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In vitro antifungal activities of luliconazole, a new topical imidazole

HIROYASU KOGA*‡, YASUKO NANJOH*, KOICHI MAKIMURA† & RYOJI TSUBOI‡

Research Center, Nihon Nohyaku Co., Ltd., Kawachi-Nagano, Osaka, †Teikyo University Institute of Medical Mycology, Hachioji, Tokyo, and ‡Department of Dermatology, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan

Luliconazole is a topical antifungal drug newly developed in Japan. The present study compares the *in vitro* antifungal activity of luliconazole against clinically important dermatomycotic fungi with that of other representative antifungal drugs. The reference drugs chosen were five classes of nine topical agents, i.e., allylamine (terbinafine), thiocarbamate (liranaftate), benzylamine (butenafine), morpholine (amorolfine), and azole (ketoconazole, clotrimazole, neticonazole, miconazole and bifonazole). The minimum inhibitory concentrations (MIC) of luliconazole and the reference drugs against *Trichophyton* spp. (*T. rubrum*, *T. mentagrophytes* and *T. tonsurans*) and *Candida albicans* were measured by the standardized broth microdilution method. Luliconazole demonstrated greater potency against *Trichophyton* spp. (MIC range: $\leq$0.00012–0.002 μg/ml) than the reference drugs, with *T. rubrum* being the most susceptible to it. Luliconazole was also highly active against *Candida albicans* (MIC range: 0.031–0.13 μg/ml), proving to be more potent than terbinafine, liranaftate, butenafine, amorolfine, and bifonazole, but less than ketoconazole, clotrimazole, neticonazole, and miconazole. Further, the MIC of luliconazole against *Malassezia restricta*, an important pathogenic agent involved in seborrhoeic dermatitis, was very low (MIC range: 0.004–0.016 μg/ml) suggesting action comparable to or stronger than that of ketoconazole. These results indicate a possible clinical role for luliconazole with its broad-spectrum antimycotic activity.

Keywords luliconazole, *in vitro* antifungal activity, *Trichophyton* spp., *Candida albicans*, *Malassezia restricta*

Introduction

Luliconazole (Fig. 1: (-)-(*E*)-[(4*R*)-4-(2,4-dichlorophenyl)-1,3-dithiolan-2-ylidene] (1*H*-imidazol-1-yl) acetonitrile), is an optically active imidazole antifungal agent created by Nihon Nohyaku Co. Ltd. (Tokyo, Japan). It has been found to have broad-spectrum of antifungal activity against pathogenic fungi, especially dermatophytes [1–3]. Luliconazole was launched in 2005 in Japan for use as a topical antifungal agent [4,5] and is presently available as 1% creams and solutions for the treatment of superficial infections such as dermatophytoses, candidiasis and pityriasis versicolor.

The *in vitro* activities of antifungal drugs constitute an important source of information for physicians seeking the most appropriate choice of topical antifungal remedies available for the treatment of superficial fungal infections. Although there is a wide variety of topical antifungal drugs already available in Japan that possess a broad spectra of potent antifungal activity, comparable data on the *in vitro* antifungal activity of these drugs are limited because measurements of the minimum inhibitory concentration (MIC) vary considerably with experimental conditions and testing facilities [6–8]. In the present study, the MICs of

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Correspondence: Hiroyasu Koga, Research Center, Nihon Nohyaku Co., Ltd., 345 Oyamada-cho, Kawachi-Nagano, Osaka 586-0094, Japan. Tel: 0721 56 9004; fax: 0721 56 9090; E-mail: koga-hiroyasu@nichino.co.jp

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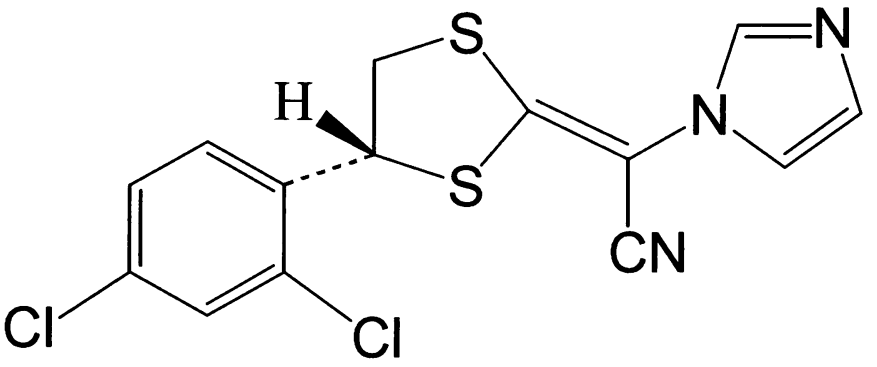

