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Synthesis of potent and highly selective nonguanidine azetidinone inhibitors of human tryptase

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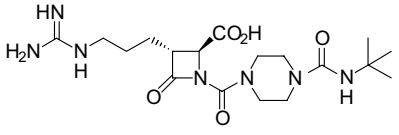
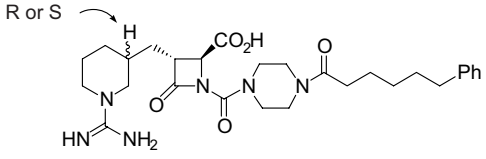
Dedicated to the memory of Steven M. Seiler, Ph.D. (deceased March 31, 2003). Steve's untimely passing will not diminish the continuing impact of his drug discovery research or the admiration of his fellow co-workers

Abstract—Azetidinones such as BMS-363131 (**2**) and BMS-363130 (**3**), which contain a guanidine group in the C-3 side chain were previously shown to be very potent inhibitors of human tryptase with high selectivity versus other serine proteases, including trypsin. In this letter, we describe the discovery of a number of potent azetidinone tryptase inhibitors in which the guanidine moiety at the ring C-3 position is replaced with primary or secondary amine or aminopyridine functionality. In particular, BMS-354326 (**4**) is a highly potent tryptase inhibitor ($IC_{50} = 1.8$ nM), which has excellent selectivity against trypsin and most other related serine proteases.

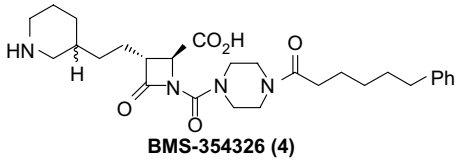
The serine protease tryptase, which is the major protein component of mast cells, is released along with histamine, chymase, and other mediators of inflammatory and allergic responses when mast cells degranulate upon stimulation. Tryptase has been implicated in inflammatory and allergic diseases and is believed to play a significant role in asthma.[1] Consequently, inhibition of tryptase has become an important therapeutic target for asthma and other inflammatory and allergic conditions.[2] We previously reported the straight-chained terminal guanidine compound BMS-262084 (**1**), which displayed potent inhibition of tryptase ($IC_{50} = 4$ nM) and moderate to good selectivity against related serine proteases with the exception of trypsin. We also demonstrated the efficacy of BMS-262084 upon intratracheal dosing in guinea pig models of bronchoconstriction and lung inflammation.[3] In a separate report, we described the conformationally constrained guanidine epimers BMS-363131 (**2**) and BMS-363130 (**3**), both of which displayed potent inhibition of tryptase (IC_{50}<1.7 nM) with excellent selectivity against other serine proteases including trypsin. BMS-363131, upon intratracheal dosing, was also efficacious in a guinea pig model of lung inflammation.[4]



BMS-262084 (1)



R-isomer BMS-363131 (2)
S-isomer BMS-363130 (3)



BMS-354326 (4)

Because a significant portion of an inhaled drug is swallowed, and considering the potential for preclinical

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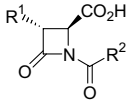
toxicity associated with trypsin inhibition,[5] selectivity against the digestive protease trypsin (>1000-fold) was considered an essential requirement for an inhaled drug candidate. We were also interested in exploring whether this guanidine-containing chemotype might be modified for oral delivery through replacement of the guanidine moiety with less basic functionality.

In this letter, we report on our efforts to search for nonguanidine compounds similar to BMS-262084 and BMS-363131. These efforts led to the discovery of a number of potent tryptase inhibitors in which a primary/secondary amine or aminopyridine functionality replace the guanidine functionality. In particular, we identified BMS-354326 (**4**), which is a highly potent tryptase inhibitor ($IC_{50} = 1.8$ nM) having excellent selectivity against trypsin and most other related serine proteases.

