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UTILITY PATENT APPLICATION TRANSMITTAL <small>(Only for new nonprovisional applications under 37 CFR 1.53(b))</small>	Attorney Docket No.	700938-052220-DIV
	First Inventor	Yoshiyuki Tatsumi
	Title	See Addendum
	Express Mail Label No.	EL 934997148 US

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APPLICATION ELEMENTS <small>See MPEP chapter 600 concerning utility patent application contents.</small>	ADDRESS TO: Mail Stop Patent Application Commissioner for Patents P.O. Box 1450 Alexandria VA 22313-1450
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1. <input checked="" type="checkbox"/> Fee Transmittal Form (e.g., PTO/SB/17) <small>(Submit an original and a duplicate for fee processing)</small> 2. <input type="checkbox"/> Applicant claims small entity status. <small>See 37 CFR 1.27.</small> 3. <input checked="" type="checkbox"/> Specification [Total Pages <u>40</u>] <small>(preferred arrangement set forth below)</small> - Descriptive title of the invention - Cross Reference to Related Applications - Statement Regarding Fed sponsored R & D - Reference to sequence listing, a table, or a computer program listing appendix - Background of the Invention - Brief Summary of the Invention - Brief Description of the Drawings (if filed) - Detailed Description - Claim(s) - Abstract of the Disclosure 4. <input checked="" type="checkbox"/> Drawing(s) (35 U.S.C. 113) [Total Sheets <u>4</u>] 5. Oath or Declaration [Total Sheets <u>4</u>] a. <input type="checkbox"/> Newly executed (original or copy) b. <input checked="" type="checkbox"/> Copy from a prior application (37 CFR 1.63(d)) <small>(for continuation/divisional with Box 18 completed)</small> i. <input type="checkbox"/> DELETION OF INVENTOR(S) <small>Signed statement attached deleting inventor(s) name in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).</small> 6. <input checked="" type="checkbox"/> Application Data Sheet. See 37 CFR 1.76	7. <input type="checkbox"/> CD-ROM or CD-R in duplicate, large table or Computer Program (Appendix) 8. Nucleotide and/or Amino Acid Sequence Submission <small>(if applicable, all necessary)</small> a. <input type="checkbox"/> Computer Readable Form (CRF) b. Specification Sequence Listing on: i. <input type="checkbox"/> CD-ROM or CD-R (2 copies); or ii. <input type="checkbox"/> Paper c. <input type="checkbox"/> Statements verifying identity of above copies
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ACCOMPANYING APPLICATION PARTS	
9. <input checked="" type="checkbox"/> Assignment Papers (cover sheet & document(s))	
10. <input type="checkbox"/> 37 CFR 3.73(b) Statement <input type="checkbox"/> Power of Attorney	
11. <input type="checkbox"/> English Translation Document (if applicable)	
12. <input checked="" type="checkbox"/> Information Disclosure Statement (IDS)/PTO-1499	<input checked="" type="checkbox"/> Copies of IDS Citations
13. <input checked="" type="checkbox"/> Preliminary Amendment	
14. <input checked="" type="checkbox"/> Return Receipt Postcard (MPEP 503) <small>(Should be specifically itemized)</small>	
15. <input type="checkbox"/> Certified Copy of Priority Document(s) <small>(if foreign priority is claimed)</small>	
16. <input type="checkbox"/> Nonpublication Request under 35 U.S.C. 122 <small>(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or its equivalent.</small>	
17. <input checked="" type="checkbox"/> Other: Return postcard; check - \$770.00; Certificate of Express Mail (1 pg.)	

18. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in the first sentence of the specification following the title, or in an Application Data Sheet under 37 CFR 1.76:

Continuation
 Divisional
 Continuation-in-part (CIP)
 of prior application No.: 10/031,929

Prior application information: Examiner Srivastava, Kailash C. Art Unit: 1651
 For CONTINUATION OF DIVISIONAL APPS only; The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

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Country		Zip Code	02110
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			617-345-1300

Name (Print/Type)	Ronald I. Eisenstein	Registration No. (Attorney/Agent)	30,628
Signature	<i>Ronald I. Eisenstein</i>	Date	10/14/03

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PTO/SB/17 (08-03)

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FEE TRANSMITTAL for FY 2003 <i>Effective 01/01/2003. Patent fees are subject to annual revision.</i>	Complete if Known	
	Application Number	TBA
	Filing Date	Herewith
	First Named Inventor	Yoshiyuki Tatsumi
	Examiner Name	TBA
	Art Unit	TBA
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27		Attorney Docket No. 700938-052220-DIV
TOTAL AMOUNT OF PAYMENT (\$) (\$) 770.00		

<p>METHOD OF PAYMENT (check all that apply)</p> <p><input checked="" type="checkbox"/> Check <input type="checkbox"/> Credit card <input type="checkbox"/> Money Order <input type="checkbox"/> Other <input type="checkbox"/> None</p> <p><input checked="" type="checkbox"/> Deposit Account:</p> <p>Deposit Account Number: <u>50-0850</u></p> <p>Deposit Account Name: <u>NIXON PEABODY LLP</u></p> <p>The Commissioner is authorized to: (check all that apply)</p> <p><input type="checkbox"/> Charge fee(s) indicated below <input checked="" type="checkbox"/> Credit any overpayments</p> <p><input checked="" type="checkbox"/> Charge any additional fee(s) during the pendency of this application</p> <p><input type="checkbox"/> Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.</p> <p style="text-align: center;">FEE CALCULATION</p> <p>1. BASIC FILING FEE</p> <table border="1"> <thead> <tr> <th>Large Entity Fee Code (\$)</th> <th>Small Entity Fee Code (\$)</th> <th>Fee Description</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>1001 750</td> <td>2001 375</td> <td>Utility filing fee</td> <td>770.00</td> </tr> <tr> <td>1002 330</td> <td>2002 165</td> <td>Design filing fee</td> <td></td> </tr> <tr> <td>1003 520</td> <td>2003 260</td> <td>Plant filing fee</td> <td></td> </tr> <tr> <td>1004 750</td> <td>2004 375</td> <td>Reissue filing fee</td> <td></td> </tr> <tr> <td>1005 160</td> <td>2005 80</td> <td>Provisional filing fee</td> <td></td> </tr> <tr> <td colspan="3" style="text-align: right;">SUBTOTAL (1)</td> <td>(\$) 770.00</td> </tr> </tbody> </table> <p>2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE</p> <p>Total Claims: <input type="text"/> -20** = <input type="text"/> X <input type="text"/> = <input type="text"/></p> <p>Independent Claims: <input type="text"/> -3** = <input type="text"/> X <input type="text"/> = <input type="text"/></p> <p>Multiple Dependent: <input type="text"/> = <input type="text"/></p> <table border="1"> <thead> <tr> <th>Large Entity Fee Code (\$)</th> <th>Small Entity Fee Code (\$)</th> <th>Fee Description</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>1202 18</td> <td>2202 9</td> <td>Claims in excess of 20</td> <td></td> </tr> <tr> <td>1201 84</td> <td>2201 42</td> <td>Independent claims in excess of 3</td> <td></td> </tr> <tr> <td>1203 280</td> <td>2203 140</td> <td>Multiple dependent claim, if not paid</td> <td></td> </tr> <tr> <td>1204 84</td> <td>2204 42</td> <td>** Reissue independent claims over original patent</td> <td></td> </tr> <tr> <td>1205 18</td> <td>2205 9</td> <td>** Reissue claims in excess of 20 and over original patent</td> <td></td> </tr> <tr> <td colspan="3" style="text-align: right;">SUBTOTAL (2)</td> <td>(\$) 0.00</td> </tr> </tbody> </table> <p><small>**or number previously paid, if greater; For Reissues, see above</small></p>	Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid	1001 750	2001 375	Utility filing fee	770.00	1002 330	2002 165	Design filing fee		1003 520	2003 260	Plant filing fee		1004 750	2004 375	Reissue filing fee		1005 160	2005 80	Provisional filing fee		SUBTOTAL (1)			(\$) 770.00	Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid	1202 18	2202 9	Claims in excess of 20		1201 84	2201 42	Independent claims in excess of 3		1203 280	2203 140	Multiple dependent claim, if not paid		1204 84	2204 42	** Reissue independent claims over original patent		1205 18	2205 9	** Reissue claims in excess of 20 and over original patent		SUBTOTAL (2)			(\$) 0.00	<p>FEE CALCULATION (continued)</p> <p>3. ADDITIONAL FEES</p> <table border="1"> <thead> <tr> <th>Large Entity Fee Code (\$)</th> <th>Small Entity Fee Code (\$)</th> <th>Fee Description</th> <th>Fee Paid</th> </tr> </thead> <tbody> <tr> <td>1051 130</td> <td>2051 65</td> <td>Surcharge - late filing fee or oath</td> <td></td> </tr> <tr> <td>1052 50</td> <td>2052 25</td> <td>Surcharge - late provisional filing fee or cover sheet</td> <td></td> </tr> <tr> <td>1053 130</td> <td>1053 130</td> <td>Non-English specification</td> <td></td> </tr> <tr> <td>1812 2,520</td> <td>1812 2,520</td> <td>For filing a request for <i>ex parte</i> reexamination</td> <td></td> </tr> <tr> <td>1804 920*</td> <td>1804 920*</td> <td>Requesting publication of SIR prior to Examiner action</td> <td></td> </tr> <tr> <td>1805 1,840*</td> <td>1805 1,840*</td> <td>Requesting publication of SIR after Examiner action</td> <td></td> </tr> <tr> <td>1251 110</td> <td>2251 55</td> <td>Extension for reply within first month</td> <td></td> </tr> <tr> <td>1252 410</td> <td>2252 205</td> <td>Extension for reply within second month</td> <td></td> </tr> <tr> <td>1253 930</td> <td>2253 465</td> <td>Extension for reply within third month</td> <td></td> </tr> <tr> <td>1254 1,450</td> <td>2254 725</td> <td>Extension for reply within fourth month</td> <td></td> </tr> <tr> <td>1255 1,970</td> <td>2255 985</td> <td>Extension for reply within fifth month</td> <td></td> </tr> <tr> <td>1401 320</td> <td>2401 160</td> <td>Notice of Appeal</td> <td></td> </tr> <tr> <td>1402 320</td> <td>2402 160</td> <td>Filing brief in support of an appeal</td> <td></td> </tr> <tr> <td>1403 280</td> <td>2403 140</td> <td>Request for oral hearing</td> <td></td> </tr> <tr> <td>1451 1,510</td> <td>1451 1,510</td> <td>Petition to institute a public use proceeding</td> <td></td> </tr> <tr> <td>1452 110</td> <td>2452 55</td> <td>Petition to revive - unavoidable</td> <td></td> </tr> <tr> <td>1453 1,300</td> <td>2453 650</td> <td>Petition to revive - unintentional</td> <td></td> </tr> <tr> <td>1501 1,300</td> <td>2501 650</td> <td>Utility issue fee (or reissue)</td> <td></td> </tr> <tr> <td>1502 470</td> <td>2502 235</td> <td>Design issue fee</td> <td></td> </tr> <tr> <td>1503 630</td> <td>2503 315</td> <td>Plant issue fee</td> <td></td> </tr> <tr> <td>1460 130</td> <td>1460 130</td> <td>Petitions to the Commissioner</td> <td></td> </tr> <tr> <td>1807 50</td> <td>1807 50</td> <td>Processing fee under 37 CFR 1.17(q)</td> <td></td> </tr> <tr> <td>1806 180</td> <td>1806 180</td> <td>Submission of Information Disclosure Stmt</td> <td></td> </tr> <tr> <td>8021 40</td> <td>8021 40</td> <td>Recording each patent assignment per property (times number of properties)</td> <td></td> </tr> <tr> <td>1809 750</td> <td>2809 375</td> <td>Filing a submission after final rejection (37 CFR 1.129(a))</td> <td></td> </tr> <tr> <td>1810 750</td> <td>2810 375</td> <td>For each additional invention to be examined (37 CFR 1.129(b))</td> <td></td> </tr> <tr> <td>1801 750</td> <td>2801 375</td> <td>Request for Continued Examination (RCE)</td> <td></td> </tr> <tr> <td>1802 900</td> <td>1802 900</td> <td>Request for expedited examination of a design application</td> <td></td> </tr> <tr> <td colspan="3">Other fee (specify) _____</td> <td></td> </tr> <tr> <td colspan="3">*Reduced by Basic Filing Fee Paid</td> <td></td> </tr> <tr> <td colspan="3" style="text-align: right;">SUBTOTAL (3)</td> <td>(\$)</td> </tr> </tbody> </table>	Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid	1051 130	2051 65	Surcharge - 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SUBMITTED BY		<i>(Complete if applicable)</i>	
Name (Print/Type)	Ronald I. Eisenstein	Registration No. (Attorney/Agent)	30,628
Signature	<i>Ronald I. Eisenstein</i>	Telephone	617-345-6054
		Date	10/14/03

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Practitioner's Docket No. 700938-052220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi et al.
Application No.: TBA (divisional of 10/031,929) Group No.: To be Assigned (1651)
Filed: Herewith Examiner: To be assigned (Srivastava,
Kailash C.)
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

EXPRESS MAIL CERTIFICATE

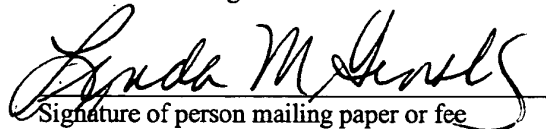
"Express Mail" Label Number: EL 934997148 US
Date of Deposit: October 14, 2003

I hereby state that the following *attached* papers and fees:

1. Check: \$770.00;
2. Return receipt postcard;
3. Certificate of Express Mail (1 pg.) EL 934997148 US;
4. Fee Transmittal for FY2003 (1 pg.);
5. Utility Patent App. Transmittal (PTO/SB05) (2 pp.);
6. Copy of Dec. & POA filed in parent case (4 pp.);
7. Preliminary Amendment (6 pp.);
8. Application Data Sheet (3 pp.);
9. Application (44 pp.): Spec: 34 pp.; 5 of Cls (Cls 1-17) and 1 pg. abstract; 4 pp. of drawings;
10. Information Disclosure Statement (3 pp.), including Refs. AA-AC and BA-BC
11. Assignment Recordation on Cover Sheet (2 pp.);
12. Executed Assignment (3 pp.)

are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. section 1.10, on the date indicated above and is addressed to Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Linda M. Ginsberg


Signature of person mailing paper or fee

ADDENDUM

Title: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

DESCRIPTION

METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
5 ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

TECHNICAL FIELD

The present invention relates to a method for detecting
pathogenic microorganism, method for evaluating an effect of an
10 antimicrobial agent on pathogenic microorganism and a method for
detecting an antimicrobial agent. The present invention also relates to
an antimicrobial agent and a therapeutic agent for onychomycosis,
which are obtained according to the above-mentioned method for
evaluating the drug effect.

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BACKGROUND ART

A method for evaluating a drug effect with an animal model is
needed in order to explore a novel antimicrobial agent (also hereinafter
referred to "drug"). Further, a method enabling a drug effect to be
20 evaluated with accuracy is needed because of grate importance in view
of predicting a clinical therapeutic efficiency thereof.

Historically, an experimental dermatophytosis model that
back, planta and interdigital of a guniea pig have been infected with
Trichophyton mentagrophytes has been used in order to evaluate an
25 effect of an antifungal agent on dermatophytosis. Such animal models
have been already employed to develop some antifungal agent. The
evaluation of the effect of such antifungal agent carried out by applying

the antifungal agent to the infected animal, by excising the skin after the certain period of time to cut into plural small pieces, by cultivating the skin pieces on the medium, and by counting the number of pieces wherein no growth of fungus is seen or the number of animals or feet
5 wherein no growth of fungus is seen in all skin pieces, as an indicator (Antimicrobial Agents and Chemotherapy, 36: 2523-2525, 1992, 39: 2353-2355, 1995). Hereinafter, the conventional method for evaluating the drug effect is referred to as "the conventional method".

Although the drug having a potent activity against
10 *Trichophyton in vitro* such as lanoconazole or amorolfine has been marketed in these days, an improvement of cure rate in a clinical use is hardly seen. As a main reason thereof, a relapse that since fungus in the skin is not completely killed after a treatment, the fungus grow again is pointed.

15 In also animal experiments, when an effect of lanoconazole on guniea pig models of tinea pedis was evaluated using the conventional method, though "fungus-negative" was observed in all feet out of 20 feet 2 days after the last treatment, a relapse was observed in 11 out of 20 feet 30 days after the last treatment, and no correlation was
20 seen between the effect 2 days after the last treatment and the effect 30 days after the last treatment (36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Louisiana, 1996, Abstr. F80).

As a reason thereof, there were followings. Since
25 lanoconazole have very potent antitrichophyton activity *in vitro*, lanoconazole persisted in the skin 2 days after the last treatment in the concentration wherein the sterilization effect was shown. Therefore,

when the skin is excised and cultivated on the medium to detect fungus, the lanconazole remaining in the skin is diffused in the medium, and therefore, no fungus was detected due to prevention of the growth regardless of the presence of viable fungus in the excised skin. On the
5 other hand, since the concentration of the drug remained in the skin is reduced 30 days after the last treatment, fungus in the skin can grow again and can be detected by culture study.

According to this hypothesis, it is ascertained that the drug remain in the skin through the inhibition of the growth of fungus around
10 the skin blocks completely, when the lanconazole-treated skin blocks were located and cultivated on the medium which contains dermatophytes.

Therefore, it became to clear that the conventional method has the problem that the drug effect can not be accurately evaluated,
15 because the apparent therapeutic effect need to be evaluated after removing the drug remaining in the skin.

Meanwhile, a kind of mycosis, dermatophytosis, is the superficial dermatosis which is caused by dermatophyte parasitizing the keratin such as skin (stratum corneum), the nail and the hair. In
20 particular, tinea unguium formed in the nail is known as the intractable disease among dermatomycoses based on dermatophytoses, and is accompanied by symptom such as opacity, tylosis, destruction and deformation of nail plate. Now the oral preparation (such as griseofulvin or terbinafine) is used for the treatment of such tinea
25 unguium. However, there are many cases where the patient stops taking the drug or that takes the drug irregularly, since they have to take the drug for a long period, for example at least a half a year in order to

completely cure tinea unguium. It is thought that this is a main cause of difficulty of curing tinea unguium completely. Furthermore, by taking the drug for a long period, griseofulvin has the problem of side effects on internal organ (gastrointestinal disorder, hepatotoxicity) and
5 hepatotoxicity is reported as the side effect in terbinafine. Therefore, in order to improve the compliance of the patient it is desired to develop a topical preparation which cure tinea unguium for a short period and has less the systemic side effect than the oral preparation.

However, in case of the simple application on nail plate_with
10 the current antifungal agent for topical use, the antifungal effect on fungus in the nail was not seen, because the drug could not sufficiently permeate the thick keratin in nail plate (Markus Niewerth and Hans C. Korting, Management of Onychomycoses, Drugs, 58: 283-296, 1999).

In addition, the therapeutic effect of a topical preparation of
15 antifungal agent on the experiment model of trichophytosis can not be evaluated using the conventional method as mentioned above. This may be a reason why the drug effect on the guinea pig model of tinea unguium has been hardly reported.

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DISCLOSURE OF INVENTION

The present invention has been accomplished based on findings that it is desirable that an effect of antimicrobial agent such as particularly antifungal agent is evaluated after removing a drug remaining in the infected site after treatment of an animal or a
25 biosample such as skin with the pathogenic microorganism. An object of the present invention is to provide a novel method for evaluating the effect of the antimicrobial agent and the antimicrobial agent obtained

according to the method for evaluating the drug effect. In detail, the present invention provides the method for detecting the viable pathogenic microorganism in the above-mentioned infected site of the animal or the biosample with the pathogenic microorganism after
5 removing the antimicrobial agent which has been administered to the animal or the biosample, and the method for evaluating the effect of antimicrobial agent which can accurately evaluate the effect of the antimicrobial agent without the influence of the antimicrobial agent remaining in the infected site of the animal or the biosample with a
10 pathogenic microorganism. In addition, the present invention provides the antimicrobial agent obtained according to the above-mentioned the method for evaluating the drug effect, and the detecting method of the antimicrobial agent which comprises detecting the existing antimicrobial agent in the infected site of the animal or the biosample
15 with the pathogenic microorganism, to which the antimicrobial agent is administered.

In more detail, according to the present invention a detection of a pathogenic microorganism and an evaluation of an effect of an antimicrobial agent can be carried out by infecting an animal or a
20 biosample with the pathogenic microorganism, administering the antimicrobial agent comprising a compound having an antimicrobial effect or a composition thereof before or after the infection, then removing the antimicrobial agent, and thereafter detecting the viable pathogenic microorganism in the infected site with the pathogenic
25 microorganism.

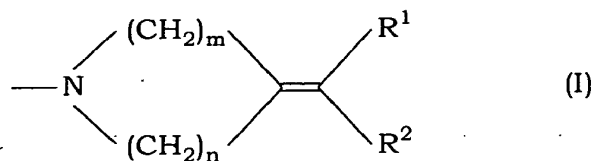
According to the present invention a detection of an existing antimicrobial agent can be carried out by infecting an animal or a

biosample with a pathogenic microorganism, administering the antimicrobial agent comprising a compound having an antimicrobial effect or a composition thereof before or after the infection; then excising the infected site with the pathogenic microorganism, placing and cultivating it on a medium containing the pathogenic microorganism, and thereafter observing a growth inhibition of the pathogenic microorganism around the infected site with the pathogenic microorganism.

Additionally, an object of the present invention is to provide the evaluation method of a drug which enables the effect of an antifungal agent to accurately evaluate in a guinea pig model of tinea unguium. Another object of present invention is to provide a therapeutic agent for onychomycosis which exhibits the effect on tinea unguium by topical application and which is capable of curing tinea unguium shorter period than that of the marketed oral preparation due to good permeability, good retention capacity and conservation of high activity in nail plate as well as the potent antifungal activity thereof based on the present invention. Another object of the present invention is to provide the effective therapeutic agent for onychomycosis exhibiting no side effect even if therapeutically effective amounts of it are administered sufficiently.

More concretely, the present invention provides a therapeutic agent for onychomycosis containing a compound having a formula (1):

25



Wherein R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,
5 m is 2 or 3,
n is 1 or 2,
or a salt thereof as active ingredient.

In addition, "presence" includes the mean of "remaining".

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BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a color copy of a photograph to identify the agent remaining in the skin which is previously evaluated by the conventional method in the detecting method of the antimicrobial agent five days after
15 last treatment in the present invention. The note (a) shows the infected control group, (b) the KP-103-treated group, (c) the lanoconazole-treated group.

Fig. 2 is a color copy of photograph to identify agent remaining in the skin which is previously evaluated by the detecting
20 method of the antimicrobial agent five days after last treatment in the present invention. The note (a) shows the infected control group, (b) the KP-103-treated group, (c) the lanoconazole-treated group.

Fig. 3 is a graph showing a distribution of the number of fungal cells in the nail of a guinea pig model of tinea unguium in each
25 treated group according to the evaluation method of the drug effect in the present invention.

Fig. 4 is a graph showing a distribution of the number of

fungal cells in the skin of a guinea pig model of tinea pedis in each treated group according to the evaluation method of the drug effect in the present invention.

5 BEST MODE FOR CARRYING OUT THE INVENTION

As an animal employed in the present invention, there includes mammal such as mice, rat, guinea pig or rabbit. As a biosample, there includes a skin of back or planta, a nail or the like, which is taken from such animal.

10 A method for infecting such animal or biosample with a pathogenic microorganism includes an inoculation percutaneously, orally, intravenously, transbronchially, transnasally or intraperitoneally. Especially in case of the skin, there includes a method for inoculating it on the skin, a method for inoculating on the exposed demis, the closed
15 patch method, intracutaneous injection or the like. In case of the nail, there includes a method for inoculating on nail, a method in which a skin of the animals' foot is infected by the above-mentioned infecting method to the skin, and thereafter the infection is moved into the nail by leaving it for several months.

20 The term "skin" means a tissue including the three layers being epidermis, demis and subcutaneous tissue, accompanied by pilus (hair), nail, glandulae sebaceae, glandulae sudoriferae and glandulae mammaria as appendages. The epidermmis is separated five layers
25 being stratum corneum, stratum lucidum, granulosum epidermidis, stratum spinosum, and stratum basale from surface in order. The stratum corneum, the stratum lucidum and the stratum granulosum epidermidis is referred to as a stratum corneum in a broad sense.

Herein, keratin substance means a part of the above-mentioned stratum corneum.

The term "nail" includes nail plate, nail bed, nail matrix, further side nail wall, posterial nail wall, eponychium and hyponychium
5 which make up a tissue around thereof.

In the present invention, the term "pathogenic microorganism" means a microorganism which causes human and animal disease in one way or another. An example of the pathogenic microorganism (hereinafter referred to "microorganism") is bacteria
10 including aerobic Gram-negative bacillus and coccus such as *Pseudomonas* and *Neisseriaceae* species; facultative anaerobic Gram-negative bacillus such as *Eschrichia*, *Salmonella* and *Enterobacter* species; Gram-positive coccus such as *Staphylococcus* and *Streptococcus* species. The other examples of microorganism are fungi
15 including Hyphomycetes such as *Trichophyton*, *Microsporum* and *Epidermophyton* species; Blastomycetes such as *Candida* and *Malassezia*; Ascomycetes such as *Aspergillus* species; Zygomycetes such as *Mucor* species; and variants thereof. Examples of such variants are resistant strain which naturally obtains drug resistance; auxotrophic
20 mutation strain which comes to have nutritious dependency; artificial mutation strain which is artificially mutated by treatment with mutagenic agent; and the like.

Mycosis means a disease which is caused by invading and proliferating in the tissue of human or animal. Usually, mycosis is
25 broadly divided into superficial mycosis and deep mycosis. A seat of the disease lie in the skin or visible mucosa in case of the former, in viscus, central nervous system, subcutaneous tissue, muscle, born or

articulation in case of the latter. Chief example of superficial mycosis is dermatophytosis which is caused by infecting with dermatophyte such as *Trichophyton*, *Microsporum* and *Epidermophyton* species, including three disease, tinea, tinea favosa and tinea imbricata. Tinea may be conventionally employed a synonymous with dermatophytosis. In addition, dermatophyte belonging to *Trichophyton* species is referred usually to as trichophytosis.

In the present invention, an antimicrobial agent means a compound having an antimicrobial effect or a composition containing the compound. The composition includes a preparation form being artificial composition and a natural composition such as a natural product.

A method for administration of the antimicrobial agent in the present invention depends on the kind thereof and includes topical application, subcutaneous administration, oral administration, intravenous administration or the like.

When the method for detecting the pathogenic microorganism, the method for evaluating the drug effect and the method for detecting the antimicrobial agent according to the present invention is carried out, either an infection with microorganism or an administration of the antimicrobial agent may be carried out first. Especially, in the method for evaluating the drug effect of the present invention (hereinafter referred to "the present evaluation method"), a therapeutic effect of the antimicrobial agent can be evaluated in case where the antimicrobial agent is administered after the infection with microorganism, meanwhile, a effect of the antimicrobial agent protecting from the infection and the retention capacity thereof can be evaluated in

case where the infection with microorganism is carried out after the administration of the antimicrobial agent. In order to evaluating the retention capacity of the antimicrobial agent, the evaluation can be carried out with varying the period until infection with microorganism
5 from the administration of the antimicrobial agent.

In the present invention, it is preferable to use dialysis or ultra filtration for removing the antimicrobial agent in view point of the usefulness, but not limited thereto as long as a microorganism to be a detecting target or a microorganism used in the present evaluation
10 method and the like is not affected by it.

In dialysis, a marketed dialysis membrane made of cellulose is convenient. A membrane made of the other material can be used without problem, as long as the microorganism to be the detecting target or the microorganism used in the present evaluation method and the
15 like can not be passed, and the antimicrobial agent can be passed through it. Since sizes of most fungi and bacteria are at least 0.2 μm , it is preferable to use the membrane having less than 0.2 μm of the pore size, particularly it is suitable to use dialysis membrane having fractional molecular weight of 1,000 to 50,000.

20 As out side solutions used in dialysis, there include physiological saline, distilled water, phosphate buffered physiological saline, the other buffer and the like.

In removing the antimicrobial agent according to the present invention, even though the infected site with the microorganism is the
25 nail, organ or the like as well as the skin, the antimicrobial agent can be efficiently removed. Usually, since there is the case where it takes longer time dialysis to remove the antimicrobial agent from nail than

skin, the following treatment with digestive enzyme may be carried out before removing it in order to enhance the removal effect.

Dialysis conditions depend on variety, dose concentration, dose term and the drug holidays (the term until evaluation from last day of treatment) of an antimicrobial agent. Therefore, it is preferable to previously investigate the dialysis conditions enabling the antimicrobial agent to be removed from the treated skin about individual cases using the following detecting method of the existing antimicrobial agent in the infected site with a microorganism in the present invention (hereinafter referred to "the present method for detecting an agent") to adjust the conditions appropriately.

Whether an antimicrobial agent has been removed can be easily determined using the following method.

The present method for detecting an agent is carried out by placing and cultivating the infected site with a microorganism which is subjected to the removing method of the antimicrobial agent (e.g. an skin piece) or a suspension obtained according to the following extraction procedure of the microorganism from the above skin piece on an agar medium containing the microorganism, and observing a growth inhibition of the microorganism found around it. When there is the remaining antimicrobial agent, the growth inhibition of the microorganism is observed.

The present evaluation method can be carried out by locating and cultivating, on a medium, the skin piece in which a removal of an antimicrobial agent has been determined using the above-mentioned present method for detecting the agent after carrying out the appropriate removal of the antimicrobial agent and observing whether there is a

growth of microorganism or not, or by smearing and cultivating a suspension obtained according to the extraction procedure of the microorganism from the skin piece on an agar medium and observing whether there is the growth of microorganism or not or counting colonies emerging on those medium.

A treatment with trypsin can be carried out in order to extract a microorganism efficiently from a biosample such as a skin or a nail. Other digestive enzyme than trypsin such as pronase or keratinase, or a keratin resolvent such as urea also can be used without limitation to trypsin as long as they have an extraction effect. It is necessary to adjust concentrations of the digestive enzyme such as trypsin and keratin resolvent in a treating solution, and reaction time to no affect range to a microorganism. The treatment with digestive enzyme such as trypsin may be carried out either before or after dialysis. When the treatment with trypsin is carried out before dialysis, it is necessary to remove the digestive enzyme sufficiently so that the microorganism is not affected on dialysis.

As a medium used for a cultivation of a microorganism in the present invention, any medium can be used as long as it can be conventionally used for the cultivation and a separation of the microorganism. In case of fungi, example of the medium is Sabouraud medium, modified Sabouraud medium, Czapek agar medium, Potato dextrose agar medium or the like. On the other hand, in case of bacteria, example of the medium is Mueller Hinton medium, modified Mueller Hinton medium, Heart Infusion agar medium, Brain Heart Infusion agar medium, normal agar medium or the like.

A reacting temperature is 10 to 40°C, preferably 20 to 40°C.

A microorganism may be cultivated with standing during a sufficient time when the microorganism can be growth, for example, 1 to 20 days in case of fungi, 1 to 5 days in bacteria.

5 The present evaluation method be utilizable as a evaluation method of a drug effect in exo vivo which comprises infecting a skin, a nail excised from an animal body with a microorganism, thereafter administering an antimicrobial agent as a test compound, then removing the antimicrobial agent and detecting and determining quantity of the microorganism in the sample.

10 The present evaluation method also can be applied to an evaluation of an antimicrobial agent such as a therapeutic agent for deep mycosis or an antibacterial agent as well as an evaluation of an effect of a therapeutic agent for superficial mycosis. That is to say, it is possible to evaluate an effect of a therapeutic agent for deep mycosis or
15 an antibacterial agent by means of administering an antimicrobial agent to an animal infected with a microorganism such as a fungus or a bacterium by inoculating percutaneously, orally, intravenously, transbronchially, transnasally, intraperitoneally, then obtaining biosample such as skin, kidney, lung or brain, and detecting the viable
20 microorganism in the biosample in which removed the remaining antimicrobial agent has been removed.

In addition, the present evaluation method enables a quantitative comparison of antimicrobial effects by means of determining the number of viable microorganisms in the treated
25 biosample.

That is to say, a significant deference test is carried out about the number of microorganisms in the infected site with the

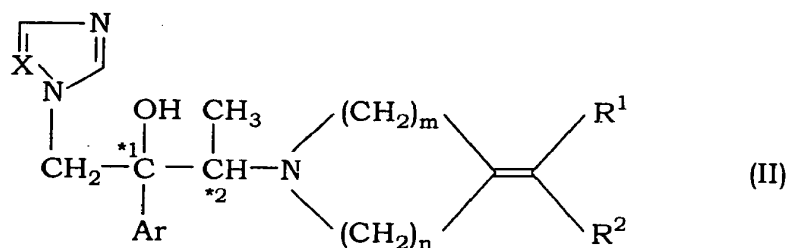
microorganism for the treated group with drug and for the reference infected group using a statistical method such as Kruskal-Wallis Test, and thereby a quantitative comparison between the groups can be done by using a multiple test such as Tukey method.

5 The present invention is useful as either a method for evaluating a drug effect or a method for detecting the antimicrobics in keratin substance or nail, after administering the antifungus to the patient infected with fungus. For example, according to the present invention, an effect of an antifungal agent can be evaluated by
10 administering it to the patient whose skin or nail is infected with fungus, obtaining the keratin substance or nail, then removing the above-mentioned antifungal agent, and thereafter detecting the viable fungus in the keratin substance or nail. Additionally, according to the present invention, a detection of an antifungal agent can be carried out by
15 administering it to the patient whose skin or nail is infected with fungus, then obtaining the keratin substance or nail, cultivating it on agar medium containing fungus, and thereafter detecting the existing antifungal agent in the keratin substance or nail through a growth inhibition of fungus observed around the keratin substance or nail.
20 Such evaluation of an antifungal agent administered to a patient with fungus and detection of the antifungal agent from the keratin substance or nail can be carried out in the same manner as in the above-mentioned evaluation method of a drug effect and detecting method of the antimicrobial agent administered to an animal or a biosample.

25 Furthermore, the present invention provides various useful antimicrobial agents according to the present evaluation method. As the antimicrobial agent obtained by the present evaluation method,

there is an antimicrobial agent comprising a compound having an eradication effect for microorganism *in vivo* or a composition for therapy of the superficial mycosis, deep mycosis or bacterial infection containing the compound; an antimicrobial agent having the true effect selected by means of showing a statistically significant effect; furthermore, an antimicrobial agent having an excellent eradication effect for microorganism *in vivo*, which is selected by means of appearing the pure antimicrobial activity thereof; or an antimicrobial agent of the complete cure type without relapse. A concrete example is a therapeutic agent for onychomycosis comprising a compound having the group represented by the above-mentioned formula (I). Among them, more preferable concrete example is a therapeutic agent for onychomycosis comprising the compound represented by the formula (II):

15



20 wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to
 25 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkinyl group, or

C_{7-12} aralkyl group,
m is 2 or 3,
n is 1 or 2,
X is nitrogen atom or CH, and

5 *1 and *2 mean an asymmetric carbon atom.

In the above-mentioned formula (I) or (II), the substituted phenyl group is a phenyl group having 1 to 3 substituents selected from a halogen atom and trifluoromethyl, and includes, for instance, 2,4-difluorophenyl, 2,4-dichlorophenyl, 4-fluorophenyl, 4-chlorophenyl, 2-10 chlorophenyl, 4-trifluoromethylphenyl, 2-chloro-4-fluorophenyl, 4-bromophenyl or the like. C_{1-6} alkyl group includes, for example, a straight chain, branched chain or cyclic alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, tert-pentyl and 15 cyclohexyl. The non-substituted aryl group includes, for example, phenyl, naphthyl, biphenyl or the like. The substituted aryl group includes, for example, 2,4-difluorophenyl, 2,4-dichlorophenyl, 4-fluorophenyl, 4-chlorophenyl, 2-chlorophenyl, 4-trifluoromethylphenyl, 2-chloro-4-fluorophenyl, 4-bromophenyl, 4-tert-butylphenyl, 4-20 nitrophenyl or the like. C_{2-8} alkenyl group includes, for example, vinyl, 1-propenyl, styryl or the like. C_{2-6} alkynyl group includes, for example, ethynyl or the like. C_{7-12} aralkyl group includes, for example, benzyl, naphthylmethyl, 4-nitrobenzyl or the like.

In addition, the most preferable compound among the 25 above-mentioned antimicrobial agent includes the compound which shows the therapeutic efficiency like the following KP-103.

The above-mentioned KP-103 means an antifungal indicated

by (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylenepiperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl)butane-2-ol. The compound can be prepared by allowing (2R,3S)-2-(2,4-difluorophenyl)-3-methyl-2-[(1H-1,2,4-triazole-1-yl)methyl]oxirane to react with 4-methylenepiperidine based on
5 Example 1 in WO94/26734.

An effectiveness of the KP-103 used as an antifungal in the present invention for onychomycosis has not been confirmed, but its antifungal activity has been already known (WO94/26734).

The antimicrobial agent obtained in such manner can be
10 used as a drug composition, the drug composition in order to sterilize a microorganism. In other words, it comes to be a drug composition which cures disease such as mycosis completely, and prevents a relapse.

Onychomycosis means a kind of the above-mentioned
15 superficial mycosis, in the other word a disease which is caused by invading and proliferating in the nail of human or an animal. *Trichophyton rubrum* and *Trichophyton mentagrophytes* mainly cause onychomycosis in human. In rare case, *Microsporium*, *Epidermophyton*, *Candida*, *Aspergillus* or *Fusarium* causes it.

20 As a disease which is susceptible to treat with a therapeutic agents for onychomycosis of the present invention, there is included tinea unguium caused by *Trichophyton* species, Onychocandidasis caused by *Candida* species or onychomycosis (sensu stricto) caused by the other fungus.

25 When a therapeutic agent for onychomycosis being a kind of antimicrobial agent in the present invention is given as topical preparation, there is liquid preparation, cream, ointment or manicure

preparation as dosage form. In this case, it can be prepared using oil vehicle, emulsion vehicle or the like. The preferable amount of active ingredient is in 0.1 to 10 % by weight. A dose amount may be appropriately aligned depending on the width of affected area and
5 condition of disease.

In case of an oral preparation, it is used as powder, tablet, granule, capsule or syrup. In addition, it is used in form of injection such as subcutaneous injection, intramuscular injection or intravenous injection.

10 In the present invention, although the dosage amount of a therapeutic agent for onychomycosis depends on age, weight and individual conditions of a patient, it is about 10 mg to about 10 g per day, preferably about 50 mg to about 5 g as amount of the active ingredient. The agent was given in the above-mentioned daily dose at once or several
15 divided portions.

