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Solid-phase synthesis and SAR of 4-carboxy-2-azetidinone mechanism-based tryptase inhibitors

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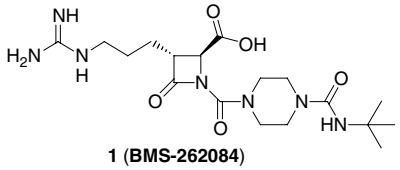
Dedicated to the memory of Steven M. Seiler, Ph.D. (deceased March 31, 2003). Steve's untimely passing will not diminish the continuing impact of his drug discovery research or the admiration of his fellow co-workers

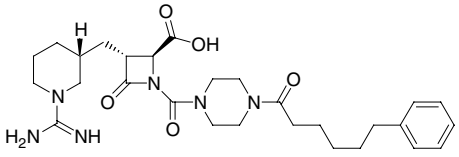
Abstract—A series of nonguanidine N1-activated C4-carboxy azetidinone tryptase inhibitors was prepared by solid-phase methodology to quickly assess the SAR associated with distal functionality on the N1-activating group. From these studies, potent inhibitors with improved specificity were discovered.

Tryptases are structurally novel heparin stabilized homotetrameric trypsin-like serine proteases produced almost exclusively by mast cells.[1] The α- and β-forms of tryptase show differential substrate recognition while sharing moderately high homology (91% α vs βI). The β-form is observed as three β-isozymes (βI, βII, and βIII) having high identity (98–99%).[2] β-tryptases, hereinafter referred to as tryptase, comprise the major protein component of mast cells where the stabilized tetramer is stored preformed. Upon stimulation of mast cells, tryptase is released along with histamine into the extracellular environment.[3] Once released, tryptase modulates inflammatory processes through numerous pathways, many of which are directly related to its proteolytic activity (see Ref. 4 for reviews). In view of the association of tryptase with mast cells and its role in inflammatory processes, inhibitors of tryptase have been investigated as novel therapeutics for the potential treatment of asthma.[4,5]

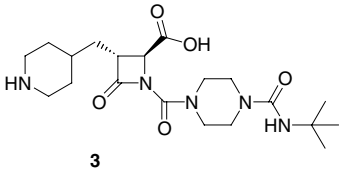
Studies using small molecule tryptase inhibitors in animal models of asthma have demonstrated amelioration of allergen-induced early-stage and late-stage bronchoconstriction, airway hyperresponsiveness and inflammatory cell infiltration into the lungs.[6,7] In human clinical studies, APC-366 reduced antigen induced late airway response in atopic asthmatics.[6a] In our laboratories, the potent inhibitor BMS-262084[7] (**1**) (tryptase $IC_{50} = 4$ nM) demonstrated efficacy in guinea pig models of bronchoconstriction and inflammatory cell recovery in broncho-alveolar lavage.[7] Although BMS-262084 had a good selectivity profile against related serine proteases,



1 (BMS-262084)



2 (BMS-363131)



3

Keywords: Tryptase inhibitor; Azetidinone.
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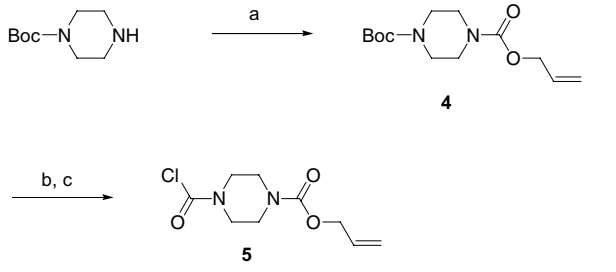
it had low selectivity against trypsin (trypsin $IC_{50} = 72\,nM$, 18-fold selectivity) and, therefore, the potential for preclinical toxicity.[8] Further elaboration of this chemotype afforded compounds with significantly improved potency for tryptase and selectivity against trypsin, for example, BMS-363131[9] (tryptase $IC_{50} < 1.7\,nM$, trypsin $IC_{50} = 5.76\,\mu M$, >3389-fold selectivity).

