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Structure–Activity Relationship of Benzo[*b*]thiophene-2-sulfonamide Derivatives as Novel Human Chymase Inhibitors

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Abstract—We have identified a new class of chymase inhibitor through a substituent analysis of MWP00965, which we previously discovered by in silico screening. TY-51076 (**7**) showed high potency ($IC_{50} = 56$ nM) and excellent selectivity for chymase compared to chymotrypsin and cathepsin G (>400-fold). The synthesis and structure–activity relationship of this class are described.

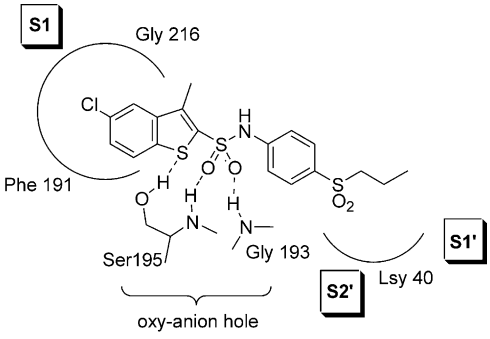
Introduction

Chymase (EC 3.4.21.39) is a chymotrypsin-like serine protease, and is a major non-ACE, angiotensin (Ang) II-generating enzyme in human tissue.[1,2] Chymase also produces transforming growth factor-beta 1 (TGF-beta 1), collagen, endothelin-1 (**1**–**31**) and inflammatory cytokines through the processing of precursors.[3–6] These chymase-induced growth factors and cytokines have been shown to play an important role in tissue remodeling and inflammation. Thus, chymase inhibitors are expected to be useful therapeutic agents in disorders such as congestive heart failure, allergy, vascular proliferation and chronic inflammation following fibrosis.[7,8]

Our research group recently used a pharmacophore hypothesis model of chymase inhibitor to discover a benzo[*b*]thiophene-2-sulfonamide compound, MWP00965.[9] Although MWP00965 shows poor chymase inhibitory activity, it is stable in human plasma. Interestingly, MWP00965 also had a unique manner of binding that is different from previously reported chymase inhibitors in a docking study (Fig. 1).[10–15] In this study, we examined the structure–activity relationships (SARs) of a class of benzo[*b*]thiophene-2-sulfonamide derivatives to identify more potent and specific chymase inhibitors.

Chemistry

Compounds **7**–**19** were generally accessed by reacting the corresponding benzo[*b*]thiophene-2-sulfonyl chlorides with aniline intermediates. Benzo[*b*]thiophene-2-sulfonyl chlorides were obtained commercially or prepared from the corresponding benzothiol via alkylation, cyclization and sulfonylchlorination



MWP00965 (1)

Figure 1. Binding mode between MWP00965 and chymase at the active site.

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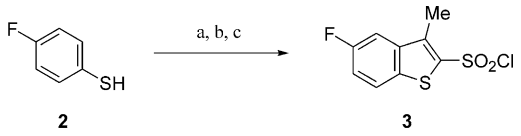
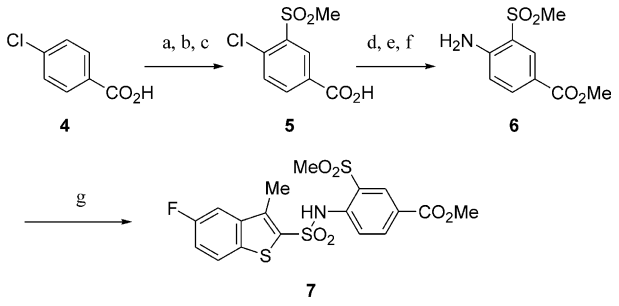


Scheme 1. Synthesis of 5-fluorobenzo[*b*]thiophene-2-sulfonylchloride **3**. Reagents and conditions: (a) chloroacetone, K_2CO_3, acetone, 94%; (b) P_2O_5, H_3PO_4, 69%; (c) $ClSO_3H$, $CHCl_3$, 74%.



Scheme 2. Synthesis of benzo[*b*]thiophene-2-sulfonamide derivative **7**. Reagents and conditions: (a) $ClSO_3H$, 130 °C; (b) 40% NaOH, 4 M Na_2SO_3; (c) MeI, 40% NaOH/MeOH/H_2O, three steps 42%; (d) $BnNH_2$, 140 °C, 55%; (e) H_2, 5% Pd/C, dioxane/MeOH, quant; (f) cH_2SO_4, MeOH, reflux, 90%; (g) **3**, 60% NaH, THF–DMF, 68%.

