Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 2199–2204

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

HUMAN CHYMASE INHIBITORS BASED ON THE 1,2,5-THIADIAZOLIDIN-3-ONE 1,1 DIOXIDE SCAFFOLD

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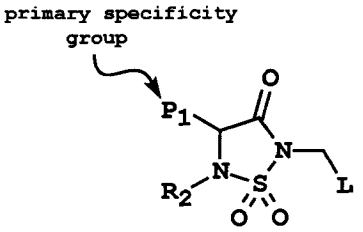
Received 19 February 1999; accepted 22 June 1999

Abstract: A series of compounds that utilize the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold was synthesized and shown to be highly effective inhibitors of recombinant human skin chymase.

Human chymase (EC 3.4.21.39) is a basic, single polypeptide glycoprotein (M_r~30,000) that is stored in the secretory granules of mast cells in active form.[1] Chymase is a serine endopeptidase with an extended binding site. The primary specificity pocket (S_1)[2] is large and hydrophobic, showing a strong preference for aromatic amino acids.[3] Furthermore, structural and substrate specificity studies indicate that remote subsite interactions on either side of the scissile bond are critical determinants of specificity.[4] Although the precise physiological function of chymase is not known, the hypothesis has been advanced that chymase may play a role in blood pressure regulation by forming angiotensin II from angiotensin I via an ACE-independent pathway.[5] Chymase has also been shown to activate interstitial collagenase,[6] and latent interleukin 1β,[7] and to degrade various components of the extracellular matrix. Thus, low molecular weight inhibitors of chymase are of potential value in probing and assessing the role of chymase in pathological states, and as potential therapeutic agents. Synthetic inhibitors of chymase reported thus far include sulfonyl fluorides,[8] peptidyl boronic acids,[9] α-ketoesters[10] and difluoromethylene ketones,[11] 3-(phenylsulfonyl)-1-phenylimidazolidine-2,4-dione derivatives[12] and others.[13]

We have recently described the structure-based design of the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold (I) and have demonstrated that the heterocyclic platform embodies a motif that renders the platform capable of binding to the active site of many serine proteases with a (chymo)trypsin-like fold in a predictable and substrate-like fashion.[14] We describe herein the use of this platform in the design of mechanism-based inhibitors of human skin chymase.

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00377-7



(I)

Materials. Compounds **1–5** were synthesized by reacting 4,5-dibenzyl-2-chloromethyl-1,2,5-thiadiazolidine-3-one 1,1 dioxide, prepared from (L)Phe-OCH_3 using synthetic methodology disclosed previously,[14] with sodium iodide in dry acetone. Evaporation of the solvent left the crude iodide that was dissolved in methylene chloride and then reacted with the appropriate carboxylic acid in the presence of DBU. The preparation of compound **2** employed mono *t*-butyl malonate, followed by deblocking with trifluoroacetic acid. Inhibitors **7–8** and **10–12** were synthesized by reacting the chloromethyl intermediate with the appropriate mercapto compound, followed by oxidation using oxone[15] or magnesium monoperphthalate.[16] Compounds **13–16** were readily obtained by reacting 4,5-dibenzyl-2-chloromethyl-1,2,5-thiadiazolidin-3-one 1,1 dioxide with the appropriate heterocyclic thiol in the presence of DBU. The crude products were purified using flash chromatography[17] and are listed in Table 1.

Table 1. Inhibitory Activity of I Toward Chymase and Cathepsin G

Compound[a]	L	k_{inact}/K_I M^{-1} s^{-1} [b]	
		Chymase	Cathepsin G
1	$OOCCH_3$	21,800	10,600
2	$OOCCH_2COOH$	45,700	22,700
3	$OOCCH_2OH$	30,460	12,800
4[c]	$OOCCHOHCH_3$	38,500	12,680
5[c]	$OOCCHOHC_6H_5$	39,930	12,330
6	SO_2CH_3	4,600	8,500
7	SO_2CH_2COOH	10,800	25,230
8	$SO_2(CH_2)_2COOH$	23,400	17,370
9	$SO_2C_6H_5$	12,460	11,210
10	SO_2(*o*-carboxy)phenyl	inactive	350
11	SO_2(*m*-carboxy)phenyl	13,500	20,710
12	SO_2(*p*-carboxy)phenyl	186,000	66,680
13	2-benzoxazolylthio (R_2 = methyl)	6,250	430
14	2-benzoxazolylthio	5,430	17,130
15	6-amino-2-benzothiazolylthio	14,680	15,740
16	5-phenyl-1,3,4-oxadiazolyl-2-thio	5,090	490

[a] P_1 = R_2 = benzyl in all cases except compound **13**.
[b] Reproducibility: ±10%; [c] mixture of diastereomers.

Biochemical Studies. The inhibitory activity of compounds **1–16** toward human chymase and leukocyte cathepsin G (Cat G) was determined using the progress curve method.[14,18] A typical progress curve for the hydrolysis of succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide by chymase in the presence of inhibitor **12** is shown in Figure 1. The release of p-nitroaniline was continuously monitored at 410 nm. The second order rate constants (k_{inact}/K_I M^{-1} s^{-1}) for the inhibition of chymase and Cat G by compounds **1–16** were determined by calculating $k_{obs}/[I]$, and correcting for the substrate concentration and Michaelis constant.[14] The k_{inact}/K_I values were determined in duplicate and are listed in Table 1.

Results and Discussion. The exquisite ability of the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold to bind to the active site of (chymo)trypsin-like serine proteases in a predictable and substrate-like mode has prompted us to (a) explore the use of suitably-embellished derivatives of (I) as inhibitors of chymase and related enzymes and (b) ascertain the utility of the heterocyclic scaffold in designing inhibitors that exhibit high selectivity toward chymase within a subset of serine proteases having similar substrate specificity, such as Cat G and α-chymotrypsin (α-CT), by exploiting subtle differences in their S_n' subsites.