Fig. 1 Chemical structure of luliconazole.

luliconazole against the dermatomycotic pathogenic fungi *Trichophyton* spp. and *Candida albicans*, were directly compared with the MICs of representative topical antifungal drugs available in Japan, using standardized broth microdilution methods [9–11]. The reference drugs chosen were five classes of nine topical antifungals including terbinafine hydrochloride (allylamine class), liranaftate (thiocalbamine class), butenafine hydrochloride (benzylamine class), amorolfine hydrochloride (morpholine class), and ketoconazole, clotrimazole, neticonazole hydrochloride, miconazole nitrate, and bifonazole (azole class).

The lipophilic yeast, *Malassezia*, is considered to be involved in seborrhoeic dermatitis [12–14], as well as the causative organism in pityriasis versicolor. The clinical efficacy of antifungal drugs in the treatment of SD has buttressed this hypothesis [15–19], and has popularized the use of ketoconazole [18–20] for the treatment of SD in Japan. Recently, the taxonomy of genus *Malassezia* was revised by molecular methods [21–23], and *Malassezia restricta* was found to be the dominant species in the SD skin lesions [24,25]. The MIC of luliconazole against *M. restricta* was also determined and comparing it to the data from the use of ketoconazole.

Materials and methods

Antifungal agents

Luliconazole (LLCZ, 99.7% purity), neticonazole hydrochloride (NCZ, 94.5% purity), butenafine hydrochloride (BTF, 100% purity), terbinafine hydrochloride (TBF, 94.5% purity) and amorolfine hydrochloride (AMO, 97.5% purity) were synthesized and purified at the Research Center at Nihon Nohyaku Co. Ltd. (Osaka, Japan). Miconazole nitrate (MCZ, 100% purity), bifonazole (BFZ, >99% purity), ketoconazole (KCZ, >99% purity) and clotrimazole (CTZ, 99.6% purity) were purchased from Sigma-Aldrich (MO, USA). Lilanaftate (LNF, 99.6% purity) was kindly provided by Pola Pharma Inc. (Tokyo, Japan). The drugs were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1,600ug/ml from which a series of two fold dilutions were then prepared. The final drug concentrations for studies with *Trichophyton* spp ranged from: 0.00012–0.016 ug/ml for LLCZ; 0.00098–0.25 μg/ml for TBF, LNF, BTF and AMO; 0.0078 to 1 μg/mL for CTZ and NCZ; and 0.002–4 μg/ml for BFZ, MCZ and KCZ. In the investigations with *C. albicans*, the range of antifungals was from: 0.016–1 μg/ml for LLCZ; 0.002–0.13 μg/ml for KCZ; 0.0039–1 μg/ml for NCZ, CTZ and MCZ; 0.016–16 μg/ml for AMO; 0.13–16 μg/ml for TBF and BFZ; and 2–16 μg/ml for LNF and BTF. For *M. restricta*, the final concentrations of LLCZ and KCZ ranged from 0.002–2 μg/ml. The solvent in the assay system was 1.0%.

Test organisms

The 10 stock cultures of *T. rubrum*, *T. mentagrophytes* and *C. albicans* were obtained from Teikyo University Institute of Medical Mycology (TIMM; Tokyo, Japan), NITE Biological Resource Center (NBRC; Chiba, Japan) and American Type Culture Collection (ATCC; Maryland, USA). Clinical isolates of *T. tonsurans* (10 isolates) and *M. restricta* (10 isolates) were collected at Teikyo University and Tokyo Medical University during 2003–2006. Identification of *T. tonsurans* and *M. restricta* were based on DNA sequence of nuclear ribosomal internal transcribed spacer 1 regions [26,27]. For *M. restricta*, catalase-negative reaction, one of the key identifying characters of this species, was also confirmed. The results of identification were compatible with morphologic features of the organisms grown on their preculture media. For quality control of the assay, *T. mentagrophytes* ATCC18748 (American Type Culture Collection, USA) and *C. parapsilosis* ATCC22019 were used as reference strains.