(Scheme 1). Compound **7** was synthesized by coupling of 5-fluorobenzo[*b*]thiophenesulfonylchloride **3** with 4-aminobenzoic acid **6**. 4-Aminobenzoic acid **6** was prepared in 6 steps as described in the literature (Scheme 2).[16] Other aniline compounds were obtained commercially or prepared by standard methods. Compounds **20**–**27** were synthesized by starting from compound **7** in a conventional sequence.

Results and Discussion

The compounds were tested for their in vitro inhibitory activity using purified recombinant human chymase.[17] The results are given as IC_{50} values, as shown in Tables 1 and 2. Based on our reported Catalyst/HypoGen model and molecular docking model,[9] we focused on investigating the effect of substituents on the *N*-phenyl ring and the benzo[*b*]thiophene ring in MWP00965. In particular, we initially changed the propylsulfonyl group at the para-position of the *N*-phenyl ring to identify an unfavorable substituent in the S1′ region. Changing the propylsulfonyl group to a propylthio-, cyano-, or nitro-group resulted in loss of activity (**8**–**10**), whereas changing it to a simple methoxycarbonyl group (**11**) dramatically enhanced the inhibitory activity to about 6-fold greater than that of MWP00965 (**1**). This improvement in the potency of a methoxycarbonyl group may be the result of increased hydrogen-bond (H-bond) accepting ability. We then examined the *ortho*- and *meta*-positions of the *N*-phenyl ring. Introduction of an electron-donating group or electron-withdrawing group at the *ortho*-position resulted in increased inhibitory activity, except for the *ortho*-methoxycarbonyl-substituted derivatives (**12**–**15**). In particular, the introduction of a methanesulfonyl group (**15**) exhibited in 8-fold higher potency than **11**. On the other hand, the introduction of substituents at the *meta*-position only reduced inhibitory activity (**16** and **17**). Next, we examined the effect of substituents at the 5-position of the benzo[*b*]thiophene ring. Replacement of the chlorine atom with a more electronegative fluorine atom (**7**) resulted in a 4-fold greater inhibitory activity than **15**, while replacement of the chlorine atom with methyl group or hydrogen atom slightly reduced the activity (**18** and **19**).

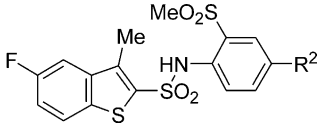
To elucidate the effect of a methoxycarbonyl group at the para-position of the *N*-phenyl ring as an H-bond acceptor subunit, several carbonyl compounds, hydroxymethyl and methoxymethyl groups were examined. As a result, replacement of a methoxycarbonyl group with a acetyl group did not alter the potency (**20**). Replacement of a methoxycarbonyl group with a hydroxymethyl group (**21**), methoxymethyl group (**22**), or carboxamide as amino acid derivatives (**23**, **24**)

Table 1. Effect of substitution on the benzo[*b*]thiophene ring and *N*-phenyl ring

Compd	X	R^1	R^2	R^3	IC_{50} (nM)[a]
					Human chymase
1	Cl	H	$SO_2(CH_2)_2CH_3$	H	10,600
8	Cl	H	$S(CH_2)_2CH_3$	H	NI[b]
9	Cl	H	CN	H	NI
10	Cl	H	NO_2	H	NI
11	Cl	H	CO_2Me	H	1647
12	Cl	NO_2	CO_2Me	H	880
13	Cl	OMe	CO_2Me	H	663
14	Cl	CO_2Me	CO_2Me	H	NI
15	Cl	SO_2Me	CO_2Me	H	203
16	Cl	H	CO_2Me	CO_2Me	4208
17	Cl	SO_2Me	CO_2Me	Me	594
18	Me	SO_2Me	CO_2Me	H	325
19	H	SO_2Me	CO_2Me	H	305
7	F	SO_2Me	CO_2Me	H	56

[a]Values are means of three experiments.
[b]No inhibition at 10 μM.

Table 2. Modifications at the para-position in the *N*-phenyl ring



Compd	R^2	IC_{50} (nM)[a]
		Human chymase
7	CO_2Me	56
20	COMe	82
21	CH_2OH	337
22	CH_2OMe	192
23	CONHGly	154
24	CONHSer	159
25	$CONH_2$	>1000
26	CO_2H	>1000
27	CHO	>1000

[a]Values are means of three experiments.

