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Dibasic Inhibitors of Human Mast Cell Tryptase. Part 2: Structure–Activity Relationships and Requirements for Potent Activity

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Abstract—Detailed structure–activity relationships (SARs) for a series of dibasic human tryptase inhibitors are presented. The structural requirements for potent inhibitory activity are remarkably broad with a range of core template modifications being well tolerated. Optimized inhibitors demonstrate potent anti-asthmatic activity in a sheep model of allergic asthma. APC-2059, a dibasic tryptase inhibitor with subnanomolar activity, has been advanced to phase II clinical trials for the treatment of both psoriasis and ulcerative colitis. © 2000 Published by Elsevier Science Ltd.

The mast cell is proposed to play a central role in modulation of the inflammatory response[1,2] and tryptase, a major mast cell secretory protease, is implicated as a key mediator of mast cell related allergic and inflammatory pathologies, including asthma.[3–6] Phase II clinical trial results with the first generation tryptase inhibitor APC-366 provide support for the involvement of tryptase in asthma pathology and possibly other inflammatory diseases.[7] As an extension of our efforts to identify novel tryptase inhibitors for development as antiinflammatory drugs, a series of analogues of the potent tryptase inhibitor APC-1390 (**7**)[8] were synthesized. A major focus was to vary the terminal nitrogen base as a range of amino-, amidino-, and guanidino- derivatives while retaining an optimal core scaffold. Herein, we highlight the structure–activity relationships (SARs) and structural requirements for potent tryptase inhibitory activity in a series of C_2-symmetrical and asymmetric dibasic analogues of APC-1390 as well as the potent antiinflammatory activity of lead analogue APC-2059 (**60**) in a sheep model of allergic asthma.

Chemistry

Scheme 1 illustrates the synthetic approach employed in the preparation of the dibasic inhibitors presented. The asymmetric inhibitors **7**–**28** (Table 1) were synthesized by stepwise conversion of *cis*-1,5-cyclooctanediol (**1**) to chloroformate (**3**) followed by reaction with deprotected guanidine (**5**)[8] to give precursor (**6**). Subsequent BOC deprotection and piperazine acylation afforded the asymmetric inhibitors. The C_2-symmetrical inhibitors **29**–**53** (Tables 2 and 3) were synthesized by the initial conversion of bis-chloroformate (**2**)[8] to piperazine derivative (**4**), which serves as a general precursor.

As before, BOC group deprotection and acylation of the diamine in this case with a twofold excess of electrophile affords the symmetrical inhibitors. In the final series (Table 4), we focused on modification of the core scaffold cyclooctane fragment. Inhibitors **54**–**65** in this series were

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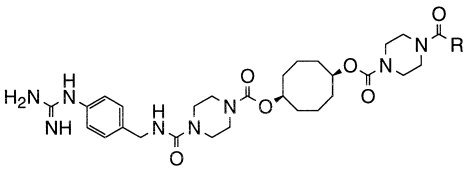
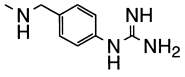
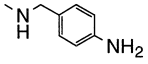
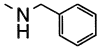
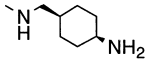
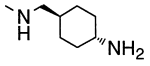
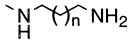
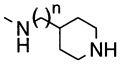
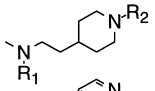
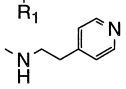
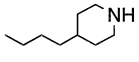
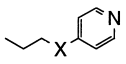
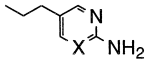
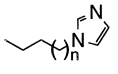
generally prepared by initial BOC-deprotection of guanidine (**5**) followed by reaction in twofold excess with a bis-chloroformate or an α,ω-diacid chloride.

