ICAAC

ABSTRACTS of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy

an Annual Meeting
of the American Society for Microbiology

September 15–18, 1996

Ernest N. Morial Convention Center
New Orleans, Louisiana

Amercan Society for Microbiology
Washington, D.C.

Printed in the United States of America
ISBN 1-55581-113-2

CONCLUSIONS

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

The anti-*Trichophyton* activities of the reference drugs were substantially duced when cultures were grown in serum-supplemented medium or hair spension, but the activity of KP-103 was less affected. KP-103 exhibited ngicidal activity comparable to LCZ and BTF against *T. mentagrophytes* nen cultures were grown on the excised human horny layer. KP-103 has low affinity with keratin as compared with LCZ and BTF. These biological aracteristics 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 cluding yeasts, dermatophytes, and *Aspergillus* spp. Since it has a low inity with the horny layer of the skin, its antifungal activity seems well kept this tissue.

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

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), lanoconazole (LCZ), and butenafine (BTF) against pathogenic fungi. MIC_{80} values (μg/ml) were shown below.

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

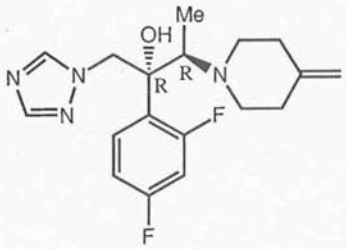
[a] A, 0.165 M MOPS-buffered RPMI 1640 medium, pH 7.0; B, Sabouraud dextrose broth; C, medium C (Faergemann, J. et al. Acta Derm. Venereol. Suppl 86: 1-23, 1979).

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. mentagropytes* 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



36th Interscience Conference on Antimicrobial Agents and Chemotherapy
New Orleans, Louisiana
15-18 September, 1996

INTRODUCTION

ral kinds of topical antifungal drugs, such as imidazoles conazole, ketoconazole, and lanoconazole), allylamine nzylamine (butenafine), and morpholine (amorolfine) eloped and introduced into the market. These are for the once-a-day treatment of dermatomycosis. hese antifungal drugs have relatively broad spectra of y in the extent of their activities against different fungi. binafine and butenafine are less active against yeast- more active against dermatophytes than are most

nly two triazole antifungal agents, fluconazole and ich are used systemically for the treatment of both nd superficial mycosis, but not used topically.

novel topical triazole having a methylenepiperizine at (Figure 1). In this study, we examined the *in vitro* of KP-103 in comparison with that of the reference clotrimazole (CTZ), neticonazole (NCZ), lanoconazole hafine (BTF).

MATERIALS AND METHODS

sting procedures.

Cryptococcus spp.

etermined by the NCCLS-based microdilution method [1]. used was RPMI 1640 medium adjusted to pH 7.0 with The test organisms were grown on yeast morphology 5°C for 2 or 3 days. The colonies were suspended in the turbidity of a 0.5 McFarland standard. The e diluted 100-fold for *Candida* spp. and 10-fold for . with RPMI 1640 medium. Aliquots of 0.1 ml of the dispensed into the wells containing 0.1 ml of two-fold ons (final inoculum size: *Candida* spp.; 0.5-2.5x10^3 *ccus* spp.; 0.5-2.5x10^4 cells/ml and all microplates were for 1-2 days (*Candida* spp.) and 3 days (*Cryptococcus*

etermined by the microdilution method using RPMI 1640 organisms were grown on potato dextrose agar (PDA) 10 days. Final inoculum size was 1x10^4 conidia/ml. All incubated at 30°C for 3 days.

etermined by the microdilution method using Sabouraud SDB). The test organisms were grown on slants of yeast-extract agar and Sabouraud dextrose agar (SDA) culum size was 1x10^4 conidia/ml or 1x10^4 hyphae/ml. All incubated at 30°C for 7 days.

etermined by the agar dilution method. Test organisms ants of PDA containing 1% yeast-extract, 1% peptone, at 30°C for 5 days. Five microliters (1x10^4 cells) of the were spotted on medium C [2] plates containing two-fold d all plates were incubated at 30°C for 5 days.

imum inhibitory and fungicidal concentrations (MICs

dida and *Cryptococcus* spp. were defined as the lowest inhibiting ≥ 80 % of growth compared with the growth *Malassezia* spp., dermatophytes, and *Aspergillus* spp. he lowest drug concentration that inhibited visible growth

matophytes were determined by subculture of 10 μl of te at 30°C for 7 days and were defined as the lowest that produced ≥ 98% reduction of the final inoculum.

and hair on anti-*T. mentagrophytes* activity.

nentagrophytes were determined by the microdilution ee tested media (SDB, SDB containing 10% human uman hair suspension in saline). MFCs were determined air on SDA plate at 30°C for 7 days and defined as the ntration that completely prevented visible growth of fungi.

y on horny layer.

f the skin were stripped from arms of five healthy male llophane tapes. The horny layer tapes were cut, put on cubated at 30°C for 24 h. Twenty microliter of two-fold ons was applied on each horny layer and spread. was wipe off. After each slide glass was incubated at for 4 h, 5 μl (5x10^3 cells) of the fungal suspension was orny layer. Each slide glass was incubated at 30°C for re determined by subculture of tape on SDA plate at and defined as the lowest drug concentration that ted visible growth of fungi.