The present invention is further explained in details based on the Examples hereinafter, but is not limited thereto.

[Pretreatment of Comparative Example 1 and Examples 1 to 3]

20 [1] Preparation of fungal solution and production of a guinea pig model of interdigital type of tinea pedis.

Millipore Filter (made by Millipore Corporation, HA, diameter 47 mm, 0.45 μ m) was placed on Brain-Heart-infusion agar medium (available from Nissui Pharmaceutical Co., LTD.), and 10^6 cells of
25 microcondium of *Trichophyton mentagrophytes* KD-04 strain were applied thereon. The cultivation was carried out at 30°C under 17 % of CO₂ for 7days. After the cultivation, just amount of physiological saline

containing 0.05 % of Tween 80 was dropped on the filter and arthroconidia were collected using a platinum loop. After a hyphal mass was removed by a filtration with a sterile gauze, the number of arthroconidia in the filtrate was calculated by hemocytometer to adjust
5 in concentration of 1×10^8 arthroconidia / ml to obtain a fungal inocula.

A guinea pig model of interdigital type of tinea pedis was prepared according to the method of Arika et al (Antimicrobial Agents and Chemotherapy, 36: 2523-2525, 1992). Concretely, in two hind
10 foots of male Hartley strain guinea pigs of 7 weeks age, the interdigital skin was lightly abraded with sandpaper. A paper disc (AAdisc available from Whatmen International Ltd cut in 8×4 mm) moisten with the above-mentioned solution of the inoculated organism was applied onto the region between the interdigital toes of the hind feet and fixed using Self-adhering-Foam Pad (Restone 1560M; available from 3M) and
15 adhesive stretch bandage (ELASTPORE; available from Nichiban Co., Ltd). The paper disc and the bandage were removed seven days after of the infection.

[2] Preparation of drug-solution and topical treatment for the guinea pig model of interdigital type of tinea pedis

20 A marketed 1 % lanoconazole solution (commercial name: Astat (trade name) solution) and a solution in which KP-103 was solved in a concentration of 1 % in polyethylene grycole #400 : ethanol (75 : 25 v/v) mixture werè used as test substance. Each solution in amount of 0.1 ml was applied to the plantar skin once a day from 10 days after the
25 infection for 10 days.

COMPARATIVE EXAMPLE 1

Conventional method for evaluating drug effect

The conventional method was described as follows. For the infected control group without an application of the drug, the KP-103-
5 treated group and the lanoconazole-treated group, 10 guinea pigs (hereinafter referred to "animal") were employed, respectively. Animals of each group were sacrificed two days after and 30 days after the last treatment. Their two hind feet were excised and wiped with the cotton swab containing alcohol sufficiently. A skin of whole sole was excised
10 and cut into 15 skin pieces in total including 12 skin pieces from plantar parts and 3 skin pieces from an interdigital part. Each skin pieces were placed on 20 ml of Sabouraud dextrose agar medium (available from Difco laboratories) containing 50 µg of chloramphenicol (available from Wako Pure Chemical Industries, Ltd.), 100 µg of gentamicin (available
15 from Schering-Plough Corporation), 50 µg of 5-fluorocytosine (available from Wako Pure Chemical Industries, Ltd.) and 1 mg of cycloheximide (available from nacalai tesque, Inc.) per ml. An antibiotic substance added to the medium was set to a condition which enable bacteria not to grow and which enable fungi to grow without problem. After 10 days
20 cultivation at 30°C, the result is described as "fungus-negative" when no growth of fungus was observed in all skin pieces, and the number of fungus-negative feet was determined. In the evaluation of the effect 30 days after last treatment, two days after the last treatment the treated feet were wiped with a cotton swab containing alcohol and fixed with the
25 bandage in order to prevent a reinfection. The bandage was changed once a week. The therapeutic effects of KP-103 and lanoconazole two days after and 30 days after the last treatment are shown in Table 1.

TABLE 1

Test substance	The number of fungus-negative feet/ Total number of infected feet	
	Two days after the last treatment	30 days after the last treatment
Infected control	0/20	0/20
KP-103	20/20	16/20
Lanoconazole	20/20	9/20

As shown in the Table 1, in the KP-103-treated group, fungus-negative was observed in all feet two days after the last treatment, and also fungus-negative was observed in 16 out of 20 feet 30 days after the last treatment. On the other hand, in the lanoconazole-treated group, fungus-negative was observed in all feet two days after the last treatment, but fungus-negative was observed in only 9 feet 30 days after the last treatment, and there is no correlation between the therapeutic effects two days after and 30 days after the last treatment. The number of fungus-negative feet decreased 30 days after the last treatment. It was thought that the therapeutic effect of lanoconazole observed two days after the last treatment resulted from the inhibition of the growth of fungus caused by an interfusion of the drug remaining in the treated skin into culture system, because lanoconazole had a potent *in vitro* antifungal activity against dermatophytes, it was eight-fold more active than KP-103 against *Trichophyton* with a growth inhibitory concentration of 15.6 ng/ml. The determination test of the remaining agent was carried out.

EXAMPLE 1

Determination of drug remaining in skin which has been already evaluated five days after the last treatment according to conventional method.

5 A model was prepared according to Comparative Example 1. Lanoconazole being a test compound was used for a therapeutic experience as 1 % solution with the same vehicle as KP-103. For the infected control group without an application of a drug, the KP-103-treated group and the lanoconazole-treated group, 20 animals were
10 employed, respectively. The two hind feet were excised from each animal five days after the last treatment in the same manner as in Comparative Example 1. A total of 20 light feet were used for an evaluation by the conventional method, and a total of 20 right feet were used in the present evaluation method.

15 The skin pieces of light foot were placed on 20 ml of Sabouraud dextrose agar medium containing *Trichophyton mentagrophytes* KD-04 strain (2×10^4 cells/ml) and the antibiotic substance described in Comparative Example 1. After the cultivation was carried out at 30°C for 3 days, a growth inhibitory-zone of fungus
20 appeared around the skin was observed and was photographed for 10 out of 20 feet. Figure 1 is an electronic date of the photograph of the skin after the cultivation in the above-mentioned condition. (a) indicates the infected control group without the drug application, (b) the KP-103-treated group and (c) the lanoconazole-treated group. One
25 plate was explained as a representative of ten plates corresponding to each animal in the infected control group (a). In Figure 1, S indicates one of 15 skin pieces of planta derived from the animal and M the

above-mentioned medium. S and M described in both the KP-103-treated group (b) and the lanoconazole-treated group (c) are also the same. In the medium, the white zone shows the growth of fungus, on the other hand, the black zone shows the inhibition of the growth of
5 fungus.

As shown Figure 1, a good growth of the fungus was observed around the skin piece of the infected control group without any drug. In the group treated with KP-103, the growth of fungus was observed in all skin pieces, although in around the skin pieces the growth of fungus
10 was slightly inhibited as compared with the infected control group. On the other hand, the growth of fungus was completely inhibited in around the skin pieces treated with lanoconazole. As these results, the therapeutic effect of lanoconazole in the conventional method shown in Table 1 was considered as an apparent therapeutic effect such that the
15 agent remaining in the skin come to be mixed in culture system to inhibit the growth of fungus.

Therefore, it came to appear that the drug effect could not be evaluated by the conventional method precisely.

20

EXAMPLE 2

Determination of remaining drug after removing drug from skin.

As Example 1, 20 right feet were excised from each animal five days after the last treatment, and sufficiently wiped with the cotton swab containing alcohol. The planta was cut off from each foot. The
25 skin mincced by a scissors was put into dialysis membrane (fractional molecular weight: 12,000-14,000, made of cellulose, available from VISKASE SALES Corporation) together with 4 ml of distilled water.

Dialysis was carried out under 3 L of distilled water at 4°C for 2 days. The dialysis water was changed twice a day 4 times in total. The content was transfer into a glass homogenizer. Thereto 4 ml double-concentration phosphate buffered saline containing 4 % of trypsin
5 derived from pig pancreas (available from BIOZYME Laboratories Limited) was added and the resulting mixture was homogenized. It was left at 37°C for one hour and was filtrated with the two-ply gauze. The resulting filtrate was centrifuged. To a precipitate obtained by removing the supernatant were added 8 ml of phosphate buffered saline
10 containing 2 % of trypsin and further it allow to react with shaking at 37°C for one hour. After a centrifugation, the precipitate obtained by removing the supernatant was washed three times by centrifuging with phosphate buffered saline in order to remove trypsin. To the precipitate 2 ml of the same saline were added to prepare a suspension thereof.

15 When dialysis and the treatment with trypsin were carried out using the same fungus used in this Example, an affect of these procedures on a survival rate of fungus could not be observed. Previously, a well was prepared in the center of Sabouraud dextrose agar medium (20 ml) containing *Trichophyton mentagrophytes* KD-04 strain
20 (2×10^4 cells/ml) and the antibiotic substance described in Comparative Example 1. Into the well 100 µl of the above-mentioned suspension were added to cultivate at 30°C for three days. After the cultivation, a growth inhibitory-circle of fungus appeared was observed and was photographed for 10 out of 20 feet. Figure 2 is an electronic date of the
25 photograph of the skin after the cultivation in the above-mentioned condition. (a) indicates the infected control group without the drug application, (b) the KP-103-treated group and (c) the lanoconazole-

treated group. One plate was explained as a representative of ten plates corresponding to each animal in the infected control group (a). In Figure 2, E indicates the skin suspension prepared from planta of the animal and M the above-mentioned medium. E and M described in both the KP-103-treated group (b) and the lanoconazole-treated group (c) are also the same. In the whole medium, the white zone shows the growth of fungus, on the other hand, the black zone around the well shows the inhibition of the growth of fungus.

In Figure 1 showing the conventional method, no growth of fungus was observed in around the skin of the lanoconazole-treated group taken five days after the last treatment and the remaining drug was determined in the skin. By contrast, in Figure 2, although few growth-inhibitory circle was observed in 2 out 10 feet suspension obtained by removing the drug using dialysis treatment of the present invention for the skin of the lanoconazole-treated group taken five days after the last treatment, the growth-inhibitory circle was never observed in residual 8 feet.

Since it came to appear that the drug remaining in treated skin could be sufficiently removed using dialysis according to the present invention, it was confirmed that the evaluation of the drug effect was not affect by the remaining drug.

EXAMPLE 3

Detection of viable fungus in skin and evaluation of drug effect

To two mediums of Sabouraud dextrose agar medium (20 ml) containing the antibiotic substance described in Comparative Example 1 were applied 100 μ l of the suspension from one right feet of each

animals obtained in Example 2. After the cultivation was carried out at 30°C for 10 days, the result is described as “fungus-negative” when a colony of fungus was not observed in two agar plates (detection limit: 10 CFU (colony forming unit)/feet). The number of fungus-negative feet was counted. On the other hand, 20 left feet were evaluated in the same manner as in Comparative Example 1. Table 2 shows the result of comparing the therapeutic effect evaluated by the conventional method with that by the present evaluation method.

10

TABLE 2

Test substance	The number of fungus-negative feet/ Total number of infected feet	
	Conventional Method	Present evaluation method
Infected control	0/20	0/20
KP-103	19/20	17/20
Lanocanazole	20/20	3/20

In case of the group treated with KP-103, no significant difference was observed in the number of fungus-negative feet, even if the number was evaluated by either the conventional method or the present evaluation method, as shown in Table 2. The rate of a fungus-negative foot evaluated by the present evaluation method is 85 % in case of KP-103. On the other hand, in the group treated with lanocanazole, although “fungus-negative” was observed in all feet by the conventional method, but “fungus-negative” was just observed in only three feet by the present evaluation method.

20

As mentioned above, it came to appear that using the present

evaluation method, a true drug effect can be substantially evaluated without an affect by the remaining drug after the treatment therewith.

Furthermore, a result in the present evaluation method correlates with a result obtained by evaluation in the conventional method described in Comparative Example 1 in 30 days after the last
5 treatment. Thereby, by using the present evaluation method, an effect of an antimicrobial agent to prevent a relapse can be estimated by the evaluation at early time after a treatment. Therefore, a complete cure type of the antimicrobial agent without the relapse can be obtained by
10 using the present evaluation method.

[Pretreatment of Examples 4 and 5]

[1] Preparation of fungal solution and production of guinea pig model of tinea unguium and tinea pedis.

15 A fungal solution was prepared in the same manner as in the pretreatment of Comparative Example 1 except for changing *Trichophyton mentagrophytes* KD-04 strain to *Trichophyton mentagrophytes* SM-110 strain.

20 A guinea pig model of tinea unguium and tinea pedis was prepared in the same manner as in the above-mentioned preparation of the guinea pig model in interdigital tinea pedis except for changing male Hartley strain guinea pigs of 7 weeks age to male Hartley strain guinea pigs of 5 weeks age and except that the paper disc and the bandage was removed 21 days after the infection changing from seven days after the
25 infection. The invasion of dermatophytes in plantar skin and nail plate was observed 60 days after the infection.

[2] Preparation of drug solution and treatment of guinea pig of tinea

unguium and tinea pedis

As test compounds, solutions were prepared by dissolving raw powders of KP-103, amorolfine and terbinafine in a concentration of 1 % thereof to mixture solution of polyethylene glycole #400 : ethanol (75 : 25 v/v), respectively. Capsule of terbinafine was prepared by crushing the marketed tablet, suspending in the concentration of 100 mg/ml into Miglyol 812 (available from Mitsuba trade Co., Ltd) with glass homogenizer uniformly, and injecting the resulting suspension into each capsule in the concentration of 40 mg/kg depending on body weight measured on administration day. A solution of KP-103, amorolfine or terbinafine in the amount of 0.1 ml was applied a plantar skin and nail of one foot once a day for 30 consecutive days. In case of terbinafine capsule, one capsule (40 mg/kg) was administered orally.

15

EXAMPLE 4

Evaluation of drug effect on tinea unguium

The effect on tinea unguium was evaluated by the following method.

Animals were sacrificed two days after the last treatment. One hind foot was excised and wiped sufficiently with the cotton swab containing alcohol. Nails (three in total) of one hind foot was excised and miced by a scissors. It was transferred into glass homogenizer and was homogenized adding 4 ml double-concentration phosphate buffered saline (Phosphate Buffered Salts, available from Takara Shuzo Co., Ltd.) containing 4 % of trypsin derived from pig pancreas (available from BIOZYME Laboratories Limited). The reaction was carried out with shaking at 37°C for one hour. After a centrifugation, the obtaining

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precipitate was washed three times by centrifuging with phosphate buffered saline in order to remove trypsin. The precipitate was suspended with 4 ml of distilled water and put into dialysis membrane (fractional molecular weight: 12,000-14,000, made of cellulose, available from VISKASE SALES Corporation). Dialysis was carried out into 3 L of distilled water at 4°C for 14 days. Dialysis water was replaced twice a day 28 times in total. After a centrifugation, 1 ml of phosphate buffered saline was added to the precipitate obtained by removing the supernatant to prepare a suspension. This suspension was defined as stock solution and was diluted by tenfold. To Sabouraud dextrose agar medium (20 ml) containing the antibiotic substance described in Comparative Example 1 were added 100 µl of the stock solution or the dilution. After the cultivation was carried out for 10 days, the result was described as "fungus-negative" when no colony of fungus was observed in all medium (detection limit: 10 CFU/feet). The number of fungus-negative feet in the nail was counted. When the colony was appeared on the medium, the number of colonies (CFU) was counted to calculate the number of colonies in the nail of one foot by the dilution rate. After Kruskal Wallis Test was carried out for the number of fungi in the nail, the multiple comparison was carried out based on Tukey method to analysis the significant difference between groups. Those results were shown in Figure 3 and Table 3 thereof was made. In Figure 3, the number of CFU in nails in each treated group was plotted and the mean number of CFU was shown by horizontal line and numerical value.

Using the above-mentioned suspension, sufficient removal of the remaining drug was determined by the present evaluation method in the same manner as in Example 2.

EXAMPLE 5

Evaluation of drug effect on tinea pedis

Skin pieces of hind feet were excised from each animal described in Example 4. A removal of the drug and a determination of the remaining drug were carried out in the same as in Example 2 except that dialysis for removing the drug carried out for 3 days and that dialysis water was changed six times in total. The sufficient removal of the remaining drug was confirmed.

Then the drug effect was evaluated in the same manner as in Example 4 (detection limit: 20 CFU/feet). Those results were shown in Figure 4 and Table 4 thereof was made. In Figure 4, the number of CFU in the skin in each treated group was plotted and the mean number of CFU was shown by horizontal line and numerical value.

15

TABLE 3

Test substance	The number of feet with fungus-negative nail/ Total number of infected feet	Mean number of fungal cells in the nail (Log CFU \pm SD)
Vehicle for topical use	0/10	3.70 \pm 0.44
KP-103 solution	0/10	2.20 \pm 0.56 **
Amorolfine solution	0/10	3.26 \pm 0.54
Terbinafine solution	0/10	3.21 \pm 0.47
Vehicle for oral use	0/10	3.76 \pm 0.67
Oral preparation of terbinafine	0/10	3.80 \pm 0.44

** : significant difference versus the vehicle for topical use, the vehicle for oral use and the oral preparation of terbinafine in 0.01 % of significant level is shown.

As shown in Figure 3 and Table 3, no foot with fungus-negative nail was observed in all groups treated with substance tested for 30 days. But, KP-103 significantly reduced the number of fungal cells in the nail as compared with the vehicle for topical use. The therapeutic effect thereof was significantly superior to the oral preparation of terbinafine. On the other hand, no significant fungicidal effect was seen in amorolfine and terbinafine (for exteranl use, oral use) as compared with the vehicle. The therapeutic effect thereof was not seen. As mentioned above, it was suggested that KP-103 exhibited the therapeutic effect on tinea unguium by topical application and that KP-103 could cure tinea unguium earlier than the oral preparation of terbinafine.

TABLE 4

Test substance	The number of feet with fungus-negative skin/ Total number of infected feet	Mean number of fungal cells in the skin (Log CFU ± SD)
Vehicle for topical use	0/10	4.37 ± 0.33
KP-103 solution	10/10 **	< 1.3 **
Amorolfine solution	4/10	1.74 ± 0.45 *
Terbinafine solution	10/10 **	< 1.3 **
Vehicle for oral use	0/10	3.85 ± 0.68
Oral preparation of terbinafine	10/10 **	< 1.3 **

15 *: significant difference versus the vehicle for topical use in 0.05 % of significant level is shown.

** : significant difference versus the vehicle for topical use and the vehicle for oral use in 0.01 % of significant level is shown.

As shown in Figure 4 and Table 4, the excellent therapeutic effect on tinea pedis was seen in all drugs, KP-103, terbinafine and amorolfine in either case where it was evaluated by the rate of fungus-negative foot or where by the number of fungal cells in the skin. On the other hand, it became clear that KP-103 exhibited the excellent fungicidal effect on tinea unguium, although terbinafine and amorolfine did not exhibited the therapeutic effect on tinea unguium as shown in Figure 3 and Table 3.

10

INDUSTRIAL APPLICABILITY

As mentioned above, recently developed drugs having an extremely potent activity against *Trichophyton in vitro* such as lanoconazole brings about the judgement of fungus-negative according to the conventional method regardless of the existence of the no-treated fungus in the skin, since the drug remaining in the treated skin inhibits a growth of the fungus in the skin.

On the contrary, according to the present invention, an effect of an antimicrobial agent can be evaluated accurately, since a remaining drug can be removed by dialyzing the infected site with a microorganism of animal or biosample such as the treated skin using a dialysis membrane. Furthermore, although it is difficult to quantitatively compare of an antimicrobial effect such as an antifungal effect in conventional method, the present evaluation method enables the antimicrobial effects to compare quantitatively, since the number of viable fungi in the infected site of an animal or a bioample such as a skin can be determined precisely. In addition, the therapeutic effect based on the present evaluation method reflect a result as to relapse in the

20

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conventional method and therefore an effect to prevent relapse can be estimated by evaluating at earlier time after the treatment according to the present evaluation method. Therefore, in the present evaluation method, a true effect of an antimicrobial agent can be evaluated and it is possible to select an antimicrobial agent having an excellent sterilization effect against fungi *in vivo* or an antimicrobial agent of complete cure type which does not bring about relapse. As mentioned above, the present evaluation method is very useful as a method for evaluating the antimicrobial agent.

10 Additionally, in onychomycosis it is the first time that it is possible to evaluate a therapeutic effect against onychomycosis on a model of tinea unguium by the present evaluation method.

 As a result of the evaluation of the therapeutic effect against onychomycosis according to the present evaluation method, it comes to clear that KP-103 exhibits the excellent therapeutic effect against onychomycosis with a simple application on which the effect is not exhibited using the conventional topical antifungal agent. Therefore, KP-103 is a beneficial agent for treating onychomycosis, industrially.

CLAIMS

1. A method for detecting a pathogenic microorganism which comprises infecting an animal or a biosample with the pathogenic
5 microorganism, administering an antimicrobial agent comprising a compound having an antimicrobial effect or a composition thereof before or after the infection, then removing the antimicrobial agent, and thereafter detecting the viable pathogenic microorganism in the infected site with the pathogenic microorganism.

10

2. The method for detecting a pathogenic microorganism of Claim 1, in which the pathogenic microorganism is a bacterium or a fungus.

15

3. The method for detecting a pathogenic microorganism of Claim 2, in which the fungus is a pathogenic fungus causing superficial mycosis or deep mycosis.

20

4. The method for detecting a pathogenic microorganism of Claim 1, in which the antimicrobial agent is a therapeutic agent for superficial mycosis, a therapeutic agent for deep mycosis and an antibacterial agent.

25

5. The method for detecting a pathogenic microorganism of Claim 1, which comprises removing the antimicrobial agent using dialysis or ultra filtration.

6. The method for detecting a pathogenic microorganism of Claim 1, in which the infected site with the pathogenic microorganism is a skin or a nail.

5 7. The method for detecting a pathogenic microorganism of Claim 1, in which the administration of the antimicrobial agent is carried out percutaneously, orally or intravenously.

8. The method for detecting a pathogenic microorganism of
10 Claim 1, which comprises treating the infected site with the pathogenic microorganism with a digestive enzyme to detect the pathogenic microorganism.

9. A method for evaluating an effect of an antimicrobial agent
15 which comprises detecting a pathogenic microorganism according to the method for detecting the pathogenic microorganism of Claim 1, 2, 3, 4, 5, 6, 7 or 8.

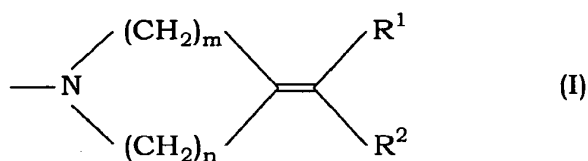
10. An antimicrobial agent obtained according to the method
20 for evaluating an effect of an antimicrobial agent of Claim 9.

11. A method for detecting an antimicrobial agent which
comprises infecting an animal or a biosample with a pathogenic
microorganism, administering an antimicrobial agent comprising a
25 compound having an antimicrobial effect or a composition thereof before or after the infection, then excising the infected site with the pathogenic microorganism, placing and cultivating it on a medium containing the

pathogenic microorganism, and thereafter detecting a existing the antimicrobial agent in the infected site with the pathogenic microorganism through a growth inhibition of the pathogenic microorganism observed around the infected site with the pathogenic
5 microorganism.

12. A therapeutic agent for onychomycosis comprising an antifungal agent compound having a group represented by the formula (I):

10



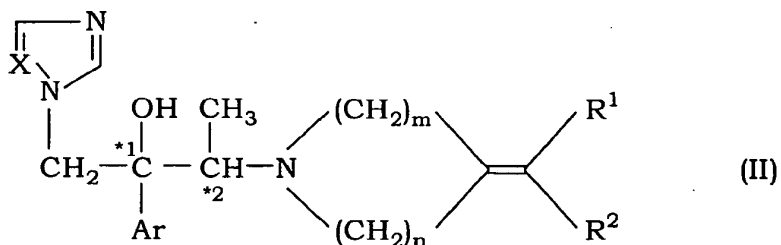
15 wherein, R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

20 m is 2 or 3,

n is 1 or 2,

or a salt thereof as an active ingredient.

13. The therapeutic agent for onychomycosis of Claim 12, in
25 which the compound is the compound represented by formula (II):



wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

10 R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

15 m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

20 14. The therapeutic agent for onychomycosis of Claim 13, in which the compound represented by the formula (II) is (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl) butane-2-ol.

25 15. A method for evaluating an effect of an antifungal agent which comprises administering the antifungal agent to a patient whose a skin or a nail is infected with fungus, thereafter excising the keratin

substance or the nail, and then detecting the viable fungus in the keratin substance or the nail after removing the antifungal agent.

16. A method for detecting an antifungal agent which
5 comprises administering the antifungal agent to a patient whose a skin
or nail is infected with fungus, thereafter excising the keratin substance
or the nail, placing and cultivating it on a medium containing the fungus,
and then detecting a existing antifungal agent in the keratin substance
or the nail through a growth inhibition of the fungus observed around
10 the keratin substance or the nail.

17. An antifungal agent obtained according to the method for
evaluating an effect of the antifungal agent of Claim 15.

ABSTRACT

A novel method for evaluating an effect of an antimicrobial agent which comprises removing the antimicrobial agent remaining in a biological sample or the like to thereby accurately evaluate the effect of
5 the antimicrobial agent without being affected by the remaining antimicrobial agent. A therapeutic agent for onychomycosis which can be obtained according to the evaluation method of the drug effect.

FIG. 1(a)

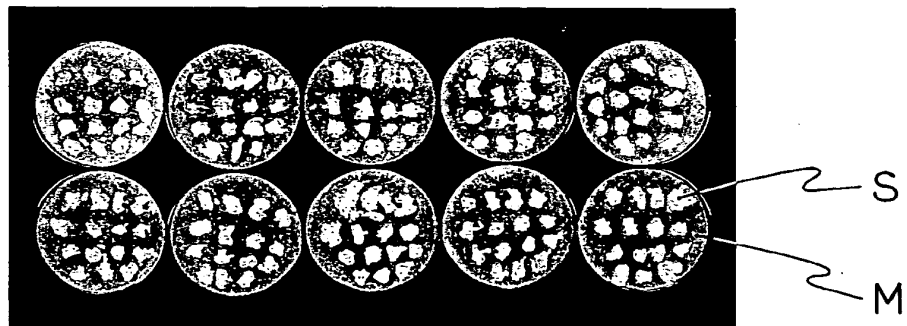


FIG. 1(b)

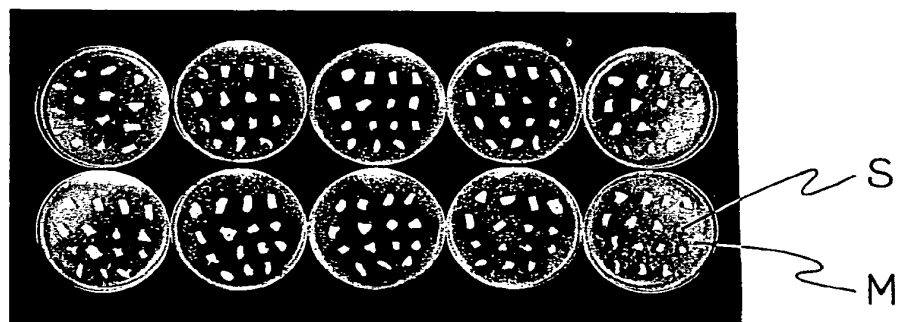


FIG. 1(c)

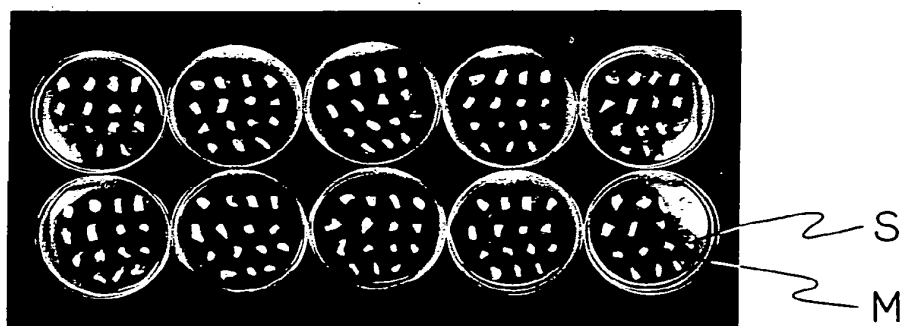


FIG. 2(a)

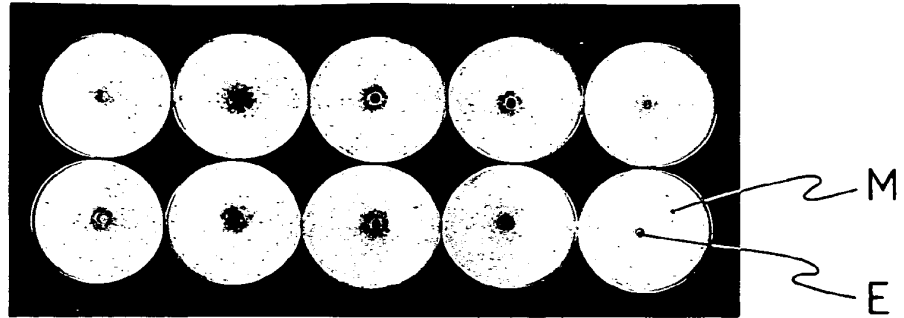


FIG. 2(b)

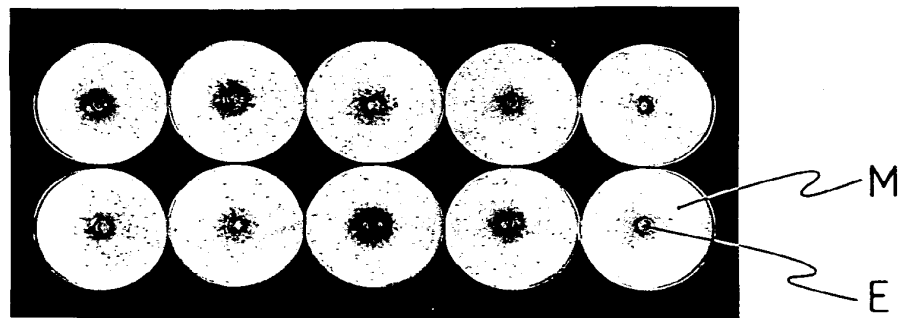


FIG. 2(c)

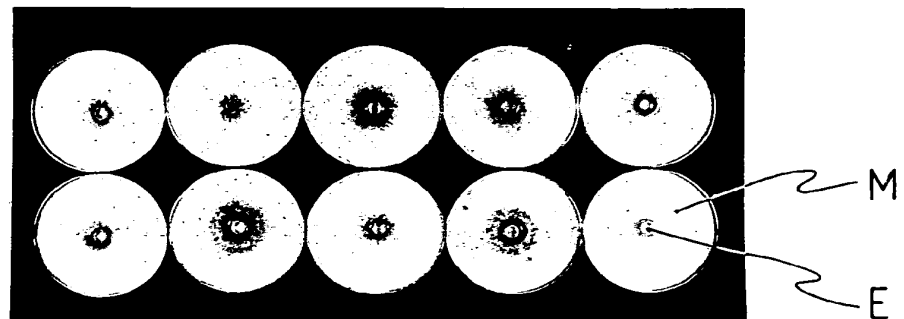
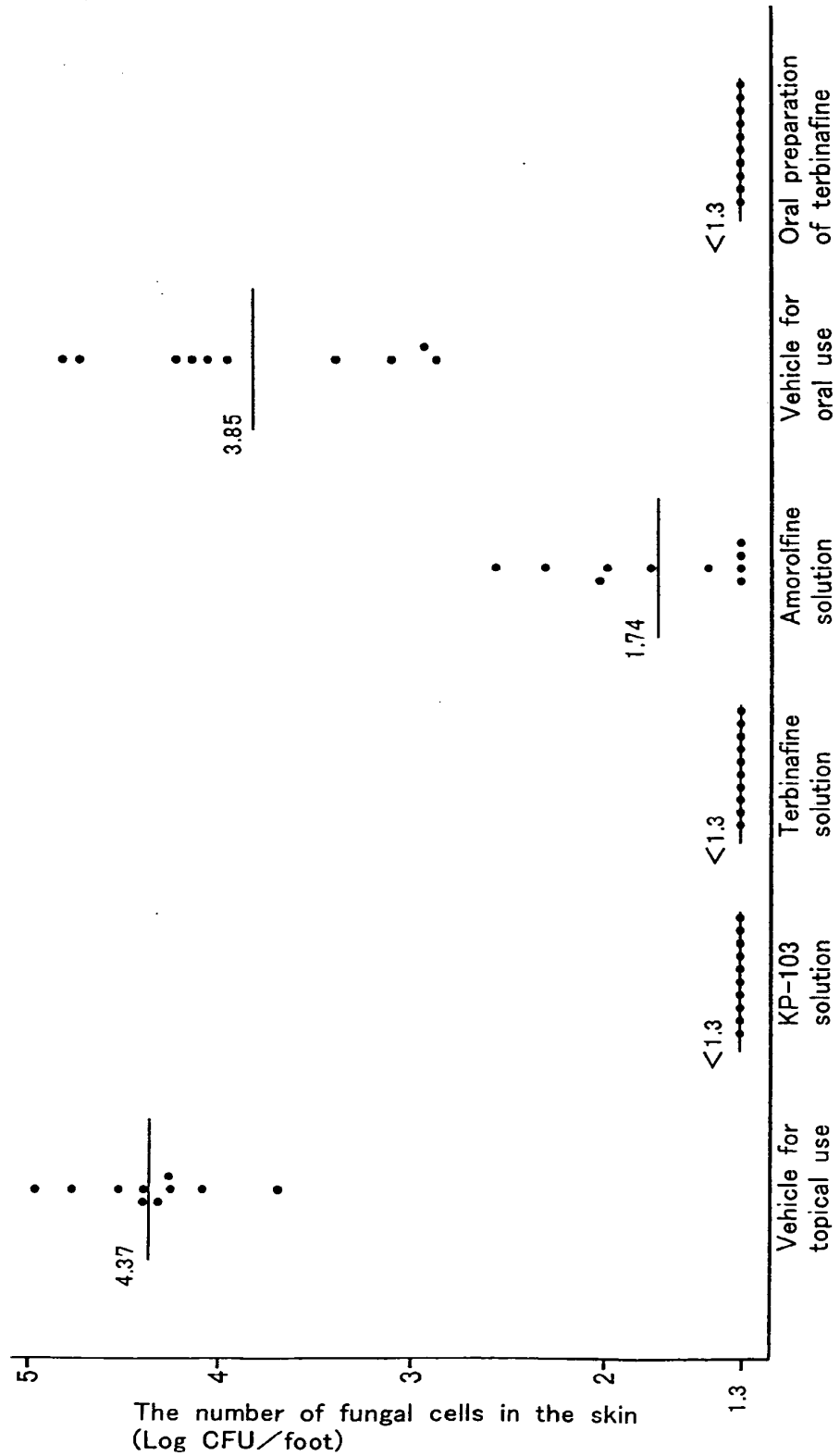


FIG. 4



DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed at 201) below or an original, first and joint inventor (if plural names are listed at 201-208 below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

which is described and claimed in:

- the specification attached hereto.
- the specification in U.S. Application Serial Number _____ filed on _____; and
- the specification in PCT international application Number PCT/JP00/0461Z, filed on July 11, 2000; and was amended on _____.

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a). I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign/PCT Applications and Any Priority Claims Under 35 U.S.C. §119:			
Application No.	Filing Date	Country	Priority Claimed Under 35 U.S.C. §119?
214369/1999	July 28, 1999	Japan	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO
			<input type="checkbox"/> YES <input type="checkbox"/> NO

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose material information as defined in 37 CFR §1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Prior U.S. Applications or PCT International Applications Designating the U.S-Benefit Under 35 U.S.C. §120				
U.S. Applications		Status (Check One)		
Application Serial No.	U.S. Filing Date	Patented	Pending	Abandoned
PCT Applications Designating the U.S.				
Application No.	Filing Date	U.S. Serial No. Assigned		

**CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. §119(e))**

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

Applicant	Provisional Application Number	Filing Date

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) with full powers of association, substitution and revocation to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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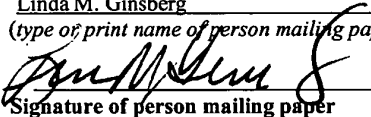
2 0 5	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY AND ZIP CODE

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signature of Inventor 201 <i>Yoshiyuki Tatsumi</i>	Date: December 28, 2001
Signature of Inventor 202 <i>Mamoru Yokoo</i>	Date: December 28, 2001
Signature of Inventor 203 <i>Kosho Nakamura</i>	Date: December 28, 2001
Signature of Inventor 204 <i>Tadashi Arika</i>	Date: December 28, 2001
Signature of Inventor 205	Date:

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Tatsumi et al.
Application No.: TBA (divisional of 10/031,929) Group No.: To be Assigned (1651)
Filed: Herewith Examiner: To be assigned (Kailash C. Srivastava)
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

CERTIFICATE OF EXPRESS MAILING (37 C.F.R. SECTION 1.10)	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" on the date shown below in an envelope addressed to Mail Stop Patent Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.	
October 14, 2003 Date	Linda M. Ginsberg <i>(type or print name of person mailing paper)</i>  Signature of person mailing paper

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**APPLICATION DATA SHEET
37 C.F.R. § 1.76
BIBLIOGRAPHIC DATA**

1. Applicant information

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Country of Citizenship: Japan
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Otsu-shi
SHIGA 520-0102
JAPAN

In re application of: Tatsumi et al.
Application No.: TBA (divisional of 10/031,929) Group No.: To be Assigned (1651)
Filed: Herewith Examiner: To be assigned (Kailash C. Srivastava)
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

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Customer No.: 26248

3. Application information

Title of Invention: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

Docket number assigned to this application: 700938-052220-DIV

Suggested Classification: Class:
Subclass:
Technology Center to which subject matter is assigned:

Total number of drawing sheets: 4
Type of application: Divisional

Application is to be published. Suggested drawing figure for publication:

Secrecy order under § 5.2:

This application is not subject matter of an application which is under a secrecy order pursuant to § 5.2.

