# Optically Active Antifungal Azoles. VII.[1] Synthesis and Antifungal Activity of Stereoisomers of 2-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolone (TAK-187)

Akihiro TASAKA, Tomoyuki KITAZAKI,* Noboru TSUCHIMORI, Yoshihiro MATSUSHITA, Ryogo HAYASHI, Kenji OKONOGI, and Katsumi ITOH

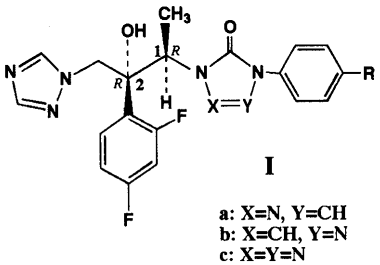
*Pharmaceutical Research Laboratories III, Pharmaceutical Research Division, Takeda Chemical Industries, Ltd., 17–85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka 532, Japan.* Received August 30, 1996; accepted October 8, 1996

**2-[(1*R*,2*R*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolone [(1*R*,2*R*)-1: TAK-187] is a new antifungal agent selected as a candidate for clinical trials. The three stereoisomers [(1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-1] of this compound were prepared to clarify the relationship between the stereochemistry and the biological activities. *In vitro* and *in vivo* assays of antifungal activity revealed that TAK-187 [(1*R*,2*R*)-1] is the most potent among the four stereoisomers. Furthermore, TAK-187 was found to exert a strong and selective inhibitory effect on the sterol synthesis in *Candida albicans* as compared with that in rat liver.**

**Key words** optically active antifungal azole; 1,2,4-triazolone; stereoisomer; stereocontrolled synthesis; antifungal activity; TAK-187

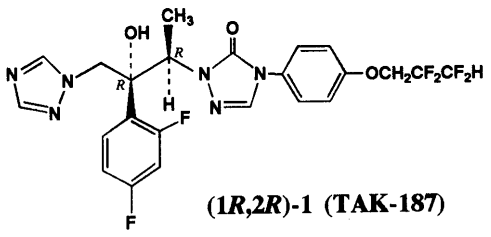
In the course of our search for new antifungal azoles, we designed optically active azolone derivatives depicted by the general formula I. In a previous report, we described the stereocontrolled synthesis of triazolone (Ia,b) and tetrazolone (Ic) derivatives, as well as their potent antifungal activity against *Candida albicans* (*C. albicans*) *in vitro* and *in vivo*.[1] Among these azolones, 2-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)-phenyl]-3(2*H*,4*H*)-1,2,4-triazolone [(1*R*,2*R*)-**1**: TAK-187] was selected as a candidate for clinical trials.

In a previous study of optically active 2,3-disubstituted-1-triazolyl-2-butanol antifungals with the general formula II (Chart 1), we clarified the relationship between stereochemistry and the antifungal activity against *C. albicans*, and reported that the target enzyme, cytochrome $P450_{14DM}$, recognized the configuration of the chiral centers: (2*R*,3*R*)-enantiomers showed the most potent activity.[2a,b] Thus, we prepared the three stereoisomers of TAK-187 [(1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-**1**; Chart 2] to examine differences in activity among the stereoisomers. In this paper, we describe the stereocontrolled synthesis of these isomers and the comparison of their *in vitro* and *in vivo* antifungal activities against *C. albicans* with those of TAK-187. In addition, the sterol biosynthesis-inhibitory activities in *C. albicans* and rat liver were measured to determine the degree of selectivity for fungal and mammalian P450. Furthermore, the four isomers [(1*R*,2*R*)-,

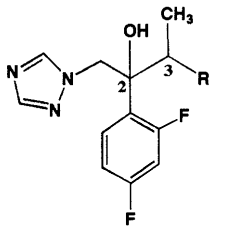



I

a: X=N, Y=CH
b: X=CH, Y=N
c: X=Y=N




**(1*R*,2*R*)-1 (TAK-187)**




II

**R= SH, 1,2,3-triazolyl, tetrazolyl**

Chart 1

* To whom correspondence should be addressed.

(1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-**1**] were assessed for growth-inhibitory activity *in vitro* using a variety of fungal species and strains to investigate the antifungal spectrum.

## Chemistry

The synthetic route to TAK-187 [(1*R*,2*R*)-**1**] and its stereoisomers [(1*S*,2*R*)-, (1*S*,2*S*)- and (1*R*,2*S*)-**1**] is illustrated in Chart 2. The starting material is the oxiranyl-(1*R*)-ethanol **2**, which is a diastereomeric mixture in the ratio of *ca.* 4:1.[2a] As reported previously,[1] TAK-187 [(1*R*,2*R*)-**1**] was prepared using the major component in **2** *via* a stereocontrolled route as follows: **2**→(1*R*,2′*R*)-**3**→(1*R*,2′*R*)-**4b**→(1*S*,2′*R*)-**4a**→(1*S*,2′*R*)-**4b**→(1*S*,2′*R*)-**4c**→(1*R*,2*S*)-**5**→(1*R*,2*R*)-**1**. We exploited this methodology of stereocontrolled synthesis in order to obtain the three stereoisomers. First, we undertook the synthesis of (1*S*,2*R*)-**1** by utilizing the (1*R*,2′*R*)-oxiranylethanol (1*R*,2′*R*)-**4b**, which is an intermediate in the synthesis of TAK-187. This alcohol was converted to the triflate (1*R*,2′*R*)-**4c** by treatment with trifluoromethanesulfonic anhydride ($Tf_2O$). *SN*2 displacement with 4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolone (H-TAZ) in the presence of sodium hydride (NaH) gave (1*S*,2*S*)-**5**, and subsequent oxirane ring-opening with the anion of 1*H*-1,2,4-triazole afforded the desired product (1*S*,2*R*)-**1**.

