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Synthesis and evaluation of 4-substituted benzylamine derivatives as β-tryptase inhibitors

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Abstract—Since β-tryptase is considered a critical mediator of asthma, potent tryptase inhibitors may be useful as new agents for the treatment of asthma. We investigated 4-substituted benzylamine derivatives and obtained M58539 (**15h**) as a potent inhibitor of β-tryptase (IC_{50} = 5.0 nM) with high selectivity against other serine proteases, low molecular weight, clog *P* value less than 5, lack of amidino and guanidino groups, and independence of Zn^{2+} ion.

β-Tryptase, a serine protease, has been implicated in allergic and inflammatory diseases like asthma.[1–4] In fact, APC-366, a tryptase inhibitor, was reported to be effective in asthmatic patients.[5] Other than APC-366, several compounds, for example, BABIM[6] and AMG-126737,[7,8] have been reported to be potent inhibitors of β-tryptase. Most, however, contain strongly basic groups such as amidino or guanidino groups. Further, AMG-126737 has relatively high molecular weight (>500). These properties are considered unfavorable for oral administration. BABIM is known to require Zn^{2+} ion for inhibition of β-tryptase (Fig. 1).

Our interest therefore focused on development of new inhibitors with the following properties.

(1) High selectivity against other serine proteases.
(2) Low molecular weight less than 500.
(3) Low clog *P* value less than 5.
(4) Lack of strongly basic groups such as amidino and guanidino groups.
(5) Independence of metal ions such as Zn^{2+} ion.

Compound **1** (Fig. 2) was found by means of high-throughput screening. It does not contain strongly basic groups and its clog *P* value of **1** is less than 5, although its molecular weight is slightly above 500. It was therefore selected as a seed compound, and further investigation was carried out in order to obtain optimal compounds.

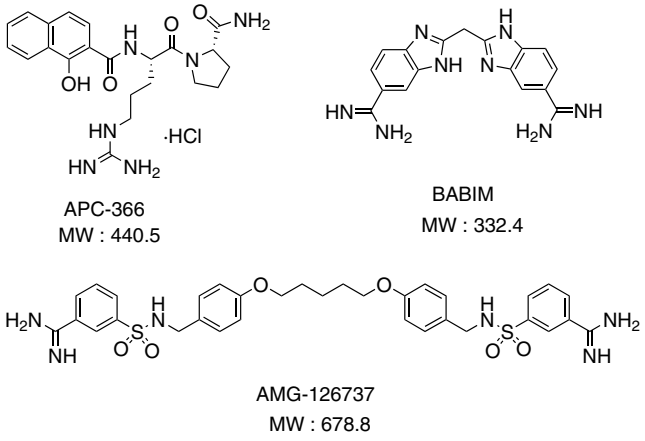

Figure 1. The chemical structure of APC-366, BABIM, and AMG-126737.

First, benzylamine derivatives containing a piperidino function at the 4-position were synthesized, as shown in Scheme 1. Condensation of **4**[10] with **3** derived from **2**[11] yielded seed compound (**1**). Reduction of the carbamoyl group in **1** with BH_3–THF complex afforded

Keywords: β-Tryptase; Serine protease.
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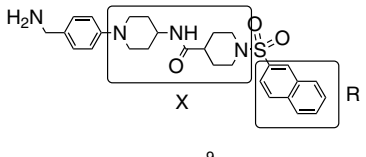


IC_{50} : 21.1μM [9]
MW : 506.7
clogP : 2.43

1

Figure 2. The chemical structure of compound **1**. See above-mentioned reference for further information.

5. 4-(4′-Hydroxymethyl)piperidinobenzonitrile (**6**)[12] was smoothly converted to the amino-aldehyde (**7**) in the usual manner. Reductive amination of **7** with 1-sulfonylpiperazine (**8**)[13] and with monosulfonyl-ethylenediamine (**10**)[14] followed by deprotection yielded compounds **9** and **11**, respectively.

