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Synthesis and SAR of 4-Carboxy-2-azetidinone Mechanism-Based Tryptase Inhibitors

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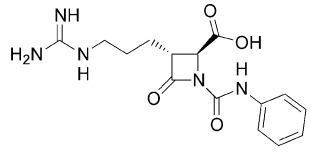
Abstract—A series of N1-activated C4-carboxy azetidinones was prepared and tested as inhibitors of human tryptase. The key stereochemical and functional features required for potency, serine protease specificity and aqueous stability were determined. From these studies compound **2**, BMS-262084, was identified as a potent and selective tryptase inhibitor which, when dosed intratracheally in ovalbumin-sensitized guinea pigs, reduced allergen-induced bronchoconstriction and inflammatory cell infiltration into the lung.

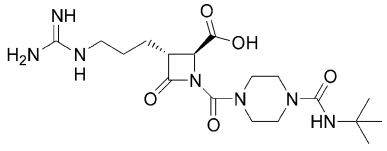
It is estimated that 14 million Americans suffer from asthma. At the current rate of increased incidence it is anticipated that 22 million Americans will suffer from asthma by the end of the decade.[1] To lessen the severity of asthma, therapeutics modulating the underlying inflammatory processes are being investigated. One target receiving attention is the trypsin-like serine protease tryptase, which is produced almost exclusively by mast cells. Tryptase accounts for a significant 20–25% of the total lung and skin mast cell protein. Active tryptase is stored in intracellular granules as a heparin stabilized tetramer and is released into the extracellular environment upon mast cell stimulation. Once in the extracellular spaces tryptase modulates inflammatory processes through a number of pathways (see ref 2 for reviews).

Through directed screening in our laboratories, racemic azetidinone (**1**)[3] was discovered to be a potent tryptase inhibitor having modest selectivity against a panel of other trypsin like serine proteases. Kinetic studies were consistent with a mechanism-based inhibition of serine protease activity wherein the N1-activated azetidinone covalently inhibited enzyme. X-ray crystal structures of azetidinone tryptase inhibitors related to **1** covalently bound at the active site of trypsin support this mechanism. At this point we embarked on a program to prepare an optimized analogue of **1**, which could be useful as an inhaled tryptase inhibitor. In this Letter we report the synthesis of a series of 4-carboxyazetidinone analogues of **1** leading to the identification of the potent tryptase inhibitor **2**, BMS-262084 (tryptase $IC_{50} = 4$ nM), having an improved selectivity profile and showing efficacy in a guinea pig model of bronchoconstriction and lung inflammation.

1 (racemic)



2 (BMS-262084)



General methods for the preparation of (3*R*,4*S*)-*trans* and racemic (3,4)-*cis* 4-carboxyazetidinones are outlined in Scheme 1. The (3*R*,4*S*)-*trans* isomers were prepared

