixed-ligand neutral, lipophilic, small-size complexes with the general formula ReO(SXS)(SR) (where X = S, O, N(R′)). The major advantage of using the '3 + 1' mixed-ligand complex concept is that it allows for the preparation of an array of compounds by modifying either the chelating tridentate ligand or the monodentate ligand [25–32]. In particular, ligands containing thiolates are of particular interest as their π-donating ability stabilizes the five-coordinate ReO^{3+} core. Several of these oxorhenium(V) '3 + 1' complexes are stable in vivo and show promise for imaging areas of the brain [33–38], heart [39] and some cancerous tumors [40]; one complex was even implicated as a steroidal hormone mimick [41].



Scheme 1. Mechanism of proteolysis by cysteine proteases.

As discussed above, the active site for cathepsin B and K is formed by a cysteine residue, a histidine residue and an asparagine residue [1]. The cysteine and histidine residues form an ion pair, which is present in the ground state of the enzyme, hence the pK_a of the active site cysteine is 3.6 resulting in a completely ionic thiolate group in the active site at physiological pH [42]. Our hypothesis was that the thiolate cysteine would be able to displace the monodentate thiol on the oxorhenium(V) '3 + 1' complexes (ReO(SSS)(SR), ReO(SOS)(SR) and ReO(SN(R′)S)(SR)) to give a reversible rhenium-bound inhibitor. In the literature, there is precedence for the reaction of non-protein thiol-containing molecules such as glutathione and free cysteine with oxorhenium(V) '3 + 1' complexes in vivo which further suggests their usefulness as potential reversible inhibitors of the cysteine proteases cathepsin B and K [34,43,44]. In this paper, we present the synthesis of several oxorhenium(V) complexes and their use as a novel class of cysteine protease inhibitors.

2. Experimental

2.1. Materials

Most of the reagents and solvents were purchased at the highest commercial quality and used without further purification. The precursor complexes $[BzEt_3N][ReOCl_4]$ [26] and ReO(SSS)Cl (SSS = $SCH_2CH_2SCH_2CH_2S$) [45] and the ligands $HN(CH_2CH_2SH)_2$ [46] and $MeN(CH_2CH_2SH)_2$ [47] were synthesized according to the literature procedures. Complexes **2**, **3**, **4**, **9**, **11**, **14**, **15**, **17**, **18**, **20**, **21**, **25**, **26**, **27**, **28**, **31** and ReO(SpyS)(SPh4OMe-*p*) were synthesized according to procedures previously reported in the literature [25–29,48,49].

^1H NMR spectra were recorded on a Bruker Avance 300 with ^1H shifts referenced to the residual proton shift of the internal deuterated solvent. IR spectra (as KBr pellets) were recorded on a Mattson Galaxy Series 5000 FTIR spectrophotometer (only the relative intense bands are reported). Electrospray mass spectra (ES-MS) were recorded on a Bruker-HP Esquire-LC Ion Trap mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). X-ray crystallographic