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SF2809 Compounds, Novel Chymase Inhibitors from *Dactylosporangium* sp.

2. Structural Elucidation

MASATO TANI[a,*], KENZO HARIMAYA[a], YASUHIRO GYOBU[a], TORU SASAKI[a], OSAMI TAKENOUCHI[b], TAKASHI KAWAMURA[b], TAKASHI KAMIMURA[b] and TOSHIAKI HARADA[b]

[a] Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd.
Morooka-Cho 760, Kohoku-Ku, Yokohama 222-8567, Japan
[b] Teijin Institute for Biomedical Research, Teijin Ltd.
Asahigaoka 4-3-2, Hino, Tokyo 191-8512, Japan

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Novel chymase inhibitors, SF2809-I, II, III, IV, V and VI, were isolated from the fermentation broth of *Dactylosporangium* sp. SF2809, and their structures were determined by spectroscopic analyses. SF2809 compounds commonly contain a substituted indole moiety and a quinolinone moiety. The two moieties are connected to a methylene carbon in SF2809-I and III. The other compounds, SF2809-II, IV, V and VI, have an additional moiety, a *p*-hydroxyphenyl group or a phenyl group. In these compounds, all of three moieties are connected to a methine carbon. Furthermore, studies concerning the stereochemistry of SF2809-V revealed that the isolated compound was racemic, and the isomer possessing (*R*)-configuration was about thirty times more potent than another isomer.

In our screening program designed to discover new chymase inhibitors from microbial products, we have isolated novel chymase inhibitors, SF2809-I (**1**), II (**2**), III (**3**), IV (**4**), V (**5**) and VI (**6**), from *Dactylosporangium* sp. SF2809 (Fig. 1). These SF2809 compounds inhibited chymase with IC_{50} values of 0.014～7.3 μM, whereas their inhibitory activities against cathepsin G and chymotrypsin were less potent. Therefore, it was suggested that SF2809 compounds are specific chymase inhibitors.

In the preceding paper[1], we described the taxonomy of strain SF2809, and the production, isolation and biological properties of SF2809 compounds. In this paper, we report the physicochemical properties and the structural elucidation of SF2809 compounds. Furthermore, the stereochemistry of the more active isomer is reported.

Results

Physicochemical Properties

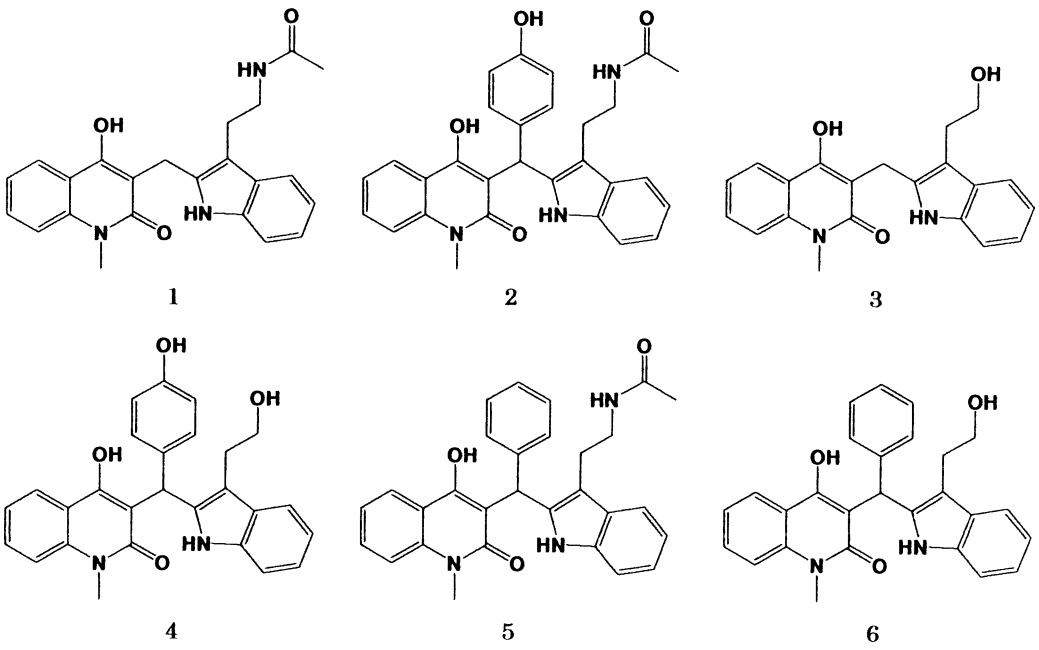
Physicochemical properties of **1**～**6** are summarized in Table 1. These compounds were soluble in methanol, ethyl acetate and dimethylsulfoxide, slightly soluble in chloroform, but insoluble in water and *n*-hexane. The similar UV spectra of these compounds indicated that they had the same chromophore. The IR spectra of these compounds showed characteristic absorption bands of hydroxyl (3400 cm^{-1}, broad absorption), conjugated olefin (1610～1630 cm^{-1}) and aromatic (748～758 cm^{-1}) groups. Optical activity was not observed in any of SF2809 compounds. ^1H and ^{13}C NMR data observed in CD_3OD are listed in Tables 2 and 3, respectively.