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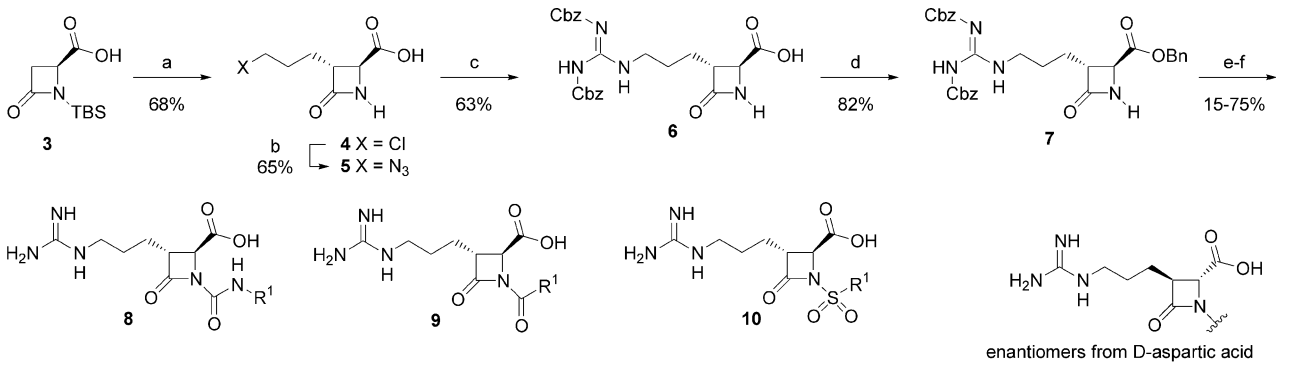
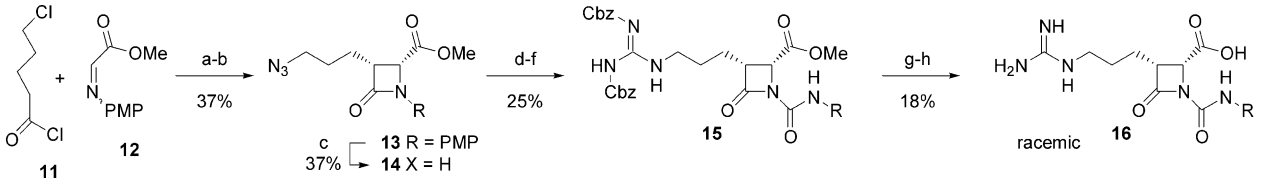


Scheme 1. (a) (i) LDA, THF, −78 to −20 °C; (ii) 1-chloro-3-iodopropane, −20 °C; (b) nBu$_4$NI, nBu$_4$NN$_3$, DMF; (c) (i) H_2, 10% Pd/C, HOAc, DMF; (ii) Et_3N, *N*,*N*′-bis-CBZ-1-guanylpyrazole; (d) BnBr, nBu$_4$NI, $NaHCO_3$, DMF; (e) (i) NaHMDS, THF, −78 to −20 °C; (ii) isocyanate, acyl chloride or sulfonyl chloride; or (iii) isocyanate, Et_3N, DMAP, CH_2Cl_2; (f) H_2, 10% Pd/C, dioxane, H_2O, HCl.

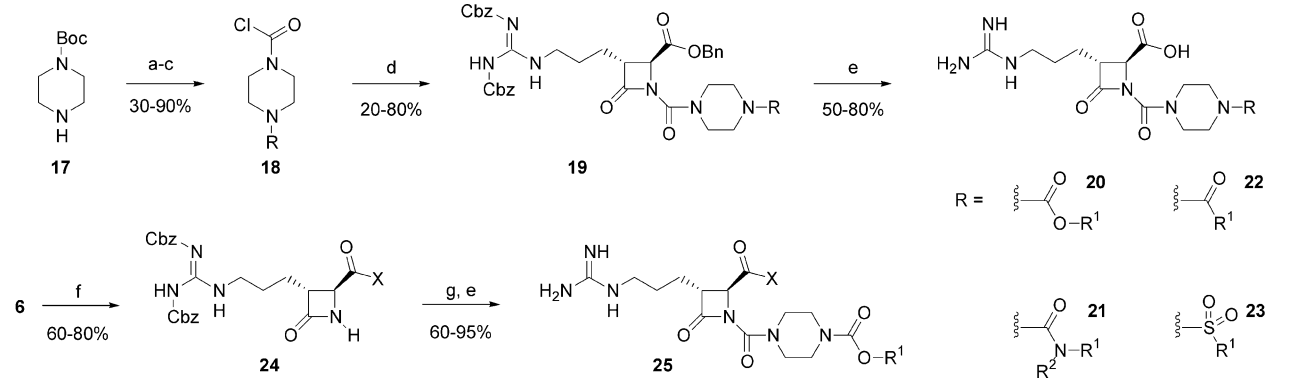
starting from azetidinone **3**, which was prepared from L-aspartic acid as described by Baldwin.[4] Alkylation of **3** with chloroiodopropane followed by azide displacement gave **5** in moderate yield. Conversion to the bis-Cbz protected guanidine compound **6** was achieved through hydrogenation of the azide followed by treatment with *N*,*N*′-bis-Cbz-1-guanylpyrazole and triethylamine. The acid was benzyl protected to provide intermediate **7**, which was transformed to N1-activated azetidinones by treatment with base followed by addition of an isocyanate, acyl chloride or sulfonyl chloride. Deprotection via hydrogenation provided acids **8**–**10**, **8a** being the active enantiomer of racemic **1**.