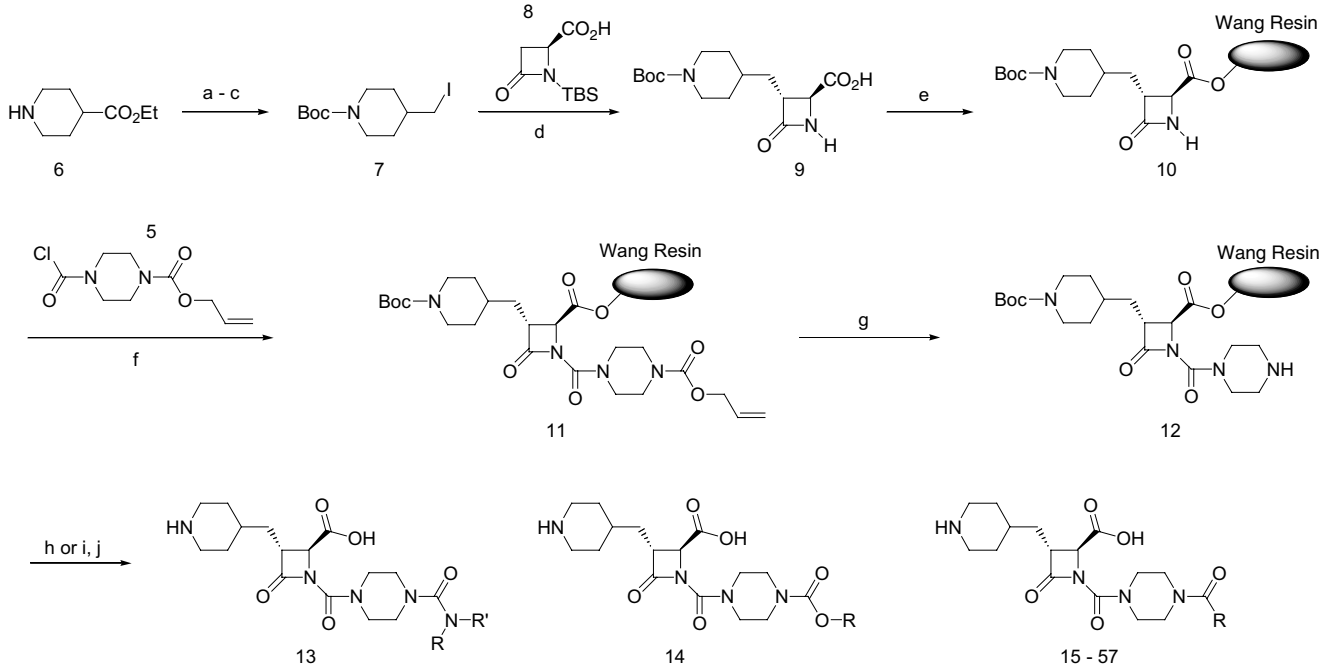
In our quest to improve upon the potency and selectivity of BMS-262084, studies investigating replacement of the guanidine P1 binding group of **1** led to the preparation of compound **3**,[10] our first potent nonguanidine tryptase inhibitor in the azetidinone series. At the time of this discovery, the importance of the distal N1 piperazine urea functionality on potency and selectivity had not yet been broadly evaluated. The piperidine functionality of **3** was anticipated to present fewer synthetic difficulties when applied in a library format than the guanidine functionality present in **1**. Therefore, solid-phase methods were developed to rapidly assess the SAR associated with the N1 position of compound **3**. In this letter we report the solid-phase parallel synthesis of a series of potent and selective piperidine containing 4-carboxy azetidinone tryptase inhibitors with improved trypsin selectivity.

The preparation of the azetidinone core and the coupling to resin is shown in Scheme 1. Boc protection of ethyl isonipecotate **6** followed by reduction of the ester group and conversion of the alcohol to the iodide provided compound **7**. Deprotonation of (4*S*)-4-carboxyazetidinone **8**[11] with 2 equiv of LDA followed by addition of iodide **7** at −20 °C gave upon mildly acidic workup the alkylated (3*R*,4*S*)-carboxyazetidinone **9**.[7] The azetidinone core was attached through the carboxylic acid to Wang Resin[12] employing MSNT ((1-mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole) and *N*-methylimidazole as the coupling reagents in CH_2Cl_2–THF (1:1) to provide **10**.