Structures of **1** and **3**

The molecular formula of **1** was established as $C_{23}H_{23}N_3O_3$ by HRFAB-MS data. The ^1H, ^{13}C NMR and

* Corresponding author: masato_tani@meiji.co.jp

Fig. 1. Structures of SF2809 compounds.



HSQC spectra of **1** revealed the presence of two methyls, three methylenes, eight sp^2 methines, seven sp^2 quaternary carbons, two enolic carbons and a carbonyl carbon. The methyl protons at δ_H 1.89 (H_3-12″) and δ_H 3.73 (H_3-9′) were assigned to an acetyl group and an *N*-methyl group, respectively. ^{1}H-^{1}H COSY and HMBC experiments suggested the existence of two di-substituted benzene rings (C-4a′～8a′ and C-3a″～7a″) as shown in Fig. 2. One of the aromatic rings was indicated to construct a 3-(1-acetylaminoethyl)indole moiety based on the long range ^{1}H-^{13}C correlations from H_2-8″ to C-2″, C-3″ and C-3a″, and from H_2-9″ to C-3″ and C-11″. Another aromatic ring was determined to be an *N*-methyl-4-hydroxy-2-quinolinone moiety as follows. The aromatic proton at δ_H 8.12 (H-5′) showed a long-range ^{1}H-^{13}C correlation with the enolic carbon C-4′ (δ_C 158.8). The *N*-methyl protons at δ_H 3.73 (H_3-9′) showed the correlations with the enolic C-2′ (δ_C 166.2) and the aromatic C-8a′ (δ_C 140.2) carbons. The remaining sp^2 quaternary carbon at δ_C 110.1 was assigned to C-3′ position based on the long-range ^{1}H-^{13}C correlation from the methylene protons at δ_H 4.17 (H_2-1). These two moieties, a 3-(1-acetylaminoethyl)indole moiety and an *N*-methyl-4-hydroxy-2-quinolinone moiety, were considered to be connected to the methylene C-1 (δ_C 22.1). As to nitrogen atoms, ^{15}N HMBC experiment confirmed the presence of three nitrogen atoms. Long range ^{1}H-^{15}N correlations from H_3-9′ to N-1′ (δ_N −245), H_2-1 to N-1″ (δ_N −245) and H_3-12″ to N-10′ (δ_N −258) were observed. Consequently, the structure of **1** was elucidated as shown in Fig. 2.

The comparison of the molecular formula ($C_{21}H_{20}N_2O_3$) and the NMR data of **3** with those of **1** suggested that the structure of **3** is an analogue of **1**, in which an acetylamino group of **1** is replaced by a hydroxyl group. The chemical shift of the C-9″ carbon at δ_C 63.8 supported the replacement by a hydroxyl group in **3**.

Structures of **2** and **4**

The molecular formula of **2** and **4** were established as $C_{29}H_{27}N_3O_4$ and $C_{27}H_{24}N_2O_4$ by HRFAB-MS, respectively. ^{1}H-^{1}H COSY and HMBC experiments of **2** suggested that a methylene proton at δ_H 4.17 (H_2-1) in **1** is substituted by a *p*-hydroxyphenyl group in **2**. The resultant methine proton at δ_H 6.31 (H-1) was connected to three olefin carbons, therefore it was observed in a relatively low field. In the same way, the structure of **4** was determined to be an analogue of **3** with a *p*-hydroxyphenyl substituent.

Structures of **5** and **6**

The molecular formula of **5** was established as

Table 1. Physico-chemical properties of SF2809 compounds.