Culture media

For *Trichophyton* spp. and *Candida* spp., Sabouraud dextrose agar (SDA;Difco, MD, USA) and RPMI1640 broth without $NaHCO_3$ or phenol red with L-glutamine (Sigma-Aldrich, MO, USA) buffered to a pH of 7.0 with 0.165M 3-[N-morpholino] propanesulfonic acid (MOPS; Wako Pure Chemical Industries, Osaka, Japan) were used for the preculturing and assay medium, respectively. For *M. restricta* preculturing modified Leeming and Notman agar (LNA) comprised of 10 g/l peptone (Difco, MD, USA), 10 g/l D-glucose (Sigma-Aldrich, MO, USA), 2 g/l yeast extract (Difco, MD, USA), 8 g/l bile salt (Oxoid, Ontario, Canada), 0.5 g/l glycerol monostearate (Tokyo Chemical Industries, Tokyo, Japan), 10 ml/l glycerol (Sigma-Aldrich, MO, USA), 5 ml/l Tween 60 (Tokyo Chemical Industries, Tokyo, Japan), 20 ml/l olive oil (Nacalai Tesque Inc, Kyoto, Japan) and 15 g/l agar (Wako Pure Chemical Industries, Osaka, Japan) was used. The assay medium was LNA without milk (LNA(-)) and contained 10 g/l peptone, 5 g/L D-glucose, 0.1 g/l yeast extract, 8 g/l bile salt, 0.5 g/l glycerol monostearate, 1 ml/l glycerol, 0.5 ml/l Tween 60, and 12 g/l agar.

Inoculum preparation

Trichophyton spp. were grown on SDA slant at 27°C for 1–3 weeks, sterile saline with 0.1% (v/v) Tween 80 was then added to the slants and conidia suspended by gently rubbing the colony with a loop. The suspension was filtrated through sterilized gauze to remove hyphal fragments. The number of conidia in the filtrate was counted using a Thoma hemacytometer and the concentration adjusted to 2.0×10^6 conidia/mL with sterile saline containing 0.1% (v/v) Tween 80. The conidia suspension was diluted 10 times with RPMI1640 broth containing 20%(v/v) Alamar Blue (Wako Pure Chemical Industries Ltd., Osaka, Japan). *Candida albicans* and *C. parapsilosis* were grown on SDA plate at 35°C for one day. Five colonies of $\geq$1 mm in diameter were suspended in 5.0 ml of sterile saline and vortexed for 15 sec. Cell density of the suspension was adjusted to 0.5 McFarland with sterile saline using a spectrophotometer, which resulted in 1.2×10^6–5.0×10^6 cells/ml, as calibrated by Thoma hemacytometer. The cell suspension was diluted 20 times with RPMI1640 broth and further diluted 100 times with RPMI1640 containing 20% (v/v) Alamar Blue. *M. restricta* was grown on a modified LNA plate at 32°C for 3–10 days. Colonies were harvested and suspended in an appropriate volume of 0.1% (v/v) Tween 80 solution and vortexed. After the suspension was allowed to stand for about 30 min to remove the aggregated cell sediment, the fine suspension was collected and the cell concentration adjusted to 1×10^8 cells/ml with sterile saline.

MIC measurement

The MICs of *Trichophyton* spp. and *C. albicans* were measured by standardized microdilution methods [9–11] with colorimetric endpoint determination [10,28]. Each drug solution prepared in DMSO was diluted 50-fold with the assay medium and a 100 µl aliquot of it was dispensed into each well on a series of 96-well plates (Multi Well Plate; Sumitomo Bakelite Co. Ltd., Tokyo, Japan). The wells were inoculated with 100 µl of the test organism and incubated at 27°C for up to 7 days for *Trichophyton* spp. and at 35°C for up to 2 days for *Candida* spp. A growth control containing a drug-free basal medium and a negative control consisting of a drug-free basal medium without inoculation were prepared for each strain. The plates were visually observed daily to ascertain the point at which the color of the medium in the growth control wells changed to a definite pink or red. Optical density (OD) at 570 nm was measured by dual wavelengths read (OD at 595 nm served as reference) with a microplate reader (THERMOmax, Molecular Devices Corporation, CA, USA). Duplicate assays were performed and their average OD was calculated. The minimum concentration of the test drug needed to lower the OD to less than 20% of the comparative growth control was defined as the MIC. Quality control (QC) was ensured each time using a set of strains according to the recommendations outlined in the methods [10,11].

MICs for *M. restricta* were measured by the agar dilution method [29] with a streak inoculation. Each drug solution prepared in DMSO was mixed with LNA(-) (1:100, 20 ml medium /9 cm plate) to provide agar plates containing serial drug concentrations. A growth control was prepared with a drug-free basal medium. One µl of each organism was inoculated on the agar plate in a 1.5 cm streak using a loop. The plates were incubated at 32°C for up to 7 days and visually observed daily until colony formation in the growth control was apparent. The minimum concentration of the drugs needed to inhibit fungal growth completely was recorded as the MIC. The assay was performed in duplicate and the higher of the two MIC values was taken as the final.

