A General Inhibitor Scaffold for Serine Proteases with a (Chymo)trypsin-Like Fold: Solution-Phase Construction and Evaluation of the First Series of Libraries of Mechanism-Based Inhibitors

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Serine proteases are involved in various physiological as well as pathological processes and, thus, are important therapeutic targets. Proteolysis by serine proteases is a highly specific process, which involves both *sequential* and *conformational* recognition of protein/peptide substrates. Current approaches to the design of specific inhibitors of serine proteases focus on the exploitation of substrate sequential specificity of target proteases, while conformational recognition has rarely been integrated into the design process.[1] Rigid inhibitor scaffolds resulting from integrated sequential and conformational design not only can provide highly specific inhibitors of target proteases but also can serve as an excellent model system for addressing fundamental issues related to protease−substrate recognition.

As part of an ongoing project related to the design of inhibitors of neutrophil-derived serine proteases involved in a range of inflammatory diseases,[2] we have recently described the structure-based design of the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold (**I** in Scheme 1) for the mechanism-based inhibition of serine proteases.[3] The design process began with the X-ray crystal structure of the human leukocyte elastase-turkey ovomucoid inhibitor (HLE-TOMI) complex.[4] With the P_1 backbone locked with a sulfamide linkage, the central segment of the substrate recognition loop (P_2-P_2')[5] was able to be conformationally frozen in the rigid five-membered ring scaffold. As suggested by many crystallographic studies of protein inhibitor−protease complexes, the active backbone conformation of substrate recognition loop P_3-P_3' is conserved among most chymotrypsin-like serine proteases;[6] consequently, inhibitor scaffold (**I**) is expected to be a general template capable of affording specific inhibitors of a wide range of serine proteases by appending the corresponding recognition elements (P_2-P_2') spatially at the three positions (P_1, R_2, L) of the cyclic template.

We wish to describe herein the results of in vitro biochemical studies, which demonstrate the generality of the aforementioned heterocyclic scaffold (**I**) for the design of specific inhibitors of a

