Synthesis and *in Vitro* Antifungal Activities of Novel Triazole Antifungal Agent CS-758

Toshiyuki Konosu,*,[a] Sadao Oida,[a] Yoshie Nakamura,[a] Shinobu Seki,[a] Takuya Uchida,[a] Atsushi Somada,[a] Makoto Mori,[a] Yoshiko Harada,[a] Yasuki Kamai,[b] Tamako Harasaki,[b] Takashi Fukuoka,[b] Satoshi Ohya,[b] Hiroshi Yasuda,[b] Takahiro Shibayama,[c] Shin-ichi Inoue,[c] Akihiko Nakagawa,[c] and Yasuo Seta[d]

Medicinal Chemistry Research Laboratories,[a] Biological Research Laboratories,[b] Pharmacokinetics and Drug Delivery Research Laboratories,[c] and Product Development Laboratories,[d] Sankyo Co., Ltd., 1–2–58 Hiromachi, Shinagawa-ku, Tokyo 140–8710, Japan.   Received July 9, 2001; accepted September 17, 2001

Synthesis and *in vitro* antifungal activities of a novel triazole antifungal agent CS-758 (former name, R-120758) are described. The minimum inhibitory concentrations (MICs) of a series of dioxane–triazole compounds related to R-102557 were examined. Variation of the length of the chain between the dioxane ring and the phenyl ring revealed that the linkage with two double bonds is the most preferable. When a cyano group was introduced to the C4 position on the benzene ring, MICs improved further. A fluorine atom was introduced to obtain CS-758. The MICs of CS-758 surpassed those of fluconazole and itraconazole against *Candida*, *Aspergillus* and *Cryptococcus* species. The precursor (*E,E*)-aldehyde was synthesized stereoselectively from 3-fluoro-4-methylbenzonitrile using the Horner–Wadsworth–Emmons reaction.

Key words   CS-758; R-120758; antifungal; triazole; Horner–Wadsworth–Emmons reaction

Recently we synthesized a series of dioxane–triazole antifungal agents, which are effective against systemic mycoses.[1] From such compounds, R-102557 (**3d**) was chosen as a candidate compound for development on the basis of its *in vivo* activities in mice. Since it was found that R-102557's high *in vivo* activities were caused by its excessively long elimination half-life in plasma,[2] we determined to search for a similar compound but with a shorter half-life. In this paper, we will describe the synthesis and *in vitro* antifungal activities of our new compound CS-758 (**3j**; former name, R-120758),[3] which is currently under development as an antifungal agent against systemic mycoses.

Starting our search, our attention was focused on minimun inhibitory concentrations (MICs) because as the elimination half-life in plasma gets shorter, the requirements for MICs become severer. We reasoned that, even if a compound with good MICs were to exhibit only fair *in vivo* activities in mice, the compound might still be able to remain sufficiently long in humans to exhibit reasonable therapeutic effects. On this assumption, some of the previous compounds, such as **3b**—**g**,[1] were re-evaluated on the basis of MICs. Their precursor **1a** was also submitted for MIC determination. Some additional compounds, such as **2a**, **3a**, **3h**—**j**, were prepared.

Preparation of the compounds **2a**, **3a**, **3h**—**j** were done in acetalization reactions of **4** with aldehydes **5a**, **6a**, **6h**—**j**, respectively (Chart 2). Previously, molecular sieves were then used to remove water,[1] but this time we conducted the reaction by concentrating a tetrahydrofuran (THF) solution containing **4**, the aldehydes, and *p*-toluenesulfonic acid in a rotary evaporator. Aldehyde **6j**, a precursor of CS-758, was
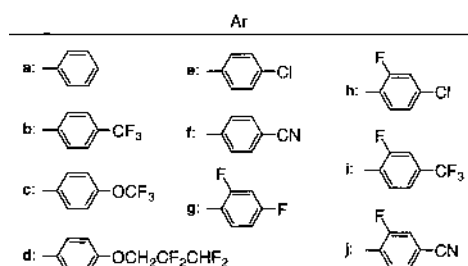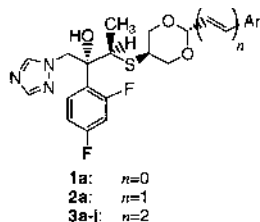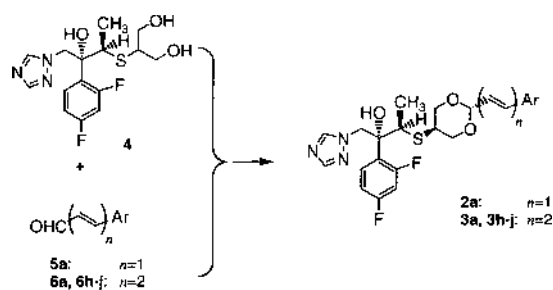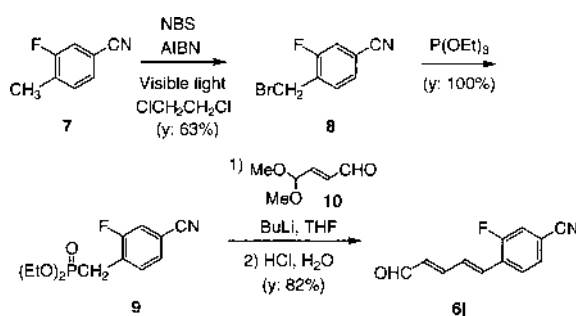