Results and Discussion

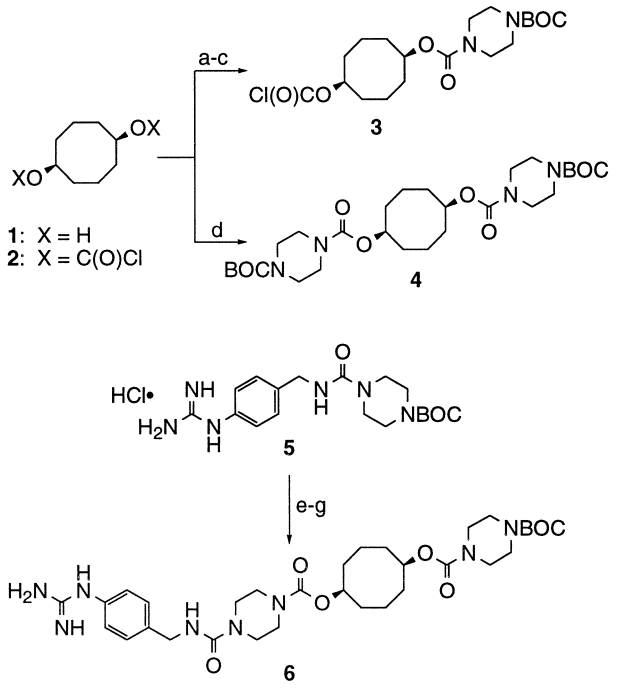
Initially, we set out to probe terminal nitrogen base structural requirements in a series of asymmetric tryptase inhibitors wherein one arylguanidine is replaced by a range of aliphatic and heterocyclic nitrogen bases (Table 1). Generally, replacement of one arylguanidine in APC-1390 (**7**) with a range of aliphatic amines and heterocyclic nitrogen bases is tolerated, although poor activity is observed for weakly basic derivatives, such as aniline **8**, pyridine **20** and the 2-aminopyrimidine **25**. A terminal nitrogen base pK_a of $\geq$6 appears to be required for potent inhibition. Both imidazole and piperidine

Table 1. Asymmetric series SAR



R	Compound	K_i (μM)[a]			
		Tryptase	Trypsin	Thrombin	Plasmin
	**7**	0.00007[b]	39	435	494
	**8**	0.820	126	>1000	>1000
	**9**	15	182	>1000	>1000
	**10**	0.078	171	274	>1000
	**11**	0.001	141	>1000	>1000
	**12** $n=1$	0.044	138	>1000	>1000
	13 $n=2$	0.004	132	>1000	>1000
	14 $n=3$	0.0005	129	>1000	>1000
	**15** $n=1$	0.070	148	>1000	>1000
	16 $n=2$	0.003	121	>1000	>1000
	17 $n=3$	0.240	80	>1000	>1000
	**18** R_1 = Me, R_2 = H	0.680	126	>1000	>1000
	19 R_1 = H, R_2 = Me	3	110	269	>1000
	**20**	0.900	131	>1000	>1000
	**21**	0.004	262	>1000	>1000
	**22** X = S	0.080	156	741	>1000
	23 X = NH	0.097	111	>1000	>1000
	**24** X = CH	0.180	156	>1000	>1000
	25 X = N	41	165	>1000	>1000
	**26** $n=1$	>1000	158	>1000	>1000
	27 $n=2$	1	144	>1000	>1000
	28 $n=3$	0.030	171	>1000	>1000

[a]Data reported is for a single determination. See ref 20 in the first paper in this series for assay protocols.[8]
[b]Value reported is the dissociation constant obtained by IC_{50} determination at variable tryptase and inhibitor concentrations. See ref 21 in the first paper in this series for experimental details.[8]



Scheme 1. Dibasic inhibitor synthetic strategy. Reagents and conditions: (a) **1**, 1.0 equiv $COCl_2$, $PhCH_3$, MeCN, K_2CO_3; (b) 1.0 equiv *tert*-butyl 1-piperazinecarboxylate, DIPEA, THF; (c) repeat (a); (d) **2**, *tert*-butyl 1-piperazinecarboxylate, DIPEA, THF; (e) TFA; (f) **3**, DIPEA, DMF; (g) aq NaOH.

serve as effective terminal nitrogen bases in the asymmetric series, but either urea or piperidine *N*-methylation (**18**–**19**) results in a substantial loss of activity. In the case of cyclohexylamines the *trans*-isomer **11** demonstrated nearly 100-fold greater potency than the *cis*-isomer **10**. For the terminal primary amines (**12**–**14**) a charge–charge distance requirement for optimal activity is apparent. In this series, a 10-fold improvement in potency is obtained for each methylene insertion on proceeding from the propylamine to the pentylamine homologue.

Inhibitory potency versus tryptase and related proteases for a series of symmetrical urea derivatives (**29**–**44**) is illustrated in Table 2. Simultaneous replacement of both guanidines in lead **7** with alternative nitrogen bases affords a SAR that is more responsive in comparison to the asymmetric series. While replacement of the guanidine moiety for amidine (**30**) is well tolerated in this series, a 100-fold loss of potency and selectivity is observed when a guanidine is replaced by an amidine (**30**). The most active non-arylguanidine analogue in this series is benzylamine **36**, which is subnanomolar versus tryptase and extremely selective. Benzylamine *N*-methylation (**37**) or extension to the phenethylamine homolog **38**, results in a dramatic loss of activity. Aromatic ring saturation (**32**–**33**) is also tolerated, although a reversal in the SAR as a function of methylene spacer length is observed when compared to the corresponding benzamidines (**30**–**31**). While the analogous ethylideneamino-piperidines (**34**–**35**) are inactive, piperidine **39** surprisingly is only fourfold less active than the corresponding guanidine **33**. Interestingly, the amino-methyl [2.2.2]bicyclooctane (**41**) is modestly active, although 20-fold less potent than the sterically less demanding cyclohexylmethylamine **40**. Potent inhibitors are also obtained by appending a simple straight-chain alkylamine onto the core scaffold as in the asymmetric series. For the symmetrical analogues **42**–**44**, the 1,5-pentanediamine derived inhibitor is optimal.

