Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1629–1633

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Dibasic Inhibitors of Human Mast Cell Tryptase. Part 3: Identification of a Series of Potent and Selective Inhibitors Containing the Benzamidine Functionality

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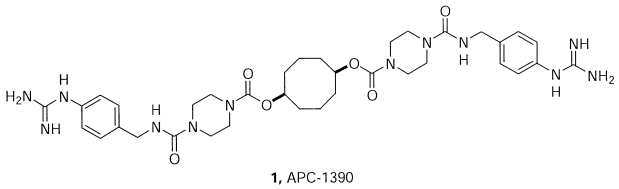
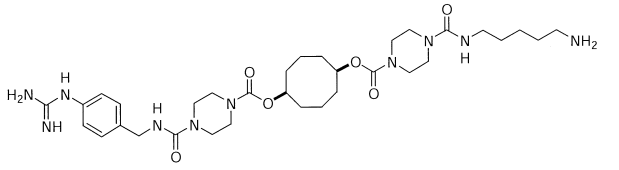
Received 8 February 2001; accepted 27 March 2001

Abstract—A survey of charged groups and linkers for a series of symmetrical and unsymmetrical dibasic inhibitors is described, leading to several classes of potent and selective inhibitors. In particular, the benzamidine functionality was identified as the most potent charged group investigated.

The development of protease inhibitors as potential human therapeutic agents is an active area of research in the pharmaceutical industry.[1] In particular, discovery of inhibitors of serine proteases such as thrombin,[2,3] factor Xa,[3] and elastase[4] has been vigorously investigated in recent years. Another serine protease, mast cell tryptase, has been implicated in certain allergic and inflammatory disease states.[5–8] Furthermore, an inhibitor of tryptase, APC-366, has been validated in a clinical setting for the modulation of the asthmatic response.[8] In previous reports, we described a series of dibasic inhibitors of tryptase, leading to the identification of APC-1390 (**1**), a potent and selective tryptase inhibitor ($K_i = 70$ pM).[9] Furthermore, variations in the cyclooctane core, charged functionality (P_1 recognition element), and unsymmetrical, dibasic analogues of **1** were also reported.[9,10] A representative potent and selective unsymmetrical derivative of **1** is the primary amine **2** ($K_i = 500$ pM). Herein we would like to present additional SARs directed toward the identification of potent and selective tryptase inhibitors related to APC-1390. In particular, our efforts focused on evaluation of different charged and linker functionalities to accomplish these objectives.



1, APC-1390



2

Syntheses of several representative side-chain fragments are shown in Scheme 1. These were incorporated into the target inhibitors according to conditions described in Scheme 2. Typically the symmetrical inhibitors were prepared by reaction of the P_1–P_2 fragments (200 mol%) under basic conditions with the bis-chloroformate of either 1,4-bis(hydroxymethyl)benzene or *cis*-(1,4-dihydroxy)cyclooctane.[9] Unsymmetrical inhibitor **23** was prepared by reaction of excess chloroformate **21**[10] with (*N*-BOC-piperidino)urea derivative **12** to give the monochloroformate **22**, which was then condensed with the benzamidine moiety **16**. Deprotection of the BOC group afforded the desired unsymmetrical derivative **23**. Alternatively, monochloroformate **24**[10] could be converted to **25** after introduction of the benzamidine moiety **7**. Subsequent BOC deprotection of **25** and