The synthesis of the isomers (1*S*,2*S*)- and (1*R*,2*S*)-**1**

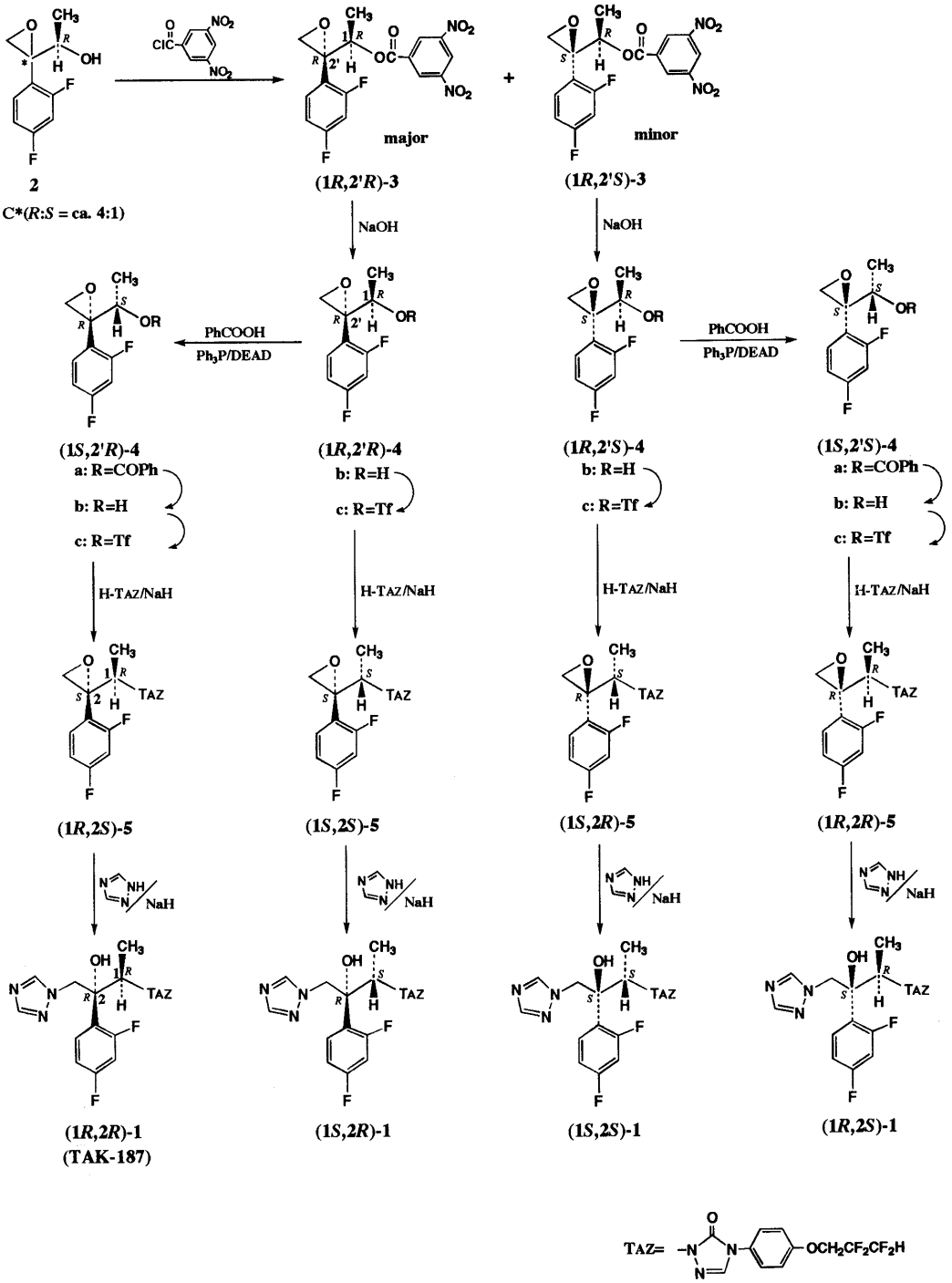



Chart 2

Table 1. 2-[2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolones (**1**)