The syntheses of the compounds shown in Tables 1–3 are described in Schemes 1 and 2, with the exception of reference compounds **19**[3] and **32**[6], which are described elsewhere. As indicated in Scheme 1, (*S*)-azetidinone **5** was alkylated with Boc-protected halides **6a**–**k** in the presence of LDA to afford intermediates **7**. N-Desilylation, followed by benzylation of the carboxylate afforded intermediates **8**. The preparation of intermediates **8l** and **8m** were described previously.[4] The preparation of several of the starting halides **6** is also shown in Scheme 1. Iodides **6c**–**f** were synthesized by reacting the corresponding chloro iodo-*n*-alkanes with potassium di-*t*-butyl iminodicarbonate followed by treatment with sodium iodide in acetone. The aminopyridine bromides **6g** and **6h** were prepared by bis-Boc protection of 2-amino-5-picoline and 2-amino-4-picoline, respectively, followed by mono bromination of the methyl groups. Reaction of α,α′-dibromo-*para*- and *meta*-xylenes with potassium di-*t*-butyl iminodicarbonate gave **6i** and **6j**, respectively. Iodides **6a**, **6b**, and **6k** were prepared by literature procedures.[7]

Table 1. Tryptase inhibition for compounds containing a 3-(4-piperidinylmethyl) group



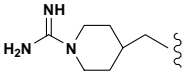
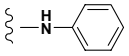
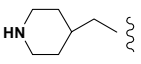
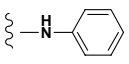
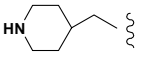
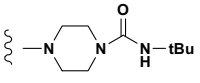
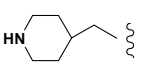
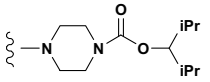
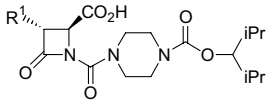
	R^1	R^2	Tryptase IC_{50} (nM)
14			515
15			147
16			30
17			29

Table 2. Tryptase inhibition for guanidine and nonguanidine compounds containing the N-1 group derived from **12**



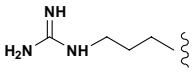
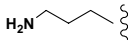
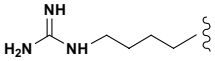
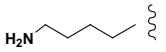
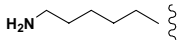
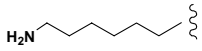
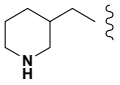
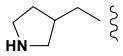
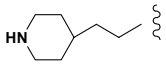
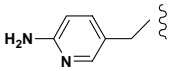
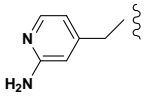
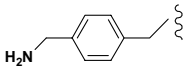
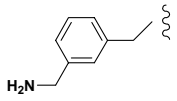
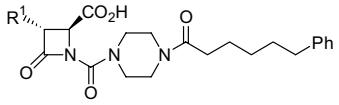
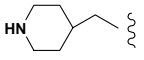
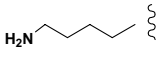
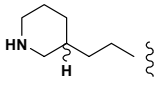
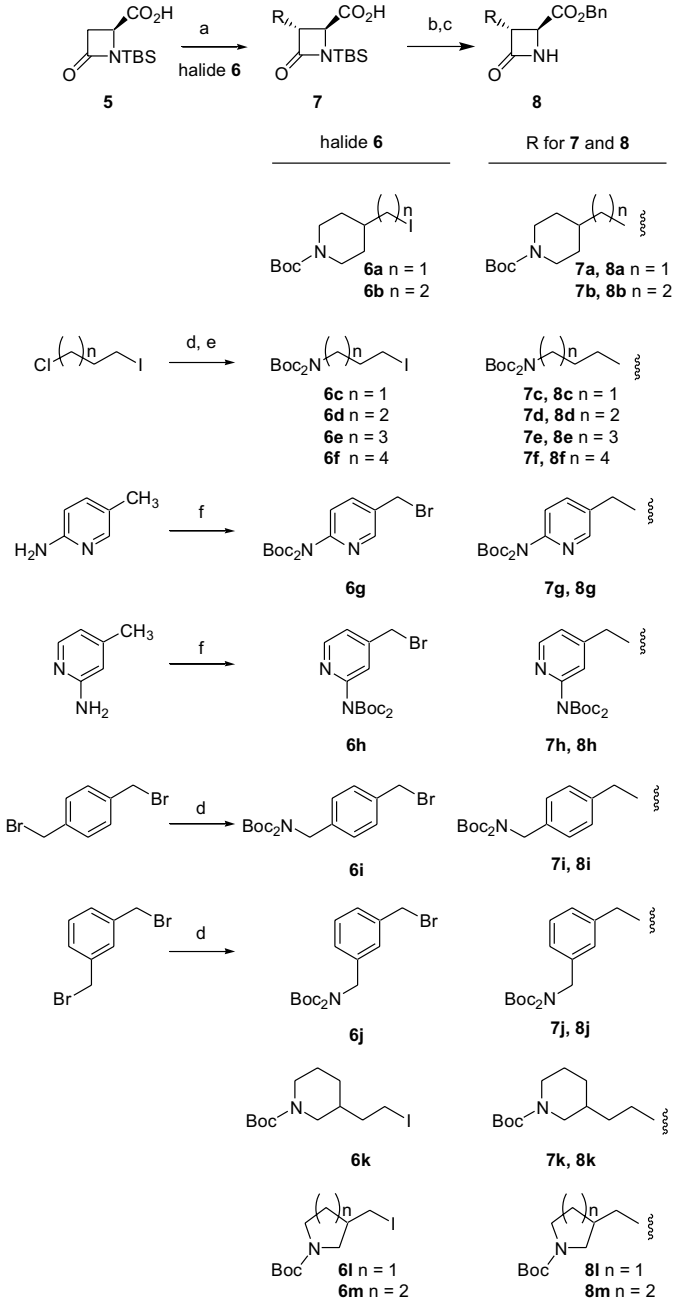
	R^1	Tryptase IC_{50} (nM)
19		4
20		>33,000
31		39
21		7
22		34
23		32
24		17,899
25		2605
26		1214
27		1053
28		61
29		211
30		19