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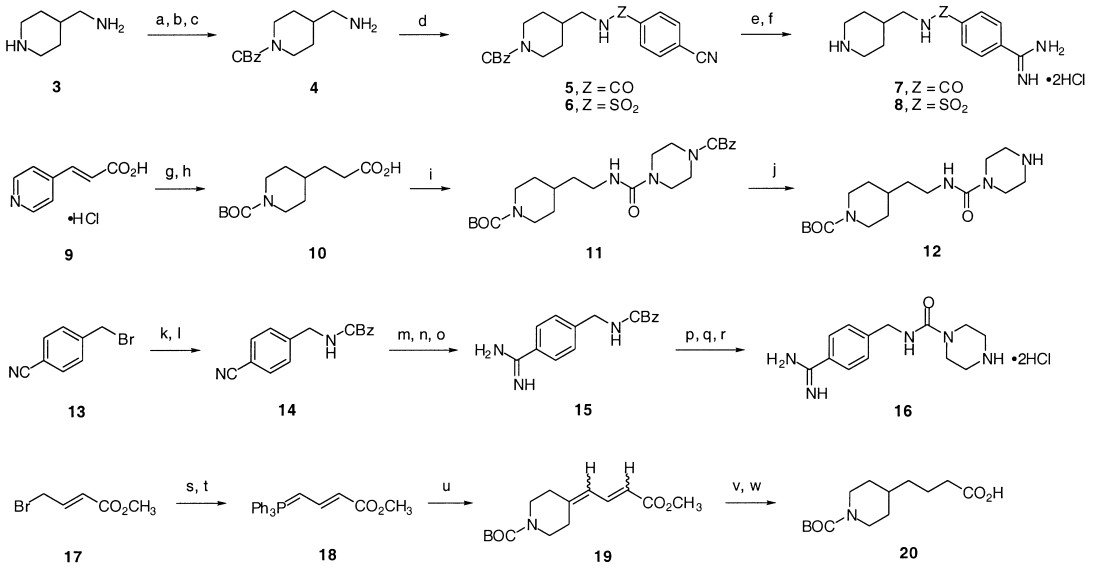
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00254-2

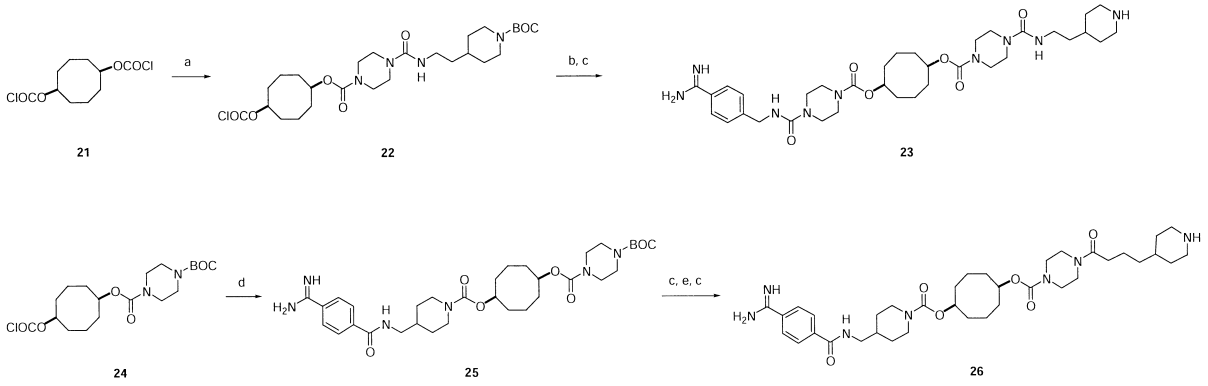
acylation of the distal piperazine with 4-(*N*-*t*-butyloxycarbonyl-4-piperidinyl)butyric acid (**20**) and BOC-deprotection gave unsymmetrical inhibitor **26**. Other derivatives were prepared in analogous fashion employing similar amine-, amidine-, and guanidine-containing fragments. Finally, all inhibitors were isolated as their HCl salts after preparative reverse-phase HPLC.

Tryptase inhibition data for a series of similar symmetrical inhibitors containing various charged and linking groups based on either a 1,4-benzenedimethanol or *cis*-(1,4-cyclooctane)diol central scaffold is reported in Table 1. K_i values for mast cell tryptase, thrombin, plasmin, and trypsin were obtained according to methods described previously.[9] Replacement of the 1,4-diaminobutane unit of **29** with the more rigid 4-aminomethylpiperidine linker in inhibitor **30** resulted in a 5-fold increase in potency. However, incubation of primary amine **30** with plasma amine oxidase (PAO) showed that rapid metabolism took place ($V_{max}/K_m = 1870$), indicating that the primary amine of the 4-aminomethylpiperidine unit was unsuitable as a charged group for in vivo applications.