In re application of: Tatsumi et al.
 Application No.: TBA (divisional of 10/031,929) Group No.: To be Assigned (1651)
 Filed: Herewith Examiner: To be assigned (Kailash C. Srivastava)
 For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
 ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
 ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

4. Representative information

The following have a power of attorney or authorization of agent in this application:

Ronald I. Eisenstein	(Reg. No. 30,628)	David S. Resnick	(Reg. No. 34,235)
Michael L. Goldman	(Reg. No. 30,727)	Nicole L.M. Valtz	(Reg. No. 47,150)
Georgia Evans	(Reg. No. 44,957)	Joseph Noto	(Reg. No. 32,163)
Gunnar G. Leinberg	(Reg. No. 35,584)	Edwin V. Merkel	(Reg. No. 40,087)
Leena Karttunen	((37 C.F.R. Sec. 10.9(b))		

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 Customer No.: 26248

5. Priority information

This application is a divisional under 35 U.S.C. § 120 of copending application 10/031,929, filed January 25, 2002, which is the National Stage under 35 U.S.C. § 371 of International Application no. PCT/JP00/04617, filed July 11, 2000, which claims the benefit under 35 U.S.C. § 119 of Japanese Application No. 214369/1999, filed July 28, 1999.


7. Assignee information

The assignee(s) of this application is/are:

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 Bunkyo-ku
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 JAPAN

Extent of interest of assignee in application: Entire

Date: October 14, 2003


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Docket No. 700938-052220-DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi et al.

Application No.: TBA (Divisional of 10/031,929) Group No.: TBA (1651)

Filed: Herewith

Examiner: TBA (Srivastava, Kailash C.)

For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

MAIL STOP PATENT APPLICATION

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

This Preliminary Amendment is being filed in the U.S. Patent and Trademark Office concurrently with the divisional application in the above-identified matter. Preliminary to the calculation of the filing fee and examination on the merits, please amend the application identified in caption as follows:

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims begin on page 3 of this paper.

Remarks begin on page 5 of this paper.

In re application of: Tatsumi et al.
Application No: TBA (divisional of 10/031,929)
Preliminary Amendment dated October 14, 2003

Group No.: TBA (1651)
Examiner: TBA (Srivastava, K.)

Amendments to the Specification:

On page 1, before line 1, please add the following heading and paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional under 35 U.S.C. § 120 of copending application 10/031,929, filed January 25, 2002, which is the National Stage under 35 U.S.C. § 371 of International Application no. PCT/JP00/04617, filed July 11, 2000, which claims the benefit under 35 U.S.C. § 119 of Japanese Application No. 214369/1999, filed July 28, 1999.

Amendments to the Claims:

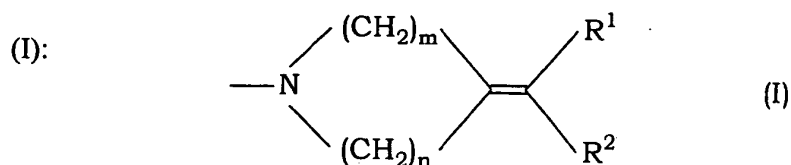
Prior to examination of the above-described application please amend the claims as follows. This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of the Claims:

Claim 1 (ORIGINAL): A method for detecting a pathogenic microorganism which comprises infecting an animal or a biosample with the pathogenic microorganism, administering an antimicrobial agent comprising a compound having an antimicrobial effect or a composition thereof before or after the infection, then removing the antimicrobial agent, and thereafter detecting the viable pathogenic microorganism in the infected site with the pathogenic microorganism.

Claims 2 – 17 (CANCELLED).

Claim 18 (NEW): A method for treating subject having onychomycosis comprising administering an effective amount of an antifungal agent compound having a group represented by the formula



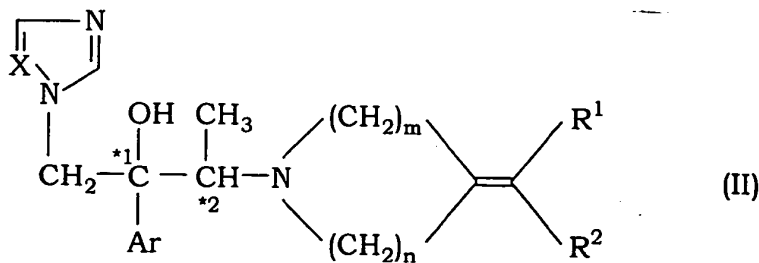
wherein, R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

or a salt thereof as an active ingredient, to a subject having onychomycosis.

Claim 19 (NEW): The method of Claim 18, in which the compound is the compound represented by formula (II):



In re application of: Tatsumi et al.
Application No: TBA (divisional of 10/031,929)
Preliminary Amendment dated October 14, 2003

Group No.: TBA (1651)
Examiner: TBA (Srivastava, K.)

wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₁₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

Claim 20 (NEW): The method of Claim 19, in which the compound represented by the formula (II) is (2R,3R)-2-(2, 4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl) butane-2-ol.

REMARKS

Applicants have amended the specification to comply with the provisions of 35 U.S.C. § 120. As such, these amendments do not constitute new matter and their entry is respectfully requested.

Applicants have rewritten claims 12 through 14 in method form. These claims are supported throughout the specification, see particularly pages 16 – 19 and the examples. As such these amendments do not constitute new matter.

In parent application U.S.S.N. 10/031,929, certain rejections of claims 12 – 14 were made. Although those claims specified that the specified use of the claimed antifungal agent was “a therapeutic agent for onychomycosis” because they were compound claims, this intended use did not effect the Examiner’s citation of art against those claims. Applicants respectfully submit that none of the references cited therein are applicable to the present method claims. Indeed, the Examiner implicitly acknowledges this at page 3 of the Office Action (mailed July 18, 2003 in the parent application), stating:

Even though the antifungal/fungicidal composition taught in the examiner-cited prior art do not refer to “therapeutic agent for onychomycosis”, to artisan of ordinary skill the claims remain anticipated by the examiner-cited art because the functional intended use of a composition does not materially change a composition and is accordingly, not given any patentable weight.

Here, however, the claims are directed to methods for treating such conditions and the failure of the Examiner-cited prior art to suggest such a method does result in patentability.

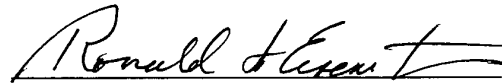
In re application of: Tatsumi et al.
Application No: TBA (divisional of 10/031,929)
Preliminary Amendment dated October 14, 2003

Group No.: TBA (1651)
Examiner: TBA (Srivastava, K.)

Accordingly, Applicants respectfully submit that the present claims are in condition for allowance. Early and favorable action is requested.

Respectfully submitted,

Date: 10/14/03



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Practitioner's Docket No. 700938-052220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): Tatsumi et al.

Group: TBA (1651)

Serial No.: TBA (Divisional of 10/031,929)

Examiner: TBA (Srivastava)

Filed: Herewith

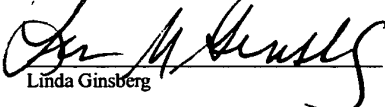
FOR: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

MAIL STOP PATENT APPLICATION

Commissioner for Patents

P.O. Box 1450

Alexandria, VA 22313-1450

CERTIFICATE OF MAILING	
I hereby certify that this correspondence, on the date shown below, is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Mail Stop Patent Application, Commissioner of Patents, P.O. Box 1450, Alexandria, VA 22313-1450.	
Date: <u>10/14/03</u>	 Linda Ginsberg

Sir:

INFORMATION DISCLOSURE STATEMENT

In accordance with the provisions of 37 C.F.R. §§1.56 and 1.97, Applicants herewith submit the publications and/or patents shown on the attached form PTO-1449, for consideration by the Examiner in connection with the examination of the above-identified patent application.

REMARKS

In accordance with the provisions of 37 C.F.R. §1.97, this statement is being filed:

- ___ (1) with the filing of this divisional utility application.

- (2) within three (3) months of the Filing Date or **before the mailing date of the First Office Action** on the merits; or
- (3) within three months of the mailing date of the PCT International Search Report; or
- (4) after the period defined in (1) but before the mailing date of a **Final Rejection** or **Notice of Allowance**, and the requisite Certification or fee under Rule 1.17(p), namely **\$240.00**, is included herein; or
- (5) after the mailing date of a **Final Rejection** or **Notice of Allowance** but before the payment of the **Issue Fee**, and the requisite Certification, petition, and petition fee are included herein.

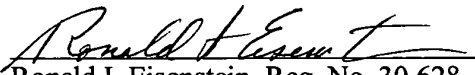
It is respectfully requested that each of the documents shown on the attached form(s) PTO-1449 be made of record in this application. Copies of these documents (CHECK ONE):

- are enclosed herewith;
 - have been cited in the parent application, and are thus not being resubmitted herein.
- Early examination and allowance of the present application are respectfully solicited.

FEE AUTHORIZATION

Should any fees associated with the submission be required, the Commissioner is authorized to charge the missing fee to our Deposit Account No. 50-0850. Any overpayments should be credited to said Deposit Account.

Respectfully submitted,


 Ronald I. Eisenstein, Reg. No. 30,628
 NIXON PEABODY LLP
 101 Federal Street
 Boston, MA 02110
 (617) 345-6054

Date: 10/14/03

FORM PTO-1449 INFORMATION DISCLOSURE STATEMENT	DOCKET NO. 700938-52220- DIV	SERIAL NO.: TBA (Divisional of 10/031,929)
	APPLICANT(S): Tatsumi et al.	
	FILING DATE: Herewith	GROUP NO.: TBA (1651)

UNITED STATES PATENT DOCUMENTS

EXAM. INITIALS	DOCUMENT NUMBER	DATE	INVENTOR/ASSIGNEE	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
AA	5,962,476	10/5/99	Naito et al.			
AB	5,716,969	2/10/98	Naito et al.			
AC	5,620,994	4/15/97	Naito et al.			

FOREIGN PATENT DOCUMENTS

	DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES/NO
BA	8-103291	4/23/96	Japan (and English abstract attached)			Y
BB	10-28597	2/3/98	Japan (and English abstract attached)			Y
BC	WO 94/26734 * U.S. Patents which are National Phase Entry of above are 5,962,476 and 5,716,969	11/24/94	PCT			*Yes

OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)

CA	

EXAMINER:	DATE:
-----------	-------

BA

L1 ANSWER 1 OF 1 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD

Full
Text

AN 1996-253890 [26] WPIDS

DNN N1996-213392 DNC C1996-080384

TI Evaluating antifungal agent quantitatively - comprises implanting fungi causing disease in skin, admin. of antifungal agent, cultivating and measuring scale of formed colony..

DC B04 C07 D16 S03

PA (POKK) POLA CHEM IND INC

CYC 1

PI JP--08103291 A 19960423 (199626)* 4p

ADT JP--08103291 A 1994JP-0239983 19941004

PRAI 1994JP-0239983 19941004

AN 1996-253890 [26] WPIDS

AB JP 08103291 A UPAB: 19960705

Evaluating an antifungal agent comprises implanting fungi causing disease in skin and administering antifungal agent, followed by cultivation, and measuring the scale of the colony formed.

ADVANTAGE - Quantitative evaluation of antifungal agent can be effected.

Dwg. 0/0

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平8-103291

(43) 公開日 平成8年(1996)4月23日

(51) Int. Cl.	識別記号	庁内整理番号	F I	技術表示箇所
C12Q 1/06		6807-4B		
G01N 33/15		B		

審査請求 未請求 請求項の数 3 O L (全4頁)

(21) 出願番号 特願平6-239983

(22) 出願日 平成6年(1994)10月4日

特許法第30条第1項適用申請有り 平成6年8月31日、
日本医真菌学会発行の「第38回日本医真菌学会総会プログラム・抄録集」に発表

(71) 出願人 000113470

ポーラ化成工業株式会社
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(74) 代理人 弁理士 有賀 三幸 (外3名)

(54) 【発明の名称】 抗真菌剤の評価法

(57) 【要約】

【構成】 動物の皮膚に表在性真菌症原因菌を移植した後又は当該移植前に被検体を投与し、しかる後に皮膚より採取した小断片を培養し、当該培地上に生育したコロニーの大きさを測定して皮膚内の真菌を定量する抗真菌剤の評価法。

【効果】 抗真菌作用の定量的比較、貯留性の比較が可能なので、抗真菌剤の開発に大変有益である。

【特許請求の範囲】

【請求項1】 動物の皮膚に表在性真菌症原因菌を移植した後、被検体を投与し、しかる後に皮膚より採取した小断片を培養し、当該培地上に生育したコロニーの大きさを測定して皮膚内の真菌を定量することを特徴とする抗真菌剤の評価法。

【請求項2】 動物に被検体を投与した後、表在性真菌症原因菌を動物の皮膚に移植し、しかる後に皮膚より採取した小断片を培養し、当該培地上に生育したコロニーの大きさを測定して皮膚内の真菌を定量することを特徴とする抗真菌剤の評価法。

【請求項3】 皮膚内の真菌の定量が、測定したコロニーの大きさを、別個に作成された検量線と対比して行うものである請求項1又は2記載の評価法。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、抗真菌剤の評価法に関し、更に詳しくは、皮膚内の真菌数を正確に定量することによる正確な抗真菌剤の評価法に関する。

【0002】

【従来の技術】本邦に於ける表在性真菌症患者は1200から1800万人と試算されている。患者が多い原因は生活環境、生活形態、生活習慣に起因すると考えられる真菌の伝播力の強さと、繰り返し感染蔓延する根治の困難さ等が考えられる。これは、有効な薬剤の開発が遅々としているためであり、その原因はかかる真菌の生育が皮膚内であるため、薬効発現に薬物動態が複雑に絡み、なおかつ皮膚内に於ける真菌の適切な定量方法がなかった為である。皮膚内の真菌の定量が正確にできないために適切な薬物の評価ができなかったのである。

【0003】従来、皮膚内の真菌の定量は皮膚を採取して裁断し、複数の小断片を取り出し、これを培地中に植え、これより真菌の生えてきた断片の数を計数し、全断片数で除した値を指標として用いていた。しかしながら、この方法では数値が1を断片数で除した値の倍数にしかならず、従って不連続であり、更に、少量しか生えてこない断片も、多量に生えた断片も同じ扱いになってしまうので、この方法では、皮膚片に於ける真菌の有無は判定できても、正しい定量はできなかつた。したがって、抗真菌剤の評価も偶然性によって揺れることが多く、たまたま真菌の存在していない部位を採取すると優れた抗真菌作用を有すると誤認されることも少なくなかつた。更に、実際には既存薬より優れた抗真菌作用を有している抗真菌剤でも有意差なしと判断されて上市にいたらなかつた可能性があつたことも否めない。更に、抗真菌剤の各種のイン・ビトロの評価法も考案されているが、強固な生体の防壁である、皮膚内に生息する真菌に対する抗真菌作用の評価方法としてイン・ビトロの評価法が適していないことは言うまでもない。

【0004】

【発明が解決しようとする課題】従って、本発明は的確な抗真菌剤の評価が行える評価法を提供することを目的とする。

【0005】

【課題を解決するための手段】上記実状を踏まえ、本発明者らは的確な抗真菌作用の評価は的確な皮膚内の真菌の定量に依存すると考え、かかる手段を求め鋭意研究を重ねた結果、皮膚中に存在する真菌数と真菌を有する皮膚を培地中で培養して得られたコロニーの大きさの間に極めて良好な相関関係があることを見いだして発明を完成させた。

【0006】すなわち、本発明は、動物の皮膚に表在性真菌症原因菌を移植した後、被検体を投与し、しかる後に皮膚より採取した小断片を培養し、当該培地上に生育したコロニーの大きさを測定して皮膚内の真菌を定量することを特徴とする抗真菌剤の評価法を提供するものである。また、本発明は、動物に被検体を投与した後、表在性真菌症原因菌を移植し、しかる後に皮膚より採取した小断片を培養し、当該培地上に生育したコロニーの大きさを測定して皮膚内の真菌を定量することを特徴とする抗真菌剤の評価法を提供するものである。

【0007】本発明において、抗真菌剤という語は、

1) 抗真菌作用を有する化合物、2) 1)の化合物を含む組成物の両者を意味し、組成物には人為的な組成物である剤形と天然抽出物など的人為的でない組成物が含まれる。

【0008】ここで、本発明の抗真菌作用の評価法の対象となる真菌は、表在性真菌症原因菌であるが、これを具体的に例示するならば、トリコフィトン属 (*Trichophyton*)、ミクロスポーラム属 (*Microsporium*)、エピデルモフィトン属 (*Epidermophyton*) 等の不完全糸状菌やキャンディダ属 (*Candida*)、マラセチア属 (*Malassezia*) の不完全酵母、及びこれらの変異株が挙げられる。この様な変異株としては、自然に薬物に対して耐性を獲得した耐性株、栄養依存性を有するようになった栄養依存性変異株、遺伝子導入などを行い人為的に変異させた人工変異株等が例示できる。

【0009】本発明方法に用いられる動物としては、哺乳類、例えばラット、モルモット、ウサギ、マウス、ブタ等が挙げられる。これらの動物の皮膚への真菌の移植方法としては、真菌を皮膚上に塗布する方法、真皮を露出させて当該真皮上に塗布する方法、クローズドパッチ法、皮内注射法等が挙げられるが、再現性の点よりクローズドパッチ法が好ましい。

【0010】被検体の投与は、被検体の種類によって異なり、経皮投与、経口投与、静脈内投与等が挙げられるが、経皮投与が好ましい。

【0011】本発明方法において、真菌の移植と被検体の投与とは、いずれを先に行ってもよい。真菌の移植後に被検体の投与を行えば、抗真菌剤の治療効果が評価で

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きる。一方、被検体の投与を行った後真菌を移植する場合は、抗真菌剤の感染予防作用あるいは抗真菌剤の皮膚貯留性が評価できる。抗真菌剤の皮膚貯留性を評価する場合には、被検体投与後、真菌移植までの期間を変化させるのが好ましい。

【0012】皮膚より小断片を採取するには、必要に応じて除毛した後、適当な大きさの皮膚を切り出した後1断片あたり1×1～20×20mmに切断すればよい。この小断片は、真菌以外の細菌を除去する目的で塩化ベンザルコニウム、ヒピテングルコネート等の殺菌剤溶液で洗浄するのが好ましい。

【0013】得られた小断片の培養に用いる培地としては、通常培養や菌分離等に用いているものであれば特に限定はなく、例えば、サブロー培地、改変サブロー培地、ツァベック寒天培地等が例示できる。

【0014】培養は、10～40℃、好ましくは20～40℃でコロニーが生育するのに十分な時間、例えば1～20日間静置培養すればよい。

【0015】培養後、培地上に生育したコロニーの大きさを測定する。コロニーの大きさの測定は、長径(1)及び短径(s)を計測し、その積(1×s)を求めて、コントロールと対比するのが簡便で好ましい。

【0016】真菌数の定量は、コロニーの大きさを、別個に作成された検量線と対比して行うことができる。この検量線はコロニーの大きさと真菌数との間の相関性を示す検量線であり、例えば所定の菌数の真菌を前記と同一の条件で培養し、生育したコロニーの大きさを測定することにより作成できる。ここで菌数の計測法としては、菌体全体をニュートラルレッドの様な染色剤で染色し、その着色度により菌数を計量しても良いし、同位体などでマークした栄養素の代謝を放射活性より定量して菌数を測定しても良い。最も好適な菌数の計数方法は、菌体と分生子を分離し、血球計数板等で分生子を計数しこれを菌数のコントロールとすることである。この方法によれば、極めて簡易に再現性良く菌数のコントロールを作ることができる。ここで用いるコントロール真菌は、標準株でも臨床分離株でも良い。

【0017】また、皮膚からの小断片の採取を経時的に2度以上行い、それぞれの皮膚内の真菌数を定量すれば、作用の持続性等の抗真菌剤投与後の動態特性を評価することができる。更に、本発明において被検体として、組成物を用いた場合には、その組成物としての評価(例えば、基剤の評価、剤形の評価など)ができる。

【0018】

【実施例】以下に実施例を挙げて更に詳しく本発明について説明するが、本発明がこれら実施例に何等限定されないことは言うまでもない。

【0019】実施例1

皮膚内真菌の測定例

(1) コントロール系列の作成

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トリコフィトンTIMM1189株(Trichophyton mentagrophytes TIMM1189)を改変サブロー寒天スラントに接種し、27℃で21日培養した。培養後、スラントに滅菌した磷酸緩衝液(0.1重量%の界面活性剤(ツイーン80)を含む)を加え、スラント表面を白金耳で擦り取った。次に菌液は滅菌ガーゼを通し菌糸を除き分生子を得た。分生子は血球計算盤でカウントし 2×10^7 cfu/mlに調整した。この後、同じ磷酸緩衝液を用いて菌液の10倍希釈液列を作成した。(分生子希釈液はサブロー寒天シャーレに100μlづつ播種し27℃で培養し、3日目、5日目、7日目にそれぞれ生菌数を算定した。)この操作と平行してサブロー寒天平板を作成した。本サブロー寒天にはシクロヘキシミド100μg/ml、シソマイシン50μg/ml、クロラムフェニコール100μg/mlを溶かし込んで作成した。これら抗生物質は細菌の生育をさせない一方、真菌の生育には支障の無い条件で設定したが、これら以外の抗生物質の組合せでサブロー寒天平板を作成しても構わない。次に、サブロー寒天に滅菌したスパーテル等で幅2mm、長さ10mmの溝を彫った(彫った溝の寒天は平板から除いた)。この溝に分生子希釈液をそれぞれ10μl入れ、27℃で5日間培養し生育コロニーを測定し長径(1)と短径(s)をノギスで計測した。これら1とsを掛け合わせた値A値を片対数グラフの正数座標に、その溝に播種した生菌数を対数座標にプロットした。この作業について、4回接種菌数を交え繰り返した。このうち2回は同一接種菌数で行った。これらの結果を図1～3に示す。これらはどれも直線上にプロットされており、非常に相関性が良いことが明白である。更に再現性も良好であることが分かる。

【0020】(2) モルモット皮膚内の真菌数の定量
ハートレー系5週令雌モルモットの背部左右2カ所を電気バリカンで除毛後、ガムテープを用いストリッピングして真皮を露出させた。次に、トリコフィトンTIMM1189株(Trichophyton mentagrophytes TIMM1189)由来分生子を 1×10^7 cfuモルモット背部皮膚露出部分(直径2cmの円形状)に接種した。菌接種13日目及び20日目にモルモットを屠殺し患部皮膚を直径2cmの円形状に切り取り、1%殺菌剤で洗浄後、滅菌水ですすぎ表面の雑菌を除き、摘出皮膚はハサミで10等分した。この皮膚切片は上の項で述べた別のサブロー寒天平板に埋め込み27℃で培養した。培養5日目にモルモット5匹左右10カ所の菌生育域を上項で述べた方法で、全ての切片につきA値を算出した。これを1部位毎10切片平均値を算出後、他の部位との平均値(Avg±SD)を求めた。モルモットに菌接種13日の菌生育域は1切片あたり平均1.764±0.231、20日のそれは0.846±0.763であった。同時に培養したコントロールとのA値の比較より、1切片当たりの菌数は450cfuと算出された。

【0021】実施例2

ケラチンの影響の確認

皮膚内の真菌の定量を行うに当たって考慮すべきことは、皮膚の構成蛋白であるケラチンが菌の定量に悪影響を与えるか否かである。そこで、実施例1の(1)の作業について、ケラチンのあり、なしでの相関性を検討した。結果を表1に示す。これより、本発明の定量法はケラチンの有無に関わらず、植えた分生子の数と生成したコロニーの大きさの間に極めて良い相関関係を有している。従って、本発明の定量法は皮膚構成蛋白に影響されることなく皮膚内の真菌を定量できることが分かる。

【0022】

【表1】

分生子数	10 ⁴	10 ³	10 ²	10 ¹	10 ⁰	10 ⁻¹	相関係数
ケラチンあり	3.6	2.5	1.7	0.6	0.1	0	0.97
ケラチンなし	4	2.2	1.6	0.7	0.1	0	0.96

【0023】実施例3

剤形による抗真菌作用の違いの評価

上記の手法に則り、抗真菌作用の知られている化合物である、ピフオナゾールの剤形による抗真菌作用の違いの評価を行った。用いた2つの剤形は親水軟膏をベヒクルとしたものとエタノール溶液をベヒクルにしたものであった。薬物投与は感染5日後から1週間0.3g/日投与した。治療後2日に屠殺し皮膚を取り出した。動物実験での従来法、即ち、コロニーを生じた皮膚片の出現率を見る方法では、表2に示した結果の如く、剤形による効果の差はもとより、抗真菌剤の抗真菌作用すら見いだせなかった。一方、本発明の評価方法によれば、剤形の差による抗真菌作用は表3に示すが如く、親水軟膏ベースの剤形の方が優れている事が明らかに判る。更に、ピフオナゾールの抗真菌作用も *in vivo* で明確に確認できる。従って、本発明の評価方法が実状を良く反映し、的確に評価できる事が明らかである。

【0024】

【表2】

検体	菌陽性率(%)
無処置	100
ピフオナゾール-親水軟膏	96
親水軟膏	100
ピフオナゾール-エタノール	99
エタノール	100

【0025】

【表3】

検体	平均菌数(cfu)
無処置	200
ピフオナゾール-親水軟膏	2
親水軟膏	100
ピフオナゾール-エタノール	5
エタノール	120

【0026】実施例4

分画中の抗真菌成分のスクリーニング例

抗真菌作用があることで知られている微生物代謝物の分画A(ブタノール分画)と分画B(水分画)について抗真菌作用を調べた。分画Aは4%濃度で、分画Bは2%と4%の濃度で実施例3と同様の処置をした。本評価方法による結果を表4に示す。抗真菌作用を有する物質はA分画に多く含有されている事が如実に判る。尚、従来の評価方法では何れも100%の菌陽性率を示し、分画方法を是非や抗真菌物質の存在を知る事が出来なかった。これより、本発明の評価方法が優れている事が明白である。

【0027】

【表4】

検体	平均菌数(cfu)
ベヒクル	200
分画A	30
分画B 4%	60
分画B 2%	200

【0028】

【発明の効果】本発明の評価法によれば、抗真菌作用の定量的比較、貯留性の比較が可能なので、抗真菌剤の開発に大変有益である。

BB

Full
Text

AN 1998-162531 [15] WPIDS

DNN N1998-129298 DNC C1998-052383

T1 Determining skin irritation - comprises treating skin model with sample substance, removing sample substance and culturing skin model in large amount of culture liquid.

DC B04 D16 S03

PA (GNZE) GUNZE KK

CYC 1

PI JP--10028597 A 19980203 (199815)* 9p

JP---3129662 B2 20010131 (200109) 8p

ADT JP--10028597 A 1996JP-0206509 19960716; JP---3129662 B2 1996JP-0206509 19960716

FDT JP---3129662 B2 Previous Publ. JP--10028597

PRAI 1996JP-0206509 19960716

AN 1998-162531 [15] WPIDS

AB JP 10028597 A UPAB: 19980410

Determining skin irritation comprises treating a skin model with a sample substance, removing the sample substance and culturing the skin model in a large amount of culture liquid.

ADVANTAGE - The method can substitute a skin irritation test using an animal. In an example, 0.5 g chloroform was added to 50 g of 3 mg/ml Type I collagen solution and homogenised at 6000 rpm for 1 minute and poured in a stainless steel frame and frozen at -40 deg. C and freeze-dried at 30 deg. C for 24 hours under 0.01 mmHg. It was further heated at 105 deg. C for 24 hours to effect dehydrative crosslinking. Then, it was immersed in 0.2 % glutaraldehyde solution for 24 hours to induce chemical crosslinking. It was again freeze-dried to give a highly crosslinked collagen sponge of a pore size of 90 mu m and 3 mm thickness. A low crosslinked collagen sponge of a pore size of 30 mu m and 1 mm thickness was also prepared. Human fibroblast was inoculated on the highly crosslinked collagen sponge and then cultured overnight and then the low crosslinked collagen sponge was placed on it and human keratinised cell was inoculated on it and cultured overnight to prepare a skin model.

Dwg. 0/6

(19) 日本国特許庁 (J P)

(12) 公開特許公報 (A)

(11) 特許出願公開番号

特開平10-28597

(43) 公開日 平成10年(1998) 2月3日

(51) Int. Cl. *	識別記号	庁内整理番号	F I	技術表示箇所
C12Q 1/02		9452-4B	C12Q 1/02	
C12N 5/06			G01N 33/15	Z
G01N 33/15			C12N 5/00	E

審査請求 未請求 請求項の数 2 F D (全 9 頁)

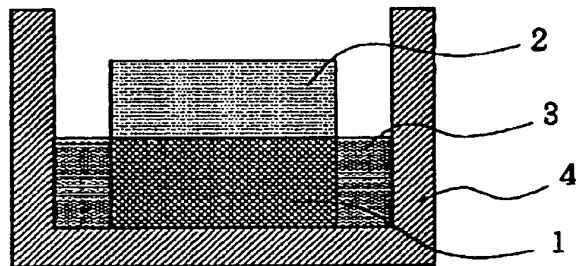
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(54) 【発明の名称】 皮膚刺激判定法

(57) 【要約】 (修正有)

【課題】 本発明は、動物を用いた皮膚刺激性試験を代替し、かつ動物実験のデータと非常によく合致する皮膚刺激判定法を提供する。

【解決手段】 皮膚細胞を含む表皮・真皮の二層構造よりなる皮膚モデルを、界面活性剤等の被検物質で処理した後、かかる被検物質を除去し、更に該皮膚モデルを大量の培養液中で培養する皮膚刺激判定法。



【特許請求の範囲】

【請求項1】 皮膚モデルを被検物質で処理した後、かかる被検物質を除去し、更に該皮膚モデルを大量の培養液中で培養することを特徴とする皮膚刺激性判定法。

【請求項2】 前記皮膚モデルが、皮膚細胞を含む表皮・真皮の二層構造よりなることを特徴とする請求項1に記載の皮膚刺激性判定法。

【発明の詳細な説明】

【0001】

【発明の属する技術分野】本発明は、皮膚モデルを用いた毒性試験に好適な皮膚刺激性判定法に関する。

【0002】

【従来の技術】近年、動物愛護運動の高まりの中で、特に化粧品等の開発における動物実験の是非が問われており、化粧品開発における動物実験を全廃しようとする動きがある。このような状況の中、動物を用いた皮膚刺激性試験を代替する方法の一つとして、細胞を組み込んだ皮膚モデルを用いた皮膚刺激性試験が提案されている。即ち、化粧品や皮膚適用製剤等の皮膚刺激性試験を、皮膚モデルを用いて行う際、通常それらの成分の中で最もヒト皮膚に刺激を与えるのは界面活性剤であり、かかる界面活性剤の皮膚刺激性をいかに予測し得るかということは重要なことであった。

【0003】しかしながら、これまでに報告されてきた方法では、皮膚モデルを用いた皮膚刺激性試験のデータと、動物実験のデータとが合致しない点がいくつかあった。例えば、強い皮膚刺激性のカチオン性界面活性剤が偽陰性を示す場合があったり、また逆にほとんど皮膚刺激性のないノニオン性界面活性剤が偽陽性を示す場合があった。

【0004】

【発明が解決しようとする課題】本発明は、上述のような実状に鑑みてなされたもので、その目的とするところは、動物を用いた皮膚刺激性試験を代替し、かつ動物実験のデータと非常によく合致する、皮膚モデルを用いた皮膚刺激性判定法を提供する点にある。

【0005】

【課題を解決するための手段】即ち、本発明は、皮膚モデルを被検物質で処理した後、かかる被検物質を除去し、更に該皮膚モデルを大量の培養液中で培養することに特徴を有する。更に、皮膚モデルが、皮膚細胞を含む表皮・真皮の二層構造よりなることに特徴を有する。

【0006】前記構成とすることにより、動物実験のデータと非常によく合致する、皮膚モデルを用いた皮膚刺激性判定法が得られた。従来の方法がなぜ動物実験のデータと合致しなかったのか、その詳細な理由は分からないが、おそらく次のようなことに起因しているものと思われる。

【0007】即ち、皮膚モデルを用いた毒性試験ではほとんどの場合、「細胞死」を指標としており、ある種の

カチオン性界面活性剤（例えばCetylpyridinium Chloride : CPC）の場合、細胞に傷害を与えてからそれが細胞死（あるいはそれに準ずる反応）として現れるまでにタイムラグがあり、それがしばしば偽陰性を示す主原因であったと思われる。

【0008】一方、ある種のノニオン性界面活性剤（例えばPolyoxyethylene Octylphenylether : TX100）の場合、細胞毒性は強いものの角質層透過性が低く、更には拡散希釈効果によって動物実験での皮膚刺激性は低いが、皮膚モデルでは拡散希釈効果が低いため、長時間作用させるうちに蓄積が進み毒性を示すようになり、これがしばしば偽陽性を示す主原因であったと思われる。

【0009】従って、従来の方法では、CPCのようなカチオン性界面活性剤に合わせて条件を設定すると、TX100のようなノニオン性界面活性剤が偽陽性を、逆にTX100のようなノニオン性界面活性剤に合わせて条件を設定すると、CPCのようなカチオン性界面活性剤が偽陰性を示すことになり、常にいずれか一方がin vivo データと合致しなかったのである。

【0010】本発明の、皮膚モデルを用いた皮膚刺激性判定法は、この二律背反する両者を同時に改善したものであり、皮膚モデルを被検物質で一定時間処理した後、かかる被検物質を除去することにより、必要以上の処理を防ぎ、ある種のノニオン性界面活性剤（例えばPolyoxyethylene Octylphenylether : TX100）のような物質が偽陽性を示すのを防ぐことができる。また、皮膚モデルを大量の培養液中で一定時間培養することにより、ある種のカチオン性界面活性剤（例えばCetylpyridinium Chloride : CPC）のような物質による傷害が細胞死として現れてくるので、偽陰性を示すのを防ぐことができる。更に、大量の培養液中で培養するので、ある程度の希釈拡散効果を持たせることができる。

【0011】前記構成において、本発明に適用できる皮膚モデルとは、皮膚細胞を含む表皮・真皮の二層構造よりなるものであればよく、例えば本出願人が既に開示している特開平8-89239号（特願平6-263047号）に記載されている培養皮膚が好適に用いられる。また、被検物質としては、界面活性剤の他に、化粧品、洗浄剤、あるいは皮膚適用製剤等が挙げられ、更に界面活性剤としては、カチオン性界面活性剤（Benzethonium Chloride : BTC、Cetylpyridinium Chloride : CPC、Stearyl Trimethyl Ammonium Chloride : STAC等）、アニオン性界面活性剤（Sodium Lauryl sulfate : SLS、Potassium Laurate : PL等）、あるいはノニオン性界面活性剤（Polyoxyethylene 23 Lauryl Ether : B35、Sucrose Fatty Acid Ester : SFAE、t-Octylphenoxy polyethoxyethanol : TX100、Polyoxyethylene sorbitan monolaurate : TW20、Polyoxyethylene sorbitan monooleate : TW80等）が挙げられる。

【0012】皮膚モデルを被検物質で処理する時間は、長すぎても短すぎても好ましくなく、該処理時間は被検物質の種類により異なるが、例えば被検物質が界面活性剤の場合、好ましくは1分間～1時間処理するのが好ましい。被検物質で処理された皮膚モデルは、被検物質を除去後、大量の培養液中で一定時間培養されるが、かかる培養液としては、MEM、DMEM等、一般的な培養液に、1～10%程度のウシ胎児血清を添加したものが好ましい。また、培養時間は培養液の種類により異なるが、好ましくは3～24時間培養するのが好ましい。

【0013】

【発明の実施の形態】

【実施例】

(実施例1)

1. 皮膚モデル(培養皮膚)の作製

(1) 高架橋スポンジの作製

3mg/mlに希釈したType Iコラーゲン溶液50gにクロロホルム0.5gを添加し、ホモジナイザーを用いて6000rpmで1分間ホモジナイズしたものをステンレス製枠に流し込み、-40℃で凍結し、これを真空減圧下(0.01mmHg)、30℃で24時間凍結乾燥した。更に、真空減圧下(0.01mmHg)、105℃で24時間熱脱水架橋を加えた後、0.2%グルタルアルデヒド溶液に24時間浸漬することにより化学架橋を導入した。これを再び凍結乾燥して孔径90 μ m、厚さ3mmの高架橋コラーゲンスポンジを得た。

(2) 低架橋スポンジの作製

0.3%水溶液(pH3)のType Iコラーゲンをエタノールで希釈し、0.285%コラーゲン、10%エタノール水溶液とした。更に、この溶液を直径9cmのシャーレに10g流し込み、-135℃で凍結し、真空度:0.1、乾燥温度:40℃、乾燥時間:24時間の条件で凍結乾燥を行い、更に真空減圧下(0.01mmHg)、105℃で24時間熱脱水架橋して、孔径30 μ m、厚さ1mmの低架橋コラーゲンスポンジを得た。

(3) 細胞の播種及び培養

24ウェル培養プレートの底面に、(1)で作製した高架橋コラーゲンスポンジを敷き詰め、クラボウ(株)から購入したヒト繊維芽細胞をMEM+10%血清培地に懸濁し、このスポンジ上に 5.0×10^4 cells/cm²の濃度で播種し、細胞が完全に接着するまで37℃、5%CO₂で一晩培養した。次に、このスポンジ上に(2)で作製した低架橋コラーゲンスポンジを重ね、クラボウ(株)から購入したヒト角化細胞をKGM培地に懸濁し、この低架橋コラーゲンスポンジ上に 5.0×10^4

cells/cm²の濃度で播種し、細胞が完全に接着するまで37℃、5%CO₂で一晩培養した。次に、かかる培養基材を24ウェル培養プレートから取り出し、6ウェル培養プレートに移した後、培地をDME+5%血清培地に変更した。ヒト角化細胞が空気中に出るように培養液の量を調整しながら5日間培養を続けた後、所望の皮膚モデル(培養皮膚)を得た。なお、上記した高架橋コラーゲンスポンジ、低架橋コラーゲンスポンジとは、それぞれ高度に架橋したコラーゲンスポンジ、低度に架橋したコラーゲンスポンジのことであり、表皮層には低架橋コラーゲンスポンジを用い、真皮層には高架橋コラーゲンスポンジを用いた。

【0016】2. 皮膚刺激判定

上記1によって作製された直径8mmの円形で筒状の皮膚モデルを以下の判定法に用いた。

(1) 準備段階

図1及び図2は、それぞれ培地の入った培養プレートに皮膚モデルを置いた時の状態を示した断面図及びその平面図であり、本発明の判定法の準備として、24ウェルプレート4に、DME+5%血清培地3を250 μ l分注し、この中に皮膚モデルを置いた。この際、表皮層2を気液界面上に、かつ真皮層1をDME+5%血清培地3中にあるようにした。