Table 3. Tryptase inhibition for nonguanidine compounds containing the N-1 group derived from **33**



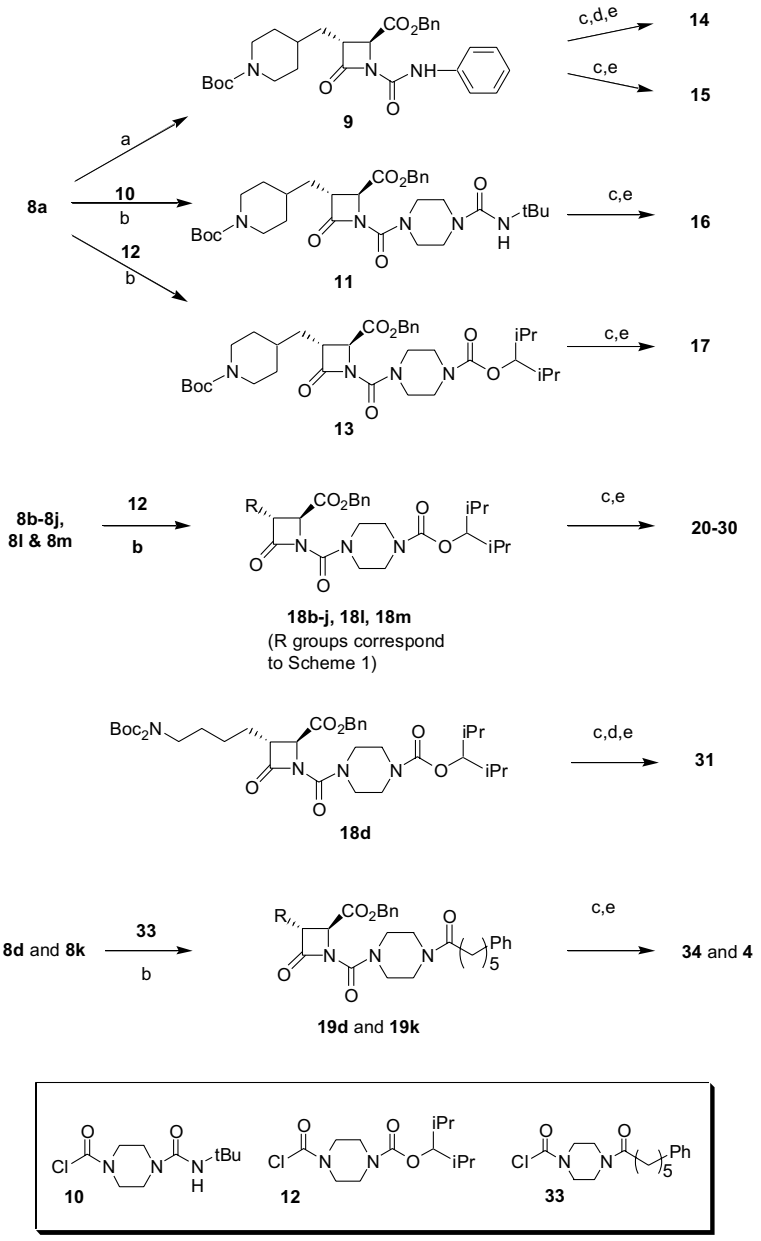
	R^1	Tryptase IC_{50} (nM)
32		1.0
34		<1.7
4 (BMS-354326)		1.8



