Available online at www.sciencedirect.com
SCIENCE DIRECT®
Archives of Biochemistry and Biophysics 436 (2005) 1–7
ABB
www.elsevier.com/locate/yabbi

1,2,5-Thiadiazolidin-3-one 1,1-dioxide-based heterocyclic sulfides are potent inhibitors of human tryptase

Tzutshin Wong [a], Christopher S. Groutas [a], Swathi Mohan [a], Zhong Lai [a], Kevin R. Alliston [a], Nga Vu [a], Norman M. Schechter [b], William C. Groutas [a,*]

[a] *Department of Chemistry, Wichita State University, Wichita, KS 67260, USA*
[b] *Department of Dermatology, University of Pennsylvania, Philadelphia, PA 19104, USA*

Received 11 August 2004, and in revised form 25 January 2005

Abstract

We describe herein the design, synthesis, and in vitro biochemical evaluation of a series of potent, time-dependent inhibitors of the mast cell-derived serine protease tryptase. The inhibitors were readily obtained by attaching various heterocyclic thiols, as well as a basic primary specificity residue P_1, to the 1,2,5-thiadiazolidin-3-one 1,1-dioxide scaffold. The inhibitors were found to be devoid of any inhibitory activity toward a neutral (elastase) or cysteine (papain) protease, however they were also fairly efficient inhibitors of bovine trypsin. The differential inhibition observed with trypsin suggests that enzyme selectivity can be optimized by exploiting differences in the S′ subsites of the two enzymes. The results described herein demonstrate the versatility of the heterocyclic scaffold in fashioning mechanism-based inhibitors of neutral, basic, and acidic (chymo)trypsin-like serine proteases.

Keywords: Tryptase; Mechanism-based inhibitors

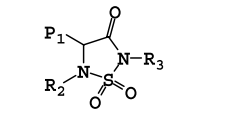
The secretory granules of mast cells contain a range of pro-inflammatory mediators, including histamine, cytokines, and proteolytic enzymes [1,2]. The latter include tryptase, chymase, and carboxypeptidase A. Upon activation by various stimuli, mast cells undergo degranulation, releasing tryptase, histamine, and other mediators into the extracellular space. The release of tryptase is associated with a range of acute inflammatory processes that comprise, among others, activation of protease-activated receptor-2 (PAR-2)[1] and prekallikrein, generation of kinins, and tissue remodeling [3]. Tryptase has thus been implicated in the pathophysiology of human inflammatory diseases characterized by the recruitment and degranulation of mast cells. These include allergic diseases such as asthma [4,5], psoriasis, and atopic dermatitis [6,7], chronic pancreatitis [8], and others [9], consequently tryptase is generally recognized to be a validated target for the development of therapeutic agents for these diseases [10,11]. Inhibitors of tryptase reported recently include benzothiazole ketones [4], 4-carboxy-2-azetidinone [12], 2-azepanone [13], and 1,2-benzisothiazol-3-one 1,1-dioxide [14] derivatives, and others [15,16].

In previous studies the 1,2,5-thiadiazolidin-3-one 1,1-dioxide template was used in the design of mechanism-based inhibitors of the neutral serine proteases human leukocyte elastase (HLE), proteinase 3 (PR 3), cathepsin G (Cat G), and chymase [17–23]. Furthermore, preliminary enzyme selectivity studies suggested that (I) constitutes a general class of mechanism-based inhibitors of (chymo)trypsin-like serine proteases [24,25], consequently it was envisaged that derivatives of the

* Corresponding author. Fax: +1 316 978 3431.
E-mail address: bill.groutas@wichita.edu (W.C. Groutas).

[1] *Abbreviations used:* PAR-2, protease-activated receptor-2; HLE, human leukocyte elastase; PR 3, proteinase 3; Cat G, cathepsin G; LDTI, Leech-derived tryptase inhibitor; TOMI, Turkey Ovomucoid inhibitor.

0003-9861/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.abb.2005.01.022



(I)

$P_1 = (CH_2)_4NH_2$, R_2=bzl

$R_3 = CH_2SHet$

Fig. 1. General structure of inhibitor (I).

heterocyclic scaffold having a *basic* primary substrate specificity residue ($P_1 = (CH_2)_4NH_2$) (Fig. 1) may function as covalent inhibitors of tryptase. We demonstrate herein that compounds represented by structure (I) are highly effective inhibitors of tryptase and related serine proteases.

Materials and methods

General

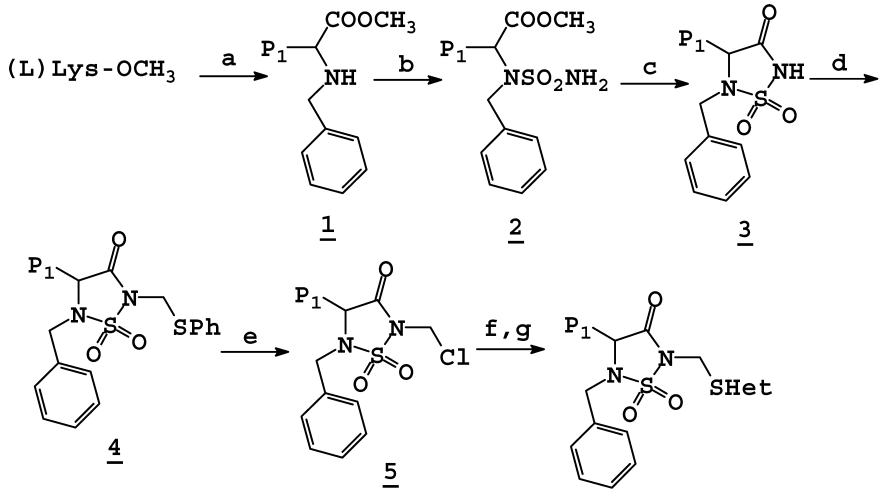
Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The ^1H and ^{13}C NMR spectra of the synthesized compounds were recorded on a Varian XL-300 or XL-400 spectrometers. *N-p*-Tosyl-Gly-Pro-Lys *p*-nitroanilide acetate salt, papain, and bovine trypsin were purchased from Sigma Chemicals, St Louis, MO. Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine vapor and/or UV light. A Hewlett–Packard diode array UV/VIS spectrophotometer was used in the enzyme assays and inhibition studies.

Chemistry

The synthesis of inhibitors **9–11** was accomplished starting with (L) Lys(ε-Cbz)-OCH_3 hydrochloride and using the reaction sequence shown in Fig. 2. The physical and spectral data of the various intermediates and final compounds are listed in Table 1.

Compound 1

To a solution of (L) Lys(ε-Cbz)methylester hydrochloride (3.31 g, 10 mmol) in 1,2-dichloroethane



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

[a] Benzaldehyde/$NaBH(OAc)_3$/HOAc; [b] $ClSO_2NH_2$/TEA

[c] NaH/THF; [d] $ClCH_2SPh$/TEA; [e] SO_2Cl_2; [f] HetSH/TEA

[i] $(CH_3)_3SiI$

Fig. 2. Synthesis of inhibitors **9–11**.