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Design and synthesis of selective keto-1,2,4-oxadiazole-based tryptase inhibitors

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Abstract—Using a scaleable, directed library approach based on orthogonally protected advanced intermediates, we have prepared a series of potent keto-1,2,4-oxadiazoles designed to explore the P_2 binding pocket of human mast cell tryptase, while building in a high degree of selectivity over human trypsin and other serine proteases.

Mast cells are involved in allergic disease by virtue of pro-inflammatory mediators stored inside the secretory granules. Upon degranulation, mucosal and respiratory mast cell subpopulations release numerous effectors including the tetrameric serine protease tryptase. With trypsin-like specificity, tryptase processes substrates with immediate and long-term effects with all of the physiological responses associated with asthma. For example, tryptase activates kininogens, leading to the potent bronchoconstrictor bradykinin, and it is capable of amplifying its own signal through stimulating mast cells to further degranulate. Tryptase also processes vasoactive intestinal peptide, or down-regulating this bronchodilatory agent, and it behaves as a potent mitogen toward smooth muscle cells, endothelial cells, and fibroblasts. This effect on smooth muscle cells is of primary concern for long-term sufferers of acute asthma.[1–14] Crystallization of tryptase in 1998 by Pereira and colleagues revealed a homotetrameric structure that was amenable to structure-guided design.[15] Previously, we have described the preparation of α-ketoheterocycles elaborated with prime-side (P′) binding moieties to afford potent and selective tryptase inhibitors.[16] The best-characterized example in this series (**1**, Fig. 1) showed potency of 5.4 nM for tryptase. Our goal in this program required that we improve selectivity over trypsin and other enzymes, and to improve drug-like properties within this series. We also sought to develop a robust synthetic strategy that would allow rapid analogue preparation and large-scale synthesis of these inhibitors to support in vivo studies (See Table 1).

Our improved synthesis of [1,2,4]oxadiazole-containing tryptase inhibitors took advantage of orthogonal protecting groups as illustrated in Scheme 1. Commercially available *N*-α-benzyloxycarbonyl-*N*-ε-*tert*-butyloxycarbonyl-L-lysine **2** was converted to its *N*-α-allyloxycarbonyl derivative **4** via the Weinreb-amide **3**. Reduction to the aldehyde **5** followed by treatment with acetone cyanohydrin afforded the diastereomeric cyanohydrins **6a**,**b**. Subsequent protection as the diastereomeric TBS ethers **7a**,**b** and then conversion to the *N*-hydroxy-amidines **8a**,**b** in the presence of 50% aqueous hydroxylamine in ethanol at 50 °C provided the needed material for analogue preparation, without the need for intermediate chromatography.

Taking advantage of our distal pocket binding knowledge on the prime side of the core, we then prepared further advanced intermediates suitable for analogue exploration. The use of the 3,4-dichlorophenethyloxyphenyl group (Scheme 2) gave us not only a modest increase in potency over the lead structure **1**, but also provided us with a more facile, higher-yielding synthesis that again avoided chromatography. Mitsunobu-based coupling between 3,4-dichlorophenylethanol **9** and

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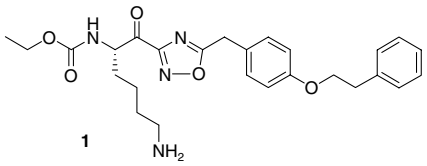
Table 1. Enzyme inhibition and pharmacokinetics data for **1**



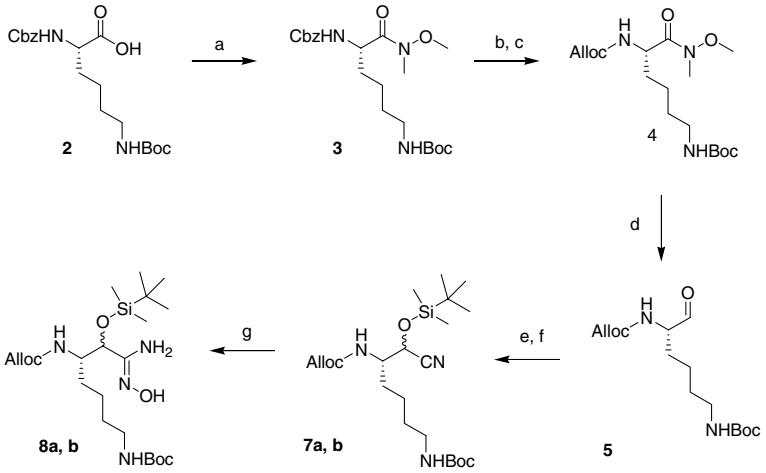
Potency (μM)	
Human B tryptase	0.0054
Selectivty (μM)	
Trypsin	0.190
Thrombin	64
Plasmin	0.43
Kallikrein	43
APC	>150
Chymotrypsin	>150
Elastase	>150
Chymase	>150
Urokinase	>150
Granzyme K	>150