Chart 1

Chart 2

Chart 3

prepared stereoselectively using the Horner–Wadsworth–Emmons reaction of fumaraldehyde monoacetal **10** as depicted in Chart 3.

The MICs of the compounds with various side chain lengths were determined against clinically important pathogenic fungi (Table 1). An unsubstituted phenyl group was first selected as the Ar group and compounds **1a** ($n=0$), **2a** ($n=1$), and **3a** ($n=2$) were compared. Compound **3a** with two olefinic double bonds ($n=2$) showed the best MICs.[4]

The difference was most clear in the activity against *Cryptococcus neoformans*.

We then fixed n to 2, and compared the MICs of the derivatives **3b**—**f** having various substituents at the C4 position on the benzene ring Ar (Table 2). The compound with a cyano group (**3f**) showed remarkable effect with excellent MICs. The difference was the most clear in the activity against *Aspergillus fumigatus*.
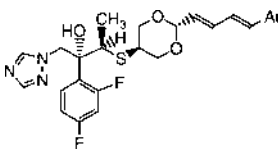
We finally introduced a fluorine atom to the C2 position on

Table 1. *In Vitro* Antifungal Activities of Compounds **1a**, **2a**, **3a**
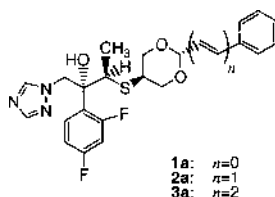
1a: $n=0$
2a: $n=1$
3a: $n=2$

Compound	n	MIC (μg/ml)			
		Candida albicans SANK 51486	*Cryptococcus neoformans* TIMM 1855	*Aspergillus fumigatus* SANK 10569	*Aspergillus flavus* SANK 18497
1a	0	≦0.008	0.25	4	>4
2a	1	≦0.008	0.031	0.25	2
3a	2	≦0.008	0.016	0.25	1

Table 2. *In Vitro* Antifungal Activites of Compounds **3a**—**j**

Compound	Ar	MIC (μg/ml)			
		Candida albicans SANK 51486	*Cryptococcus neoformans* TIMM 1855	*Aspergillus fumigatus* SANK 10569	*Aspergillus flavus* SANK 18497
3a	(phenyl)	≦0.008	0.016	0.25	1
3b	—⟨⟩—CF₃	0.016	0.016	0.25	2
3c	—⟨⟩—OCF₃	0.031	0.031	0.5	1
3d (R-102557)	—⟨⟩—OCH₂CF₂CHF₂	0.016	0.031	0.125	0.25
3e	—⟨⟩—Cl	≦0.008	0.016	0.125	0.25
3f	—⟨⟩—CN	≦0.008	0.016	0.063	0.25
3g	—⟨⟩—F (F)	0.016	≦0.008	0.25	0.5
3h	—⟨⟩—Cl (F)	0.016	0.016	0.25	0.5
3i	—⟨⟩—CF₃ (F)	0.016	0.031	0.25	1
3j (CS-758)	—⟨⟩—CN (F)	≦0.008	0.016	0.063	0.25

Table 3. *In Vitro* Antifungal Activites of CS-758, Fluconazole, and Itraconazole

