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Dibasic Inhibitors of Human Mast Cell Tryptase. Part 1: Synthesis and Optimization of a Novel Class of Inhibitors

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Abstract—The synthesis and optimization of a novel class of reversible and active-site directed dibasic inhibitors of human mast cell tryptase are described. The compounds were shown to be both remarkably potent and selective for tryptase with K_i values for optimized inhibitors in the picomolar range. © 2000 Published by Elsevier Science Ltd.

Recent advances in cell biology have led to an improved understanding of the mast cell as a multifunctional, key effector cell in the immune system.[1,2] Emerging data suggest that tryptase, a major mast cell secretory protease, is a potent mediator of mast cell related allergic and inflammatory pathologies, including most notably asthma.[3,4] Tryptase may function in this context through several possible mechanisms including the regulation of potent neuropeptides such as bradykinin,[5] the stimulation of surrounding tissue proliferation and secretion of secondary cellular mediators by activation of the protease-activated receptor (PAR) family[6–8] and possibly through direct amplification of the inflammatory response.[9] Phase II clinical trials with our first generation tryptase inhibitor APC-366 further support the involvement of tryptase in asthma pathology.[10] Herein, we describe the evolution of SAR for a novel second generation series of highly potent and selective dibasic inhibitors of human tryptase.

Several classes of modestly active dibasic inhibitors of tryptase have been reported earlier by Sommerhoff and Tidwell,[11,12] however, these compounds lack the potency and selectivity to be of significant biological or pharmaceutical utility. Recent reports have described classes of dibasic inhibitors with improved potency and selectivity.[13,14] Efforts in our lab commenced with the serendipitous discovery of *p*-xylylenediamine oligomer **8**, a very potent, selective, competitive and reversible tryptase inhibitor. Our goal was the design and optimization of tryptase inhibitors based on this template with an aim toward the identification of novel anti-inflammatory drugs suitable for clinical development.

Chemistry

Scheme 1 illustrates the synthetic strategy employed in the preparation of compounds **7**–**17**. Reaction of a two-fold excess of the appropriate acid chloride with *p*-xylylenediamine efficiently gave diesters **3**–**6**. Direct formation of the external amide in the presence of an excess of *p*-xylylenediamine or 4-aminobenzylamine at elevated temperature afforded the desired diamines **7**–**11** and **12**–**14**, respectively. In the latter case, completely selective acylation of the benzylamine over the aniline nitrogen was observed. Guanidines **15**–**17** are prepared by treatment with either cyanamide or pyrazolecarboxamidine.

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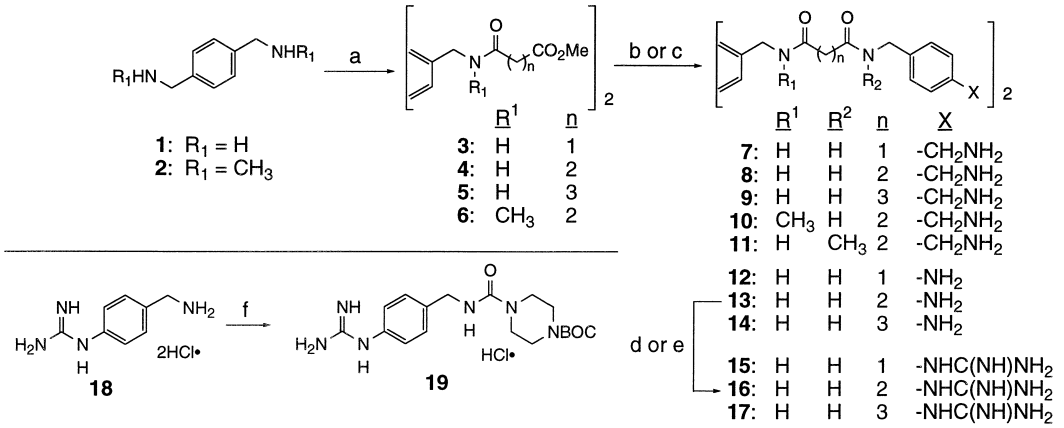


Scheme 1. Synthesis of the dibasic inhibitors. Reagents: (a) $CH_3O_2C(CH_2)_nCOCl$, DCM, DIPEA; (b) *p*-xylylenediamine, 40 °C; (c) 4-aminobenzylamine, 40 °C; (d) cyanamide, 4 M HCl in dioxane, 60 °C; (e) *N*,*N*′-di(BOC)pyrazolecarboxamidine, DMF; TFA; (f) *tert*-butyl (1-piperazinecarboxylate)-4-carbonyl chloride, DIPEA, DCM, MeOH.