(2) 被検物質処理

表皮層にのみ作用するように、表皮層上に被検物質100 μ lを滴下し、一定時間作用させた。

(3) 洗浄

表皮層に残った被検物質を、PBSバッファで洗い落とした。

(4) インキュベーション

24ウェルプレートにDME+5%血清培地1.5mlを分注し、この中に皮膚モデル(培養皮膚)を沈め、一晩培養した。

(5) MTT法により生細胞数を測定した。

(6) 上記の方法で被検物質作用時間を10分間に設定し、被検物質として10種類の界面活性剤10%溶液(溶媒:PBS)についてそれぞれ試験を行った。その結果を表1に示す。

なお、試験結果はMTT発色後570nmの吸光度を測定し、PBSのみを作用させた場合の吸光度を100%として次式より比を求め、それを細胞生存率(%)とした。

【0017】

【数1】

$$\text{細胞生存率(\%)} = \frac{\Delta A_{570}(\text{被検物質-ブランク}^{\circ})}{\Delta A_{570}(\text{コントロール-ブランク}^{\circ})} \times 100$$

※ブランク：細胞の全く入っていないスポンジに
MTT溶液を作用させた後の吸光度

【0018】一方in vivo 動物実験としては、日本白色種ウサギの健常皮膚を用い、10%濃度に調整した各被検物質を4時間閉塞貼付し、除去後1時間、24時間および48時間後にドレイズ基準により判定した。得られたin vivo 動物実験データを表1及び表2に示す。また、本発明の判定法により得られたデータと、in vivo 動物実験データとの比較図を図3に示す。図3から明らかなように、両者は非常に良い相関を示していることが判る。

【0019】(比較例1)実施例1の1で作製した皮膚モデル(培養皮膚)を用い、被検物質として実施例1で用いたものと同じ10種類の界面活性剤について従来法で試験を行った。方法としては、被検物質を1時間および24時間作用後インキュベーションを行わずそのままMTT法で生細胞数を測定した。得られたデータを、実施例1と同様in vivo 動物実験データと比較した。その結果を表2に示す。また、従来法により得られたデータと、in vivo 動物実験データとの比較図を図4の(a)、(b)に示す。これらの図より、1時間処理ではカチオン系のCPCおよびSTACが偽陰性を、24時間処理ではノニオン系のB35およびTX100が偽陽性を示すことが判る。

【0020】(実施例2)被検物質が、化粧品(シャンプー、ウォッシング)であるという以外は、実施例1と同様の条件で行った。in vivo 動物実験のデータと比較した結果を表3に示す。また、本発明の判定法により得られたデータと、in vivo 動物実験データとの比較図を図5に示す。図5から明らかなように、両者は非常に良い相関を示していることが判る。

【0021】(比較例2)被検物質が、化粧品(シャン

プー、ウォッシング)であるという以外は、比較例1と同様の条件で行った。in vivo 動物実験のデータと比較した結果を表3に示す。また、従来法により得られたデータと、in vivo 動物実験データとの比較図を図6の(a)、(b)に示す。これらの図から明らかなように、両者は1時間処理及び24時間処理ともほとんど合致していないことが判る。

【0022】

【発明の効果】以上説明したように本発明によれば、動物を用いた皮膚刺激性試験を代替し、かつ動物実験のデータと非常によく合致する皮膚刺激判定法を提供できる。

【図面の簡単な説明】

【図1】培地の入った培養プレートに皮膚モデルを置いた時の状態を示した断面図である。

【図2】図1の平面図である。

【図3】実施例1における相関関係を示した図である。

【図4】(a)比較例1の1時間処理における図である。

(b)比較例1の24時間処理における図である。

【図5】実施例2における相関関係を示した図である。

【図6】(a)比較例2の1時間処理における図である。

(b)比較例2の24時間処理における図である。

【符号の説明】

1. 真皮層
2. 表皮層
3. DME+5%血清培地
4. 24ウェルプレート

【表1】

被検物質		in vivo刺激点	細胞生存率(%)
カチオン性 界面活性剤	Benzethonium Chloride : BTC	4.0	3.5
	Cetylpyridinium Chloride : CPC	5.0	5.6
	Stearyl Trimethyl Ammoni um Chloride : STAC	4.7	25.0
アニオン性 界面活性剤	Sodium Lauryl sulfate : SLS	6.0	0.0
	Potassium Laurate : PL	5.3	32.4
ノニオン性 界面活性剤	Polyoxyethylene 23 Laury l Ether : B35	0.3	98.2
	Sucrose Fatty Acid Ester : SFAE	0.3	102.3
	t-Octylphenoxy polyethoxy ethanol : TX100	0.0	104.1
	Polyoxyethylene sorbitan monolaurate : TW20	0.0	109.8
	Polyoxyethylene sorbitan monoolate : TW80	0.3	88.1

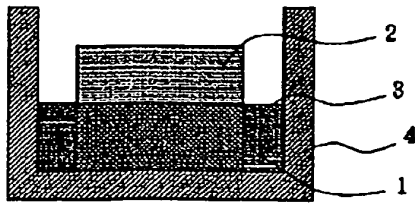
【表2】

被検物質		in vivo 刺激点	細胞生存率(%)	
			1時間 処理	24時間 処理
カチオン性 界面活性剤	Benzethonium Chloride : BTC	4.0	33.6	10.5
	Cetylpyridinium Chloride : CPC	5.0	111.3	10.9
	Stearyl Trimethyl Ammonium Chloride : STAC	4.7	111.0	16.3
アニオン性 界面活性剤	Sodium Lauryl sulfate : SLS	6.0	36.2	5.2
	Potassium Laurate : PL	5.3	19.2	10.5
ノニオン性 界面活性剤	Polyoxyethylene 23 Lauryl Ether : BS5	0.3	121.2	10.5
	Sucrose Fatty Acid Ester : SFAE	0.3	110.5	136.4
	t-Octylphenoxypolyethoxy ethanol : TX100	0.0	128.2	6.3
	Polyoxyethylene sorbitan monolaurate : TW20	0.0	104.1	131.3
	Polyoxyethylene sorbitan monooleate : TW80	0.3	115.3	142.1

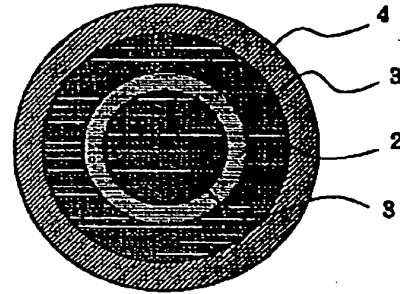
【表3】

被検物質		in vivo 刺激点	(実施例2) 細胞生存率(%)	(比較例2) 細胞生存率(%)	
				1時間 処理	24時間 処理
シャンプー	PTS	6.0	5.3	32.5	3.6
	STS	1.0	90.3	30.3	7.7
ウォッシング	HA	0.3	90.6	48.7	9.6
	BC	1.7	75.1	73.0	7.5
	MW	0.0	116.9	105.3	10.9

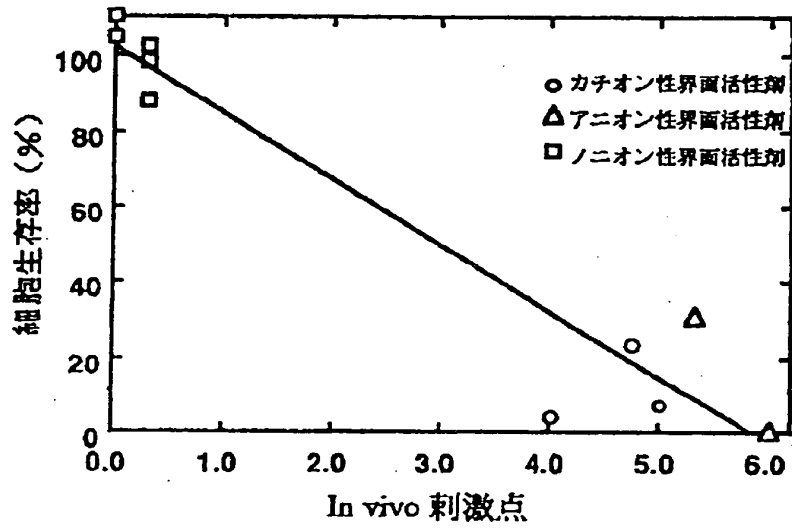
【図1】



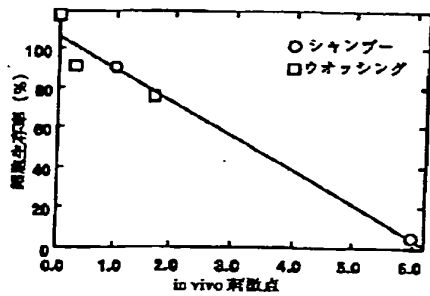
【図2】



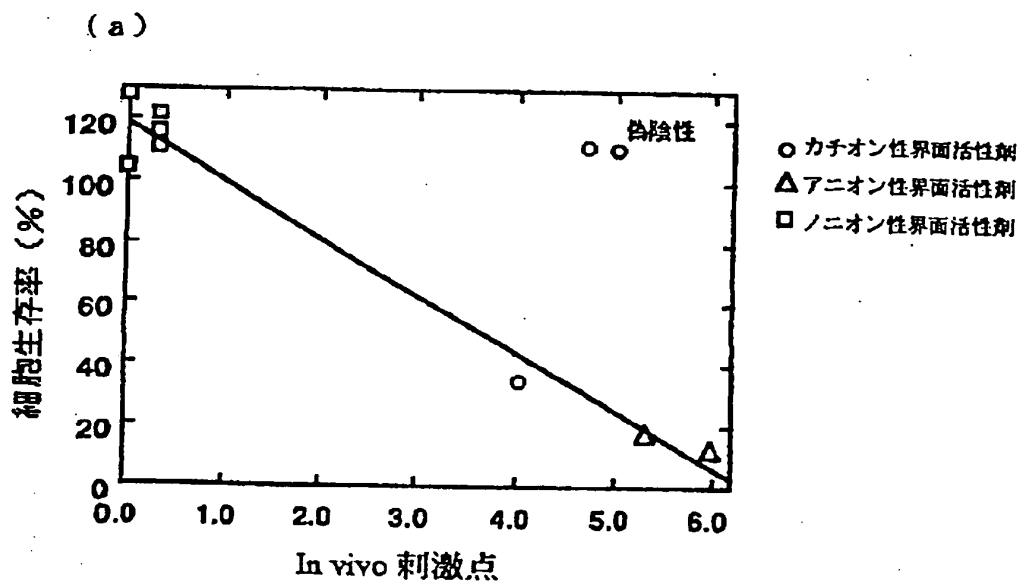
【図3】



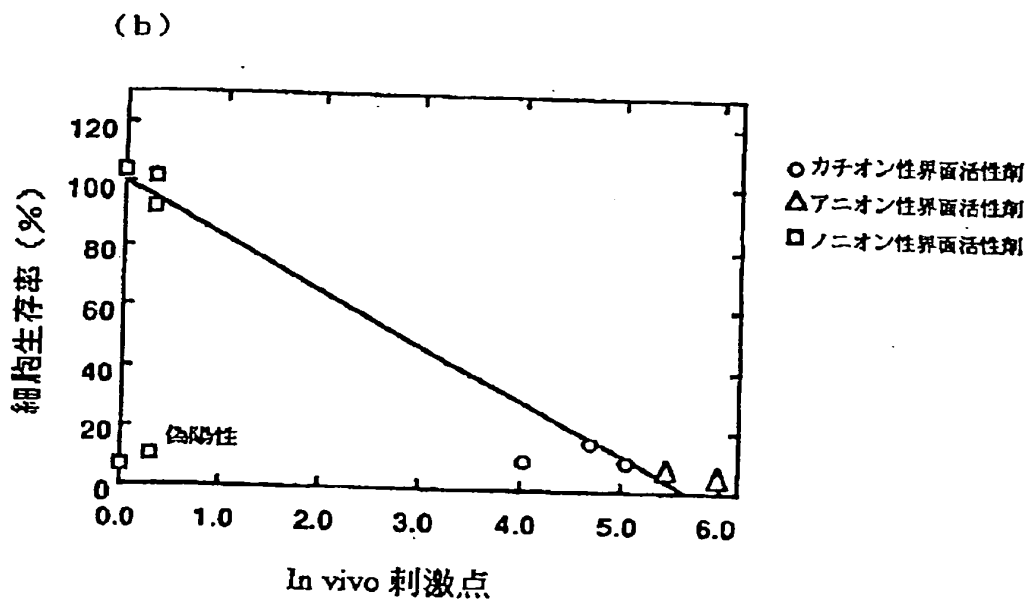
【図5】



【図4】

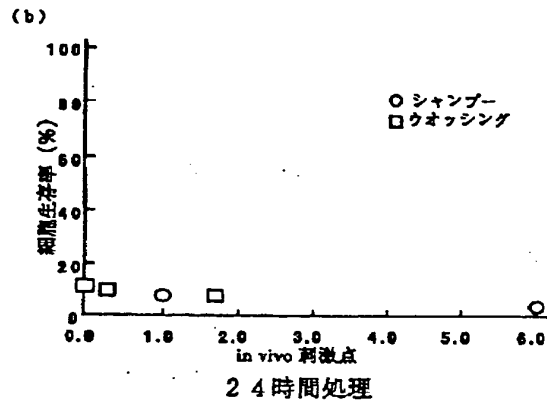
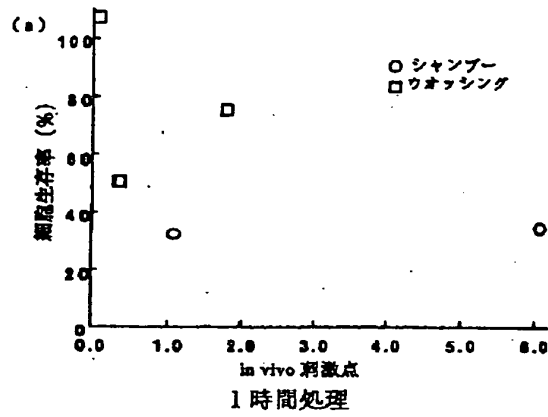


1 時間作用での試験結果



24 時間作用での試験結果

【図6】



BC

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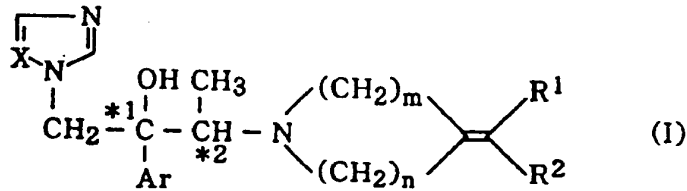
PCT

特許協力条約に基づいて公開された国際出願

<p>(51) 国際特許分類 5 C07D 401/06, 403/06, A61K 31/40, 31/445</p>	<p>A1</p>	<p>(11) 国際公開番号 WO 94/26734 (43) 国際公開日 1994年11月24日(24.11.94)</p>
<p>(21) 国際出願番号 PCT/JP94/00737 (22) 国際出願日 1994年5月28日(02.05.94) (30) 優先権データ 特願平5/132931 1993年5月10日(10.05.93) JP (71) 出願人 (米国外を除くすべての指定国について) 科研製薬株式会社 (KAKEN PHARMACEUTICAL CO., LTD.)(JP/JP) 〒113 東京都文京区本駒込二丁目28番8号 Tokyo, (JP) (72) 発明者; および (75) 発明者/出願人 (米国外についてのみ) 内藤隆信 (NAITO, Takanobu)(JP/JP) 小林潤仁 (KOBAYASHI, Haruhito)(JP/JP) 小倉浩輔 (OGURA, Hironobu)(JP/JP) 永井基美 (NAGAI, Kiyoshi)(JP/JP) 西田時子 (NISHIDA, Tokiko)(JP/JP) 有可正 (ARIKA, Tadashi)(JP/JP) 横尾守 (YOKOO, Mamoru)(JP/JP) 中橋聡子 (NAKABASHI, Satoko)(JP/JP) 〒607 京都府京都市山科区四ノ宮南河原町14番地 科研製薬株式会社 中央研究所内 Kyoto, (JP) (74) 代理人 弁護士 朝日薬業太. 外 (ASAHINA, Sohta et al.) 〒540 大阪府大阪市中央区谷町二丁目2番22号 NSビル Osaka, (JP)</p>	<p>(81) 指定国 AU, BB, BG, BR, BY, CA, CN, CZ, FI, GE, HU, JP, KG, KR, KZ, LK, LV, MD, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SI, SK, TJ, TT, UA, US, UZ, VN, 欧州特許(AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI特許(BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). 添付公開書類 国際調査報告書</p>	
<p>(54) Title : AZOLYLAMINE DERIVATIVE (54) 発明の名称 フゾリルアミン誘導体</p> <div style="text-align: center;"> <p>(I)</p> </div> <p>(57) Abstract An antifungal agent containing as the active ingredient a compound represented by general formula (I) or an acid-addition salt thereof, in particular, one having an absolute configuration of (R, R) at the asymmetric carbon atoms or an acid-addition salt thereof.</p>		

(57) 要約

一般式 (I) :



で表される化合物またはその酸付加塩、とくにその不斉炭素の絶対配置が (R, R) である化合物またはその酸付加塩を有効成分として含有する抗真菌剤が開示されている。

情報としての用途のみ

PCTに基づいて公開される国際出願のパンフレット第1頁にPCT加盟国を特定するために使用されるコード

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CF	中央アフリカ共和国	GR	ギリシャ	ML	マリ	TC	トリニダード
CG	コンゴ	HU	ハンガリー	MN	モンゴル	TJ	タジキスタン
CH	スイス	IE	アイルランド	MR	モーリタニア	TT	トリニダードトバゴ
CI	コートジボワール	IT	イタリア	MW	マラウイ	UA	ウクライナ
CM	カメルーン	JP	日本	NE	ニジェール	US	米国
CN	中国	KE	ケニア	NL	オランダ	UZ	ウズベキスタン共和国
CS	セルビア	KG	キルギスタン	NO	ノルウェー	VN	ベトナム

(1)

明 細 書

アゾリルアミン誘導体

技術分野

5 本発明は、人および動物の真菌疾患にたいして有効であり、さらに農園芸用または工業用防カビ剤としても有効であるアゾリルアミン誘導体に関する。

背景技術

10 分子中にトリアゾリル基あるいはイミダゾリル基などのアゾリル基とピペリジノ基、ピロリジノ基あるいはモルホリノ基などのアミン環をともに有するアゾリルアミン誘導体については特開昭57-140768号公報および英国特許GB2159148A号公報に記載されているが、いずれも抗真菌作用などの面から医薬品として
15 十分な効果を有しているとはいいがたい。またアミン環上にメチレン基あるいは置換メチレン基を有する化合物についての開示はない。

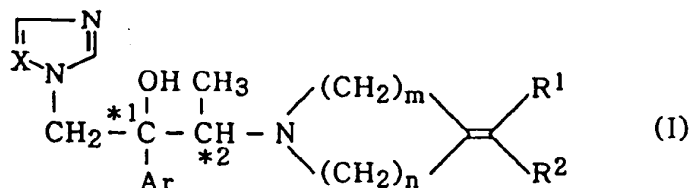
本発明はアミン環上にメチレン基あるいは置換メチレン基を有することを特徴とする、強い抗真菌活性を示す新規アゾリルアミン誘導体を提供することにある。

発明の開示

20

本発明は一般式(1)：

(2)



(式中、Arは無置換またはハロゲン原子およびトリフルオロメチルから選ばれた置換基1～3個で置換されたフェニル基を示し、R¹およびR²は、同一または異なって水素原子、低級アルキル基、無置換またはハロゲン原子および低級アルキル基から選ばれた置換基1～3個で置換されたアリール基、アルケニル基、アルキニル基、またはアラルキル基を示し、mは2または3を示し、nは1または2を示し、Xは窒素原子またはCHを示し、^{*}1、^{*}2は不斉炭素を示す)で表される化合物またはその酸付加塩を提供するものである。

前記一般式(I)で示される化合物としては、とりわけ^{*}1、^{*}2の不斉炭素の絶対配置が(R,R)であるもの、^{*}1、^{*}2の不斉炭素の絶対配置が(R,S)である一般式(I)で表される化合物またはその酸付加塩を含み、他の光学異性体を含んでいる混合体であるものが好ましい。

また、本発明は前記一般式(I)で示される化合物またはその酸付加塩を有効成分として含有する抗真菌剤、さらに、前記化合物を用いた真菌感染症の治療方法を提供するものである。

発明を実施するための最良の形態

前記一般式(I)において、置換されたフェニル基と

(3)

してはハロゲン原子およびトリフルオロメチルから選ばれた1~3個の置換基を有するフェニル基であり、たとえば2,4-ジフルオロフェニル、2,4-ジクロロフェニル、4-フルオロフェニル、4-クロロフェニル、5
2-クロロフェニル、4-トリフルオロメチルフェニル、2-クロロ-4-フルオロフェニルまたは4-プロモフェニルなどがあげられ、低級アルキル基としてはたとえばメチル、エチル、n-プロピル、イソプロピル、n-
10 プチル、イソプチル、sec-プチル、tert-プチル、n-ペンチル、イソペンチル、ネオペンチル、tert-ペンチルなどの炭素数1~6の直鎖、分岐鎖または環状アルキル基があげられ、無置換アリアル基としては、たとえばフェニル、ナフチルまたはビフェニルなどがあげられ、置換アリアル基としては、たとえば2,4
15 -ジフルオロフェニル、2,4-ジクロロフェニル、4-フルオロフェニル、4-クロロフェニル、2-クロロフェニル、4-トリフルオロメチルフェニル、2-クロロ-4-フルオロフェニル、4-プロモフェニル、4-tert-プチルフェニルまたは4-ニトロフェニルなど
20 などがあげられ、アルケニル基としては、たとえばビニル、1-プロペニルまたはスチリルなどがあげられ、アルキニル基としては、たとえばエチニルなどがあげられ、アラルキル基としては、たとえばベンジル、ナフチルメチルまたは4-ニトロベンジルなどがあげられる。

25 一般式(1)で表される本発明化合物は分子内に不斉炭素を2個以上有しており光学異性体およびジアステレオマーが存在する。光学異性体は一般的な光学分割の手法により、あるいは不斉合成の手法により両対掌体をえ

(4)

ることができる。またジアステレオマーの分離は分別再結晶やクロマトグラフィーなどの通常の方法を用いてそれぞれの異性体をえることができる。一般式(1)はこれらの異性体の一つまたは混合物を包含する。

5 これらの中でも、不斉炭素の絶対配置が(R, R)であるものがとくに強力な抗真菌作用を有しており、とりわけ好ましく用いられる。

一般式(1)で示される本発明の化合物の代表例としては、たとえば(2R, 3R) - 2 - (2, 4 - ジフル
10 オロフェニル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 - (1H - 1, 2, 4 - トリアゾール - 1 - イル) ブタン - 2 - オール、(2S, 3S) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリ
15 ジン - 1 - イル) - 1 - (1H - 1, 2, 4 - トリアゾール - 1 - イル) ブタン - 2 - オール、(2RS, 3RS) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 - (1H - 1, 2, 4 - トリアゾール - 1 - イル) ブタン - 2 - オール、
20 (2R, 3R) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 - (1H - イミダゾール - 1 - イル) ブタン - 2 - オール、(2S, 3S) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 - (1H - イミダゾール - 1 - イル) ブタン - 2 - オール、
25 (2RS, 3RS) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 - (1H - イミダゾール - 1 - イル) ブタン - 2 - オール、(2R, 3R) - 2 - (4 - クロロフェニル) - 3

(5)

- (4-メチレンピペリジン-1-イル) - 1 - (1 H
 - 1, 2, 4-トリアゾール-1-イル) ブタン-2-
 オール、(2 S, 3 S) - 2 - (4-クロロフェニル)
 - 3 - (4-メチレンピペリジン-1-イル) - 1 -
 5 (1 H - 1, 2, 4-トリアゾール-1-イル) ブタン
 - 2 - オール、(2 R S, 3 R S) - 2 - (4-クロロ
 フェニル) - 3 - (4-メチレンピペリジン-1-イル)
 - 1 - (1 H - 1, 2, 4-トリアゾール-1-イル)
 ブタン-2-オール、(2 R, 3 R) - 2 - (4-クロ
 10 ロフェニル) - 3 - (4-メチレンピペリジン-1-イ
 ル) - 1 - (1 H - イミダゾール-1-イル) ブタン-
 2 - オール、(2 S, 3 S) - 2 - (4-クロロフェニ
 ル) - 3 - (4-メチレンピペリジン-1-イル) - 1
 - (1 H - イミダゾール-1-イル) ブタン-2-オー
 15 ル、(2 R S, 3 R S) - 2 - (4-クロロフェニル)
 - 3 - (4-メチレンピペリジン-1-イル) - 1 -
 (1 H - イミダゾール-1-イル) ブタン-2-オール、
 (2 R, 3 R) - 2 - (4-トリフルオロメチルフェニ
 ル) - 3 - (4-メチレンピペリジン-1-イル) - 1
 20 - (1 H - 1, 2, 4-トリアゾール-1-イル) ブタ
 ン-2-オール、(2 S, 3 S) - 2 - (4-トリフル
 オロメチルフェニル) - 3 - (4-メチレンピペリジン
 - 1-イル) - 1 - (1 H - 1, 2, 4-トリアゾール
 - 1-イル) ブタン-2-オール、(2 R S, 3 R S)
 25 - 2 - (4-トリフルオロメチルフェニル) - 3 - (4
 -メチレンピペリジン-1-イル) - 1 - (1 H - 1,
 2, 4-トリアゾール-1-イル) ブタン-2-オール、
 (2 R, 3 R) - 2 - (4-トリフルオロメチルフェニ

(6)

ル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1
 - (1 H - イミダゾール - 1 - イル) ブタン - 2 - オール、
 (2 S, 3 S) - 2 - (4 - トリフルオロメチルフェ
 ニル) - 3 - (4 - メチレンピペリジン - 1 - イル)
 5 - 1 - (1 H - イミダゾール - 1 - イル) ブタン - 2 -
 オール、(2 R S, 3 R S) - 2 - (4 - トリフルオロ
 メチルフェニル) - 3 - (4 - メチレンピペリジン - 1
 - イル) - 1 - (1 H - イミダゾール - 1 - イル) ブタ
 ン - 2 - オール、(2 R, 3 R) - 2 - (2, 4 - ジク
 10 ロロフェニル) - 3 - (4 - メチレンピペリジン - 1 -
 イル) - 1 - (1 H - 1, 2, 4 - トリアゾール - 1 -
 イル) ブタン - 2 - オール、(2 S, 3 S) - 2 - (2,
 4 - ジクロロフェニル) - 3 - (4 - メチレンピペリジ
 ン - 1 - イル) - 1 - (1 H - 1, 2, 4 - トリアゾー
 15 ル - 1 - イル) ブタン - 2 - オール、(2 R S, 3 R S)
 - 2 - (2, 4 - ジクロロフェニル) - 3 - (4 - メチ
 レンピペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4
 - トリアゾール - 1 - イル) ブタン - 2 - オール、(2
 R, 3 R) - 2 - (2, 4 - ジクロロフェニル) - 3 -
 20 (4 - メチレンピペリジン - 1 - イル) - 1 - (1 H -
 イミダゾール - 1 - イル) ブタン - 2 - オール、(2 S,
 3 S) - 2 - (2, 4 - ジクロロフェニル) - 3 - (4
 - メチレンピペリジン - 1 - イル) - 1 - (1 H - イミ
 ダゾール - 1 - イル) ブタン - 2 - オール、(2 R S,
 25 3 R S) - 2 - (2, 4 - ジクロロフェニル) - 3 -
 (4 - メチレンピペリジン - 1 - イル) - 1 - (1 H -
 イミダゾール - 1 - イル) ブタン - 2 - オール、(2 R,
 3 R) - 2 - (2, 4 - ジフルオロフェニル) - 3 -

(7)

(4-エチリデンピペリジン-1-イル) - 1 - (1H
 - 1, 2, 4-トリアゾール-1-イル) ブタン-2-
 オール、(2S, 3S-2-(2, 4-ジフルオロフェ
 ニル) - 3 - (4-エチリデンピペリジン-1-イル)
 5 - 1 - (1H-1, 2, 4-トリアゾール-1-イル)
 ブタン-2-オール、(2RS, 3RS) - 2 - (2,
 4-ジフルオロフェニル) - 3 - (4-エチリデンピペ
 リジン-1-イル) - 1 - (1H-1, 2, 4-トリア
 ザール-1-イル) ブタン-2-オール、(2R, 3R)
 10 - 2 - (2, 4-ジフルオロフェニル) - 3 - (4-プ
 ロピリデンピペリジン-1-イル) - 1 - (1H-1,
 2, 4-トリアゾール-1-イル) ブタン-2-オール、
 (2S, 3S) - 2 - (2, 4-ジフルオロフェニル)
 - 3 - (4-プロピリデンピペリジン-1-イル) - 1
 15 - (1H-1, 2, 4-トリアゾール-1-イル) ブタ
 ン-2-オール、(2RS, 3RS) - 2 - (2, 4-
 ジフルオロフェニル) - 3 - (4-プロピリデンピペリ
 ジン-1-イル) - 1 - (1H-1, 2, 4-トリアゾ
 ール-1-イル) ブタン-2-オール、(2R, 3R)
 20 - 2 - (2, 4-ジフルオロフェニル) - 3 - (4-n
 -ブチリデンピペリジン-1-イル) - 1 - (1H-1,
 2, 4-トリアゾール-1-イル) ブタン-2-オール、
 (2S, 3S) - 2 - (2, 4-ジフルオロフェニル)
 - 3 - (4-n-ブチリデンピペリジン-1-イル) -
 25 1 - (1H-1, 2, 4-トリアゾール-1-イル) ブ
 タン-2-オール、(2RS, 3RS) - 2 - (2, 4-
 ジフルオロフェニル) - 3 - (4-n-ブチリデンピ
 ペリジン-1-イル) - 1 - (1H-1, 2, 4-トリ

(8)

アゾール-1-イル)ブタン-2-オール、(2R, 3
 R)-2-(2,4-ジフルオロフェニル)-3-(4
 -n-ペンチリデンピペリジン-1-イル)-1-(1
 H-1,2,4-トリアゾール-1-イル)ブタン-2
 5 -オール、(2S, 3S)-2-(2,4-ジフルオロ
 フェニル)-3-(4-n-ペンチリデンピペリジン-
 1-イル)-1-(1H-1,2,4-トリアゾール-
 1-イル)ブタン-2-オール、(2RS, 3RS)-
 2-(2,4-ジフルオロフェニル)-3-(4-n-
 10 ペンチリデンピペリジン-1-イル)-1-(1H-1,
 2,4-トリアゾール-1-イル)ブタン-2-オール、
 (2R, 3R)-2-(2,4-ジフルオロフェニル)
 -3-(4-n-ヘキシリデンピペリジン-1-イル)
 -1-(1H-1,2,4-トリアゾール-1-イル)
 15 ブタン-2-オール、(2S, 3S)-2-(2,4-
 ジフルオロフェニル)-3-(4-n-ヘキシリデンピ
 ペリジン-1-イル)-1-(1H-1,2,4-トリ
 アゾール-1-イル)ブタン-2-オール、(2RS,
 3RS)-2-(2,4-ジフルオロフェニル)-3-
 20 (4-n-ヘキシリデンピペリジン-1-イル)-1-
 (1H-1,2,4-トリアゾール-1-イル)ブタン
 -2-オール、(2R, 3R)-2-(2,4-ジフル
 オロフェニル)-3-(4-シクロプロピルメチレンピ
 ペリジン-1-イル)-1-(1H-1,2,4-トリ
 25 アゾール-1-イル)ブタン-2-オール、(2S, 3
 S)-2-(2,4-ジフルオロフェニル)-3-(4
 -シクロプロピルメチレンピペリジン-1-イル)-1
 -1-(1H-1,2,4-トリアゾール-1-イル)ブタ

(9)

ン - 2 - オール、(2 R S, 3 R S) - 2 - (2, 4 -
ジフルオロフェニル) - 3 - (4 - シクロプロピルメチ
レンピペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4
- トリアゾール - 1 - イル) ブタン - 2 - オール、(2
5 R, 3 R) - 2 - (2, 4 - ジフルオロフェニル) - 3
- (4 - シクロヘキシルメチレンピペリジン - 1 - イル)
- 1 - (1 H - 1, 2, 4 - トリアゾール - 1 - イル)
ブタン - 2 - オール、(2 S, 3 S) - 2 - (2, 4 -
ジフルオロフェニル) - 3 - (4 - シクロヘキシルメチ
10 レンピペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4
- トリアゾール - 1 - イル) ブタン - 2 - オール、(2
R S, 3 R S) - 2 - (2, 4 - ジフルオロフェニル)
- 3 - (4 - シクロヘキシルメチレンピペリジン - 1 -
イル) - 1 - (1 H - 1, 2, 4 - トリアゾール - 1 -
15 イル) ブタン - 2 - オール、(2 R, 3 R) - 2 - (2,
4 - ジフルオロフェニル) - 3 - (4 - ベンジリデンピ
ペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4 - トリ
アゾール - 1 - イル) ブタン - 2 - オール、(2 S, 3
S) - 2 - (2, 4 - ジフルオロフェニル) - 3 - (4
20 - ベンジリデンピペリジン - 1 - イル) - 1 - (1 H -
1, 2, 4 - トリアゾール - 1 - イル) ブタン - 2 - オ
ール、(2 R S, 3 R S) - 2 - (2, 4 - ジフルオロ
フェニル) - 3 - (4 - ベンジリデンピペリジン - 1 -
イル) - 1 - (1 H - 1, 2, 4 - トリアゾール - 1 -
25 イル) ブタン - 2 - オール、(2 R, 3 R) - 2 - (2,
4 - ジフルオロフェニル) - 3 - (4 - イソプロピリデ
ンピペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4 -
トリアゾール - 1 - イル) ブタン - 2 - オール、(2 S,

(10)

3 S) - 2 - (2 , 4 - ジフルオロフェニル) - 3 -
 (4 - イソプロピリデンピペリジン - 1 - イル) - 1 -
 (1 H - 1 , 2 , 4 - トリアゾール - 1 - イル) ブタン
 - 2 - オール、 (2 R S , 3 R S) - 2 - (2 , 4 - ジ
 5 フルオロフェニル) - 3 - (4 - イソプロピリデンピペ
 リジン - 1 - イル) - 1 - (1 H - 1 , 2 , 4 - トリア
 ザール - 1 - イル) ブタン - 2 - オール、 (2 R , 3 R)
 - 2 - (2 , 4 - ジフルオロフェニル) - 3 - (4 - ジ
 フェニルメチレンピペリジン - 1 - イル) - 1 - (1 H
 10 - 1 , 2 , 4 - トリアゾール - 1 - イル) ブタン - 2 -
 オール、 (2 S , 3 S) - 2 - (2 , 4 - ジフルオロフ
 ェニル) - 3 - (4 - ジフェニルメチレンピペリジン -
 1 - イル) - 1 - (1 H - 1 , 2 , 4 - トリアゾール -
 1 - イル) ブタン - 2 - オール、 (2 R S , 3 R S) -
 15 2 - (2 , 4 - ジフルオロフェニル) - 3 - (4 - ジフ
 ェニルメチレンピペリジン - 1 - イル) - 1 - (1 H -
 1 , 2 , 4 - トリアゾール - 1 - イル) ブタン - 2 - オ
 ール、 (2 R , 3 R) - 2 - (2 , 4 - ジフルオロフェ
 ニル) - 3 - (4 - プロペニリデンピペリジン - 1 - イ
 20 ル) - 1 - (1 H - 1 , 2 , 4 - トリアゾール - 1 - イ
 ル) ブタン - 2 - オール、 (2 S , 3 S) - 2 - (2 ,
 4 - ジフルオロフェニル) - 3 - (4 - プロペニリデン
 ピペリジン - 1 - イル) - 1 - (1 H - 1 , 2 , 4 - ト
 リアゾール - 1 - イル) ブタン - 2 - オール、 (2 R S ,
 25 3 R S) - 2 - (2 , 4 - ジフルオロフェニル) - 3 -
 (4 - プロペニリデンピペリジン - 1 - イル) - 1 -
 (1 H - 1 , 2 , 4 - トリアゾール - 1 - イル) ブタン
 - 2 - オール、 (2 R , 3 R) - 2 - (2 , 4 - ジフル

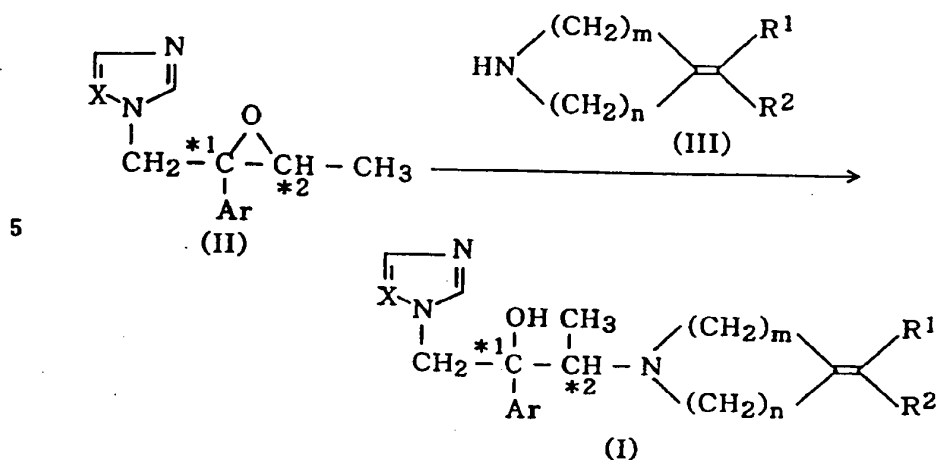
(11)