Acylation of N1 of the resin-bound azetidinone with the carbamoyl chloride of Alloc-piperazine, **5** (preparation in Scheme 2), under mild conditions with triethylamine and DMAP in dichloromethane, cleanly provided the activated azetidinone **11**. Removal of the Alloc group with palladium tetrakistriphenylphosphine and phenylsilane in CH_2Cl_2 afforded resin-bound **12**. Treatment of the amine with isocyanates or chloroformates in the presence of Et_3N and DMAP in CH_2Cl_2, followed by TFA cleavage from the resin, gave ureas **13** and carboxamides **14**. Coupling the amine with carboxylic acids



Scheme 2. (a) Allylchloroformate, Et_3N, CH_2Cl_2, 89%; (b) HCl (g), THF–Et_2O (1:4), 89%; (c) 20% phosgene in toluene, $NaHCO_3$, CH_2Cl_2, 100%.



Scheme 1. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2, 98%; (b) $LiAlH_4$, THF, 0 °C, 94%; (c) (i) Ph_3P, imidazole, CH_2Cl_2, (ii) I_2, 0 °C, 74%; (d) (i) LDA, THF, −78 to −20 °C, (ii) add compound **7**, −78 to −20 ° C, 43%; (e) Wang Resin, MSNT (1-mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole), *N*-methyl-imidazole, CH_2Cl_2, THF; (f) compound **5**, Et_3N, DMAP, CH_2Cl_2; (g) $Pd(Ph_3P)_4$, $PhSiH_3$, CH_2Cl_2; (h) isocyanate or chloroformate, Et_3N, DMAP, CH_2Cl_2; (i) carboxylic acid, DIC, HOAT, CH_2Cl_2–dimethylacetamide (1:1); (j) 20% TFA in CH_2Cl_2.

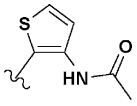
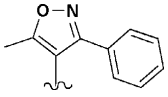
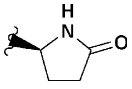
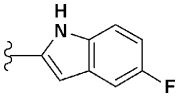
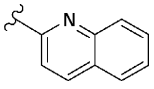
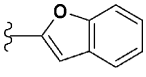
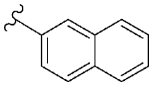
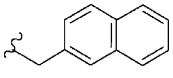
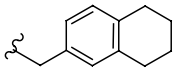
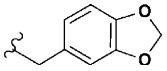
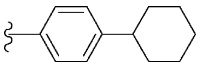
using DIC and HOAT in CH_2Cl_2, followed by TFA cleavage, provided the series of amides **15–57**. Compounds were then purified by reverse phase preperative HPLC.

Compounds were screened for activity against human tryptase and bovine trypsin.[13–15] General tryptase screening was performed employing recombinant human tryptase[14] in a spectrophotometric assay; however, when the inhibitory activity was less than 2 nM, compounds were assayed under fluorometric conditions with a lower limit of 0.03 nM. Trypsin IC_{50}'s were obtained for the more active compounds (tryptase $IC_{50} < 30$ nM). Compounds not meeting this criterion had a single-point percent tryptase inhibition measured at 0.2 μM. Select compounds were screened against a broader panel of related serine proteases,[13] including trypsin, thrombin, plasmin, factor Xa, urokinase (uPA), and tissue plasminogen activator (tPA).

Over 200 compounds were prepared using the solid-phase methodology described. All compounds prepared showed excellent to moderate tryptase inhibitory activity (tryptase IC_{50} range = 0.5–500 nM), with 70% of the compounds having activity at concentrations below 15 nM. Trypsin inhibition was notably less with IC_{50}'s in the range of 50 nM to 3 μM. From the compounds prepared, the linker (amide, urea, carbamate) off the piperazine did not appear to be a critical factor for potency or selectivity. Therefore this letter will focus on the amide compounds **15–57**, as a broader array of these were prepared.

The data in Table 1 show the tryptase IC_{50}, the trypsin IC_{50} and the selectivity ratio (trypsin IC_{50}/tryptase IC_{50}) for a subset of compounds prepared. Comparison of the analogous guanidine- and piperidine-containing compounds **1** and **3** show the piperidine compound to be 7.5-fold less active against tryptase and 100-fold less active

Table 1. Tryptase inhibition and trypsin selectivity for compounds **1**, **3**, and **16–57**

