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Identification of 6-substituted 4-arylsulfonyl-1,4-diazepane-2,5-diones as a novel scaffold for human chymase inhibitors

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Abstract—A novel series of 6-substituted 4-sulfonyl-1,4-diazepane-2,5-diones were designed, synthesized and evaluated as human chymase inhibitors. Structure–activity relationship studies led to the identification of a potent inhibitor, (6*S*)-6-(5-chloro-2-methoxybenzyl)-4-[(4-chlorophenyl)sulfonyl]-1,4-diazepane-2,5-dione, with an IC_{50} of 0.027 μM.

Chymase (EC 3.4.21.39) is a chymotrypsin-type serine protease localized in the secretory granules of mast cells, and is known as a major non-ACE, angiotensin-II (Ang-II)-generating enzyme in human tissue.[1] In addition to Ang-II formation, chymase mediates the production of endothelin-1 (1–31)[2] and modification of apolipoproteins,[3] and several reports have suggested that these functions of chymase are involved in cardiovascular diseases such as atherosclerosis,[4] restenosis,[5] and heart failure.[6] On the other hand, chymase has also been shown to participate in the production of collagen,[7] activation of metalloproteases,[8] and processing of cytokines such as interleukin-1β,[9] transforming growth factor-β1,[10] and stem cell factor.[11] In addition to such activities, the localization of chymase in mast cells has prompted the study of its role in allergy and fibrosis. It has been demonstrated that chymase is involved in the progression of dermatitis[12] and chronic inflammation following cardiac[6,13] and pulmonary fibrosis.[14] Thus, inhibition of chymase is expected to provide therapeutic means for the treatment of cardiovascular diseases, allergic inflammation, and fibrotic disorders.[15]

In a previous paper, we have reported on novel human chymase inhibitors possessing a 3-phenylsulfonylquinazoline-2,4-dione scaffold.[16] Although several compounds identified in that report are effective in some pathological animal models,[15] these compounds are not sufficiently efficacious for use as therapeutic agents. Thus, we continued to explore a new class of human chymase inhibitors, based on the insights from docking studies of these quinazoline derivatives with human chymase. The docking structure of the active site of human chymase with compound **1**[16] revealed that the inner part of the S1 pocket is not completely filled with the aromatic part of the quinazoline moiety of compound **1**, and it seems that the hydrophobic interaction of **1** at the S1 pocket is not fully efficient. On the basis of these findings, we designed benzyl-substituted monocyclic lactams to increase the flexibility of the aromatic part of the inhibitors, in order to sufficiently interact with the S1 pocket, and identified a novel scaffold, 6-substituted 4-arylsulfonyl-1,4-diazepane-2,5-diones (**2**), for human chymase inhibitors (Fig. 1). In this paper, we describe the synthesis and the pharmacological evaluation of this new class of human chymase inhibitors.

Racemic compounds **2** were synthesized as shown in Scheme 1. The Baylis–Hillman reaction of substituted benzaldehydes **3** with ethyl acrylate followed by acetylation gave adducts **4**. S_N2'-type substitution of the acetoxy groups of **4** by sodium azide, and subsequent reduction of the introduced azide groups, led to amines

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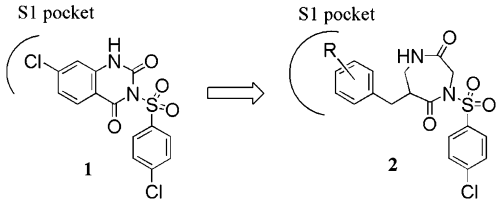
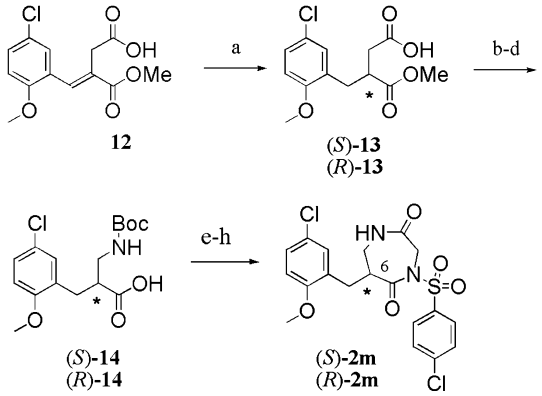


