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Synthesis of Potent and Highly Selective Inhibitors of Human Tryptase

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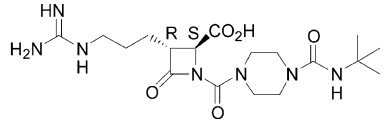
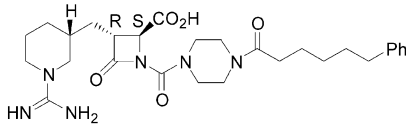
Abstract—The serine protease tryptase has been implicated in allergic and inflammatory diseases and associated with asthma. The synthesis and SAR of a series of N1-activated-4-carboxy azetidinones are described, resulting in identification of BMS-363131 (**2**) as a potent inhibitor of human tryptase ($IC_{50} < 1.7$ nM) with high selectivity (> 3000-fold) for tryptase versus related serine proteases including trypsin.

Tryptase is a serine-like protease that is the major protein component found in mast cells. Upon activation, mast cells degranulate, releasing tryptase, histamine, chymase, and other mediators of allergic and inflammatory conditions. Tryptase has been implicated in allergic and inflammatory diseases as well as autoimmune diseases and is believed to play an important role in inflammatory, bronchoconstrictive, and remodeling processes associated with asthma.[1–3] It is therefore believed that inhibition of tryptase may offer a therapeutic benefit for the treatment of asthma. Our preceding report[4] described the identification of BMS-262084 (**1**) as a potent inhibitor of tryptase ($IC_{50} = 4$ nM) with moderate to good selectivity against related serine proteases but not for trypsin.

In ovalbumin-sensitized guinea pig models, intratracheally dosed BMS-262084 demonstrated efficacy in preventing allergen-induced bronchoconstriction and in protecting against inflammatory cell infiltration into the lung. It was recognized that a significant portion of any inhaled drug would be swallowed; consequently, good selectivity against the gastric protease trypsin was considered highly desirable. In this Letter, we report on our efforts to improve upon BMS-262084 by exploring the effects of conformationally constrained guanidino groups at C-3 and new substituents at N-1 of the azetidinone nucleus. These investigations led to the discovery of BMS-363131 (**2**) as a potent inhibitor of tryptase having excellent selectivity versus other serine proteases including trypsin and having improved hydrolytic stability.



BMS-262084 (1)



BMS-363131 (2)

The general Scheme for preparation of compounds **9b–f** in Table 1 is outlined in Scheme 1. Alkylation of (*S*)-azetidinone **4**[5] with iodides **3** gave compounds **5**, which were converted in two steps to **6**. Removal of the Boc group and then guanylation gave the bis-Cbz protected guanidine compounds **7**. Subsequent acylation with chlorocarbonylpiperazine **8a** and hydrogenation provided (3*R*,4*S*) compounds **9b–f**.

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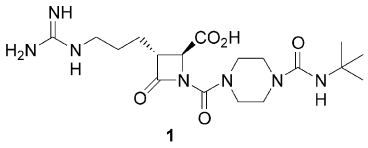
Table 1. Tryptase inhibition, selectivities and chemical stability for compounds **1** and **9**



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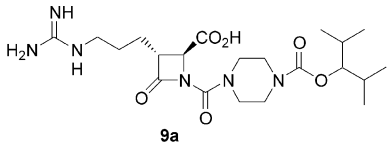
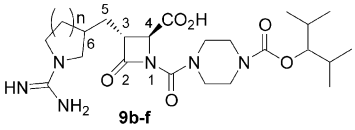