MIC range, the geometric mean MIC (MIC_{GM}), and MICs at which 50% and 90% of the isolates were inhibited (MIC_{50} and MIC_{90}) were recorded for each strain and species.

Results

The MICs of LLCZ and the reference drugs against 30 strains of *Trichophyton* spp. and 10 strains of *C. albicans* are indicated in Table 1 and Table 2, respectively. The MIC range and the MIC_{GM} of the tested agents are summarized in Fig. 2 in order to illustrate the antifungal spectrum of the drugs. LLCZ exhibited extremely strong activity against *Trichophyton* spp. (total 30 strains) in terms of the MICs ranges. The MIC_{GM} of LLCZ against *T. rubrum*, *T. mentagrophytes* and *T. tonsurans* were the lowest among all of the tested drugs. *Trichophyton rubrum* was the most susceptible for LLCZ, in that one isolate had a low off-scale MIC ($\leq$0.00012 μg/ml) and the MIC for the other nine isolates were at the same range of 0.00024 μg/ml. Although the MIC of LLCZ for *C. albicans* (MIC_{GM}: 0.055 μg/ml) was 125 times higher than that for *Trichophyton* spp. (MIC_{GM}: 0.00044 μg/ml), the antifungal activity of LLCZ against *C. albicans* was still high in comparison with the reference drugs. The MIC_{GM} of LLCZ against *C. albicans* was lower than those of TBF, LNF, BTF, AMO, and BFZ, but higher than those of KCZ, CTZ, NCZ, and MCZ.

Among the reference drugs, TBF of the allylamine class, LNF of the thiocalbamine class and BTF of the benzylamine class showed potent activity against *Trichophyton* spp., but not against *C. albicans*. In contrast to TBF, LNF and BTF, azole compounds such as KCZ, CTZ, NCZ, MCZ and BFZ were more active against *C. albicans*. KCZ was the most potent against *C. albicans* in the azole class. For *Trichophyton* spp., these reference azoles also exhibited potent activity, however the MIC_{GM} was apparently higher than that of TBF, LNF or BTF. The MIC_{GM} of the AMO of the morpholine class for *Trichophyton* spp. and for *C. albicans* were almost equivalent to that of the reference azoles.

Because antifungal susceptibility testing for the genus *Malassezia* has not been standardized, we employed the agar dilution method [29] commonly used for this species. The MICs of LLCZ and KCZ against 10 strains of *M. restricta* are listed in Table 3. The MIC range of LLCZ was almost comparable to that of KCZ, whereas the MIC_{GM}, MIC_{50} and MIC_{90} of LLCZ were lower than those of KCZ.

Discussion

Dermatomycoses are by far the most common superficial fungal infection. An epidemiological survey of dermatomycoses conducted in Japan in 2002 [30] reported that 12.3% of outpatients who visited the dermatology clinic were suffering from dermatomycoses. *Trichophyton rubrum* and *T. mentagrophytes* were the main causative agents of the dermatophytoses reported. *Trichophyton rubrum* was the dominant species in conditions like tinea pedis, tinea corporis, tinea cruris and tinea unguium [30,31]. *Trichophyton tonsuran*, known as the main causative organism of tinea capitis and tinea corporis in Europe and America, was introduced into Japan by athletes in the past decade [32–35]. Recent outbreaks of *T. tonsurans*-tinea capitis and tinea corporis among young athletes have become a major concern for physicians in Japan [36,37]. Against these clinically important dermatophytes (*T. rubrum*, *T. mentagrophytes* and *T. tonsurans*), LLCZ exerted the strongest antifungal activity in comparison with the other five classes of drugs. *T. rubrum* was the most susceptible to LLCZ. A MIC_{GM} was noted which was lower than that of TBF which is known as a strong anti-dermatophytes drug. The results reflect favorably on the clinical efficacy of 1% LLCZ cream for short-term (2 weeks) treatment of tinea pedis involving one application per day [4,5]. Candidiasis caused by *C. albicans* is the second most common fungal skin infection in Japan after dermatophytoses. LLCZ showed potent activity against this organism as well. The MIC of LLCZ, was lower than that of TBF, BTF, LNF and BFZ, but higher than that of KCZ, NCZ, CTZ and MCZ. However, it was sufficient to provide a favorable clinical assessment of the efficacy of 1% LLCZ cream against candidiasis.