Racemic 3,4-*cis* azetidinones were prepared via the Staudinger reaction[5] between 5-chlorovaleryl chloride (**11**) and the *para*-methoxyphenyl (PMP) protected imine of methylglyoxylate (**12**), Scheme 2. Subsequent azide displacement of the intermediate alkylchloride provided compound **13**. Removal of the PMP group with ceric ammonium nitrate followed with transformations described above for the *trans*-azetidinones gave compound **15**. Hydrogenation to remove the Cbz protecting groups followed by hydrolysis of the methyl ester under mild conditions in a pH 7.4 aqueous buffer gave acid **16**.

The preparation of a series of N1-piperazine carbonyl analogues is described in Scheme 3. Reaction of 1-Boc-piperazine (**17**) with isocyanates, acyl chlorides or sulfonyl chlorides followed by Boc deprotection and treatment with phosgene in CH_2Cl_2 over solid $NaHCO_3$ provided 4-substituted piperazinecarbonyl chlorides (**18**) in moderate to good yields over two steps.



Scheme 2. (a) Et_3N, hexane, toluene, 3 Å MS; (b) NaN_3, nBu$_4$NI, DMF; (c) CAN, CH_3CN, H_2O; (d) H_2, 10% Pd/C, dioxane, 1 N HCl; (e) 1,3-bis-CBZ-2-methyl-2-thiopseudourea, $HgCl_2$, Et_3N, DME, MeOH; (f) (i) NaHMDS, THF, −78 to −20 °C; (ii) isocyanate; (g) H_2, 10% Pd/C, Dioxane, H_2O, HCl; (h) pH 7.4 phosphate buffer.



Scheme 3. (a) (i) iPr$_2$NEt, DMAP, CH_2Cl_2; (ii) isocyanate, chloroformate, carbamoylchloride, acyl chloride or sulfonyl chloride; (b) TFA, CH_2Cl_2; (c) phosgene, CH_2Cl_2, $NaHCO_3$; (d) **7**, Et_3N, DMAP, CH_2Cl_2; (e) H_2, 10% Pd/C, Dioxane, H_2O, HCl; (f) primary or secondary amine, BOP, THF; (g) 4-BOC-1-piperazinecarbonyl chloride, Et_3N, DMAP, CH_2Cl_2.

Table 1. Tryptase inhibition, selectivity and aqueous stability for compounds **8**–**10** and **16**

Activating group	Entry	R^1	Tryptase	Selectivity ratio					Stability, $t_{1/2}$ at 37 °C (h)	
			IC_{50} (nM)	Plasmin	Thrombin	uPA	tPA	Trypsin	Buffer pH 7	Buffer pH 9
Carbamoyl	**8a**	Phenyl	6	52	1548	3	117	1	>48	2.2
	ent-8a	Phenyl	5380	>6	>6	0.7	>6	0.3		
	16a (cis)	Phenyl	992							
	8c	Cyclohexyl	18	34	>611	2	64	1	>48	9.1
	8d	Benzyl	71	45	465	16	465	2	>48	6.8
Acyl	**9a**	Phenyl	9	17	142	3	128	1		
	9b	2-Thiophene	1	12	190	5	109	3	8.7	<0.1
	9c	4-Biphenyl	1	8	65	3270	1610	2	11.1	<0.1
Sulfonyl	**10**	4-Biphenyl	1	47	7	44	4370	1	10.6	<0.1