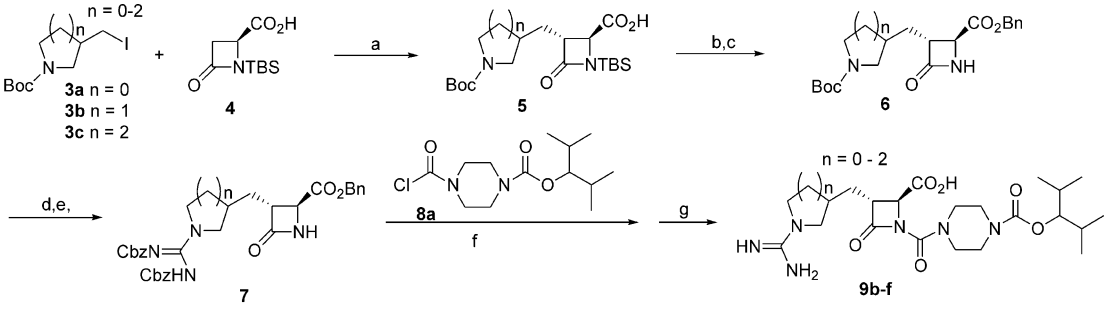
9a



9b-f

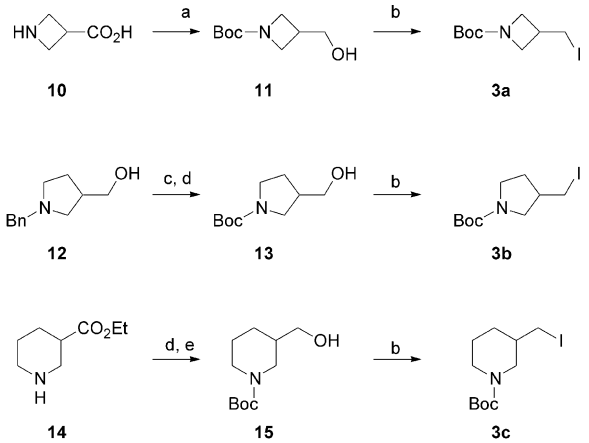
Compd	*n*	Tryptase	Selectivity ratio					Stability, $t_{1/2}$ at 37 °C (h)	
		IC_{50} (nM)	Plasmin	Thrombin	uPA	tPA	Trypsin	pH 7	pH 9
1		4	430	2625	135	>8250	18	>48	8.0
9a		4	364	>9170	843	9170	5	>48	7.3
9b	0	12	394	>2845	862	2845	7	>48	14.4
9c[a]	1	9					4		
9d[a]	2	71	>465	>465	>465	>465	258	>48	>48
9e[b]	2	63	>465	>465	>465	>465	364		
9f[b]	2	204	>162	>162	>162	>162	>162		

[a]Mixture of diastereomers at C-6.
[b]Single diastereomer at C-6, compounds **9e** and **9f** are the isolated diastereomers of **9d**.



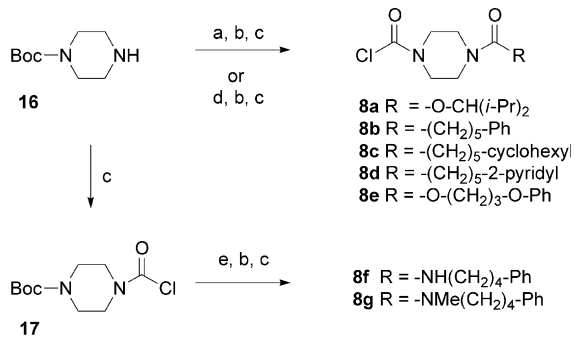
Scheme 1. (a) LDA, THF; (b) TBAF, THF; (c) BnBr, $NaHCO_3$, DMF; (d) TFA, CH_2Cl_2; (e) 1-[C(NCbz)(NHCbz)]pyrazole, TEA, DMF or MeSC(NCbz)(NHCbz), $Hg(OAc)_2$, TEA, DMF; (f) TEA, DMAP, DMF; (g) Pd/C, H_2, dioxane, aq 1 N HCl.

The iodo compounds **3a–c** employed in Scheme 1 were prepared from the intermediate Boc protected amino alcohols **11**, **13**, and **15** (Scheme 2), which could be obtained from the commercially available amino acid, amino alcohol, or amino ester. Chlorocarbonyl-piperazines (**8a–g**) used in either Scheme 1 or Scheme 4 were prepared as shown in Scheme 3. Compounds **8a–e** were prepared from Boc-piperazine (**16**) by acylation, removal of the Boc group, and finally treatment with phosgene. The synthesis of compounds **8f–g** involved the formation of the carbamoylchloride of Boc-piperazine (**16**) followed by sequential treatment with an amine, removal of the Boc group, and treatment with phosgene.

