Synthesis and Antifungal Activity of Novel Thiazole-Containing Triazole Antifungals

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A new series of thiazole-containing triazole antifungals was synthesized and evaluated for antifungal activity against a variety of clinically isolated pathogenic fungi *in vitro* and against systemic candidosis *in vivo*. Among these compounds, (±)-1-(2,4-difluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (ER-24161) showed the most potent and well-balanced *in vitro* activities and excellent *in vivo* efficacy. We also achieved an enantioselective synthesis of the more potent enantiomer of ER-24161.

Key words thiazole-containing triazole antifungal; antifungal activity; ER-24161; enantioselective synthesis

During the past two decades, life-threatening, deep-seated fungal infections have increased dramatically. Immunocompromised patients who have received cancer chemotherapy and immunosuppressive therapy for organ transplants, patients under long-term treatment with broad-spectrum antibiotics or glucocorticosteroids, diabetics, and patients with AIDS and AIDS-related complex are susceptible to fungal infections. *Aspergillus fumigatus* (*A. fumigatus*), *Candida* spp., and *Cryptoccocus neoformans* (*C. neoformans*) are three major pathogens that cause opportunistic fungal infection in compromised hosts.[1] For the treatment of these infections, orally active antifungal azoles, such as fluconazole[2] (FLCZ), and itraconazole[3] (ITCZ) are in clinical use, but recently, resistance to FLCZ in *Candida albicans* (*C. albicans*), in other Candida spp. and in *C. neoformans* has been reported.[4] Therefore, we have started an azole antifungal discovery program to search for more effective, broader-spectrum, and safer drugs. The azole antifungals act by inhibiting the cytochrome P-450 monooxygenase, lanosterol 14α-demethylase, a key enzyme in fungal ergosterol biosynthesis.[5] Our initial screening studies of thiazole-containing triazole derivatives represented by the general formula **1** as racemates (Chart 1) revealed potent antifungal activities against *A. fumigatus*, *C. albicans*, *C. neoformans*, and *Candida glabrata* (*C. glabrata*) *in vitro* and against *C. albicans in vivo*.[6] For further evaluation of the *in vitro* and *in vivo* activities, as well as for preclinical pharmacokinetic and toxicological studies, we required the optical isomers of these compounds. Thus, we examined a synthetic route to the more potent enantiomer by using Sharpless' asymmetric dihydroxylation (AD) reaction.[7] In this paper, we describe in detail the synthesis and antifungal activities of a series of compounds **1**.

Chemistry A series of (±)-1-(2,4-difluorophenyl)-1-(4-substituted thiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanols (**5a—h**) was synthesized from α-haloketones (**2a—h**) in two steps as shown in Chart 2. 4-Substituted thiazoles (**3a—h**) were prepared from α-haloketones (**2a—h**) and thioformamide[8] by using Hantzsh's method.[9] 4-Substituted thiazoles (**3a—h**) were treated with *n*-butyllithium to give the corresponding 2-lithiated intermediates, which reacted with 2-chloro-2′,4′-difluoroacetophenone in tetrahydrofuran at −78 °C. The resultant chlorohydrins (**4**), without purification, were reacted with 1*H*-1,2,4-triazole in the presence of sodium hydride (NaH) in *N*,*N*-dimethylformamide (DMF) at 60 °C to afford the triazoles (**5a—h**), which contain a 4-substituted thiazole moiety, in 46—77% yield. Tetrazole substituents (**5i—k**) were derived from the nitrile group of **5h** as follows. Reaction of **5h** with sodium azide in DMF gave **5i**. Compound **5i** was reacted with iodomethane in the presence of cesium carbonate in DMF to afford **5j** and **5k** in 30% and 18% yields, respectively. In the same way, **6a—g** were synthesized by reaction with the corresponding imidazole and 2,4-dichlorophenyl, 4-fluorophenyl or 4-chlorophenyl moiety as shown in Chart 3.

A series of (±)-1-(2,4-difluorophenyl)-1-(2-substituted thiazol-5-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanols (**9a—g**) was synthesized from thiobenzamide derivatives (**7a—d**) in two steps as shown in Chart 4. 2-Substituted thiazoles (**8a—d**) were prepared from thiobenzamide derivatives (**7a—d**) and bromoacetoaldehyde dimethylacetal by using Hantzsh's method.[9] In the same way as mentioned above, the triazoles (**9a—d**) having a 2-substituted thiazole moiety were synthesized in 33—69% yields. Compounds **9e**, **9g**

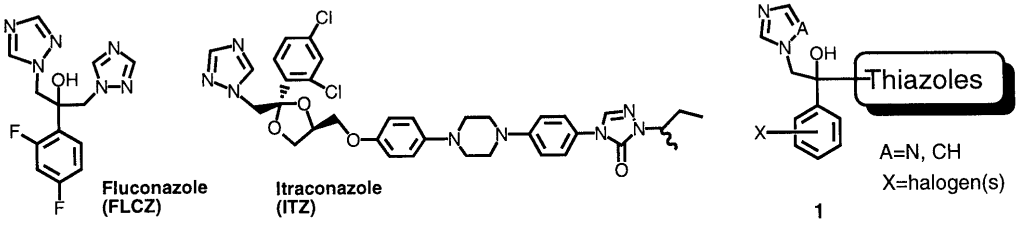

Chart 1

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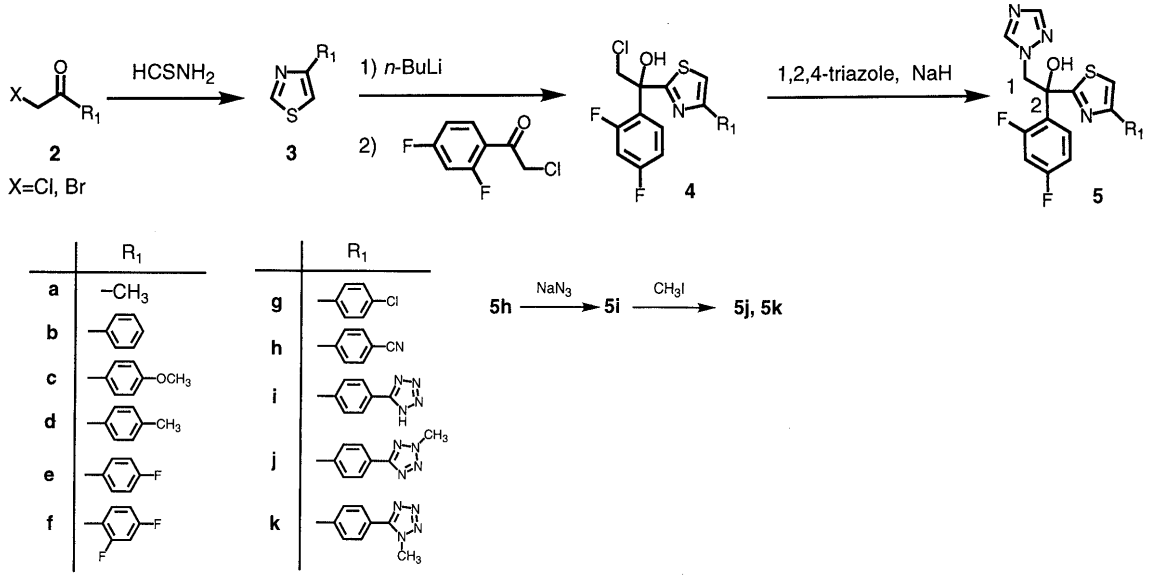