Table 2. Tryptase inhibition and selectivities for compounds **20**–**25**

Entry	X	$R^1(R^2)$	Tryptase	Selectivity ratio					Stability, $t_{1/2}$ at 37 °C (h)	
			IC_{50} (nM)	Plasmin	Thrombin	uPA	tPA	Trypsin	pH 7	pH 9
20a	OH	*t*-Butyl	4	295	>8250	218	>8250	8	>48	6.2
20b	OH	Methyl	9	210	683	70	>1200	2	>48	5.6
20c	OH	2-Phenethyl	0.7	302	3323	926	14,246	8	>48	7
2(21a)	OH	*t*-Butyl (H)	4	430	2625	135	>8250	18	>48	8
21b	OH	Ethyl (Ethyl)	5	227	>6600	520	>6600	3	>48	6.4
22a	OH	*t*-Butyl	7	232	>1500	154	>1500	3	>48	2.5
22b	OH	Neopentyl	11	314	>1000	8	>1000	1.5	>48	6.5
22c	OH	Phenyl	5.2	332	286	142	>2000	1	>48	3.9
23	OH	Phenyl	13	147	556	43	>2500	14	>48	1.7
25a	NH_2	*t*-Butyl	0.4	227	105	82	8300	6	42	0.3
25b	$NHCH_3$	*t*-Butyl	0.7	172	177	75	6800	3	31.8	0.3
25c	Piperidine	*t*-Butyl	3	205	155	246	4433	1	>48	0.8

Coupling of carbamoyl chlorides (**18**) to azetidinone (**7**) in the presence of Et_3N and DMAP gave adducts **19**. Subsequent hydrogenation then gave the series of compounds **20**–**23**. Amine couplings to acid **6** followed by condensation with the carbamoyl chloride of *N*-Boc-piperazine and subsequent hydrogenation provided a series of C4-amides, **25a**–**c**.

Compounds were screened for activity against human tryptase and a panel of related serine proteases;[6] including trypsin, thrombin, plasmin, urokinase (uPA), and tissue plasminogen activator (tPA). Stabilities in aqueous buffer at pH 7 and pH 9 were also evaluated. Aqueous stabilities of these compounds were found to mirror stability in human plasma (data not shown).

The data in Table 1 show that the tryptase inhibitory activity of the C4-carboxy azetidinone series resides predominantly in the 3*R*,4*S*-*trans* stereoisomer, demonstrated by comparison of compounds **8a** (tryptase $IC_{50} = 6$ nM), its enantiomer ***ent*-8a** (tryptase $IC_{50} = 5.38$ μM) and the racemate of the *cis* isomer, **16a**, (tryptase $IC_{50} = 999$ nM). However, the inhibitory activity against most of the other serine proteases also resided in the 3*R*,4*S*-*trans* stereoisomer. Replacement of the N1-phenylcarbamoyl group of **8a** with cyclohexyl- or benzyl-carbamoyl groups provided analogues with diminished potency but somewhat enhanced aqueous stability, as assessed at pH 9. Analogues with acyl (**9a**–**9c**) or sulfonyl (**10**) activating groups were highly potent tryptase inhibitors ($IC_{50} = 1$–9 nM) with mixed selectivity, but had relatively poor aqueous stability, likely the result of the higher degree of activation of the azetidinone.

The superior aqueous stability of the C4-carboxy azetidinones bearing the N1-carbamoyl activating group focused attention on this series. The data in Table 2 shows the tryptase activity and selectivity profiles of a series of related compounds. The Boc compound **20a** showed a greatly improved selectivity with the exception of selectivity against trypsin. Replacement of the Boc group of **20a** provided functionalized carbamates **20b**–**c**, ureas **21a**–**b**, amides **22a**–**c**, and sulfonamide **23** exhibiting low nanomolar tryptase inhibitory activity ($IC_{50} = 0.7$–13 nM) and generally good selectivity profiles. The C4-amides (**25a**–**c**) were very potent tryptase inhibitors ($IC_{50} = 0.4$–3 nM) but in general had somewhat poorer selectivity against thrombin, compared to the C4-carboxylic acids. Compound **2** (**21a**), BMS-262084, a potent tryptase inhibitor ($IC_{50} = 4$ nM) with a moderate to good selectivity profile except for trypsin, and having relatively good aqueous stability was chosen for in vivo evaluation.