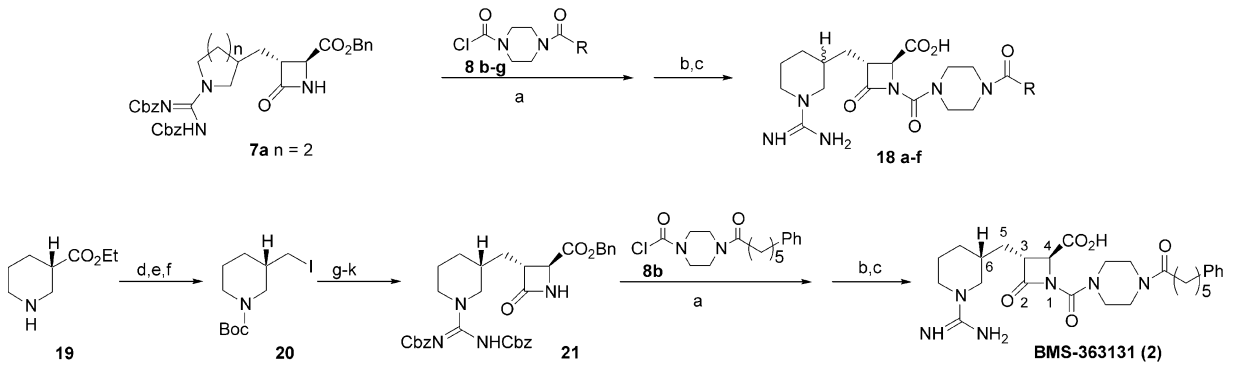


Scheme 2. (a) Procedures described in ref 7; (b) I_2, PPh_3, imidazole, CH_2Cl_2; (c) Pd/C, H_2, MeOH or *i*PrOH; (d) Boc_2O, THF; (e) LAH, THF.

The synthesis of diastereomeric mixtures **18a–f** and the single diastereomer BMS-363131 (**2**), which are listed in Table 2, are outlined in Scheme 4. The mixtures **18a–f** were prepared by acylation of diastereomeric mixtures **7a** with chlorocarbonylpiperazines **8b–g** and deprotection. BMS-363131 (**2**), however, was prepared from the single diastereomer **21** by acylation with **8b** and deprotection. Compound **21** was derived from (*R*)-ethyl



Scheme 3. (a) HO_2C-R, EDAC, DIEA, CH_2Cl_2; (b) TFA, CH_2Cl_2; (c) Cl_2CO. CH_2Cl_2, TEA or $NaHCO_3$; (d) ClOC-R, TEA, DMF; (e) $H_2NR_1(CH_2)_4Ph$ R_1 = H or Me, $NaHCO_3$ or TEA, CH_2Cl_2.



Scheme 4. (a) TEA, cat. DMAP, DMF, 82%; (b) Pd/C, H_2, dioxane, aq 1 N HCl, (c) polyvinylpyridine resin, H_2O, 92% 2 steps; (d) Boc_2O, CH_2Cl_2, 87%; (e) LAH, THF, 87%; (f) I_2, PPh_3, imidazole, CH_2Cl_2, 77%; (g) **4**, LDA, THF, $-70 \rightarrow -20\,^{\circ}C$; (h) TBAF, THF; (i) BnBr, $NaHCO_3$, DMF, 76% 3 steps; (j) TFA, CH_2Cl_2; (k) 1-[C(NCbz)(NHCbz)]pyrazole, TEA, DMF, 73% 2 steps. Yields are for the synthesis of BMS-363131 (**2**).

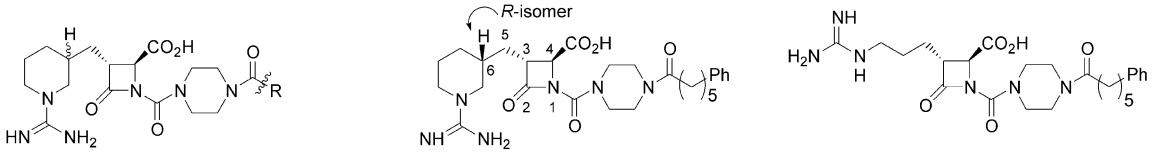
nipecotate (**19**), which along with the (*S*)-enantiomer was obtained by resolution of racemic ethyl nipecotate.[6] Boc protection of **19**, followed by reduction and iodination gave compound **20.** Alkylation of (*S*)-azetidinone **4** with **20** and sequential desilylation, benzylation, removal of the Boc group, and guanylation provided intermediate **21**. BMS-363130 (**22**), which has the (*S*)-configuration at C-6 in contrast to BMS-363131 (**2**) which has the (*R*)-configuration at C-6, was prepared in the same manner as BMS-363131, however, starting from (*S*)-ethyl nipecotate in place of **19**.

Guanidinylpropyl compounds **9a** (Table 1) and **23** (Table 2) were prepared using chlorocarbonylpiperazines **8a** and **8b**, respectively, following methodology described in the previous Letter to prepare BMS-262084.