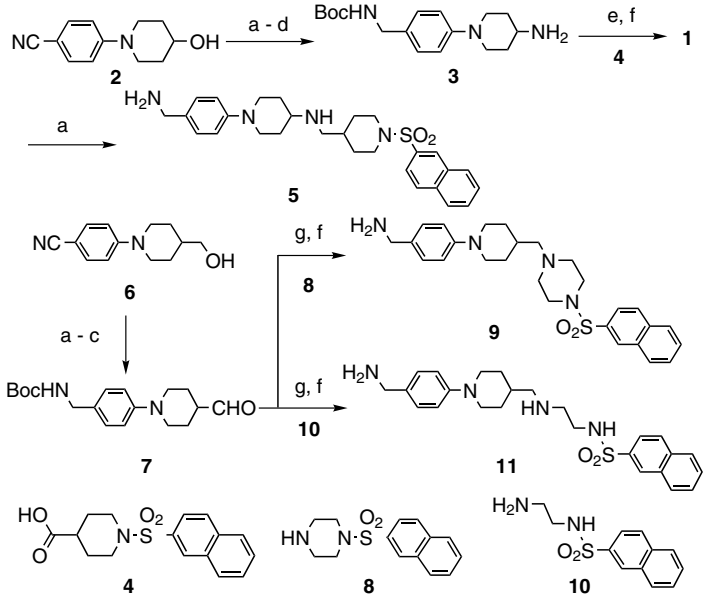
Second, compounds containing an open chain structure in the central part of the molecule were synthesized as illustrated in Scheme 2. Thus, N-protected 4-iodo-benzylamine (**12**) was allowed to react with allyl alcohol in the presence of $Pd(OAc)_2$ to afford aldehyde (**13**). Reductive amination of **13** with **10** yielded **14**, which was deprotected to obtain **15a**. Similarly, compounds **15b**–**h** were prepared from **13** by sulfonylation with arylsulfonyl chlorides (**19b**–**h**) and deprotection via **18**.[19] In addition, *N*-methyl derivative (**16**) of **15a** was prepared from **14** by means of Leukart type reductive methylation. Compound **21** was synthesized from 4-cyanophenylacetic acid (**20**)[15] by condensation with **10** and subsequent conversion of an amide carbonyl group to a methylene group. Further, compound (**25**) containing a carbonyl group on the ethylenediamine moiety was prepared from the protected 4-aminomethylbenzaldehyde (**22**)[16] via **23**, as shown in Scheme 2.

Finally, a benzylamine derivative (**29**) with an ether type side chain instead of the secondary amine group in **15a** was synthesized. Compound **27**, prepared by DIBAL reduction of **26**,[18] was alkylated by bromoacetonitrile in the presence of a phase-transfer catalyst, and subsequent reduction of the cyano group by $NaBH_4/CoCl_2$ afforded the amine (**28**). The amine (**28**) was easily converted into the corresponding sulfonamide, which was deprotected to the ether (**29**). The spectral data of the products shown in Schemes 1–3 are not contradicted by the proposed structures.

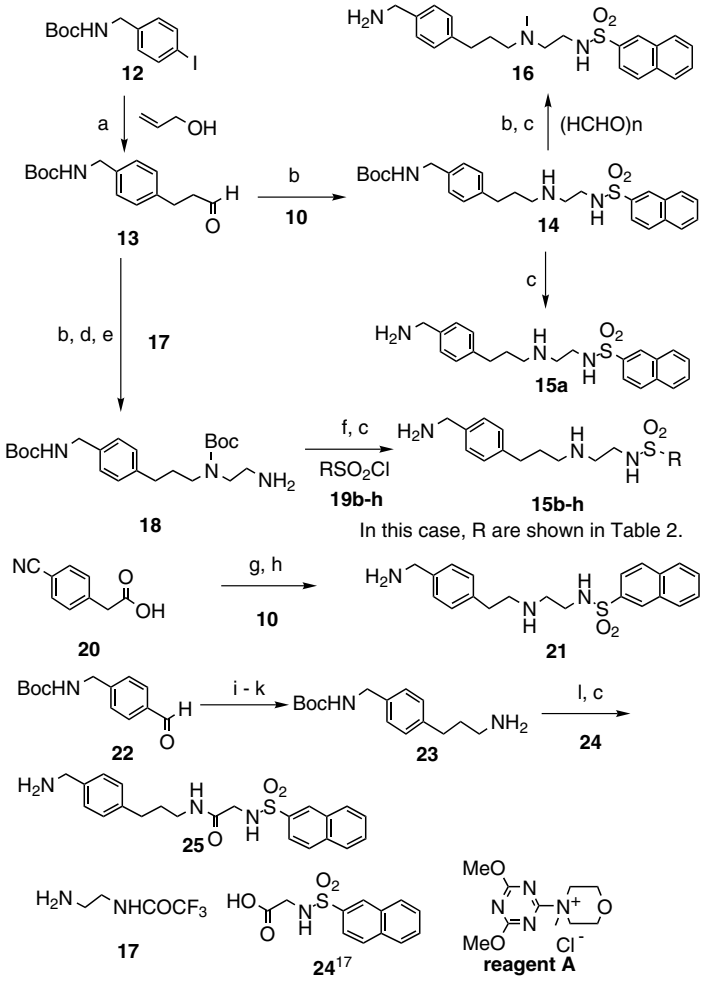
Optimization was performed from the middle region (X) of **1**. The results of modification of the region are listed in Table 1. Activity was about 4-fold stronger than for **1**, when reduction of the amide moiety (**5**) or replacement of the piperidine ring of the sulfonamide region with a piperazine ring (**9**) was performed.