The effect of BMS-262084 on the pulmonary mechanics of ovalbumin-sensitized guinea pigs[7] following allergen-induced bronchoconstriction were evaluated. Ovalbumin challenge increased lung resistance (R_{lung}) approximately

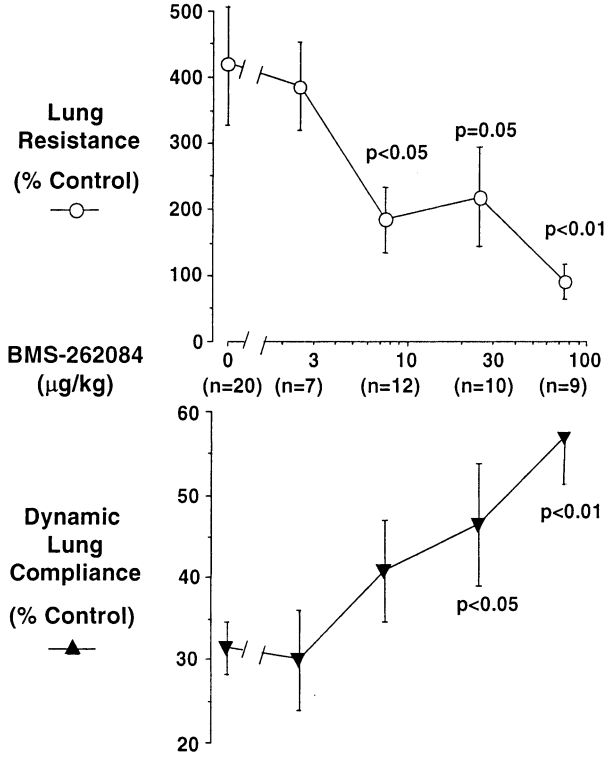


Figure 1. Effect of BMS-262084, **2**, on the pulmonary mechanics in ovalbumin-sensitized guinea pigs after allergen-induced bronchoconstriction.

4-fold ($R_{lung}\%\Delta = 421 \pm 98$, $n = 20$), which reflects constriction of the large airways. Ovalbumin also decreased dynamic lung compliance (C_{dyn}) by two-thirds ($C_{dyn}\%\Delta = -69 \pm 3$, $n = 20$), which corresponded to narrowing of the small airways and diminished pulmonary elasticity. Intratracheal (i.t.) administration of BMS-262084 (75 μg/kg) 10 min prior to ovalbumin challenge inhibited the increase in resistance by 79% ($R_{lung}\%\Delta = 89 \pm 26\%$, $n = 9$) and the decrease in dynamic lung compliance by 37% ($C_{dyn}\%\Delta = -43 \pm 6\%$, $n = 9$), Figure 1.

BMS-262084 also showed a protective effect against inflammatory cell infiltration in the delayed phase of the immediate hypersensitivity response to allergen.[8] BMS-262084 produced a dose-dependent inhibition of the cellular inflammatory response as shown in Figure 2. At the 225 μg/kg i.t. dose all cell counts were reduced by 70–80% to the level observed in sham-treated animals. BAL protein concentration was also decreased 22% from 238±14 μg/mL to 186±27 μg/mL ($p < 0.05$), a level equal to that observed in sham control animals (182±22 μg/mL). Both the cell counts and the BAL protein concentration following the 225 μg/kg i.t. dose of BMS-262084 and ovalbumin challenge were not significantly different from those of ovalbumin-sensitized guinea pigs studied 24 h after sham operation without ovalbumin challenge. These results are consistent with compound BMS-262084 exerting a strong protective effect against cellular activation that accompanies allergen-induced pulmonary inflammation.