	1	2	3
Appearance	pale yellow powder	pale red powder	pale yellow powder
Molecular weight	389	481	348
Molecular formula	$C_{23}H_{23}N_3O_3$	$C_{29}H_{27}N_3O_4$	$C_{21}H_{20}N_2O_3$
HR-FABMS (*m/z*)			
calcd:	390.1818 $(M+H)^+$	482.2080 $(M+H)^+$	349.1552 $(M+H)^+$
found:	390.1820	482.2090	349.1566
UV λ_{max} (MeOH) nm (ε)			
with 0.01N HCl	227 (45900)	227 (68600)	227 (47000)
	277 (10900)	278 (15500)	276 (10800)
	284 (10900)	286 (15500)	284 (11000)
	319 (6420)	322 (10600)	319 (6450)
	332 (5250)	335 (7730)	332 (5050)
with 0.01N NaOH	206 (54900)	207 (93200)	205 (44300)
	222 (54500)	223 (81600)	223 (46300)
	257s (14000)	260s (29000)	257s (12200)
	292 (12100)	283 (23200)	292 (10500)
	310 (11700)	292 (22700)	310 (10100)
		310s (18800)	
IR ν_{max} (KBr) cm^{-1}	3400, 1630, 1610,	3400, 1632, 1610,	3380, 1628, 1608,
	1589, 1572, 1460,	1572, 1510, 1460,	1576, 1460, 1336,
	1338, 1238, 748,	1240, 754	1236, 1093, 758
$[\alpha]^{25}_D$ (*c* 0.2, MeOH)	0°	0°	0°

	4	5	6
Appearance	pale red powder	colorless powder	colorless powder
Molecular weight	440	465	424
Molecular formula	$C_{27}H_{24}N_2O_4$	$C_{29}H_{27}N_3O_3$	$C_{27}H_{24}N_2O_3$
HR-FABMS (*m/z*)			
calcd:	441.1815 $(M+H)^+$	466.2131 $(M+H)^+$	425.1865 $(M+H)^+$
found:	441.1823	466.2130	425.1878
UV λ_{max} (MeOH) nm (ε)			
with 0.01N HCl	228 (57400)	227 (62800)	227 (50200)
	280 (14500)	278 (14400)	278 (11900)
	323 (8020)	284 (14000)	322 (7490)
	334 (6170)	323 (9120)	334 (5960)
		335 (6980)	
with 0.01N NaOH	205 (75700)	207 (83700)	205 (51700)
	223 (58000)	222 (75300)	223 (51100)
	260s (16700)	257s (23700)	258s (13200)
	286 (16000)	284 (17700)	293 (10500)
	293 (16000)	292 (18100)	310 (9790)
	310 (13600)	310 (15800)	
IR ν_{max} (KBr) cm^{-1}	3410, 1630, 1610,	3425, 1632, 1611,	3430, 1624, 1610,
	1574, 1510, 1460,	1589, 1572, 1460,	1589, 1574,1460,
	1338, 1240, 756	1338, 1216, 754	1338, 1091, 756
$[\alpha]^{25}_D$ (*c* 0.2, MeOH)	0°	0°	0°

$C_{29}H_{27}N_3O_3$ by HRFAB-MS data. The molecular formula and the NMR data of **5** indicated that **5** is a 4‴-dehydroxy analogue of **2**. The structure of **6** was also identified to be a 4‴-dehydroxy analogue of **4** based on the molecular formula ($C_{27}H_{24}N_2O_3$) and the NMR data.

Stereochemistries

Compounds **2**, **4**, **5** and **6** possess one asymmetric carbon at C-1, however, these compounds did not show optical activity. It seemed that SF2809 compounds were racemic. Actually, HPLC analysis of **5** with a chiral column exhibited two peaks. It prompted us to separate the optical

Table 2. 400 MHz ^{1}H NMR data for SF2809 compounds in CD_3OD (δ [ppm], J [Hz]).