In order to give more flexibility to the substrates, **11**, which featured replacement of the piperidine ring in X region with an ethylenediamine, was examined. Compound **11** was 13-fold more potent than **1**. In order to make X region even more flexible, **15a**, which featured replacement of two piperidine rings with open chains, was examined and was significantly more potent (ca. 300-fold) than the seed compound **1**.

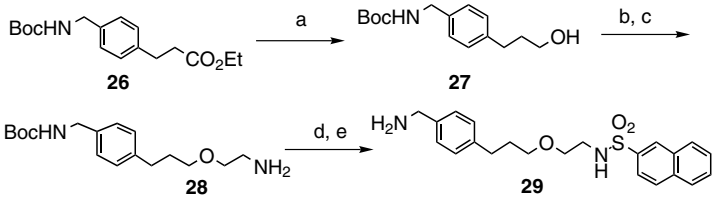
Based on **15a**, the middle region was optimized further. The focus of this optimization was the length of this region, the addition of substituent at the amine, and the need for the amine. Compound **21**, the length of which was shortened by one methylene, was 11-fold less potent than **15a**. N-methylation of the amine (**16**) reduced activity 1.5-fold. The amide (**25**) was also significantly



Scheme 1. Synthesis of 4-piperidinobenzylamine derivatives. Reagents: (a) BH_3-THF; (b) Boc_2O; (c) $(COCl)_2$, DMSO, Et_3N; (d) $AcONH_4$, $NaBH_3CN$; (e) WSC–HCl; (f) HCl; (g) $NaBH(OAc)_3$, AcOH.



Scheme 2. Synthesis of benzylamine derivatives. Substituted with open chain function at 4-position. Reagents: (a) $Pd(OAc)_2$; (b) $NaBH(OAc)_3$, AcOH; (c) HCl; (d) Boc_2O; (e) K_2CO_3; (f) Et_3N; (g) WSC–HCl; (h) BH_3-THF; (i) $(EtO)_2P(O)CH_2CN$, NaH; (j) $NaBH_4$; (k) H_2, PtO_2; (l) reagent A. See above-mentioned reference for further information.



Scheme 3. Synthesis of derivative (**29**) containing an ethereal linkage on side chain. Reagents: (a) DIBAL; (b) $BrCH_2CN$, NaOH, Bu_4NHSO_4; (c) $NaBH_4$, $CoCl_2$; (d) 2-naphthalenesulfonyl chloride, Et_3N; (e) HCl.

less potent than compound **15a**. Compound **29**, which featured replacement of the nitrogen atom by an oxygen atom, was 80-fold less potent than compound **15a**.

Thus, compound **15a** was evaluated as the starting point for further optimization.

Modification of the naphthalene ring moiety (R) was performed. The results of this modification are shown in Table 2. The naphthalene in **15a** was replaced by other aromatic ring systems, that is, benzene (**15b**), indan (**15c**), benzo[*b*]thiophene (**15d**, **e**), 1,2,3,4-tetrahydroiso-quinoline (**15f**), or benzothiazole (**15g**). Bicyclic rings seemed superior to monocyclic rings, and **15h**[20] was 15-fold more potent than **15a**. These results suggested that the shape of 1-chloronaphthalene fits the binding site exactly. Compound **15h** was 4220-fold more potent than seed compound **1**.

The most potent compound **15h**, M58539, was tested for selectivity against trypsin, factor Xa, plasmin, thrombin, and elastase. Among them, M58539 displayed exceedingly high selectivity against tryptase as shown in Table 3.