A SAR for a related series of symmetrical amide linked derivatives is illustrated in Table 3. In general, removal of the external carbamyl nitrogen present in the urea series is very well tolerated. Notably, both the phenylacetic and the hydrocinnamic benzamidines **47** and **48** are remarkably potent and more active than the corresponding carbamyl analogues **30** and **31**. In contrast, phenylguanidines **45** and **46** exhibit a 400-fold loss in activity on proceeding to the hydrocinnamic case. The amidinopiperidines **49** and **50**, as well as the phenethylamine **51**, are also substantially more active than their corresponding carbamyl derivatives. Although the optimal piperidine in this series (**52**) is moderately potent, imidazole **53** is nearly inactive.

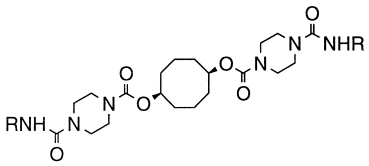
Table 4 summarizes inhibitory potency for tryptase and related proteases of the core scaffold analogues **54**–**65**. Both alicyclic, heterocyclic and aromatic templates as well as straight chain alkylene variations are well tolerated. 1,3-Adamantanedimethanol derivative **58** illustrates the remarkable tolerance for steric bulk in this region. In the case of straight chain α,ω-diacid derived analogues **61**–**65**, a clear SAR as a function of chain length is evident with the six methylene homologue (**63**) affording optimal subnanomolar activity.

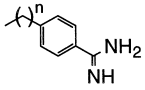
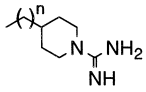
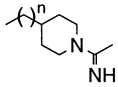
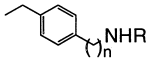
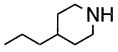
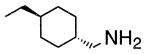
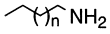
An inter-active-site bridging mechanism has been proposed for dibasic tryptase inhibitors of this general type wherein the two terminal nitrogen bases of the inhibitor dock into the S1 sites of adjacent A and D monomer subunits.[6,9] The core scaffold then serves to span the active-site gap of roughly 20 Å. These highly flexible inhibitors have low energy conformers well suited to span this distance with the central core of the scaffold making minimal van der Waals contact with the enzyme surface. This binding model provides a rationale for the range of core scaffold variations tolerated in our survey.

In preclinical studies APC-2059 (**60**) demonstrated optimal pharmacokinetics and safety. Additionally, in a sheep model of allergic asthma,[10] **60** was effective at blocking both the late phase and hyperresponsive phase as determined by measuring specific lung resistance as a function of time after antigen challenge with inhaled *Ascaris suum* (Fig. 1).

In summary, a remarkably broad SAR exists for the optimized series of potent, selective, competitive and reversible inhibitors of human tryptase described herein, with a range of core template and terminal nitrogen base modifications being well tolerated. With the exception of the rapidly metabolized primary amines, we have found that in the symmetrical classes only guanidine and amidine nitrogen base analogues are of

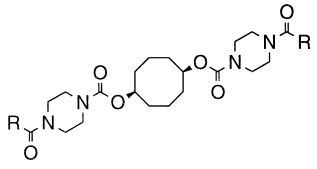
Table 2. Symmetrical urea series SAR

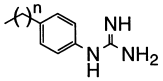
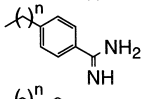
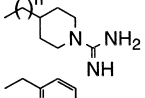
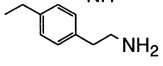
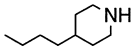
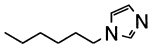


R	Compound	K_i (μM)[a]			
		Tryptase	Trypsin	Thrombin	Plasmin
	**29**	101	44	14	>1000
	**30** $n=1$	0.009	25	60	134
	31 $n=2$	0.440	66	36	60
	**32** $n=1$	0.610	>1000	146	>1000
	33 $n=2$	0.030	850	112	632
	**34** $n=1$	>1000	>1000	>1000	>1000
	35 $n=2$	255	>1000	>1000	>1000
	**36** $n=1$, R = H	0.0001	219	>1000	>1000
	37 $n=1$, R = Me	6	>1000	>1000	>1000
	38 $n=2$, R = H	1	>1000	>1000	570
	**39**	0.115	>1000	>1000	>1000
	**40**	0.016	>1000	>1000	>1000
	**41**	0.300	95	>1000	>1000
	**42** $n=2$	0.510	>1000	>1000	>1000
	43 $n=3$	0.011	754	>1000	>1000
	44 $n=4$	1	>1000	>1000	>1000