Table 3. Enzyme selectivity and stability of compounds **7**, **15** and **20**

Compd	Stability[b] (%)	IC_{50} (nM)[a]			
		Human chymase	Bovine chymotrypsin	Human cathepsin G	Human elastase
7	74.3	56	> 10,000	> 10,000	> 10,000
15	93.9	203	> 10,000	> 10,000	> 10,000
20	96.2	82	> 10,000	> 10,000	> 10,000

[a]Values are means of three experiments.
[b]Stability was defined as the percent remaining in rat plasma after incubation for 60 min at 37 °C.

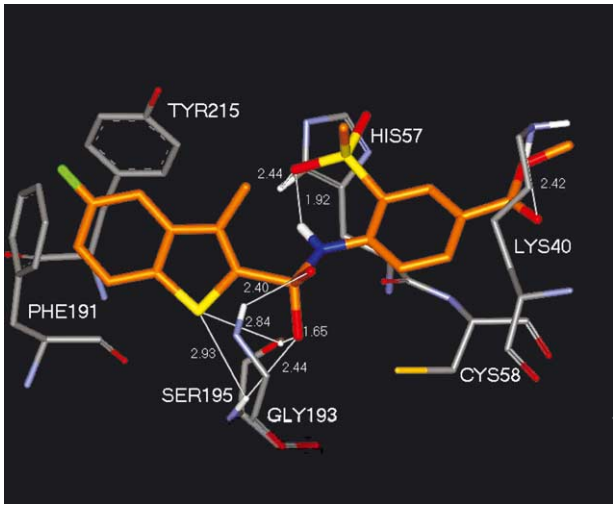


Figure 2. Proposed binding of **7** with chymase at the active site. Red, blue, yellow and green represent oxygen, nitrogen, sulfur, and fluorine atoms, respectively.

decreased the inhibitory activity to 3- to 7-fold less than that of **7**. In addition, replacement of a methoxycarbonyl group with a carbamoyl, carboxyl, or formyl group dramatically decreased the potency (**25**–**27**).

The enzyme selectivity and stability of selected compounds in rat plasma are shown in Table 3. All of the compounds tested (**7**, **15**, **20**) showed excellent selectivity versus chymotrypsin, cathepsin G and elastase (> 400-fold), and were sufficiently stable in rat plasma.

To predict the binding mode of this class of inhibitor, an original hypothesis docking model for **7** with chymase was constructed (Fig. 2).[9] As shown in Figure 2, a benzo[*b*]thiophene ring occupies the S1 specificity pocket, and a sulfonamide moiety is located in the oxyanion hole and forms strong H-bond interactions with Ser195 and Gly193 in a manner similar to MWP00965. In addition, two H-bond interactions are observed between basic amino acid residues and substituents of the *N*-phenyl ring. (1) The methoxycarbonyl group at the 4-position was found close to the S1′–S2′ region and formed a H-bond interaction with Lys40. (2) The methanesulfonyl group at the 2-position formed an H-bond interaction with His57. These interactions are important for maintaining potency, respectively, and H-bond interactions in oxy-anion hole especially would be related to enzyme specificity. To determine the actual shape of the complex, we will need to obtain further experimental evidence including the result of an X-ray crystal structure analysis.

These SAR studies and the docking modeling suggest that H-bond acceptors at the *ortho*- and *para*-positions of the *N*-phenyl ring are necessary to increase the inhibitory activity against chymase. In particular, the ability to interact with the Lys40 region is sensitively dependent on the property of the H-bond acceptor at the *para*-position. Furthermore, we found that substitution at the *meta*-position of the *N*-phenyl ring could sterically hinder the S′ subsite. We also observed that the introduction of electron-withdrawing groups at the 5-position of the benzo[*b*]thiophene ring and the *ortho*-position of the *N*-phenyl ring enhanced interaction with the enzyme. This effect may be due to an increase in the H-bond-accepting ability of the sulfonamide–NH and sulfonyl group.

In conclusion, based on our hypothesis modeling, we improved the potency of lead compound **1**. Compounds **7**, **15** and **20** showed increased potency and greater selectivity than chymotrypsin and cathepsin G, and were stable in plasma. Thus, these compounds should enable more precise studies of the biological roles of chymase, and may lead to new drugs against cardiovascular diseases and inflammation. Additional studies are currently underway, and further modifications and examinations of potency in vivo will be reported in the near future.

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