Inhibitors **31** and **32**, containing the more rigid P_1–P_2 units (R fragment), are clearly potent and selective for tryptase (Table 1). Furthermore, a 60-fold preference for the benzamidine **31** over the primary amine **30** was noted. The benzamidine **32** possessed inhibitory potency comparable to inhibitor **31**, indicating that this charged group/linker combination is critical for optimum

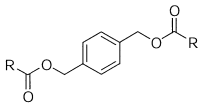


Scheme 1. Preparation of key charged fragment-linker and distal element-linker moieties. Reagents and conditions: (a) PhCHO, toluene, reflux; (b) CBz-Cl, DIPEA; (c) $KHSO_4$, H_2O, then NaOH; (d) 4-cyanobenzoyl chloride or 4-cyanobenzenesulfonyl chloride, DIPEA, CH_2Cl_2; (e) $H_2NOH{\cdot}HCl$, DIPEA, EtOH, reflux; (f) H_2, 10% Pd–C, EtOH–AcOH (1:1), then HCl; (g) H_2, PtO_2, AcOH–H_2O; (h) NaOH, $(BOC)_2O$, then $KHSO_4$; (i) DPPA, Et_3N, benzene, 90 °C, then *N*-CBz-piperazine·HCl, Et_3N; (j) H_2, 10% Pd–C, EtOH; (k) NaN_3, aqueous acetone, reflux; (l) Ph_3P, 5% H_2O in THF, *N*-CBz-benzotriazole; (m) H_2S, Et_3N, pyridine; (n) CH_3I, acetone, reflux; (o) NH_4OAc, MeOH, reflux, then NaOH; (p) 30% HBr in AcOH; (q) 4-(*tert*-butyloxycarbonyl)piperazine-1-carbonyl chloride, DIPEA, DMF; (r) 4 N HCl in 1,4-dioxane; (s) triphenylphosphine, toluene; (t) NaOH, H_2O; (u) 4-*N*-BOC-piperidinone, toluene, 95–105 °C; (v) H_2, 10% Pd–C, EtOH; (w) NaOH, THF–H_2O–MeOH (3:1:1), then $KHSO_4$.

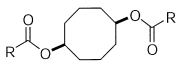


Scheme 2. Representative syntheses of unsymmetrical tryptase inhibitors. Reagents and conditions: (a) **12**, DIPEA, CH_2Cl_2; (b) **16**, DIPEA, DMF; (c) HCl or TFA; (d) **7** (100 mol%), DIPEA, DMF, then NaOH; (e) **20**, EDC, HOBt, DMF.

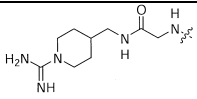
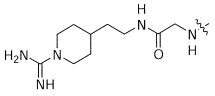
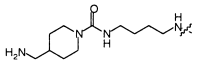
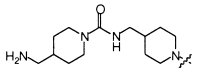
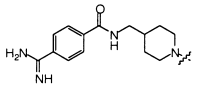
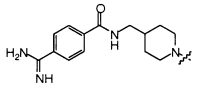
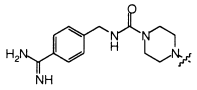
Table 1. Inhibition constants of tryptase and other serine proteases for symmetrical dibasic inhibitors derived from scaffold **A** or **B**



A



B

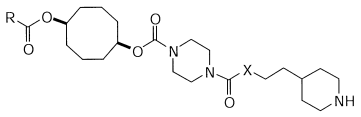
Compound	Scaffold	R	K_i (μM)			
			Tryptase	Thrombin	Plasmin	Trypsin
27	A		0.155	386	> 1000	466
28	A		0.110	423	> 1000	> 1000
29	A		0.112	> 1000	> 1000	> 1000
30	A		0.024	> 1000	> 1000	515
31	A		0.0004	42.2	0.006	4.4
32	B		0.00085	20.8	62.2	4.67
33	B		0.0087	59.5	134	25

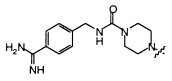
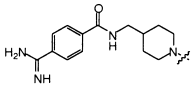
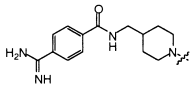
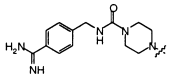
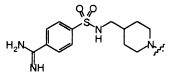
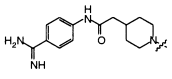
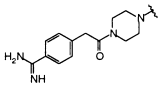
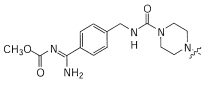
activity. In the cyclooctane series, preference for the amidinobenzamide/aminomethylpiperidinyl unit **7** over amidinobenzyl/piperazinyl fragment **16** was observed, as indicated by the 10-fold potency increase for **32** relative to benzamidine **33**. Lastly, the alkyl amines and guanidines represented in Table 1 were much less active against tryptase than the corresponding benzamidine derivatives. Next, additional SAR studies of unsymmetrical inhibitors similar to compound **2** were undertaken, employing the benzamidine motif incorporated in inhibitors **31** and **32**.