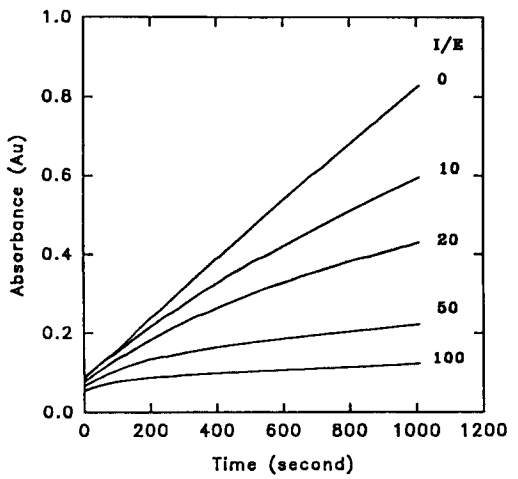


Figure 1. Progress curves for the inhibition of human chymase by compound **12**. Absorbance was recorded at 410 nm for reaction solutions containing human chymase (3.0 nM), N-succinyl Ala-Ala-Pro-Phe-pNA (1.0 mM), and the indicated concentrations of inhibitor in 0.45 M Tris buffer, pH 8.03, containing 1.8 M NaCl, and 2% DMSO. The temperature was maintained at 25 °C and the reactions were initiated by the addition of enzyme.

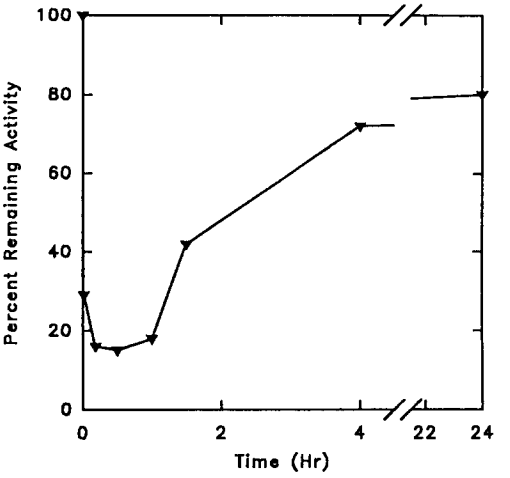


Figure 2. Interaction of compound **3** with α-chymotrypsin. A 50-fold excess of inhibitor **3** (6.0 μM) was incubated with α-chymotrypsin (120 nM) in 0.1 M Tris buffer containing 0.01 M $CaCl_2$, pH 7.83. Aliquots were withdrawn at different time intervals, and enzyme activity was assayed using N-succinyl Ala-Ala-Pro-Phe-pNA at 25 °C.

The binding of **I** to the active site places the P_1 and R_2 groups in the S_1 and S_2 subsites, respectively, and orients L toward the S_n' subsites. Thus, based on (a) the known substrate specificity of chymase, namely, its strong preference for an aromatic residue (P_1) at S_1 and a hydrophobic residue (Pro or proline surrogate) at S_2 and (b) the fact that the S_n' subsites of chymase are polar[19] (vide infra), a series of compounds having an acidic or polar group as part of the leaving group (L) with $P_1 = R_2$ = benzyl and various leaving groups (L) was synthesized, and their *in vitro* inhibitory activity against chymase and Cat G determined. Based on the X-ray crystal structures of chymase[19] and Cat G,[20] insights gained from the docking of ecotin to the active site of human chymase[19] and modeling studies, it was envisioned that the polar functionality in an inhibitor might interact with Arg-143 (located in the S_2' subsite of chymase).

The results summarized in Table 1 clearly indicate that the incorporation of appropriate recognition and reactivity elements into the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold yields compounds that are potent, time-dependent inhibitors of chymase and Cat G. In general, with the exception of compound **10**, inhibitors with polar leaving groups (**2–5**, **7–8**, **11–12**, and **15**) are fairly efficient inhibitors of chymase. The high potency of **12** suggests that the carboxylate group interacts directly with Arg-143. That these compounds are also effective inhibitors of Cat G is not surprising, since the S_2' residue in Cat G is also Arg-143. The ten-fold selectivity shown by compound **16** suggests that there are subtle differences in the S_n' subsites of chymase and cathepsin G which can be exploited. Importantly, the range of leaving groups (carboxylates, sulfones, heterocyclic sulfides) that can be tolerated will greatly facilitate the future design of selective chymase inhibitors.

In contrast to chymase and Cat G, α-CT was transiently inhibited by compounds **1–16**. As illustrated in Figure 2, initial rapid acylation of the enzyme is followed by rapid regain of enzymatic activity. The reason(s) for the lower stability of the acyl enzymes derived from α-CT and these compounds is not intuitively obvious.

In conclusion, the results reported herein demonstrate that the motif embodied in the 1,2,5-thiadiazolidin-3-one 1,1 dioxide platform is ideally-suited for (a) the design of inhibitors of serine proteases in general, and human chymase in particular and (b) the optimal exploitation of subtle differences in the active site topography of closely-related serine proteases.

Acknowledgements: This work was generously supported by grants from SPARTA Pharmaceuticals, Inc. and the National Institutes of Health (HL 57788 and AR 42931 to W.C.G and N.M.S., respectively).

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2. Nomenclature: S_1,S_2,S_3,...S_n and S_1',S_2',S_3',...S_n' correspond to the enzyme subsites on either side of the scissile bond (-P_1-P_1'-). Each subsite accommodates a corresponding amino acid side chain designated P_1,P_2,P_3,...P_n and P_1',P_2',P_3',...P_n' of the substrate or inhibitor. S_1 is the primary specificity site.

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