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ABSTRACTS
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CONCLUSIONS

A new triazole, KP-103 had potent activity against *C. albicans* and moderate activity against dermatophytes. The anti-*Candida* activity of KP-103 was higher than that of all the reference drugs, CTZ, NCZ, LCZ and BTF. On the other hand, the anti-*Trichophyton* activity of KP-103 was equal to or higher than that of CTZ and NCZ but lower than that of LCZ and BTF.

The anti-*Trichophyton* activities of the reference drugs were substantially reduced when cultures were grown in serum-supplemented medium or hair suspension, but the activity of KP-103 was less affected. KP-103 exhibited fungicidal activity comparable to LCZ and BTF against *T. mentagrophytes* when cultures were grown on the excised human horny layer. KP-103 has low affinity with keratin as compared with LCZ and BTF. These biological characteristics of KP-103 might be reflected by its favorable *in vivo* efficacies.

In summary, KP-103 is active against a wide variety of pathogenic fungi including yeasts, dermatophytes, and *Aspergillus* spp. Since it has a low affinity with the horny layer of the skin, its antifungal activity seems well kept in this tissue.

Abstract No. F792

In vitro Activity of KP-103, a Novel Topical Antifungal Triazole.

Y. Tatsumi, M. Yokoo, T. Arika, H. Ogura, K. Nagai, and T. Naito.
Development Research Laboratories, Kaken Pharmaceutical Co., Ltd., Kyoto, Japan.
H. Yamaguchi, Teikyo Univ., Tokyo, Japan.

ABSTRACT

The *in vitro* activity of KP-103, a triazole having 4-methylenepiperidine moiety at the C-3 position, was compared with that of clotrimazole (CTZ), neticonazole (NCZ), itraconazole (LCZ), and butenafine (BTF) against pathogenic fungi. MIC₈₀ values (μg/ml) were shown below.

Fungi (No. of strains)	MIC ₈₀ (μg/ml)					Media ^a
	KP-103	CTZ	NCZ	LCZ	BTF	
<i>C. albicans</i> (44)	0.002	0.0313	0.0625	0.25	>8.0	A
<i>M. furfur</i> (6)	0.025	6.25	3.13	0.78	12.5	C
<i>Aspergillus</i> spp. (15)	0.0625	2.0	0.25	0.002	0.25	A
<i>T. rubrum</i> (39)	0.125	0.5	0.125	0.0078	0.0078	B
<i>T. mentagrophytes</i> (28)	0.25	0.25	0.25	0.0313	0.0156	B

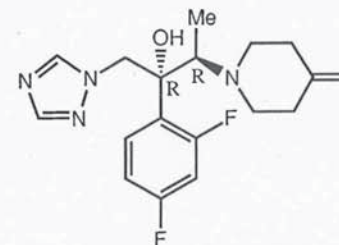
^a A, 0.165 M MOPS-buffered RPMI 1640 medium, pH 7.0; B, Sabouraud dextrose broth; C, medium C (Faergemann, J. et al. Acta Derm. Venereol. Suppl 86: 1-23, 1979).

KP-103 was the most active against *C. albicans* and *M. furfur* among the tested drugs. Its activity against *Trichophyton* spp. was almost equal to those of CTZ and NCZ, but was weaker than those of LCZ and BTF.

Anti-*T. mentagrophytes* activities of the reference drugs were reduced by the addition of human serum and horny materials as reported, while that of KP-103 was not affected. Furthermore, anti-*T. mentagrophytes* activity of KP-103 on the stripped human horny layer was equal to those of LCZ and BTF. These results reflected *in vivo* efficacies.

In summary, KP-103 has a broad antifungal spectrum and could keep a high activity in the horny layer where fungi reside.

Figure 1. Chemical structure of KP-103



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For further information, please contact the following.

TAIRA OKAMOTO
Manager
International Operation and Licensing Department
KAKEN PHARMACEUTICAL CO., LTD.

HINODE 1, URAYASU-SHI, CHIBA, 279, JAPAN
PHONE: 81-473-90-6140
FAX : 81-473-90-6161

INTRODUCTION

Various kinds of topical antifungal drugs, such as imidazole conazole, ketoconazole, and lanocanazole, allylamine nylamine (butenafine), and morpholine (amorolfine) eloped and introduced into the market. These are for the once-a-day treatment of dermatomycosis. These antifungal drugs have relatively broad spectra of activity in the extent of their activities against different fungi. Butenafine and butenafine are less active against yeast-like more active against dermatophytes than are most

only two triazole antifungal agents, fluconazole and which are used systemically for the treatment of both and superficial mycosis, but not used topically. novel topical triazole having a methylenepiperazine at (Figure 1). In this study, we examined the *in vitro* activity of KP-103 in comparison with that of the reference clotrimazole (CTZ), neticonazole (NCZ), lanocanazole (LCZ), and butenafine (BTF).