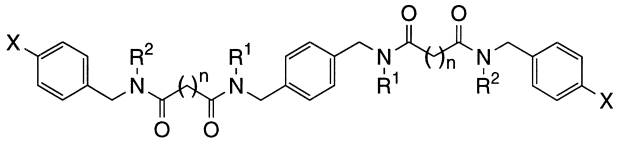
The general synthetic approach employed in the preparation of carbamate linked inhibitors **20**–**26** was analogous. The sequence was carried out through reaction of 1,4-phenylenedimethylene bis-chloroformate[15] with the amino acid of choice under Schotten–Baumann conditions followed by coupling of the resulting diacid with a twofold excess of either 4-guanidinobenzylamine hydrochloride (**18**)[16] or *mono*-*N*-(BOC)-*p*-xylylenediamine[17] followed by BOC group removal in the latter case. Piperazine derived analogue **27** was synthesized by the acylation of **18** with *tert*-butyl (1-piperazinecarboxylate)-4-carbonyl chloride[18] to give guanidine intermediate **19**. Subsequent BOC deprotection and reaction in twofold excess with *cis*-1,5-cyclooctylene bis-chloroformate[19] in aqueous THF at pH 8–9 optimally afforded the symmetrical bis-arylguanidine **27**.

Results and Discussion

Inhibitory potency versus tryptase and related proteases for a series of dibasic polyamides is summarized in Table 1.[20] Succinyl derivatives **8** and **16** demonstrated optimal potency versus tryptase as a function of methylene chain length, although the arylguanidine analogue was approximately 10-fold less active than the corresponding benzylamine ($K_i = 3.0$ and 40 nM, respectively). In both cases $>10^3$-fold selectivity over related proteases screened was observed. While internal amide *N*-methylation is well tolerated in the benzylamine series (compound **10**), *N*-methylation of the external amide, as in the case of **11**, resulted in a dramatic potency loss.

A second series of dibasic inhibitors was designed to resist metabolism at the internal amide through replacement with a carbamate linker. Inhibitory potency and selectivity for this series of analogues is shown in Table 2. Here the glycyl derivatives **20** and **22** were the most potent. While *N*-methylation of the carbamate nitrogen is well tolerated in this series, substitution at the adjacent methylene, as in the case of alanine analogues **24** and **25**, resulted in a significant potency loss with the (*R*,*R*)-diastereomer demonstrating approximately 10-fold greater activity than the (*S*,*S*)-diastereomer. In the bis-arylguanidine series replacement of the core scaffold aromatic ring with alicyclic functionality is well tolerated and a fivefold improvement in activity was observed in the case of cyclooctylene derivative **26**. As a further

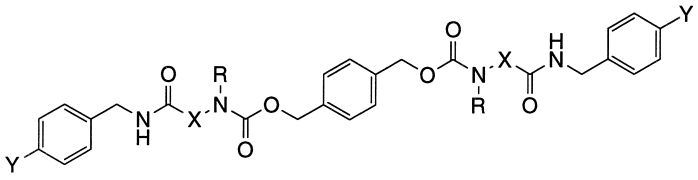
Table 1. Amide series SAR



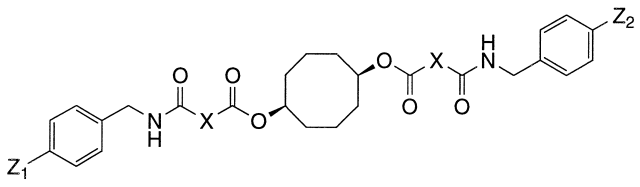
Compound	R^1	R^2	n	X	K_i (μM)[a] Tryptase	Trypsin	Thrombin	Plasmin
7	H	H	1	$-CH_2NH_2$	0.150	317	>1000	>1000
8	H	H	2	$-CH_2NH_2$	0.003	237	>1000	769
9	H	H	3	$-CH_2NH_2$	0.009	274	>1000	>1000
10	Me	H	2	$-CH_2NH_2$	0.005	162	>1000	214
11	H	Me	2	$-CH_2NH_2$	2	>1000	>1000	>1000
15	H	H	1	$-NHC(NH)NH_2$	0.680	73	>1000	>1000
16	H	H	2	$-NHC(NH)NH_2$	0.040	146	>1000	>1000
17	H	H	3	$-NHC(NH)NH_2$	0.100	70	492	>1000

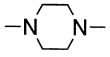
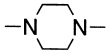
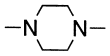
[a]Data reported is for a single determination. See ref 20 for assay protocols.

Table 2. Carbamate series SAR



Compound	R	X	Y	K_i (μM)[a] Tryptase	Trypsin	Thrombin	Plasmin
20	H	-CH_2-	-NHC(NH)NH_2	0.010	56	>1000	>1000
21	H	-$(CH_2)_2$-	-NHC(NH)NH_2	0.090	72	>1000	>1000
22	H	-CH_2-	-CH_2NH_2	0.0006	84	>1000	>1000
23	Me	-CH_2-	-CH_2NH_2	0.005	50	>1000	>230
24	H	-(*R*)-CH(CH_3)-	-CH_2NH_2	0.031	38	>1000	>1000
25	H	-(*S*)-CH(CH_3)-	-CH_2NH_2	0.366	273	>1000	83



Compound	X	Z_1	Z_2	K_i (μM)[a] Tryptase	Trypsin	Thrombin	Plasmin
26	-$NHCH_2$-	-NHC(NH)NH_2	-NHC(NH)NH_2	0.002	78	>1000	>1000
27		-NHC(NH)NH_2	-NHC(NH)NH_2	0.00007[b]	39	435	494
28		-NHC(NH)NH_2	-H	15	182	>1000	>1000
29		-H	-H	101	44	14	>1000