Ergosterol is essential to membrane integrity and cell growth in fungi. All of the drugs tested in the present study functioned as ergosterol inhibitors, although the inhibitory point in the biosynthesis pathway and the antifungal properties are different in each class [38]. The azole class that is potent against both *Trichophyton* spp. and *C. albicans* inhibits C14α-lanosterol demethylase in the ergosterol biosynthesis pathway. In contrast to the azole class, the allylamine (TBF), thiocarbamate (LNF) and benzylamine (BTF) classes, which inhibit squalene epoxidase, an early step in the pathway, showed stronger anti-dermatophyte action with lower MICs against *Trichophyton* spp., but less efficacy against *C. albicans*. On the other hand, the morpholine class (AMO), which inhibits both of C14-reductase and C7- C8 isomerase later stages in the pathway, showed the same range of MICs against both of *Trichophyton* spp., and *C. albicans* compared with the azole class. It is noteworthy that LLCZ, though an azole class agent, was extremely potent against dermatophytes while also effective against *C. albicans*. The MIC_{GM} of LLCZ against *T. rubrum, T. mentagrophytes,* and *T. tonsurans* was the lowest among the 5 classes of drugs, while its MIC_{GM} for *C. albicans* fell into the same range as that of the azole class. The typical antifungal property of

Table 1 Antifungal activities of luliconazole and reference drugs against *Trichophyton* spp. as determined by broth microdilution method.

		MIC(μg/mL)									
Species (no. of strain test)		LLCZ	NCZ	CTZ	BFZ	MCZ	KCZ	TBF	LNF	BTF	AMO
T. rubrum	Range	≦0.00012	≦0.0078	0.031	0.0078	0.031	0.016	0.002	0.002	0.0039	0.016
(10)		∼0.00024	∼0.031	∼0.063	∼0.25	∼0.25	∼0.13	∼0.0078	∼0.016	∼0.031	∼0.031
	MIC_{GM}	0.00022	0.017	0.051	0.028	0.073	0.037	0.0037	0.0049	0.010	0.027
	MIC_{50}	0.00024	0.016	0.063	0.016	0.063	0.031	0.0039	0.0039	0.0078	0.031
	MIC_{90}	0.00024	0.031	0.063	0.13	0.13	0.13	0.0078	0.016	0.016	0.031
T. mentagrophytes	Range	0.00049	0.016	0.016	0.13	0.13	0.25	0.002	0.0039	0.0078	0.031
(10)		∼0.002	∼0.13	∼0.5	∼.4	∼2	∼1	∼0.031	∼0.063	∼0.063	∼0.25
	MIC_{GM}	0.0011	0.055	0.090	0.47	0.66	0.5	0.01	0.019	0.027	0.14
	MIC_{50}	0.00098	0.063	0.13	0.5	1	0.5	0.016	0.031	0.031	0.13
	MIC_{90}	0.002	0.13	0.25	1	2	1	0.016	0.031	0.063	0.25
T. tonsurans	Range	0.00024	0.031	0.13	0.063	0.13	0.13	≦0.00098	0.002	0.002	0.031
(10)		∼0.00049	∼0.13	∼0.25	∼ 1	∼ 1	∼ 1	∼0.016	∼0.016	∼0.031	∼0.13
	MIC_{GM}	0.00037	0.051	0.15	0.27	0.41	0.27	0.0030	0.0042	0.0068	0.059
	MIC_{50}	0.00049	0.063	0.13	0.25	0.5	0.25	0.002	0.0039	0.0039	0.063
	MIC_{50}	0.00049	0.063	0.25	1	1	0.5	0.0078	0.016	0.031	0.063
Trichophyton spp.	Range	≦0.00012	≦0.0078	0.016	0.0078	0.031	0.016	≦0.00098	0.002	0.002	0.016
(30)		∼0.002	∼0.13	∼0.5	∼.4	∼2	∼1	∼0.031	∼0.063	∼0.063	∼0.25
	MIC_{gm}	0.00044	0.036	0.088	0.15	0.27	0.17	0.0049	0.0073	0.012	0.06
	MIC_{90}	0.00049	0.031	0.13	0.13	0.25	0.25	0.0039	0.0039	0.0078	0.063
	MIC_{90}	0.002	0.13	0.25	1	1	1	0.016	0.031	0.031	0.25

LLCZ: luliconazole, NCZ: neticonazole hydrochloride, CTZ: clotrimazole, BFZ: bifonazole, MCZ: miconazole nitratete, KCZ: ketoconasole, TBF: terbinafine hydrochloride, LNF: liranaftate, BTF: butenafme hydrochloride and AMF: amorolfme hydrochloride.

Table 2 Antifungal activities of luliconazole and reference drugs against *Candida albicans* as determined by broth microdilution method.