MATERIALS AND METHODS

Straining procedures.

Cryptococcus spp. determined by the NCCLS-based microdilution method [1], used was RPMI 1640 medium adjusted to pH 7.0 with NaOH. The test organisms were grown on yeast morphology medium at 37°C for 2 or 3 days. The colonies were suspended in sterile distilled water to a turbidity of a 0.5 McFarland standard. The suspension was diluted 100-fold for *Candida* spp. and 10-fold for *Cryptococcus* spp. with RPMI 1640 medium. Aliquots of 0.1 ml of the suspension were dispensed into the wells containing 0.1 ml of two-fold dilutions (final inoculum size: *Candida* spp.; 0.5–2.5x10³ cells/ml; *Cryptococcus* spp.; 0.5–2.5x10⁴ cells/ml) and all microplates were incubated for 1–2 days (*Candida* spp.) and 3 days (*Cryptococcus* spp.)

determined by the microdilution method using RPMI 1640 medium. The test organisms were grown on potato dextrose agar (PDA) for 10 days. Final inoculum size was 1x10⁴ conidia/ml. All microplates were incubated at 30°C for 3 days.

determined by the microdilution method using Sabouraud dextrose agar (SDB). The test organisms were grown on slants of yeast-extract agar and Sabouraud dextrose agar (SDA) for 10 days. Final inoculum size was 1x10⁴ conidia/ml or 1x10⁴ hyphae/ml. All microplates were incubated at 30°C for 7 days.

determined by the agar dilution method. Test organisms were grown on PDA containing 1% yeast-extract, 1% peptone, and 1% glucose for 5 days. Five microliters (1x10⁴ cells) of the suspension were spotted on medium C₂ plates containing two-fold dilutions of the suspension. All plates were incubated at 30°C for 5 days.

Minimum inhibitory and fungicidal concentrations (MICs)

Minimum inhibitory concentrations (MICs) were defined as the lowest drug concentration that inhibited visible growth of the test organisms.

Minimum fungicidal concentrations (MFCs) were defined as the lowest drug concentration that produced > 99% reduction of the final inoculum.

Anti-T. mentagrophytes activity. The anti-T. mentagrophytes activity was determined by the microdilution method using Sabouraud dextrose agar (SDB) containing 10% human hair suspension in saline. MFCs were determined by the agar dilution method. MFCs were determined by the agar dilution method. MFCs were determined by the agar dilution method.

Activity on horny layer. The activity on horny layer was determined by the agar dilution method. MFCs were determined by the agar dilution method. MFCs were determined by the agar dilution method.

Affinity with keratin.

Aliquot of 0.1 ml of each drug solution (1 mg/ml) was dispensed into 9.9 ml of 5% keratin suspension in saline to give a final concentration of 10 µg/ml. Each tube was incubated at 37°C for 1 h with shaking. After incubation, the mixture was centrifuged and two 150 µl portions of the supernatant were taken to determine the adsorption rate of drug to keratin. The drug-bound keratin was washed 10 times by shaking in saline at 37°C for 10 min. After each wash, the mixture was centrifuged and two 150 µl portions of the supernatant were taken to determine the release rate of drug from keratin. The drug concentration in the supernatant was determined by the conventional agar-well diffusion assay using *A. niger* for LCZ, *T. mentagrophytes* for BTF, and *C. kelyr* for KP-103 as the test organism.

Figure 2. Chemical structures of reference drugs

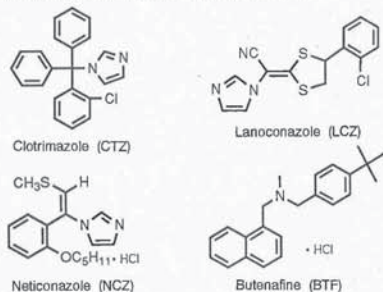


Table 1. Antifungal activity of KP-103 against C. albicans, T. rubrum, and T. mentagrophytes