Strain	MIC (μg/ml)		
	CS-758	Fluconazole	Itraconazole
C. albicans ATCC 24433	0.016	0.5	0.125
C. albicans SANK 51486	$\leqq$0.008	0.25	0.031
C. albicans TIMM 3164	0.063	>4	0.25
C. albicans ATCC 64550	0.5	>4	1
C. parapsilosis ATCC 90018	0.016	0.5	0.125
C. glabrata ATCC 90030	1	>4	1
C. krusei ATCC 6258	0.25	>4	0.5
C. tropicalis ATCC 750	0.25	2	0.5
C. neoformans TIMM 1855	0.016	>4	0.25
A. fumigatus ATCC 26430	0.063	>4	0.25
A. fumigatus SANK 10569	0.063	>4	0.25
A. flavus SANK 18497	0.25	>4	0.5

the benzene ring to obtain CS-758 (**3j**). The aim of the fluorine atom was to improve the solubility and chemical/metabolic stability.[5] CS-758 (**3j**) showed comparable MICs to those of **3f**. Analogs **3g**—**i**, with a fluorine atom at the C2 position but without a cyano group at the C4 position, showed lower activities. The excellent MIC of **3g** against *Cryptococcus neoformans* was an exception.

The MICs of CS-758, fluconazole, and itraconazole against 12 fungal strains are listed in Table 3. The MICs of CS-758 were excellent and surpassed those of the reference drugs. Further evaluations are under way.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. IR spectra were recorded on a Nic 5SCX spectrometer. ^{1}H-NMR spectra were recorded either on a JEOL GX-270 (270 MHz), on a Varian Mercury 400 (400 MHz), or on a JNM GSX-400 (400 MHz) spectrometer using tetramethylsilane as an internal standard. The abbreviations are as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; dt, doublet of triplets; td, triplet of doublets; tt, triplet of triplets; q, quartet; m, multiplet; br, broad. MS and high-resolution MS (HR-MS) were recorded either on a JEOL JMS D300, on a JEOL BU30, or on a JEOL JMS-700 spectrometer. Column chromatography was carried out on silica gel (Kieselgel 60, 0.040—0.063 mm, Merck). The amounts of silica gel used are shown in parentheses.

(2R,3R)-2-(2,4-Difluorophenyl)-3-[[*trans*-2-[(*E*)-styryl]-1,3-dioxan-5-yl]thio]-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (2a) and (2R,3R)-2-(2,4-Difluorophenyl)-3-[[*trans*-2-[(2*E*,4*E*)-5-aryl-2,4-pentadienyl]-1,3-dioxan-5-yl]thio]-1-(1*H*-1,2,4-triazol-1-yl)-2-butanols (3a, 3h, 3i) As a typical example, the preparation of **2a** is described. A solution of (2*R*,3*R*)-2-(2,4-difluorophenyl)-3-[[2-hydroxy-1-(hydroxymethyl)ethyl]thio]-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (**4**; 1.10 g, 2.78 mmol),[1] (*E*)-cinnamaldehyde (**5a**; 404 mg, 3.06 mmol), and *p*-toluenesulfonic acid monohydrate (581 mg, 3.05 mmol) in THF (10 ml) was allowed to stand at room temperature for 5 min, and then concentrated *in vacuo* using a rotary evaporator to remove water azeotropically. Similar processes (addition of THF and concentration) were repeated two more times. The residual oil was dissolved in THF and cautiously poured into a stirred aqueous solution of NaHCO$_3$ at 0 °C. The mixture was partitioned between EtOAc and water. The organic layer was washed with brine and dried over MgSO$_4$. Evaporation of the solvent afforded an oily residue, which was chromatographed on silica gel (60 g). Elution with a mixture of EtOAc and hexane (1:1 then 4:1, v/v) afforded **2a** (796.5 mg, 61%) as a colorless, amorphous solid. $[\alpha]_D^{25}$ −86.1° (*c*=1.64, CHCl$_3$). IR (KBr) cm^{-1}: 1617, 1499, 1388, 1274, 1137, 1050, 966, 852. ^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.19 (3H, d, *J*=7 Hz), 3.34 (1H, q, *J*=7 Hz), 3.42 (1H, tt, *J*=11, 5 Hz), 3.65 (1H, t, *J*=11 Hz), 3.67 (1H, t, *J*=11 Hz), 4.35 (1H, ddd, *J*=11, 5, 2 Hz), 4.44 (1H, ddd, *J*=11, 5, 2 Hz), 4.83 (1H, d, *J*=14 Hz), 5.02 (1H, s, *J*=14 Hz), 5.04 (1H, d, *J*=14 Hz), 5.13 (1H, d, *J*=5 Hz), 6.18 (1H, dd, *J*=16, 5 Hz), 6.7-6.8 (2H, m), 6.80 (1H, d, *J*=16 Hz), 7.25-7.42 (6H, m), 7.79 (2H, s). HR-MS (FAB) *m/z*: Calcd for C$_{24}$H$_{26}$F$_2$N$_3$O$_3$S (M+H)$^+$:

474.1663. Found: 474.1672.

Compounds **3a**, **3h**, and **3i** were prepared similarly from aldehydes **6a**, **6h**, and **6i**, respectively.

3a: Amorphous solid, 69%. $[\alpha]_D^{25}$ −80.3° (*c*=1.13, CHCl$_3$). ^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.18 (3H, d), 3.33 (1H, q, *J*=7 Hz), 3.39 (1H, tt, *J*=11, 5 Hz), 3.62 (1H, t, *J*=11 Hz), 3.64 (1H, t, *J*=11 Hz), 4.30 (1H, ddd, *J*=11, 4, 2 Hz), 4.41 (1H, ddd, *J*=11, 4, 2 Hz), 4.83 (1H, d, *J*=14 Hz), 5.00 (1H, d, *J*=1 Hz), 5.03 (1H, d, *J*=14 Hz), 5.05 (1H, d, *J*=14 Hz), 5.77 (1H, dd, *J*=15, 4 Hz), 6.59 (1H, dd, *J*=15, 11 Hz), 6.63 (1H, d, *J*=15 Hz), 6.7—6.8 (2H, m), 6.78 (1H, dd, *J*=15, 11 Hz), 7.20—7.25 (1H, m), 7.3—7.4 (3H, m), 7.41 (2H, d, *J*=5 Hz), 7.79 (2H, s). IR (KBr) cm^{-1}: 1617, 1597, 1499, 1450, 1420, 1388, 1275, 1140, 1050, 966, 899, 852. HR-MS (FAB) *m/z*: Calcd for C$_{26}$H$_{28}$F$_2$N$_3$O$_3$S (M+H)$^+$: 500.1819. Found: 500.1814.

3h: Amorphous solid, 83%. $[\alpha]_D^{25}$ −73.6° (*c*=0.96, CHCl$_3$). IR (KBr) cm^{-1}: 1617, 1602, 1499, 1488, 1416, 1388, 1275, 1141, 1050, 967, 900, 854. ^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.18 (3H, d, *J*=7 Hz), 3.33 (1H, q, *J*=7 Hz), 3.39 (1H, tt, *J*=11, 5 Hz), 3.62 (1H, t, *J*=11 Hz), 3.64 (1H, t, *J*=11 Hz), 4.30 (1H, ddd, *J*=11, 5, 2 Hz), 4.41 (1H, ddd, *J*=11, 5, 2 Hz), 4.83 (1H, d, *J*=14 Hz), 5.01 (1H, s), 5.03 (1H, d, *J*=14 Hz), 5.05 (1H, d, *J*=4 Hz), 5.81 (1H, dd, *J*=15, 4 Hz), 6.58 (1H, dd, *J*=15, 11 Hz), 6.7—6.8 (2H, m), 6.70 (1H, d, *J*=15 Hz), 6.82 (1H, dd, *J*=15, 11 Hz), 7.05—7.12 (2H, m), 7.3—7.45 (2H, m), 7.79 (2H, s). HR-MS (FAB) *m/z*: Calcd for C$_{26}$H$_{26}$ClF$_3$N$_3$O$_3$S (M+H)$^+$: 552.1335. Found: 552.1328.