| No. | Yield (%) | mp (°C) (Solv.)[a] | Formula | Analysis (%) Calcd (Found) C | H | N | $^1$H-NMR (in $CDCl_3$) $\delta$ | IR (KBr) $cm^{-1}$ | $[\alpha]_D$ {°C} MeOH (*c*) | de[b] (%) | ee[c] (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (1*R*,2*R*)-**1**[d] | 67 | 154—155 (EA–IPE) 150—151 (IPE) | $C_{23}H_{20}F_6N_6O_3$ | 50.93 (50.91 | 3.72 3.84 | 15.49 15.47) | 1.30 (3H, d, *J*=7 Hz), 4.37 (1H, d, *J*=15 Hz), 4.40 (2H, tt, *J*=11.8, 1.4 Hz), 5.02 (1H, d, *J*=15 Hz), 5.09 (1H, q, *J*=7 Hz), 5.47 (1H, s), 6.07 (1H, tt, *J*=53, 4.8 Hz), 6.75—6.88 (2H, m), 7.07 (2H, dt, *J*=9, 2.2 Hz), 7.53 (2H, dt, *J*=9, 2.2 Hz), 7.50—7.64 (1H, m), 7.69 (1H, s), 7.75 (1H, s), 7.95 (1H, s) | 1716, 1697, 1618, 1558, 1517, 1506 | −22.0° {20} (1.0) | >99.9 | >99.9 |
| (1*S*,2*S*)-**1** | 63 | 153—154 (EA–IPE) | $C_{23}H_{20}F_6N_6O_3$ | 50.93 (51.04 | 3.72 3.72 | 15.49 15.49) | 1.30 (3H, d, *J*=7 Hz), 4.37 (1H, d, *J*=15 Hz), 4.40 (2H, tt, *J*=11.8, 1.4 Hz), 5.02 (1H, d, *J*=15 Hz), 5.09 (1H, q, *J*=7 Hz), 5.47 (1H, s), 6.07 (1H, tt, *J*=53, 4.8 Hz), 6.75—6.88 (2H, m), 7.07 (2H, dt, *J*=9, 2.2 Hz), 7.53 (2H, dt, *J*=9, 2.2 Hz), 7.50—7.64 (1H, m), 7.69 (1H, s), 7.75 (1H, s), 7.95 (1H, s) | 1716, 1695, 1619, 1558, 1517, 1502 | +21.8° {20} (1.0) | 99.5 | 99.9 |
| (1*R*,2*S*)-**1** | 87 | Oil | $C_{23}H_{20}F_6N_6O_3$ | 50.93 (50.63 | 3.72 3.80 | 15.49 15.30) | 1.62 (3H, d, *J*=7 Hz), 4.36 (2H, tt, *J*=11.8, 1.4 Hz), 4.61 (1H, d, *J*=14 Hz), 4.92 (1H, d, *J*=14 Hz), 5.08 (1H, q, *J*=7 Hz), 5.91 (1H, s), 6.03 (1H, tt, *J*=53.2, 4.6 Hz), 6.62—6.80 (2H, m), 6.98 (2H, dt, *J*=9, 2.2 Hz), 7.24 (2H, dt, *J*=9, 2.2 Hz), 7.25—7.41 (1H, m), 7.40 (1H, s), 7.70 (1H, s), 8.09 (1H, s) | 1708, 1681, 1616, 1558, 1516 | +56.5° {20} (1.0) | 99.9 | 99.7 |
| (1*S*,2*R*)-**1** | 75 | Oil | $C_{23}H_{20}F_6N_6O_3$ | 50.93 (50.51 | 3.72 3.86 | 15.49 15.34) | 1.62 (3H, d, *J*=7 Hz), 4.35 (2H, tt, *J*=11.8, 1.4 Hz), 4.61 (1H, d, *J*=14 Hz), 4.91 (1H, d, *J*=14 Hz), 5.07 (1H, q, *J*=7 Hz), 5.90 (1H, s), 6.03 (1H, tt, *J*=53.2, 4.6 Hz), 6.60—6.80 (2H, m), 6.98 (2H, dt, *J*=9, 2.2 Hz), 7.24 (2H, dt, *J*=9, 2.2 Hz), 7.25—7.42 (1H, m), 7.43 (1H, s), 7.69 (1H, s), 8.08 (1H, s) | 1708, 1681, 1616, 1558, 1517 | −57.0° {20} (1.0) | 99.7 | 99.8 |

*a*) Recrystallization solvent: EA, ethyl acetate; IPE, diisopropyl ether. *b*) Determined by the HPLC method using an ODS column (YMC, A-303). *c*) Determined by the HPLC method using Chiralpak AD. *d*) Reported in reference 1.

was then carried out using the minor component in **2**, which was isolated in the form of the 3,5-dinitrobenzoate (1*R*,2′*S*)-**3** with high purity by silica gel chromatography followed by recrystallization. This dinitrobenzoate was hydrolyzed with an aqueous solution of sodium hydroxide to give the (1*R*,2′*S*)-oxiranylethanol (1*R*,2′*S*)-**4b**. The enantioisomer of TAK-187, (1*S*,2′*S*)-**1**, was synthesized from this oxiranylethanol *via* a route similar to that described above, *i.e.*, (1*R*,2*S*)-**4b**→(1*R*,2′*S*)-**4c**→(1*S*,2*R*)-**5**→(1*S*,2*S*)-**1**. Isomer (1*R*,2*S*)-**1** was prepared from (1*R*,2′*S*)-**4b** *via* substantially the same reactions as reported for TAK-187: (1*R*,2′*S*)-**4b**→(1*S*,2′*S*)-**4a**→(1*S*,2′*S*)-**4b**→(1*S*,2′*S*)-**4c**→(1*R*,2*R*)-**5**→(1*R*,2*S*)-**1**. The analytical results for the three stereoisomers are shown in Table 1 together with those for TAK-187.

To compare accurately the biological activities of the stereoisomers, it was considered essential that each isomer had to be of the highest possible diastereomeric (% de) and enantiomeric (% ee) purity. The % de and % ee were determined by HPLC on an octadecyl silica (ODS) column and a chiral stationary column, respectively. As shown in Table 1, the four stereoisomers each showed high diastereomeric and enantiomeric purity.