Chart 2

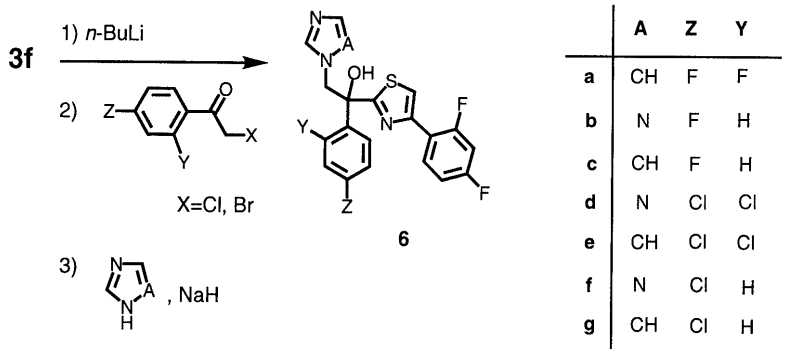


Chart 3

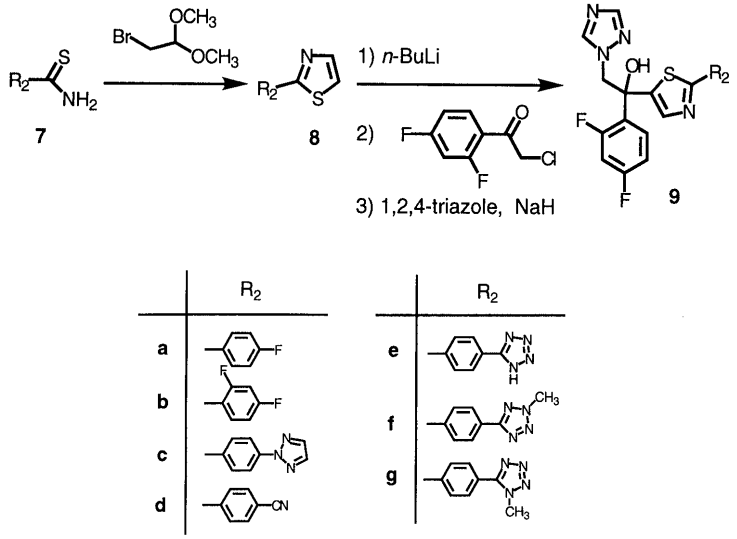


Chart 4

and **9f** were synthesized by using the same procedure as described for **5i**, **5j** and **5k**.

A series of (±)-1-(2,4-difluorophenyl)-1-(6-substituted benzothiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanols (**11a**—**d**) was synthesized in 22—52% yields from benzothiazoles (**10a**—**d**), prepared by using a known method,[9b,10] according to a procedure similar to that described above (Chart 5). Compounds **11e** and **11f** were synthesized by using the same procedure as described for **5i**, **5j** and **5k**.

Antifungal Activity Antifungal activity of the thiazole-containing triazoles (**5a**—**k**, **9a**—**g**, **11a**—**f**) was examined against clinical isolates of *A. fumigatus*, *C. albi-*

cans, *C. neoformans*, and *C. glabrata*. Minimum antibiotic concentrations (MACs) were determined on Sabouraud dextrose agar incubated at 37 °C for 48 h. MAC was determined as the lowest drug concentration which showed clear inhibition of fungal growth compared with the control fungal growth. We also investigated the therapeutic effects of the compounds against experimental candidosis infection caused by *C. albicans* MCY8622 *in vivo*. *C. albicans* MCY8622 (2×10^6 cells/mouse) was given intravenously to mice and compounds were orally administered one time at 1 h after infection. Efficacy was expressed in terms of the mean survival days calculated based on termination of the experiment 7 d after infection.

The results of *in vitro* and *in vivo* studies on the triazoles containing a 4-substituted thiazole (**5a**—**k**) are shown in Table 1. Compound **5b**, with the phenyl substituent at the 4-position of the thiazole moiety showed significantly increased activity compared with the corresponding 4-methylthiazole **5a** *in vitro*. Compounds **5c**, **5d**, **5e**, **5f**, **5g**, **5h**, and **5j** also showed excellent activity, whereas the tetrazole (**5i**) showed weak activity, and the 1-methyltetrazole (**5k**) showed poor activity except against *C. albicans*, *in vitro*.

In the *in vivo* evaluation, compounds **5f**, **5g**, **5h**, **5j**, and **5k**, which have an electron-withdrawing substituent such as a fluoro group, chloro group, cyano group, or methyltetrazolyl group on the phenyl group at the 4-position of the thiazole moiety showed potent protective effects against candidosis, comparable to that of fluconazole. On the other hand, compounds **5c** and **5d**, which have an electron-donating group such as a methoxyl group or methyl group on the phenyl group, and compound **5b**, which has an unsubstituted phenyl group, showed poor activity.

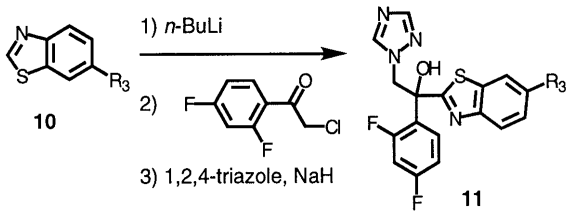


Chart 5

Next, we evaluated the activities of **5f** and its derivatives (**6a**—**g**), in which the triazole and 2,4-difluorophenyl moieties in the basic structure of **5f** were replaced with imidazole and 2,4-dichlorophenyl, 4-fluorophenyl, or 4-chlorophenyl, respectively, as shown in Table 2. In the *in vitro* assay, the activities of **6a**, **6b**, **6d**, and **6e** were comparable to that of **5f**, and the activities of **6c**, **6f**, and **6g** were weaker than that of **5f**. In the *in vivo* assay, all of the compounds (**6a**—**g**) showed weaker activities than **5f**. Based on these results, the order of potency for the azole and phenyl parts in the basic structure of **5f** and its derivatives is as follows: triazole > imidazole; 2,4-difluorophenyl > 2,4-dichlorophenyl > 4-chlorophenyl, 4-fluorophenyl.

The results of *in vitro* and *in vivo* studies on the triazoles having a 2-substituted thiazole (**9a**—**g**) and a 6-substituted benzothiazole (**11a**—**f**) are shown in Table 3. Compounds **9a**, **9b**, **9c**, **9d**, **9f**, and **9g**, which have a fluoro, triazole, cyano, or methyltetrazole group on the phenyl group connected to the 2-position of the thiazole moiety, showed potent *in vitro* activities against *C. albicans* and *C. glabrata*, like the triazoles having a 4-substituted thiazole. However, these compounds showed weak *in vitro* activities against *A. fumigatus* and *C. neoformans* compared with the triazoles having a 4-substituted thiazole. Compounds **11a**, **11b**, **11c**, **11d**, and **11f** showed potent activity against *C. albicans* and *C. glabrata*, while the activity against *A. fumigatus* and *C. neoformans* was weak. All compounds, except the 2*H*-1,2,3,4-tetrazole **9e** and unsubstituted benzothiazole **11a**, showed good efficacy against systemic candidosis in mice *in vivo*. Though these compounds (**9a**—**g**, **11a**—**f**) showed potent activity against *C. albicans*

Table 1. Antifungal Activity of (±)-1-(2,4-Difluorophenyl)-1-(4-substituted thiazol-2-yl)-2-(1*H*-1,2,4-triazol-2-yl)ethanols (**5a**—**k**)

Compound No.	*In vitro* MAC (μg/ml)						*In vivo* Murine systemic candidosis (mean survival days)	
	A. fumigatus TIMM0069	*A. fumigatus* TIMM0070	*C. albicans* MCY8622	*C. albicans* M1012	*C. neoformans* AJK4290	*C. glabrata* MCY86111	2.5 mg/kg	10 mg/kg
5a	3.13	6.25	0.4	0.4	6.25	0.8	2.6	4.0
5b	0.4	0.4	0.05	<0.05	0.2	<0.05	3.4	5.2
5c	0.4	0.4	<0.05	<0.05	0.2	0.1	5.6	5.2
5d	0.2	0.4	<0.05	<0.05	<0.05	<0.05	2.0	3.0
5e	0.4	0.8	<0.05	<0.05	0.2	0.1	4.6	5.2
5f	0.2	0.2	<0.05	<0.05	<0.05	<0.05	6.6	7.0
5g	0.4	0.4	<0.05	<0.05	0.1	<0.05	5.8	6.8
5h	0.4	0.4	<0.05	<0.05	0.2	0.1	7.0	7.0
5i	6.25	6.25	0.8	1.56	6.25	6.25	3.4	3.2
5j	0.4	0.4	<0.05	<0.05	0.4	0.1	6.2	7.0
5k	12.5	12.5	0.1	0.2	12.5	6.25	6.0	7.0
Fluconazole	100	100	0.4	0.8	12.5	12.5	7.0	7.0
Itraconazole	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	3.4	5.6
Control							2.6	

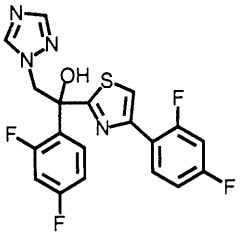
Table 2. *In Vitro* Activities of **5f** and Its Derivatives (**6a—g**)

