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Monocharged Inhibitors of Mast Cell Tryptase Derived from Potent and Selective Dibasic Inhibitors

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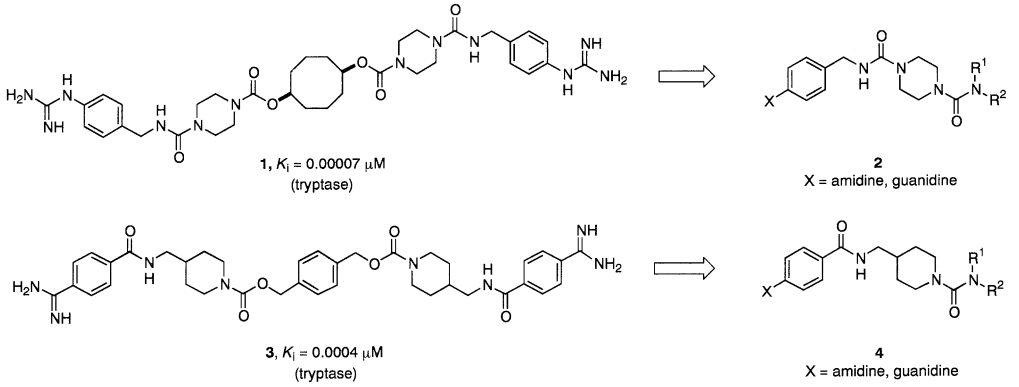
Abstract—Truncation of potent and selective dibasic inhibitors afforded monocharged inhibitors of human mast-cell tryptase. Using two classes of analogues as lead structures, several monocharged derivatives were identified with K_i values ranging from 0.084 to 0.21 μM against the enzyme.

Human mast-cell tryptase is a trypsin-like serine protease that comprises up to 23% of the protein in the mast cell.[1] The enzyme is believed to play a central role in mediating both allergic and inflammatory responses[2–4] and has recently been shown in the clinic to be an important therapeutic target for treatment of asthma.[5] Structurally, the active form of human β-tryptase is a glycosylated tetramer, requiring heparin to stabilize this tetrameric structure.[6,7] Several in vitro and in vivo mechanisms of tryptase inactivation have appeared in the literature. Both neutrophil myeloperoxidase (MPO)[8] and lactoferrin[9] have been shown to inactivate the tetramer by interfering with the heparin-mediated stabilization. In addition, secretory leukocyte protease inhibitor (SLPI) may regulate tryptase activity in vivo through inhibition of the tryptase-dependent degradation of vasoactive intestinal peptide (VIP).[10]

Recently, examples of synthetic inhibitors of this unusual tetrameric protease have appeared in the literature,[11–17] including APC-366, which was the first tryptase inhibitor to enter clinical trials for asthma.[5,12] Furthermore, a series of potent and selective symmetrical and unsymmetrical dibasic inhibitors of human β-tryptase has been reported.[16,17] As part of a program to identify tryptase inhibitors with optimum therapeutic utility, including oral bioavailability, we describe our efforts to develop monocharged analogues based on dibasic inhibitors **1** and **3**, which are potent and selective for tryptase (Scheme 1).[18–20] Our initial synthetic efforts



Scheme 1. General approach to monocharged tryptase inhibitors.

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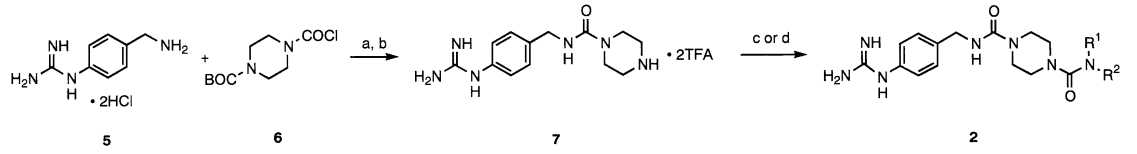
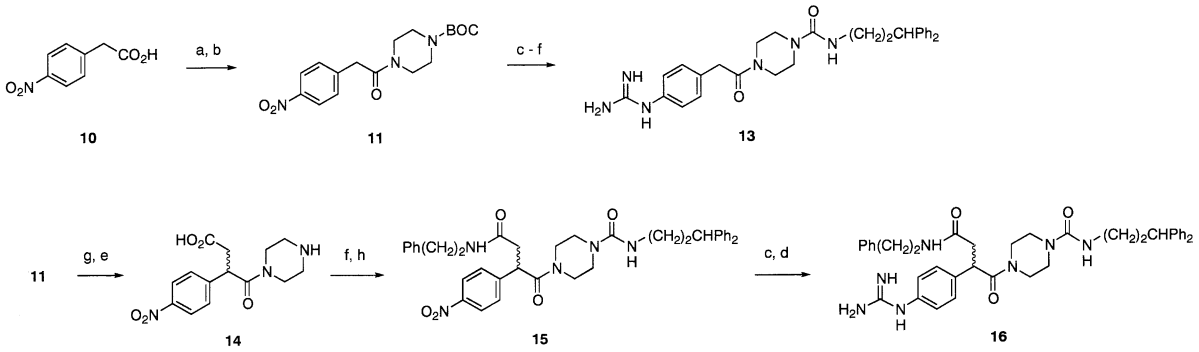