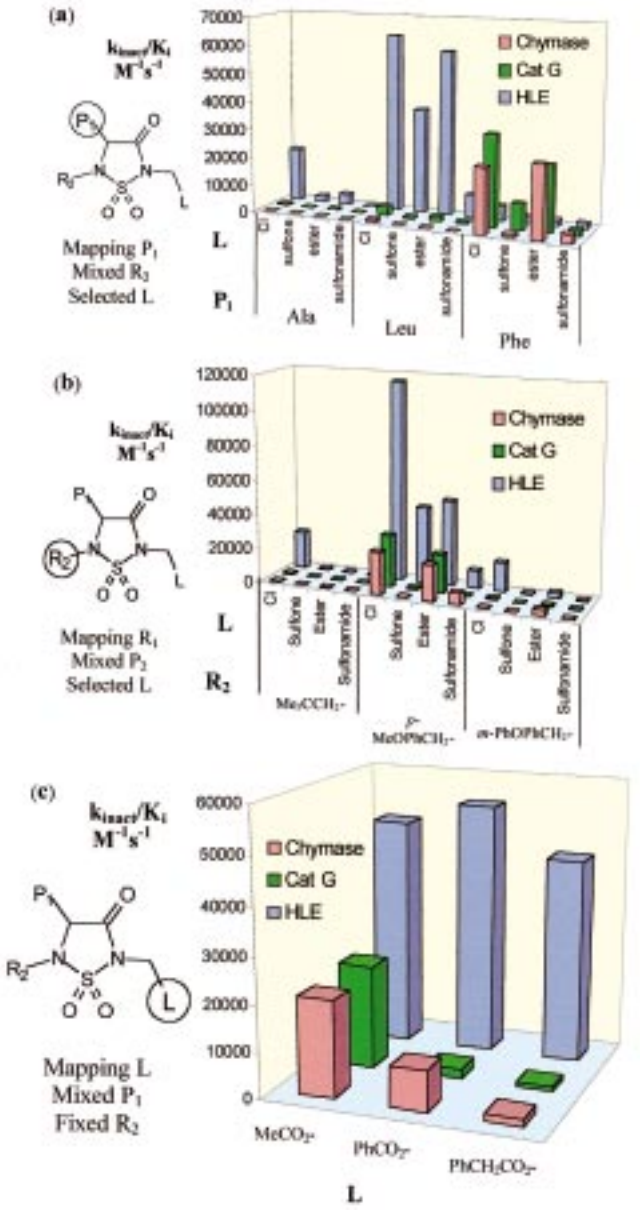

Figure 1. Inhibitory activity of P_1, R_2, and L libraries toward human leukocyte elastase, cathepsin G, and chymase: (a) each P_1 sublibary is derived from a single racemic amino acid (Ala, Leu, Phe), mixed aldehydes (R_2 is Me_3CCH_2-, (*p*-methoxy)benzaldehyde, (*m*-phenoxy)-benzaldehyde), and selected L: Cl, sulfone (*p*-chloro)benzenesulfonyl), and sulfonamide ($MeSO_2N(COOCH_3)$; (b) each R_2 sublibrary is derived from a single aldehyde, mixed racemic amino acids and selected L; (c) each L sublibrary is derived from *p*-anisaldehyde (R_2), mixed racemic amino acids and single carboxylic acid (acetate, benzoate, or phenylacetate).

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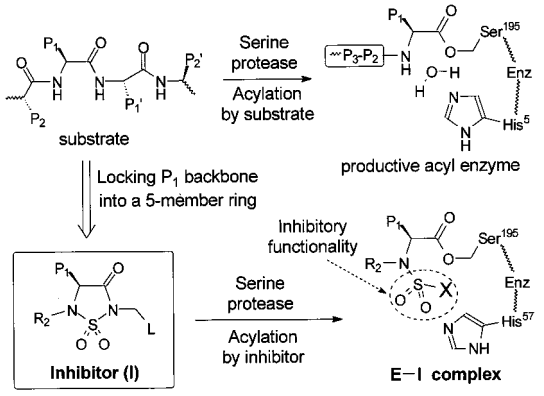
Table 1. Selectivity of Inhibitors (I) Against a Panel of Serine Proteases

compounds[a]			k_{inact}/K_i $M^{-1}s^{-1}$								
			neutral					basic			acidic
no.	P_1	R_2	PR-3[b]	HLE[b]	Cat G[b]	chymase	α-CT	trypsin	tryptase	plasmin	granz B
1	*n*-propyl	methyl	1830	780	na[c]	na	na	na	na	na	na
2	*n*-propyl	benzyl	4960	7260	130	60	na	na	na	na	na
3	isobutyl	methyl	2250	9490	na	na	na	na	na	na	na
4	isobutyl	benzyl	5200	95200	110	na	na	na	na	na	na
5	benzyl	benzyl	na	na	11200	12500	500[d]	na	na	na	na
6	$(CH_2)_4NH_2$	methyl	na	na	na	na	na	13500	6380	850	na
7	$(CH_2)_4NH_2$	benzyl	na	na	na	na	na	30700	12900	1310	na
8	CH_2CO_2H	benzyl	na	na	na	na	na	na	na	na	160[e]

[a] L = phenylsulfonyl. [b] Reference 2. [c] No activity. A compound was classified as being inactive when it gave less than 50% inhibition following incubation with an inhibitor-to-enzyme ratio of 250. [d] $k_{obs}/[I]$ M^{-1} s^{-1} determined by the incubation method. [e] $k_{obs}/[I]$ M^{-1} s^{-1} determined by the progress curve method.