## Antifungal Activity

*In vitro* and *in vivo* antifungal activities of TAK-187 [(1*R*,2*R*)-**1**] and its three stereoisomers [(1*S*,2*S*)-, (1*R*,2*S*)- and (1*S*,2*R*)-**1**] against two strains of *C. albicans*, IFO 0583 and TA, are shown in Table 2. The *in vitro* assay using *C. albicans* was carried out by a paper disc method (Disc) using yeast nitrogen base (YNB) medium and by an agar-dilution method using YNB and RPMI 1640[3] media. In the case of the agar-dilution method using RPMI 1640 medium, the agar plates were incubated under an atmosphere containing 20% $CO_2$.[4] The activities are expressed as the diameter (mm) of the growth inhibition zone around a paper disc soaked in a 1 mg/ml solution of the test compound and as the minimum inhibitory concentration (*MIC*, μg/ml). The ability to inhibit the hyphal outgrowth of *C. albicans* IFO 0583 in serum was measured *in vitro* and is expressed in terms of *MICH* (μg/ml). *C. albicans* TA-infected mice were used for the *in vivo* assay, and the activity is expressed in terms of $ED_{50}$ (mg/kg, the dose of the test compound which allows 50% of infected mice to survive after a single oral dose). In addition, the ability of these four stereoisomers to inhibit sterol 14α-demethylase of *C. albicans* TA (cell lysate) and rat liver (cell lysate) was examined *in vitro*. The results ($IC_{50}$, μg/ml) of this assay are also shown in Table 2.

All the stereoisomers prepared inhibited the growth of *C. albicans* IFO 0583 and TA in the paper disc assay. The observed *MIC* values for *C. albicans* IFO 0583 and TA on YNB medium were 50—100 μg/ml. No clear differences

Table 2. Biological Activities of Stereoisomers

| Compound | Antifungal activity against *C. albicans* | | | | | | | | Sterol biosynthesis-inhibitory activity (Cell lysate) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *In vitro* | | | | | | | *In vivo* | | |
| | Disc (mm) | | *MIC* (μg/ml) | | | | *MICH* (μg/ml) | $ED_{50}$ | $IC_{50}$ (μg/ml) | |
| | IFO 0583 YNB | TA YNB | IFO 0583 YNB | TA YNB | IFO 0583 RPMI 1640[a] | TA RPMI 1640[a] | IFO 0583 | TA *p.o.* (mg/kg)[b] | *C. albicans* TA | Rat liver |
| (1*R*,2*R*)-**1** | 41 | 42[c] | 50 | 50[c] | 0.004 | 0.008 | <0.05[c] | 0.32[c] | 0.0020 | 6.60 |
| (1*S*,2*S*)-**1** | 26 | 25 | 50 | 50 | 2 | 2 | 5 | >16 | 0.036 | 31.0 |
| (1*R*,2*S*)-**1** | 20 | 20 | 100 | 50 | 8 | 8 | 20 | >16 | 0.97 | 207 |
| (1*S*,2*R*)-**1** | 30 | 28 | 100 | 100 | 1 | 0.5 | 1.25 | 10.4 | 0.0049 | 38.4 |

*a*) Determined under 20% $CO_2$. *b*) Administered in the form of 0.2% carboxymethylcellulose (CMC) suspension. *c*) Reported in reference 1.

Table 3. *In Vitro* Antifungal Activity of Stereoisomers

| Species and strain | | *MIC* (μg/ml, RPMI 1640 agar)[a] | | | |
|---|---|---|---|---|---|
| | | Compound **1** | | | |
| | | (1*R*,2*R*) | (1*S*,2*S*) | (1*R*,2*S*) | (1*S*,2*R*) |
| *C. albicans* | TIMM 1756 | 0.016 | 4 | 16 | 2 |
| *C. albicans* | TIMM 1850 | 0.008 | 2 | 16 | 2 |
| *C. albicans* | TIMM 0239 | 0.016 | 4 | 32 | 2 |
| *C. albicans* | CA 382 | 0.25 | 32 | >64 | 32 |
| *C. albicans* | CA 383 | 4 | >64 | >64 | >64 |
| *Candida tropicalis* | IFO 0587 | 0.25 | >64 | >64 | 32 |
| *Candida tropicalis* | IFO 10241 | 0.25 | >64 | >64 | 32 |
| *Candida glabrata* | IFO 0622 | 2 | 16 | >64 | 8 |
| *Candida krusei* | IFO 0584 | 1 | >64 | >64 | 64 |
| *Candida krusei* | IFO 1162 | 4 | >64 | >64 | >64 |
| *Candida utilis* | IFO 0619 | 0.25 | 8 | >64 | 1 |
| *Cryptococcus neoformans* | TIMM 1740 | 0.13 | 32 | 64 | 32 |
| *Cryptococcus neoformans* | TIMM 1855 | 0.25 | 64 | >64 | 32 |
| *Saccharomyces cerevisiae* | IFO 0209 | 0.5 | 32 | >64 | 16 |
| *Aspergillus fumigatus* | 437[b] | 2 | >64 | >64 | >64 |
| *Aspergillus fumigatus* | TIMM 1728[b] | 2 | >64 | >64 | >64 |
| *Aspergillus fumigatus* | IFO 6344[b] | 2 | >64 | >64 | >64 |
| *Aspergillus niger* | IFO 4414[b] | 2 | >64 | >64 | >64 |

*a*) Determined under 20% $CO_2$. *b*) Determined under air.

in activity were seen among the stereoisomers in these *in vitro* assays, although (1*R*,2*R*)-**1** seemed somewhat more potent than the others in the paper disc assay.