Entry	R	Tryptase IC_{50} (μM)	Trypsin IC_{50} (μM)/Selectivity ratio[a]
1		0.004	0.072/18
3		0.030	1.9/63
16		0.19	68% @ 0.2 μM
17		0.29	31% @ 0.2 μM
18		0.17	69% @ 0.2 μM
19		0.45	63% @ 0.2 μM
20		0.11	51% @ 0.2 μM
21		0.021	89% @ 0.2 μM
22		0.030	72% @ 0.2 μM
23		0.07	22% @ 0.2 μM
24		0.030	18% @ 0.2 μM
25		0.055	19% @ 0.2 μM
26		0.040	76% @ 0.2 μM

(*continued on next page*)

Table 1. (*continued*)

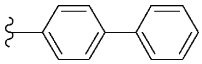
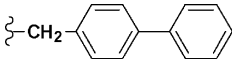
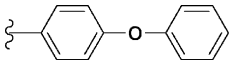
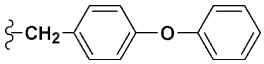
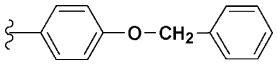
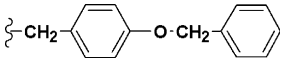
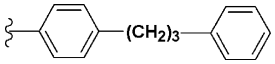
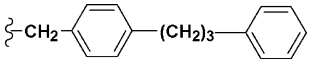
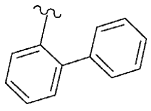
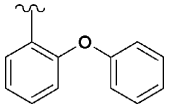
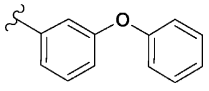
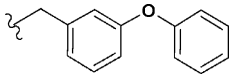
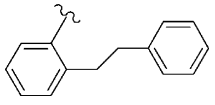
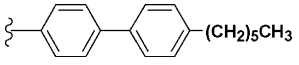
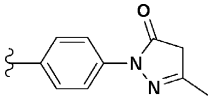
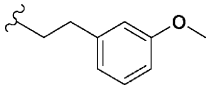
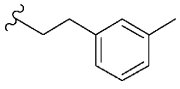
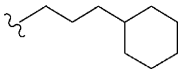
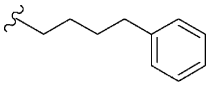
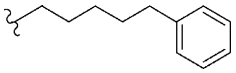
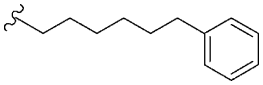
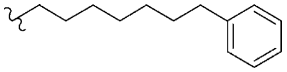
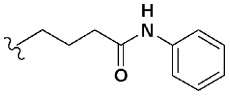
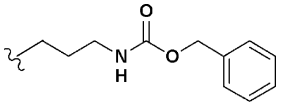
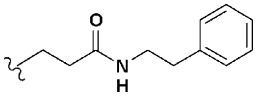
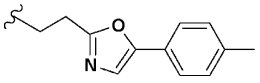
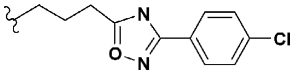
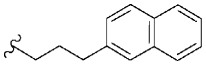
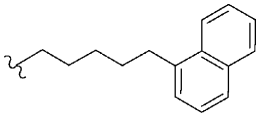
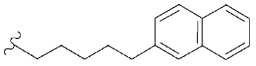
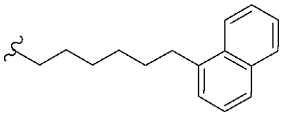
Entry	R	Tryptase IC_{50} (μM)	Trypsin IC_{50} (μM)/Selectivity ratio[a]
27		0.006	0.12/20
28		0.005	0.74/148
29		0.007	0.14/20
30		0.005	0.95/190
31		0.004	0.12/30
32		**0.001**[b,c]	**0.44/440**
33		0.006	0.31/51
34		0.009	1.58/176
35		0.042	46% @ 0.2 μM
36		0.12	2% @ 0.2 μM
37		0.015	78% @ 0.2 μM
38		0.017	0.97/57
39		0.14	60% @ 0.2 μM
40		0.12	1.20/10
41		0.38	91% @ 0.2 μM
42		0.014	0.81/57
43		0.013	1.20/92

Table 1. (*continued*)

Entry	R	Tryptase IC_{50} (μM)	Trypsin IC_{50} (μM)/Selectivity ratio[a]
44		0.024	40% @ 0.2 μM
45		0.006	0.81/135
46		**0.001**[b,c]	**0.31/310**
47		0.005	1.3/260
48		0.005	1.3/260
49		0.012	1.35/112
50		0.006	1.20/200
51		0.017	1.9/111
52		**0.005**	**1.8/361**
53		0.005	0.15/30
54		0.010	0.60/60
55		**0.001**[b,c]	**0.24/267**
56		**0.001**[b]	**0.32/320**
57		**0.001**[b]	**0.32/320**