Scheme 1. (a) LDA, THF; (b) TBAF, THF; (c) BnBr, $NaHCO_3$; (d) Boc_2NK, DMF; (e) NaI, acetone; (f) (i) Boc_2O, CH_2Cl_2, DMAP, iPr$_2$EtN; (ii) NBS, benzoyl peroxide, CCl_4.

As shown in Scheme 2, compound **8a** was N-acylated with phenylisocyanate in the presence of LDA to afford **9**. Removal of the Boc group of **9** with TFA, followed by conversion of the piperidine amine to a bis-CBZ protected guanidine function using *N*,*N*′-bis-CBZ-1-guanylpyrazole, and finally reductive removal of the benzyl and CBZ groups afforded **14**. The corresponding nonguanidine piperidine analog **15** was prepared by sequential deprotection of the Boc and benzyl groups of **9**. Intermediate **8a** was also converted to compounds **16** and **17** by reaction with the chlorocarbonyl reagents **10** and **12** in the presence of TEA/DMAP, followed by removal of the Boc and benzyl groups. Intermediates **8b**–**j**, **8l**, and **8m** were acylated with **12**, and afforded compounds **20**–**30** following deprotection. Compound **31**, the guanidine analog of **21**, was prepared from **18d** by sequential removal of the Boc group, reaction of the primary amine with *N*,*N*′-bis-CBZ-1-guanylpyrazole, and reductive removal of the CBZ and benzyl groups. Intermediates **8d** and **8k** were converted to compounds **34** and **4** by N-acylation with the chlorocarbonyl reagent **33**, followed by deprotection of the Boc and benzyl groups.

During the course of our SAR studies of guanidine-containing azetidinone tryptase inhibitors we prepared compound **14**, a conformationally constrained guanidine analog of one of our early lead compounds. While



Scheme 2. (a) LDA, THF, phenylisocyanate; (b) TEA, DMAP, DMF; (c) TFA, CH_2Cl_2; (d) TEA, *N*,*N*′-bis-CBZ-1-guanylpyrazole; (e) Pd/C, H_2, dioxane.

compound **14** had only modest potency ($IC_{50} = 515$ nM), we were intrigued when we discovered that the corresponding nonguanidine piperidine analog **15** showed improved potency ($IC_{50} = 147$ nM). Keeping the C-3 4-(piperidinylmethyl) substituent constant, we next prepared analog **16**, which contained the *t*-butylaminocarbonylpiperizinylcarbonyl N-1 group found in BMS-262084 (**1**), as well as analog **17**, containing the diisopropylmethoxypiperizinylcarbonyl N-1 group. These two N-1 groups had previously been recognized to be superior to the phenylaminocarbonyl N-1 group in the C-3 guanidinylpropyl series, and in the C-3 piperidine series afforded more potent tryptase inhibitors: **16** displayed an IC_{50} of 30 nM and **17** displayed an IC_{50} of 29 nM. The selectivity of **17** was profiled in a panel of related serine proteases (Table 4). While **17** is highly selective versus thrombin, factor Xa, uPA, and tPA and is modestly selective for plasmin, it is poorly selective for trypsin (32-fold).