The tryptase inhibition, selectivities, and chemical stability for the new conformationally constrained guanidino compounds are shown in Tables 1 and 2. Table 1 displays several constrained guanidino analogues of guanidinylpropyl compound **9a**. Compound **9a** and BMS-262084 (**1**), which differ in their N-1 substituent, were equally potent against tryptase ($IC_{50} = 4$ nM) and exhibited similar hydrolytic stability profiles. However, the overall selectivity profile of **9a** offered an improved template to investigate constrained guanidine analogues. The amidinoazetidinyl analogue **9b** showed potent activity against tryptase ($IC_{50} = 12$ nM) but no significant improvement in selectivity against plasmin, thrombin, urokinase (uPA), tissue plasminogen activator (tPA), or trypsin. The amidinopyrrolidinyl analogue **9c**, although potent against tryptase, similarly showed poor selectivity against trypsin. Activity against tryptase was substantially reduced for the unsymmetrical amidinopiperidinyl diastereomeric mixture **9d** (diastereomeric at the piperidinylmethyl juncture) and the single diastereomers **9e** and **9f**. However, **9d** displayed greatly improved selectivity for tryptase over trypsin (258-fold), as well as enhanced aqueous stability ($t_{1/2} > 48$ h at pH 9). The diastereomers **9e** and **9f**, comprising the mixture **9d**, were similarly selective.

SAR in the amidinopiperidinyl series was studied further by examining substituents on N-4 of the piperazine

Table 2. Tryptase inhibition, selectivities and chemical stability for compounds **2**, **18**, **22**, and **23**



Compd	R	Tryptase	Selectivity ratio					Stability, $t_{1/2}$ at 37 °C (h)	
		IC_{50} (nM)	Plasmin	Thrombin	uPA	tPA	Trypsin	pH 7	pH 9
23		< 1.7	> 26	> 16,500	> 226	> 16,500	> 8		
18a	$-(CH_2)_5-Ph$	< 1.7	> 16,280	> 19,410	> 17,480	> 19,410	> 1941	> 48	> 17.5
18b	$-NH-(CH_2)_4-Ph$	< 1.7	> 16,070	> 19,410	> 8785	> 19,410	> 1364	> 48	12
18c	$-NMe-(CH_2)_4-Ph$	6	3621	> 5500	> 5500	> 5500	2166	> 48	23.9
18d	$-(CH_2)_5-$cyclohexyl	1.9	8421	> 17,370	12,100	> 17,370	1684		
18e	$-(CH_2)_5-$2-pyridyl	4.6	> 7147	> 7147	5000	> 7147	739		
18f	$-O-(CH_2)_3-O-Ph$	< 1.7	> 92,476	> 19,410	> 19,410	> 19,410	> 1676		
2		< 1.7	> 12,900	> 19,410	> 11,820	> 19,410	> 3389	> 48	> 17.5
22		< 1.7	> 19,410	> 19,410	> 19,410	> 19,410	> 3279	> 48	> 17.5

in **9d**. This led to a series of diastereomeric mixtures (**18a–f**) of highly potent inhibitors of tryptase with excellent selectivity against related serine proteases including trypsin as shown in Table 2. The diastereomeric mixture **18a** was comparable to the guanidinylpropyl analogue **3** in potency versus tryptase but exceedingly more selective against plasmin, uPA, and trypsin. Additionally, **18a** exhibited improved hydrolytic stability at pH 9 ($t_{1/2} > 17.5$ h) compared to BMS-262084 (**1**) in Table 1 ($t_{1/2} = 8.0$ h). The other amidinopiperidinyl diastereomeric mixtures in Table 2 displayed potent activity versus tryptase and high selectivity against other serine proteases including trypsin (most > 1000-fold).

Potency versus tryptase and selectivity for the two single diastereomers BMS-363131 (**2**) and BMS-363130 (**22**) that comprise the mixture **18a** are also shown in Table 2. Each of the diastereomers had an $IC_{50} < 1.7$ nM against tryptase with selectivity $> 3{,}000$-fold versus trypsin and $> 10{,}000$-fold versus plasmin, thrombin, uPA, and tPA. BMS-363131 was selected over BMS-363130 for further evaluation following comparisons of other biological properties of the two compounds. In the ovalbumin-sensitized guinea pig model of lung inflammation,[4] intratracheal dosing of BMS-363131 effected a reduction in inflammatory cell count in the lungs compared to control.

In summary our investigation of the SAR at N-1 and C-3 of the azetidinone nucleus of BMS-262084 (**1**) has shown that conformationally constrained guanidinyl groups at C-3 and extended substituents appended to the piperazine at N-1 provide potent inhibitors of typtase with highly improved selectivity versus other serine proteases including trypsin. One of the most potent and selective compounds, BMS-363131 (**2**), was shown to be efficacious in a guinea pig model of asthma.

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