On the other hand, the *MIC* values determined on RPMI 1640-agar medium under 20% $CO_2$ and the *MICH* values measured in serum were clearly dependent on the configuration. The (1*R*,2*R*)-isomer (TAK-187) showed the lowest *MIC* (0.004—0.008 μg/ml) on RPMI 1640 and the strongest inhibitory activity (*MICH*, <0.05 μg/ml) against hyphal outgrowth. The order of potency was (1*R*,2*R*)≫(1*S*,2*R*)>(1*S*,2*S*)>(1*R*,2*S*).

In the *in vivo* assay, activity was also found to be dependent on the configuration. The potency substantially paralleled the *MIC* (RPMI 1640) and *MICH* values, and the order was (1*R*,2*R*)≫(1*S*,2*R*)>(1*S*,2*S*), (1*R*,2*S*).

A definite difference among the stereoisomers was observed in the inhibition of sterol biosynthesis in *C. albicans* TA. The order of the inhibitory potency was (1*R*,2*R*)≫(1*S*,2*R*)>(1*S*,2*S*)>(1*R*,2*S*), which parallels the antifungal activities described above. The (1*R*,2*R*)-isomer caused the most potent inhibition ($IC_{50}$, 0.002 μg/ml). On the other hand, the effects of the four isomers on the sterol synthesis in rat liver were weak ($IC_{50}$, 6.6→207 μg/ml) compared with those in *C. albicans*. Thus, the (1*R*,2*R*)-isomer (TAK-187) provides the most potent and selective inhibition of fungal cytochrome $P450_{14DM}$.

The antifungal spectra of the stereoisomers are shown in Table 3. The *MIC* values for yeast type fungi such as *Candida*, *Cryptococcus* and *Saccharomyces* species were determined by an agar dilution method using RPMI 1640 medium under 20% $CO_2$, and *MIC* values for *Aspergillus* species were measured using the same medium under ordinary air. As can be seen in Table 3, the (1*R*,2*R*)-isomer (TAK-187) has the most potent antifungal activity against various fungi. These results indicate that the (1*R*,2*R*)-configuration provides the best fit for the binding site of the target enzyme P450 in a broad range of fungi.

In conclusion, we confirmed that the (1*R*,2*R*)-configuration of TAK-187 is a key factor for potent antifungal activity as well as for a broad antifungal spectrum. Further data on the biological activities of this new antifungal agent will be reported in a separate paper.[5]

## Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. IR spectra were measured with a JASCO IR-810 spectrometer. $^1$H-NMR spectra were taken on a Varian Gemini-200 spectrometer with tetramethylsilane as an internal standard. The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. The secondary ion mass spectra (SIMS) were measured with a Hitachi M-80A mass spectrometer. The optical rotations were recorded with a JASCO DIP-181 or DIP-370 digital polarimeter.

Reactions were run at room temperature unless otherwise noted and followed by TLC on Silica gel 60 $F_{254}$ precoated TLC plates (E. Merck) or by HPLC using an ODS column (A-303, 4.6 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co.). Standard work-up procedures were as follows. The reaction mixture was partitioned between the indicated solvent and water. Organic extracts were combined and washed in the indicated order using the following aqueous solutions; water, 5% aqueous sodium bicarbonate solution (aqueous $NaHCO_3$) and saturated NaCl solution (brine). Extracts were dried over $MgSO_4$, filtered and evaporated *in vacuo*.

Chromatographic separations were carried out on Silica gel 60 (0.063—0.200 mm, E. Merck) using the indicated eluents.

The % de of the four stereoisomers was determined by HPLC using an ODS column under the following conditions: mobile phase, methanol (MeOH)–$H_2O$–acetic acid, 7:3:0.02, v/v; flow rate, 0.8 ml/min; detection, UV at 262 nm.

The % ee of the four stereoisomers was determined by HPLC using a chiral stationary phase column (Chiralpak AD 4.6 mm i.d. × 250 mm, Daicel Chemical Industries, Tokyo, Japan) under the following conditions: mobile phase, hexane–isopropyl alcohol, 1:1, v/v; flow rate, 1.0 ml/min; detection, UV at 262 nm.

The *in vitro* antifungal activity (Disc on YNB,[2a] *MIC* on YNB[2c] and *MICH* assay[2b]), *in vivo* antifungal activity against candidiasis[2a] and sterol biosynthesis-inhibitory activity for cell lysate[2b] were measured by the methods described in our preceding reports.

**[(1*R*)-1-[(2*R*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethyl] 3,5-Dinitrobenzoate [(1*R*,2′*R*)-3] and [(1*R*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethyl] 3,5-Dinitrobenzoate [(1*R*,2′*S*)-3]** (1*R*)-1-[2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol[1] (**2**, 18.5 g) and 3,5-dinitrobenzoyl chloride (24.5 g) were dissolved in $CH_2Cl_2$ (300 ml). Triethylamine ($Et_3N$, 10.7 g) was added dropwise to this solution at 0 °C over a period of 10 min. After having been stirred for 1 h, the mixture was washed (water, brine), concentrated *in vacuo* and diluted with a mixture of $CH_2Cl_2$ and diisopropyl ether ($isoPr_2O$). The precipitated crystals were collected by filtration and purified by silica gel column chromatography ($CH_2Cl_2$) followed by recrystallization from $CH_2Cl_2$–$isoPr_2O$ to give (1*R*,2′*R*)-**3**[1] (14.48 g, 40%) as colorless needles. The mother liquor obtained upon filtration was evaporated *in vacuo* and submitted to chromatography on silica gel (hexane–$isoPr_2O$, 1:2, v/v). The fractions containing (1*R*,2′*S*)-**3** were combined and concentrated *in vacuo*. The residue was recrystallized from $CH_2Cl_2$–$isoPr_2O$ to give (1*R*,2′*S*)-**3** (2.7 g, 7.4%) as pale yellow plates. From the second eluate, an additional amount of (1*R*,2′*R*)-**3** (6.59 g, 18%) was obtained.

