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SYNTHESES AND EVALUATION OF AMIDINOBENZOFURAN DERIVATIVES AS TRYPTASE INHIBITORS

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Abstract: We report the syntheses and evaluation of amidinobenzofuran derivatives as tryptase inhibitors. Among the compounds we evaluated, 1,5-Bis[4-(5-amidinobenzofuran-2-ylcarbonyl)piperazinylcarbonylmethoxy]cyclooctane **26** (AY0068) was found to be a selective and potent non-peptide inhibitor. **26** was effective in PCA reaction in rats without showing anti-histaminic activity.

Keyword: Enzyme inhibitors

Tryptase, a serine protease with trypsin-like substrate specificity, is a major protein component of human mast cells. Recently, tryptase has been used as a biochemical marker of mast cell activation, and elevated levels of the enzyme have been detected in a number of disease-indicating allergic and inflammatory conditions.[1,2] Of special note, the tryptase inhibitor APC366, which was developed by Axys Pharm. Inc., was reportedly effective in asthmatic patients.[3]

A search of our in-house compound collection yielded the amidinobenzofuran derivatives 1 ($Ki'=2.1\times10^{-7}$ M) and 2 ($Ki'=4.6\times10^{-7}$ M) as tryptase inhibitors. Compounds **1** and **2** share a common substructure, 4-(5-amidinobenzofuran-2-ylcarbonyl)piperazine, which is considered a necessary component of inhibitory activity. Modification of these derivatives resulted in the following compounds. In addition to the evaluation of tryptase inhibition,[4] all compounds were also tested for thrombin inhibitory activity[5] to test the selectivity.

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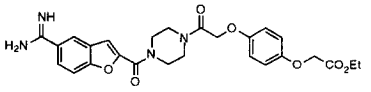

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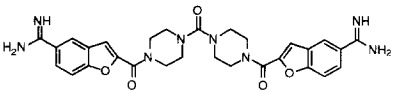

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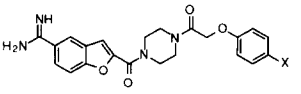
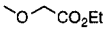
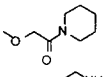
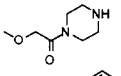
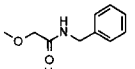
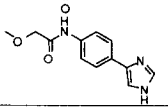
Inhibitory activities of analogs **1** are collected in Table 1. It can be seen from the Table that the longer the length of the molecule, the higher the degree of tryptase inhibition.

Table 1. SAR of Amidinobenzofurans derivatives

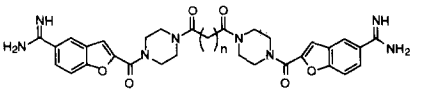


Compound No.	X	Ki'(M)	
		Tryptase	Thrombin
3	H	3.0×10^{-6}	N.D.[a]
1		2.1×10^{-7}	N.D.[a]
4		6.0×10^{-8}	N.D.[a]
5		6.7×10^{-8}	N.D.[a]
6		3.7×10^{-8}	2.8×10^{-5}
7		6.8×10^{-9}	5.3×10^{-6}

[a]N.D.: denotes a value more than 1×10^{-4}

The fact that the presence of an imidazole group is favorable to activity as in **7** suggests that the presence of a basic moiety at both ends of the molecule may be a contributing factor. The SARs of di-basic compounds may be found in Table 2. Compounds **11** to **14** showed stronger inhibition than that of the derivatives listed in Table 1 and the longer the length of the molecule, the higher the degree of activity attained. These results implicate the presence of basic moieties such as amidino group that can interact with the acidic function contributes positively for the activity. A similar result was reported in tryptase inhibition.[3]

Table 2. SAR of Bis-amidinobenzofuran derivatives

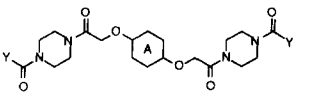


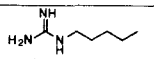
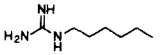
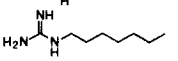
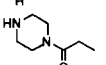
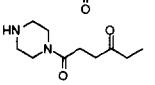
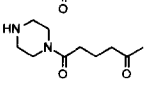
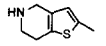
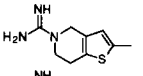
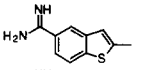
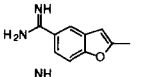
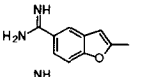
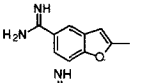
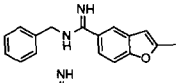
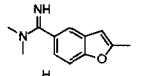
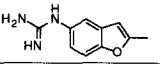
Compound No.	n	Ki[a](M) Tryptase	Thrombin
8	1	6.5×10^{-7}	8.2×10^{-5}
9	2	1.5×10^{-8}	5.1×10^{-5}
10	3	8.8×10^{-9}	3.2×10^{-5}
11	4	8.6×10^{-10}	2.3×10^{-5}
12	5	1.2×10^{-9}	8.5×10^{-5}
13	6	1.2×10^{-10}	N.D.[a]
14	7	1.9×10^{-11}	2.3×10^{-4}