オロフェニル) - 3 - (4 - プロピニリデンピペリジン
 - 1 - イル) - 1 - (1 H - 1, 2, 4 - トリアゾール
 - 1 - イル) ブタン - 2 - オール、(2 S, 3 S) - 2
 - (2, 4 - ジフルオロフェニル) - 3 - (4 - プロピ
 5 ニリデンピペリジン - 1 - イル) - 1 - (1 H - 1, 2,
 4 - トリアゾール - 1 - イル) ブタン - 2 - オール、
 (2 R S, 3 R S) - 2 - (2, 4 - ジフルオロフェニ
 ル) - 3 - (4 - プロピニリデンピペリジン - 1 - イル)
 - 1 - (1 H - 1, 2, 4 - トリアゾール - 1 - イル)
 10 ブタン - 2 - オール、(2 R, 3 R) - 2 - (2, 4 -
 ジフルオロフェニル) - 3 - (3 - メチレンピペリジン
 - 1 - イル) - 1 - (1 H - 1, 2, 4 - トリアゾール
 - 1 - イル) ブタン - 2 - オール、(2 S, 3 S) - 2
 - (2, 4 - ジフルオロフェニル) - 3 - (3 - メチレ
 15 ンピペリジン - 1 - イル) - 1 - (1 H - 1, 2, 4 -
 トリアゾール - 1 - イル) ブタン - 2 - オール、(2 R
 S, 3 R S) - 2 - (2, 4 - ジフルオロフェニル) -
 3 - (3 - メチレンピペリジン - 1 - イル) - 1 - (1
 H - 1, 2, 4 - トリアゾール - 1 - イル) ブタン - 2
 20 - オール、(2 R, 3 R) - 2 - (2, 4 - ジフルオロ
 フェニル) - 3 - (3 - メチレンピロリジン - 1 - イル)
 - 1 - (1 H - 1, 2, 4 - トリアゾール - 1 - イル)
 ブタン - 2 - オール、(2 S, 3 S) - 2 - (2, 4 -
 ジフルオロフェニル) - 3 - (3 - メチレンピロリジン
 25 - 1 - イル) - 1 - (1 H - 1, 2, 4 - トリアゾール
 - 1 - イル) ブタン - 2 - オール、および (2 R S, 3
 R S) - 2 - (2, 4 - ジフルオロフェニル) - 3 -
 (3 - メチレンピロリジン - 1 - イル) - 1 - (1 H -

(12)

1, 2, 4-トリアゾール-1-イル)ブタン-2-オール、などがあげられる。

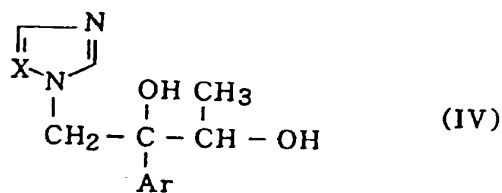
一般式(I)で表される本発明化合物は以下に示す方法によって製造することができる。



(上記式中、Ar、R¹、R²、X、mおよびnは前述したものと同意義を示す。)

すなわち、一般式(II)で表されるエポキシ化合物と一般式(III)で表されるアミン誘導体を反応させることにより一般式(I)で表される化合物に導くことができる。一般式(II)で表されるエポキシ化合物は特開平2-191262号公報などに記載されているとき、たとえば一般式(IV)：

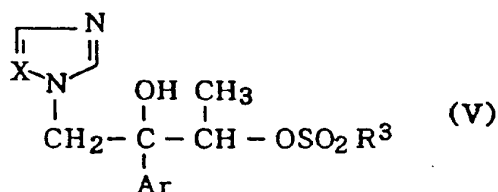
10



15 (式中、ArおよびXは前述したものと同意義を示す)

(13)

で表される化合物を塩基存在下に式 $R^3 SO_2 - O - SO_2 R^3$ または $R^3 SO_2 - Z$ (式中、 R^3 は低級アルキル基、ハロゲン化低級アルキル基または置換されていてもよいフェニル基を示し、 Z はハロゲン原子などの脱離基を示す。) を有する化合物と反応させることにより、
5 化合物 (V) :



をえ、これに塩基を反応させる方法などによりえられる。

10 一般式 (I I I) で表されるアミン誘導体は公知の合成法 (たとえば Chem. Pharm. Bull. 41 (11) 1971-1986 (1993)) により、あるいは本発明の参考例に記載されている方法などによりえることができる。

15 なお、アミン誘導体が塩基などの酸との塩のばあいは水酸化ナトリウムなどの無機塩基あるいはトリエチルアミンなどの有機塩基であらかじめ、あるいは反応液中で中和し遊離のアミンとして使用される。

20 反応は通常、水または有機溶媒あるいは水と有機溶媒の混合溶液を用いて、あるいは無溶媒で行われる。有機溶媒としては出発化合物と反応しないものであればよく、たとえばメタノール、エタノール、*n*-プロパノール、イソプロパノール、*n*-ブタノール、*tert*-ブタノール、エチレングリコール、プロピレングリコール、グリセリンまたはメチルセルソルブなどのアルコール類、テトラヒドロフラン、ジオキサンまたはジメトキシエタ

(14)

ンなどのエーテル類、N，N-ジメチルホルムアミドまたはN，N-ジメチルアセトアミドなどのアミド類、ジメチルスルホキシドなどが単独であるいは混合液として使用される。

- 5 上記反応系においては、有機溶媒のみよりも、反応系に1～80 v/v%の水を添加すると反応が円滑に進行する。

反応溶液中の各原料の使用量は、化合物(11)に対して1～20倍モルの化合物(111)を使用する。

- 10 反応温度は室温～200℃、好ましくは50～150℃である。反応時間は1時間～72時間である。

反応終了後、溶媒を留去し再結晶またはクロマトグラフィーなどの手段により精製することにより一般式(1)で表される本発明化合物が単離される。

- 15 一般式(1)で表される本発明化合物は必要に応じて、医薬として許容される塩、たとえば塩酸、硫酸、硝酸、磷酸または臭化水素酸などとの無機酸塩、フマル酸、マレイン酸、酢酸、リンゴ酸、酒石酸、クエン酸、メタンスルホン酸またはトルエンスルホン酸などとの有機酸塩とすることができる。

つぎに、上記一般式(1)で表される本発明化合物の抗真菌活性について述べる。なお、以下の試験に使用する被験化合物番号は後に記載する実施例番号を引用した。

1. 最小発育阻止濃度(MIC)の測定

- 25 カンジダ・アルビカンス(*Candida albicans* ATCC-10259)については合成アミノ酸培地(SAAMF培地)を用いた液体培地希釈法により被験化合物のMICを測定した。すなわち2倍段階希釈系列の薬液3μlに、最終

(15)

菌濃度 1×10^3 cells/ml になるように接種した S A A M F 培地 300 μ l を加え、35℃ で 2 日間培養後、菌の発育を阻止する被験化合物の最小濃度をもって M I C とした。カンジダ・アルビカンス以外の菌についてはサプロー寒天培地を用いた寒天平板希釈法により M I C を測定した。すなわちジメチルスルホキシドに溶解して 10 mg/ml の濃度になるように調製した被験薬剤溶液を、ジメチルスルホキシドでさらに 2 倍段階希釈した液 0.1 ml を滅菌シャーレにとり、サプロー寒天培地 9.9 ml を加え十分に混和し薬剤添加平板を作成し、 10^6 cells/ml に調製した菌浮遊液 5 μ l をマイクロプランター（株式会社佐久間製作所製）を用い接種した。アスペルギルス・フミガタス (*Aspergillus fumigatus* NI-5561) とクリプトコッカス・ネオホルマンス (*Cryptococcus neoformans* NI-7496) は 30℃ で 48 時間、トリコフィトン・メンタグロフィテス (*Trichophyton mentagrophytes* KD-01) は 30℃ で 7 日間培養後、菌の発育を阻止する被験化合物の最小濃度をもって M I C とした。それらの結果を表 1 に示す。比較対照化合物としてクロトリマゾールおよびフルコナゾールを用いた。

20 供試菌名略号は下記の通りである。

菌名	略号
<i>Candida albicans</i> ATCC 10259	C. a.
<i>Cryptococcus neoformans</i> NI-7496	Cr. n.
<i>Aspergillus fumigatus</i> NI-5561	A. f.
<i>Trichophyton mentagrophytes</i> KD-01	T. m.

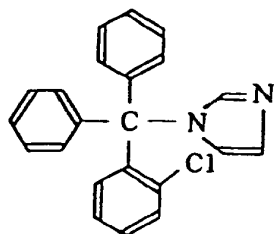
表 1 に本発明の実施例の化合物の各種真菌に対しての抗真菌力（最小発育阻止濃度 M I C）を示す。

(16)

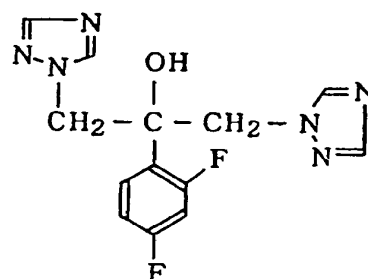
表 1

被験化合物 (実施例番号)	最小発育阻止濃度 (MIC ($\mu\text{g/ml}$))			
	試験菌			
	C.a.	Cr.n.	A.f.	T.m.
1	< 0.025	0.05	0.05	0.39
2	< 0.025	0.1	0.1	0.39
3	0.39	0.78	> 100	50
4	< 0.025	< 0.025	0.05	< 0.025
5	< 0.025	0.025	0.05	0.1
6	< 0.0125	0.2	6.25	3.13
7	0.025	0.05	0.39	0.39
8	< 0.025	0.1	0.2	0.78
10	< 0.025	0.025	0.1	0.39
12	< 0.025	0.1	0.2	0.78
13	0.1	0.39	0.78	1.56
14	< 0.025	0.39	0.39	0.78
クロトリマゾール	0.025	0.2	0.78	0.39
フルコナゾール	0.39	12.5	> 100	> 100

クロトリマゾール:



フルコナゾール:



(17)

以上の結果から、一般式(1)で示される本発明の化合物、とりわけ絶対配置が(R, R)であるものが、従来の抗真菌剤に比べてきわめて高い活性を有することがわかる。

- 5 また、クロトリマゾールおよびフルコナゾールとの比較から、本発明化合物のごとき、メチレン基を有するアミン環が結合した化合物が、驚くべき高い活性を示すことがわかる。

2. 感染治療実験

- 10 (1) モルモット白癬感染モデルに対する効果

Hartley系雄性モルモット(体重400-500g)の背部の皮膚の毛を抜き軽くサンドペーパーで皮膚を摩擦した。トリコフィトン・メンタグロフィテス(*Trichophyton mentagrophytes* KD-04)の小分生子浮遊液

- 15 (10⁷ cells/ml)の0.1mlを皮膚面に滴下し、ガラス棒で擦り込み感染させた。被験化合物はポリエチレングリコール400-エタノール(75:25)に1%溶液となるように溶解し、感染3日目から1日1回、10日間、0.2mlを塗布治療した。最終治療2
20 日後に動物をエーテル麻酔で屠殺し、感染部位から10個の皮膚組織片を切り出し、サブロー寒天培地上で7日間培養した。抑制率は次式より算出した。

抑制率(%)

$$= (1 - \text{菌陽性組織片数} / \text{全組織片数}) \times 100$$

- 25 その結果を表2に示す。対照化合物としてクロトリマゾールを用いた。

(18)

表 2

群	抑制率 (%)
感染無処置対照群	0
基剤対照群	0
実施例1の化合物	98
クロトリマゾール	20

(2) モルモット皮膚カンジダ感染モデルに対する治療効果

Hartley 系雄性モルモット (体重 400 - 500 g) の背部の皮膚の毛を抜きカンジダ・アルビカンス (*Candida albicans*; KC-36) 孢子浮遊液 (5×10^7 cells / ml) の 0.1 ml を皮膚面に滴下し、ガラス棒で擦り込み感染させた。感染を容易にするためプレドニソロン 30 mg / kg を感染 1 日前、感染当日および感染 4 日後に皮下投与した。被験化合物はポリエチレングリコール 400 - エタノール (75 : 25) に 1 % 溶液となるように溶解し、感染 2 日目から 1 日 1 回、3 日間、0.2 ml を塗布治療した。最終治療 2 日後に動物をエーテル麻酔で屠殺し、感染部位から 10 個の皮膚組織片を切り出し、カンジダ GS 培地栄研 (栄研化学株式会社製) 上で 7 日間培養した。抑制率は前記と同様の式より算出した。その結果を表 3 に示す。対照化合物としてクロトリマゾールを用いた。

[以下余白]

(19)

表 3

群	抑制率 (%)
感染無処置対照群	4
基剤対照群	8
実施例1の化合物	98
クロトリマゾール	96

以上1、2の試験から本発明化合物は広範囲でかつ強力な抗真菌作用を有することが認められた。

3. マウス急性毒性試験

- 5 5週令のICR雄性マウスを使用し、実施例1の化合物をポリエチレングリコール200に溶解し皮下および経口投与した。結果を表4に示す。

表 4

投与量	死亡数/使用匹数	
	皮下	経口
1000mg/kg	0/3	0/3
500mg/kg	0/3	0/3
250mg/kg	0/3	-
125mg/kg	0/3	-

10 上表に示されるように本発明の化合物の毒性は低いと考えられる。

本発明化合物は強い抗真菌活性を有しているうえ、毒性も低いものである。一般式(I)で表される本発明化

(20)

化合物を有効成分とする抗真菌剤は、ヒトを含む哺乳動物において、とくにカンジダ属、トリコフィトン属、ミクロスポラム属、エピデルモフィトン属、マラセジア属、クリプトコッカス・ネオフォルマンズ、アスペルギルス属、コクシジオイデス属、パラコクシジオイデス属、ヒストプラズマ属またはプラストミセス属の菌種による局所および全身性の真菌感染症の治療に用いることができる。本発明の化合物を有効成分とする抗真菌剤は、ヒトおよび動物の真菌感染症に有用であるばかりでなく、農園芸用防カビ剤または工業用防カビ剤などとしても有用である。

一般式(1)で表される本発明の化合物を有効成分とする抗真菌剤は化合物単独またはこれと液体または固体の製剤上の補助成分、たとえば賦形剤、結合剤、希釈剤と混合してなるもので、外用塗布、経口または非経口的に投与することができる。また、必要に応じて他の薬剤を調合させてもよい。

外用剤として投与するばあい、クリーム剤、液剤、軟膏剤、眼軟膏剤、座剤、腫剤、パウダー、乳剤などの剤形が調製可能である。調製するにあたっては、油性基剤または乳剤性基剤などを用いて調製することができ、有効成分の好ましい含量は0.1~10重量%である。投与量は患部の広さおよび症状によって適宜調節すればよい。

経口投与のばあい、粉末、錠剤、顆粒剤、カプセル剤またはシロップとして使用され、さらには皮下、筋肉内または静脈内注射剤などの注射剤としても使用される。

投与量は患者の年齢、体重および個々の条件により異

(21)

なるが、成人1日あたり有効成分として10mg～10g、好ましくは50mg～5g程度であり、投与方法としては上記1日あたりの投与量を1回ないし数回にわけて投与する。

- 5 以下に実施例および参考例をあげて本発明についてさらに詳しく説明するが、本発明はかかる実施例にのみ限定されるものではない。

なお、¹H-NMRスペクトルは重クロロホルム(CDC1₃)溶液中、内部標準としてテトラメチルシラン
 10 を使用し、JNM-EX270型スペクトルメーター(日本電子株式会社製)により測定し、ケミカルシフト(δ)値はppmで示した。また、高速液体クロマトグラフィー(以下、HPLCと略す)は光学活性カラム、キラルセル(CHIRALCEL)OJ(4.6mm×
 15 25cm、ダイセル化学工業(株)製)を用い、LC-6A(HPLC装置、(株)島津製作所製)にて測定した。

実施例1

(2R, 3R) - 2 - (2, 4 - ジフルオロフェニル)
 20 - 3 - (4 - メチレンピペリジン - 1 - イル) - 1 -
 (1H - 1, 2, 4 - トリアゾール - 1 - イル) ブタン
 - 2 - オール

4 - メチレンピペリジン塩酸塩 1.336g に 50%
 水酸化カリウム水溶液 11.2ml を加え、攪拌溶解後
 25 エチルエーテル 20ml で抽出し、水層をさらにエチル
 エーテル 10ml で抽出し、有機層を合わせエチルエー
 テルを留去した。残留物にエタノール 3ml、(2R,
 3S) - 2 - (2, 4 - ジフルオロフェニル) - 3 - メ

(22)

チル-2-[(1H-1, 2, 4-トリアゾール-1-
 イル)メチル]オキシラン 251 mg、蒸留水 3 ml を
 順次加え、85℃油浴上で24時間加熱還流した。反応
 後反応液を室温まで冷却し、酢酸エチル 20 ml および
 5 蒸留水 20 ml を加え有機層を分離した。水層をさらに
 酢酸エチル 10 ml で抽出し先の有機層と合わせ飽和食
 塩水で洗浄、無水硫酸マグネシウム上で乾燥後溶媒を留
 去した。残留物をシリカゲル 8 g を用いた HPLC に付
 10 媒で溶出し表題の化合物 188 mg をえた。収率 54.
 0%。エーテル/ヘキサン混合溶媒から再結晶を行い、
 融点 86-87℃を有する純品をえた。

HPLC: 移動層にヘキサン/イソプロピルアルコ
 ール = 9/1 を用い、流速 1.0 ml/分、室
 15 温、UV (254 nm) で検出する条件で分析
 したところ、保持時間 6.6 分に単一のピーク
 を示した。

比旋光度: $[\alpha]_D^{28} - 93^\circ$ (C = 1.00,
 CHCl₃)

20 元素分析: C₁₈H₂₂F₂N₄O として

計算値: C、62.15; H、6.36;
 N、16.02

実測値: C、62.05; H、6.37;
 N、16.08

25 ¹H-NMR スペクトル (CDCl₃) δ ppm:
 0.96 (3H, dd)、2.1-2.5 (6H, m)、
 2.6-2.8 (2H, m)、2.91 (1H, q)、4.64 (2H, s)、
 4.80 (1H, d)、4.89 (1H, d)、5.48 (1H, brs)、

(23)

6.7 - 6.8 (2H, m)、7.47 - 7.63 (1H, m)、
7.79 (1H, s)、8.03 (1H, s)。

実施例 2

(2RS, 3RS) - 2 - (2, 4 - ジフルオロフェニ
5 ル) - 3 - (4 - メチレンピペリジン - 1 - イル) - 1
- (1H - 1, 2, 4 - トリアゾール - 1 - イル) プタ
ン - 2 - オール

(2R, 3S) - 2 - (2, 4 - ジフルオロフェニル)
- 3 - メチル - 2 - [(1H - 1, 2, 4 - トリアゾ
10 ル - 1 - イル) メチル] オキシランの代わりにそのラセ
ミ体である (2RS, 3SR) - 2 - (2, 4 - ジフル
オロフェニル) - 3 - メチル - 2 - [(1H - 1, 2,
4 - トリアゾール - 1 - イル) メチル] オキシランを使
って実施例 1 と同様にして表題の化合物をえた。

15 HPLC : 移動層にヘキサン / イソプロピルアルコ
ール = 9 / 1 を用い、流速 1.0 ml / 分、室
温、UV (254 nm) で検出する条件で分析
したところ、保持時間 6.6 分と 5.8 分に面
積比 1 : 1 の 2 本のピークを示した。

20 ¹H - NMR スペクトル (CDCl₃) δ ppm :
0.96 (3H, dd, J=3Hz, 7Hz)、2.1 - 2.5 (6H, m)、
2.6 - 2.8 (2H, m)、2.91 (1H, q, J=7Hz)、
4.64 (2H, s)、4.80 (1H, d, J=15Hz)、
4.89 (1H, d, J=15Hz)、5.47 (1H, br s)、
25 6.7 - 6.8 (2H, m)、7.5 - 7.6 (1H, m)、
7.79 (1H, s)、8.02 (1H, s)

実施例 3

(2S, 3S) - 2 - (2, 4 - ジフルオロフェニル)

(24)

− 3 − (4 − メチレンピペリジン − 1 − イル) − 1 −
(1 H − 1 , 2 , 4 − トリアゾール − 1 − イル) ブタン
− 2 − オール

(2 R , 3 S) − 2 − (2 , 4 − ジフルオロフェニル)
5 − 3 − メチル − 2 − [(1 H − 1 , 2 , 4 − トリアゾール − 1 − イル)
メチル] オキシランの代わりにそのエナンチオマーである (2 S , 3 R) − 2 − (2 , 4 − ジフル
ロフェニル) − 3 − メチル − 2 − [(1 H − 1 , 2 ,
4 − トリアゾール − 1 − イル) メチル] オキシランを使
10 って実施例 1 と同様にして表題の化合物をえた。

H P L C : 移動層にヘキサン / イソプロピルアルコ
ール = 9 / 1 を用い、流速 1 . 0 m l / 分、室
温、U V (2 5 4 n m) で検出する条件で分析
したところ、保持時間 5 . 8 分に単一のピーク
を示した。

15 ¹H − N M R スペクトル (C D C l ₃) δ p p m :
0 . 9 6 (3 H , d d , J = 3 H z , 7 H z) 、 2 . 1 − 2 . 5 (6 H , m) 、
2 . 6 − 2 . 8 (2 H , m) 、 2 . 9 1 (1 H , q , J = 7 H z) 、
4 . 6 4 (2 H , s) 、 4 . 8 0 (1 H , d , J = 1 5 H z) 、
20 4 . 8 9 (1 H , d , J = 1 5 H z) 、 5 . 4 8 (1 H , b r s) 、
6 . 7 − 6 . 8 (2 H , m) 、 7 . 5 − 7 . 6 (1 H , m) 、
7 . 7 8 (1 H , s) 、 8 . 0 3 (1 H , s)

実施例 4 ~ 1 4

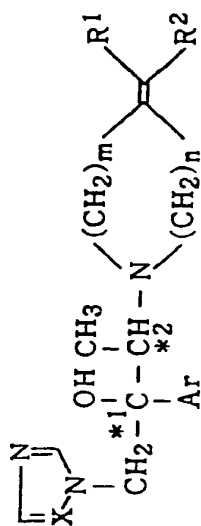
実施例 1 と同様にして、表 5 に示される原料を使用し
25 て表 6 に示される実施例 4 から 1 4 の化合物を合成した。

表 5

実施例 番号	エポキシ化合物 (II)				アミン誘導体 (III)			
	X	*1	*2	Ar	R ¹	R ²	m	n
4	CH	R	S	2,4-ジフルオロ フェニル	H	H	2	2
5	CH	RS	SR	2,4-ジフルオロ フェニル	H	H	2	2
6	N	R	S	4-クロロフェニル	H	H	2	2
7	N	R	S	2,4-ジクロロ フェニル	H	H	2	2
8	N	RS	SR	2,4-ジフルオロ フェニル	H	CH ₃	2	2
9	N	R	S	2,4-ジフルオロ フェニル	H	n-C ₅ H ₁₁	2	2
10	N	RS	SR	2,4-ジフルオロ フェニル	H	Ph*	2	2
11	N	R	S	2,4-ジフルオロ フェニル	Ph*	Ph*	2	2
12	N	R	S	2,4-ジフルオロ フェニル	H	CH=CH ₂	2	2
13	N	R	S	2,4-ジフルオロ フェニル	H	H	3	1
14	N	R	S	2,4-ジフルオロ フェニル	H	H	2	1

* Phはフェニル基を表す

表 6



実施例 番号	えられた化合物					¹ H - NMR スペクトル (CDCl ₃)		
	R1	R2	X	m	n		絶対配置 Ar	
4	H	H	CH	2	2	R	2,4-ジフル オロフェニル	1.03 (3H, dd, J = 5Hz, 7Hz), 2.2 - 2.4 (8H, m), 2.77 (1H, q, J = 7Hz), 4.35 (1H, d, J = 14Hz), 4.54 (1H, d, J = 14Hz), 4.64 (2H, s), 6.7 - 6.8 (3H, m), 6.92 (1H, s), 7.45 (1H, s), 7.5 - 7.6 (1H, m)
5	H	H	CH	2	2	RS RS	2,4-ジフル オロフェニル	1.02 (3H, dd, J = 5Hz, 7Hz), 2.2 - 2.4 (8H, m), 2.77 (1H, q, J = 7Hz), 4.35 (1H, d, J = 14Hz), 4.54 (1H, d, J = 14Hz), 4.64 (2H, s), 6.7 - 6.8 (3H, m), 6.92 (1H, s), 7.45 (1H, s), 7.5 - 7.6 (1H, m)

(27)

6	H	H	N	2	2	R	R	4-クロロフェニル	1.07 (3H,d,J = 7Hz)、2.0 - 2.4 (8H,m)、2.55 (1H,q,J = 7Hz)、4.41 (1H,d,J = 14Hz)、4.61 (2H,s)、4.70 (1H,d,J = 14Hz)、5.80 (1H,s)、7.2 - 7.5 (4H,m)、7.84 (1H,s)、8.17 (1H,s)
7	H	H	N	2	2	R	R	2,4-ジクロロフェニル	0.80 (3H,d,J = 7Hz)、2.2 - 2.4 (4H,m)、2.4 - 2.6 (2H,m)、2.9 - 3.1 (2H,m)、3.55 (1H,q,J = 7Hz)、4.66 (2H,s)、4.83 (1H,d,J = 15)、4.91 (1H,s)、5.52 (1H,d,J = 15Hz)、7.09 (1H,dd,J = 9Hz,2Hz)、7.27 (1H,d,J = 2Hz)、7.56 (1H,d,J = 9Hz)、7.74 (1H,s)、7.93 (1H,s)、8.04 (1H,s)
8	H	CH ₃	N	2	2	RS	RS	2,4-ジフルオロフェニル	0.97 (3H,dd,J = 3Hz,7Hz)、1.56 (3H,d,J = 7Hz)、2.1 - 2.5 (6H,m)、2.5 - 2.8 (2H,m)、2.87 (1H,q,J = 7Hz)、4.79 (1H,d,J = 15Hz)、4.88 (1H,d,J = 15Hz)、5.17 (1H,q,J = 7Hz)、5.62 (1H,s)、6.7 - 6.9 (2H,m)、7.27 (1H,s)、7.5 - 7.6 (1H,m)、7.79 (1H,s)、8.05 (1H,s)

(28)

9	H	C ₅ H ₁₁	N	2	2	R	R	2,4-ジフル オロフェニル	0.88 (3H,t,J = 7Hz)、0.97 (3H,dd,J = 7Hz、 4Hz)、1.2 - 1.4 (6H,m)、1.9 - 2.0 (2H,m)、 2.1 - 2.3 (6H,m)、2.6 (2H,m)、2.86 (1H,q、 J = 7Hz)、4.78 (1H,d,J = 15Hz)、4.88 (1H、 d,J = 16Hz)、5.10 (1H,t,J = 7Hz)、5.6 (1H、 brs)、6.7 - 6.8 (2H,m)、7.3 - 7.6 (1H,m)、 7.79 (1H,s)、8.05 (1H,s)
10	H	Ph	N	2	2	RS	RS	2,4-ジフル オロフェニル	0.97 (3H,dd,J = 3Hz,7Hz)、1.56 (3H,d,J = 7Hz)、2.6 - 2.7 (1H,m)、2.7 - 2.8 (1H,m)、 2.91 (1H,q,J = 7Hz)、4.82 (1H,d,J = 15Hz)、 4.91 (1H,d,J = 15Hz)、5.5 (1H,brs)、 6.26 (1H,s)、6.7 - 6.8 (2H,m)、7.1 - 7.2 (3H,m)、7.3 (2H,m)、7.5 - 7.6 (1H,m)、 7.79 (1H,s)、8.03 (1H,s)
11	Ph	Ph	N	2	2	R	R	2,4-ジフル オロフェニル	1.00 (3H,dd,J = 7Hz)、2.35 (6H,s)、2.6 - 2.7 (2H,m)、2.89 (1H,q,J = 7Hz)、4.76 - 4.90 (2H,dd,J = 9Hz,15Hz)、6.7 - 6.8 (2H、 m)、7.1 - 7.3 (10H,m)、7.5 (1H,m)、7.78 (1H,s)、8.05 (1H,s)

(8)

12	H	CHCH ₂	N	2	2	R	R	2,4-ジフル オロフェニル	0.95, 1.01 (3H, each dd, J = 3Hz, 7Hz), 1.76 (2H, d), 2.1 - 2.2 (2H, m), 2.3 - 2.4 (2H, m), 2.6 - 2.7 (1H, m), 2.9 (1H, m), 3.0 - 3.3 (1H, m), 4.6 - 5.2 (3H, m), 5.51 (1H, brs), 5.4 - 5.7 (1H, m), 5.82, 6.05 (1H, each d, J = 11Hz, 16Hz), 6.7 - 6.8 (1H, m), 7.5 - 7.6 (1H, m), 7.78 (1H, s), 8.02 (1H, s)
13	H	H	N	3	1	R	R	2,4-ジフル オロフェニル	1.02 (3H, dd, J = 3, 7Hz), 1.5 - 1.8 (2H, m), 2.0 - 2.2 (2H, m), 2.3 - 2.5 (2H, m), 2.6 - 2.9 (3H, m), 3.15 (1H, d, J = 10Hz), 4.65 (1H, s), 4.70 (1H, s), 4.75 (1H, J = 15Hz), 5.53 (1H, s), 6.7 - 6.9 (2H, m), 7.5 - 7.6 (1H, m), 7.78 (1H, s), 8.04 (1H, s)
14	H	H	N	2	1	R	R	2,4-ジフル オロフェニル	0.90 (3H, dd, J = 2Hz, 7Hz), 2.43 (2H, brs), 2.7 - 2.9 (2H, m), 3.2 - 3.4 (2H, m), 4.81 (1H, d, J = 15Hz), 4.86 (1H, d, J = 2Hz), 4.90 (1H, d, J = 15Hz), 6.7 - 6.8 (2H, m), 7.4 - 7.5 (1H, m), 7.77 (1H, s), 7.95 (1H, s)

(30)

実施例 15 (実施例 1 の化合物の別途合成法)

(2 R , 3 S) - 2 - (2 , 4 - ジフルオロフェニル)
- 3 - メチル - 2 - [(1 H - 1 , 2 , 4 - トリアゾール - 1 - イル) メチル] オキシラン 17 . 59 g (70
5 mmol) を 4 - メチレンピペリジン水溶液 (含有量 6
1 %) 113 g に溶かし、90℃で21時間加熱還流した。反応後、過剰の 4 - メチレンピペリジンを減圧下留
去し、残留物をイソプロピルアルコール 140 ml に溶かし、イソプロピルアルコール 50 ml に溶かした p -
10 トルエンスルホン酸一水和物 13 . 32 g (70 mmol) を加えた。室温で1時間、冷蔵庫で一晩放置後、析
出した結晶を濾取しイソプロピルアルコール 50 ml で
洗浄後、乾燥すると (2 R , 3 R) - 2 - (2 , 4 - ジ
フルオロフェニル) - 3 - (4 - メチレンピペリジン -
15 1 - イル) - 1 - (1 H - 1 , 2 , 4 - トリアゾール -
1 - イル) ブタン - 2 - オールの p - トルエンスルホン
酸塩の結晶 32 . 20 g がえられた。

上記でえられた p - トルエンスルホン酸塩 18 . 3 g
にエチルエーテル 40 ml および 1 N - 水酸化ナトリウム
20 ム水溶液 35 ml を加え有機層を分取し、無水硫酸マグ
ネシウム 5 g で乾燥後溶媒を留去した。残留液に n - ヘ
キサン 40 ml を加え、析出した結晶を濾取し、乾燥す
ると目的とする (2 R , 3 R) - 2 - (2 , 4 - ジフル
オロフェニル) - 3 - (4 - メチレンピペリジン - 1 -
25 イル) - 1 - (1 H - 1 , 2 , 4 - トリアゾール - 1 -
イル) ブタン - 2 - オールが 9 . 43 g えられた。この
ものは実施例 1 の化合物と ¹H - NMR が一致した。

参考例 1

(31)

4-ベンジリデンピペリジン塩酸塩の合成

(1) アルゴン気流下、塩化ベンジルトリフェニルホスホニウム 49.0 g (126 mmol) を無水テトラヒドロフラン 100 ml に懸濁し、氷冷下、ブチルリチウム 86 ml を滴下した。室温で 1 時間攪拌後、1-ベンジルー 4-ピペリドンの無水テトラヒドロフラン溶液を氷冷下滴下し、15 時間加熱還流した。反応溶液を濾過し、濾液にジエチルエーテル、水を加え有機層を分取した。有機層を水、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥した。溶媒を減圧下留去してえられた油状物をシリカゲル 1 kg を用いたカラムクロマトグラフィーに付し、酢酸エチルーヘキサン (1:100-3:100) で溶出して 1-ベンジルー 4-ベンジリデンピペリジン 22.6 g をえた。

¹H-NMR スペクトル (CDCl₃) δ ppm :
2.4 - 2.5 (4H, m) 、 2.5 - 2.6 (4H, m) 、
3.52 (2H, s) 、 6.27 (1H, s) 、 7.1 - 7.4 (10H, m)

(2) 1-ベンジルー 4-ベンジリデンピペリジン 24.6 g (96 mmol) をジクロロエタン 200 ml に溶かし、氷冷下、クロロギ酸 1-クロロエチル 11.1 ml (102 mmol) を滴下した。反応液を 30 分加熱還流し、その後室温で 1.5 時間攪拌した。減圧下溶媒を留去して反応液を 80 ml に濃縮し、メタノール 200 ml を加え 12 時間加熱還流した。溶媒を減圧下留去してえられた残留物にイソプロピルエーテル 100 ml を加え沈殿物を濾取し、表題化合物 8.6 g をえた。

¹H-NMR スペクトル (CDCl₃) δ ppm :
2.74 (2H, t, J=6Hz) 、 2.84 (2H, t, J=6Hz) 、

(32)

3.18 (2H, brs)、3.31 (2H, brs)、6.47 (1H, s)、
7.1 - 7.4 (5H, m)、9.8 (2H, brs)

参考例 2

4-ジフェニルメチレンピペリジンの合成

5 (1) イソニコチン酸エチルエステル 102 g (650 mmol) をジオキササン 100 ml に懸濁し、氷冷下 t-ブトキシカルバメート 213 g (974 mmol) を加え 15 時間攪拌した。溶媒を減圧下留去し、1-
10 t-ブトキシカルボニル-4-エトキシカルボニルピペリジン 234 g をえた。

$^1\text{H-NMR}$ スペクトル (CDCl_3) δ ppm :

1.27 (3H, t, $J=7\text{Hz}$)、1.46 (9H, s)、
1.6 - 1.7 (2H, m)、1.8 - 1.9 (2H, m)、
2.3 - 2.5 (1H, m)、2.8 - 2.9 (2H, m)、
15 3.7 - 4.0 (2H, m)、4.14 (2H, q, $J=7\text{Hz}$)

(2) アルゴン気流下、1-t-ブトキシカルボニル-4-エトキシカルボニルピペリジン 26.4 g (72 mmol) を乾燥テトラヒドロフラン 100 ml に溶かし、氷冷下 2 mol / mol フェニルマグネシウムブロマイド
20 108 ml を滴下して 2 日間攪拌した。反応溶液を飽和塩化アンモニウム溶液 200 ml、酢酸エチル 200 ml 中に注ぎ、有機層を分取した。有機層を水、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥した。溶媒を減圧下留去し、1-t-ブトキシカルボニル-4-(ヒ
25 ドロキシジフェニル)メチルピペリジン 34.7 g をえた。

$^1\text{H-NMR}$ スペクトル (CDCl_3) δ ppm :

1.2 (4H, m)、1.42 (9H, s)、2.5 - 2.7 (3H, m)、

(33)

4.1 - 4.2 (2H, m) 、 7.2 - 7.5 (10H, m)

(3) 1-*t*-ブトキシカルボニル-4-(ヒドロキシジフェニル)メチルピペリジン 20 g (54 mmol) をフェノール 12.8 g、48%臭化水素水 210 ml に溶かし、140℃で5時間、室温で15時間攪拌した。有機層を分取し、ジエチルエーテルを加え析出した沈澱を濾取した。沈澱にジエチルエーテル、水酸化カリウム溶液を加え有機層を分取し、水酸化カリウムで乾燥した。溶媒を減圧下留去し、表題化合物 6.1 g をえた。

¹H-NMR スペクトル (CDCl₃) δ ppm :
2.0 (1H, br s) 、 2.32 (4H, t, J=6 Hz) 、
2.91 (4H, t, J=6 Hz) 、 7.1 - 7.3 (10H, m)

参考例 3

4-プロペニリデンピペリジン塩酸塩の合成

(1) アルゴン気流下、臭化アリルトリフェニルホスホニウム 2.9 g (7.5 mmol) を無水テトラヒドロフラン 10 ml に懸濁し、氷冷下、ブチルリチウム 4.3 ml を滴下した。室温で30分攪拌後、1-ベンジル-4-ピペリドン 1 g (5.3 mmol) の無水テトラヒドロフラン溶液を氷冷下滴下し、室温で15時間攪拌した。反応溶液を濾過し、濾液に酢酸エチル、水を加え有機層を分取した。有機層を水、飽和食塩水で洗浄し、無水硫酸マグネシウムで乾燥した。溶媒を減圧下留去してえられた油状物をシリカゲル 40 g を用いたカラムクロマトグラフィーに付し、酢酸エチル-ヘキサン (1:1-1:3) で溶出して1-ベンジル-4-プロピリデンピペリジン 200 mg をえた。

¹H-NMR スペクトル (CDCl₃) δ ppm :

(34)

2.2 - 2.3 (2H, m) 、 2.4 - 2.5 (6H, m) 、
 3.50 (2H, s) 、 4.98 (1H, d, J=10Hz) 、
 5.11 (1H, d, J=17Hz) 、 5.82 (1H, d, J=11Hz) 、
 6.5 - 6.6 (1H, m) 、 7.2 - 7.3 (5H, m)

- 5 (2) 1-ベンジル-4-プロピリデンピペリジン 4.5 g (21 mmol) をジクロロエタン 20 ml に溶かし、氷冷下、クロロギ酸 1-クロロエチル 2.8 ml (25 mmol) を滴下した。反応液を室温で 30 分間攪拌し、その後 30 分間加熱還流した。減圧下溶媒を留去して反応液を 10 ml に濃縮し、メタノール 60 ml を加え 12 時間加熱還流した。溶媒を減圧下留去して表題化合物 3.7 g をえた。

- 15 ¹H-NMR スペクトル (CDCl₃) δ ppm :
 2.3 - 3.0 (4H, m) 、 3.0 - 3.6 (4H, m) 、
 4.7 - 6.3 (4H, m)

製剤例 1 液剤

- 20 200 ml のマクロゴール 400 をエタノール 750 ml に溶かし、これに実施例 1 の化合物、(2R, 3R) - 2 - (2, 4-ジフルオロフェニル) - 3 - (4-メチレンピペリジン-1-イル) - 1 - (1H-1, 2, 4-トリアゾール-1-イル) ブタン-2-オール 5 g を加えて溶かした。さらにエタノールにて全量を 1000 ml とし、液剤として供した。

製剤例 2 軟膏剤

- 25 白色ワセリン 400 g、セタノール 180 g、パラオキシ安息香酸プロピル 1 g およびセスキオレイン酸ソルビタン 50 g を水浴上にて 80℃ に保ちながら溶かし、ついで実施例 1 の化合物、(2R, 3R) - 2 - (2,

(35)

4 - ジフルオロフェニル) - 3 - (4 - メチレンピペリ
ジン - 1 - イル) - 1 - (1 H - 1 , 2 , 4 - トリアゾ
ール - 1 - イル) ブタン - 2 - オール 5 g を加えて溶か
した。一方、パラオキシ安息香酸メチル 1 g に水を加え
5 8 0 ° C に加温して溶かした液を前述の液に徐々に加え混
合した。冷却後軟膏剤として供した。

製剤例 3 クリーム剤

白色ワセリン 1 5 g 、流動パラフィン 2 0 0 g 、ステ
アシルアルコール 5 0 g 、モノステアリン酸グリセリン
1 0 4 0 g 、プロピレングリコール 1 4 5 g およびパラオキシ
1 0 安息香酸プロピル 1 g よりなる混合物を水浴上で 8 0
° C に保ちながら溶解し、ついで実施例 1 の化合物、(2
R , 3 R) - 2 - (2 , 4 - ジフルオロフェニル) - 3
- (4 - メチレンピペリジン - 1 - イル) - 1 - (1 H
1 5 - 1 , 2 , 4 - トリアゾール - 1 - イル) ブタン - 2 -
オール 1 0 g を加えて溶かし、さらに 4 0 g のステアリン
酸ポリオキシ 4 0 および 1 g のパラオキシ安息香酸メ
チルに精製水 4 9 8 g を加え、8 0 ° C に加温して溶解し
た液を加えたのち充分攪拌した。攪拌後、冷却水を用い
2 0 て冷却しながら固まるまでさらに充分攪拌し、クリーム
剤として供した。

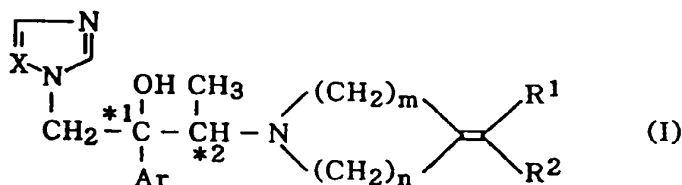
産業上の利用可能性

本発明化合物は強い抗真菌活性を有している。したが
って、一般式 (1) で表される本発明化合物を有効成分
2 5 とする抗真菌剤は、ヒトおよび動物の真菌疾患に対し、
その予防および治療に有効であり、また、農園芸用防カ
ビ剤および工業用防カビ剤などとしても有用である。

(36)

請求の範囲

1. 一般式 (I) :



- (式中、Arは無置換またはハロゲン原子およびトリ
 5 フルオロメチルから選ばれた置換基1～3個で置換さ
 れたフェニル基を示し、R¹およびR²は、同一また
 は異なって水素原子、低級アルキル基、無置換または
 ハロゲン原子および低級アルキル基から選ばれた置換
 基1～3個で置換されたアリール基、アルケニル基、
 10 アルキニル基、またはアラルキル基を示し、mは2ま
 たは3を示し、nは1または2を示し、Xは窒素原子
 またはCHを示し、*1、*2は不斉炭素を示す)で
 表される化合物またはその酸付加塩。
2. *1、*2の不斉炭素の絶対配置が(R, R)であ
 15 る請求の範囲第1項記載の化合物またはその酸付加塩。
3. *1、*2の不斉炭素の絶対配置が(R, R)であ
 る一般式(I)で表される化合物またはその酸付加塩
 を含み、他の光学異性体を含んでいる混合体である請
 求の範囲第1項記載の化合物またはその酸付加塩。
- 20 4. Xが窒素原子である請求の範囲第1項、第2項また
 は第3項記載の化合物またはその酸付加塩。
5. XがCHである請求の範囲第1項、第2項または第
 3項記載の化合物またはその酸付加塩。

(37)

6. *1、*2の不斉炭素の絶対配置が(R, R)である一般式(1)で示される化合物が(2R, 3R)-2-(2,4-ジフルオロフェニル)-3-(4-メチレンピペリジン-1-イル)-1-(1H-1,2,4-トリアゾール-1-イル)ブタン-2-オールである請求の範囲第2項または第3項記載の化合物またはその酸付加塩。
7. 請求の範囲第1項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
8. 請求の範囲第2項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
9. 請求の範囲第3項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
10. 請求の範囲第4項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
11. 請求の範囲第5項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
12. 請求の範囲第6項記載の化合物またはその酸付加塩を有効成分として含有する抗真菌剤。
13. 請求の範囲第1項記載の化合物またはその酸付加塩を用いた真菌感染症の治療方法。
14. 請求の範囲第2項記載の化合物またはその酸付加塩を用いた真菌感染症の治療方法。
15. 請求の範囲第3項記載の化合物またはその酸付加塩を用いた真菌感染症の治療方法。
16. 請求の範囲第4項記載の化合物またはその酸付加塩を用いた真菌感染症の治療方法。
17. 請求の範囲第5項記載の化合物またはその酸付加塩

(38)

を用いた真菌感染症の治療方法。

18. 請求の範囲第6項記載の化合物またはその酸付加塩を用いた真菌感染症の治療方法。

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP94/00737

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl⁵ C07D401/06, 403/06, A61K31/40, 31/445
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
Int. Cl⁵ C07D401/00, 403/00, A61K31/00
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP, A, 59-33271 (Pfizer Corp.), February 23, 1984 (23. 02. 84), Claim & US, A, 4507484	1-18

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:
 "A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier document but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reasons (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed
 "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
 "A" document member of the same patent family

Date of the actual completion of the international search
June 17, 1994 (17. 06. 94)

Date of mailing of the international search report
July 12, 1994 (12. 07. 94)

Name and mailing address of the ISA/
Japanese Patent Office
Facsimile No.