[a]Data reported is for a single determination. See ref 20 in the first paper in this series for assay protocols.[8]

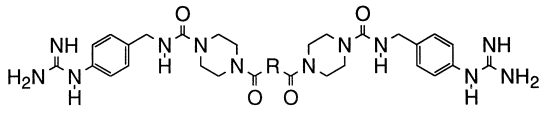
Table 3. Symmetrical amide series SAR

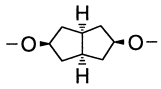
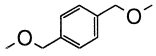
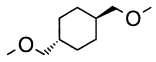
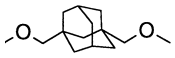
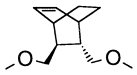
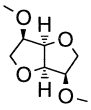


R	Compound	K_i (μM)[a]			
		Tryptase	Trypsin	Thrombin	Plasmin
	**45** $n=1$	0.0005	82	255	>1000
	46 $n=2$	0.210	>1000	537	>1000
	**47** $n=1$	0.0002	11	34	155
	48 $n=2$	0.0004	21	29	66
	**49** $n=1$	0.005	445	130	>1000
	50 $n=2$	0.002	171	213	>1000
	**51**	0.002	>1000	>1000	>1000
	**52**	0.100	>1000	>1000	>1000
	**53**	46	>1000	>1000	>1000

[a]Data reported is for a single determination. See ref 20 in the first paper in this series for assay protocols.[8]

Table 4. Core scaffold series SAR



Compound	R	K_i (μM)[a]			
		Tryptase	Trypsin	Thrombin	Plasmin
54		0.028	4	>1000	>1000
55		0.0004	20	123	3
56		0.0004	17	>1000	>1000
57		0.003	28	>1000	>1000
58		0.010	17	363	576
59		0.008	15	138	700
60		0.0001[b]	15	138	700
61	$-(CH_2)_4-$	0.044	54	>1000	316
62	$-(CH_2)_5-$	0.004	52	>1000	>1000
63	$-(CH_2)_6-$	0.0001	34	>1000	>1000
64	$-(CH_2)_7-$	0.0005	29	>1000	>1000
65	$-(CH_2)_8-$	0.0007	20	>1000	>1000

[a]Data reported is for a single determination. See ref 20 in the first paper in this series for assay protocols.[8]
[b]Value reported is the dissociation constant obtained by IC_{50} determination at variable tryptase and inhibitor concentrations. See ref 21 in the first

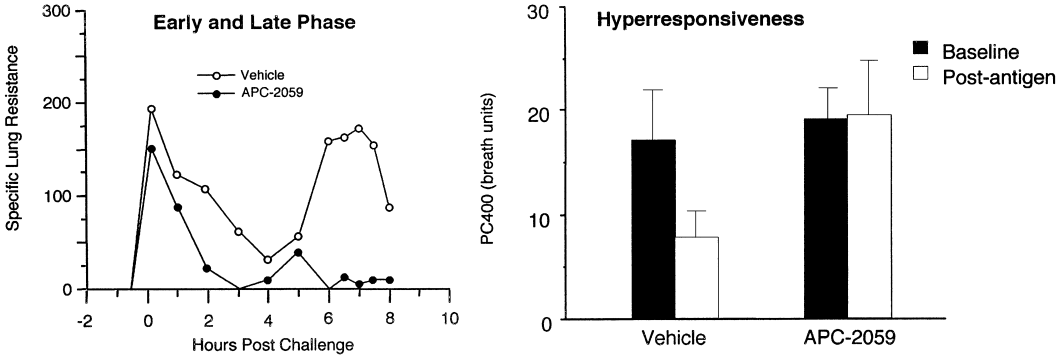


Figure 1. Sheep model efficacy data for APC-2059. Allergic sheep ($n=2$) were administered (by aerosol in 3 mL PBS buffer) a dose of APC-2059 (500 mg) or vehicle control at 0.5 h before, and 4 and 24 h following inhalation challenge with *Ascaris suum* antigen. Specific lung resistance was measured at time points indicated in the graph on the left. Twenty-four hours after antigen challenge, airway responsiveness was measured, defined as the cumulative carbamylcholine dose in breath units required to increase specific lung resistance by 400% (right). Airway resistance of each treated animal was compared with control (in which the same animal was dosed with vehicle only).

sufficient inhibitory potency to merit further evaluation as potentially valuable clinical or biological tools. Optimized inhibitors demonstrate potent antiinflammatory activity in a sheep model of allergic asthma. APC-2059 (**60**) has been advanced to phase II clinical trials for the treatment of both psoriasis and ulcerative colitis.

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