

Inhibition of Human Tryptase by 1, 2, 5 – Thiadiazolidin-3-one 1, 1 Dioxide-Based Heterocyclic Sulfides.

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1. Introduction

Proteases are biological tools used in degrading proteins. The human body contains a number of proteases to help in metabolism of food particles and may sometimes aid in fighting certain diseases. But in certain cases these biological tools may be responsible for some ailments. One such example is where the secretory granules present in mast cells when activated by stimuli release a range of enzymes that include histamine, tryptase and some other proteolytic enzymes involved in inflammatory reactions [1]. These enzymes are released into the space around the cells. The enzyme tryptase, has been shown to be involved in certain human inflammatory diseases and allergic diseases such as asthma, psoriasis, etc [2]. So it is important to develop therapeutic agents or drugs that will help treat these diseases. Several inhibitors (compounds that reduce the biological activity of the enzyme) have been reported recently in the literature.

Our previous studies using the 1,2,5 – thiadiazolidin-3-one-1,1-dioxide template (**Fig 1**) were performed using the enzymes human leukocyte elastase (HLE), chymase and Cathepsin G [3]. Preliminary enzyme selectivity studies showed that this template is a part of a general class of inhibitors of a class of enzymes called (chymo) trypsin-like serine proteases [4]. So using this template and modifying it slightly we were able to show that compounds based on this template bearing a heterocyclic group are effective inhibitors of tryptase and related serine proteases [5].

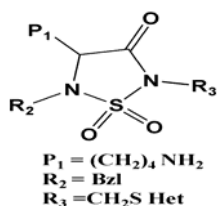


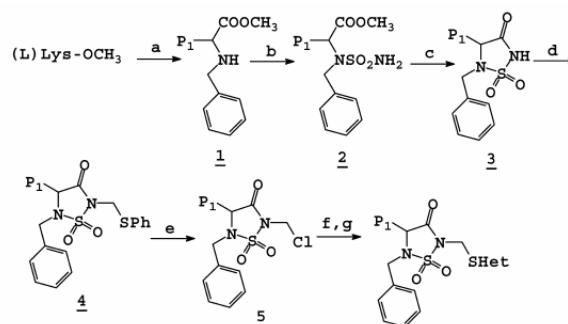
Fig 1: General structure of inhibitor

2. Experimental data

All the compounds were synthesized using the reaction sequence shown in **Fig 2**. The compounds were purified using standard protocol and the pure compounds were verified using Nuclear Magnetic Resonance Spectroscopy (NMR) and High-resolution Mass Spectrometry (HRMS).

Biochemical Studies:

The biochemistry of the compounds was studied using human tryptase enzyme solution stored in dilute form in standard buffer (0.05M Tris buffer containing 0.12M NaCl and 0.1mg/mL heparin at pH 7.62). The substrate used to assay the enzyme is N-p-Tosyl-Gly-Pro-Lys-pNA (a molecule containing the amino acids glycine, proline and lysine along with a chromophore (pNA) para-nitro anilide).



^aBenzaldehyde/ $NaBH(OAc)_3$ /HOAc; ^b $ClSO_2NH_2$ /TEA
^cNaH/THF; ^d $ClCH_2SPh$ /TEA; ^e SO_2Cl_2 ; ^fHetSH/TEA
ⁱ $(CH_3)_3SiI$

P_1	SHet
ZNH(CH ₂) ₄	2-mercaptobenzoxazolyl 6
ZNH(CH ₂) ₄	5-phenyl-1,3,4-oxadiazole-2-mercapto 7
ZNH(CH ₂) ₄	3-phenyl-1,2,4-oxadiazole-5-mercapto 8
NH ₂ (CH ₂) ₄	2-mercaptobenzoxazolyl 9
NH ₂ (CH ₂) ₄	5-phenyl-1,3,4-oxadiazole-2-mercapto 10
NH ₂ (CH ₂) ₄	3-phenyl-1,2,4-oxadiazole-5-mercapto 11

Fig 2: Synthesis of inhibitors

The biochemical assay is performed using a UV/Vis-spectrophotometer that monitors the release of the chromophore at 410 nm. All biochemical experiments are performed either in duplicates or triplicates to verify the results.

There are specific biochemical methods for studying the response of the enzyme when mixed with inhibitors. They are called time-dependent loss of enzyme activity assay and progress curve method [5].

The inhibitors were also tested with another enzyme called trypsin in order to compare the activity of the compounds with the two enzymes. Similar biochemical procedures were followed.

3. Results and Discussion

Human tryptase is a trypsin-like serine protease that is stored in the mast cells. Similar to trypsin, tryptase prefers a lysine or arginine residue as its recognition unit. But unlike trypsin, tryptase is a tetrameric glycoprotein with four identical subunits and each subunit bears an active site. Human lung tryptase shows 50% homology with trypsin and has similar preference for recognition units. Unlike trypsin, the body has no endogenous inhibitors for tryptase. Based on certain studies using leech-derived tryptase inhibitor (LDTI) and turkey ovomucoid inhibitor (TOMI) the template was designed and the inhibitors were synthesized. These inhibitors of tryptase are mechanism based inhibitors, i.e., the enzyme is completely inactivated by these inhibitors using a certain mechanism of action. From the various biochemical studies these compounds are highly effective inhibitors of tryptase. The enzyme selectivity of these inhibitors is low when comparing the activity of the compounds between tryptase and trypsin (**Table 1**). But the subtle differences in their active sites can be exploited to optimize the selectivity.

Table 1: Inhibitory activity of compounds 9-11

Inhibitory activity is represented as $k_{\text{inact}}/K_{\text{I}} \text{ M}^{-1} \text{ s}^{-1}$
(Larger numbers indicate better inhibitory activity of the compound)

Compound	$k_{\text{inact}}/K_{\text{I}} \text{ M}^{-1} \text{ s}^{-1}$	
	Tryptase	Trypsin
9	49,600	4,180
10	69,200	10,600
11	112,000	36,500

Inactivation occurs when the formation of acyl enzyme arises from the nucleophilic attack of the active site serine (Ser-195 residue) on the carbonyl carbon of the inhibitor to form a tetrahedral intermediate which collapses and is subsequently hydrolyzed to the inactive acyl enzyme. (**Fig 3**)

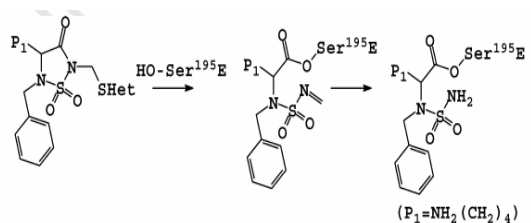


Fig 3: Postulated mechanism of action

4. Acknowledgements

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5. References

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