Organisms (No. of strains)	Compounds	MIC (µg/ml)		
		Range	50 %	80 %
<i>C. albicans</i> (44)	KP-103	0.0005–0.0156	0.002	0.002
	CTZ	0.0078–0.25	0.0156	0.0313
	NCZ	0.0313–>1.0	0.0625	0.0625
	LCZ	0.0313–>1.0	0.125	0.25
	BTF	>8.0	>8.0	>8.0
<i>T. rubrum</i> (39)	KP-103	0.0156–0.5	0.0625	0.125
	CTZ	0.0625–1.0	0.25	0.5
	NCZ	0.0156–0.5	0.0625	0.125
	LCZ	0.0005–0.0313	0.0039	0.0078
	BTF	0.0039–0.0156	0.0039	0.0078
<i>T. mentagrophytes</i> (28)	KP-103	0.0625–0.5	0.25	0.25
	CTZ	0.125–0.25	0.25	0.25
	NCZ	0.0313–0.25	0.125	0.25
	LCZ	0.001–0.0625	0.0156	0.0313
	BTF	0.0039–0.0156	0.0078	0.0156

Table 2. Fungicidal activity of KP-103 against T. rubrum and T. mentagrophytes

Organisms (No. of strains)	Compounds	MIC (µg/ml)		
		Range	50 %	80 %
<i>T. rubrum</i> (39)	KP-103	0.0156–1.0	0.125	0.25
	CTZ	0.0625–2.0	0.25	0.5
	NCZ	0.0156–1.0	0.25	0.25
	LCZ	0.0005–0.0625	0.0078	0.0313
	BTF	0.0039–0.0156	0.0078	0.0078
<i>T. mentagrophytes</i> (28)	KP-103	0.125–1.0	0.25	0.25
	CTZ	0.125–0.5	0.25	0.5
	NCZ	0.0625–1.0	0.25	0.25
	LCZ	0.0039–0.0625	0.0313	0.0313
	BTF	0.0039–0.0313	0.0078	0.0156

Table 3. Antifungal activity of KP-103 against various pathogenic fungi

Organisms (No. of strains)	Geometric mean MIC (µg/ml)				
	KP-103	CTZ	NCZ	LCZ	BTF
<i>C. tropicalis</i> (4)	0.0157	0.1249	0.2973	0.3536	>2.3784
<i>C. krusei</i> (2)	0.0442	0.125	0.25	1.4142	1.4142
<i>C. parapsilosis</i> (3)	0.0197	0.125	1.2599	1.5874	>2.5198
<i>C. guilliermondii</i> (1)	0.0039	0.0625	0.5	0.25	4.0
<i>C. stellatoidea</i> (1)	0.0313	0.125	0.25	0.125	0.5
<i>C. utilis</i> (1)	0.0313	0.125	0.25	0.0625	0.25
<i>C. glabrata</i> (6)	0.0124	0.2227	0.0156	0.0197	>4.0
<i>C. neoformans</i> (4)	0.0039	0.1768	0.3536	0.25	0.5
<i>C. laurentii</i> (1)	0.0625	0.5	1.0	0.5	>4.0
<i>M. furfur</i> (6)	0.025	3.9415	2.4816	0.6191	9.9213
<i>M. pachydermatis</i> (2)	<0.006	1.56	0.78	0.1	1.1031
<i>T. violaceum</i> (2)	0.0156	0.0884	0.0221	0.0014	0.0039
<i>T. ajelloi</i> (1)	0.0313	0.125	0.0625	0.0078	0.0078
<i>M. canis</i> (1)	0.0313	0.25	0.0625	0.0078	0.0078
<i>M. gypseum</i> (2)	0.0422	0.1768	0.0625	0.0028	0.0078
<i>E. floccosum</i> (1)	0.0078	0.0625	0.0156	0.001	0.0078
<i>A. fumigatus</i> (3)	0.0496	1.2599	0.1984	0.0010	0.1984
<i>A. flavus</i> (5)	0.0413	0.6598	0.25	0.0011	0.0825
<i>A. niger</i> (4)	0.0625	2.0	0.3536	0.0024	0.1768
<i>A. terreus</i> (2)	0.0625	2.0	0.3536	0.0020	0.1768
<i>A. nidulans</i> (1)	0.125	2.0	0.25	0.001	0.5

Table 4. Effect of human serum on anti-T. mentagrophytes activity of KP-103

Organisms (No. of strains)	Compounds	Geometric mean MIC (µg/ml)		
		SDB	SDB with 10% serum	
<i>T. mentagrophytes</i> (8)	KP-103	0.1487	0.1621(x1)	
	CTZ	0.1051	0.5946(x6)	
	NCZ	0.1363	0.3536(x3)	
	LCZ	0.0066	0.1768(x27)	
	BTF	0.0078	0.0442(x6)	