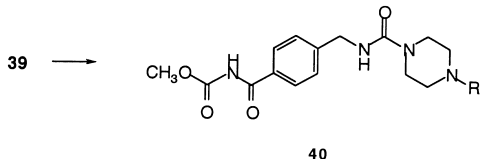
The piperidine moiety, the most potent terminal nitrogen base identified in our previous report,[10] was used for these studies since other charged groups incorporated at the distal site followed trends observed for compounds reported in the phenylguanidine series. Tryptase inhibition data for these benzamidine derivatives are shown in Table 2. Replacement of one benzamidine moiety of symmetrical inhibitor **32** with (piperidino)amide and (piperidino)urea fragments afforded compounds **26** and **34** that were 19-fold less active than the parent inhibitor **32**. Furthermore, compounds **26** and **34** demonstrate that the nature of the linkage (amide or urea) at the distal site does not significantly contribute to binding of the inhibitor to the enzyme. In addition, fragment **16** was the preferred charged group in the asymmetric series (cf. **23** to **34**, Table 2), whereas fragment **7** provided the more potent symmetric inhibitor **32** (Table 1). Table 2 also shows further variations in the charged group and linker fragments. Benzamidines **23** and **35** were the most potent and selective tryptase inhibitors in this series. Furthermore **23** was about 10 times more potent than the parent symmetrical inhibitor **33**. Other variations reported in this table include the retro-inverso amide analogue **37** and sulfonamide **36**, both of which were less potent than either **23** or **35**. Lastly, the carbamate derivative of amidine **23** (**39**) was prepared as a potential prodrug[11,12] and possessed modest activity against tryptase. Studies addressing the viability of **39** as a prodrug were not pursued since this material, isolated as the bis-HCl salt, proved to be unstable in aqueous solution. We believe that hydrolysis of **39** to the corresponding acyl methylcarbamate **40** was taking place (Scheme 3), based on LC–MS data and control experiments with the parent *N*-(methoxycarbonyl)benzamidine.[13,14]

In summary, we have identified potent and selective dibasic inhibitors of mast cell tryptase, including **23**, **31**, **32**, and **35**, each of which features a benzamidine charged group. Future reports will outline additional modifications based on these inhibitors, including monocharged derivatives.

Table 2. Inhibition constants of tryptase and other serine proteases for P_1–P_2 variations of unsymmetrical dibasic inhibitors



Compound	R	X	K_i (μM)			
			Tryptase	Thrombin	Plasmin	Trypsin
23		NH	0.00091	111	287	47.2
26		CH_2	0.0164	31.9	320	37.4
34		NH	0.016	41.2	279	38.8
35		CH_2	0.00112	102	310	52.1
36		NH	0.028	>1000	>1000	16.8
37		NH	0.0089	52.9	293	21.1
38		NH	0.0067	94.4	>1000	63.1
39		NH	0.0406	561	>1000	554



Scheme 3. Hydrolytic instability of methoxycarbonylbenzamidine prodrug **39**.

Acknowledgements

The authors would like to thank Mark Dreyer and Liling Fang for analytical support, and Mike Venuti and Heinz Gschwend for their guidance with this project. Bayer AG is also acknowledged for financial support of the tryptase research program at Axys Pharmaceuticals, Inc.

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10. Part 2: Rice, K. D.; Wang, V. R.; Gangloff, A. R.; Kuo, E. Y.-L.; Dener, J. M.; Newcomb, W. S.; Young, W. B.; Putnam, D.; Cregar, L.; Wong, M.; Simpson, P. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2361.
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13. Control experiments with *N*-(methoxycarbonyl)benzamidine indicate that aqueous solutions containing this functionality at

low pH may lead to this hydrolysis product. Solutions of *N*-carboxymethylphenylamidine in 50% aqueous acetonitrile were stirred overnight at either pH 2.0 (with added hydrochloric acid) or pH 6.8 (no added acid). HPLC–MS analysis of both solutions showed that hydrolysis to the corresponding acyl carbamate had occurred only in the presence of acid.

14. After these studies were completed, a similar observation for an *N*-(ethoxycarbonyl)benzamidine prodrug was reported: Shahrokh, Z.; Lee, E.; Olivero, A. G.; Matamoros, R. A.; Robarge, K. D.; Lee, A.; Weise, K. J.; Blackburn, B. K.; Powell, M. F. *Pharm. Res.* **1998**, *15*, 434