[a]N.D.: denotes a value more than 1×10^{-4}

Further modifications were performed on di-basic compounds as shown in Table 3. Neither guanidinoalkyl chains nor piperazinyl groups contributed to the activity as shown in compounds **15** to **20**. Furthermore, the lower activity observed in substituted amidinobenzofuran derivatives (compounds **27** and **28**) and guanidinobenzofuran (compound **29**) could indicate that a specific structure regarding basic function is needed for high activity. In particular, amidinobenzofuran (**13**, **14**, **24** and **25**), amidinobenzothiophene (**23**) and amidino-tetrahydrothienopyridine (**22**) had the clearest functions of the evaluated compounds. In contrast to the basic function described above, tether groups such as those described in A in Table 3, appear not to be critical for the activity as shown in **24**, **25**, **26**, **13** and **14**. This result indicates that any function which is capable of separating amidino-function at an appropriate distance is available for high activity. Recently Burgess group[6] reported that similar dimeric benzamidine inhibitors might bridge two adjacent active sites of human tryptase which is reported as a homotetrameric structure.[7] The fact that representative compounds of the burgess report and our compounds shows similar distances of di-basic function could indicate that our compounds might interact with the enzyme in a similar manner.

Table 3. SAR of di-basic compounds



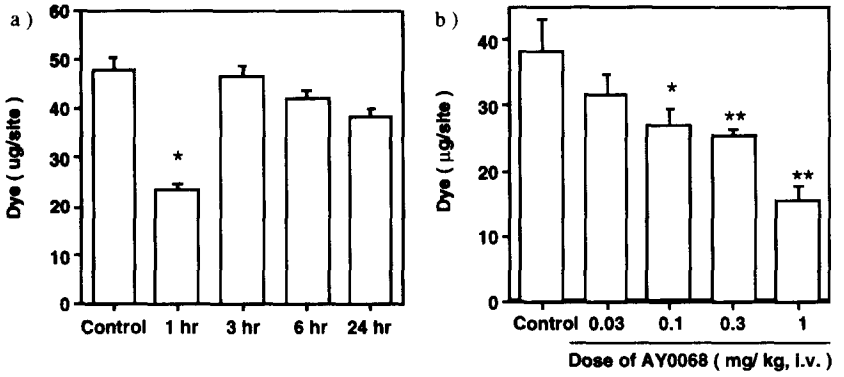
no.	Y	A	Ki'(M)	
			Tryptase	Thrombin
15			8.0×10^{-5}	N.D.[a]
16			2.3×10^{-5}	N.D.[a]
17			1.3×10^{-5}	N.D.[a]
18			1.3×10^{-5}	N.D.[a]
19			N.D.[a]	N.D.[a]
20			N.D.[a]	N.D.[a]
21			9.7×10^{-7}	N.D.[a]
22			4.6×10^{-11}	2.9×10^{-5}
23			2.8×10^{-11}	2.2×10^{-5}
24			5.7×10^{-11}	3.2×10^{-5}
25			1.1×10^{-9}	8.8×10^{-5}
26			2.9×10^{-11}	1.5×10^{-4}
27			1.1×10^{-6}	N.D.[a]
28			6.6×10^{-6}	N.D.[a]
29			1.1×10^{-6}	2.4×10^{-5}

[a]N.D.: denotes the value more than 1×10^{-4}

Among the evaluated compounds, **26** was selected for further evaluations because of its good metabolic stability when administered intravenously to rats (data not shown). **26** inhibited 51% at 1 mg/kg intravenously dosing, 1hr prior to antigen challenge on the PCA reaction (Fig. 1).[8] In addition, dose-dependency was observed as shown in Fig. 2. On the other hand, the lack of

inhibition on the vascular permeability induced by histamine in rats (data not shown) could indicate that **26** inhibits PCA reaction via its tryptase inhibition.