Authorized officer
Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

A. 発明の属する分野の分類 (国際特許分類 (IPC))
**Int. Cl.⁸ C07D401/06, 403/06,
 A61K31/40, 31/445**

B. 調査を行った分野

調査を行った最小限資料 (国際特許分類 (IPC))
**Int. Cl.⁸ C07D401/00, 403/00,
 A61K31/00**

最小限資料以外の資料で調査を行った分野に含まれるもの

国際調査で使用了電子データベース (データベースの名称、調査に使用した用語)
CAS ONLINE

C. 関連すると認められる文献

引用文献の カテゴリー*	引用文献名 及び一部の箇所が関連するときは、その関連する箇所の表示	関連する 請求の範囲の番号
A	JP, A, 59-33271 (ファイザー・コーポレーション), 23. 2月. 1984 (23. 02. 84), 特許請求の範囲 & US, A, 4507484	1-18

C欄の続きにも文献が列挙されている。 パテントファミリーに関する別紙を参照。

* 引用文献のカテゴリー
 「A」 特に関連のある文献ではなく、一般的技術水準を示すもの
 「E」 先行文献ではあるが、国際出願日以後に公表されたもの
 「L」 優先権主張に拠るを提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献 (理由を付す)
 「O」 口頭による開示、使用、展示等に関する文献
 「P」 国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
 「T」 国際出願日又は優先日後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
 「X」 特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
 「Y」 特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
 「&」 同一パテントファミリー文献

国際調査を完了した日 **17. 06. 94** 国際調査報告の発送日 **12.07.94**

名称及びあて先 日本国特許庁 (ISA/JP) 郵便番号100 東京都千代田区霞が関三丁目4番3号	特許庁審査官 (権限のある職員) 池田 正人 電話番号 03-3581-1101 内線 3454
--	--

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

10/21/2003 DTESSEM1 00000064 10685266

01 FC:1001

770.00 DP

PTO-1556
(5/87)

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2003

Application or Docket Number

10685266

CLAIMS AS FILED - PART I

(Column 1) (Column 2)

TOTAL CLAIMS	4	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	4 minus 20 = *	0
INDEPENDENT CLAIMS	2 minus 3 = *	0
MULTIPLE DEPENDENT CLAIM PRESENT		<input type="checkbox"/>

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY

TYPE

OTHER THAN SMALL ENTITY

RATE	FEE	OR	RATE	FEE
BASIC FEE	385.00	OR	BASIC FEE	770.00
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL		OR	TOTAL	770

CLAIMS AS AMENDED - PART II

(Column 1) (Column 2) (Column 3)

AMENDMENT A		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus	**	=
	Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					<input type="checkbox"/>

SMALL ENTITY

OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1) (Column 2) (Column 3)

AMENDMENT B		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus	**	=
	Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					<input type="checkbox"/>

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

(Column 1) (Column 2) (Column 3)

AMENDMENT C		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus	**	=
	Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM					<input type="checkbox"/>

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
 *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Same

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2003

Application or Docket Number

10685266

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	4	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	0 minus 20 = *	0
INDEPENDENT CLAIMS	2 minus 3 = *	0
MULTIPLE DEPENDENT CLAIM PRESENT		<input type="checkbox"/>

SMALL ENTITY TYPE <input type="checkbox"/>		OR	OTHER THAN SMALL ENTITY	
RATE	FEE		RATE	FEE
BASIC FEE	385.00	OR	BASIC FEE	770.00
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL		OR	TOTAL	770

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	**
	Total	*	=
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

SMALL ENTITY TYPE <input type="checkbox"/>		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	**
	Total	*	=
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

SMALL ENTITY TYPE <input type="checkbox"/>		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	**
	Total	*	=
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR
		Minus	***
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

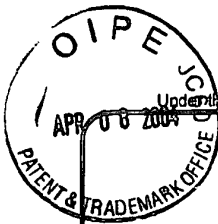
SMALL ENTITY TYPE <input type="checkbox"/>		OR	OTHER THAN SMALL ENTITY	
RATE	ADDITIONAL FEE		RATE	ADDITIONAL FEE
X\$ 9=		OR	X\$18=	
X43=		OR	X86=	
+145=		OR	+290=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
 *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
 The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

**MULTIPLE DEPENDENT CLAIM
FEE CALCULATION SHEET
(FOR USE WITH FORM PTO-875)**

SERIAL NO. 10685266 FILING DATE _____
 APPLICANT(S) _____

CLAIMS							*		*		*	
AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT		IND.	DEP.	IND.	DEP.	IND.	DEP.	
IND.	DEP.	IND.	DEP.	IND.	DEP.							
1		1										
2		—										
3		—										
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TOTAL DEP.	↓	2	↓	↓		TOTAL DEP.	↓	↓	↓			
TOTAL CLAIMS		4				TOTAL CLAIMS						



Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM <small>(to be used for all correspondence after initial filing)</small>	Application Number	10/685,266
	Filing Date	October 14, 2003
	First Named Inventor	TATSUMI, Y.
	Art Unit	1651
	Examiner Name	To be assigned
Total Number of Pages in This Submission	Attorney Docket Number	700938-52220-DIV

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form	<input type="checkbox"/> Drawing(s)	<input type="checkbox"/> After Allowance Communication to Group
<input type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
<input type="checkbox"/> Amendment/Reply	<input type="checkbox"/> Petition	<input type="checkbox"/> Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
<input type="checkbox"/> After Final	<input type="checkbox"/> Petition to Convert to a Provisional Application	<input type="checkbox"/> Proprietary Information
<input type="checkbox"/> Affidavits/declaration(s)	<input checked="" type="checkbox"/> Change of Attorney/Delegation	<input type="checkbox"/> Status Letter
<input type="checkbox"/> Extension of Time Request	<input type="checkbox"/> Change of Correspondence Address	<input checked="" type="checkbox"/> Other Enclosure(s) (please identify below):
<input type="checkbox"/> Express Abandonment Request	<input type="checkbox"/> Terminal Disclaimer	Certificate of Mailing and Return Receipt Postcard
<input type="checkbox"/> Information Disclosure Statement	<input type="checkbox"/> Request for Refund	
<input type="checkbox"/> Certified Copy of Priority Document(s)	<input type="checkbox"/> CD, Number of CD(s) _____	
<input type="checkbox"/> Response to Missing Parts/Incomplete Application	Remarks	
<input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53	The Commissioner is hereby authorized to charge any additional fees associated with this communication to Deposit Account No. 50-0850.	

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT	
Firm or Individual name	Ronald I. Eisenstein (30,628) Nixon Peabody LLP 100 Summer Street, Boston, MA 02110
Signature	<i>Ronald I. Eisenstein</i>
Date	April 6, 2004

CERTIFICATE OF TRANSMISSION/MAILING	
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below. Washington, DC 20231 on this date:	
Typed or printed name	Linda M. Ginsberg
Signature	<i>Linda M. Ginsberg</i>
Date	4/6/2004

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



Docket No. 700938-052220-DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi et al.
Application No.: 10/685,266 (divisional of 10/031,929) Group No.: 1651 (1651)
Filed: 10/14/2003 Examiner: To be assigned
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CERTIFICATE OF MAILING/TRANSMISSION (37 C.F.R. § 1.8(a) and 1.10)

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- 2. Transmittal (1 pg.);
- 3. Change of Attorney's Address (1 pg.); and
- 4. Return Receipt Postcard.

is on the date shown below being:

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deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450.

FACSIMILE

transmitted by facsimile to the Patent and Trademark Office.

Date: April 6, 2004



Signature

Linda M. Ginsberg

(type or print name of person certifying)



Docket No. 700938-052220-DIV

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Tatsumi et al.
Application No.: 10/685,266(divisional of 10/031,929) Group No.: 1651 (1651)
Filed: 10/14/2003 Examiner: To be assigned
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

CHANGE OF ATTORNEY'S ADDRESS IN APPLICATION

Please send all correspondence for this application as follows:

Ronald I. Eisenstein
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110

Please direct telephone calls to:

(617) 345-6054

SIGNATURE OF PRACTITIONER
Ronald I. Eisenstein (Reg. No. 30,628)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110
Tel. No. (617) 345-6054

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



B1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification⁶ : A61K 6/00, 7/00, 7/04</p>	<p>AI</p>	<p>(11) International Publication Number: WO 99/39680 (43) International Publication Date: 12 August 1999 (12.08.99)</p>
<p>(21) International Application Number: PCT/US99/02628 (22) International Filing Date: 8 February 1999 (08.02.99) (30) Priority Data: 60/074,025 9 February 1998 (09.02.98) US (71) Applicant: MACROCHEM CORPORATION [US/US]; 110 Hartwell Avenue, Lexington, MA 02421-3134 (US). (72) Inventors: SAMOUR, Carlos, M.; 8 Emery Road, Bedford, MA 01730 (US). KRAUSER, Scott, F.; 4 Bridgeview Circle #41, Tyngsboro, MA 01879 (US). (74) Agent: STEINBERG, Richard, A.; Sherman & Shalloway, 413 North Washington Street, Alexandria, VA 22314 (US).</p>	<p>(81) Designated States: BR, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i></p>	
<p>(54) Title: ANTIFUNGAL NAIL LACQUER AND METHOD USING SAME (57) Abstract A nail lacquer effective for the treatment or prevention of fungal infections, such as, onychomycosis, includes fungicidally effective amount of ciclopirox, econazole, or other antifungal agent in a clear, stable, film-forming lacquer vehicle which includes a water-insoluble film-forming polymer, 2-n-nonyl-1,3-dioxolane or similar penetration enhancer; and volatile solvent. A plasticizer for the film-forming polymer which is also compatible with the other components may be included although the preferred penetration enhancers may also function as plasticizer. The composition, when applied to the nails provides a hard, clear, water-resistant film containing the antifungal agent. The film is resistant to multiple washings and is effective in the treatment of onychomycosis.</p>		

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ANTIFUNGAL NAIL LACQUER AND METHOD USING SAMEBackground of the Invention

(1). Field of Invention

This invention relates to antifungal nail lacquer compositions and to the treatment of onychomycoses or other
5 fungal infestations affecting toe nails or finger nails using the nail lacquer composition. More particularly, the invention relates to antifungal nail lacquers which when applied to nails form strongly adherent, water-resistant, clear films; and to the method for treating or preventing
10 fungal infestations of animal nails by applying the antifungal composition to the infected nail or to the fungal susceptible nail.

(2). State of the Prior Art

Fungal infection of the nails, commonly referred to as
15 onychomycosis, is most frequently caused by dermatophytes but also can be caused by molds and *Candida*. Mixed infections also occur. Onychomycosis includes dermatophyte infection of the nail plate and includes infection of nails by any fungus, including yeast or molds. Thus, for example,
20 onychomycosis serves as a reservoir for dermatophytes and contributes to treatment failure and recurrence of tinea pedis.

Most common causes of tinea unguium are *Trichophyton rubrum* (most frequent), *T. mentagrophytes*, and
25 *Epidermophyton floccosum*. Onychomycosis due to nondermatophytes is usually caused by *Candida* species.

Nail lacquers for the treatment of onychomycoses and similar fungal infections affecting nails (toe nails and/or finger nails) of humans, in particular, or other animals, are known. Representative examples are described in the patent literature, of which the following U.S. patents can be mentioned: 4,957,730 (1-hydroxy-2-pyridone in water-insoluble film-former); 5,120,530 (amorolfine in quaternary ammonium acrylic copolymer); 5,264,206 (tioconazole, econazole, oxiconazole, miconazole, tolnaftate, naftifine hydrochloride, in water-insoluble film-former); 5,346,692 (with urea and dibutyl phthalate plasticizer); 5,487,776 (griseofulvin as colloidal suspension).

Other U.S. patents which relate to antifungal products include, for example, 4,636,520 (combination of imidazole and pyrrolnitrin); 5,002,938 (gel, combination of imidazole and 17-ester corticosteroid antiinflammatory agent); 5,110,809 (antifungal gel plus steroid); 5,219,877 (gel product with imidazole antifungal optionally with steroidal antiinflammatory, in a vehicle system that includes lauryl alcohol); 5,391,367 (aqueous alcoholic gel with tioconazole); 5,464,610 (salicylic acid plaster); 5,696,105 (mometasone furoate).

Effectiveness of nail lacquers as a delivery vehicle for topically administering the antifungal agent amorolfine is described by Jean-Paul L. Marty, J. of the European Academy of Dermatology and Venereology, 4(Suppl. 1), pp.S17-S21 (1995). As described by the author, the film-generating solution as the lacquer base for the active

principle basically consists of volatile solvent (ethanol, ethyl/butyl/methyl acetate, methylene chloride, methyl ethyl ketone, isopropanol), and a non-water-soluble polymer (methacrylic acid copolymers, vinyl polymers) which leaves a
5 thin continuous film following evaporation of the solvent. Plasticizers (triacetin, dibutyl phthalate) impart sufficient mechanical flexibility to prevent flaking and removal. Marty further notes the similarity of the film-generating solution to the nail lacquers used in cosmetics.

10 It is further explained that the specific aims addressed in formulating the film-generating solution of the anti-fungal nail lacquer include obtaining maximal affinity of the active principle to the nail keratin and obtaining the highest possible thermodynamic activity compatible with
15 maintaining the active principle in true or supersaturated solution.

Differences in diffusion characteristics between nail and skin are also discussed in the Marty article. The nail structure is characterized as a water-gel in which water
20 facilitates diffusion of at least polar compounds. In contrast, the skin tends to more readily facilitate diffusion of lipophilic, non-polar molecules, through the extracellular lipids of the stratum corneum. Thus, since the absolute transmission of water vapor through nails is

about 10 times that through skin, and since nails are approximately 100 times as thick as stratum corneum, the permeability of nails to water vapor is about 1000 times greater.

5 Therefore, Marty reports that "excipients developed for use on skin are thus inappropriate for releasing active principles on the nail, as shown by the inefficacy of diffusion promoters such as DMSO" (citing Walters KA, Penetration of chemicals into, and through, the nail plate. 10 Pharm Int. 1985; April, p. 85-89).

 It has also been suggested in the literature (Mast, "Nail Products"....) that "[a]s a working hypothesis, it should be assumed that nails are, in general, quite permeable to polar and semipolar low molecular weight 15 chemicals." See also, Walters KA and Flynn GL, "Permeability characteristics of the human nail plate" Intl J. of Cosmetic Science, 5, 231-246 (1983) for a review of the structure and characteristics of the nail and a discussion of permeation through the nail plate of various 20 chemicals and permeation coefficients of C₁-C₁₂-alcohols.

 These authors conclude, on the basis of the accumulated data that in connection with the successful formulation of drugs used in the treatment of nail infections, "that solvents with proven efficacy as skin 'penetration 25 enhancers' show little promise as enhancers of nail plate permeability" (citing to Walters, KA and Flynn GL, J. Pharm. Pharmac. 33 6P (1981) and Kligman, AM J. Amm. Med. Ass. 193 796-804 (1965)).

Nevertheless, there remains a need for more effective and more durable (longer lasting) nail lacquer formulations which incorporate an antifungal agent.

There also remains a need for an antifungal nail
5 lacquer formulation which provides clear and glossy films which are capable of resisting multiple washings.

It is also known in the art, as indicated by several of the patent documents discussed above, that the overall effectiveness of antimycotic products for treating fungal
10 infections of the skin may often be improved by combining the antifungal agent with a steroidal antiinflammatory agent. To date however such combination products have not been formulated into a lacquer type product for the
15 treatment of onychomycosis but, rather, have been limited to gels, lotions, creams and other topically applied solutions.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a graphical presentation of release rate ($\mu\text{g}/\text{h}$) of econazole as a function of time from the invention lacquer of Example 2; and

20 Figure 2 is a graphical presentation of the release rate (% dose) of econazole as a function of time from the invention lacquer of Example 2.

SUMMARY OF INVENTION

The present invention aims to solving the above needs.
25 Thus, according to the present invention there is provided a stable, nail lacquer formulation incorporating an antifungal agent, which formulation, when applied to nails yields a

hard, durable, substantially clear, long lasting film, effective in the treatment or prevention of fungal infestations or infections on or associated with nails.

In particular, the present invention provides a composition effective for the treatment or prevention of fungal infections of nails, comprising:

(a) at least one antifungal agent effective in the treatment or prevention of onychomycoses;

(b) penetration enhancing agent selected from the group consisting of C₇-C₁₄-hydrocarbyl substituted 1,3-dioxolane, C₇-C₁₄-hydrocarbyl substituted 1,3-dioxane and C₇-C₁₄-substituted acetal;

(c) water-insoluble, film-forming polymer; and,

(d) volatile solvent,

the composition, when applied to nails, forming, upon evaporation of the volatile solvent, a hard, water-resistant film from which the antifungal agent is releasable and becomes available to treat or prevent fungal infection.

In a particular embodiment of the invention a nail lacquer composition is provided which includes a combination of an antifungal or antimycotic agent and a steroidal antiinflammatory agent in a solution of film-forming polymer in at least one volatile solvent; the composition may also include at least one penetration enhancing agent selected from the group consisting of C₇-C₁₄-hydrocarbyl substituted 1,3-dioxolane, C₇-C₁₄-hydrocarbyl substituted 1,3-dioxane and C₇-C₁₄-substituted acetal. A plasticizer for the film-forming polymer may also be included.

The invention also provides lacquer compositions effective for providing long-lasting, water-resistant adherent films on animal (e.g., human) skin and nails comprising a substantially non-aqueous solution of water-resistant, film-forming polymer, and plasticizing effective amount of at least one compound selected from the group consisting of C₇-C₁₄-hydrocarbyl substituted 1,3-dioxolane, C₇-C₁₄-hydrocarbyl substituted 1,3-dioxane and C₇-C₁₄-substituted acetal in volatile solvent.

10 The resulting water-resistant, adherent films provide novel products especially suitable as a delivery matrix for drugs, including the antifungal agents and others. When such film with drug incorporated therein, is deposited on animal, especially human or other mammal, skin or nail, the
15 drug will leach from the film and will be capable of being absorbed by or transported into and through the skin or nail.

DETAILED DESCRIPTION AND PREFERRED EMBODIMENTS

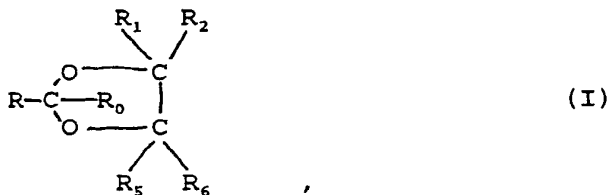
The present invention provides still further
20 improvements in the physical properties (e.g., durability, water-resistance, flexibility) of water-insoluble adherent films provided upon evaporation of the volatile solvent from the film-generating solution of nail lacquer composition, as well as improved diffusion characteristics of active
25 principle(s) included in the lacquer composition from the resulting film.

The present invention makes it possible to effectively incorporate two, generally chemically dissimilar active principles: an antifungal agent and a steroidal antiinflammatory agent in a nail lacquer effective in
5 treatment of onychomycosis.

The improvement in nail lacquer products according to the present invention is, in part, made possible by the incorporation into the film-generating solution of a specific class of penetration enhancing agent, namely,
10 C₇ - C₁₄-hydrocarbyl substituted 1,3-dioxolanes, 1,3-dioxanes and acetals, which have previously been described as enhancers for penetration of various pharmacologically active principles through the skin, and commercially available from MacroChem Corporation, Lexington,
15 Massachusetts, under the SEPA® trademark. The SEPA® skin penetration enhancers (hereinafter may be referred to as SPE's) are the subject matter of several issued U.S. patents, including, 4,861,764, 5,391,567, 4,910,020, and 5,620,980, issued to one or more of the current inventors,
20 and the disclosures of which are incorporated herein by reference thereto.

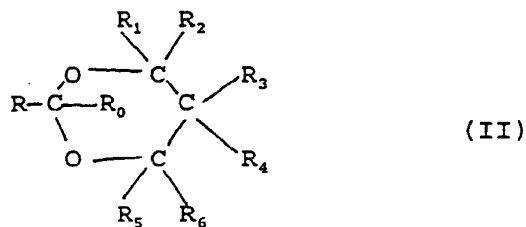
The preferred SPE's for use in the present invention may be represented by the following general formulas:
2-substituted 1,3-dioxolanes of the formula (I):

25



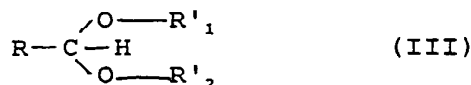
2-substituted 1,3-dioxanes of the formula (II):

5



substituted-acetals of the formula (III):

10



In the above formulas (I), (II) and (III) R preferably
15 represents a C₇ to C₁₄ hydrocarbyl group,

R₀, R₁, R₂, R₃, R₄, R₅, and R₆, each, independently,
represent hydrogen or a C₁ to C₄ alkyl group.

R'₁ and R'₂, each, independently, represent C₁ to C₄
alkyl group.

20 The hydrocarbyl group for R may be a straight or
branched chain alkyl, alkenyl or alkynyl group, especially
alkyl or alkenyl. Preferably, R represents a C₇ to C₁₂
aliphatic group; especially C₇ to C₁₀ aliphatic group.
Examples of suitable alkyl groups include, for example,
25 n-heptyl, n-octyl, n-nonyl, n-decyl, n-undecyl, n-dodecyl,
2-methyl-octyl, 4-ethyl-decyl, 8-methyl-decyl, and the like.
The straight chain alkyl groups, such as n-heptyl, n-octyl,
n-nonyl and n-decyl, are especially preferred. Examples of
alkenyl groups include, for example, 2-hexenyl,
30 2-heptenyl, 2-octenyl, 2-nonenyl, 2',6'-dimethyl-2',6'-

heptadienyl, 2'6'-dimethyl-2'heptaenyl, and the like. The R group may also be substituted by, for example, halo, hydroxy, carboxy, carboxamide and carboalkoxy.

The C₁ to C₄ alkyl group may be, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, tert-butyl, and the like. The preferred alkyl groups for R₀, and for R₁ to R₆ and for R'₁ and R'₂ are alkyl having 1 or 2 carbon atoms, most especially ethyl. R₀, and R₁ to R₆ may also, preferably, all be hydrogen.

Specific enhancer compounds include, for example, 2-n-heptyl-1,3-dioxolane, 2-n-nonyl-1,3-dioxolane, 2-n-undecyl-1,3-dioxolane, 2-n-nonyl-1,3-dioxane, 2-n-undecyl-1,3-dioxane, 2-n-heptylaldehyde-acetal, 2-n-octyl-aldehyde-acetals, e.g., 2-n-octyl-aldehyde-dimethylacetal; 2-n-nonylaldehyde-acetals, 2-n-decylaldehyde-acetals, 3,7-dimethyl-2,6-octadienal (citral) acetals, citronal acetals and the like. 2-n-nonyl-1,3-dioxolane (2-NND), and decanal dimethyl or diethyl acetals are especially preferred. Mixtures of these compounds may also be used.

The amount of enhancer compound is selected to provide the desired delivery rate for the active compound but, taking into consideration such additional factors as, product stability, side effects, carrier system and the like. Generally, depending on the particular antifungal agent and film-forming polymer, amounts of the enhancer compound in the range of from about 0.5 to 35%, preferably from about 2 or 3 up to about 25 or 30 percent, especially from about 5 to 20 or 25 percent, by weight of the total

composition, will provide optimal transungal delivery of the active principle over the duration of the film on the nail. From a practical matter, using the preferred enhancer compounds and film-forming polymers, optimum results (release and skin permeation characteristics) may usually be achieved without incorporating additional co-solvents or plasticizers, using amount of enhancer in the range of from about 12% to about 24% by weight, especially, from about 15% to about 20% by weight, based on the total weight of the composition, of the enhancer compound.

In this regard, it has been found that the SEPA® SPE's are not only effective to facilitate diffusion of the active agent(s) transungually but, quite surprisingly, in addition, the SEPA® family of compounds, function as adhesion promoters and, as plasticizers, for the film-forming polymer of the subject nail lacquer compositions, especially for compatible acrylate and methacrylate copolymers and copolymers of maleate esters with vinyl ethers. Compatibility between the film-forming polymer and the SEPA enhancer compounds may be readily determined by one of ordinary skill in the art, such as, for example, by formation of a single homogenous phase when the polymer and enhancer are mixed together. As will be appreciated by those skilled in the art, various factors, such as, for example, polarity of "mer" units of the polymer, molecular weight, and the like, will be considered for compatibility.

Although the reason for the enhanced transungual diffusion has not yet been fully elucidated, it is hypothesized that the SEPA® compounds function as plasticizing agents for the film-forming polymer and as
5 solubilizing agent for the antifungal agent and other active principles, if any, upon evaporation of the volatile solvent, thereby making it easier for the active agent(s) to diffuse through and be released from the dry lacquer film. At the interface between the lacquer film and the nail the
10 combination of SPE and active agent becomes available to penetrate into and through the nail.

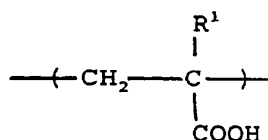
The plasticizing and adhesion promoting functions, of the subject hydrocarbyl substituted 1,3-dioxolanes, 1,3-dioxanes and acetals are not, of course, restricted to the
15 resulting films incorporating antifungal agent used as antifungal nail lacquers, but also are more generally exhibited with the below-described film-forming polymers, for virtually any drug which may be dissolved in the polymer/enhancer compound matrix, with or without the
20 assistance of solvents or co-solvents. Thus, drugs which may be topically administered to the skin as well as drugs which are adapted for use in treating nails for onychomycoses or other ailments, may be incorporated into the nail and skin-adherent polymer plus enhancer compound
25 film-forming composition of this invention.

The film-forming polymers which may be used in the present invention are not particularly limited and may be chosen from among any of the film-forming polymers previously used in or useful for nail lacquer film-forming polymers and which are compatible with the SPE and which have good adhesion to nail keratin (and/or skin) and form water-insoluble and/or water-resistant films which permit release of the antifungal agent and also the steroidal antiinflammatory agent, if present.

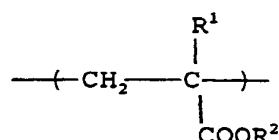
10 Examples of water-insoluble, film-forming polymers which may be used in the nail lacquer compositions of this invention, include, for example, polyvinyl acetate, mixed polymers (or copolymers) of vinyl acetate with acrylic or methacrylic acid, copolymers of (meth)acrylic acid and
15 (meth)acrylate esters, copolymers of (meth)acrylic acid esters with amino group and/or quaternary ammonium group-containing comonomers, and the like. These polymers may be used alone or in mixtures with each other or with other film-forming polymers that will not impair the objectives of
20 this invention.

As used in this application, the term "lower" in connection with "alkyl", etc., refers generally to carbon chain lengths of up to 6 carbon atoms, however, the preferred lower alkyl groups typically have from 1 to 4
25 carbon atoms.

Especially preferred film-forming polymers include acrylate (co)polymers, methacrylate (co)polymers, and copolymers of alkyl vinyl ether and maleic anhydride. For example, a preferred acrylic copolymer comprises recurring units of at least one of the following moieties (IV) and (V):



(IV)

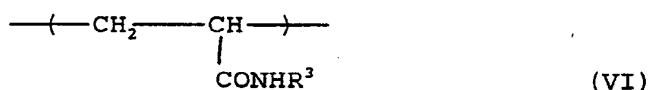


(V)

10

wherein R^1 represents H or CH_3 ; and R^2 represents an alkyl group of from 1 to about 12 carbon atoms, preferably from about 2 to about 12 carbon atoms, especially preferably, from about 4 to about 10 carbon atoms. The alkyl group may be linear or branched. Examples of alkyl groups for R^2 include methyl, ethyl, propyl, isopropyl, t-butyl, isobutyl, n-butyl, n-pentyl, 4-methyl-n-pentyl, n-hexyl, n-heptyl, n-octyl, 2-methyloctyl, n-nonyl, n-decyl, n-dodecyl, and the like.

Another useful acrylic copolymer comprises recurring units of a moiety of formula (VI)



wherein R^3 represents an alkyl group, such as, for example, the alkyl groups described above for R^2 ; preferably an alkyl group of at least two and up to about 12 carbon atoms, especially preferably C_4 to C_{10} alkyl.

Acrylic copolymers which comprise recurring units of formula (V) or formula (VI) or both formulas (V) and (VI), and, optionally, recurring units of formula (IV), as defined above, wherein at least one of R^2 and R^3 represents an alkyl group having at least 4 carbon atoms, are particularly preferred.

Another preferred class of acrylic copolymer comprises recurring units of acrylic and/or methacrylic acid esters and recurring units of a moiety containing a cationic amine and/or quaternary ammonium group, such as, for example, carboethoxy-*t*-butyl ammonium. As is well known in the art, the cationic amine group may be quaternized by reaction of the amine with an alkylating agent or other appropriate reagent to form a salt.

For example, any of the water-insoluble quaternary ammonium group containing acrylic copolymers disclosed in the aforementioned U.S. 5,120,530, the disclosure of which is incorporated herein by reference thereto, may be used as the film-forming copolymer in the compositions of the present invention.

Another preferred example of the water-insoluble, film-forming polymer comprises a copolymer of alkyl vinyl ether, such as, for example, methyl vinyl ether or ethyl vinyl ether, and at least one comonomer of a monoester of a dicarboxylic acid. Examples of such comonomer of a monoester of a dicarboxylic acid are shown by the following formula (VII):

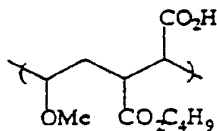


wherein R⁴ represents a lower alkyl group, especially an alkyl group of from 1 to 4 carbon atoms, such as, for example, methyl, ethyl, propyl.

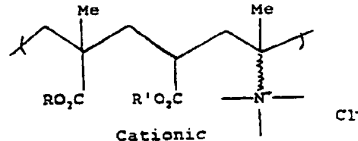
See also the film-forming polymers disclosed in the aforementioned U.S. Patent Nos., 5,264,206, and the other patents mentioned above, which may also be used in this invention.

10 Film-forming polymers useful in the present invention are commercially available, such as, for example, the acrylic copolymers sold by National Starch Co. under the tradename Dermacryl, e.g., Dermacryl 79, Dermacryl LT; the amine or quaternary ammonium group containing acrylic
15 copolymers sold by Rohm (a division of Huls Group) under the tradename Eudragit, e.g., Eudragits E, RS, RL,; the methylvinyl ether copolymers sold by ISP Corp. under the tradename Gantrez, e.g., Gantrez ES-335I, Gantrez ES-425, ES-435; the quaternary ammonium acrylic copolymers sold by
20 National Starch Co. under the tradename Amphomer, e.g., Amphomer LV-71. Particularly good results have been obtained with each of the following commercially available products:

Gantrez ES-425:

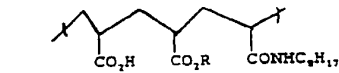


Eudragit RL:

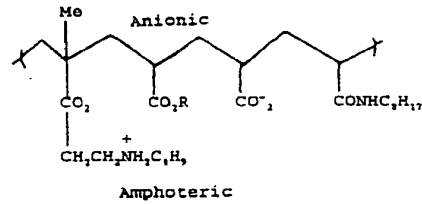


Dermacryl 79 &

Dermacryl LT :



Amphomer LV-71:



5 The amount of film-forming polymer will depend on such factors as, for example, the molecular weight of the polymer, the desired thickness of the resulting film, the degree of water-resistance and the intended duration and delivery rate of the active agent(s), the compatibility with
10 the other ingredients, and the like. Usually, however, satisfactory results are obtained when the amount of film-forming polymer is in the range of from about 10 to about 70 percent, preferably from about 15 to about 50 percent, especially from about 20 to 40 percent by weight of the
15 total nail lacquer composition.

 In terms of weight ratio between film-forming polymer and penetration enhancing (and plasticizing) dioxolane, dioxane or acetal compound, suitable values of polymer:enhancer/plasticizer generally range from about 4:1
20 to about 1:1, preferably from about 3:1 to about 1.2:1, especially preferably from about 2:1 to about 1.2:1. The plasticizing function of the enhancer compounds is exhibited over generally the same or somewhat higher concentrations as the skin penetration enhancing function. Therefore, when

other plasticizing additives, as described below, are included in the compositions of this invention, the ratio of polymer to enhancer may be somewhat higher than the above ranges, for example, from about 5:1 to about 1:1.

5 Conventional plasticizers compatible (e.g., forming a homogenous solution) with film-forming polymers may be included in the compositions of this invention to provide additional flexibility to the dried polymer film upon evaporation of the solvent, and/or additional releasability
10 of the antifungal agent (and antiinflammatory, when present) as well as for the SPE compound. Suitable plasticizers include, for example, 1,2,3-propanetriol triacetate (triacetin), dibutyl phthalate, dioctyl phthalate, dibutoxy ethyl phthalate, diamyl phthalate, sucrose acetate
15 isobutyrate, butyl acetyl ricinoleate, butyl stearate, triethyl citrate, dibutyl tartrate, polyethylene glycol, dipropylene glycol, polypropylene glycols, propylene glycol, glycol fatty acid esters, such as, propylene glycol dipelargonate, and the like.

20 Particularly preferred plasticizers are glycols, such as propylene glycol and dipropylene glycol, glycol esters, phthalate esters, citrate esters, polyethylene glycols, and polypropylene glycols.

 The type and amount of plasticizer, when present in the
25 formulation, affects resistance of the dried polymer film to water and also affects the release rate of the active drug ingredients as well as that of the SPE. Those skilled in the art will recognize that the degree of water resistance

can also be controlled by the type and amount of the plasticizer(s), the nature of the active principles, the choice of polymer (e.g., amount of acid groups in the polymer, etc.), the amount of the polymer, and the like.