Compound No.	*In vitro* MAC (μg/ml)						*In vivo* Murine systemic candidosis (mean survival days)	
	A. fumigatus TIMM0069	*A. fumigatus* TIMM0070	*C. albicans* MCY8622	*C. albicans* M1012	*C. neoformans* AJK4290	*C. glabrata* MCY86111	2.5 mg/kg	10 mg/kg
5f	0.05	0.1	0.013	0.013	0.05	0.025	6.6	7.0
6a	0.1	0.2	0.025	0.025	0.2	0.2	4.8	5.4
6b	0.2	0.2	0.05	0.05	0.2	0.2	5.0	5.4
6c	0.8	0.8	0.4	0.4	0.8	0.8	4.0	3.8
6d	0.2	0.2	0.006	0.006	0.05	0.05	3.8	6.4
6e	0.2	0.2	0.013	0.013	0.1	0.1	4.2	4.2
6f	0.8	1.56	0.05	0.05	0.1	0.1	5.6	6.0
6g	3.13	1.56	0.2	0.2	0.4	0.8	5.6	3.6

Table 3. Antifungal Activity of (±)-1-(2,4-Difluorophenyl)-1-(2-substituted thiazol-2-yl)-2-(1*H*-1,2,4-triazol-2-yl)ethanols (**9a—g**) and (±)-1-(2,4-Difluorophenyl)-1-(6-substituted benzothiazol-2-yl)-2-(1*H*-1,2,4-triazol-2-yl)ethanols (**11a—f**)

Compound No.	*In vitro* MAC (μg/ml)						*In vivo* Murine systemic candidosis (mean survival days)	
	A. fumigatus TIMM0069	*A. fumigatus* TIMM0070	*C. albicans* MCY8622	*C. albicans* M1012	*C. neoformans* AJK4290	*C. glabrata* MCY86111	2.5 mg/kg	10 mg/kg
9a	12.5	12.5	<0.05	<0.05	12.5	<0.05	7.0	6.8
9b	12.5	6.25	<0.05	<0.05	6.25	<0.05	3.6	7.0
9c	3.13	6.25	<0.05	<0.05	6.25	0.2	6.6	7.0
9d	6.25	6.25	<0.05	<0.05	12.5	<0.05	7.0	7.0
9e	>100	>100	0.2	0.2	100	12.5	2.8	2.2
9f	6.25	3.13	<0.05	<0.05	12.5	0.2	5.6	7.0
9g	50	50	<0.05	0.2	>100	6.25	4.0	6.8
11a	6.25	6.25	0.2	0.2	25	0.2	4.4	3.4
11b	3.13	6.25	<0.05	<0.05	6.25	0.1	7.0	7.0
11c	0.4	0.4	0.1	0.1	12.5	0.2	5.2	7.0
11d	12.5	12.5	<0.05	0.1	100	0.8	7.0	7.0
11f	3.13	3.13	0.2	0.4	50	0.8	3.6	6.4
Fluconazole	100	100	0.4	0.8	12.5	12.5	7.0	7.0
Itraconazole	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	3.4	5.6
Control							2.6	

Table 4. Comparison of *in Vitro* Antifungal Activities of the Enantiomers **5fA** and **5fB**



5fA and 5fB

Test organism	*In vitro* MAC (μg/ml) of enantiomers	
	5fA	**5fB**
C. albicans MCY8622	<0.05	0.8
C. neoformans Ando	<0.05	3.13
A. fumigatus TIMM0070	0.1	0.2

in vitro and *in vivo*, their antifungal potency against *A. fumigatus* and *C. neoformans in vitro* was lower than that of the 4-phenyl-substituted thiazoles (**5f** and its derivatives).

Among a series of thiazoles containing triazole derivatives, **5f** (ER-24161) showed a potent *in vivo* activity against *C. albicans*, and also has well-balanced and strong activity *in vitro*. Since the stereoisomers of azole antifungals show different antifungal activity,[11-13] we resolved racemic **5f** by using an optical resolution column and evaluated the *in vitro* activity of the enantiomers. It was found that the activity of **5fA** was 2 to 63 times greater than that of **5fB** (see Table 4). In general, azole antifungals which have an asymmetric center combined with a tertiary hydroxyl group, such as D-0870[12] and SCH-42427,[13] are more potent in the (*R*)-configuration than in the (*S*)-configuration (see Chart 6). Thus, we chose **5f** (ER-24161) as a candidate for further evaluation and set out to synthesize (*R*)-**5f**.

Asymmetric Synthesis Our synthetic strategy for optically active **5f** is shown in Chart 7 as a retrosynthesis. Compound **5f** should be obtainable by the introduction of 1*H*-1,2,4-triazole into an optically active diol **14** corresponding to the racemic chlorohydrin **4**. The key reaction of this synthetic plan is the AD reaction of the alkene **13** using Sharpless' AD-mix reagent.[7] If the 2,4-difluorophenyl group is considered the smaller group and the 4-phenylthiazole moiety the larger, (*R*)-configuration would be expected when AD-mix-α is used.

A Wittig reaction was performed on the ketone **12**

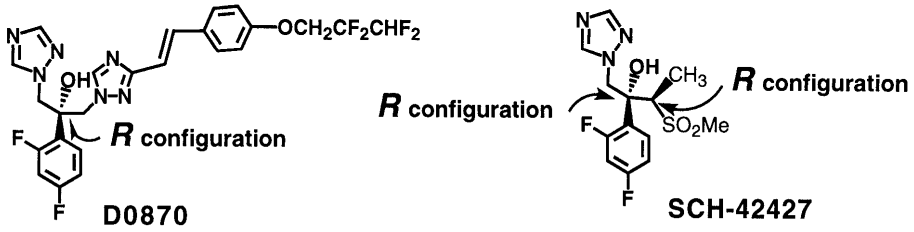


Chart 6

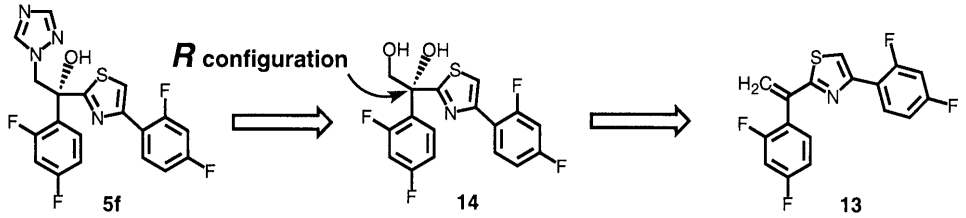
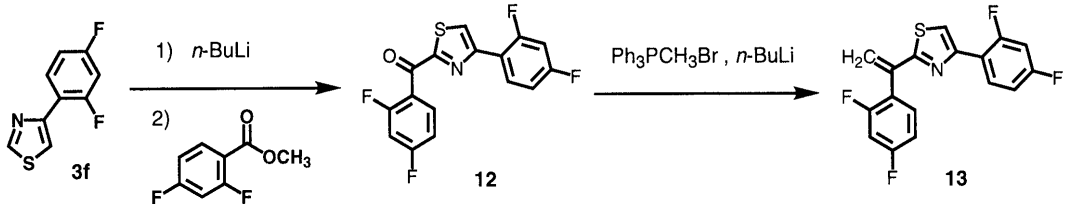


Chart 7



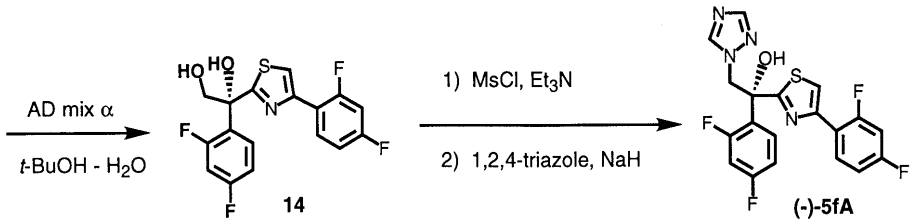


Chart 8

derived from **3f** and methyl 2,4-difluorophenyl acetate to form the olefin **13** in 25% yield. The olefin **13** was treated with AD-mix-α in *tert*-butyl alcohol and water at room temperature for 22 h to give the diol **14** in 57% yield. Mesylation of **14** gave the mesylate, which was without purification, reacted with 1*H*-1,2,4-triazole in the presence of NaH in DMF at 60 °C to afford optically active **5f** in 63% yield (see Chart 8). The enantiomeric purity of **5f**A was determined, by HPLC using a chiral column, to be 81% enantiomeric excess (ee). The retention time on HPLC and the antifungal activity of the major product obtained in this asymmetric synthesis were identical with those of **5f**A. The resultant **5f**A was recrystallized to afford optically pure **5f**A (99.2% ee). In general, high enantioselectivity of the AD reaction in the case of an *exo*-methylene moiety will be achieved when one of the groups is distinctly larger than the other.[7] In this case, it is not clear whether the sizes of the 2,4-difluorophenyl group and the 4-phenylthiazole moiety are sufficiently different. Thus, the optical purity may be lower than would be typical. From the results, the configuration of **5f**A is considered to be *R*. Therefore, this procedure should allow us to obtain the more potent optical isomers of this series of racemic thiazole-containing triazole derivatives **1**.