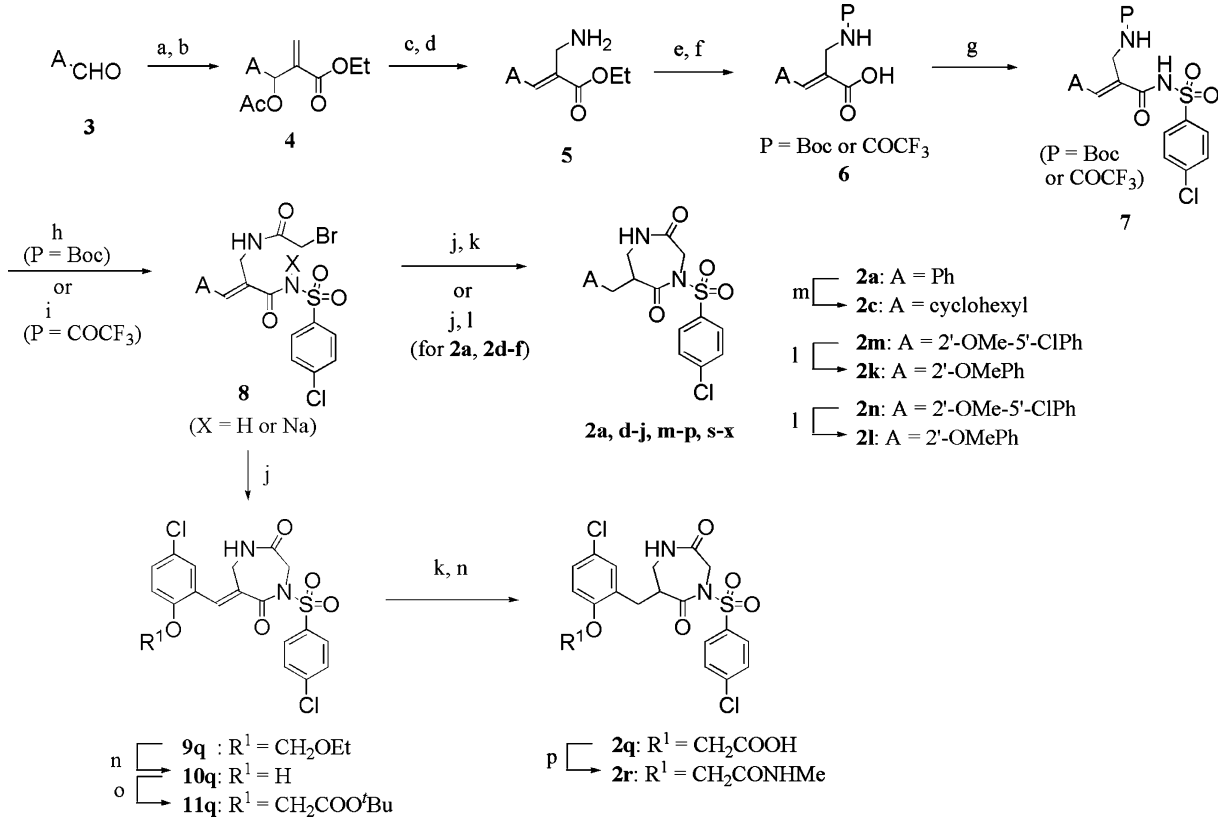
Figure 1. Design of 1,4-diazepane-2,5-dione scaffold.

5, which were converted to β-amino acids **6**[17] by standard methods. Condensation of **6** with *p*-chlorobenzensulfonamide gave acylsulfonamides **7**, which were transformed to bromoacetamides **8** or their sodium salts. Ring closure of **8** to a 7-membered ring by intramolecular alkylation, followed by catalytic hydrogenation, afforded compounds **2**.[18] Compounds **2c**, **2k**, and **2l** were prepared by reduction of precursor compounds **2a**, **2m**, and **2n**, respectively. Compounds **2q** and **2r** were synthesized from **9q**, which was obtained by a similar procedure to that described above. Scheme 2 shows the synthesis of the optically active compounds (*S*)- and (*R*)-**2m**. Optically active β-amino acids (*S*)- and (*R*)-**14** were synthesized[19a] from compounds (*S*)- and (*R*)-**13**, which were obtained by asymmetric hydrogenation of itaconic acid **12** using (*S*,*S*)- or (*R*,*R*)-phenyl-