(1*R*,2′*S*)-**3**: mp 91 °C. $^1$H-NMR ($CDCl_3$) $\delta$: 1.46 (3H, d, $J$ = 6.4 Hz), 2.95 (1H, d, $J$ = 5 Hz), 3.28 (1H, d, $J$ = 5 Hz), 5.41 (1H, q, $J$ = 6.4 Hz), 6.78—7.00 (2H, m), 7.51 (1H, dt, $J$ = 6.4, 8.4 Hz), 9.08 (2H, d, $J$ = 2.2 Hz), 9.23 (1H, t, $J$ = 2.2 Hz). *Anal.* Calcd for $C_{17}H_{12}F_2N_2O_7$: C, 51.79; H, 3.10; N, 7.10. Found: C, 51.71; H, 3.12; N, 7.04. IR (KBr): 1715, 1625, 1612, 1540, 1502, 1340 cm$^{-1}$. $[\alpha]_D^{20}$ +14.4° ($c$ = 1.0, $CHCl_3$).

**(1*R*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol [(1*R*,2′*S*)-4b, Table 4]** A 1 N aqueous NaOH solution (30.4 ml) was added dropwise to a solution of (1*R*,2′*S*)-**3** (6 g) in MeOH (120 ml). The mixture was stirred for 40 min, then 1 N HCl (15.2 ml) was added. The whole was concentrated *in vacuo* and worked up (AcOEt; brine) to afford a residue, which was purified by silica gel column chromatography [hexane–ethyl acetate (AcOEt), 2:1, v/v] to give (1*R*,2′*S*)-**4b** (3.04 g, quantitative) as a pale yellow oil.

**[(1*S*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethyl] Benzoate [(1*S*,2′*S*)-4a]** Triphenylphosphine ($Ph_3P$, 6.55 g), benzoic acid (Ph-COOH, 3.05 g) and diethyl azodicarboxylate (DEAD, 4.35 g) were added to an ice-cooled solution of (1*R*,2′*S*)-**4b** (2.0 g) in tetrahydrofuran (THF, 50 ml). The mixture was stirred for 16 h, then worked up (AcOEt; water), and the residue was chromatographed on silica gel (hexane–AcOEt, 30:1→10:1, v/v) to give (1*S*,2′*S*)-**4a** (2.91 g, 96%) as a colorless oil. $^1$H-NMR ($CDCl_3$) $\delta$: 1.38 (3H, dd, $J$ = 6.6, 1.4 Hz), 2.90 (1H, d, $J$ = 5 Hz), 3.24 (1H, d, $J$ = 5 Hz), 5.40 (1H, q, $J$ = 6.6 Hz), 6.80—7.00 (2H, m), 7.40—7.65 (4H, m), 8.00—8.08 (2H, m). IR (neat): 1720, 1616, 1600, 1508, 1270 cm$^{-1}$. $[\alpha]_D^{24}$ +48.7° ($c$ = 2.0, MeOH).

**(1*S*)-1-[(2*S*)-2-(2,4-Difluorophenyl)-2-oxiranyl]ethanol [(1*S*,2′*S*)-4b, Table 4]** A 28% sodium methoxide (NaOMe)–MeOH (1.84 g) solution was added to an ice-cooled solution of (1*S*,2′*S*)-**4a** (2.9 g) in MeOH (50 ml). The mixture was stirred for 4 h, then 1 N HCl (9.5 ml) was added. The whole was concentrated *in vacuo* and worked up (AcOEt; water, brine) to afford a residue, which was submitted to silica gel column chromatography (hexane–AcOEt, 2:1, v/v) to give (1*S*,2′*S*)-**4b** (1.83 g, 96%) as a colorless oil.

**2-[(1*S*,2*R*)-2-(2,4-Difluorophenyl)-2,3-epoxy-1-methylpropyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolone [(1*S*,2*R*)-5: Table 5]** $Tf_2O$ (0.93 ml) was added dropwise to a stirred solution of (1*R*,2′*S*)-**4b** (1.0 g) and diisopropylethylamine ($isoPr_2NEt$, 0.96 ml) in $CH_2Cl_2$ (20 ml) over a period of 3 min at −78 °C under a nitrogen atmosphere. The resulting mixture was stirred for 20 min at −60 °C and then for 20 min at −20 °C. It was diluted with hexane (20 ml) and submitted to flash chromatography on silica gel ($CH_2Cl_2$–hexane, 1:1, v/v). The eluates containing the triflate [(1*R*,2′*S*)-**4c**] were combined and concentrated to about 7 ml.[7] This solution was added to a stirred mixture of H-Taz (1.16 g), NaH (60% in oil, 150 mg) and *N*,*N*-dimethylformamide (DMF, 30 ml) at −15 °C. The resulting mixture was stirred at −15 °C for 20 min and then at 0 °C for 20 min. The mixture was worked up (AcOEt; water, brine). The residue was purified by column chromatography on silica gel (hexane–AcOEt, 2:1, v/v) to give (1*S*,2*R*)-**5** (0.94 g, 40%) as a colorless oil.