Position	1	2	3
1	4.17 (2H, s)	6.31 (H, s)	4.21 (2H, s)
5'	8.12 (H, dd, J=8.1, 1.4)	8.07 (H, dd, J=8.1, 1.4)	8.05 (H, dd, J=8.1, 1.2)
6'	7.30 (H, ddd, J=8.1, 7.1, 1.0)	7.32 (H, ddd, J=8.1, 7.1, 1.0)	7.28 (H, ddd, J=8.1, 7.1, 1.2)
7'	7.62 (H, ddd, J=8.5, 7.1, 1.4)	7.63 (H, ddd, J=8.8, 7.1, 1.4)	7.63 (H, ddd, J=8.5, 7.1, 1.2)
8'	7.54 (H, dd, J=8.5, 1.0)	7.55 (H, dd, J=8.8, 1.0)	7.55 (H, dd, J=8.5, 1.2)
9'	3.73 (3H, s)	3.70 (3H, s)	3.75 (3H, s)
4"	7.44 (H, dd, J=7.8, 1.2)	7.52 (H, d, J=7.8)	7.41 (H, dd, J=7.1, 1.4)
5"	6.92 (H, ddd, J=7.8, 7.1, 1.2)	6.97 (H, dd, J=7.8, 7.1)	6.92 (H, ddd, J=7.1, 7.1, 1.2)
6"	6.96 (H, ddd, J=7.8, 7.1, 1.2)	7.03 (H, dd, J=8.0, 7.1)	6.96 (H, ddd, J=7.1, 7.1, 1.4)
7"	7.20 (H, dd, J=7.8, 1.2)	7.28 (H, d, J=8.0)	7.18 (H, dd, J=7.1, 1.2)
8"	3.09 (2H, t, J=7.1)	3.00 (H, m)	3.09 (2H, t, J=6.6)
		3.14 (H, m)	
9"	3.48 (2H, t, J=7.1)	3.33 (H, m)	3.82 (2H, t, J=6.6)
		3.50 (H, m)	
12"	1.89 (3H, s)	1.72 (3H, s)	
2"', 6"'		6.87 (2H, d, J=8.8)	
3"', 5"'		6.63 (2H, d, J=8.8)	
Position	4	5	6
1	6.27 (H, s)	6.42 (H, s)	6.41 (H, s)
5'	8.08 (H, dd, J=8.1, 1.4)	8.11 (H, dd, J=8.1, 1.2)	8.12 (H, dd, J=8.1, 1.4)
6'	7.33 (H, ddd, J=8.1, 7.1, 1.0)	7.23 (H, ddd, J=8.1, 7.1, 1.0)	7.25 (H, ddd, J=8.1, 7.1, 1.0)
7'	7.65 (H, ddd, J=8.5, 7.1, 1.4)	7.56 (H, ddd, J=8.5, 7.1, 1.0)	7.57 (H, ddd, J=8.1, 7.1, 1.4)
8'	7.57 (H, dd, J=8.5, 1.0)	7.47 (H, dd, J=8.5, 1.0)	7.49 (H, dd, J=8.5, 1.0)
9'	3.71 (3H, s)	3.67 (3H, s)	3.68 (3H, s)
4"	7.52 (H, d, J=7.8)	7.52 (H, d, J=7.6)	7.51 (H, dd, J=7.8, 1.2)
5"	6.98 (H, dd, J=7.8, 7.1)	6.95 (H, dd, J=7.6, 7.1)	6.96 (H, ddd, J=7.8, 7.1, 1.2)
6"	7.04 (H, dd, J=7.8, 7.1)	7.01 (H, dd, J=8.1, 7.1)	7.01 (H, ddd, J=7.8, 7.1, 1.2)
7"	7.29 (H, d, J=7.8)	7.27 (H, d, J=8.1)	7.27 (H, dd, J=7.8, 1.2)
8"	3.05 (H, m)	3.04 (H, m)	3.08 (H, m)
	3.13 (H, m)	3.18 (H, m)	3.18 (H, m)
9"	3.73 (2H, m)	3.33 (H, m)	3.73 (2H, m)
		3.55 (H, m)	
12"		1.68 (3H, s)	
2"', 6"'	6.83 (2H, d, J=8.8)	7.02 (2H, m)	7.00 (2H, m)
3"', 5"'	6.63 (2H, d, J=8.8)	7.14 (2H, m)	7.14 (2H, m)
4"'		7.06 (H, m)	7.06 (H, m)

Chemical shifts are shown with reference to TMS as an internal standard.

isomers, and examine their inhibitory activities against chymase as well as cathepsin G and chymotrypsin.

Optical isomers of **5** were separated by preparative HPLC, and two fractions containing each isomer were obtained. These fractions were evaporated to dryness, then their optical rotation and biological activities were measured. Initially eluted isomer showed an optical rotation at $[\alpha]_D$ +84.1° (c 0.2, methanol) and inhibited chymase with the IC_{50} value of 0.007 μM. On the other hand, subsequently eluted isomer exhibited an optical rotation at $[\alpha]_D$ −82.2° (c 0.2, methanol) and inhibited chymase with the IC_{50} value of 0.23 μM. Therefore, it was suggested that the potency of chymase inhibition depends on the configurations of the optical isomers. Their inhibitory activities against other proteases are listed in Table 4.

In order to determine the absolute configurations of the active components, crystallographic studies were carried out. Prior to the studies, **5** was artificially synthesized

Table 3. 100 MHz ^{13}C NMR data for SF2809 compounds in CD_3OD (δ [ppm]).