Finally, the influence of Zn^{2+} ion on the inhibitory effect of M58539 was tested. BABIM exhibited very low inhibitory activity (1/670) in the absence of Zn^{2+} ion, while

Table 1. SAR for X region modification

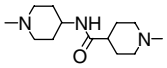
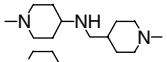
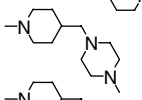
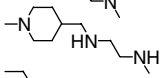
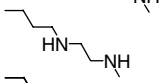
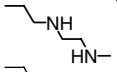
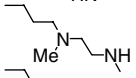
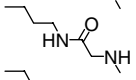
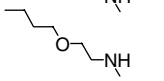
Compound	X	IC_{50} (μM)
1		21.1
5		5.1
9		5.6
11		1.6
15a		0.073
21		0.81
16		0.12
25		1.3
29		5.7

Table 2. SAR for naphthalene ring moiety modification

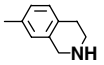
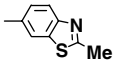
Compound	X	IC_{50} (μM)
15a		0.073
15b		0.76
15c		0.20
15d		0.13
15e		0.21
15f		0.19
15g		0.27
15h M58539		0.005

Table 3. Selectivity of M58539 and BABIM against serine proteases

Compound	Enzyme	IC_{50} (μM)	Selectivity
M58539	Tryptase	0.005	
	Trypsin	>100	>20,000
	FXa	>100	>20,000
	Plasmin	>100	>20,000
	Thrombin	>100	>20,000
	Elastase	>100	>20,000
BABIM	Tryptase	0.051	
	Trypsin	4.4	86
	FXa	4.5	88
	Plasmin	26	510
	Thrombin	31	608
	Elastase	>100	>1960

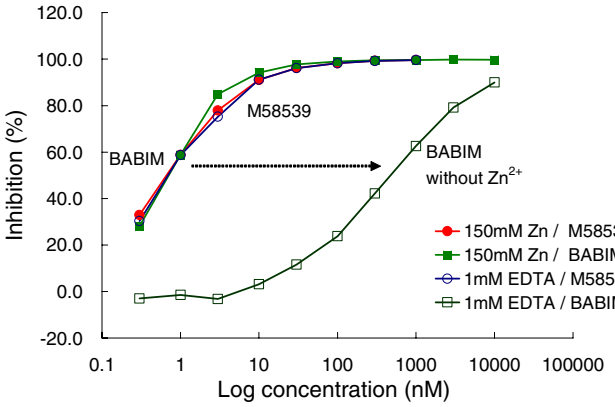


Figure 3. The influence of zinc ion on inhibitory effect.

M58539 exhibited high inhibitory activity independent of Zn^{2+} ion (Fig. 3).

As described in this paper, through modification of 4-substituted benzylamine derivatives, we obtained a very active compound, M58539, as a potent inhibitor of β-tryptase (IC_{50} = 5.0 nM) with the desired properties of high selectivity against other serine proteases (>20,000), low molecular weight (432.0), low clog *P* value (4.04), lack of amidino and guanidino groups, and independence of Zn^{2+} ion.

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9. Inhibition assay of human β-tryptase: Human β-tryptase was purchased from PROMEGA (WI, U.S.A.). The enzymatic assay was performed at 25 °C in 100 mM Tris–HCl, pH 7.5, 200 ng/mL heparin, and 0.1 mM substrate (BocPhe-Ser-Arg-MCA). The final concentration of β-tryptase was 20 ng/mL. The reaction was stopped with 20% AcOH before hydrolysis of the substrate by tryptase was monitored with fluorometric measurement of hydrolyzed AMC under the condition of an excitation wavelength of 355 nm and an emission wavelength of 460 nm.

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19. Spectroscopic data of **18**: ^{1}H NMR ($CDCl_3$) δ: 7.20 (2H, d, J = 8 Hz), 7.13 (2H, d, J = 8 Hz), 4.79 (1H, s), 4.28 (2H, d, J = 5 Hz), 3.33–3.10 (4H, m), 2.80 (2H, d, J = 7 Hz), 2.58 (2H, d, J = 8 Hz), 1.93–1.75 (2H, m), 1.46 (9H, s), 1.44 (9H, s).
20. Spectroscopic data of **15h**: ^{1}H NMR (DMSO-d_6) δ: 8.92 (2H, s), 8.61 (1H, d, J = 2 Hz), 8.35–8.15 (3H, m), 8.15–8.05 (1H, m), 8.12 (1H, d, J = 9 Hz), 7.90 (1H, dd, J = 2, 9 Hz), 7.40 (2H, d, J = 8 Hz), 7.26 (2H, d, J = 8 Hz), 4.05–3.90 (2H, m), 3.15–2.80 (6H, m), 2.87 (3H, s), 2.64 (2H, t, J = 8 Hz), 1.95–1.80 (2H, m).