Species (no. of strain test)		MIC(μg/mL)									
		LLCZ	NCZ	CTZ	BFZ	MCZ	KCZ	TBF	LNF	BTF	AMO
C. albicans (10)	Range	0.031 ~0.13	0.016 ~0.063	≦0.0039 ~0.031	0.25 ~1	0.016 ~0.063	≦0.002 ~0.016	2 ~8	>16	>16	0.063 ~4
	MICGM	0.055	0.039	0.012	0.71	0.029	0.006	3.2	32	32	0.23
	MIC_{50}	0.063	0.031	0.016	1	0.031	0.0039	4	>16	>16	0.25
	MIC_{90}	0.13	0.063	0.016	1	0.063	0.0078	4	>16	>16	2

LLCZ: luliconazole, NCZ: neticonazole hydrochloride, CTZ: clotrimazole, BFZ: bifonazole, MCZ: miconazole nitratete, KCZ: ketocunazole, TBF: terbinafine hydrochloride, LNF: liranaftate, BTF: butenafine hydrochloride and AMF: amorolfine hydrochloride.

LLCZ might be due to its unique chemical structure (Fig. 1) augmented by the introduction of an imidazole moiety into a ketene dithioacetate structure [39]. The ketene dithioacetate structure is responsible for a variety of bioactivities, as attested by the fact that chemicals derived from this structure have been used to treat rice blast disease [40] and certain liver conditions in humans [41] and livestock [42].

Seborrhoeic dermatitis is a common and chronic inflammatory disorder occurring in areas of the skin rich in sebaceous glands, such as the eyebrows, eye-lids and the nose. Although the etiology of SD seems to involve multiple factors including the genetic, environmental and/or hormonal, the role of the lipophilic yeast, *Malassezia,* in its pathogenesis has been strongly corroborated by the efficacy of antifungal drugs against this disease [15–19,43]. As *M. restricta* has been considered a pathogenic factor of SD [24,25], susceptibility of *M. restricta* to LLCZ was compared with that of KCZ which is the only drug clinically available for SD treatment in Japan [18–20]. The anti-*Malassezia* activity of LLCZ has been documented [29] and this compound has been used clinically to treat *Malassezia* infections, such as pityriasis versicolor. However, susceptibility of *M. restricta* in the new taxonomy of the species has not been determined. LLCZ showed activity comparable to or stronger than that of KCZ against *M. restricta*. These results underscore the utility of LLCZ for the management of SD.

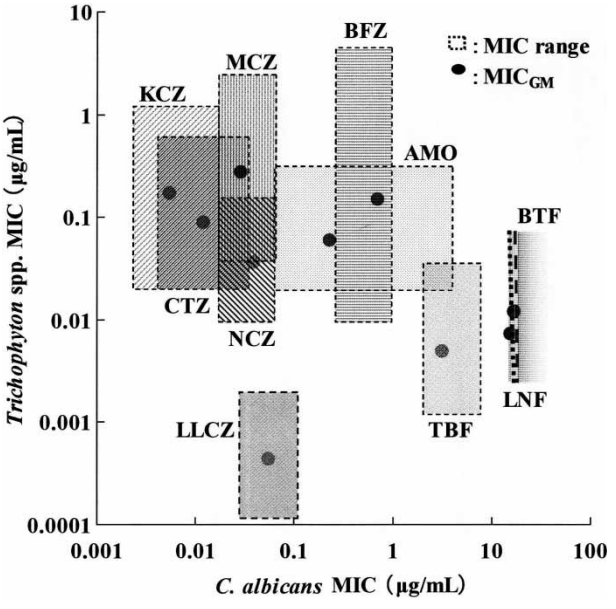

Fig. 2 Comparison of antifungal activities of luliconazole and reference drugs against *Trichophyton* spp. and *Candida albicans.* LLCZ, luliconazole; NCZ, neticonazole; CTZ, clotrimazole; BFZ, bifonazole; MCZ, miconazole nitratete; KCZ, ketoconazole; TBF, terbinafine hydrochloride; LNF, lilanaftate; BTF, butenafine hydrochloride; AMF, amorolfine hydrochloride.

In conclusion, LLCZ is a potent antifungal drug for dermatomycotic fungi. The *in vitro* antifungal potency of LLCZ, because of its extremely strong anti-dermatophytic properties, is different from those of other azoles. The MICs of LLCZ against *T. rubrum, T. mentagrophytes*, and *T. tonsurans* were lowest among the representative drugs clinically available in Japan. Furthermore, LLCZ demonstrates high *in vitro* potency against *M. restricta,* an important pathogenic factor in seborrheic dermatitis. These results underscore the clinical utility of luliconazole as a potent, broad-spectrum antimycotic agent.

Table 3 Antifungal activities of luliconazole and ketoconazole against *Malassezia restricta* as determined by agar dilution method.