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 150μl 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. kefyr* for KP-103 as the test organism.

Figure 2. Chemical structures of reference drugs

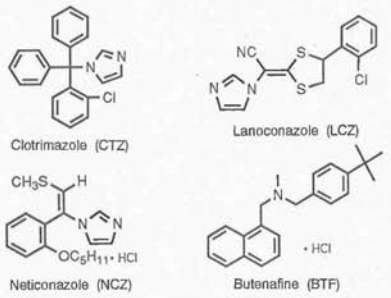


Table 1. Antifungal activity of KP-103 against *C. albicans*, *T. rubrum*, and *T. mentagrophytes*

Organisms (No. of strains)	Compounds	MIC (μg/ml) Range	50 %	80%
C. albicans (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
T. rubrum (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
T. mentagrophytes (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 %
T. rubrum (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
T. mentagrophytes (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
C. tropicalis (4)	**0.0157**	0.1249	0.2973	0.3536	>2.3784
C. krusei (2)	**0.0442**	0.125	0.25	1.4142	1.4142
C. parapsilosis (3)	**0.0197**	0.125	1.2599	1.5874	>2.5198
C. guilliermondii (1)	**0.0039**	0.0625	0.5	0.25	4.0
C. stellatoidea (1)	**0.0313**	0.125	0.25	0.125	0.5
C. utilis (1)	**0.0313**	0.125	0.25	0.0625	0.25
C. glabrata (6)	**0.0124**	0.2227	0.0156	0.0197	>4.0
C. neoformance (4)	**0.0039**	0.1768	0.3536	0.25	0.5
C. laurentii (1)	**0.0625**	0.5	1.0	0.5	>4.0
M. furfur (6)	**0.025**	3.9415	2.4816	0.6191	9.9213
M. pachydermatis (2)	**<0.006**	1.56	0.78	0.1	1.1031
T. violaceum (2)	**0.0156**	0.0884	0.0221	0.0014	0.0039
T. ajelloi (1)	**0.0313**	0.125	0.0625	0.0078	0.0078
M. canis (1)	**0.0313**	0.25	0.0625	0.0078	0.0078
M. gypseum (2)	**0.0422**	0.1768	0.0625	0.0028	0.0078
E. floccosum (1)	**0.0078**	0.0625	0.0156	0.001	0.0078
A. fumigatus (3)	**0.0496**	1.2599	0.1984	0.0010	0.1984
A. flavus (5)	**0.0413**	0.6598	0.25	0.0011	0.0825
A. niger (4)	**0.0625**	2.0	0.3536	0.0024	0.1768
A. terreus (2)	**0.0625**	2.0	0.3536	0.0020	0.1768
A. nidulans (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
T. mentagrophytes (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 MIC(μg/ml)	SDB MFC(μg/)ml	5% hair suspension MIC(μg/ml)	5% hair suspension 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)

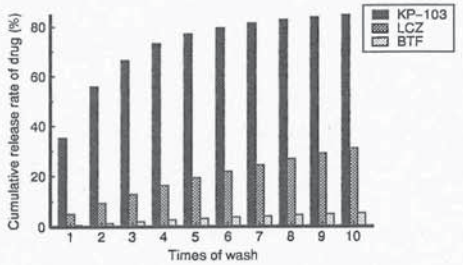
Table 6. Fungicidal activity of KP-103 against *C. albicans* and *T. mentagrophytes* grown on human horny layer.

Organisms	Compounds	MFC (%) Human horny layer pieces No.1	No.2	No.3	No.4	No.5
C. albicans 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
T. menta. 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	0.125	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 MFC_{80} value of KP-103 was equal to or two times higher than its MIC_{80} 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).

REFERENCES

1) Yamaguchi, H., K. Uchida, H. Kume, T. Shinoda, K. Watanabe, T. Kusunoki, M. Hiruma, and H. Ishizaki. 1995. Report of committee of clinical laboratory standards-1994. Jpn. J. Med. Mycol. 36:61-86.

2) Faergemann, J. and S. Bernander. 1979. Tinea versicolor and *Pityrosporum orbiculare*: mycological investigations, experimental infection and epidemiological surveys. Acta Derm. Venereol. Suppl 86: 1-23.