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SYNTHESIS AND ANTIFUNGAL ACTIVITIES OF OPTICALLY ACTIVE ISOMERS OF SM-8668

Hiroshi MIYAUCHI*, Tomoharu TANIO, and Naohito OHASHI

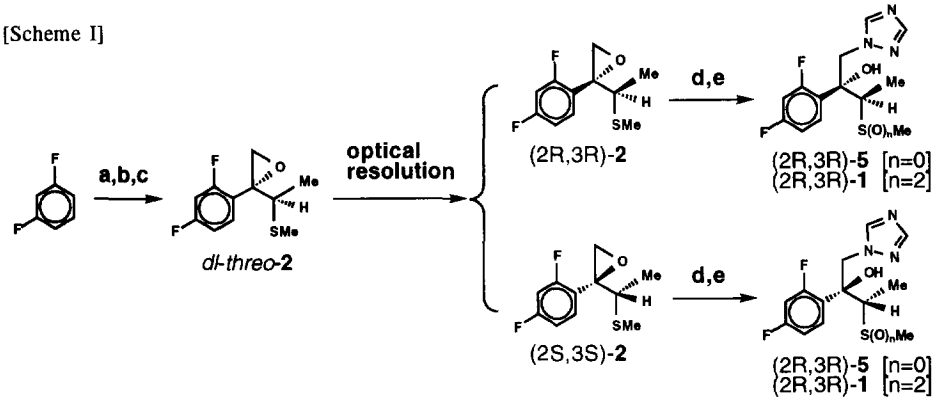
Research Center, Sumitomo Pharmaceuticals Co., Ltd.,

1-98, Kasugadenaka 3-chome, Konohana-ku, Osaka 554, Japan

Abstract: Synthesis and antifungal activities of optically active isomers of SM-8668 (**1**) are described. These isomers were prepared in eight steps from *m*-difluorobenzene. The crucial step was optical resolution of a synthetic intermediate *dl-threo*-2-(2,4-difluorophenyl)-2-(1-methylthio)ethyloxirane (**2**). Only (2R,3R)-isomer of **1** showed potent antifungal activities both *in vitro* and *in vivo*.

During the course of our search for antifungal azole compounds, we found that *dl-threo*-2-(2,4-difluorophenyl)-3-methylsulfonyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanol (SM-8668) (**1**)[1] had excellent antifungal activities with oral administration on various deep fungal infection models.[2] We previously reported the synthesis of racemic **1** in a regioselective manner.[3] As part of our interest in the biologically active constituent of *dl*-**1**, we prepared both enantiomers of **1**[4] and found that only a (2R, 3R)-isomer of **1** showed potent antifungal activities both *in vitro* and *in vivo*. Moreover, (2R,3R)-**1** had 20-fold higher solubility in water than *dl*-**1**, which enabled us to administer antifungal agent **1** by intravenous injection. We herein report the synthesis of optically active isomers of **1** and their antifungal activities. These isomers were prepared in eight steps from *m*-difluorobenzene as outlined in Scheme I.

[Scheme I]



Reagents:
a) $AlCl_3$, α-bromopropionyl bromide (96.0 %) b) 15 % aq NaSMe, $(CH_2Cl)_2$ (91.1 %)
c) $Me_3S(O)Cl$, 48 % aq NaOH, CH_2Cl_2 (95.0 %, *threo*/*erythro*=7:1)
d) 1*H*-1,2,4-triazol, NaOH, DMSO (76.0 %) e) 30 % aq H_2O_2, conc HCl, Na_2WO_4, H_2O (92.1 %)