3i: Amorphous solid, 63%. $[\alpha]_D^{25}$ −70.3° (*c*=1.00, CHCl$_3$). IR (KBr) cm^{-1}: 1618, 1500, 1429, 1331, 1276, 1171, 1138, 1050, 993. ^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.19 (3H, d, *J*=7 Hz), 3.33 (1H, q, *J*=7 Hz), 3.40 (1H, tt, *J*=11, 5 Hz), 3.62 (1H, t, *J*=11 Hz), 3.64 (1H, t, *J*=11 Hz), 4.31 (1H, ddd, *J*=11, 5, 2 Hz), 4.42 (1H, ddd, *J*=11, 5, 2 Hz), 4.83 (1H, d, *J*=14 Hz), 5.00 (1H, s), 5.03 (1H, d, *J*=14 Hz), 5.07 (1H, d, *J*=4 Hz), 5.87 (1H, dd, *J*=15, 4 Hz), 6.62 (1H, dd, *J*=15, 11 Hz), 6.7—6.8 (2H, m), 6.77 (1H, d, *J*=16 Hz), 6.93 (1H, dd, *J*=16, 11 Hz), 7.31 (1H, d, *J*=10 Hz), 7.33—7.39 (2H, m), 7.59 (1H, t, *J*=8 Hz), 7.79 (2H, s). HR-MS (FAB) *m/z*: Calcd for C$_{27}$H$_{26}$F$_6$N$_3$O$_3$S (M+H)$^+$: 586.1599. Found: 586.1602.

4-(Bromomethyl)-3-fluorobenzonitrile (8)[6] In a 2000 ml round-bottomed Pyrex glass flask fitted with a condenser, a mixture of 3-fluoro-4-methylbenzonitrile (**7**; 96 g, 710 mmol), *N*-bromosuccinimide (NBS; 126 g, 708 mmol), 2,2′-azobis(isobutyronitrile) (AIBN; 1.2 g, 7 mmol) and 1,2-dichloroethane (1000 ml) was stirred with a magnetic stirrer and irradiated with a tungsten lamp (375 W). The mixture was allowed to warm by the light of the lamp. When the mixture started to reflux, the lamp was occasionally turned off to prevent violent refluxing. After 20 min of refluxing, the red-brown color of the bromine disappeared and the solution became yellow. The mixture was irradiated for 5 min further, and then allowed to cool to room temperature. The mixture was then cooled with ice, and the precipitated succinimide was removed by filtration. The filtrate was partitioned between EtOAc and 10% (w/w) NaCl. The organic layer was dried over MgSO$_4$ and solvents were removed *in vacuo* to afford a crude solid, which was recrystallized from hexane–EtOAc to afford **8** (96 g, 63% yield) as colorless needles, mp 71—74 °C. *Anal.* Calcd for C$_8$H$_5$BrFN: C, 44.89; H, 2.36; N, 6.54. Found: C, 45.01; H, 2.36; N, 6.67. IR (KBr) cm^{-1}: 2238, 1573, 1502, 1417, 1264, 954, 895, 841. ^{1}H-NMR (270 MHz, CDCl$_3$) δ: 4.49 (2H, s), 7.38 (1H, dd, *J*=9, 1 Hz), 7.45 (1H, dd, *J*=8, 1 Hz), 7.54 (1H, t, *J*=8 Hz). MS [electron impact (EI)] *m/z*: 215, 213 (M$^+$), 134 (100%), 107. HR-MS (EI) *m/z*: Calcd for C$_8$H$_5$BrFN (M$^+$): 214.9569. Found: 214.9542.

Diethyl 4-Cyano-2-fluorobenzylphosphonate (9) A mixture of **8** (1.5 g, 7.0 mmol) and triethyl phosphite (1.4 g, 8.4 mmol) was heated at 150 °C for 2 h. The mixture was then concentrated under reduced pressure, and the residue was heated at 100 °C while being suctioned with a vacuum pump to remove volatile materials. The crude oil thus obtained (1.97 g, quantitative yield) was used for the next reaction without further purification. IR (CHCl$_3$) cm^{-1}: 2237, 1262, 1054, 1029. ^{1}H-NMR (270 MHz, CDCl$_3$) δ: 1.27 (6H, t, *J*=7.1 Hz), 3.24 (2H, d, *J*=22.3 Hz), 4.00—4.05 (4H, m), 7.37 (1H, d, *J*=9.2 Hz), 7.43 (1H, d, *J*=7.9 Hz), 7.51 (1H, td, *J*=9.2, 2.6 Hz). MS (EI) *m/z*: 271 (M$^+$) 139, 109, 93.