In conclusion, we have synthesized a series of thiazole-containing triazole derivatives. Initial screening revealed that, among these racemic compounds, **5f** (ER-24161) showed the most potent, well-balanced *in vitro* activities against a variety of pathogenic fungi, as well as excellent *in vivo* efficacy against systemic candidosis. We synthesized the more potent optical isomer, **5f**A, for further biological, pharmacokinetic and toxicological evaluation.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. Infrared spectra were measured with a Nicolet 205 FT-IR spectrometer. The proton nucler magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) as an internal reference. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra (MS) were obtained on a JEOL JMS-HX100 mass spectrometer. The optical rotations were recorded with a JASCO DIP 1000 digital polarimeter.

Compounds **3a—h**, **8a—d** and **10a—d** were prepared from commercially available materials by using a known method.[8,9] Silica gel

(Kieselgel 60, Merck) was used for column chromatography, silica gel (Kieselgel 60 F_{254}, layer thickness 0.25 mm, Merck) for analytical thin layer chromatography (TLC) and silica gel (Kieselgel 60 F_{254}, layer thickness, 2 mm, Merck) for preparative TLC (PTLC). All organic extracts were dried over anhydrous $MgSO_4$, and the solvent was removed with a rotary evaporator under reduced pressure.

1-(2,4-Difluorophenyl)-1-(4-methylthiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5a) A 1.6 mol solution of *n*-BuLi in *n*-hexane (1.54 ml, 2.47 mmol) was added dropwise to a solution of **3a** (245 mg, 2.47 mmol) in THF (6 ml), while stirring at −78 °C. After 5 min, a solution of 2-chloro-2′,4′-difluoroacetophenone (447 mg, 2.35 mmol) in THF (5 ml) was added dropwise to the stirred mixture at the same temperature. Stirring was continued for 1 h at the same temperature. After addition of water, the mixture was extracted with AcOEt. The organic extract was washed with brine, dried, and evaporated to afford the chlorohydrin **4a**. A solution of the crude **4a** in DMF (10 ml) was added to a solution of sodium triazolide, prepared from 1*H*-1,2,4-triazole (551 mg, 7.41 mmol) and NaH (60% mineral oil dispersion, 247 mg, 6.18 mmol) in DMF (5 ml). The mixture was then stirred at 60 °C for 3 h. After addition of water, the mixture was extracted with AcOEt. The organic extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (20 g, 1% MeOH–CH_2Cl_2) to afford **5a** (495 mg, 65%) as colorless prisms, mp 142—143 °C. IR ($CHCl_3$): 1616, 1501, 1277, 1139 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.41 (3H, d, J=0.9 Hz), 5.19 (2H, s), 5.75 (1H, s), 6.75—6.90 (2H, m), 6.85 (1H, br s), 7.55—7.65 (1H, m), 7.83 (1H, s), 8.07 (1H ,s). *Anal.* Calcd for $C_{14}H_{12}F_2N_4OS$: C, 52.17; H, 3.75; N, 17.38. Found: C, 52.22; H, 3.72; N, 17.38.

1-(2,4-Difluorophenyl)-1-(4-phenylthiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5b) In the same manner as described for the preparation of **5a**, **5b** was obtained as colorless needles (77%), mp 160—161 °C. IR ($CHCl_3$): 1616, 1501, 1277, 1141 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.93 (2H, s), 5.89 (1H, s), 6.78—6.88 (2H, m), 7.32—7.37 (1H, m), 7.40—7.46 (2H, m), 7.45 (1H, s), 7.64—7.70 (1H, m), 7.85—7.88 (2H, m), 7.87 (1H, s), 8.11 (1H, s). *Anal.* Calcd for $C_{19}H_{14}F_2N_4OS$: C, 59.37; H, 3.67; N, 14.57. Found: C, 59.57; H, 3.65; N, 14.56.

1-(2,4-Difluorophenyl)-1-[4-(4-methoxyphenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5c) In the same manner as described for the preparation of **5a**, **5c** was obtained as colorless needles (75%), mp 159—160 °C. IR ($CHCl_3$): 1614, 1500, 1278, 1251, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.85 (3H, s), 5.26 (2H, s), 5.87 (1H, s), 6.78—6.87 (2H, m), 6.93—6.97 (2H, m), 7.31 (1H, s), 7.63—7.69 (1H, m), 7.77—7.81 (2H, m), 7.86 (1H, s), 8.10 (1H, s). *Anal.* Calcd for $C_{20}H_{16}F_2N_4O_2S$: C, 57.96; H, 3.89; N, 13.52. Found: C, 57.92; H, 3.80; N, 13.67.

1-(2,4-Difluorophenyl)-1-[4-(4-methylphenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5d) In the same manner as described for the preparation of **5a**, **5d** was obtained as colorless needles (49%), mp 124—126 °C. IR ($CHCl_3$): 1616, 1501, 1277, 1141 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.39 (3H, s), 5.26 (2H, s) 5.86 (1H, s), 6.77—6.87 (2H, m), 7.23 (2H, br d, J=8.0 Hz), 7.38 (1H, s), 7.62—7.70 (1H, m), 7.75 (2H, br d, J=8.0 Hz), 7.85 (1H, s), 8.10 (1H, s). *Anal.* Calcd for $C_{20}H_{16}F_2N_4OS$: C, 60.29; H, 4.05; N, 14.06. Found: C, 60.59; H, 4.12; N, 13.93.

1-(2,4-Difluorophenyl)-1-[4-(4-fluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5e) In the same manner as described for the preparation of **5a**, **5e** was obtained as colorless prisms (68%), mp 177—179 °C. IR ($CHCl_3$): 1616, 1607, 1500, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.26 (2H, s) 5.93 (1H, s), 6.78—6.90 (2H, m), 7.11 (2H, br t, J=8.7 Hz), 7.38 (1H, s), 7.65—7.72 (1H, m), 7.80—7.86 (2H, m), 7.87 (1H, s), 8.10 (1H, s). *Anal.* Calcd for $C_{19}H_{13}F_3N_4OS$: C, 56.76; H, 3.26; N, 13.92. Found: C, 56.90; H, 3.28; N, 13.89.

1-(2,4-Difluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5f) In the same manner as described for the preparation of **5a**, **5f** was obtained as colorless needles (58%), mp 148—150 °C. IR ($CHCl_3$): 1617, 1598, 1278, 1102 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.23 (1H, d, J=14.1 Hz), 5.28 (1H, d, J=14.1 Hz), 5.97 (1H, s), 6.8—7.0 (4H, m), 7.66 (2H, d, J=2.2 Hz), 7.69 (1H, td, J=6.4, 9.5 Hz), 7.86 (1H, s), 8.10 (1H, s), 8.14 (1H, td, J=6.6, 9.5 Hz). *Anal.* Calcd for $C_{19}H_{12}F_4N_4OS$: C, 54.29; H, 2.88; N, 13.33. Found: C, 54.35; H, 2.99; N, 13.44.

1-(2,4-Difluorophenyl)-1-[4-(4-chlorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5g) In the same manner as described for the preparation of **5a**, **5g** was obtained as colorless needles (70%), mp 168—169 °C. IR ($CHCl_3$): 1616, 1501, 1278, 1140, 1093 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.26 (2H, s), 5.95 (1H, s), 6.77—6.88 (2H, m), 7.39 (2H, br d, J=8.8 Hz), 7.43 (1H, s), 7.65—7.71 (1H, m), 7.79 (1H, br d, J=8.8 Hz), 7.86 (1H, s), 8.09 (1H, s). *Anal.* Calcd for $C_{19}H_{13}ClF_2N_4OS$: C, 54.48; H, 3.13; N, 13.38. Found: C, 54.56; H, 3.16; N, 13.24.