5 When the additional plasticizer is present it will generally be used in amounts which depend on the types and amounts of the film-forming polymer and the SPE, most usually in the range of from about 0.5 to about 20 percent, preferably from about 2 to 10 percent, especially, from
10 about 4 to 8 percent, based on the total weight of the composition.

 While additional plasticizers may be incorporated in the invention compositions, as noted above, in view of the surprising plasticizing effect of the subject skin
15 penetration enhancing compounds, sufficient flexibility and adhesion, as well as compatibility (both wet and dry) between the respective ingredients, is usually achieved without the addition of conventional plasticizers.

 Solvents which may be used in the nail lacquer
20 compositions of this invention are also not particularly critical but may be selected from among the usual physiologically safe organic solvents for lacquer compositions, so long as the active principles and film-forming polymers are soluble therein and so
25 long as the lacquer is easy to apply and sufficiently volatile to provide acceptable drying times, usually dry to the touch in less than about 5 minutes, preferably less than about 2 minutes. As examples of such solvents mention may

be made of lower alkanols, e.g., ethanol, propanol, isopropanol, butanol, isobutanol; lower alkyl esters of lower carboxylic acids, e.g., ethyl acetate, propyl acetate, n-butyl acetate, n-amyl acetate; lower alkyl ethers, e.g., methyl ether, methyl ethyl ether; lower alkyl ketones, e.g., methyl ethyl ketone; halogenated hydrocarbons, e.g., methylene chloride, methyl chloroform; aromatic hydrocarbons, e.g., toluene; cyclic ethers, such as, tetrahydrofuran, 1,4-dioxane; and mixtures thereof.

10 Anhydrous ethanol (EtOH) is especially preferred.

The solvents used in the nail lacquer formulations of this invention are generally and preferably non-aqueous. However, in some cases small amounts of water, generally less than about 10%, preferably less than about 5 % by weight of total solvents, may be used if not substantially impairing the homogeneity, clarity and solubility of the various ingredients in the lacquer solution. For example, ethanol when used may sometimes be added in the form of a 95% ethanol solution.

20 Again, in view of the good compatibility between the film-forming polymer and the dioxolane, dioxane and acetal enhancer/plasticizer compounds, use of co-solvents, such as propylene glycol, in addition to solvent, e.g., ethanol, are usually not required and, therefore, may be omitted.

25 On the other hand, however, it may be desirable and, in some cases, preferred, to decrease the water-resistance of the dried polymer film, for example, to facilitate removal of the film after release of all or most of the active

ingredients. Thus, it is envisioned that in addition to a lacquer film from which the active ingredients are released over periods of several days to about 1 week or longer, lacquer films from which the active ingredient is released over shorter periods of time, such as one day, may be desirable since many individuals are accustomed to and prefer treatments requiring applications of a drug on a daily basis.

Techniques for increasing the availability of the active ingredients for transungual delivery have been described above. When the active ingredient is exhausted from the film or mostly exhausted the film may be removed by application of suitable solvents, such as those described above, such as alcohols, acetone, ketones, etc., and/or by scraping or brushing, as also well known in the nail lacquer art.

Often, mixtures of volatile solvents of different boiling points, usually a low boiling solvent in the range of from about 40°C to about 100°C with a medium boiling solvent (boiling point up to about 150°C) may be selected to provide drying times of no more than a few minutes or less, with uniform evaporation rates, good flow and viscosity characteristics and other desirable lacquer parameters, as well known in the cosmetic art. In some cases, high boiling point solvents, such as, for example, cellosolve,

butylcellosolve acetate, butyl cellosolve, ethyl cellosolve, and the like, may be added in small amounts provided they do not impede the fast drying property and other desired characteristics.

5 In this connection, one of the important features of the compositions of the present invention is that all of the volatile and non-volatile ingredients are compatible with each other and form upon mixing clear solutions which are stable against phase separation over a wide temperature
10 range above and below room temperature, such as, for example, from temperatures within the range of from about -10°C to about +135°C.

 Another important characteristic of the invention compositions is that the films formed upon evaporation of
15 the solvent(s) and any other volatile components are strongly adherent to the nail and are water-resistant, namely, capable of withstanding repeated normal washing with soapy water for at least 1 day, usually up to about 5 or more days, preferably, at least one week, depending on the
20 amount of antifungal agent with or without antiinflammatory agent in the film and upon the release rate of the active principles from the film. That is, it is possible to formulate the lacquer composition to remain strongly adherent and water-resistant for sufficiently long so as to
25 last between applications and provide a therapeutically effective amount of the active ingredient(s) present in the dried lacquer film.

In addition, the dry films, for cosmetic appearance, should be substantially clear and transparent.

However, it is also within the scope of the invention to include colorants, such as pigments and/or dyestuffs, nacreous agents, pearlescent agents, fillers, and the like, to cover the nail, for example, to hide any unsightly manifestations of the fungal, yeast or other infection, or otherwise as may be cosmetically desirable.

Other conventional additives customarily present in cosmetic or medicinal nail lacquers may be included in the present formulations in their usual amounts so long as they do not interfere with the diffusion of the active principles and other parameters of the lacquer composition and dried polymer-film. Examples of such additives include, sedimentation retarders, chelating agents, antioxidants, silicates, aroma substances, wetting agents, lanolin derivatives, light stabilizers, antibacterial substances, and the like.

The lacquer compositions of this invention, with or without antifungal agent, may be prepared following any of the procedures normally employed in the nail lacquer field, noting that most of the ingredients are added as mobile liquids such that normal mixing techniques are available, with no particular order of addition of the respective ingredients being required. Generally, however, the polymer film-former, if in powder form, should be added gradually to

some or all of the liquid components in such manner as to avoid clumping and resulting protracted dissolution times. Other ingredients may be added as convenient, as will be readily apparent to the practitioner.

5 The antifungal agent films obtained from the nail lacquers of this invention are effective in treating onychomycoses and other fungal infections. Usually, repeated applications of the antifungal lacquer will be made over a period of several weeks to several months, depending
10 on the severity of the infection, the amount of active agent, and the condition of the nails of the patient. Since the antifungal agent containing film will contain sufficient active principle to be diffused through the nail over a period of at least 1 day, and up to about 7 days and,
15 since the film will remain in place usually for the entire period of diffusion, applications of the antifungal nail lacquer need be repeated only about once per day to about once per week. For example, it may be desired to provide formulations for daily application during the initial period
20 of usage until the patient observes substantial reduction in the degree and extent of infection and thereafter to provide different formulations designed for less frequent applications, such as every other day, weekly, etc.

 In addition to treating an existing infection or fungal
25 infestation, the nail lacquers of this invention may also be applied prophylactically to the nails of a healthy individual who is or who believes he or she may be at risk for a mycotic infection, as a result, for example, of

occupation, geographical location or otherwise. The manner of use is otherwise identical to the use in treating an existing infection, however, smaller dosages, but still at least above the MIC of the antifungal agent, may be
5 sufficient in many cases to prevent the onset of fungal infection in the event of fungal contamination or infestation.

There is no particular limitation on the antifungal agents used in the compositions of this invention; any of
10 the agents known to be effective for this purpose may be used and a listing of such compounds may be found, for example, in any current edition of The Merck Index under the headings "Antifungal (Antibiotic)" and "Antifungal (Synthetic)" in the Therapeutic Category and Biological
15 Activity Index section.

As examples of suitable antifungal agents mention may be made of, for example, polyenes, e.g., Natamycin, Nystatin; allylamines, e.g., Naftifine, Terbinafine; imidazoles, e.g., Bifonazole, Chlotrimazole, Econazole,
20 Fenticonazole, Ketocanazole, Miconazole, Oxiconazole; triazoles, e.g., Fluconazole, Itraconazole, Terconazole; tolinaftate, ciclopirox, undecylenic acid, sulbentine, and morpholines, e.g., amorolfine, and the related morpholines disclosed in the aforementioned U.S. 5,120,530. The
25 1-hydroxy-2-pyridone compounds disclosed in U.S. 4,957,730,

the disclosure of which is incorporated herein, by reference thereto, may also be used, as may the antifungal agents disclosed in any of the other patent documents discussed in the Background of the Invention.

5 In the present invention, the antifungal agents are, preferably, present in the free form, e.g., as acid or base, rather than in the form of their salts. In this regard, the free form of antifungal agent will usually have a higher diffusion rate through the nail than a salt of the same
10 agent; or, the salt form of a drug may impair the water-resistance of the lacquer film.

 The amount of the active antifungal agent or mixture of such agents in the composition will depend on such factors as its structure and antimicrobial activity, release rate
15 from the polymer film, diffusion characteristics and penetration behavior in the nail. Generally, any amount effective to kill the infecting microorganism, which will generally be several to several tens to hundreds of times greater than the Mean Inhibitory Concentration (MIC), may be
20 included in the nail lacquer (as applied) composition. Typically, amounts of active antifungal agent in the range of from about 0.5 to 20 percent by weight, preferably from about 1 to 10 percent, by weight, of the total composition (including solvents, film-forming polymer, enhancer, etc.)
25 will suffice for compositions for treatment as well as compositions for prevention. The amount of antifungal agent in the dried film will, therefore, depend on the amount of agent in the lacquer solution and by the thickness of the

applied film. The thickness of the film can be controlled by, for example, controlling the viscosity of the lacquer solution, such as by the type and amount of polymer, types and amounts of solvents, etc.

5 Conversely, on the basis of the non-volatile components of the composition, the amount of active agent is generally about 1 to 50%, preferably about 2 to 35%, more preferably, from about 2 to 30%, especially preferably from about 5 to 20%, by weight of the composition (film-forming polymer(s),
10 active(s), plasticizer(s) and other non-volatile additives).

The antifungal nail lacquers according to this invention, by virtue of the incorporation of the penetration enhancer/plasticizer, as described above, provide therapeutically effective concentrations of antifungal agent
15 deep into the nail bed. Although a precise minimum value of the therapeutically effective amount of antifungal agent will depend on several factors, primarily the particular antifungal agent and the degree and severity and cause of onychomycoses or other fungal infection, generally
20 concentrations of antifungal agent greater than at least about 150 ppm in deep nail bed should be reached to attain favorable clinical results.

The compositions of this invention may also include a steroidal antiinflammatory agent in addition to the
25 antifungal agent. While combinations of antifungal agent and steroidal antiinflammatory agent have been known in the past, there have been no known uses of such combinations in a nail lacquer compositions.

The steroidal antiinflammatory agent may be selected from among any of the known steroidal antiinflammatory agents, including, for example, any of those disclosed in The Merck Index or in any of the aforementioned U.S. Patent
5 Nos. 5,002,938, 5,110,809, 5,219,877, the disclosures of which are incorporated herein by reference thereto. As examples of steroidal antiinflammatory agents useful in the compositions of the present invention mention may be made of, for example, 21-acetoxypregnenolone, alclometasone or
10 its dipropionate salt, algestone, amcinonide, beclomethasone or its dipropionate salt, betamethasone and salts thereof, including, for example, betamethasone benzoate, betamethasone dipropionate, betamethasone sodium phosphate, betamethasone sodium phosphate and acetate, and
15 betamethasone valerate; clobetasol or its propionate salt, clocortolone pivalate, hydrocortisone and salts thereof, including, for example, hydrocortisone acetate, hydrocortisone butyrate, hydrocortisone cypionate, hydrocortisone phosphate, hydrocortisone sodium phosphate,
20 hydrocortisone sodium succinate, hydrocortisone tebutate and hydrocortisone valerate; cortisone acetate, desonide, desoximetasone, dexamethasone and salts thereof, for example, acetate and sodium phosphate; diflorasone diacetate, fludrocortisone acetate, flunisolide,
25 fluocinolone acetonide, fluocinonide, fluorometholone, flurandrenolide, halcinonide, medrysone, methylprednisolone and salts thereof, e.g., acetate, sodium succinate; mometasone furoate, paramethasone acetate, prednisolone and

salts thereof, e.g., acetate, diethylaminoacetate, sodium phosphate, sodium succinate, tebutate, trimethylacetate; prednisone, triamcinolone and derivatives thereof, e.g., acetonide, benetonide, diacetate, hexacetonide. Other
5 glucocorticoid steroids reported in the literature, including The Merck Index, or otherwise approved by the local drug regulatory agency, e.g., Food and Drug Administration, may also be used.

Particularly preferred steroidal antiinflammatory
10 agents include clobetasol and its salts, e.g., propionate salt; betamethasone and its salts, hydrocortisone and its salts, and triamcinolone and its salts.

Although not particularly limited, the antiinflammatory agent will usually be present in the lacquer composition in
15 an amount within the range of 0.01 to about 5 percent, preferably from about 0.1 to 2 percent, based on the total weight of the solution.

The total amount of antifungal agent and antiinflammatory agent will usually range from about 0.5 to
20 about 30 percent, by weight, preferably from about 1 to 25 percent by weight, especially from about 1.5 to about 12 percent by weight, based on the total weight of the lacquer composition, i.e., the lacquer solution.

The following examples illustrate various compositions according to the invention but are not intended to and should not be construed to in any manner limit the scope of the invention.

5 Example 1

The nail lacquer compositions shown in the following table were prepared. Each composition was observed for compatibility. The results of the observations are shown in the table. In addition, each nail lacquer composition was
10 applied to a glass substrate and allowed to dry in air and the state (homogeneity) of the dried lacquer films were observed. The results are also reported in the following Table 1.

TABLE 1
SEPA ANTIFUNGAL LACQUERS

HC No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	16017A	18229B	16070A	16071D	16074A	16074B	16074C	18234A	18234B	18234C	18234D	18240A	18236E	18242A
1. Econazole	2	2	5	5	5	5	5	5	5	5	5	5	5	5
2. Miconazole	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3. Clotrioxolone	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4. Hydrocortisone	6	6	6	6	6	6	6	6	6	6	6	6	6	12
5. 2-Nonyl-1,3-dioxolane	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. Citral ethylene glycol acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Decanal dimethylacetal	20	20	20	24	24	24	24	24	24	24	24	24	24	24
8. Propylene glycol	24	-	-	-	-	-	-	-	-	-	-	-	-	-
9. Amphomer LV-71	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10. Amphomer	-	-	24	-	-	-	-	-	-	-	-	-	-	-
11. Dermacryl LF	-	24	-	-	-	-	-	-	-	-	-	-	-	-
12. Dermacryl 79	48	48	45	65	-	34	49	59	59	59	59	58	59	53
13. Ethanol	-	-	-	-	59	-	-	-	-	-	-	-	-	-
14. Acetone	-	-	-	-	-	25	10	-	-	-	-	-	-	-
15. Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16. Diethyl ether	-	-	-	-	-	-	-	6	6	6	6	6	6	6
17. Urea	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18. PEG 200	-	-	-	-	-	-	-	-	-	6	6	6	6	6
19. PPG 1K	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20. Dipropylene glycol	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Compatibilities*:
Wet*
Dry*

*C = Clear/compatible; H = Hazy; S = Slightly; V = Very
* Complete lacquer, * Air dried film

(a) crystallized
+ 2-(2',6'-Dimethyl-2',6'-heptadienyl)-1,3-dioxolane

TABLE 1 (cont.)

SEPA Antifungal Lacquers

	15	16	17	18	19	20	21	22	23	24	25	26	27
HC No.	18242B	18242C	18242D	18245A	18245D	18245D	18245F	18236B	18246A	18246B	18245C	16908A	17529B
1. Econazole	5	5	5	5	5	5	5	8	10	20	-	-	-
2. Miconazole	-	-	-	-	-	-	-	-	-	-	5	-	-
3. Clotrimox	-	-	-	-	-	-	-	-	-	-	-	8	0
4. Hydrocortisone	-	-	-	-	-	-	-	-	-	-	-	-	0
5. 2-Honyl-1,3-dioxolane	24	12	24	-	-	-	-	6	6	6	6	6	6
6. Citral ethylene glycol acetate	-	-	-	6	-	-	-	-	-	-	-	-	-
7. Paranal dimethylacetal	-	-	-	-	6	-	-	-	-	-	-	-	-
8. Propylene glycol	6	-	-	-	-	-	6	6	6	6	-	6	6
9. Amphomer LV-71	24	24	24	24	24	24	24	24	24	24	24	24	24
10. Amphomer	-	-	-	-	-	-	-	24	-	-	-	-	-
11. Dermacryl I.T	-	-	-	-	-	-	-	-	-	-	-	-	-
12. Dermacryl 79	-	-	-	-	-	-	-	-	-	-	-	-	-
13. Ethanol	41	59	47	65	65	71	65	56	54	44	65	20	20
14. Acetone	-	-	-	-	-	-	-	-	-	-	-	20	-
15. Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	20
16. Diethyl ether	-	-	-	-	-	-	-	-	-	-	-	-	-
17. Urea	-	-	-	-	-	-	-	-	-	-	-	-	-
18. PEG 200	-	-	-	-	-	-	-	-	-	-	-	-	-
19. PPG 1K	-	-	-	-	-	-	-	-	-	-	-	-	-
20. Dipropylene glycol	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100	100
Compatibility:													
Met*	C	C	C	C	C	C	C	C	C	C	C	C	C
Dry*	VII	C	SII	C	C	C	C	C	C	C	C	C	C

* C = Clear/compatible; II = Hazy; S = slightly; V = Very Complete lacquer, * NR dried film

(a) crystallized
 † 2-(2',6'-dimethyl-2',6'-heptadienyl)-1,3-dioxolane

TABLE 1 (cont.)

Ingredient	28	29	30	31	32	33	34
Econazole	5	1	5	5	5	5	5
2-heptyl-1,3-dioxolane		6		18			
2-nonyl-1,3-dioxolane	6	6	5				18
Citral ethylene glycol acetal					18		
Decanal dimethylacetal						18	
Amphomer LV-71	24	24					
Eudragit RL			24	24	24	24	24
Ethanol	59	63	66	53	53	53	47
PEG 200	6						
Triacetin							6

The compositions of Run Nos. 28-34 were also compatible and clear under wet and dry conditions.

Furthermore, in any of these examples, the lacquers with or without the antifungal agent will form flexible films which are strongly adherent to nails and other hard surfaces, including glass and metal substrates.

Moreover, these results (see, e.g., Run Nos. 23 and 24) show that the antifungal agent is very highly compatible in the invention films, such that crystallization, even at very high drug levels, is greatly inhibited. Thus, the 10% lacquer remained clear for more than a month after casting and drying and even a 20% (corresponding to 35% in the dry film) lacquer did not fully crystallize after drying.

Accordingly, the SEPA plasticization effect will increase bioavailability of drugs through decrease of diffusional barriers to release.

Example 2

The following compositions were prepared and used in the tests described below:

	<u>Ingredient</u>	<u>Wt.%</u>
5	Econazole	1-10
	2-n-nonyl-1,3-dioxolane	18
	Eudragit® RL	24
	Ethanol	q.s. 100 (57-48)

Using the above formulation with 5% Econazole and 53% ethanol, stability testing was performed. There was no decomposition as indicated by lack of color changes of lacquers stored in clear or light protected containers under accelerated conditions. In addition, gas chromatography quantitative analysis was conducted on samples stored in glass containers for 50 days at 40°C/75%RH at varying pH (5.2, 6.83, 12.2; by addition of acid or base, as necessary).

The analytical test procedure involves a simple direct dilution and injection method for determining levels of both antifungal agent and enhancer compound in the same chromatogram, i.e., without separation steps. The test procedure detects a known primary degradant of econazole (i.e., 1-(2,4-dichloro- β -hydroxyphenethyl)imidazole) and a known primary degradant of the enhancer (i.e., the corresponding aldehyde, e.g., decanal for 2-n-nonyl-1,3-dioxolane). Specifically, a Hewlett-Packard Model

5890 Chromatograph with a Hewlett-Packard 50+ (crosslinked 50% phenylmethylsiloxane), 30m, 0.32mm ID, 0.50 μ film (Cat. #19091L) column and Model 7673 Autoinjector, operating in split mode (split flow 0.7 mL/min; split ratio 0.652:1), using methanol as wash solvent and hexanophenone as internal standard, was used for the analysis. The results are shown in the following Table 2. In Table 2 the results are reported for the average of six injections.

TABLE 2

10

<u>Run No.</u>	<u>pH</u>	<u>SEPA assay (%)</u>	<u>Econazole Assay (%)</u>
1	5.2	93.85	94.29
2	6.83	98.41	96.67
3	12.2	99.36	97.60

15

The following additional test procedures were used to evaluate the release and penetration characteristics of compositions according to the invention.

In Vitro Release Test for Lacquers

20 Using a 50 μ l micropipette (VWR) set on 11 μ l, approximately 10 mg of lacquer are applied homogeneously on frosted glass tile squares, 1 cm². This corresponds to the amount deposited on nails in the nail permeation method described below. Each tile is weighed out before and after applications of the lacquer and weights are recorded. The exact amount of lacquer applied is determined from the difference in the weight of the tile before and after treatment. Tiles are then placed on an orbital shaker set at 180 rpm at room temperature over the duration of the experiment.

30

Aliquots of 1 ml are collected from each vial 2, 4, 6, and 24 hours after the beginning of the agitation. Samples are poured into 2 ml HPLC vials and analyzed by the HPLC method for econazole (see below). Results are expressed as the amount of Econazole released in the milieu over time ($\mu\text{g/h}$) and as the cumulative amount of drug released expressed as percentage of drug and are shown in the accompanying Figures 1 and 2.

A satisfactory release profile shows 60% antifungal agent released to the milieu within 6 hours.

In Vitro Drug Delivery

HPLC Analysis of Econazole

The HPLC assay used is a reverse phase assay system using a Whatman RTF column: 40:55:5 (ACN:pH=3.01, 10mM $\text{KH}_2\text{PO}_4:\text{CH}_3\text{OH}$); injection = $24\mu\text{L}$ ($20\mu\text{L}$ sample + $4\mu\text{LH}_3\text{PO}_4$), temperature = 50°C , flow = 0.9mL/min ; Samples in 80:20 ethanol:phosphate buffered saline (PBS). The assay is suitable for measuring econazole at low levels in analyte fluids. The HPLC software reports the final results in units of micrograms per ml of test solution.

Example 3

Optimization Studies in Human Skin

Studies are performed in human organ transplant donor skin to optimize the release and subsequent skin permeation characteristics of lacquers of varying composition. These studies are designed to determine whether the characteristic advantageous drug delivery properties of the invention SPE's

are retained when formulated into lacquers. The results demonstrate optimum release and permeation between 12 and 24% w/w SPE.

In Vitro Studies: Porcine Nail

5 Nail procedure: single application

Pig feet are obtained from an abattoir and are cleaned and washed with tap water. Nails are excised using scalpel and nail bed tissue is removed. Circlets are punched with a 1.2 cm diameter metallic punch. Each nail, depending on its size, provides an average of 3-4 circles. Nail circlets are packed in groups of 6 in gauze, soaked with phosphate buffered saline solution, and stored in a refrigerator at 2-8°C until needed.

Petri dishes are prepared by filling with a gel, e.g., phosphate buffered saline:polyethylene glycol 200 (90:10) carbomer thickened gel (pH 5.13). The gel is spread evenly on the bottom of the petri dishes and is of sufficient consistency to support the nail circlets for the duration of the studies. Each dish could contain up to 6 nails.

20 Lacquer, approximately 10 mg, is applied evenly to each nail with an adjustable 50 μ l micropipette (VWR) set to 11 μ l. Each nail is weighed before and after application of the lacquer and weights are recorded. The exact amount of lacquer applied is determined from the difference in the weight of the nail before and after treatment. Nail

circlets are then placed in groups of 6 on the gel and allowed to air dry for 10-15 minutes before covering the dishes. Dishes are subsequently placed in an incubator, set at 40-45°C, for the duration of the experiment.

5 At the end of the exposure time frame, the nails are removed, rinsed with deionized water, and placed individually into 20 ml vials. Ethanol (2 ml of 95%) is added and the vials are agitated for 15 minutes using an orbital shaker at 150-200 rpm. Supernatants are then
10 collected into 4 ml vials. This washing is repeated with 2 ml of fresh ethanol and the supernatants combined. A 100 µl aliquot is added to an HPLC vial containing 900 µl PBS to a final 1/10 dilution and analysed by HPLC.

Nail circlets are then blotted dry and the thickness
15 measured using a Digimatic Micrometer. Five representative measurements for each nail are taken. Nails are then secured to a wooden dowel using cyanoacrylate adhesive and allowed to fix for a minimum of 30 minutes. Three successive 10 mg nail scrapings are taken from each nail
20 using a single-edge razor blade or Exacto knife. Each scraping is accurately weighed on an analytical balance and placed individually into a 4 ml vial. Ethanol (2 ml of 95%) is added to the vials which are then shaken overnight (orbital shaking, 150-200 rpm). Subsequently, a 100 µl
25 aliquot of the supernatant is added to an HPLC vial containing 900 µl PBS to a final 1/10 dilution and analysed by HPLC.

Nails are removed from the dowels and thickness measured by Digimatic Micrometer. The depth of nail scraping is determined by the difference in the thickness of the nails before and after scraping. For porcine nails, the average thickness before scraping (24 nail samples, 5 measurement each) is 1.062 ± 0.134 mm. The average nail thickness after scraping is 0.670 ± 0.138 (corresponding to a nail depth of 0.392 ± 0.14 mm. The weight of each nail scraping ranged between 0.950 to 13.00 mg (first scraping), 9.70 to 14.40 mg (second scraping) and 10.00 to 15.30 mg (third scraping) for an average value of all three scrapings of 33.54 ± 2.02 mg. In contrast, human toe nails (3 samples, 5 measurements) had an average thickness before and after scraping of 0.845 ± 0.022 and 0.385 ± 0.051 mm, respectively. The average weight (total) of the 3 scrapings was 22.23 ± 0.90 mg.

Nail procedure: four multiple applications with wash off between applications

For nails prepared as described immediately above the subsequent dosage regimen is as follows:

Day one: Lacquer, approximately 10 mg, is applied evenly to each nail with an adjustable 50 μ l micropipette (VWR) set to 11 μ l. Each nail is weighed before and after application of the lacquer and weights are recorded. The exact amount of lacquer applied is determined from the difference in the weight of the nail before and after treatment. Nail circlets are then placed as a group of 4 or 6 (nails 1-6) on

the gel and allowed to air dry for 10-15 minutes before covering the dish. The dish is subsequently placed in an incubator set at 40-45°C.

Day two: The day one procedure is repeated with a new group
5 of 4 or 6 nails (7-12). Nails 1-6 are removed from the petri dish and the underside of each nail is rinsed with deionized water to remove adhering gel. Then nails are washed with 2 ml 95% ethanol with orbital shaking as previously described. Samples of the supernatants are
10 stored and the nails treated with fresh lacquer exactly as described for day one. Both sets of nails are placed in the incubator set at 40-45°C.

Days three and four: The day one and day two procedures are repeated with wash-off and re-application, with new groups
15 of 4 or 6 nails (13-18; 19-24).

Day five: All four Petri dishes are removed from the incubator. The nails are removed, rinsed with deionized water, and placed individually into 20 ml vials. Ethanol (2 ml of 95%) is added and the vials are agitated for 15
20 minutes using an orbital shaker at 150-200 rpm. Supernatants are collected into 4 ml vials. This washing is repeated with 2 ml of fresh ethanol and all of the washing supernatants are combined (collective resultant volumes of washings are 10 ml for nails 1-6; 8 ml for nails 7-12;
25 6 ml for nails 13-18; 4 ml for nails 19-24). Subsequently,

a 50 μ l aliquot of the collective washings is added to an HPLC vial containing 950 μ l PBS to a final 1/20 dilution and analysed by HPLC. This provides washing recovery data for mass balance determination.

5 All nail circlets are subsequently treated to determine the levels of econazole in each nail scraping layer, as previously described.

Nail procedure: four multiple applications without wash off between applications

10 Nails are prepared as described above. The subsequent dosage regimen is as follows:

Day one: Lacquer, approximately 10 mg, is applied evenly to each of 24 nails with an adjustable 50 μ l micropipette (VWR) set to 11 μ l. Each nail is weighed before and after
15 application of the lacquer and weights are recorded. The exact amount of lacquer applied is determined from the difference in the weight of the nail before and after treatment. Nail circlets are then placed on the gel (as described) and allowed to air dry for 10-15 minutes before
20 covering the dish. The dish is subsequently placed in an incubator set at 40-45°C.

Day two: The Petri dishes are removed from the incubator. Nail samples 1-18 are treated as on day one. The exact amount of lacquer applied is determined by the difference in
25 the weight of the Petri dish before and after application. The dishes are then returned to the incubator. Nail samples 19-24 were removed from the gel, rinsed with deionized water

and washed with 95% ethanol. The nails are then scraped according to the procedure described above. Scrapings are stored.

Day three: Lacquer is re-applied to nail samples 1-12.

5 Nail samples 13-18 are removed from the gel, rinsed with deionized water and washed with 95% ethanol. The nails are then scraped according to the procedure described above. Scrapings are stored.

Day four: Lacquer is re-applied to nail samples 1-6. Nail
10 samples 7-12 are removed from the gel, rinsed with deionized water and washed with 95% ethanol. The nails are then scraped according to the procedure described above. Scrapings are stored.

Day five: Nail samples 1-6 are removed from the incubator
15 and treated according to the procedure described above. All washings and scrapings are treated and analysed for econazole as described above.

In Vitro Validation: Human Nail

Human toenails are obtained from a regional organ bank.
20 After debridement and cleaning of the underneath surface, partially hydrated nails are punched out and prepared exactly as described above for porcine nails. The method used for the validation study used "four multiple applications with wash off between applications" method
25 described above.

Example 4

Following the general procedure for the single application nail procedure described above the following composition is tested for absorption of Econazole through
 5 porcine nail.

	<u>Ingredient</u>	<u>Amount (wt%)</u>
	Econazole	5.0
	2-n-Nonyl-1,3-dioxolane	6.0
10	Amphomer	24.0
	Ethanol	65.0

In this test, a phosphate buffered saline (PBS): ethanol (95:5) hydroxypropyl cellulose (2%) thickened gel (pH 7.45), is used as a nail support/receptor fluid. 5.6
 15 mg of the formulation is applied (T=40°C) to 4 nail circlets. Measurement of econazole penetration (avg. for 4 nails) is measured after 48 hours.

The results are shown below in Table 3:

TABLE 3

20	<u>Nail Layer</u>	<u>Amount of Econazole ($\mu\text{g}/\text{mg}$)</u>
	1	1.04
	2	0.07
	3	0.06

This corresponds to a concentration of about 1170 ppm
 25 of econazole.

Example 5

The procedure of Example 4 is repeated except that the pH of the receptor fluid is increased to 7.7, the amount of lacquer is changed as shown in the following Table 4, and

the penetration is measured after 120 hours. The following econazole containing antifungal nail lacquers, as shown in Table 4, are tested by the single application procedure described above.

5

TABLE 4

Ingredient	260A	260B	260F
Econazole	5	5	20
2-n-Nonyl-1,3-dioxolane		6	6
Propylene glycol	6	6	6
Dermacryl 79	24	24	24
Ethanol	65	59	44
Amt. formulation Applied (mg)	8.75	9.60	9.55
Amt. Econazole (μ g)	437.5	480.0	1910.0

10

15

The results are shown in the following Table 5 for the average penetration for each layer of the four treated porcine nail circlets.

TABLE 5

20

<u>Sample No.</u>	<u>Amount of Econazole (μg/mg of nail)</u>		
	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>
260A	0.66	0.05	0.04
260B	0.72	0.07	0.03
260F	2.42	0.25	0.02

25

From these results it is seen that Sample 260B with enhancer was not significantly improved relative to the control Sample 260A and that the penetration of econazole, in Sample 260F, measured as percent of dose was only comparable to Sample 260A and 260B. For subsequent results, it is presumed that the duration of the study (120 hours)

30

was too long, namely, the antifungal agent from Sample 260B substantially completely passed through the nail. In addition, there may have been insufficient fluidization of the antifungal agent.

5 Example 6

In this example the same procedure as described in Example 5 was used except that the test duration is reduced to 96 hours. The following antifungal lacquer formulations are tested for econazole absorption:

10

TABLE 6

Ingredient	303A	274A	274C	249A
Econazole	5	5	5	5
2-n-nonyl-1,3-dioxolane	-	6	12	6
Propylene Glycol	6	6	6	6
Eudragit RL	24	24	24	-
Dermacryl 79	-	-	-	24
Ethanol	65	59	53	65
Amt. Applied (mg)	6.98	6.83	7.98	7.58
Drug Amt. (μ g)	348.75	341.25	398.75	378.75

The results (average of four porcine nail circlets) are shown in Table 7.

25

TABLE 7

Amount of Econazole (μ g/mg)

<u>Sample No.</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>
303A	0.7	0.18	0.15
274A	0.72	0.22	0.15
274C	0.74	0.18	0.12
249A	0.34	0.16	0.13

From the results of Table 7 it is seen that the econazole absorption from the Eudragit polymer lacquer is greater than from the Dermacryl polymer lacquer. It is also seen that there is no significant difference between the 6% and 12% enhancer levels, again suggesting the test duration may be overly long.

Example 7

This example is another 96 hour test for different concentrations of enhancer in a series of lacquer formulations containing 5% econazole and 24% Eudragit RL. The amount (wt%) of enhancer (2-n-nonyl-1,3-dioxolane) and alcohol in each formulation is shown below.

	<u>Sample No.</u>	<u>Enhancer:Ethanol</u>
15	318A	0:71
	318C	5:66
	318D	12:59
	318E	18:53
	318F	24:47

The procedure used is the same as described in Example 5 except that the receptor fluid (gel support) is 90% PBS/10% PEG 200, pH 4.8. The amount of lacquer applied in this series of runs varied between 6.38 mg to 7.65 mg.

The results are shown in Table 8.

TABLE 8

	<u>Amount of Econazole ($\mu\text{g}/\text{mg}$)</u>			
	<u>Sample No.</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>
30	318A	0.75	0.14	0.03
	318C	0.64	0.16	0.08
	318D	0.56	0.07	0
	318E	0.5	0	0.05
	318F	0.45	0.09	0.07

Based on the inverse correlation of enhancer concentration and antifungal agent absorption it is concluded that the study duration (96 hours) is too long, namely the antifungal agent has already substantially passed through the nail thickness.

Accordingly, the same procedure as above is reported but for a test duration of only 48 hours and using 6 porcine nail circlets. Also, the amount of lacquer applied was slightly increased, on average, ranging from 7.10 mg to 8.52 mg. The results are shown in Table 9.

TABLE 9
Amount of Econazole ($\mu\text{g}/\text{mg}$)

<u>Sample No.</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
15 318A	0.52	0.06	0	0.58
318C	0.41	0.13	0.02	0.56
318D	0.56	0.08	0.08	0.72
318E	0.79	0.09	0.03	0.91

Based on the control sample (318A, 0% SEPA) the change in enhancement is as follows: (the amount of SEPA is shown in parentheses).

% Enhancement vs. Control

318C (5%) vs. 318A (0%)	-6%
318D (12%) vs. 318A (0%)	+23%
318E (18%) vs. 318A (0%)	+56%

To further determine the effect of test duration on the same sample formulations (separately prepared) as used above with 0, 12, 18 and 24% SEPA, the same procedure described above is again carried out but only for a 24 hour period. The results are shown in Table 10.

TABLE 10

Ingredient	338A	338B	338C	338D
Econazole	5	5	5	5
Enhancer	-	12	18	24
Eudragit RL	24	24	24	24
Ethanol	71	59	53	47
Amount Econazole Absorbed ($\mu\text{g}/\text{mg}$)				
First Layer	0.91	1.2	0.75	0.84
Second Layer	0.14	0.11	0.09	0.08
Third Layer	0.06	0.16	0.08	0.08
Total (ppm)	1102	1462	943	1006
Enhancement vs. Control	----	+33%	-14%	-9%

While this example shows significant enhancement using 12% concentration of enhancer (2-n-nonyl-1,3-dioxolane), based on other tests, as described below, it is concluded that the 24 hour test duration for the single application is too short.

Example 8

This example is designed to show the effect of various excipients.

Using the same single application procedure as described in Example 7 except that the test duration is 48 hours, the following four samples were compared:

TABLE 11

	Ingredient	353A	353B	353C	353D
	Econazole	5	5	5	5
	Eudragit RL	24	24	24	24
5	2-n-nonyl-1,3-dioxolane	18	18	18	18
	Propylene Glycol	--	6	--	--
	Triacetin	--	--	6	--
10	Citroflex*	--	--	--	6
	Ethanol	53	47	47	47

* - acylated triesters of citric acid (Morflex, Inc.)
The results are shown in Table 12.

TABLE 12

15

Econazole, Amount ($\mu\text{g}/\text{mg}$)

<u>Sample No.</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
353A	1.36	0.17	0	1.534
353B	1.76	0.35	0.07	2.176
353C	1.15	0.08	0.07	1.304
20 353D	0.49	0.07	0.09	0.647

Example 9

This example is designed to show the effect of increasing the concentration of antifungal agent for a single dose application under the same conditions described in Example 7. The following lacquer samples are prepared.

25

	Ingredient	353-B	357-B	357-C	357-D
	Econazole	5	5	10	20
	Eudragit RL	24	24	24	24
5	2-n-nonyl- 1,3-dioxolane	18	18	18	18
	Propylene Glycol	6	--	--	--
	Ethanol	47	53	48	38

The results for absorption of econazole in each nail
10 layer (average of six nails) is shown in Table 13.

TABLE 13

Amount Econazole Absorbed ($\mu\text{g}/\text{mg}$) (48h)

	<u>Sample No.</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
	353B	1.61	0.06	0.09	1.769
15	357B	1.23	0.07	0.09	1.392
	357C	1.87	0.09	0.02	1.984
	357D	1.51	0.15	0.01	1.675

These results suggest that no significant benefit is
achieved by increasing the dose of antifungal agent from 10%
20 to 20%.

In order to test the effect of antifungal agent doses
below 5% the following antifungal nail lacquers were
prepared and tested by the same procedure as above. The
formulations of each sample and the results are shown in
25 Table 14.

TABLE 14

Ingredient	906A	906B	906C	906D
Econazole	1	2	5	10
Eudragit RL	24	24	24	24
2-n-nonyl- 1,3-dioxolane	18	18	18	18
Ethanol	57	56	53	48
Amount Econazole Absorbed ($\mu\text{g}/\text{mg}$) (48h)				
First Layer	0.31	0.49	0.76	1.09
Second Layer	0.45	0.17	0.15	0.3
Third Layer	0.22	0.27	0.16	0.77
Total	0.986	0.920	1.067	2.166

Example 10

This and the following examples are designed to show the effect of multiple lacquer applications. In this example the test procedure for multiple applications with washoff (using ethanol) as described above is applied to six porcine nail circlets using as the nail support/receptor fluid PBS:PEG200 (90:10) (pH, 5.13), and the following nail lacquer:

Econazole	5%
Eudragit RL	24%
2-n-nonyl-1,3-dioxolane	18%
Propylene Glycol	6%
Ethanol	47%

The results are shown in Table 15.