Scheme 2. Synthesis of 4-(guanidinophenyl)urea derivatives (**2**). Reagents and conditions: (a) MeOH, CH_2Cl_2, DIPEA; (b) TFA; (c) R^1–N=C=O (**8**), DIPEA, DMF; (d) R^1R^2NCOCl (**9**), DIPEA, DMF.



Scheme 3. Preparation of 4-(guanidinophenyl)acetylpiperazine derivatives. Reagents and conditions: (a) $SOCl_2$, reflux; (b) *tert*-butyl 1-piperazinecarboxylate, DIPEA, THF, −78 °C; (c) 10% Pd/C, H_2, MeOH; (d) NH_2CN, 4 N HCl in 1,4-dioxane, 60 °C; (e) TFA; (f) $Ph_2CH(CH_2)_2N$=C=O (**12**), DIPEA, DMF; (g) NaH, *t*-butyl bromoacetate, 0 °C; (h) $Ph(CH_2)_2NH_2$, EDC, HOBt, DMF.

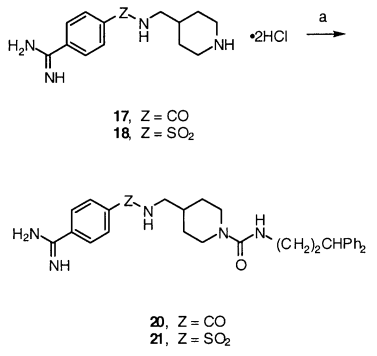
began with replacement of the central core and the second charged group of both **1** and **3** with small molecular fragments to give inhibitors of general structure **2**, containing the piperazine scaffold, and **4**, which possesses a 4-(aminomethyl)piperidine moiety.

Scheme 2 outlines the chemistry employed to prepare the piperazine-containing inhibitors of general structure **2**, varying substitution on the urea nitrogen, whose activity is shown in Table 1. Commercially available 4-aminobenzylamine was converted to 4-guanidinobenzylamine dihydrochloride **5** according to the literature procedure.[21] Formation of the urea **7** was accomplished by reaction of **5** with carbamoyl chloride **6**[22] under basic conditions, followed by BOC deprotection. (4-Guanidinophenyl)piperazine **7** was reacted with either isocyanates (**8**) to give trisubstituted ureas of **2** (R^2 = H), or carbamoyl chlorides (**9**) to afford tetrasubstituted urea derivatives of **2**, where R^2 is alkyl.

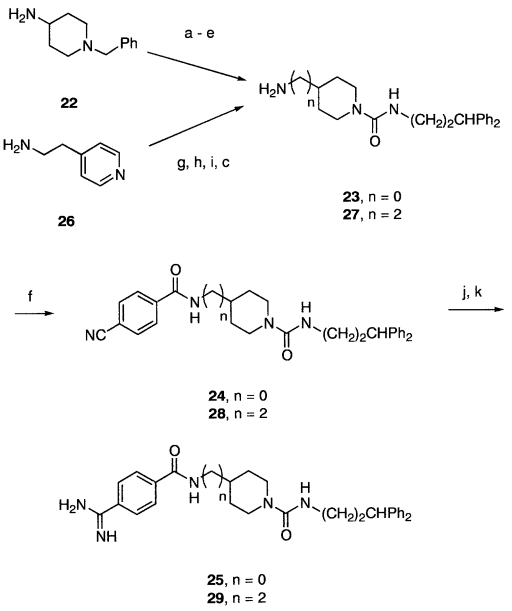
Scheme 3 outlines representative chemical routes used to prepare the inhibitors shown in Table 2, which contain the 4-(guanidinophenyl)acetylpiperazine motif. 4-Nitrophenylacetic acid (**10**) was converted to the acid chloride and reacted with *tert*-butylpiperazine 1-carboxylate to afford piperazine amide **11**. This material was then converted to **13** by reduction of the nitro functionality, followed by guanidine formation, BOC-deprotection and urea formation with 3,3-diphenylpropylisocyanate (**12**). Substituted derivative **16** was prepared from **11** by alkylation with *t*-butyl bromoacetate using sodium hydride as base to give amino acid **14** after treatment with TFA. The monosubstituted piperazine **14** was acylated with isocyanate **12** then the carboxylic acid functionality was converted to *N*-phenethyl amide **15** under standard conditions. The synthesis of **16** was completed by introduction of the guanidine functionality, employing conditions similar to those described for **13**.