The oxiranylethanols (1*R*,2′*R*)-**4b**[1] and (1*S*,2′*S*)-**4b** were converted to the corresponding triflates, (1*R*,2′*R*)-**4c** and (1*S*,2′*S*)-**4c**, which were allowed to react with H-Taz in a manner similar to that described above to give (1*S*,2*S*)-**5** and (1*R*,2*R*)-**5** (Table 5), respectively.

**2-[(1*S*,2*S*)-2-(2,4-Difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolone [(1*S*,2*S*)-1: Table 1]** A solution of (1*S*,2*R*)-**5** (0.93 g)

Table 4. 1-[2-(2,4-Difluorophenyl)-2-oxiranyl]ethanols (**4b**)

| No. | Yield (%) | Appearance | $^1$H-NMR (in $CDCl_3$) $\delta$ | IR neat (cm$^{-1}$) | $[\alpha]_D$ ($CHCl_3$, $c$) {°C} |
|---|---|---|---|---|---|
| (1*S*,2′*R*)-**4b**[a] | 92 | Oil | 1.20 (3H, dd, $J$ = 6.4, 1 Hz), 2.24 (1H, d, $J$ = 2 Hz), 2.92 (1H, d, $J$ = 5 Hz), 3.28 (1H, d, $J$ = 5 Hz), 4.12 (1H, dq, $J$ = 6.4, 2 Hz), 6.77—6.95 (2H, m), 7.32—7.44 (1H, m) | 3420, 2980, 1615, 1600, 1500, 1425 | −33.3° (2.4)[b] {24} |
| (1*R*,2′*S*)-**4b** | 100 | Oil | 1.20 (3H, dd, $J$ = 6.2, 1 Hz), 2.22 (1H, d, $J$ = 2 Hz), 2.92 (1H, d, $J$ = 5 Hz), 3.28 (1H, d, $J$ = 5 Hz), 4.12 (1H, dq, $J$ = 6.2, 2 Hz), 6.77—6.95 (2H, m), 7.32—7.43 (1H, m) | 3420, 2980, 1619, 1508, 1427 | +30.2° (2.1) {22} |
| (1*S*,2′*S*)-**4b** | 96 | Oil | 1.16 (3H, dd, $J$ = 6.6, 1.2 Hz), 1.85 (1H, d, $J$ = 8 Hz), 2.80 (1H, d, $J$ = 5 Hz), 3.30 (1H, d, $J$ = 5 Hz), 4.00—4.20 (1H, m), 6.74—6.94 (2H, m), 7.35—7.47 (1H, m) | 3440, 1636, 1618, 1604, 1508, 1425 | +52.7° (2.2) {25} |
| (1*R*,2′*R*)-**4b** | 100 | Oil | 1.17 (3H, dd, $J$ = 6.6, 1.2 Hz), 1.83 (1H, d, $J$ = 8 Hz), 2.80 (1H, d, $J$ = 5.2 Hz), 3.30 (1H, d, $J$ = 5.2 Hz), 4.01—4.17 (1H, m), 6.75—6.93 (2H, m), 7.36—7.48 (1H, m) | 3420, 1615, 1600, 1505, 1425 | −52.3° (2.6)[c] {25} |

*a*) Reported in reference 1. *b*) −26.4° (Reported in reference 6). *c*) −54.9° (Reported in reference 6).

Table 5. 2-[2-(2,4-Difluorophenyl)-2,3-epoxy-1-methylpropyl]-4-[4-(2,2,3,3-tetrafluoropropoxy)phenyl]-3(2*H*,4*H*)-1,2,4-triazolones (**5**)