Scheme 1

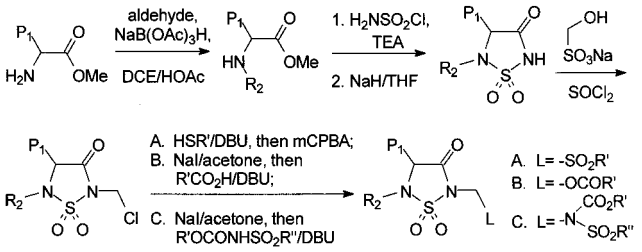


panel of serine proteases with widely differing specificities (neutral, basic, acidic), as well as the development of a combinatorial approach for the rapid establishment of the inhibitory profile of this template.

According to the design rationale, the nature of the P_1 residue, accommodated at the primary specificity site S_1 of a target serine protease, determines which type of protease will be inhibited. This is indeed the case as suggested by the data in Table 1. For instance, compounds with a basic P_1 group (compounds 6 and 7) are fairly effective inhibitors of trypsin, tryptase, and plasmin. Likewise, an acidic P_1 residue leads to inhibition of granzyme B only, while a medium size alkyl or aromatic P_1 residue gives rise to inhibitors of proteinase 3 (PR 3) and human leukocyte elastase (HLE), and chymase and α-chymotrypsin, respectively, in accordance with the known substrate specificity of each enzyme. Thus, *absolute* specificity between neutral, basic, and acidic serine proteases can be achieved by choosing an appropriate P_1 residue. Most importantly, the heterocyclic platform docks to the active site of these serine proteases in a predictable fashion, namely, with the P_1 residue accommodated at the primary specificity site (S_1) and the R_2 and L groups oriented toward the S_2 and S_n' subsites, respectively.

After establishing the generality of the 1,2,5-thiadiazolidin-3-one 1,1 dioxide scaffold, we then pursued a library approach to rapidly obtain the inhibitory profile of this template, with emphasis on the exploitation of secondary recognition elements (P_2, P_1' and P_2') to achieve highly selective inhibition of members of a *subset* of serine proteases with *similar* substrate specificity (for example, chymase vs α-chymotrypsin or cathepsin G (Cat G); PR 3 vs HLE; trypsin vs tryptase, etc.). Thus, as a first step toward this goal, the solution phase construction of a series of small biased libraries was carried out using Scheme 2 with diversity elements derived from three racemic amino acids (Ala, Leu, and Phe) as P_1, three aldehydes (pivalaldehyde, *p*-methoxybenzaldehyde, *m*-phenoxybenzaldehyde) for R_2, and a range of leaving groups for L. The amino acid ester precursors used in the synthesis of

Scheme 2



individual inhibitors and libraries were chosen on the basis of the known primary substrate specificities of HLE (Val, Leu),[7] Cat G (Phe),[8] and human chymase (Phe, Tyr, Leu)[9] (vide infra). All steps proceeded in high yield, and each library, consisting of three pairs of racemic components, had a >95% purity.

Each library was assayed against HLE, Cat G, and chymase using the progress curve method.[2−3,10] The apparent second-order rate constants k_{inact}/K_i (M^{-1} s^{-1}) serve as an index of inhibitory potency and are summarized in Figure 1. As suggested by the data, all three enzymes prefer the same R_2 group (*p*-MeOPh-CH_2-). However, there are substantial differences in their tolerance of leaving group L (Figure 1c), which implies that the S_1' and S_2' subsites are more valuable secondary subsites for optimizing selectivity than the S_2 subsite. Indeed, the presence of an Arg-143 residue in the S_2' subsite of chymase, as opposed to a Leu-143 residue in the S_2' subsite of α-chymotrypsin, led to the design of compound **9** (**I**, P_1 = R_2 = benzyl, L = (*p*-carboxyphenyl)-sulfonyl), which was found to be a highly efficient inhibitor of recombinant chymase[11] (k_{inact}/K_i =186 000 M^{-1} s^{-1}) but was devoid of any inhibitory activity toward α-chymotrypsin.

In conclusion, the results presented herein demonstrate that the 1,2,5-thiadiazolidin-3-one 1,1 dioxide platform embodies a general 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 fashion and is amenable to the facile construction of libraries for lead identification and optimization.

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