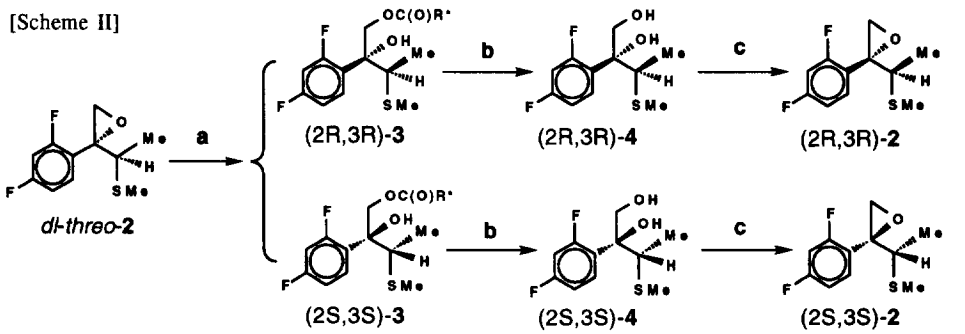
Synthesis

A racemate of *threo*-epoxide **2** which was prepared from *m*-difluorobenzene in three steps as described in previous report[3] could be separated into each enantiomer by the reaction of epoxide **2** with carboxylic acid,[5] as shown in Scheme II. Actually, *dl-threo*-**2** was reacted with various chiral carboxylic acids (R*COOH) to give a 1:1 diastereomeric mixture of **3** which was easily separated by silica-gel column chromatography or fractional crystallization. In the case of reaction of *dl-threo*-**2** with R^1COOH, the mixture could be separated by silica-gel column chromatography, when R^1COOH was chosen from (+)-2-phenylpropionic acid, (+)-2-phenylbutylic acid, N-Boc-L-proline, (-)-menthoxyacetic acid, N-benzoyl-D-phenylglycine[6] and their enantiomers. In contrast, in the case of reaction with R^2COOH, the mixture could be separated by fractional crystallization, when R^2COOH was chosen from L-mandelic acid, (-)-pyroglutamic acid, N-benzoyl-D-phenylglycine and their enantiomers. The resulting single isomer of **3** could be regenerated to optically active epoxide **2** by alkaline hydrolysis of ester **3** followed by regioselective mesylation and alkaline treatment. These steps proceeded with complete retention of absolute configuration of **3.**

For typical example, the reaction of *dl*-**2** (*threo*/*erythro*=7:1) with L-mandelic acid (1.2 eq.) in toluene at reflux temperature for 2.5 h afforded a 1:1 diastereomeric mixture of ester **3**, one of which was crystallized in toluene-heptane (1:1). The crystalline powder was then recrystallized in toluene-heptane (1:1) to give L-mandelate of (2R,3R)-**3** as a single isomer[7] [30.6 % yield, $[\alpha]_D^{25}$ -0.8° (c=0.5, $CHCl_3$)]. Next, this ester was hydrolyzed with aqueous NaOH in toluene to give (2R,3R)-**4** [quantitative yield, $[\alpha]_D^{25}$ -24.2° (c=0.5, $CHCl_3$)]. Then, it was treated with metanesulfonyl chloride in toluene and aqueous NaOH in presence of a catalytic amount of benzyltriethylammonium chloride[8] to afford (2R,3R)-**2** [quantitative yield, $[\alpha]_D^{25}$ -54.4° (c=0.5, $CHCl_3$)]. Thus, racemic **2** was resolved into optically active **2**. Such direct optical resolution method of epoxide hasn't been reported, while *dl*-epoxides are usually resolved after transformed to corresponding *dl*-diols by ring-cleavage reaction.[9] This methodology is valuable in the field of optical resolution.

Thus obtained (2R,3R)-**2** was then treated with *1H*-1,2,4-triazole in presence of sodium hydroxide at 80 °C to give (2R,3R)-**5** as a crystalline powder [76.0 % yield, $[\alpha]_D^{25}$ -126.7° (c=0.5, $CHCl_3$), mp 137-138°C].

[Scheme II]



Reagents:
a) R*COOH, then separated b) NaOH, EtOH c) MsCl, NaOH, $BnNEt_3Cl$, CH_2Cl_2-H_2O

Finally, sulfide (2R,3R)-**5** was oxidized with hydrogen peroxide in presence of a catalytic amount of sodium tungstate under acidic conditions to afford sulfone (2R,3R)-**1** [92.1 % yield, $[\alpha]_D^{25}$ -38.5° (c=1.0, MeOH), mp 147-148°C].[10,11] The enantiomer (2S,3S)-**1** was also obtained by the same method using D-mandelic acid as a resolving agent.

Interestingly, the solubility of (2R,3R)-**1** in water was measured to be 3.0 mg/ml at 20 °C, while that of *dl*-**1** was 0.16 mg/ml under the same conditions. This result suggests that it is possible to use the optical isomer of antifungal agent **1** by intravenous administration.

Antifungal activities

The *in vitro* and *in vivo* antifungal activities of (2R,3R)-**1**, (2S,3S)-**1** and racemic **1** are shown in Tables I and II, respectively. (2R,3R)-**1** was twice more potent than racemic **1** against both *Candida albicans* KB-8 and *Aspergillus fumigatus* MTU6001 *in vitro*. On the other hand, (2S,3S)-**1** showed only 500-fold less activity than (2R,3R)-**1** against *C. albicans* and failed to inhibit the growth of *A. fumigatus* at 800 μg/ml.

The prophylactic efficiencies of (2R,3R)-**1** against murine systemic candidiasis and aspergillosis were also higher than that of racemic **1**. The oral treatment of (2R,3R)-**1** was as efficient as the intravenous treatment. In contrast, (2S,3S)-**1** showed no efficacy against both systemic candidasis and systemic aspergillosis either by oral treatment or by intravenous treatment.

These data suggest that the potent antifungal activity of racemic **1** only depends on (2R,3R)-isomer.

[Table I] The *in vitro* antifungal activity of (2R,3R)-**1**, (2S,3S)-**1** and racemic **1**.[12]

compound	MIC (μg/ml)	
	C. albicans KB-8	*A. fumigatus* MTU6001
(2R,3R)-1	0.10	6.25
(2S,3S)-1	50	> 800
dl-1	0.20	12.5

[Table II] The prophylactic efficiencies of (2R,3R)-**1**, (2S,3S)-**1** and racemic **1** against murine systemic candidiasis and aspergillosis.[13]

compound	route	ED_{50} (mg/kg)	
		C. albicans KB-8	*A. fumigatus* MTU6001
(2R,3R)-1	p.o.	0.27	4.1
	i.v.	0.26	5.3
(2S,3S)-1	p.o.	> 100	> 125
	i.v.	> 25 *1	> 25 *1
dl-1	p.o.	0.38	18

*1 ; Maximum injectable dose in mice.