Compounds described in Table 3 were prepared from amidines **17** and **18**,[20] which were then acylated with either **12** or 4-nitrophenylcarbamate **19**,[23] to give the urea derivatives **20** and **21**, as shown in Scheme 4.

Scheme 5 outlines the preparation of homologues of the 4-(aminomethyl)piperidine unit of **4**, reported in Table 4. 4-Amino-1-benzylpiperidine (**22**) was protected as the phthalimide under standard conditions and the benzyl group was removed by hydrogenolysis. The 3,3-diphenylpropyl-1-aminocarbonyl moiety was introduced with either **12** or **19** as described previously, then the phthalimide group removed with hydrazine in ethanol. Finally, acylation of **23** with 4-cyanobenzoyl chloride and conversion of the nitrile to the amidine moiety afforded inhibitor **25**. The two-carbon homologue of **25**, inhibitor **29**, was prepared from 4-(2-aminoethyl)pyr-



Scheme 4. Representative syntheses of tryptase inhibitors containing the 4-(aminomethyl)piperidine scaffold. Reagents and conditions: (a) **12** or $Ph_2CH(CH_2)_2NHCO_2C_6H_4NO_2$ (**19**), DIPEA, DMF.



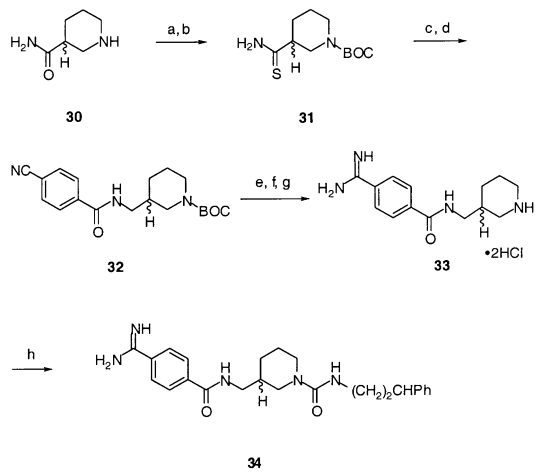
Scheme 5. Preparation of inhibitors containing homologues of 4-(aminomethyl)piperidine. Reagents and conditions: (a) *N*-Carboethoxyphthalimide, Na_2CO_3, CH_2Cl_2; (b) $(Boc)_2O$, H_2, Pd–C, EtOH; (c) TFA, CH_2Cl_2; (d) **12**, DIPEA, CH_2Cl_2; (e) H_2NNH_2, EtOH, reflux; (f) 4-cyanobenzoyl chloride, DIPEA, CH_2Cl_2; (g) $(Boc)_2O$, MeOH; (h) H_2, PtO_2, MeOH/AcOH (7.5:1); then HCl; (i) **12** or **19**, DIPEA, CH_2Cl_2; (j) $H_2NOH{\cdot}HCl$, DIPEA, EtOH, reflux; (k) H_2, 10% Pd–C, EtOAc/EtOH (1:1).

idine (**26**), first by protection of the free amine followed by reduction of the pyridine ring. Acylation of the resulting secondary amine with isocyanate **12** afforded the urea derivative, which was deprotected to give (2-aminoethyl)urea **27**. Acylation of **27** with 4-cyanobenzoyl chloride gave **28**, which was converted to the amidine **29** under identical conditions employed for **24**.

Lastly, Scheme 6 shows the preparation of the 3-isomer of **20**. Nipecotamide (**30**) was converted to the Boc-protected thioamide **31** first by Boc-protection followed by reaction of the amide with Lawesson's reagent.[24] Reduction of the thioamide to the amine was performed using nickel boride[25] and the crude amine was acylated with 4-cyanobenzoyl chloride in the presence of DIPEA to give 4-cyanobenzamide **32**. Conversion of **32** to the inhibitor **34** was accomplished using reaction conditions developed for benzamidines **25** and **29** (Scheme 5).