Scheme 2. Reagents and conditions: (a) H_2, [RhCl(cod)$_2$], (*R*,*R*)- or (*S*,*S*)-Ph-CAPP, Et_3N, MeOH, 40 °C, 3 h; (b) PhI(OH)OTs, MeCN, reflux, 1 h; (c) $(Boc)_2O$, aq NaOH, THF, rt, 2 h; (d) aq KOH, MeOH, rt, 3 h; (e) EDCI HCl, DMAP, *p*-chlorobenzensulfonamide, CH_2Cl_2, rt, 1 h; (f) 1 M HCl/AcOH, rt, 30 min; (g) bromoacetyl chloride, aq NaOH, CH_2Cl_2, 0 °C, 30 min (isolated as sodium salt); (h) DMF, rt, 72 h.

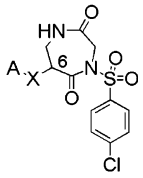
CAPP,[19b,9c] respectively, as a chiral ligand. Using the same procedure described in Scheme 1, (*S*)- and (*R*)-**14** were converted to optically active (*R*)-**2m** and (*S*)-**2m**, respectively.



Scheme 1. Reagents and conditions: (a) ethyl acrylate, DABCO, La(OTf)$_3$, N(CH$_2$CH$_2$OH)$_3$, rt, 24–48 h or ethyl acrylate, DABCO, MeOH (3 equiv), rt, 24–48 h; (b) AcCl, Et_3N, CH_2Cl_2, 0 °C, 1 h; (c) NaN_3, DMSO, rt, 1 h; (d) PPh_3, H_2O, THF, rt, 18 h; (e) aq NaOH, EtOH, rt, 1 h; (f) $(Boc)_2O$, aq NaOH, THF, 0 °C, 1 h or $(CF_3CO)_2O$, pyridine, THF, rt, 1 h; (g) EDCI HCl, DMAP, *p*-chlorobenzensulfonamide, CH_2Cl_2, rt, 1 h; (h) i—1 M HCl/AcOH, rt, 1 h, ii— bromoacetyl chloride, Et_3N, CH_2Cl_2, H_2O, 0 °C, 30 min; (i) i—aq NaOH, MeOH, rt, 1 h, ii—bromoacetyl chloride, aq NaOH, CH_2Cl_2, 0 °C, 30 min; (j) NaH, DMF, 60 °C, 18 h (in the case of X = H) or DMF, 60 °C, 18 h (in the case of X = Na); (k) H_2, Pt(sulfided)–C, THF, rt, 18 h; (l) H_2, Pd–C, THF, rt, 14 h; (m) H_2, PtO_2, AcOH, rt, 8 h; (n) TFA, CH_2Cl_2, 0 °C, 1 h; (o) $BrCH_2COO^tBu$, NaI, $NaHCO_3$, DMF, rt, 20 h; (p) $MeNH_2$ HCl, Et_3N, 2-chloro-1,3-dimethylimidazolinium chloride, CH_2Cl_2, rt, 2 h.