References and notes

1) a) Saji, I.; Tamoto, K.; Tanio, T.; Okuda, T.; Atsumi, T., *Abstracts of 8th Medicinal Chemistry Symposium*, Osaka, Nov. 1986, pp.9-12. b) Saji, I.; Tanaka, Y.; Ichise, K.; Tanio, T.; Okuda, T.; Atsumi, T., *European Patent*, **1986**, 178533; *Chem. Abstr.*, **1986**, *105*, 35613.

2) Tanio, T.; Ichise, K.; Nakajima, T.; Okuda, T., *Antimicrob. Agents Chemother.*, **1990**, *34*, 980.

3) Saji, I.; Tamoto, K.; Tanaka, Y.; Miyauchi, H.; Fujimoto, K.; N. Ohashi, *Bull. Chem. Soc. Jpn.*, **1994**, *67*, 1427.

4) Girijavallabhan, V. M.; Ganguly, A. K.; Pinto, P. A.; Sarre, O. Z., *Bioorg. Med. Chem Lett.*, **1991**, *1*, 349.

5) Miyauchi, H.; Nakamura, T.; N. Ohashi, manuscript in preparation.

6) Cohen, S. G.; Schultz, R. M.; Weinstein, S. Y., *J. Am. Chem. Soc.*, **1966**, *88*, 5315.

7) The ratio of diastereomers was determined to be 100 % d.e. by HPLC in following conditions: column; Sumipax ODS A-212 (5 μm, 6 mmϕ x 25 cm), mobile phase; acetonitrile/water = 55/45, flow rate; 1.0 ml/min., detection; UV 254 nm: The peak at 15.4 min. corresponded to (2R,3R)-**3** (R*=L-mandelate), and the peak at 14.5 min. corresponded to its diastereomer (2S,3S)-**3** (R*=L-mandelate).

8) Szeja, W., *Synthesis*, **1979**, 822

9) a) Tanaka, Y.; Yuasa, T.; Kawakami, Y.; Terashima, K.; Morita, T.; Nishikawa, A.; Bando, K.; Kawashima, M., *European Patent*, **1993**, 552974; *Chem. Abstr.*, **1993**, *120*, 134499. b) Tomari, M.; Takagi, J.; Asahara, N.; Sakuma, O.; Ooto, H.; Iwase, Y., *Jpn. Kokai Tokkyo Koho*, 1993, 05202005; *Chem. Abstr.*, **1993**, *120*, 134482.

10) The absolute configuration of (-)-**1** was determined to be (2R,3R) by X-ray crystallographic analysis.

11) The optical purity of **1** was determined to be 100 % e.e. by HPLC in following conditions: column; Sumipax OA-4400 (5 μm, 4 mmϕ x 25 cm), mobile phase; hexane/1,2-dichloromethane/ethanol/acetic acid = 1000/200/16/1, flow rate; 1.0 ml/min., detection; UV 260 nm: The peak at 20.7 min. corresponded to (2R,3R)-**1**, and the peak at 18.0 min. corresponded to its enantiomer (2S,3S)-**1**.

12) The *in vitro* activity was tested as follows: *C. albicans* KB-8 was grown at 37 °C on Sabouraud dextrose agar (SDA) for 24 h and transferred in glucose polypeptone yeast-extractbroth for 24 h. *A. fumigatus* MTU6001 was grown at 30 °C on potatodextrose agar for 5 days. Approximately 10^3 saline-washed cells of *C. albicans* or conidia of *A. fumigatus* were inoculated into 1 ml of synthetic amino acid medium fungal (GIBCO) contained serially diluted (2R,3R)-**1**, (2S,3S)-**1** or racemic **1**, and those were incubated at 37 °C for 24 h for *C. albicans* or 30 °C for 2 days for *A. fumigatus*. The MIC was determined based on the lowest concentration of compound preventing visible fungal growth.

13) The prophylactic efficiencies against murine systemic candidiasis and aspergillosis were tested as follows: Male albino ddY mice, five-weeks old, were inoculated via tail vein with 2.0 x 10^6 cells of *C. albicans* KB-8 or 2 x 10^7 conidia of *A. fumigatus* MTU6001. Appropriate doses of (2R,3R)-**1**, (2S,3S)-**1** or racemic **1** in 0.5 % methylcellulose or saline were orally or intravenously administered to groups of 10 mice at 0, 24 and 48 h after infection. The survival rates were recorded for a period of 10 days. The 50 % effective dose (ED_{50}) values were calculated from the survival rates of each group on the final day by using probit analysis.

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