1-(2,4-Difluorophenyl)-1-[4-(4-cyanophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5h) In the same manner as described for the preparation of **5a**, **5h** was obtained as colorless prisms (46%), mp 195—198 °C. IR ($CHCl_3$): 2230, 1610, 1501, 1278, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.24 (1H, d, J=14.5 Hz), 5.28 (1H, d, J=14.5 Hz), 6.05 (1H, s), 6.78—6.90 (2H, m), 7.60 (1H, s), 7.68—7.74 (1H, m), 7.71 (2H, br d, J=8.5 Hz), 7.89 (1H, s), 7.97 (2H, br d, J=8.5 Hz), 8.11 (1H, s). *Anal.* Calcd for $C_{20}H_{13}F_2N_5OS$: C, 58.67; H, 3.20; N, 17.11. Found: C, 58.44; H, 3.23; N, 17.07.

1-(2,4-Difluorophenyl)-1-[4-[4-(1*H*-1,2,3,4-tetrazol-5-yl)phenyl]thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5i) NaN_3 (191 mg, 2.93 mmol) and triethylamine hydrochloride (404 mg, 2.93 mmol) were added to a solution of **5h** (400 mg, 0.98 mmol) in DMF (10 ml), and the reaction mixture was heated at 100 °C for 14 h, then cooled to room temperature and filtered. EtOH, acetone and water were added to the filtrate, and the mixture was adjusted to pH 5 with concentrated HCl. The precipitate was collected by filtration, washed with water, and dried to afford **5i** as a solid (380 mg, 85%), which was used for the next step without further purification. ^{1}H-NMR (DMSO-d_6) δ: 5.27 (2H, s), 7.0—7.05 (1H, m), 7.18—7.25 (1H, m), 7.43 (1H, s), 7.50—7.57 (1H, m), 7.72 (1H, s), 8.12 (2H, br d, J=8.5 Hz), 8.20 (2H, br d, J=8.5 Hz), 8.28 (1H, s), 8.33 (1H, s).

1-(2,4-Difluorophenyl)-1-[4-[4-(2-methyl-2*H*-1,2,3,4-tetrazol-5-yl)-phenyl]thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5j) and 1-(2,4-Difluorophenyl)-1-[4-[4-(1-methyl-1*H*-1,2,3,4-tetrazol-5-yl)phenyl]thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (5k) Cesium carbonate (231 mg, 0.71 mmol) was added to a solution of **5i** (320 mg, 0.71 mmol) in DMF (3 ml), and the mixture was heated at 60 °C for 30 min, then cooled to room temperature. Iodomethane (0.048 ml, 0.78 mmol) was added, and the whole was stirred at the same temperature for 15 h. After addition of water, the mixture was extracted with AcOEt. The organic extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (40 g, 1%—2% MeOH in CH_2Cl_2) to give **5j** as colorless prisms (200 mg, 30%) and **5k** as a colorless powder (60 mg, 18%). Compound **5j**: mp 195—198 °C. IR ($CHCl_3$): 1617, 1506, 1420, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.42 (3H, s), 5.27 (1H, d, J=14.4 Hz), 5.32 (1H, d, J=14.4 Hz), 5.94 (1H, s), 6.79—6.89 (2H, m), 7.55 (1H, s), 7.65—7.72 (1H, m), 7.88 (1H, s), 7.99 (2H, br d, J=8.6 Hz), 8.13 (1H, s), 8.20 (2H, br d, J=8.6 Hz). *Anal.* Calcd for $C_{21}H_{16}F_2N_8OS$: C, 54.07; H, 3.46; N, 24.02. Found: C, 54.21; H, 3.49; N, 24.26. Compound **5k**: mp 185—187 °C. IR ($CHCl_3$): 1616, 1506, 1457, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.22 (3H, s), 5.28 (2H, br s), 6.02 (1H, s), 6.79—6.91 (2H, m), 7.61 (1H, s), 7.69—7.76 (1H, m), 7.82 (2H, br d, J=8.2 Hz), 7.89 (1H, s), 8.06 (2H, br d, J=8.2 Hz), 8.14 (1H, s). *Anal.* Calcd for $C_{21}H_{16}F_2N_8OS$: C, 54.07; H, 3.46; N, 24.02. Found: C, 54.10; H, 3.47; N, 24.10.

1-(2,4-Difluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-imidazol-1-yl)ethanol (6a) In the same manner as described for the preparation of **5a**, **6a** was obtained as a colorless powder (70%), mp 191—192 °C. IR ($CHCl_3$): 1618, 1598, 1500, 1268, 1140 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.98 (2H, br s), 6.67 (1H, br s), 6.81 (1H, br s), 7.0—7.08 (1H, m), 7.18—7.26 (2H, m), 7.30 (1H, br s), 7.35—7.42 (1H, m), 7.39 (1H, s), 7.55—7.62 (1H, m), 7.91 (1H, d, J=2.8 Hz), 8.12—8.20 (1H, m). *Anal.* Calcd for $C_{20}H_{13}F_4N_3OS$: C, 57.28; H, 3.12; N, 10.02. Found: C, 57.29; H, 3.09; N, 9.90.

1-(4-Fluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (6b) In the same manner as described for the preparation of **5a**, **6b** was obtained as a colorless powder (59%), mp 158—160 °C. IR ($CHCl_3$): 1616, 1506, 1457, 1277, 1140 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.08 (2H, br s), 7.22—7.28 (1H, m), 7.35—7.42 (1H, m), 7.37 (1H, s), 7.60—7.66 (2H, m), 7.75 (1H, s), 7.86 (1H, d, J=2.8 Hz), 8.22 (1H, s), 8.24—8.3 (1H, m). *Anal.* Calcd for $C_{19}H_{13}F_3N_4OS$: C, 56.71; H, 3.26; N, 13.92. Found: C, 56.61; H, 3.32; N, 13.90.

1-(4-Fluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-imidazol-1-yl)ethanol (6c) In the same manner as described for the preparation of **5a**, **6c** was obtained as a colorless powder (64%), mp 184—186 °C. IR ($CHCl_3$): 1622, 1602, 1507, 1457, 1139 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.79 (1H, d, J=14.5 Hz), 4.87 (1H, d, J=14.5 Hz), 6.66 (1H, br s), 6.81 (1H, br s), 7.10—7.18 (2H, m), 7.22—7.28 (1H, m), 7.30 (1H, br s), 7.32 (1H, s), 7.35—7.42 (1H, m), 7.86 (1H, d, J=2.5 Hz), 8.25—8.32 (1H, m). *Anal.* Calcd for $C_{20}H_{14}F_3N_3OS$: C, 59.84; H, 3.52; N, 10.47. Found: C, 59.96; H, 3.55; N, 10.53.

1-(2,4-Dichlorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (6d) In the same manner as described for the preparation of **5a**, **6d** was obtained as a colorless powder (68%), mp 188—189 °C. IR ($CHCl_3$): 1621, 1589, 1505, 1467, 1278, 1266, 1103 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.35 (1H, d, J=14.4 Hz), 5.50 (1H, d, J=14.4 Hz), 7.2—7.26 (1H, m), 7.35—7.44 (3H, m), 7.54—7.58 (2H, m), 7.68 (1H, s), 8.00 (1H, d, J=2.5 Hz), 8.15—8.22 (1H, m), 8.31 (1H, s). *Anal.* Calcd for $C_{19}H_{12}Cl_2F_2N_4OS$: C, 50.34; H, 2.67; N, 12.36. Found: C, 50.21; H, 2.62; N, 12.27.

1-(2,4-Dichlorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-imidazol-1-yl)ethanol (6e) In the same manner as described for the preparation of **5a**, **6e** was obtained as a colorless powder (71%), mp 238—239 °C. IR (Nujor): 1496, 1139 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.05 (1H, d, J=14.4 Hz), 5.26 (1H, d, J=14.4 Hz), 6.67 (1H, br s), 6.73 (1H, br s), 7.2—7.25 (1H, m), 7.23 (1H, s), 7.37 (1H, s), 7.37—7.42 (2H, m), 7.57 (1H, d, J=2.5 Hz), 7.64 (1H, d, J=8.8 Hz), 7.98 (1H, d, J=2.5 Hz), 8.14—8.2 (1H, m). *Anal.* Calcd for $C_{20}H_{13}Cl_2F_2N_3OS$: C, 53.11; H, 2.90; N, 9.29. Found: C, 53.08; H, 2.91; N, 9.20.