The compounds described above were tested for their in vitro inhibitory activity against recombinant human chymase[20] using Suc-Ala-Ala-Pro-Phe-MCA as a substrate.[21] We initially examined the substituents on the 6-position of the 1,4-diazepane-2,5-dione core, which would interact with the S1 pocket (Table 1). Compound **2b** possessing a benzylidene substituent was less potent than the corresponding benzyl derivative **2a**. Similar activity trends were observed in the other benzylidene series corresponding to **2d**–**2m** (data not shown), suggesting that the benzene ring in the fixed benzylidene substituent is not located in the proper position in the S1 pocket. The more bulky cyclohexylmethyl (**2c**) and 2-naphthylmethyl (**2d**), or more hydrophilic pyridylmethyl groups (**2e** and **2f**), did not show high inhibitory activity. Introduction of a chlorine atom to the benzene ring of **2a** retained or slightly increased activity (**2g**–**2i**), and the 2′-Cl substituent (**2i**) was the most preferable. Other substituents at the 2′-position such as fluoro (**2j**) and alkoxy (**2k** and **2l**) also conferred high activity. The combination of 2′-methoxy and 5′-chloro substituents (**2m**) synergistically enhanced the activity, so that the compound was 10- or fivefold more potent than **2h** or **2k**, respectively. Next, we investigated the effect of the substituents at the 2′- and 5′-positions of **2m** (Table 2). Elongation of the methoxy moiety of **2m** decreased the activity (**2n**–**2p**), whereas addition of a carboxyl (**2q**) or carboxamide (**2r**) group resulted in the same level of activity as **2m**. The fact that **2q** and **2r** having bulky and hydrophilic substituents at the 2′-position showed high inhibitory activity indicated that substituents at the 2′-position were not located inside the S1 pocket, which is space-restricted and hydrophobic, and the carboxyl or methylcarbamoyl group was situated at a hydrophilic region and formed a favorable interaction, such as hydrogen bonding, with another enzyme site (e.g., S2 or S3). The lower activity of compounds **2n**–**2p**, which have longer, hydrophobic alkyl chains at the 2′-alkoxy group, supports this idea. Replacement of the chlorine atom at the 5′-position with a fluoro (**2s**) or cyano (**2t**) group did not alter the potency, but replacement with a more hydrophilic hydroxy (**2u**) or methoxy (**2v**) group decreased the activity remarkably. These results support the idea that substituents at the 5′-position lie in the inner part of the hydrophobic S1 pocket. Introduction of an additional chlorine atom at the 4′-position did not affect the activity (**2m** vs **2w**), but introduction at the 3′-position did reduce the activity (**2s** vs **2x**), probably due to restriction of the space around the 3′-position in the S1 pocket. As shown in Table 3, the representative compound **2m** exhibited high selectivity against other serine proteases,

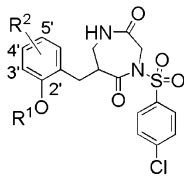
Table 1. Inhibitory activity of compounds **2a**–**2m** against recombinant human chymase



Compound[a]	X	A	IC_{50}[b] (μM)
Chymostatin	—	—	0.043
1	—	—	0.018
2a	–CH_2–	Ph	0.42
2b	–CH=[c]	Ph	1.5
2c	–CH_2–	Cyclohexyl	>10
2d	–CH_2–	2-Naphthyl	>10
2e	–CH_2–	3-Pyridyl	>10
2f	–CH_2–	4-Pyridyl	>10
2g	–CH_2–	4-Cl–Ph	0.46
2h	–CH_2–	3-Cl–Ph	0.31
2i	–CH_2–	2-Cl–Ph	0.11
2j	–CH_2–	2-F–Ph	0.15
2k	–CH_2–	2-OMe–Ph	0.14
2l	–CH_2–	2-OEt–Ph	0.18
2m	–CH_2–	2-OMe-5-Cl–Ph	0.034

[a] Compounds **2a**, **2c**–**2m** are racemates at the 6-position.
[b] For details of the assay conditions, see Ref. 21.
[c] (*E*)-Isomer.

Table 2. Inhibitory activity of compounds **2m**–**2x** against recombinant human chymase



Compound[a]	R^1	R^2	IC_{50}[b] (μM)
2m	Me	5′-Cl	0.034
2n	Et	5′-Cl	0.083
2o	^{n}Pr	5′-Cl	0.22
2p	^{n}Bu	5′-Cl	0.28
2q	CH_2COOH	5′-Cl	0.039
2r	$CH_2CONHMe$	5′-Cl	0.042
2s	Me	5′-F	0.026
2t	Me	5′-CN	0.027
2u	Me	5′-OH	1.1
2v	Me	5′-OMe	7.4
2w	Me	4′,5′-Cl_2	0.023
2x	Me	3′-Cl, 5′-F	4.2

[a] Compounds **2m**–**2x** are racemates at the 6-position.
[b] For details of the assay conditions, see Ref. 21.