Our investigation into monocharged derivatives of **1** began by synthesis of urea derivatives of general structure **2**, which were prepared according to the chemical routes described above.

Apparent inhibition constants (K_i values)[18] and selectivity over trypsin[26] for these inhibitors, using the guanidine as the charged group, are reported in Table 1. The data given in Table 1 exhibit some important trends. First, urea derivatives containing hydrophobic residues based on the *gem*-diphenyl motif (compounds **2k** and **2m**) were the most potent in the series, possessing K_i values around 0.8–0.9 μM. In addition, the 2-naphthylmethyl derivative **2i** also exhibited modest

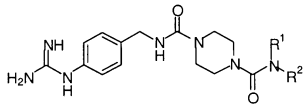


Scheme 6. Preparation of the 3-aminopiperidine analogue of inhibitor **20**. Reagents and conditions: (a) $(Boc)_2O$, MeOH; (b) Lawesson's reagent, THF, reflux; (c) $NiCl_2{\cdot}6H_2O$, $NaBH_4$, THF/MeOH, 0 °C; (d) 4-cyanobenzoyl chloride, DIPEA, CH_2Cl_2; (e) $H_2NOH{\cdot}HCl$, DIPEA, EtOH, reflux; (f) H_2, 10% Pd–C, EtOAc/EtOH (1:1); (g) 4 N HCl in 1,4-dioxane; (h) **12**, DIPEA, DMF.

activity. Restricting rotation of the two phenyl groups of **2k**, as found in fluorenyl derivative **2n** reduced activity 13-fold. In general, tetrasubstituted urea derivatives **2g** and **2h** were slightly less active (1.7–2×) than their trisubstituted analogues (e.g., **2b** and **2d**).

The data presented in Table 1 shows the 3,3-diphenylpropyl motif to be the optimal binding fragment attached to the urea functionality found in **2**. Based on these results, a series of derivatives containing the [(3,3-diphenylpropylamino)carbonyl]piperazine motif was synthesized, varying the 4-guanidinophenyl charged group (arginine mimetic) found in **2** and compounds **2a**–**n**. These variations included amide and α-substituted carboxamide analogues of the guanidinophenyl moiety

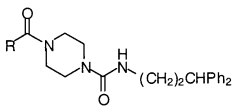
Table 1. Tryptase inhibition data for (4-guanidinophenyl)urea derivatives **2**

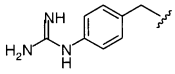
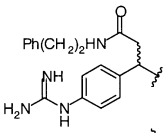
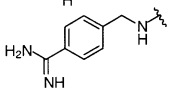
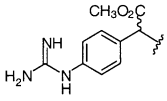


Compd	R^1	R^2	Tryptase (K_i, μM)[a]	Selectivity[b]
2a	CH_2CH_3	H	370	0.38
2b	CH_2Ph	H	16	12
2c	$(CH_2)_2Ph$	H	42	3.6
2d	$(CH_2)_3Ph$	H	7.3	21
2e	$(CH_2)_4Ph$	H	11	10
2f	CH_2Ph	CH_3	80	0.80
2g	CH_2Ph	CH_2Ph	29	2.5
2h	$(CH_2)_3Ph$	CH_2Ph	12	4.2
2i	2-Naphthymethyl	H	1.3	170
2j	2-(1-Naphthyl)ethyl	H	7.4	14
2k	$(CH_2)_2CHPh_2$	H	0.87	360
2l	$(CH_2)_2CPh_3$	H	6.9	>150
2m	(4,4-Diphenyl)-1-cyclohexyl	H	0.89	170
2n	2-(9-Fluorenyl)ethyl	H	6.9	48

[a]Data reported is for a single determination. See ref 18 for assay protocols.
[b]Ratio of K_i(trypsin)/K_i(tryptase).

Table 2. Tryptase inhibition data for various charged group derivatives of **2k**

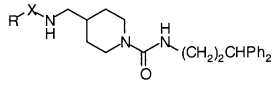


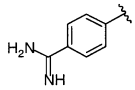
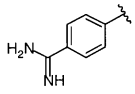
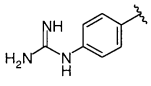
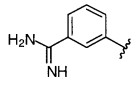
Compd	R	Tryptase (K_i, μM)	Selectivity
13		7.0	>140
16		0.21	>4800
35		0.40	200
36		0.16	>6300

of **2k**, as well as the corresponding benzamidine analogue of **2k**. The structure–activity relationships for these compounds are shown in Table 2.