[a] (Bovine trypsin IC_{50})/(tryptase IC_{50}).
[b] Re-purified and assayed under fluorometric conditions.
[c] Assayed below 1 nM.

against trypsin, with an improved selectivity ratio of 62-fold versus 18-fold for **1**. Replacing the *tert*-butylamine group of **3** with heterocyclic R groups provided no significant improvement in tryptase activity and in many cases proved to be deleterious to tryptase activity, as can be seen with compounds **16**–**18** (tryptase $IC_{50} = 0.17$–0.29 μM). Likewise, fused biaryl and heterobiaryl substitutions linked to the amide carbonyl through a single bond or a methylene generally gave no improvement in activity, with a few examples, **19** and **20**, showing a notable loss of tryptase activity (tryptase $IC_{50} = 0.45$ and 0.11 μM, respectively).

Significant improvements in activity were observed for amides bearing both proximal and distal aromatic rings, as shown by compounds **27**–**34**. The benzamide compounds **27**, **29**, **31**, and **33** showed improved activity against tryptase (tryptase IC_{50}'s 4–7 nM) but trypsin selectivity was unremarkable (trypsin IC_{50}'s 0.12–0.31 μM). Interestingly, addition of a methylene group next to the amide carbonyl provided compounds **28**, **30**, **32**, and **34**, which showed comparable improvements in activity against tryptase but reduced potency against trypsin (trypsin IC_{50}'s 0.44–1.58 μM), thus providing significant improvements in selectivity. Compound **32** exhibited the best selectivity (440-fold). Linking the distal phenyl group through the *para* position of the proximal phenyl was consistent with improved tryptase inhibition (comparison of **29** to **36**–**37**, and **30** to **38**). In addition, continued propagation of the hydrophobic group as shown by compound **40** did not lead to additional gains in potency or selectivity. Introduction of polar functionality at the terminus was generally not well tolerated as exemplified by compound **41**.

Linking the distal hydrophobic phenyl group through a simple alkyl chain, as in compounds **42**–**48**, provided compounds with potent tryptase inhibition and similar selectivity against trypsin when compared to compound **32**. Comparison of compounds **42**–**46** shows that stepwise improvements in tryptase inhibitory activity can be obtained by incremental insertion of methylene groups, to a maximum of five, between the carbonyl of the amide and phenyl group. The four, six, and seven methylene analogs **45**, **47**, and **48** exhibited slightly higher tryptase IC_{50}'s than **46** ($IC_{50} = \sim 5$ nM vs 1 nM, respectively). Comparison of **46** to compounds **49**–**53** showed that polar functionality in the form of amides, carbamates, oxazoles, and oxadiazoles were tolerated within the link to the terminal aryl group without significant effect on activity. A modest change of the terminal phenyl to a naphthyl afforded compounds **54**–**57**, which showed activity and selectivity comparable to **46**.

To obtain an understanding of the SAR observed, a crystal structure of compound **1** in bovine trypsin[16] (Fig. 1) was compared to the structure of compound **46** modeled into the crystal structure of tryptase[17] (Fig. 2). Compound **1** binds to the active site of trypsin with covalent attachment of the former beta-lactam C2 carbonyl to Ser195, and with the guanidine occupying the S1 pocket of trypsin in a classical salt bridge to Asp189. The N1-*tert*-butylaminocarbonylpiperidine group is directed to a relatively small pocket extending over the Cys58-Cys42 disulfide bridge and terminating at a boundary formed by the side chains of Lys60-Tyr39, which are hydrogen bonded. In this region of tryptase, a four amino acid residue insertion before the corresponding Lys60D, redirects this Lys to form a salt bridge with the inserted Asp60B. In addition, Tyr39 in trypsin is replaced by a Met in tryptase. Together these differences result in a much more open and highly lipophilic binding groove above the disulfide bridge in tryptase into which the lipophilic tail of **46** appears to fit nicely, assuming a similar orientation of binding. The differences between tryptase and trypsin in this binding region may account for much of the SAR and selectivity observed.