Position	1	2	3	4	5	6
1	22.1 (t)	37.7 (d)	22.2 (t)	38.1 (d)	38.2 (d)	38.4 (d)
2'	166.2 (s)	165.7 (s)	166.2 (s)	165.6 (s)	166.1 (s)	166.2 (s)
3'	110.1 (s)	114.9 (s)	110.0 (s)	115.1 (s)	113.5 (s)	113.4 (s)
4'	158.8 (s)	161.0 (s)	159.7 (s)	159.9 (s)	165.3 (s)	165.4 (s)
4a'	118.5 (s)	118.9 (s)	118.2 (s)	118.1 (s)	120.8 (s)	120.9 (s)
5'	124.7 (d)	124.5 (d)	124.4 (d)	124.5 (d)	125.1 (d)	125.3 (d)
6'	122.9 (d)	123.1 (d)	123.1 (d)	123.3 (d)	122.6 (d)	122.6 (d)
7'	131.8 (d)	132.1 (d)	132.0 (d)	132.2 (d)	131.6 (d)	131.6 (d)
8'	115.7 (d)	115.8 (d)	115.6 (d)	115.8 (d)	115.5 (d)	115.4 (d)
8a'	140.2 (s)	140.2 (s)	140.0 (s)	140.2 (s)	140.5 (s)	140.5 (s)
9'	30.2 (q)	30.2 (q)	30.3 (q)	30.3 (q)	30.1 (q)	30.1 (q)
2"	136.5 (s)	137.9 (s)	135.6 (s)	137.7 (s)	138.7 (s)	138.8 (s)
3"	108.6 (s)	110.6 (s)	108.8 (s)	110.2 (s)	110.2 (s)	109.6 (s)
3a"	129.7 (s)	129.3 (s)	129.6 (s)	129.4 (s)	129.2 (s)	129.4 (s)
4"	118.5 (d)	118.9 (d)	118.4 (d)	118.9 (d)	118.9 (d)	118.8 (d)
5"	119.4 (d)	119.5 (d)	119.4 (d)	119.6 (d)	119.4 (d)	119.3 (d)
6"	121.5 (d)	122.0 (d)	121.5 (d)	122.1 (d)	121.9 (d)	121.7 (d)
7"	111.6 (d)	111.9 (d)	111.5 (d)	111.9 (d)	111.8 (d)	111.7 (d)
7a"	137.1 (s)	136.9 (s)	137.1 (s)	136.9 (s)	136.9 (s)	136.8 (s)
8"	24.8 (t)	25.0 (t)	28.3 (t)	28.7 (t)	24.9 (t)	28.8 (t)
9"	41.5 (t)	41.7 (t)	63.8 (t)	63.8 (t)	41.7 (t)	63.9 (t)
11"	173.6 (s)	173.4 (s)			173.4 (s)	
12"	22.6 (q)	22.5 (q)			22.4 (q)	
1"'		134.6 (s)		134.3 (s)	144.9 (s)	144.9 (s)
2"', 6"'		129.2 (d)		129.1 (d)	128.0 (d)	128.0 (d)
3"', 5"'		115.9 (d)		115.9 (d)	128.9 (d)	128.8 (d)
4"'		156.4 (s)		156.5 (s)	126.3 (d)	126.3 (d)

Chemical shifts are shown with reference to CD_3OD as 49.8 ppm.

Fig. 2. NMR correlations for **1** and **2**.