Generally only the 4-isomer of phenylguanidine possessed significant activity against tryptase (K_i values less than 1 μM). In addition, several alkyl guanidine derivatives of **2k** were also prepared and were less potent (K_i values = 14–29 μM; structures not shown) than the 4-phenylguanidine and benzamidine derivatives reported in Table 3.

Table 3. Tryptase inhibition data for benzamidine and phenylguanidine derivatives of **4** containing the 3,3-diphenylpropylurea moiety from **2k**



Compd	R	X	Tryptase (K_i, μM)	Selectivity
20		CO	0.084	950
21		SO_2	9.7	3.5
37		CO	9.8	>100
38		CO	24	6

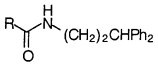
The most potent compounds included benzamidine **35** (0.40 μM), guanidinophenylsuccinic diamide **16** (0.21 μM) and carboxymethyl derivative **36** (0.16 μM). Furthermore, the latter two compounds possessed excellent selectivity over trypsin. However, kinetic analysis of **36** suggested that this compound was a substrate and not an inhibitor of tryptase.

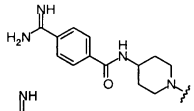
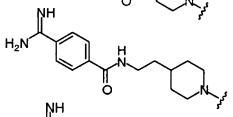
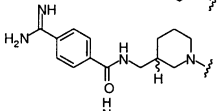
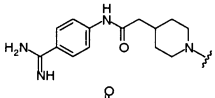
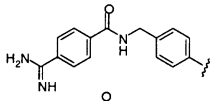
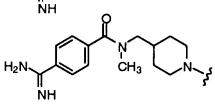
With the optimal urea substituent identified, we then prepared charged group variants using the 4-(aminomethyl)piperidine unit found in dibasic inhibitor **3**. These derivatives are shown in Table 3.

The inhibition data for monocharged inhibitors derived from **3** indicates that arylamidines are preferred over arylguanidines as the arginine mimetic, with *para*-substitution on the benzamide being optimal. This preference for the benzamidine moiety is more dramatic in the (aminomethyl)piperidine series by a factor of 110, as seen by comparison of benzamidine **20** to guanidine **37**. In contrast, benzamidine **35** was only twice as potent as guanidine **2k** in the piperazine series (Tables 1 and 2). Interestingly 4-(amidinobenzene)sulfonamide analogue **21** was considerably less potent (ca. 100-fold) than the corresponding 4-amidinobenzamide **20**.

Table 4 shows some additional variations in the charged moiety (arginine mimetic) and (4-aminomethyl)piperidine motif of **20**. These results confirm that the 4-(aminomethyl)piperidine is required for optimum activity. Simple *N*-methylation of the benzamide nitrogen of **20** (compound **41**) reduced activity almost 600-fold. Furthermore, variation of the chain length of the aminomethyl fragment, or incorporation of the 3-

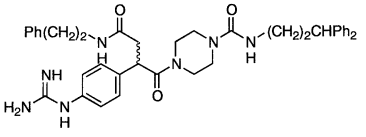
Table 4. Arginine mimetic and scaffold analogues of benzamidine **20**



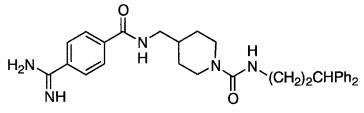
Compound	R	Tryptase (K_i, μM)	Selectivity
25		2.6	44
29		11	6.8
34		13	0.7
39		0.30	490
40		0.34	270
41		50	16

aminomethyl isomer of piperidine was not tolerated. These variations, represented by compounds **25**, **29**, and **34**, afforded inhibitors with inhibition constants in the low micromolar range. The best compounds in this series, the 'retro-inverso' amide **39** and the 4-aminomethylbenzamide derivative **40**, were 3.5–4.0 times less active than **20**.

In summary, we have shown that novel dibasic inhibitors **1** and **3** can be used as lead structures to design monocharged inhibitors of tryptase. From these derivatives, preparation of a series of urea derivatives led to the identification of the 3,3-diphenylpropyl fragment as a potent binding motif. Optimization of the charged binding fragment afforded compounds with K_i values ranging from 0.084 to 0.21 μM, including guanidinophenylsuccinamide **16** and benzamidine **20**. Being the most potent mast cell tryptase inhibitor in this series, compound **20** was selected for pharmacokinetic analysis employing a rat model. Unfortunately **20** exhibited low bioavailability (< 1%) after id administration at 6 mg/kg.



16
K_i = 0.21 μM



20
K_i = 0.084 μM

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