Table 4. Tryptase inhibition and selectivities for **17** and **4**

	17		**4** (BMS-354326)	
	IC_{50} (nM)	Selectivity	IC_{50} (nM)	Selectivity
Tryptase	29	—	1.8	—
Trypsin	935	32X	10,005	5583X
Thrombin	>33,000	>1138X	>33,000	>18,333X
FXa	>33,000	>1138X	>33,000	>18,333X
Plasmin	6220	214X	309	169X
uPA	6999	241X	>33,000	>18,333X
tPA	>33,000	>1138X	>33,000	>18,333X

Keeping the diisopropylmethoxypiperizinylcarbonyl N-1 group constant, we compared two additional guanidine-containing beta-lactams with their corresponding nonguanidine counterparts, namely **19** and **20**, having guanidinylpropyl and aminopropyl C-3 substituents, respectively, and **31** and **21**, having guanidinylbutyl and aminobutyl C-3 substituents, respectively (Table 2). Whereas guanidine **19** is quite potent ($IC_{50} = 4$ nM), the corresponding amino compound **20** completely lacks potency. In contrast, the analogs in the *N*-butyl series are both fairly potent, with the amino analog **21** sixfold *more* potent than the corresponding guanidine **31** ($IC_{50} = 39$ nM vs 7 nM, respectively). It was evident that simple amine functionality at the azetidinone C-3 could afford very potent tryptase inhibitors, and that the SAR was different from the corresponding guanidine analogs having the same alkyl linker. This latter observation is not surprising given the assumption that both the guanidine and the amine C-3 functional groups are salt-bridging to aspartate 189 at the bottom of the tryptase S_1 pocket[8], and that the proper placement of the positive charge is a critical determinant of potency.

Further survey of amino functionality at the azetidinone C-3 position showed the aminopentyl (**22**, $IC_{50} = 34$ nM) and aminohexyl (**23**, $IC_{50} = 32$ nM) analogs to be somewhat less potent than aminobutyl **21**. In contrast to the 4-piperidinylmethyl compound **17**, the 3-piperidinylmethyl (**24**), 2-pyrrolidinylmethyl (**25**), and 4-piperidinlyethyl (**26**) analogs were all weakly potent. Whereas the 4-amino-3-pyridinylmethyl analog **27** was only modestly potent ($IC_{50} = 1053$ nM), the isomeric 3-amino-4-pyridinylmethyl analog **28** had relatively good potency ($IC_{50} = 61$ nM). The pK_a of 2-aminopyridine is around 7.0, and thus **28** is the least basic inhibitor of this series having good potency. Finally, 4-aminomethylbenzyl analog **29** had an IC_{50} of 211 nM while its 3-aminomethyl analog **30** was 10-fold more potent ($IC_{50} = 19$), an interesting positional effect somewhat mirroring the relative potencies of the aminopyridine isomeric pair, **27** and **28**.

The more recent discovery of the potency- and selectivity-enhancing properties of certain lipophilic N-1 groups such as the phenyl-*n*-pentylcarbonyl-piperizinylcarbonyl group[4,6] prompted us to survey select nonguanidine analogs in that N-1 series. The C-3 4-piperidinylmethyl analog **32** has been reported separately[6], and is shown in Table 3 for comparison. Thus, **32** is about 30-fold more potent than the corresponding N-1 diisopropyl-methoxycarbonyl analog **17** (IC_{50}'s 1 nM versus 29 nM, respectively). Analog **34** containing the C-3 aminobutyl group is also more potent than the corresponding analog **21** (IC_{50}'s < 1.7 nM versus 7 nM, respectively). Additionally we prepared an analog in this N-1 series having a 3-piperidinylethyl group at C-3 (**4**, BMS-354326), a group not surveyed in earlier N-1 series. BMS-354326 was found to be a very potent tryptase inhibitor ($IC_{50} = 1.8$ nM), and, except for only moderate selectivity versus plasmin (169-fold), the selectivity of BMS-354326 versus a panel of related serine proteases is excellent. In particular, it is over 5000-fold selective against trypsin. By comparison the selectivity of **32** against trypsin is 310-fold.[3] BMS-354326 also demonstrated good aqueous hydrolytic stability, with a half-life of >48 h at pH 7 and 35 h at pH 9.

In summary, we have identified a number of potent, nonguanidine azetidinone inhibitors of human tryptase. One of them, BMS-354326 has an IC_{50} for tryptase of 1.8 nM, and displays excellent selectivity against trypsin and most other related serine proteases.

References and notes

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