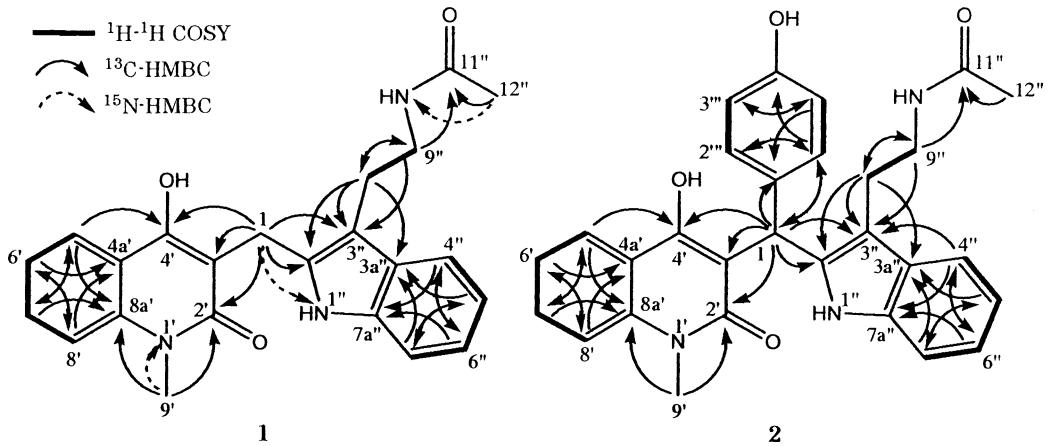
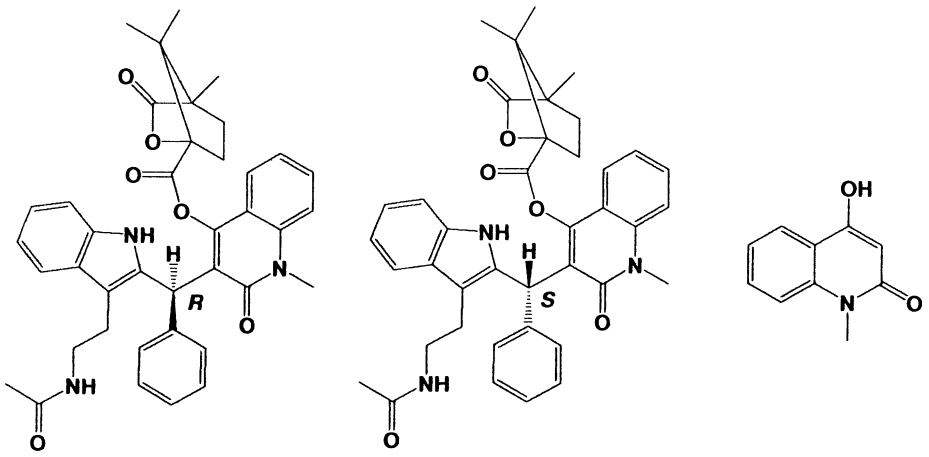


Fig. 3. Structures of **7**, **8** and **9**.

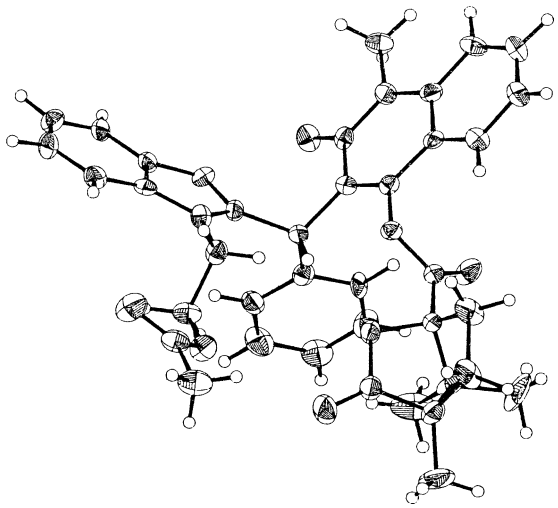


7 **8** **9**

Table 4. Enzyme inhibitory activities of 5 and its optical isomers.

	IC_{50}, μM		
	Chymase	CathepsinG	Chymotrypsin
(±) 5	0.043	1.0	>20
(+) 5	0.007	0.31	>20
(−) 5	0.23	19.0	>20

Fig. 4. ORTEP picture of **8**.



from 3-(1-acetylaminoethyl)indole, *N*-methyl-4-hydroxy-2-quinolinone and benzaldehyde to obtain enough amounts of the sample for the crystallization. The physicochemical properties (^{1}H NMR, ^{13}C NMR, UV, IR and $[\alpha]_D$) of the synthesized **5** were identical to those of the natural compound. The synthetic method is reported elsewhere[2].

Racemic compound **5** was converted to camphanic acid esters (**7** and **8**, Fig. 3), and after separation of the obtained two diastereomers by pTLC, the polar ester (**8**) was crystallized from a mixture of methanol and chloroform. A single crystal X-ray diffraction analysis of **8** proved the absolute stereochemistry at C1 to be (*S*)-configuration (Fig. 4).

Subsequently, **8** was hydrolyzed with sodium hydroxide, and then optical properties of the hydrolysate were analyzed. As a result, the optical rotation ($[\alpha]_D$) of the hydrolysate was −78.0° (*c* 0.3, methanol), and the retention time of the hydrolysate in HPLC analysis was identical to that of (−)-**5**. On the other hand, the hydrolysate of **7**, which was considered to have (*R*)-configuration, exhibited $[\alpha]_D$ +72.0° (*c* 0.3, methanol) and HPLC retention time identical to that of (+)-**5**. Consequently, it was concluded that the more potent inhibitor, (+)-**5**, possessed (*R*)-configuration.