1-(4-Dichlorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (6f) In the same manner as described for the preparation of **5a**, **6f** was obtained as a colorless powder (61%), mp 167—168 °C. IR ($CHCl_3$): 1506, 1493, 1279, 1265, 1139 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.09 (2H, br s), 7.22—7.28 (1H, m), 7.35—7.40 (2H, m), 7.42 (1H, s), 7.6—7.64 (2H, m), 7.76 (1H, s), 7.87 (1H, d, J=2.8 Hz), 8.24 (1H, s), 8.24—8.3 (1H, m). *Anal.* Calcd for $C_{19}H_{13}ClF_2N_4OS$: C, 54.48; H, 3.13; N, 13.38. Found: C, 54.53; H, 3.19; N, 13.42.

1-(4-Dichlorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-2-(1*H*-imidazol-1-yl)ethanol (6g) In the same manner as described for the preparation of **5a**, **6g** was obtained as a colorless powder (63%), mp 201—203 °C. IR ($CHCl_3$): 1622, 1596, 1507, 1492, 1139, 1102 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 4.81 (1H, d, J=14.5 Hz), 4.86 (1H, d, J=14.5 Hz), 6.67 (1H, s), 6.83 (1H, s), 7.22—7.28 (1H, m), 7.31 (1H, s), 7.35—7.42 (4H, m), 7.62—7.66 (2H, m), 7.87 (1H, d, J=2.5 Hz), 8.25—8.32 (1H, m). *Anal.* Calcd for $C_{20}H_{14}ClF_2N_3OS$: C, 57.49; H, 3.38; N, 10.06. Found: C, 57.72; H, 3.43; N, 10.13.

1-(2,4-Difluorophenyl)-1-[2-(4-fluorophenyl)thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9a) In the same manner as described for the preparation of **5a**, **9a** was obtained as a colorless powder (43%), mp 156—157 °C. IR ($CHCl_3$): 1617, 1604, 1500, 1444, 1276, 1237 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.89 (1H, d, J=14.2 Hz), 5.22 (1H, d, J=14.2 Hz), 5.84 (1H, s), 6.78—6.90 (2H, m), 7.11 (2H, br t, J=9.0 Hz), 7.61 (1H, d, J=1.6 Hz), 7.69—7.75 (1H, m), 7.84—7.89 (2H, m), 7.88 (1H, s), 8.05 (1H, s). *Anal.* Calcd for $C_{19}H_{13}F_3N_4OS$: C, 56.71; H, 3.26; N, 13.92. Found: C, 56.97; H, 3.27; N, 14.11.

1-(2,4-Difluorophenyl)-1-[2-(2,4-difluorophenyl)thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9b) In the same manner as described for the preparation of **5a**, **9b** was obtained as colorless prisms (33%), mp 162—163.5 °C. IR ($CHCl_3$): 1617, 1500, 1277, 1139, 1102 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.91 (1H, d, J=14.1 Hz), 5.23 (1H, d, J=14.1 Hz), 5.86 (1H, s), 6.78—7.02 (4H, m), 7.68—7.76 (2H, m), 7.87 (1H, s), 8.05 (1H, s), 8.18—8.25 (1H, m). *Anal.* Calcd for $C_{19}H_{12}F_4N_4OS$: C, 54.29; H, 2.88; N, 13.33. Found: C, 54.49; H, 2.89; N, 13.28.

1-(2,4-Difluorophenyl)-1-[2-[4-(2*H*-1,2,3-triazol-2-yl)phenyl]thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9c) In the same manner as described for the preparation of **5a**, **9c** was obtained as a colorless powder (38%), mp 170—171 °C. IR ($CHCl_3$): 1617, 1608, 1500, 1410, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.92 (1H, d, J=14.1 Hz), 5.24 (1H, d, J=14.1 Hz), 5.87 (1H, s), 6.80—6.95 (2H, m), 7.67 (1H, d, J=1.5 Hz), 7.71—7.77 (1H, m), 7.85 (2H, s), 7.89 (1H, s), 7.99—8.03 (2H, m), 8.07 (1H, s), 8.14—8.18 (2H, m). *Anal.* Calcd for $C_{21}H_{15}F_2N_7OS \cdot 0.2H_2O$: C, 55.43; H, 3.41; N, 21.55. Found: C, 55.68; H, 3.47; N, 21.33.

1-(2,4-Difluorophenyl)-1-[2-(4-cyanophenyl)thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9d) In the same manner as described for the preparation of **5a**, **9d** was obtained as a colorless powder (69%), mp 87—90 °C. IR ($CHCl_3$): 2231, 1617, 1608, 1500, 1420, 1277, 1101 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.90 (1H, d, J=14.1 Hz), 5.22 (1H, d, J=14.1 Hz), 5.96 (1H, s), 6.79—6.91 (2H, m), 7.70—7.77 (4H, m), 7.89 (1H, s), 7.99 (2H, br d, J=8.5 Hz), 8.06 (1H, s). *Anal.* Calcd for $C_{20}H_{13}F_2N_5OS$: C, 58.67; H, 3.20; N, 17.11. Found: C, 58.47; H, 3.15; N, 16.96.

1-(2,4-Difluorophenyl)-1-[2-[4-(1*H*-1,2,3,4-tetrazol-5-yl)phenyl]thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9e) In the same manner as described for the preparation of **5a**, **9e** was obtained as a colorless powder (84%). Compound **9e** was used for the next step without further purification, mp 129—131 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.08 (1H, d, J=14.3 Hz), 5.18 (1H, d, J=14.3 Hz), 6.98—7.05 (1H, m), 7.18—7.25 (1H, m), 7.25 (1H, s), 7.45—7.52 (1H, m), 7.73 (1H, s), 8.02 (1H, d, J=0.7 Hz), 8.11 (4H, br s), 8.34 (1H, s).

1-(2,4-Difluorophenyl)-1-[2-[4-(2-methyl-2*H*-1,2,3,4-tetrazol-5-yl)-phenyl]thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9f) and 1-(2,4-Difluorophenyl)-1-[2-[4-(1-methyl-1*H*-1,2,3,4-tetrazol-5-yl)phenyl]thiazol-5-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (9g) In the same manner as described for the preparation of **5j** and **5k**, **9f** was obtained as colorless prisms (61%) and **9g** was obtained as a colorless powder (8%). **9f**: mp 147—149 °C. IR ($CHCl_3$): 1617, 1500, 1439, 1418, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.42 (3H, s), 4.92 (1H, d, J=14.1 Hz), 5.24 (1H, d, J=14.1 Hz), 5.89 (1H, s), 6.79—6.91 (2H, m), 7.68 (1H, d, J=1.5 Hz), 7.70—7.77 (1H, m), 7.88 (1H, s), 8.01 (2H, br d, J=8.2 Hz), 8.07 (1H, s), 8.20 (2H, br d, J=8.2 Hz). *Anal.* Calcd for $C_{21}H_{16}F_2N_8OS$: C, 54.07; H, 3.46; N, 24.02. Found: C, 54.15; H, 3.49; N, 24.23. **9g**: mp 113—115 °C. IR ($CHCl_3$): 1616, 1499, 1453, 1278, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.42 (3H, s), 4.93 (1H, d, J=14.1 Hz), 5.24 (1H, d, J=14.1 Hz), 5.99 (1H, s), 6.80—6.92 (2H, m), 7.72 (1H, d, J=1.6 Hz), 7.72—7.78 (1H, m), 7.83 (2H, br d, J=8.6 Hz), 7.89 (1H, s), 8.08 (1H, s), 8.08 (2H, br d, J=8.6 Hz). *Anal.* Calcd for $C_{21}H_{16}F_2N_8OS$: C, 54.07; H, 3.46; N, 24.02. Found: C, 54.12; H, 3.42; N, 24.03.