Species (no. of strain test)		MIC (μg/mL)	
		LLCZ	KCZ
M. restricta (10)	Range	0.004 ~0.016	0.008 ~0.016
	MICGM	0.008	0.013
	MIC_{50}	0.008	0.016
	MIC_{90}	0.008	0.016

LLCZ: luliconazole and KCZ: ketoconazole.

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References

1 Koga H, Tsuji Y, Inoue K, *et al*. *In vitro* antifungal activity of luliconazole against clinical isolates from patients with dermatomycoses. *J Infect Chemother* 2006; **12**: 163–165.
2 Uchida K, Nishiyama Y, Yamaguchi H. *In vitro* antifungal activity of luliconazole (NND-502), a novel imidazole antifungal agent. *J Infect Chemother* 2004; **10**: 216–219.
3 Niwano Y, Kuzuhara N, Kodama H, *et al*. *In vitro* and *in vitro* antidermatophyte activities of NND-502, a novel optically active imidazole antimycotic agent. *Antimicrob Agents and Chemother* 1998; **42**: 967–970.
4 Watanabe S, Takahashi H, Nishikawa T, *et al*. Dose-finding comparative study of 2 weeks of luliconazole cream treatment for tinea pedis – comparison between three groups (1%, 0.5%, 0.1%) by a multi-center randomized double-blind study. *Mycoses* 2007; **50**: 35–40.
5 Watanabe S, Takahashi H, Nishikawa T, *et al*. A comparative clinical study between 2 weeks of luliconazole 1% cream treatment and 2 weeks of bifonazole 1% cream treatment for tinea pedis. *Mycoses* 2006; **49**: 236–241.
6 Uchida K, Yamaguchi H. Status and issues of preclinical evaluation of the therapeutic effects of newly developed antifungal agents. *Jpn J Med Mycol* 2004; **45**: 83–91.
7 Brennan B, Leyden J. Overview of topical therapy for common superficial fungal infections and the role of new topical agents. *J Am Acad Dermatol* 1997; **36**: s3–s8.
8 Rex JH, Pfller MA, Rinaldi MG, Polak A, Galgiani JN. Antifungal susceptibility testing: *Clin Microbiol Rev* 1993; **6**: 367–381.
9 Clinical Laboratory Standards Institute/National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi*. Approved Standard M38-A. Wayne, PA: National Committee for Clinical Laboratory Standards, 2002.
10 Standardization Committee of Japanese Society of Medical Mycology. Report of the Standardization Committee of Japanese Society of Medical Mycology 1995–1997. *Jpn J Med Mycol* 1999; **40**: 239–257.
11 Clinical Laboratory Standards Institute/National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing of yeasts.* Approved Standard M27-A. Wayne, PA: National Committee for Clinical Laboratory Standards, 1997.
12 Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000; **38**: 337–341.
13 Ashbee HR, Evans EG. Immunology of diseases associated with Malassezia species. *Clin Microbiol Rev* 2002; **15**: 21–57.
14 Midgley G. The lipophilic yeasts: state of the art and prospects. *Med Mycol* 2000; **38**(Suppl. I): 9–16.
15 Sei Y, Hamguti T, Ninomiya J, Nakabayasgi A, Takiuchi I. Seborrhoeic dermatitis: treatment with anti-mycotic agents. *J Dermatol* 1994; **21**: 334–340.
16 Segal R, David M, Ingber A, Lurie R, Sandbank M. Treatment with bifonazole shampoo for seborrhoeic dermatitis: a randomized, double-blind study. *Acta Derm Venereol* 1992; **72**: 454–455.
17 Faergemann J. Seborrhoeic dermatitis and *Pityrosporum orbiculare*: treatment of seborrhoeic dermatitis of the scalp with miconazole and hydrocortisone. *Br J Dermatol* 1986; **114**: 695–700.
18 Skinner RB, Noah PW, Taylor RM, *et al*. Double-blind treatment of seborrheic dermatitis with 2% ketoconazole cream. *J Am Acad Dermatol* 1985; **12**: 852–856.
19 Katsambas A, Antoniou C, Frangouli E, *et al*. A double-blind trial of treatment of seborrheic dermatitis with 2% ketoconazole cream compared with 1% hydrocortisone cream. *Br J Dermatol* 1989; **121**: 353–357.
20 Hammer KA, Carson CF, Riley TV. *In vitro* activities of ketoconazole, econazole, miconazole and *Melaleuca alternifolia* (tea tree) oil against *Malassezia* species. *Antimicrob Agents Chemother* 2000; **44**: 467–469.
21 Gueho E, Midgley G, Guillot J. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek* 1996; **69**: 337–355.
22 Hirai A, Kano R, Makimura K, *et al*. *Malassezia nana* sp. nov., a novel lipid-dependent yeast species isolated from animals. *Int J Syst Evol Microbiol* 2004; **54**: 623–627.
23 Sugita T, Takashima M, Shinoda H, *et al*. New yeast species, *Malassezia* dermatis isolated from patient with atopic dermatitis. *J Clin Microbiol* 2002; **40**: 1363–1367.
24 Tajima M, Sugita T, Nishikawa A, Tsuboi R. Molecular analysis of *Malassezia* microflora in seborrheic dermatitis patients: Comparison with other diseases and healthy subjects. *J Invest Dermatol* 2008; **128**: 345–351.
25 Sugita T, Tajima M, Ito T, *et al*. Antifungal activities of tacrolimus and azole agents against the eleven currently accepted *Malassezia* species. *J Clin Microbiol* 2005; **43**: 2824–2829.
26 Makimura K, Tamura Y, Mochizuki T, *et al*. Phylogenetic classification and species identification of dermatophytes strains based on DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J Clin Microbiol* 1999; **37**: 920–924.
27 Makimura K, Tamura Y, Kudo M, *et al*. Species identification and strain typing of *Malassezia* species stock strains and clinical isolates based on the DNA sequences of nuclear ribosomal internal transcribed spacer 1 regions. *J Med Microbiol* 2000; **49**: 29–35.
28 Pfaller MA, Buschelman B, Bale MJ *et al*. Multicenter comparison of a colorimetric microdilution broth method with the reference macrodilution method for *in vitro* susceptibility testing of yeast isolates. *Microbiol Infect Dis* 1994; **19**: 9–13.
29 Uchida K, Nishiyama Y, Tanaka T, Yamaguchi H. *In vitro* activity of novel imidazole antifungal agent NND-502 against *Malassezia* species. *Int J Antimicrobial Agents* 2003; **21**: 234–238.
30 Committee for Epidemiology, The Japanese Society for Medical Mycology. An epidemiological survey of dermatomycoses in Japan, 2002. *Jpn J Med Mycol* 2006; **47**: 103–111.
31 Odom RB. Updated on topical therapy for superficial fungal infections: focus on butenafine. *J Am Acad Dermatol* 1997; **36**: S1–2.
32 Chan YC, Friedlander SF. New treatments for tinea captis. *Curr Opin Infect Dis* 2004; **17**: 97–103.
33 Adams BB. Tinea corporis gladiatorum. *J Am Acad Dermatol* 2002; **47**: 286–290.