Table 3. IC_{50} values[a] of inhibition of human chymase and other serine proteases for compound **2m**

Enzyme	IC_{50}[a] (μM)
Human chymase	0.034
Bovine α-chymotrypsin	0.3
Human cathepsin G	2.9
Bovine trypsin	>10
Human elastase	>10

[a] For details of the assay conditions, see Refs. 21,22.

Table 4. Inhibitory activity of compounds (*S*)-**2m** and (*R*)-**2m** against recombinant human chymase

Compound	IC_{50}[a] (μM)
(*S*)-**2m**	0.027
(*R*)-**2m**	>10

[a] For details of the assay conditions, see Ref. 21.

including chymotrypsin and chymotrypsin-type protease cathepsin G. Finally, we examined the effect of chirality at the 6-position of the 1,4-diazepane core, and found that only (*S*)-**2m** exhibited inhibitory activity (IC_{50}, 0.027 μM) (Table 4).

In conclusion, we have described here the design, synthesis, and evaluation of 6-substituted 1,4-diazepane-2,5-diones as novel and potent human chymase inhibitors. From insights obtained from the docking of compound **1** with the active site of human chymase, we designed 6-benzyl-substituted 4-sulfonyl-1,4-diazepane-2,4-diones to interact sufficiently with the S1 pocket. Modification of the benzyl moiety of a prototype such as **2a** led to the development of a potent inhibitor such as (*S*)-(**2m**), which exhibited an IC_{50} of 0.027 μM.

Acknowledgment

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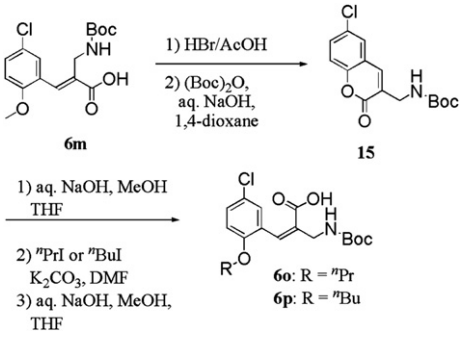
17. Compounds **6o** and **6p** for the synthesis of **2o** and **2p** were synthesized from **6m** via intermediate **15** by a five-step sequence of reactions, as shown below.



18. In the synthesis of compound **2u** (R^1, 2-OMe, 5-OH), the final deprotection reaction (TFA, CH_2Cl_2) was needed to remove the methoxymethyl group which protected the phenolic hydroxy group during the reaction sequence.
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20. The expression and purification of recombinant human chymase are described in Ref. 11b.
21. The human chymase assay was performed as follows: recombinant human chymase was preincubated with the test compounds for 10 min in 50 mM Tris/HCl buffer (pH 7.5) containing 1 M NaCl and 0.01% Triton X-100. The enzyme reaction was initiated with substrate Suc-Ala-Ala-Pro-Phe-MCA (Peptide Institute), supplied at 100 μM, and was stopped with 30% acetic acid after 10 min incubation. The intensity of the fluorescence of the AMC produced by chymase was measured (extinction 380 nm, emission 460 nm), and the IC_{50} value was calculated from the inhibition of AMC formation at each concentration of the tested compound.
22. The inhibitory effects on the enzymatic activity of serine proteases were evaluated by a similar procedure as in Ref. 21. The enzymes and substrates used for the assays were as follows: Suc-Ala-Ala-Pro-Phe-MCA (Peptide Institute) for bovine pancreatic α-chymotrypsin (Sigma) and human neutrophil cathepsin G (Calbiochem); Boc-Gln-Ala-Arg-MCA (Peptide Institute) for bovine pancreatic trypsin (Nacalai Tesque); MeOSuc-Ala-Ala-Pro-Val-pNA (Bachem) for human neutrophil elastase (Calbiochem). The assay buffer was as follows: 0.1 M Tris/HCl (pH 7.5) containing 1 M KCl and 0.01% Triton X-100 for α-chymotrypsin; 50 mM Tris/HCl buffer (pH 7.5) containing 1 M NaCl and 0.01% Triton X-100 for cathepsin G and elastase; 50 mM Tris/HCl buffer (pH 7.5) containing 20 mM $CaCl_2$ for trypsin.