3-Fluoro-4-[(1*E*,3*E*)-5-oxo-1,3-pentadienyl]benzonitrile (6j) A solution of butyllithium (1.53 N in hexane, 0.5 ml, 0.77 mmol) was added in drops to a stirred solution of **9** (209 mg, 0.77 mmol) in dry THF (4 ml) at −78 °C. After the solution was stirred at −78 °C for 30 min, a solution of fumaraldehyde mono(dimethylacetal) (**10**; 100 mg, 0.77 mmol) in dry THF (2 ml) was added in drops. The whole was stirred for 2 h at −78 °C, and then stirred in an ice bath for 15 min. Then, the reaction was quenched by adding an aqueous solution of 0.1 N HCl (3.9 ml). The mixture was stirred again in the ice bath for 30 min, and then at room temperature for 1 h. Then the mixture was cooled with ice and treated with a saturated NaHCO$_3$ solution. The

mixture was partitioned between water and EtOAc. The organic layer was dried over $MgSO_4$ and the solvents were removed *in vacuo* to afford a crystalline residue, which was recrystallized from hexane–AcOEt to afford **6j** (127 mg, 82%) as a yellow solid, mp 174—177 °C. *Anal.* Calcd for $C_{12}H_8FNO$: C, 71.64; H, 4.01; N, 6.96. Found: C, 71.84; H, 4.27; N, 6.83. IR (KBr) cm^{-1}: 2230, 1681, 1672, 1621, 1421, 1159, 1124. ^{1}H-NMR (270 MHz, CDCl$_3$) δ: 6.36 (1H, dd, $J=15$, 8 Hz), 7.14 (1H, d-like, $J=3$ Hz), 7.16 (1H, d, $J=8$ Hz), 7.28 (1H, ddd, $J=15$, 8, 3 Hz), 7.40 (1H, dd, $J=10$, 1 Hz), 7.47 (1H, dd, $J=8$, 1 Hz), 7.67 (1H, t, $J=8$ Hz), 9.68 (1H, d, $J=8$ Hz). MS *m/z* 201 (M$^+$), 172 (100%), 158, 145.

4-[(1*E*,3*E*)-4-[*trans*-5-[[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]thio]-1,3-dioxan-2-yl]-1,3-butadienyl]-3-fluorobenzonitrile (CS-758, 3j) A mixture of **4** (8.73 g, 24.3 mmol), **6j** (4.63 g, 23.0 mmol) and *p*-toluenesulfonic acid monohydrate (5.07 g, 26.7 mmol) in dry THF (200 ml) was allowed to stand at room temperature for 30 min. Then the solvent was removed using a rotary evaporator. The residue was suctioned using a vacuum pump at room temperature to afford an oily residue. Similar evaporation cycles (addition of THF, allowing the mixture to stand, and evaporation) were repeated two more times. To the residue, THF (150 ml) was added, and the solution was poured slowly into a stirred aqueous solution of saturated $NaHCO_3$ at 0 °C. The product was extracted with EtOAc. The organic layer was washed with brine. The extract was dried over $MgSO_4$ and solvents were removed *in vacuo* to afford a pale yellow oil, which was chromatographed on silica gel (500 g, hexane–AcOEt, 2:1, v/v) to afford CS-758 (**3j**) (9.35 g, 74%) as a yellow amorphous solid. An analytical sample, mp 127—128 °C, was obtained by recrystallization from EtOAc–hexane. $[\alpha]_D^{25}$ −78.3° (*c*=1.8, CHCl$_3$). *Anal.* Calcd for $C_{27}H_{25}F_3N_4O_3S$: C, 59.77; H, 4.64; F, 10.50; N, 10.33; S, 5.91. Found: C, 59.66; H, 4.62; F, 10.38; N, 10.24; S, 5.92. IR (KBr) cm^{-1}: 2232, 1616, 1499, 1140. ^{1}H-NMR (400 MHz, CDCl$_3$) δ: 1.19 (3H, d, $J=7$ Hz), 3.33 (1H, q, $J=7$ Hz), 3.40 (1H, tt, $J=11$, 5 Hz), 3.62 (1H, t, $J=11$ Hz), 3.64 (1H, t, $J=11$ Hz), 4.30 (1H, ddd, $J=11$, 5, 2 Hz), 4.43 (1H, ddd, $J=11$, 5, 2 Hz), 4.83 (1H, d, $J=14$ Hz), 5.01 (1H, s), 5.03 (1H, d, $J=14$ Hz), 5.07 (1H, d, $J=4$ Hz), 5.90 (1H, dd, $J=15$, 4 Hz), 6.62 (1H, dd, $J=15$, 11 Hz), 6.7—6.8 (2H, m), 6.73 (1H, d, $J=16$ Hz), 6.95 (1H, dd, $J=16$, 11 Hz), 7.3—7.4 (1H, m), 7.34 (1H, d, $J=9$ Hz), 7.40 (1H, d, $J=8$ Hz), 7.58 (1H, t, $J=8$ Hz), 7.79 (2H, s). MS (FAB) *m/z* 543 (M+H)$^+$.