34 Gupta AK, Summerbell RC. Tinea capitis. *Med Mycol* 2000; **38**: 255–287.
35 Aly R, Hay RJ, Del Palacio A, Galimbertis R. Epidemiology of tinea captis. *Med Mycol* 2000; **38**: 183–188.
36 Mochizuki T, Kawasaki M, Tanabe H, *et al*. Molecular epidemiology of *Trichophyton tonsurans* isolated in Japan using RFLP analysis of non-transcribed spacer regions of ribosomal RNA genes. *Jpn J Infect Dis* 2007; **60**: 188–192.
37 Kasai T. Epidemiological survey of *Trichophyton tonsurans* infection in Tohoku district and its clinical problems. *Jpn J Med Mycol* 2005; **46**: 87–91.
38 Gupta AK, Einarson TR, Summerbell RC, Shear NH. An over view of topical antifungal therapy in dermatomycoses. *Drugs* 1998; **55**: 645–674.
39 Niwano Y, Ohmi T, Seo A, *et al*. Lanoconazole and its related optically active compound NND-502: novel antifungal imidazoles with a ketene dithioacetal structure. *Curr Med Chem-Anti-Infective Agents* 2003; **2**: 147–160.
40 Nakamura H. Isoprothiolane, a new systemic pesticide for the control of rice blast and plant hoppers. *Rev Plant Prot Res* 1977; **10**: 1–5.
41 Takase S, Takada A, Yasuhara M, Sato H, Matsuda Y. Effects of malotilate treatment on the serum markers of hepatic fibrogenesis in liver cirrhosis. *Gastroenterol Jpn* 1988; **23**: 639–645.
42 Oka A, Yamasaki T, Shibatani M, Suzuki T, Saito T. Efficacy of isoprothiolane for the treatment of fat necrosis in cattle. *Br Vet J* 1988; **144**: 507–514.
43 Ro BI, Dawson TL. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. *J Invest Dermatol Symp Proc* 2005; **10**: 194–197.

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