methyl 4-hydroxyphenylacetate **10**, followed by saponification of the ester, extraction of neutral by-products, acidification, and filtration, permitted the product **11** to be isolated in near quantitative yield. **11** was converted to its *N*-hydroxysuccinimidoyl ester **12**, which was then coupled with **8a**,**b** under neutral conditions to give the intermediate esters **13a**,**b** and then cyclized via heating in toluene, utilizing a Dean–Stark type apparatus. The diastereomeric 1,2,4-oxadiazoles, **14a**,**b**, were then N-α-deprotected via Pd-catalyzed reductive cleavage using tributyl stannane to give **15a**,**b**, which were thus set up for the final stages of the synthesis. Although the diastereomeric nature of the intermediates (via the silyloxy group) complicated intermediate analysis, we were fortunate in discovering that one of the diastereomers of **14a**,**b** could in fact be isolated by crystallization, although at this point we did not care which one. Subsequently, the silyl group was to be removed and the secondary alcohol oxidized, thus removing the chiral center. For ease of preparation in subsequent steps, we used the crystalline material (stereochemistry unassigned) and at a later stage repeated the sequence with a mixture of **14a**,**b** and obtained similar results.

With the α-amino group now available for derivatization, we prepared a library of compounds to explore the P_2 residue of tryptase (Scheme 3). Through coupling with the appropriate acylating agent (acid chloride, aminocarbonyl chloride, isocyanate, etc.) in the presence of appropriate bases, **15a**,**b** derivatives of type **16a**,**b** were made. Deprotection of the silyl group with tetrabutylammonium fluoride gave alcohols **17a**,**b**, which were then oxidized using either Dess–Martin periodinane or through a Swern procedure to give the Boc protected, penultimate intermediate **18**. Finally, HCl-mediated deprotection of the N-ε group yielded inhibitors **19**–**44**.

Compounds **19**–**44** were assayed against tryptase and trypsin according to conditions outlined in Ref. 17. Table 2 shows the results as organized by P_2 binding moiety, represented by R^2, as attached through linker L.

While good potency was inherent in this series, thanks to the distal pocket binding moiety on the prime side, the goal of achieving several 100-fold selectivity over human trypsin required an extensive analysis of different binding elements elsewhere in the active site. In this series, we explored the effects of aliphatic and aromatic amides, carbamates, and ureas as linkers between the lysine α-amine group and the P_2-targeting group. Simple aliphatic amides (compounds **19**–**25**) showed the lowest selectivity, with only the sterically hindered (and possibly anomalous, within this series) pivalamide **36** displaying the targeted selectivity. Carbamates, both simple and extended (**26**, **27**, **30**, and **32**) showed modest (80- to 130-fold) selectivity, suggesting that the atom next to the linker carbonyl should bear minimal substituents. The ureas began to show an improvement in selectivity (**29**, **31**, and **33**–**35**) but we still felt that the intrinsic potency against trypsin was too high. When we introduced an



Scheme 1. Reagents and conditions: (a) *N*,*O*-Dimethylhydroxylamine, DCC, Et_3N, CH_2Cl_2, rt; (b) H_2/10% Pd, EtOH; (c) Alloc-Cl, Et_3N, THF, rt; (d) $LiAlH_4$, THF, 0 °C; (e) acetone cyanohydrin, NEt_3, CH_2Cl_2, rt; (f) TBSCl, imidazole, DMAP, CH_2Cl_2, rt; (g) NH_2OH 50 wt% in H_2O, EtOH, 50 °C.