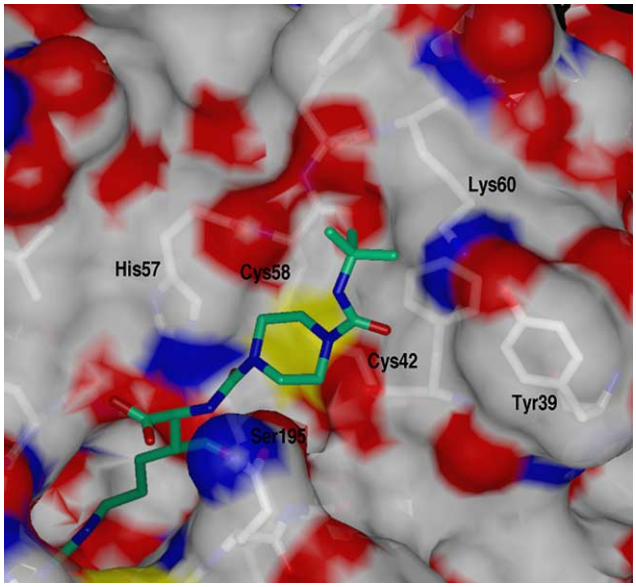


Figure 1. Connolly surface map of the X-ray crystal structure of the trypsin compound **1** complex at 1.7 Å resolution.

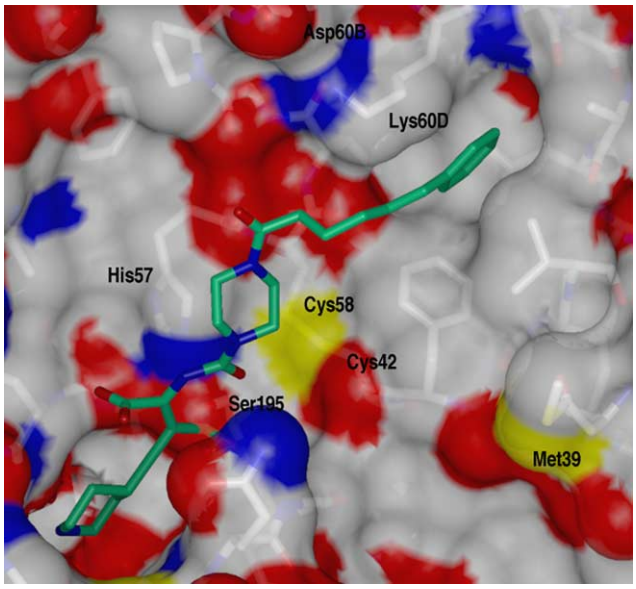


Figure 2. Compound **46** docked into the X-ray crystal structure of tryptase.

Selectivity screening of select compounds against trypsin, thrombin, plasmin, factor Xa, urokinase (uPA), and tissue plasminogen activator (tPA) showed the piperidine containing compounds **3**, **32**, and **46** to be highly selective against thrombin, factor Xa and tPA (Table 2), as was the case with the guanidine-containing compound **1**. Potency toward plasmin was variable, with **32** and **46** being more potent against plasmin, but due to their potency against tryptase they retained a moderate level of selectivity. When compared to compounds **1** and

Table 2. Tryptase inhibition and selectivities for select compounds

	Compound			
	1	**3**	**32**	**46**
Selectivity ratio				
Tryptase IC_{50} (nM)	4	30	1[a,b]	1[a,b]
Trypsin	4.5	62	440	310
Thrombin	2625	>1100	>33,000	>33,000
Plasmin	430	250	380	170
Factor Xa	>8250	>1100	>33,000	>33,000
uPA	135	34	1210	970
tPA	>8250	>1100	>33,000	>33,000

[a] Re-purified and assayed under fluorometric conditions.
[b] Assayed below 1 nM.

3, compounds **32** and **46** showed improved selectivity against trypsin and uPA.

In summary, we have studied the solid-phase synthesis and SAR of a series of piperidine containing N1-activated C4-carboxy azetidinone tryptase inhibitors, which show good selectivity against related serine proteases, with notably improved selectivity against trypsin compared to the previously reported guanidine series. Although the SAR provided above is incomplete, the trends observed provide insight into structural features that can be applied to obtain compounds with improved tryptase specificity. The SAR obtained from the series of compounds described in this paper was successfully applied, in conjunction with additional structural changes, to provide highly potent and selective tryptase inhibitors, described in our previous letter.[9]

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