| No. | Yield[a] (%) | Appearance | Formula | Analysis (%) Calcd (Found) C | H | N | $^1$H-NMR (in $CDCl_3$) $\delta$ | IR neat ($cm^{-1}$) | $[\alpha]_D$ {°C} MeOH (*c*) |
|---|---|---|---|---|---|---|---|---|---|
| (1*R*,2*S*)-**5**[b] | 57 | Oil | $C_{21}H_{17}F_6N_3O_3$ [SIMS 474 ($MH^+$)] | 53.28 (52.88) | 3.62 (3.68) | 8.88 (8.65) | 1.47 (3H, d, *J*=7 Hz), 2.88 (1H, d, *J*=4.8 Hz), 3.16 (1H, d, *J*=4.8 Hz), 4.38 (2H, t, *J*=11.8 Hz), 4.94 (1H, q, *J*=7 Hz), 6.07 (1H, tt, *J*=53, 4.8 Hz), 6.75—6.90 (2H, m), 6.95—7.12 (2H, m), 7.28—7.55 (3H, m), 7.63 (1H, s) | 1716, 1705, 1616, 1558, 1516, 1257 | +14.6° {20} (1.0) |
| (1*S*,2*R*)-**5** | 40 | Oil | $C_{21}H_{17}F_6N_3O_3$ | 53.28 (53.31) | 3.62 (3.57) | 8.88 (9.15) | 1.46 (3H, d, *J*=7 Hz), 2.88 (1H, d, *J*=4.8 Hz), 3.16 (1H, d, *J*=4.8 Hz), 4.37 (2H, tt, *J*=11.8, 1.4 Hz), 4.94 (1H, q, *J*=7 Hz), 6.05 (1H, tt, *J*=53, 4.8 Hz), 6.74—6.90 (2H, m), 6.95—7.05 (2H, m), 7.28—7.48 (3H, m), 7.61 (1H, s) | 1714, 1704, 1618, 1558, 1517, 1400 | −15.6° {22} (1.1) |
| (1*R*,2*R*)-**5** | 25 | Oil | $C_{21}H_{17}F_6N_3O_3$ | 53.28 (52.85 | 3.62 3.36 | 8.88 9.04) | 1.51 (3H, d, *J*=7 Hz), 2.85 (1H, d, *J*=4.8 Hz), 3.34 (1H, d, *J*=4.8 Hz), 4.37 (2H, t, *J*=11.8 Hz), 4.69 (1H, q, *J*=7 Hz), 6.06 (1H, tt, *J*=53, 4.6 Hz), 6.70—6.89 (2H, m), 6.95—7.10 (2H, m), 7.30—7.50 (3H, m), 7.55 (1H, s) | 1714, 1706, 1700, 1616, 1558, 1519 | +87.8° {23} (1.7) |
| (1*S*,2*S*)-**5** | 38 | Oil | $C_{21}H_{17}F_6N_3O_3$ | 53.28 (53.14 | 3.62 3.67 | 8.88 8.99) | 1.51 (3H, d, *J*=7 Hz), 2.85 (1H, d, *J*=4.8 Hz), 3.33 (1H, d, *J*=4.8 Hz), 4.37 (2H, tt, *J*=11.8, 1.4 Hz), 4.68 (1H, q, *J*=7 Hz), 6.06 (1H, tt, *J*=53, 4.6 Hz), 6.70—6.90 (2H, m), 6.95—7.05 (2H, m), 7.32—7.46 (3H, m), 7.55 (1H, s) | 1712, 1706, 1700, 1618, 1556, 1515 | −89.0° {22} (1.6) |

*a*) Based on **4b**. *b*) Reported in reference 1.

in DMF (5 ml) was added to a stirred mixture of 1*H*-1,2,4-triazole (0.34 g), NaH (60% in oil, 0.16 g) and DMF (5 ml). The resulting mixture was stirred for 8 h at 50 °C and worked up (AcOEt; water,brine) to afford a residue, which was purified by silica gel column chromatography (hexane–AcOEt, 1:1→1:4, v/v) followed by recrystallization from AcOEt–isoPr$_2$O to give (1*S*,2*S*)-**1** (0.673 g, 63%) as colorless crystals.

The reaction of (1*S*,2*S*)-**5** and (1*R*,2*R*)-**5** with 1*H*-1,2,4-triazole was carried out in a manner similar to that described above to give (1*S*,2*R*)-**1** and (1*R*,2*S*)-**1** (Table 1), respectively.

**Acknowledgment** We thank Dr. S. Kishimoto and Dr. H. Ono for their encouragement throughout this work. We also thank Ms. J. Obita, Mr. K. Fukuda, Dr. K. Jono and Mr. T. Yoshida for the biological assay.

**References and Notes**

1) Part VI: Kitazaki T., Tamura N., Tasaka A., Matsushita Y., Hayashi R., Okonogi K., Itoh K., *Chem. Pharm. Bull.*, **44**, 314—327 (1996).
2) *a*) Tasaka A., Tamura N., Matsushita Y., Teranishi K., Hayashi R., Okonogi K., Itoh K., *Chem. Pharm. Bull.*, **41**, 1035—1042 (1993); *b*) Tasaka A., Tsuchimori N., Kitazaki T., Hiroe K., Hayashi R., Okonogi K., Itoh K., *ibid.*, **43**, 441—449 (1995); *c*) Tasaka A., Tamura N., Matsushita Y., Hayashi R., Okonogi K., Itoh K., *ibid.*, **41**, 1043—1048 (1993).
3) RPMI 1640 was purchased from Gibco BRL.
4) The agar dilution method for *in vitro* susceptibility testing of antifungal azoles under 20% $CO_2$ was developed in our laboratories. This susceptibility test gave *MIC* values nearly equal to those determined by the broth dilution method proposed by the National Committee for Clinical Laboratory Standards (NCCLS protocol, NCCLS Document M27-P, Vol. 12, No. 25). Details of the experimental procedure will be described in a separate paper; Yoshida T., Jono K., Okonogi K., *Antimicrob. Agents Chemother.*, submitted.
5) Okonogi K., Hayashi R., Obita J., Tsuchimori N., Tasaka A., Kitazaki T., Itoh K., *Antimicrob. Agents Chemother.*, in preparation.
6) An alternative method for the synthesis of the (1*S*,2′*R*)- and (1*R*,2′*R*)- oxiranylethanols has already been reported: *a*) Konosu T., Miyaoka T., Tajima Y., Oida S., *Chem. Pharm. Bull.*, **39**, 2241—2246 (1991); *b*) Sankyo Co., European Patent 0332387A1 (1989) [*Chem. Abstr.*, **113**, 231379*y* (1990)]; *c*) Sankyo Co., Japan. Patent, laid open to the public, 3-128338 (1991) [*Chem. Abstr.*, **115**, 231852*p* (1991)].
7) Considerable decomposition was observed during attempted isolation of the triflate. Therefore, the concentrated eluate containing the triflate was used directly in the subsequent nucleophilic displacement reaction.