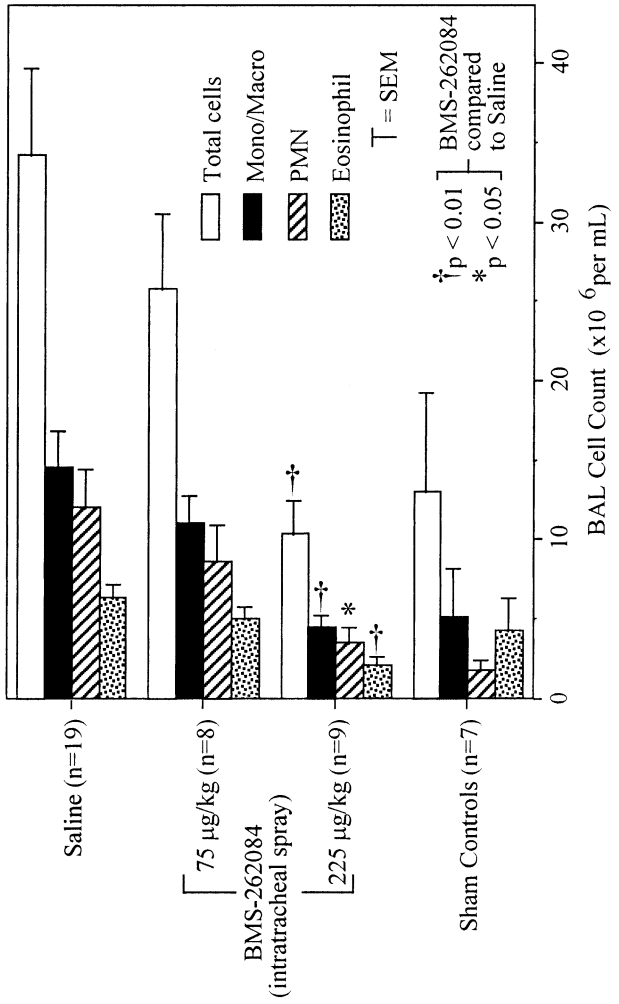


Figure 2. BMS-262084, **2**, produces a dose-dependent inhibition of the cellular inflammatory response in ovalbumin-sensitized guinea pigs.

In summary, we have studied the synthesis and SAR of a series of N1-activated C4-carboxy azetidinone tryptase inhibitors which show moderate selectivity against related serine proteases with the exception of trypsin. One of the more potent, selective and aqueous stable compounds BMS-262084 (**2**) reduced the bronchoconstriction and the cellular inflammatory response in ovalbumin-sensitized guinea pigs.

References and Notes

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7. Ovalbumin-sensitized guinea pigs were prepared in the following manner; ip injections of ovalbumin (10 μg) with aluminum hydroxide (100 mg/kg) given on days 0 and 14, cyclophosphamide (30 mg/kg) on day 12 and utilized between day 35 and 65. On the day of the experiment, the animals were anesthetized with Na-pentobarbital and placed on a mechanical ventilator. Intratracheal flow and pressures were recorded and processed in real time with a pulmonary mechanics computer (Model PMAG, Buxco, Troy, NY) providing measurement of airways resistance (R_{lung}) and dynamic lung compliance (C_{dyn}). Ten min prior to ovalbumin challenge (50 μg/kg, iv) animals were disconnected from the ventilator and 50 μL of BMS-262084 (dose range 2.5–75 μg/kg) was administered as an aerosol intratracheal spray using a Model 1A needle (PenCentury, Philadelphia, PA) producing 25 μm median particle diameter. The trachea was reconnected to the ventilator and changes in pulmonary mechanics were followed over time.
8. In this experiment ovalbumin-sensitized guinea pigs were anesthetized with isoflurane and the trachea was exposed by a small skin incision. The Model 1A PenCentury spray injector was advanced through a small cut between adjacent tracheal rings up to the bronchial bifurcation. Saline or BMS-262084 (50 μL at 75 or 225 mg/kg) was administered as a spray and the tracheal incision was closed immediately. Ovalbumin (50 μg/kg) was injected into the jugular vein ~6 min after intratracheal dosing. The skin incision was then closed, anesthesia discontinued and the animals allowed to recover. Twenty-four h later, the guinea pigs were anesthetized with pentobarbital and subjected to bronchoaveolar lavage (BAL). Differential cell counts and protein concentration in the BAL were determined by a co-worker blinded to the treatment protocol.