Discussion

Six novel compounds have been isolated as human

chymase inhibitors and their structures have been elucidated. All SF2809 compounds possess *N*-methyl-4-hydroxy-2-quinolinone moiety and 3-substituted indole moiety. In the structures of **1** and **3**, two moieties are linked by a methylene, whereas in the structures of **2**, **4**, **5** and **6**, this methylene is substituted by a phenyl or a *p*-hydroxyphenyl group. In addition to these three patterns, there is a difference in the substitutional group at 9″-position of the 3-substituted indole moiety, a hydroxyl group or an acetylamino group, which makes six analogues in total. As for the production of quinolinone derivatives, several plant alkaloids were reported[3,4], however, only a few quinolinone compounds produced by actinomycete are known[5,6].

Studies of stereochemistories have revealed that **5** is racemic. It is quite unique that a natural product is racemic. Considering the high reactivity of 3-H of *N*-methyl-4-hydroxy-2-quinolinone and 2-H of 3-(1-acetylamino)ethylindole, spontaneous condensation of the constituent moieties could be expected. Actually, we have isolated a possible precursor, *N*-methyl-4-hydroxy-2-quinolinone (**9**), from the fermentation broth of strain SF2809. This compound had been found easily by HPLC analysis with a photodiodearray detector, because it had the same UV absorption as SF2809 compounds. At present, however, biosynthetic pathway of SF2809 compounds remains unrevealed.

Compound **9** inhibited chymase with the IC_{50} value of 300 μM. Although the potency of this compound was low, the addition of an indole moiety improved the potency about 100 times. Compounds **1** and **3** presented the IC_{50} values of 7.3 μM and 2.1 μM, respectively. Furthermore, the addition of a phenyl group also drastically improved the potency. Compounds **2**, **4**, **5** and **6** showed 100 times higher potency than **1** and **3** (IC_{50}=0.041, 0.081, 0.043 and 0.014 μM, respectively). The difference between a hydroxyl group and an acetylamino group at 9″ position, *i.e.* **2** and **4**, or **5** and **6**, seems to be less important. The substitution of 4‴ proton by a *p*-hydroxyl group, which corresponds to the relation between **2** and **5**, or **4** and **6**, seems to slightly reduce the potency.

Recently, several chymase inhibitors have been reported[7]. Some of them possess three independent aromatic groups in their molecules, such as 2,4-quinazolinedione derivatives containing two phenyl moieties[8], 5-amino-6-oxo-1,6-dihydropyrimidine derivatives containing phenylalanine and a phenyl substituent[9], and benzimidazole derivatives containing naphthalene and benzoic acid moieties[10]. These functional groups are considered to interact with an active site of chymase as discussed by NIWATA *et al.*[11] In our studies, an isomer of (*R*)-configuration was 30 times as potent as another isomer of (*S*)-configuration. This finding implies that phenyl moiety could be recognized stereoselectively by the active site of chymase, possibly P_1 pocket. The detail analysis of a three dimensional complex model is now in progress.

Experimental

Generals

NMR spectra were obtained on a JEOL JNM-LA400 spectrometer. The chemical shifts of ^1H were given in ppm using TMS as an internal standard, and the chemical shifts of ^{13}C were given with reference to CD_3OD as 49.8 ppm. In the case of ^{15}N NMR, sodium nitrate was used as an external standard. UV and IR absorption spectra were recorded on Shimadzu UV-260 and Shimadzu FTIR-8100 spectrophotometers, respectively. FAB-MS and HR-FABMS were measured using a JEOL JMS-700 mass spectrometer. Optical rotations were determined on a JASCO DIP-370 digital polarimeter using a 10 cm cell.

HPLC Analysis and Separation of a Racemic Compound

The analysis of (±) **5** was performed on a Sumichiral OA-7000 column (250 mm×4.6 mm i.d., Sumitomo chem.). A mixture of methanol and aqueous 0.02 M KH_2PO_4 (60 : 40) was used as a mobile phase at a flow rate of 1.0 ml/minute at 40°C. The elution was monitored photometrically at 280 nm. In this analysis, (+)-**5** and (−)-**5** were eluted at 27.2 and 25.0 minutes respectively. Preparative HPLC for the separation of optical isomers was performed using a Chiralpak AS column (250 mm×10 mm i.d., Daicel Chem. Ind.). Six mg of **5** was injected and eluted with a mixture of *n*-hexane, isopropanol and trifluoroacetic acid (100 : 100 : 0.1) at a flow rate of 1.8 ml/minute and at room temperature, monitoring the UV absorption at 320 nm. Eluates at 9.9 minutes and 13.9 minutes were collected and evaporated to obtain 2.9 mg of (+)-**5** and 2.4 mg of (−)-**5**, respectively.