1-(2,4-Difluorophenyl)-1-(benzothiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)-ethanol (11a) In the same manner as described for the preparation of **5a**, **11a** was obtained as colorless crystals (31%), mp 130—131 °C. IR ($CHCl_3$): 1616, 1501, 1436, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.32 (2H, s), 6.05 (1H, s), 6.77—6.89 (2H, m), 7.26—7.40 (1H, m), 7.46—7.50 (1H, m), 7.70 (1H, dt, J=6.4, 8.9 Hz), 7.83—7.85 (1H, m), 7.86 (1H, s), 7.89 (1H, s), 7.99—8.02 (1H, m), 8.15 (1H, s), 8.14—8.18 (2H, m). *Anal.* Calcd for $C_{17}H_{12}F_2N_4OS$: C, 55.31; H, 3.60; N, 15.18. Found: C, 55.21; H, 3.36; N, 15.03.

1-(2,4-Difluorophenyl)-1-(6-fluorobenzothiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol (11b) In the same manner as described for the preparation of **5a**, **11b** was obtained as colorless prisms (52%), mp 139—140 °C. IR ($CHCl_3$): 1616, 1604, 1501, 1457, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.28 (1H, d, J=14.1 Hz), 5.32 (1H, d, J=14.1 Hz), 6.16 (1H, br s), 6.72—6.82 (1H, m), 6.83—6.90 (1H, m), 7.20 (1H, ddd, J=0.2, 0.9, 9.0 Hz), 7.50 (1H, dd, J=2.8, 8.4 Hz), 7.64—7.74 (1H, m), 7.84 (1H, s), 7.93 (1H, dd, J=5.2, 9.0 Hz), 8.13 (1H, s). *Anal.* Calcd for $C_{17}H_{11}F_3N_4OS$: C, 54.25; H, 2.95; N, 14.89. Found: C, 54.13; H, 2.89; N, 14.84.

1-(2,4-Difluorophenyl)-1-[6-(2*H*-1,2,3-triazol-2-yl)benzothiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (11c) In the same manner as described for the preparation of **5a**, **11c** was obtained as colorless crystals (22%), mp 148—149 °C. IR ($CHCl_3$): 1615, 1607, 1501, 1411, 1277, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.29 (1H, d, J=14.2 Hz), 5.34 (1H, d, J=14.2 Hz), 6.24 (1H, br s), 6.76—6.83 (1H, m), 6.84—6.90 (1H, m), 7.68—7.75 (1H, m), 7.83 (2H, br s), 7.85 (1H, s), 8.07 (1H, d, J=8.8 Hz), 8.16 (1H, br s), 8.24 (1H, dd, J=2.0, 8.8 Hz), 8.54 (1H, d, J=2.0 Hz). *Anal.* Calcd for $C_{19}H_{13}F_2N_7OS \cdot 0.1H_2O$: C, 53.42; H, 3.11; N, 22.95. Found: C, 53.60; H, 3.09; N, 22.67.

1-(2,4-Difluorophenyl)-1-(6-cyanobenzothiazol-2-yl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol (11d) In the same manner as described for the preparation of **5a**, **11d** was obtained as colorless prisms (31%), mp 170—172 °C. IR ($CHCl_3$): 2232, 1617, 1501, 1278, 1141 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.26 (1H, d, J=14.0 Hz), 5.35 (1H, d, J=14.0 Hz), 6.33 (1H, s), 6.78—6.83 (1H, m), 6.87—6.92 (1H, m), 7.72 (1H, dd, J=1.4. 8.3 Hz), 7.89 (1H, s), 8.07 (1H, dd, J=0.4, 8.8 Hz), 8.16 (1H, s), 8.18 (1H, dd, J=0.4, 1.4 Hz). *Anal.* Calcd for $C_{18}H_{11}F_2N_5OS$: C, 56.39; H, 2.89; N, 18.29. Found: C, 56.24; H, 2.95; N, 18.09.

1-(2,4-Difluorophenyl)-1-[6-(2*H*-1,2,3,4-tetrazol-5-yl)benzothiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (11e) In the same manner as described for the preparation of **5i**, **11e** was obtained as a colorless powder (88%). Compound **11e** was used for the next step without further purification. ^{1}H-NMR (DMSO-d_6) δ: 5.27 (2H, s), 6.90—7.05 (1H, m), 7.18—7.25 (1H, m), 7.43 (1H, s), 7.50—7.57 (1H, m), 7.72 (1H, s), 8.12 (2H, br d, J=8.5 Hz), 8.21 (2H, br d, J=8.5 Hz), 8.28 (1H, s), 8.33 (1H, s).

1-(2,4-Difluorophenyl)-1-[6-(2-methyl-2*H*-1,2,3,4-tetrazol-5-yl)benzothiazol-2-yl]-2-(1*H*-1,2,4-triazol-1-yl)ethanol (11f) In the same manner as described for the preparation of **5j**, **11f** was obtained as a colorless powder (24%), mp 182—186 °C. IR ($CHCl_3$): 1616, 1501, 1418, 1278, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.42 (3H, s), 5.30 (1H, d, J=14.0 Hz), 5.36 (1H, d, J=14.0 Hz), 6.18 (1H, s), 6.78—6.88 (2H, m), 7.48—7.55 (1H, m), 7.72 (1H, s), 7.78 (1H, s), 8.19 (1H, dd, J=0.4, 8.8 Hz), 8.31 (1H, dd, J=1.6, 8.8 Hz), 8.66 (1H, d, J=0.4 Hz). *Anal.* Calcd for

$C_{19}H_{14}F_2N_8OS$: C, 51.81; H, 3.20; N, 25.44. Found: C, 51.53; H, 3.15; N, 25.16.

Resolution of 5f Compound **5f** (1000 mg, 2.38 mmol) was resolved by using an optical resolution column (CHIRAL CEL OD; Daicel Chemical Industries, Tokyo, Japan; *n*-hexane–EtOH = 9/1 v/v) to give **5f**A (490 mg) and **5f**B (494 mg), mp 61—63 °C.

2,4-Difluorophenyl [4-(2,4-Difluorophenyl)thiazol-2-yl] Ketone (12) A 1.6 M solution of *n*-BuLi in *n*-hexane (33.1 ml, 52.9 mmol) was added dropwise to a solution of **3f** (10.2 g, 50.8 mmol) in tetrahydrofran (THF, 90 ml), while stirring at −78 °C. After 20 min, a solution of methyl 2,4-difluorophenylacetate (8.9 g, 51.8 mmol) in THF (75 ml) was added dropwise to the stirred mixture at the same temperature. Stirring was continued for 1 h at the same temperature. After addition of an aqueous solution of NH_4Cl, the mixture was extracted with AcOEt. The organic extract was washed with brine, dried, and evaporated. The resulting solid was crystallized from CH_2Cl_2–*n*-hexane to give **12** (13.9 g, 81%, colorless needles). Compound **12** was used for the next step without further purification. IR ($CHCl_3$): 1662, 1611, 1485, 1272, 1141 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.91—7.00 (3H, m), 7.03—7.07 (1H, m), 8.08—8.15 (3H, m).

1-(2,4-Difluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]ethene (13) A 1.6 M solution of *n*-BuLi in *n*-hexane (3.7 ml, 5.9 mmol) was added dropwise to a solution of methyltriphenylphosphonium bromide (2.3 g, 6.3 mmol) in THF (25 ml), while stirring at −50 °C. After 10 min, a solution of **12** (1.4 g, 4.2 mmol) in THF (15 ml) was added dropwise to the stirred mixture at 0 °C. Stirring was continued for 1 h at room temperature. After addition of water, the mixture was extracted with AcOEt. The organic extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (50 g, 0.5% AcOEt–*n*-hexane) to afford **13** (350 mg, 25%) as a solid. Compound **13** was used for the next step without further purification. IR ($CHCl_3$): 3140, 3064, 2401, 1662, 1633, 1506, 1425 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 5.60 (1H, s), 6.39 (1H, s), 6.86—6.96 (4H, m), 7.39—7.45 (1H, m), 7.66 (1H, d, J = 2.2 Hz), 8.14—8.20 (1H, m).