Figure 1. Effect of **26** (AY0068) on PCA reaction in rats



a) Time course; **26** was administrated at 1mg/kg, *i.v.* prior to challenge with antigen. *; $p<0.05$ vs control group (Student's t-test). b) Dose dependency; **26** was administrated at 0.03 - 1 mg/kg, *i.v.* and 1 hr prior to challenge with antigen. * ; $p<0.05$, ** ; $p<0.01$ vs control group (Dunnet's method).

Results are expressed as means ± s.e.

In summary, following the preparation and evaluation of new benzofuran derivatives as tryptase inhibitors, structural optimization yielded **26** (AY0068) as a potent and selective tryptase inhibitor.

References and Notes:

1. Clark, J. M.; Moore, W. R.; Tanaka, R. D. *Drugs of the Future.* **1996**, 21, 811.

2. McEuen, A. R.; He, S.; Brander, M. L.; Walls, A. F. *Biochem Pharm.* **1996**, 52. 331.

3. Rice, K. D.; Tanaka, R. D.; Katz, B. A.; Numerof, R. P.; Moore, W. R. *Current Pharmaceutical Design,* **1998**, 4, 381.

4. The Ki' values represent the average of three separate determinations (n=3).

Tryptase Inhibiton : The ability (Ki') of compounds to act as inhibitors of tryptase catalytic activity was assessed by determination of the residual concentration of test substance and the residual tryptase activity to cleave the chromogenic substrate (Tos-Gly-Pro-Arg-p-nitroanilide). Tryptase as human mast cell lysate cultured from human umbilical cord blood in 10 mM MES (pH6.1), 2 mM $CaCl_2$, 20% glycerol and 50 µg/ml heparin, assay buffer (0.05 M Tris-HCl (pH8.0), 0.1 M NaCl, 0.1% Triton X-100 and 50 µg/ml heparin) and test substance in DMSO (2% final) were preincubated for 10 min at 37℃. To this mixture was added the chromogenic substrate (0.5mM final) and the initial rate of the chromogenic substrate hydrolysis

was measured by observing the changes in absorbance (OD_{405nm}).

5. **Thrombin Inhibiton :** The ability (Ki') of compounds to act as inhibitors of tryptase catalytic activity was assessed by determination of the residual concentration of test substance and the residual thrombin activity to cleave the chromogenic substrate (H-D-Phe-Pip-Arg-p-nitroanilide). Typically thrombin in 10 mM sodium acetate (pH6.5), 20 mM $CaCl_2$ and 0.1% Triton X-100, assay buffer (0.1 M Tris-HCl (pH8.0), 20 mM $CaCl_2$, 0.1% Triton X-100) and test substance in DMSO (2% final) were preincubated for 10 min at 37℃. To this mixture was added the chromogenic substrate (0.5mM final) and the initial rate of the chromogenic substrate hydrolysis was measured by observing the changes in absorbance (OD_{405nm}).

6. Burgess, L. E.; Newhouse, B. J.; Ibrahim, P.; Rizzi, J.; Kashem, M. A.; Hartman, A.; Brandhuber, B. J.; Wright, C.; Thomson, D. S.; Vigers, G.P.; Koch, K. *Proc. Natl. Acad. Sci. USA*, **1999**, 96, 8348.

7. Pereira, P. J. B.; Bergner, A.; Macedo-Ribeiro, S.; Huber, R.; Matschiner, G.; Fritz, H.; Sommerhoff, C. P.; Bode, W. *Nature*, **1998**, 392, 306-311.

8. Effects on 48 hr homologous passive cutaneous anaphylaxis (PCA) in rats. Rat reaginic antibody (IgE) raised against ovalbumin (OA) was prepared by the method of Stotland and Share.[9] Male SD rats were used; 0.05 mL of anti-OA rat serum, diluted 1:20 with 0.9% saline, was injected intradermally at two points on the dorsum. After 48 hr, PCA reaction was induced by intravenous administration of an aqueous solution (5 mL/kg) containing 2 mg of OA and 5 mg of Evans blue (antigen challenge). A test compound solved in PBS was injected intravenously before antigen challenge. After 20 min, the animals were sacrificed using CO_2 gas and the dorsal skin removed to measure the extravasated dye at each reaction site. The amount of dye was determined by the method of Katayama et al.[10] The percentage inhibition was then calculated from the amount of dye extravasated compared with the control group.

9. Scotland L.M., Share N.N. *Can. J. Physiol. Pharmacol.*, **1974**, *52*, 1114-1118.

10. Katayama S., Shionoya H, Ohtake S. *Microbiol. Immunol.*, **1978**, 22, 89-101.