Measurement of Biological Activities

Enzyme inhibitory analyses of recombinant human chymase, human cathepsin G and bovine pancreatic chymotrypsin were measured as described in the preceding paper[1].

Synthesis of Camphanic Acid Ester (**7** and **8**)

To a solution of chemically synthesized (±) **5** (232.5 mg) in 10 ml of pyridine were added 217 mg of 1-(*S*)-(−)-

camphanic chloride, then the reaction mixture was stirred for 19.5 hours at room temperature. After addition of water, the solution was extracted with ethyl acetate, and the organic layer was evaporated to obtain 388 mg of the residue. An aliquot (90 mg) of the residue was purified on a silica gel pTLC (chloroform - methanol, 20 : 1) to give two diastereomers, 17.4 mg of a less polar substance (**7**) and 24.7 mg of a polar substance (**8**). The yields were calculated to be 23.1% and 33.0%, respectively. Compound **8** was crystallized twice from a mixture of chloroform and methanol (20 : 1).

7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, s), δ 1.15 (3H, s), δ 1.22 (3H, s), δ 1.72 (2 H, m), δ 2.11 (1H, m), δ 2.44 (1H, m), δ 2.94 (1H, m), δ 3.12 (1H, m), δ 3.42 (2H, m), δ 3.66 (3H, s), δ 5.85 (1H, s), δ 7.01 (2H, m), δ 7.05 (1H, m), δ 7.12 (2H, m), δ 7.17 (2H, m), δ 7.25 (2H, m), δ 7.37 (1H, m), δ 7.46 (1H, m), δ 7.56 (1H, m), δ 7.71 (1H, m), δ 9.94 (1H, s); FAB-MS *m*/*z* 646 $(M+H)^+$.

8: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, s), δ 1.07 (3H, s), δ 1.10 (3H, s), δ 1.86 (2 H, m), δ 2.44 (1H, m), δ 2.56 (1H, m), δ 2.94 (1H, m), δ 3.10 (1H, m), δ 3.46 (2H, m), δ 3.65 (3H, s), δ 5.87 (1H, s), δ 6.97 (2H, m), δ 7.03 (1H, m), δ 7.09 (2H, m), δ 7.13 (2H, m), δ 7.23 (2H, m), δ 7.37 (2H, m), δ 7.57 (1H, m), δ 7.73 (1H, m), δ 9.95 (1H, s); FAB-MS *m*/*z* 646 $(M+H)^+$.

Isolation of *N*-Methyl-4-hydroxy-2-quinolinone (**9**)

Compound **9** was isolated in the same method as **1**～**6** described in the preceding paper[1]. At the final step of the purification by preparative HPLC, **9** was eluted prior to **1**.

9: ^{1}H NMR (400 MHz, DMSO) δ 3.52 (3H, s), δ 5.85 (1H, s), δ 7.21 (1H, dd), δ 7.46 (1H, d), δ 7.61 (1H, dd), δ 7.89 (1H, d); FAB-MS *m*/*z* 176 $(M+H)^+$.

Hydrolysis of **7** and **8**

To a methanol solution (3.5 ml) containing 32 mg of **7** or **8**, 43 mg of sodium hydroxide was added. After stirring for one hour at room temperature, dry ice and water were added to the reaction mixture under cooling on the ice bath. The hydrolysates were extracted with chloroform following neutralization with 1-N hydrochloric acid, and then chloroform layers were evaporated to obtain 27.7 mg and 25.0 mg of residues from **7** and **8**, respectively. The residues were analyzed by a chiral HPLC.

X-Ray Crystallographic Analysis of Compound **8**

X-Ray diffraction measurement was performed on a Rigaku AFC5R diffractometer using graphite monochromated Cu$K\alpha$ radiation and rotating anode generator.

The crystal data of **8** are as follows: Empirical formula; $C_{39}H_{39}N_3O_6$. Formula weight; 645.75. Crystal system; orthorhombic. Space group; $P2_12_12_1$. Lattice parameters; a=18.701 Å, b=18.858 Å, c^-=9.548 Å, α=90°, β=90°, γ=90°, Z=4, V=3370 Å^3, d=1.27 g/cm^3. Final R and weighted R values were 0.050 and 0.064, respectively.

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