1-(2,4-Difluorophenyl)-1-[4-(2,4-difluorophenyl)thiazol-2-yl]-1,2-ethanediol (14) AD-mix-α (1.1 g) was added to a solution of **13** (90 mg, 0.27 mmol) in *tert*-BuOH (7 ml) and water (3.5 ml) and then the mixture was stirred at room temperature for 22 h. After addition of Na_2SO_3 (1 g) and water, the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was chromatographed on silica gel (10 g, 5% AcOEt–*n*-hexane) to afford **14** (57 mg, 57%) as an oil. Compound **14** was used for the next step without further purification. IR ($CHCl_3$): 3531, 2401, 1619, 1601, 1502, 1425, 1274 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.71 (1H, dd, J = 4.4, 10.0 Hz), 3.87 (1H, dd, J = 10.0, 11.6 Hz), 4.44 (1H, s), 4.78 (1H, ddd, J = 0.6, 4.4, 11.6 Hz), 6.77—6.82 (1H, m), 6.79—7.00 (3H, m), 7.70 (1H, s), 7.84—7.88 (1H, m), 8.07—8.13 (1H, m).

(−)-5fA Mesyl chloride (0.036 mg, 0.46 mmol) and triethylamine (0.065 mg, 0.46 mmol) were added to a solution of **14** (57 mg, 0.15 mmol) in chloroform (3 ml) at 0 °C. The mixture was stirred at room temperature for 1.5 h. After addition of water, the mixture was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. A solution of the residue in DMF (1 ml) was added to a solution of sodium triazolide, prepared from 1*H*-1,2,4-triazole (64 mg, 0.9 mmol) and NaH (60% mineral oil dispersion, 31 mg, 0.75 mmol) in DMF (2 ml). The mixture was then stirred at 60 °C for 1 h. After addition of water, the whole was extracted with AcOEt. The extract was washed with brine, dried, and evaporated. The residue was purified by PTLC (10% MeOH–CH_2Cl_2) to afford (−)-**5f**A (41 mg, 63%). The ^{1}H-NMR data were identical with those of the racemic compound **5f**A. The enantiomeric purity of (−)-**5f**A was measured by HPLC under the following conditions: Chiralcel OD (Daicel Chemical Industries, Tokyo, Japan), mobile phase (*n*-hexane–EtOH, 9 : 1, v/v; flow rate, 1 ml/min; detection, UV at 254 nm). The enantiomeric excess (ee) value was determined to be 80.6%. The resultant (−)-**5f**A (100 mg, 0.24 mmol, 80.6% ee) was crystallized from EtOH–*n*-hexane to give (−)-**5f**A (17 mg), whose enantiomeric excess was determined to be 31.5% ee. The resultant mother liquid was left to stand and afforded crystals of (−)-**5f**A (43 mg), whose enantiomeric excess was determined to be 99.2% ee, $[\alpha]_D$ −85.2° (c = 0.5, MeOH).

Determination of Minimum Antibiotic Concentrations (MACs) Minimum antibiotic concentrations (MACs) were determined by the two-fold agar dilution method with Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, Mich.). Yeasts were grown on SDA at 30 °C for 24 to 48 h and diluted to a final concentration of 10^5 cells per ml with sterilized saline. Filamentous fungi were grown on potato dextrose agar (PDA; Eiken Chemical Co., Tokyo, Japan) at 30 °C for 1 to 2 weeks, and diluted to a final concentration of 10^5 cells per ml with sterilized saline containing 0.05% Tween 80. Five microliters of each fungal suspension was spotted with a multiple-inoculum replicator (Microplanter; Sakuma Seisakusho, Tokyo, Japan) onto agar plates that contained twofold serial dilutions of antifungals. Fungal growth was observed 48 h after incubation at 37 °C. MAC was determined as the lowest drug concentration which visibly inhibited fungal growth compared with the control fungal growth.

Investigation of Therapeutic Effect in Experimental Systemic Infection by *C. albicans* *C. albicans* MCY8622 was incubated on SDA plates at 30 °C for 24 h, and challenge organisms were prepared in sterilized saline. Mice (age 4.5 weeks) (n = 5) were infected *via* the tail vein with 2×10^6 cells. Drugs were orally administered in a volume of 0.2 ml per dose, 1 h after infection. Control groups received 10% dimethyl sulfoxide (DMSO) in 0.5% carboxymethyl cellulose (CMC). Doses of drugs were 2.5 and 10 mg/kg. The mean survival days were calculated based on termination of the experiment 7 d after infection.

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References

1) *a*) Georgiev V. St., *Ann. N. Y. Acad. Sci.*, **544**, 1—3 (1988); *b*) Lyman C. A., Walsh T. J., *Drugs*, **44**, 9—35 (1992); *c*) Georgopapadakou N. H., Walsh T. J., *Antimicrob. Agents Chemother.*, **40**, 279—291 (1996).
2) *a*) Richardson K., Brammer K. W., Marriott M. S., Troke P. F., *Antimicrob. Agents Chemother.*, **27**, 832—835 (1985); *b*) Richardson K., Cooper K., Marriott M. S., Tarbit M. H., Troke P. F., Whittle P. J., *Ann. N. Y. Acad. Sci.*, **544**, 12—31 (1988); *c*) Washton H., *Dign. Microbiol. Infec. Dis.*, **12**, 229S (1989).
3) *a*) Heeres J., Backx L. J. J., Cutsem Van J., *J. Med. Chem.*, **27**, 894—900 (1984); *b*) Espinel-Ingroff A., Shadomy S., Gebhart R. J., *Antimicrob. Agents Chemother.*, **26**, 5—9 (1984).
4) *a*) Cokker R. J., Harris J. R. W., *J. Infect.*, **23**, 101—103 (1991); *b*) Odds F. C., *J. Antimicrob. Chemother.*, **31**, 463—471 (1993); *c*) Hitchcock C. A., *Biochem. Soc. Trans.*, **21**, 10—39 (1993); *d*) Millon L., Manteaux A., Reboux G., Drobacheff C., Monod M., Barale T., Michel-Briand Y., *J. Clin. Microbiol.*, **32**, 1115—1118 (1994); *e*) Johnson E. M., Warnock D. W., Luker J., Porter S. R., Scully C., *J. Antimicrob. Chemother.*, **35**, 103—114 (1995); *f*) Rex J. H., Rinaldi M. G., Pfaller M. A., *Antimicrob. Agents Chemother.*, **39**, 1—8 (1995).
5) *a*) Bossche H. V., Willemsens G., Cools W., Lauwers J. W. F., Jeune L. le, *Chem. Biol. Interacts.*, **21**, 59—78 (1978); *b*) Bossche H. V., Marichal P., "Recent Progress in Antifungal Chemotherapy," ed. by Yamaguchi H., Kobayashi G. S., Takahashi H., Marcel Dekker, Inc., New York, 1992, pp. 25—40.
6) Naito T., Kaku Y., Tsuruoka A., Kakinuma H., Tsukada I., Yanagisawa M., Nara K., Abstracts of Papers, The 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Sep. 1995, F91.
7) Sharpless K. B., Amberg W., Bennani Y. L., Crispino G. A., Hartung J., Jeong K-S., Kwong H-L., Morikawa K., Wang Z-M., Xu D., Zhang X-L., *J. Org. Chem.*, **57**, 2768—2771 (1992).
8) Erlenmeyer H., Menzi K., *Helv. Chim. Acta*, **31**, 2065—2075 (1948).
9) *a*) Hantzsch, *Justus Liebigs Ann. Chem.*, **249**, 1—6 (1888); *b*) Sprague J. M., Land A. H., "Heterocyclic Compounds," Vol. 5, ed. by Elderfield R. C., John Wiley & Sons, Inc., New York, pp. 484—722.
10) *a*) Stuckwisch C. G., *J. Am. Chem. Soc.*, **71**, 3417 (1949); *b*) Erlenmeyer H., Ueberwasser H., *Helv. Chim. Acta*, **25**, 515—521 (1942).
11) *a*) Yoshida Y., Aoyama Y., Takano H., Kato T., *Biochem. Biophys. Res. Commun.*, **137**, 513—519 (1986); *b*) Yoshida Y., Aoyama Y., *Chirality*, **2**, 10—15 (1990).
12) Yamada H., Tsuda T., Watanabe T., Ohashi M., Murakami K., Mochizuki H., *Antimicrob. Agents Chemother.*, **37**, 2412—2417 (1993).
13) Loebenberg D., Cacciapuoti A., Parmegiani R., Moss E. L., Jr., Menzel F., Jr., Antonacci B., Norris C., Yarosh-Tomaine T., Hare R. S., Miller G. H., *Antimicrob. Agents Chemother.*, **36**, 498—501 (1992).