[a]Data reported is for a single determination. See ref 20 for assay protocols.
[b]Value reported is the dissociation constant obtained by IC_{50} determination at variable tryptase and inhibitor concentrations.[21]

modification of the core scaffold we focused on replacement of the external amide. The piperazine ring was chosen as a glycyl isostere that would serve to introduce a urea linkage in place of the external amide. Arylguanidine **27** demonstrated a marked improvement in potency with a K_i value of 0.07 nM[21] and selectivity over trypsin and thrombin approaching 10^5. Asymmetric derivative **28**, which differs from the optimized lead only in the absence of a second guanidine, illustrates the importance of both basic moieties for potent tryptase inhibition. Removal of the second guanidine affords a nearly complete loss of activity for the intact core scaffold analogue **29**. The dramatic loss in potency that is observed on sequential removal of the terminal guanidines from the inhibitor suggests that core scaffold interactions contribute little to the overall binding energy of lead **27**.

The X-ray crystal structure of tryptase has been recently determined[22,23] and revealed a structure comprised of four quasi-equivalent monomers arranged in a flat frame-like structure, with each of the four subunits contributing a functional catalytic site. A spatially constricted central pore defines the active site domain with directly adjacent S1 pockets of the order of 20 Å apart. A binding mechanism for the dibasic inhibitors described herein could be envisioned where the central pore is spanned with the terminal nitrogen bases docked into adjacent S1 pockets.

In summary, we have designed and optimized a series of remarkably potent, selective, competitive and reversible tryptase inhibitors, exemplified by compound **27**. Although limited by poor oral absorption, the optimized dibasic inhibitors outlined herein represent valuable biological and clinical tools aimed at the further elucidation of tryptase pathology in asthma and related allergic and inflammatory diseases. Further SAR development leading to the selection of an optimized clinical candidate is described in an additional report following this paper.

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18. To triphosgene (25 g, 84.2 mmol) in DCM (200 mL) at 0 °C was slowly added a solution of *tert*-butyl 1-piperazinecarboxylate (40 g, 214.8 mmol) and pyridine (35 mL, 432.7 mmol) in DCM (100 mL). The mixture was warmed to room temperature over 30 min followed by addition of aq HCl (0.1 N, 200 mL) then drying the organic layer over anhydrous magnesium sulfate. Filtration and concentration gave *tert*-butyl 1-piperazinecarboxylate-4-carbonyl chloride (45.6 g, 85%) as a pale-yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.70 (m, 2H), 3.60 (m, 2H), 3.50 (m, 4H), 1.50 (s, 9H).
19. *cis*-1,5-Cyclooctanediol (20.2 g, 0.14 mol) and potassium carbonate (41.4 g, 0.3 mol) were taken into acetonitrile (250 mL) then cooled to 0 °C followed by dropwise addition of phosgene (1.9 M in toluene, 220 mL, 0.42 mol) over 1 h followed by warming to rt and stirring over 12 h. Ether (1 L) was added and the suspension filtered. Concentration followed by recrystallization from hexane gave *cis*-1,5-cycloocetylene bis-chloroformate as a colorless crystalline solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.00–4.85 (m, 2H), 2.20–1.60 (m, 12H).
20. Stock solutions of enzyme (0.06 mg/mL) were prepared with 10 mM 2-(*N*-morpholino)ethane sulfonic acid (MES) containing 2 mM calcium chloride, 20% glycerol and 0.05 mg/mL heparin. Approximately 1 mg of inhibitor was dissolved in DMSO (0.2 mL) and diluted 10-fold into buffer containing 50 mM Tris hydrochloride (pH 8.2), 100 mM sodium chloride, and 0.05% Tween-20. Seven additional threefold dilutions were made from the initial dilution into the same buffer supplemented with 10% DMSO. Aliquots (0.05 mL) from each of the eight dilutions were transferred to individual wells in a 96-well U-bottom microtiter plate. Enzyme stock solution (25 μL) was added to each well and incubated for 1 h at rt. The enzymatic reaction was initiated with addition of tosyl-Gly-Pro-Lys-*p*-nitroanilide (25 μL, 0.5 mM final concentration) and the reaction was followed spectrophotometrically at 405 nm. Initial velocity measurements calculated from the progress curves by a kinetic analysis program (*BatchKi*; Peter Kuzmic, University of Wisconsin, Madison, WI) were used to determine apparent inhibition constants. The standard error for single determinations is a factor of 2.
21. K_i value for the tight binding inhibitor **27** was determined by the following procedure. Tryptase (0.25, 0.50, 1.0, and 2.0 nM) and inhibitor (40, 110, 340, and 680 pM) were varied in preincubation mixtures for 1 h at rt followed by addition of substrate as before. The IC_{50} of the compound was then determined at each concentration of tryptase. The *y*-intercept of a replot of IC_{50} (ordinate) versus tryptase concentration (abscissa) produces a value for the apparent dissociation constant from which the true dissociation constant of the tight binding competitive inhibitor was calculated.
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