Table 5. Effect of human hair on anti-T. mentagrophytes activity of KP-103

Compounds	SDB		5% hair suspension	
	MIC(µg/ml)	MFC(µg/ml)	MIC(µg/ml)	MFC(µg/ml)
KP-103	0.2	0.2	0.2 (x1)	0.39(x2)
CTZ	0.2	0.39	6.25(x32)	12.5 (x32)
NCZ	0.1	0.1	1.56(x16)	3.13(x32)
LCZ	0.006	0.025	0.1 (x16)	0.39(x16)
BTF	0.006	0.006	0.2 (x32)	0.39(x64)

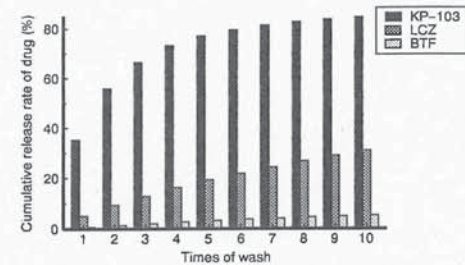
Table 6. Fungicidal activity of KP-103 against C. albicans and T. mentagrophytes grown on human horny layer.

Organisms	Compounds	MFC (%)				
		Human horny layer pieces				
		No.1	No.2	No.3	No.4	No.5
<i>C. albicans</i> KC-36	KP-103	0.008	0.008	0.008	0.008	0.008
	CTZ	>1.0	>1.0	0.5	>1.0	0.5
	NCZ	>1.0	>1.0	>1.0	>1.0	>1.0
	LCZ	>1.0	>1.0	>1.0	>1.0	>1.0
	BTF	>1.0	>1.0	>1.0	>1.0	>1.0
<i>T. menta.</i> KD-04	KP-103	0.016	0.016	0.016	0.016	0.008
	CTZ	>1.0	>1.0	>1.0	>1.0	0.5
	NCZ	0.25	0.25	1.25	0.5	0.125
	LCZ	0.016	0.016	0.016	0.032	0.008
	BTF	0.016	0.016	0.008	0.016	0.008

Table 7. The rate of adsorption of KP-103 to keratin

Drug / keratin ratio (w/w) in the incubation mixture	Adsorption rate (% of)		
	KP-103	LCZ	BTF
1:5000	60.3	94.9	95.7

Figure 3. The rate of release of KP-103 from the drug-bound keratin



RESULTS

1) Against *C. albicans*, CTZ, NCZ, LCZ, and BTF were 16-, 32-, 126-, >4096-fold, respectively, less active than KP-103 (Table 1).

2) Against *T. rubrum*, KP-103 was 16-fold less active than LCZ and BTF but as active as NCZ and twofold more active than CTZ.

Against *T. mentagrophytes*, KP-103 was 8- to 16-fold less active than LCZ and BTF but as active as CTZ and NCZ (Table 1).

3) The MFC₅₀ value of KP-103 was equal to or two times higher than its MIC₅₀ value for *Trichophyton* spp., which indicates that KP-103 was fungicidal against this fungi (Tables 1 and 2).

4) Against *Candida*, *Cryptococcus*, and *Malassezia* spp. KP-103 was the most active among the tested drugs (Table 3).

5) Against *Aspergillus* spp. KP-103 was less active than LCZ but more active than CTZ, NCZ, and BTF (Table 3).

6) Against dermatophytes other than *T. rubrum* and *T. mentagrophytes*, KP-103 was less active LCZ and BTF but more active than CTZ and NCZ (Table 3).

7) The anti-*T. mentagrophytes* activities of the reference drugs were 3- to 27-fold reduced by the addition 10% human serum to the assay medium, but the activity of KP-103 was little affected (Table 4).

8) The anti-*T. mentagrophytes* activities of the reference drugs in SDB were 16- to 32-fold more lower than those of the reference drugs in 5% human hair suspension in saline, but the activity of KP-103 was the same in both media (Table 5).

9) KP-103 had fungicidal activity against *C. albicans* and *T. mentagrophytes* grown on human horny layer. Among the drugs tested, KP-103 was the most active against *C. albicans*. Against *T. mentagrophytes*, the activity of KP-103 was stronger than that of CTZ and NCZ and comparable to that of LCZ and BTF (Table 6).

10) KP-103 showed low adsorption to keratin and high release from keratin as compared with LCZ and BTF (Table 7 and Figure 3).

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