Determination of MICs MICs were determined by the broth microdilution method according to the guidelines in National Committee for Clinical Laboratory Standard (NCCLS) documents 1997 M27-A, 1995 M27-T, and 1998 M38-P, Wayne, PA. Roswell Park Memorial Institute (RPMI) 1640 medium (GIBCO) (for *C. neoformans*: yeast nitrogen base) buffered with 0.165 M 3-(morpholino)propanesulfonic acid (MOPS) (pH 7.0) was used. Microplates (96 wells) were inoculated at a concentration of $1—5\times10^3$

cells/ml for *Candida* species, *ca.* 1×10^4 cells/ml for *C. neoformans*, and 2.5×10^4 spores/ml for *Aspergillus* species, and incubated at 35 °C (for *Aspergillus* species: 30 °C). The MICs were read when obvious growth was observed in compound-free control wells. The MICs were defined as the lowest compound concentrations causing at least 80% growth inhibition compared with the control.

References and Notes
1) Oida S., Tajima Y., Konosu T., Nakamura Y., Somada A., Tanaka T., Habuki S., Harasaki T., Kamai Y., Fukuoka T., Ohya S., Yasuda H., *Chem. Pharm. Bull.*, **48**, 694—707 (2000).
2) The elimination half-life of R-102557 in plasma was 37.4 h in rats.
3) A part of this work was presented by the authors at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Poster No. 1087, Sep. 17—20, 2000, Toronto.
4) We did not investigate derivatives with longer ($n\geqq3$) side chains because their syntheses would require more steps. A separate experiment we carried out showed that a compound having three double bonds ($n=3$) with a 4-(trifluoromethyl)phenyl group (Ar=4-(CF$_3$)C$_6$H$_4$) had lower activities than **3b** ($n=2$) *in vivo* in mice (unpublished data).
5) The effects of the fluorine atom can be seen in the results of the preliminary experiments. The concentration of CS-758 reached 13 μg/ml when a capsule (No. 2) containing CS-758 (50 mg) and polysorbate 80 (300 mg) was stirred in a JP-2 solution (900 ml) at 37 °C. For **4f**, the maximum concentration was 4—5 μg/ml. The half-life of CS-758 in HCl (0.007 mol/l) solution in CH$_3$CN–H$_2$O (3:7, v/v) at 37 °C was 6.40 min, whereas that of **3f** was 3.12 min. The absolute bioavailability (BA) of CS-758 in rats after oral administration (20 mg/kg) of its poly(ethylene glycol) 400 solution was 113%, whereas that of **3f** was 50.7%. The elimination half-life of CS-758 in plasma was 9.1 h in rats, whereas that of **3f** was 5.7 h.
6) Xue C.-B., Wityak J., Sielecki T. M., Pinto. D. J., Batt D. G., Cain G. A., Sworin M., Rockwell A. L., Roderick J. J., Wang S., Orwat M. J., Frietze W. E., Bostrom L. L., Liu J., Higley C. A., Rankin F. W., Tobin A. E., Emmett G., Lalka G. K., Sze J. Y., Meo S. V. D., Mousa S. A., Thoolen M. J., Racanelli A. L., Hausner E. A., Reilly T. M., Degrado W. F., Wexler R. R., Olson R. E., *J. Med. Chem.*, **40**, 2064—2084 (1997); Lee K., Jung W.-H., Hwang S. Y., Lee S.-H., *Bioorg. Med. Chem. Lett.*, **9**, 2483—2486 (1999).