TABLE 15

Amount of Econazole ($\mu\text{g/ml}$)

<u>Application</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
5 1	1.25	0.25	0.2	1.693
2	1.64	0.42	0.16	2.228
3	2.37	0.78	0.21	3.365
4	2.69	0.58	0.38	3.654

From Table 15 it is seen that there is a significant
 10 dose response with multiple daily applications, however,
 steady state appears to occur after the third application.

Example 11

This example shows the effects of multiple (once daily)
 applications similarly to Example 10 but without washing
 15 between applications. In this example, the nail lacquer was
 similar to that used in Example 10, except that propylene
 glycol is not used, and is replaced with an equivalent
 amount of ethanol, namely, 5% econazole, 24% Eudragit RL,
 18% enhancer (2-n-nonyl-1,3-dioxolane) and 53% ethanol.

20 The results are reported in Table 16.

TABLE 16

Amount of Econazole ($\mu\text{g/mg}$)

<u>Application</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
25 1	0.69	0.12	0.27	1.157
2	1.57	0.34	0.22	2.135
3	1.4	0.29	0.29	1.986
4	2.32	0.71	0.47	3.493

As compared to Example 10 where the lacquer is removed by washing between applications, it is seen that without washing the dose response curve achieves a statistically significant maximum after the fourth application.

5 Example 12

This example is similar to Example 10 (wash off after each 24 hour application) using the same antifungal nail lacquer used in Example 10. The results are shown in Table 17.

10

TABLE 17

Amount of Econazole ($\mu\text{g}/\text{mg}$)

<u>Application</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
1	0.53			
2	0.48	0.34	0.61	1.492
15 3	0.81	0.53	0.61	1.625
4	1.03	0.57	0.61	1.983
		0.84	0.75	2.620

Example 13

This example is similar to Example 12 (four daily applications without washoff between applications) but using the same antifungal nail lacquer as used in Example 7, Sample 353B. The results are shown in Table 18.

20

TABLE 18

Amount of Econazole ($\mu\text{g}/\text{mg}$)

<u>Application</u>	<u>First Layer</u>	<u>Second Layer</u>	<u>Third Layer</u>	<u>Total</u>
25 1 (24h)	1.36	0.17	0	1.534
2 (48h)	1.76	0.35	0.07	2.176
3 (72h)	1.15	0.08	0.07	1.304
4 (96h)	0.49	0.07	0.09	0.647

Example 14

This example is designed to compare the effects of several enhancers according to this invention.

Using the same procedures as described in Example 8, the following antifungal nail lacquers are tested for econazole absorption after 48 hours.

TABLE 18

Ingredient	911-A	911-B	911-C	911-D
Eudragit RL	24	24	24	24
Enhancer:				
2-n-nonyl-1,3-dioxolane	18	--	--	--
2,6-dimethyl-2,7-heptadienyl-1,3-dioxolane	--	18	--	--
2-n-heptyl-1,3-dioxolane	--	--	18	--
decanal dimethyl acetal	--	--	--	18
Ethanol	53	53	53	53

The results are shown in Table 19.

TABLE 19

Econazole Absorption (48h) (µg/mg)

Sample No.	First Layer	Second Layer	Third Layer	Total
25 911-A	1.03	0.05	0.02	1.115
911-B	0.78	0.01	0	0.791
911-C	0.78	0.05	0.03	0.855
911-D	0.76	0.01	0.05	0.813

Example 15

This is an *in vitro* validation study using human toenail specimens in a procedure similar to that described above using four consecutive daily applications of the test sample with wash off between applications, except that the
 5 PBS/PEG200 support gel is replaced by a PBS/Ethanol (80:20) gel (pH, 7.7). The same formulation as used in Example 10 (separately prepared) is used in this example. The results after the fourth application (96 hours) are shown in Table
 10 20, as the average of six replicates.

TABLE 20Amount of Econazole ($\mu\text{g}/\text{mg}$)

	Layer 1	0.82
	Layer 2	0.90
15	Layer 3	1.49
	Total	3.210

Example 16

This example shows the percutaneous absorption of econazole through human skin using lacquer compositions with
 20 or without the skin penetration enhancing compound.

In a first series of experiments conducted for 96 hours using the static cell method (receptor fluid PBS/ethanol (80:20), pH 7.7, temperature 32°C) the following lacquer formulations were tested to determine the effect on
 25 percutaneous absorption of antifungal agent (econazole, 5%) of enhancer (2-n-nonyl-1,3-dioxolane, 0%, 6% or 12%) and various polymeric film-formers, as follows:

TABLE 21

		<u>Sample No. (wt.%)</u>							
<u>Ingredient</u>		<u>303-A</u>	<u>274-D</u>	<u>274A</u>	<u>274C</u>	<u>249A</u>	<u>249B</u>	<u>242C</u>	<u>242A</u>
5	Econazole	5	5	5	5	5	5	5	5
	Enhancer	0	6	6	12	6	6	12	12
	Propylene Glycol	6	0	6	6	0	6	0	6
	Eudragit RL	24	24	24	24	-	-	-	-
10	Dermacryl 79	-	-	-	-	24	24	-	-
	Amphomer LV71	-	-	-	-	-	-	24	24
	Ethanol	65	65	59	53	65	65	59	59

The results are shown in the following Table 22 (average (for 6 or 5 replicates) cumulative (96h) percutaneous absorption through the skin, i.e., amount in receptor) as well as in the epidermis and dermis); Table 23 (cumulative (96h) delivery of antifungal agent as percent of dose for receptor, epidermis and dermis).

TABLE 22

<u>Sample No.</u>		<u>Receptor Amt. (µg)</u>	<u>Epidermis (µg)</u>	<u>Dermis (µg)</u>
20	303-A (n=6)	1.58±0.78	2.13±2.42	0.83±1.48
	274-D (n=5)	5.59±0.75	6.08±2.14	1.82±1.21
	274-A (n=6)	7.06±1.25	9.14±3.43	2.37±1.67
	274-C (n=6)	13.25±2.20	13.50±6.61	6.09±1.90
	249-A (n=6)	1.74±0.88	9.76±4.05	1.34±1.17
25	249-B (n=6)	1.58±0.71	9.30±6.70	0.96±1.29
	242-C (n=6)	3.25±0.45	11.83±7.31	2.17±1.19
	242-A (n=5)	3.64±0.41	9.14±3.10	2.63±0.70

TABLE 23

<u>Sample No.</u>		<u>Receptor</u>	<u>Epidermis</u>	<u>Dermis</u>
30	303-A	0.31±0.18	0.40±0.46	0.17±0.31
	274-D	1.07±0.26	1.13±0.38	0.33±0.21
	274-A	1.43±0.31	1.83±0.67	0.48±0.35
	274-C	2.51±0.50	2.52±1.17	1.15±0.35
	249-A	0.39±0.18	2.17±0.81	0.30±0.27
35	249-B	0.31±0.14	1.92±1.27	0.20±0.27
	242-C	0.66±0.14	2.24±1.17	0.42±0.23
	242-A	0.78±0.06	1.91±0.59	0.55±0.16

WHAT WE CLAIM IS:

1 Claim 1. A composition effective for the treatment or
2 prevention of fungal infections of nails, comprising:

3 (a) at least one antifungal agent effective in the
4 treatment or prevention of onychomycoses;

5 (b) a penetration enhancing agent selected from the
6 group consisting of C₇-C₁₄-hydrocarbyl substituted 1,3-
7 dioxolane, C₇-C₁₄-hydrocarbyl substituted 1,3-dioxane and
8 C₇-C₁₄-substituted acetal;

9 (c) water-insoluble, film-forming polymer; and,

10 (d) volatile solvent;

11 the composition, when applied to nails, forming, upon
12 evaporation of the volatile solvent, a hard, water-resistant
13 film from which the antifungal agent is releasable and
14 becomes available to treat or prevent fungal infection.

1 Claim 2. The composition of claim 1 wherein the
2 antifungal agent is selected from the group consisting of
3 polyenes, allylamines, imidazoles, triazoles, ciclopirox,
4 undecylenic acid, and amorolfine.

1 Claim 3. The composition of claim 1 wherein the
2 antifungal agent comprises at least one of amorolfine,
3 ciclopirox and econazole.

1 Claim 4. The composition of claim 1 wherein the
2 antifungal agent comprises ciclopirox.

1 Claim 5. The composition of claim 1 wherein the
2 antifungal agent comprises econazole.

1 Claim 6. The composition of claim 1 which further
2 comprises antiinflammatory effective amount of steroidal
3 antiinflammatory agent.

1 Claim 7. The composition of claim 4 wherein the
2 steroidal antiinflammatory agent comprises at least one of
3 hydrocortisone, triamcinolone, betamethasone, or clobestol
4 or the salts thereof.

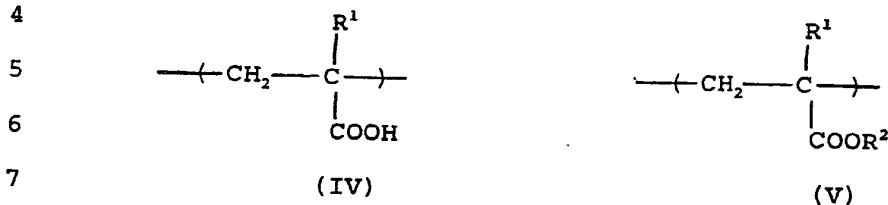
1 Claim 8. The composition of claim 1 which further
2 comprises a plasticizer for the film-forming polymer.

1 Claim 9. The composition of claim 8 wherein the
2 plasticizer is at least one plasticizer selected from the
3 group consisting of glycols, glycol esters, phthalate
4 esters, citrate esters, polyethylene glycols,
5 dipropyleneglycol and polypropylene glycols.

1 Claim 10. The composition of claim 1 wherein the film-
2 forming polymer comprises a water-insoluble film-forming
3 polymer selected from the group consisting of acrylate
4 polymers, methacrylate polymers, and copolymers of alkyl
5 vinyl ether and maleic anhydride.

1 Claim 11. The composition of claim 1 wherein the film-
2 forming polymer comprises an acrylic copolymer.

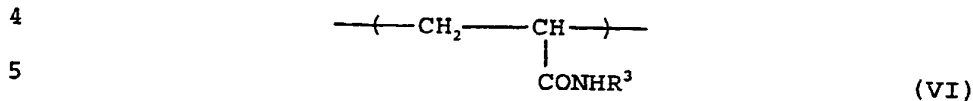
1 Claim 12. The composition of claim 11 wherein the
 2 acrylic copolymer comprises recurring units of at least one
 3 of the following moieties (IV) and (V):



8 wherein R¹ represents H or CH₃; and R² represents an
 9 alkyl group.

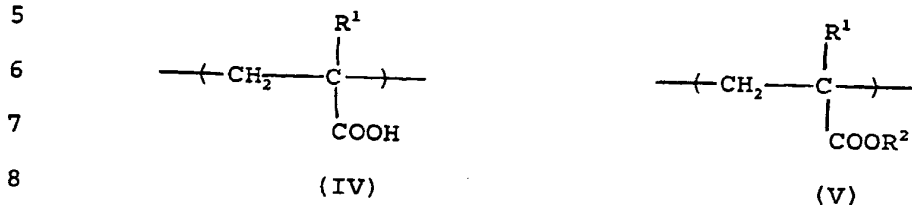
1 Claim 13. The composition of claim 11 wherein the
 2 acrylic copolymer comprises recurring units of the moiety
 3 (V) and wherein R² is an alkyl of at least 4 carbon atoms.

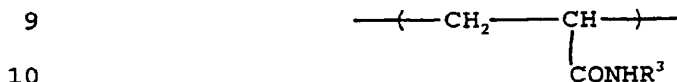
1 Claim 14. The composition of claim 11 wherein the
 2 acrylic copolymer comprises recurring units of a moiety of
 3 formula (VI)



6 wherein R³ represents an alkyl group.

1 Claim 15. The composition of claim 11 wherein the
 2 acrylic copolymer comprises recurring units of formula (V)
 3 or formula (VI) or both formulas (V) and (VI), and,
 4 optionally, recurring units of formula (IV):





(VI)

12 wherein R represents H or CH₃; R² represents an alkyl
13 group, and

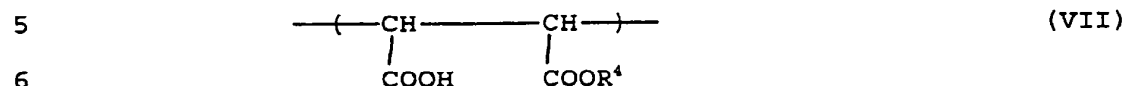
14 R³ represents an alkyl group;

15 at least one of R² and R³ representing an alkyl group having
16 at least 4 carbon atoms.

1 Claim 16. The composition of claim 11 wherein the
2 acrylic copolymer comprises recurring units of a moiety
3 containing a cationic amine group.

1 Claim 17. The composition of claim 15 wherein the
2 cationic amine group is carboethoxy-*t*-butyl amine.

1 Claim 18. The composition of claim 1 wherein the
2 water-insoluble, film-forming polymer comprises a copolymer
3 of methyl vinyl ether or ethyl vinyl ether and at least one
4 comonomer of formula (VII):



7 wherein R⁴ represents a lower alkyl group.

1 Claim 19. The composition of claim 18 wherein said
2 copolymer comprises recurring units of formula (VII) wherein
3 R^{2'} is an alkyl group of at least 2 carbon atoms.

1 Claim 20. The composition of claim 1 wherein the
2 volatile solvent is selected from the group consisting of
3 lower alkanols, lower alkyl esters of lower carboxylic
4 acids, lower alkyl ethers, lower alkyl ketones, and mixtures
5 thereof.

1 Claim 21. The composition of claim 1 wherein the
2 penetration enhancer is 2-n-nonyl-1,3-dioxolane, decanal
3 diethylacetal or decanal dimethylacetal.

1 Claim 22. The composition of claim 1 which comprises:

2 (a) at least one antifungal agent selected from the
3 group consisting of amorolfine, ciclopirox and econazole;

4 (b) a penetration enhancing agent selected from the
5 group consisting of 2-n-nonyl-1,3-dioxolane, decanal
6 diethylacetal and decanal dimethylacetal;

7 (c) water-insoluble, film-forming polymer selected from
8 the group consisting of (meth)acrylate copolymer and alkyl
9 vinyl ether copolymer;

10 (d) volatile solvent selected from the group consisting
11 of ethanol, n-propanol, isopropanol, n-butanol, iso-butanol,
12 acetone, ethyl acetate, propyl acetate, n-butyl acetate, n-
13 amyl acetate, methyl ether, methylethyl ether, methylethyl
14 ketone, methylene chloride, methyl chloroform, toluene,
15 tetrahydrofuran, 1,4-dioxane, and mixtures thereof;

16 (e) plasticizer for the film-forming copolymer selected
17 from the group consisting of glycols, glycol esters,
18 phthalate esters, citrate esters, polyethylene glycol,
19 dipropylene glycol, polypropylene glycols, and mixtures
20 thereof.

1 Claim 23. The composition of claim 1 which comprises:

2 from about 0.5 to about 20 percent (a) antifungal
3 agent;

4 from about 0.5 to about 35 percent (b) penetration
5 enhancing agent;

6 from about 0.5 to about 40 percent (c) film-forming
7 polymer; and

8 from about 10 to about 70 percent (d) volatile solvent.

1 Claim 24. The composition of claim 23 which further
2 comprises from about 0.5 to about 20 percent (e) plasticizer
3 for the film-forming copolymer.

1 Claim 25. A method for the treatment of a fungal
2 infection which comprises applying to an infected nail a
3 nail lacquer composition as defined in claim 1.

1 Claim 26. A method for preventing a fungal infection
2 from developing which comprises applying to the nail of a
3 person in need thereof a nail lacquer composition as defined
4 in claim 1.

1 Claim 27. A nail lacquer composition effective for
2 applying a water-resistant adherent film to animal nails,
3 comprising a substantially non-aqueous solution of water-
4 resistant, film-forming polymer, and plasticizing effective
5 amount of at least one compound selected from the group
6 consisting of C₇-C₁₄-hydrocarbyl substituted 1,3-dioxolane,
7 C₇-C₁₄-hydrocarbyl substituted 1,3-dioxane and C₇-C₁₄-
8 substituted acetal in volatile solvent.

1 Claim 28. The nail lacquer composition of claim 27
2 further comprising in said solution at least one additional
3 plasticizer for the water-resistant film-forming polymer.

1 Claim 29. An antifungal nail lacquer composition
2 comprising a substantially non-aqueous solution of water-
3 resistant, film-forming polymer, antifungal agent effective
4 in the treatment or prevention of onychomycoses, and
5 steroidal antiinflammatory agent in volatile solvent.

1 Claim 30. A plasticized film-forming composition
2 comprising
3 water-insoluble film-forming polymer, and plasticizing
4 effective amount of a compound selected from the group
5 consisting of C₇-C₁₄ hydrocarbyl substituted 1,3-dioxolane,
6 C₇-C₁₄-hydrocarbyl substituted 1,3-dioxolane and C₇-C₁₄
7 hydrocarbyl substituted acetal.

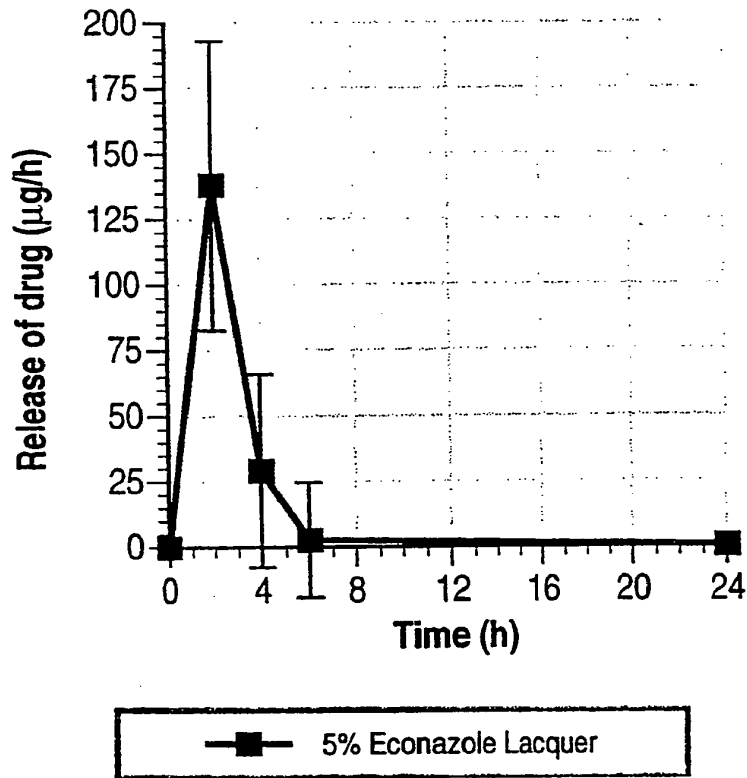


FIGURE 1

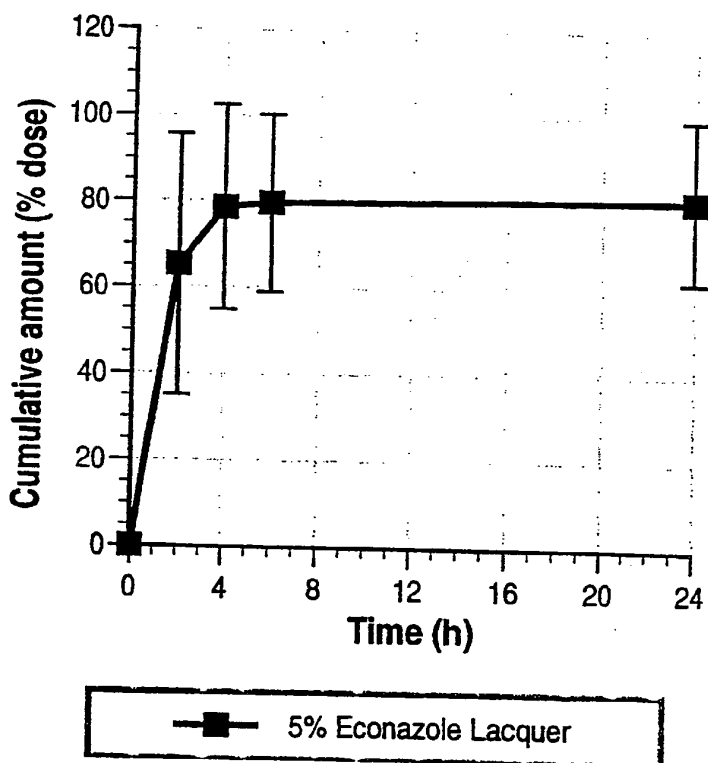


FIGURE 2

INTERNATIONAL SEARCH REPORT


International application No.
PCT/US99/02628

A. CLASSIFICATION OF SUBJECT MATTER IPC(6) : A61K6/00, 7/00, 7/04 US CL : 424/61, 401 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/61, 401 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) NONE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,696,164 A (SUN et al) 09 December 1997, see entire document.	1-30
Y	US 5,219,877 A (SHAH et al) 15 June 1993, see entire document.	1-30
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
A	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E	earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O	document referring to an oral disclosure, use, exhibition or other means	*Z* document member of the same patent family
P	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 10 MAY 1999		Date of mailing of the international search report 28 MAY 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer <i>SH</i> SHARON HOWARD Telephone No. (703) 308-1235 JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX

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B2

①9  **Europäisches Patentamt**
European Patent Office
Office européen des brevets

①1 Publication number:

0 402 989
A2

①2 **EUROPEAN PATENT APPLICATION**

②1 Application number: 90201447.1

⑤1 Int. Cl.⁵: **C07D 405/14, A61K 31/495**

②2 Date of filing: 01.06.90

③0 Priority: 09.06.89 US 363795

B-2340 Beerse(BE)

④3 Date of publication of application:
19.12.90 Bulletin 90/51

⑦2 Inventor: Heeres, Jan
Leemskuilen 18

⑥4 Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

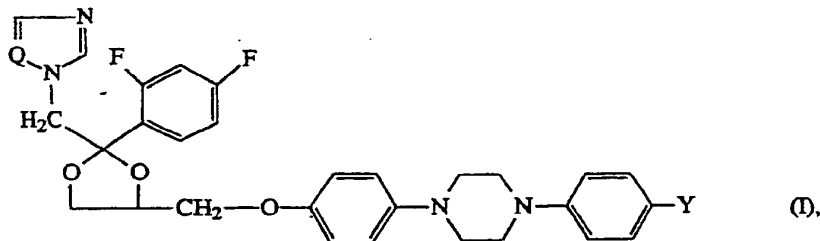
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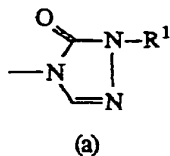
⑤4 **Antifungal**
4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-azolylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]triazolones and imidazolones.

⑤7 4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-azolylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]triazolones and imidazolones of formula

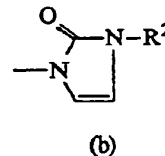


wherein
Q is CH or N;
Y is a radical of formula

EP 0 402 989 A2



or



R¹ is C₅-₇cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl; and
R² is C₁₋₆alkyl, C₅-₇cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl,
the acid addition salts and stereoisomeric forms thereof; said compounds having antifungal properties.
Pharmaceutical compositions containing such compounds as an active ingredient; methods of preparing

said compounds and pharmaceutical compositions.

descriptors R and S, this R and S notation corresponding to the rules described in Pure Appl. Chem 1976, 45, 11-30. The relative configuration of the asymmetric centers in diastereomeric racemates of formula (I) is denoted by the descriptors cis and trans according to the rules described in J. Org. Chem. 1970, 35 (9), 2849-2867. Stereochemically isomeric forms of the compounds of formula (I) are obviously intended to be embraced within the scope of the invention.

The compounds of formula (I) have basic properties and, consequently, they may be converted to their therapeutically active non-toxic acid addition salt forms by treatment with appropriate acids, such as, for example, inorganic acids, e.g. hydrochloric, hydrobromic and the like acids, sulfuric acid, nitric acid, phosphoric acid and the like; or organic acids, such as, for example, acetic, propanoic, hydroxyacetic, 2-hydroxypropanoic, 2-oxopropanoic, ethanedioic, propanedioic, butanedioic, (Z)-2-butenedioic, (E)-2-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxybutanedioic, 2-hydroxy-1,2,3-propanetricarboxylic, methanesulfonic, ethanesulfonic, benzenesulfonic, 4-methylbenzenesulfonic, cyclohexanesulfamic, 2-hydroxybenzoic, 4-amino-2-hydroxybenzoic and the like acids. Conversely the salt form can be converted by treatment with alkali into the free base form.

The term pharmaceutically acceptable acid addition salts also comprises the solvates which the compounds of formula (I) may form and said solvates are intended to be included within the scope of the present invention. Examples of such solvates are e.g. the hydrates, alcoholates and the like.

Interesting compounds are those compounds of formula (I) wherein Q is N; and/or the substituents on the dioxolane nucleus have a cis configuration.

Particularly interesting compounds are those interesting compounds wherein R¹ and R² are mono-, di-, tri-, tetra- or pentafluoroC₁₋₄alkyl, cyclohexyl or cyclopentyl, or R² is C₁₋₄alkyl.

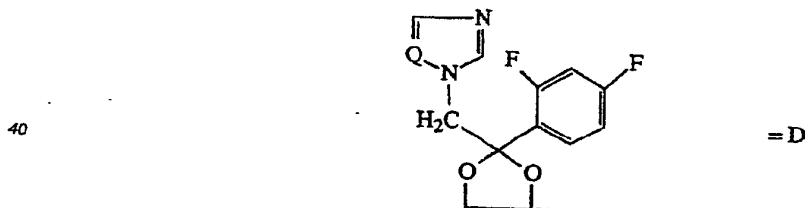
Preferred compounds are those particularly interesting compounds wherein R¹ and R² are 2,2,2-trifluoroethyl, 2,2,3,3-tetrafluoropropyl, cyclopentyl, or R² is propyl, 1-methylpropyl, 2-methylpropyl or butyl.

The most preferred compounds are cis-2-cyclopentyl-4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-3H-1,2,4-triazol-3-one;

cis-4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(2,2,2-trifluoroethyl)-3H-1,2,4-triazol-3-one; and cis-1-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-1,3-dihydro-3-(1-methylpropyl)-2H-imidazol-2-one, the pharmaceutically acceptable salts thereof and the stereochemically isomeric forms thereof.

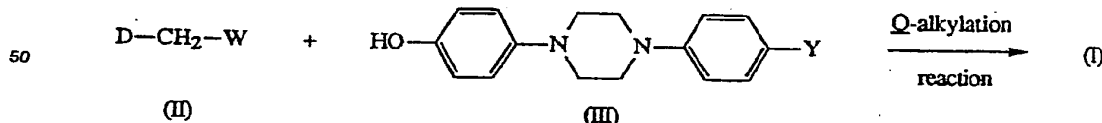
In order to simplify the structural representations of the compounds of formula (I) and of certain starting materials and intermediates used in the preparation thereof, the 2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl group will hereafter be represented by the symbol D:

35



40

The compounds of formula (I) can be prepared by Q-alkylating an appropriately substituted phenol of formula (III) with an alkylating reagent of formula (II).



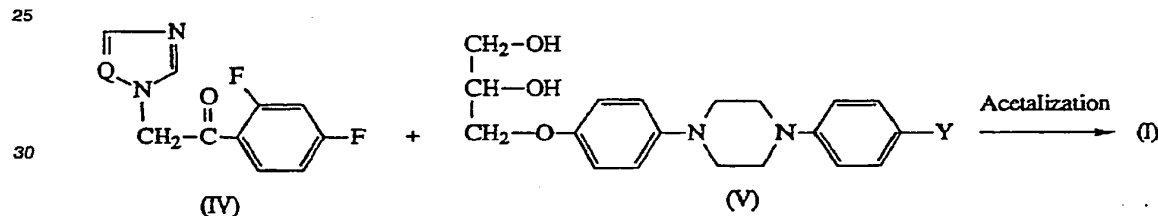
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In formula (II) and in a number of the following intermediates, W represents a reactive leaving group such as, for example, halo, preferably chloro, bromo or iodo, or a sulfonyloxy group such as, for example, methanesulfonyloxy, 2-naphtalenesulfonyloxy or 4-methylbenzenesulfonyloxy and the like.

The alkylation reaction of (II) with (III) can be carried out under art-known conditions of performing Q-

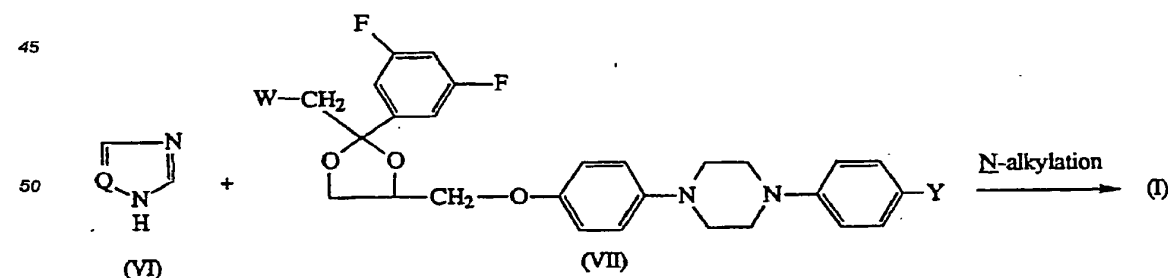
alkylations. Said O-alkylation reaction can conveniently be conducted in a suitable reaction-inert solvent in the presence of an appropriate base. A suitable reaction-inert solvent is, for example, an aromatic hydrocarbon, e.g., benzene, methylbenzene, dimethylbenzene and the like; a halogenated hydrocarbon, e.g., dichloromethane, trichloromethane and the like; a lower alkanol, e.g., methanol, ethanol, 1-butanol and the like; a ketone, e.g., 2-propanone, 4-methyl-2-pentanone and the like; an ether, e.g., 1,4-dioxane, 1,1-oxybisethane, tetrahydrofuran and the like; a dipolar aprotic solvent, e.g., N,N-dimethylformamide, N,N-dimethylacetamide, hexamethylphosphoric triamide, dimethyl sulfoxide, nitrobenzene, 1-methyl-2-pyrrolidinone and the like, or a mixture of said solvents. The acid which is liberated during the course of the reaction may be picked up by an appropriate base such as, for example, an alkali or an earth alkaline metal carbonate, hydrogen carbonate, hydroxide, alkoxide, hydride or amide, e.g., sodium carbonate, potassium carbonate, sodium hydroxide, sodium methoxide, sodium hydride, sodium amide and the like, or an organic base such as, for example, an amine, e.g., N,N-diethylethanamine, N(1-methylethyl)-2-propanamine, 4-ethylmorpholine, and the like. In some instances it may be advantageous to convert the substituted phenol (III) first into a metal salt thereof, preferably the sodium salt, e.g., by the reaction of (III) with a metal salt such as sodium hydride, sodium hydroxide and the like, and to use said metal salt subsequently in the reaction with (II). Stirring and somewhat elevated temperatures may enhance the rate of the reaction; more particularly the reaction may be conducted at a temperature from about 50 °C to about 60 °C. Additionally, it can be advantageous to conduct said O-alkylation under an inert atmosphere such as, for example, oxygen-free nitrogen or argon gas.

Alternatively, the compounds of formula (I) may be prepared following the procedures described in U.S. Pat. No. 4,101,666, which is incorporated herein by reference, for instance, by the acetalation reaction of a ketone of formula (IV) with a 2,3-dihydroxypropyl ether derivative of formula (V) in the presence of an acid such as, for example, benzenesulfonic acid, 4-methylbenzenesulfonic acid, methanesulfonic acid and the like acids.



35 Said acetalization reaction can conveniently be conducted in a reaction-inert solvent such as, an aromatic hydrocarbon, e.g., benzene, methylbenzene, a halogenated hydrocarbon, e.g., trichloromethane; an alkanol, e.g., ethanol, propanol, butanol and the like, or a mixture of such solvents. Preferably, the water which is liberated during the course of the reaction, is removed by azeotropical distillation. In a similar way, the compounds of formula (I) may also be obtained by transacetalation of (IV) with the acetone acetal of (V), or by acetalation of (IV) with the 2,3-epoxypropyl ether derivative corresponding to (V), in the presence of an acid and a reaction-inert solvent as described hereinbefore.

40 Or, the compounds of formula (I) may also be synthesized by N-alkylating an azole (VI) with an intermediate of formula (VII).



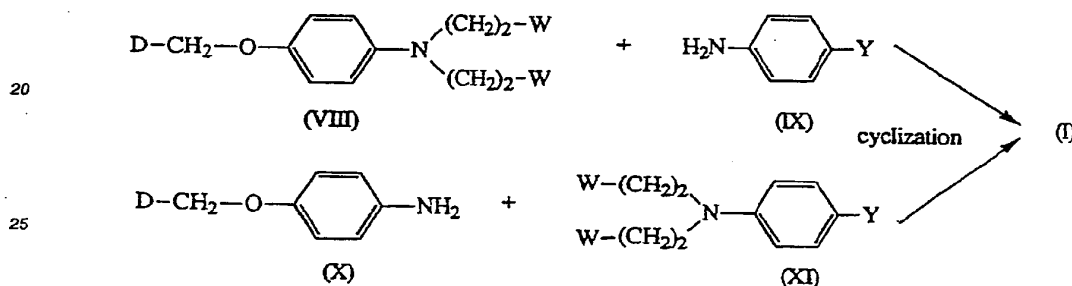
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Said N-alkylation reaction can conveniently be conducted in a suitable reaction-inert solvent or a mixture of such solvents in the presence of an appropriate base. Suitable reaction-inert solvents are, for example, an aromatic hydrocarbon, e.g., benzene, methylbenzene, dimethylbenzene, and the like; a lower

alkanol, e.g., methanol, ethanol, 1-butanol and the like; a ketone, e.g., 2-propanone, 4-methyl-2-pentanone and the like; an ether, e.g., 1,4-dioxane, 1,1'-oxybisethane, tetrahydrofuran and the like; a dipolar aprotic solvent, e.g., *N,N*-dimethylformamide, *N,N*-dimethylacetamide, dimethyl sulfoxide, nitrobenzene, 1-methyl-2-pyrrolidinone, and the like; a halogenated hydrocarbon, e.g., dichloromethane, trichloromethane and the like.

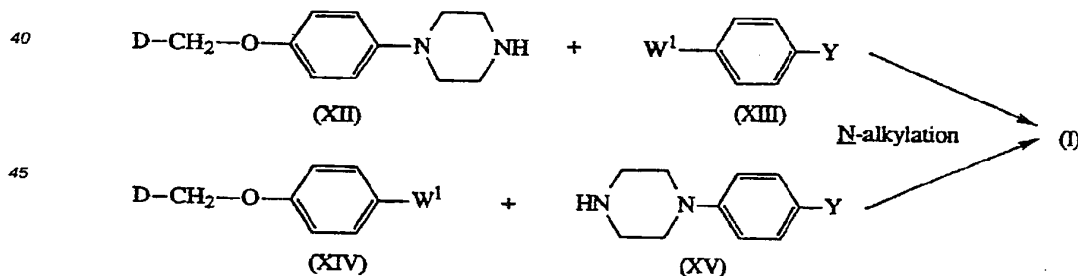
The addition of an appropriate base such as, for example, an alkali or an earth alkaline metal carbonate, hydrogen carbonate, hydroxide, amide or hydride, e.g., sodium hydroxide, potassium hydroxide, potassium carbonate, sodium hydride and the like or an organic base such as, for example, *N,N*-dimethyl-4-pyridinamine, *N,N*-diethylethanamine or *N*-(1-methylethyl)-2-propanamine may be employed to pick up the acid which is liberated during the course of the reaction. In some instances it may be advantageous to use an excess of the azole (VI) or to convert it to its metal salt form, in particular its alkali metal salt form following art-known procedures such as, e.g. by treatment of the azole (VI) with an alkali metal hydroxide, alkoxide, amide or hydride.

The compounds of formula (I) may also be obtained by cyclizing an intermediate of formula (VIII) with an appropriately substituted benzenamine of formula (IX), or by cyclizing a benzenamine of formula (X) with a reagent of formula (XI).



Said cyclization reaction may be carried out by stirring the reactants in the presence of an appropriate polar solvent, e.g. water, in admixture with an appropriate water-miscible organic solvent, such as, for example, 2-propanol, 2-propanone and the like, preferably at an elevated temperature and most preferably, in the presence of an alkali or earth alkaline metal iodide such as, e.g., potassium iodide.

Furthermore, the compounds of formula (I) may be prepared by *N*-alkylating a piperazine of formula (XII) with a benzene of formula (XIII), or by *N*-alkylating a piperazine of formula (XV) with a benzene of formula (XIV) following standard *N*-alkylating procedures. In formulae (XIII) and (XIV) W¹ represents an appropriate reactive leaving group, such as, for example, halo, e.g., chloro or bromo and in particular fluoro, or a sulfonyloxy group, e.g. trifluoromethanesulfonyloxy.



Said *N*-alkylation may be carried out by stirring the reactants, preferably at somewhat elevated temperatures, in an appropriate organic solvent such as, for example, *N,N*-dimethylacetamide, dimethyl sulfoxide and the like, in the presence of an appropriate base such as, for example, an alkali metal hydride or carbonate and the like bases.

The compounds of formula (I) wherein Y is a radical of formula (a), said compounds being represented by formula (I-a), can generally be prepared by cyclizing an intermediate of formula (XVI) with an appropriate reagent of formula (XVII) and the compounds wherein Y is a radical of formula (b), said compounds being represented by formula (I-b), can generally be prepared by cyclizing and intermediate of formula (XVI) with

depending on the desired mode of administration. These pharmaceutical compositions are preferably in unitary dosage form suitable for administration orally, rectally or by parenteral injection. For example, in preparing the compositions in oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols and the like in the case of oral liquid preparations such as suspensions, syrups, elixirs and solutions; or solid carriers such as starches, sugars, kaolin, lubricants, binders, disintegrating agents and the like in the case of powders, pills, capsules and tablets. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. For parenteral compositions, the carrier will usually comprise sterile water, at least in large part, though other ingredients, for example, to aid solubility, may be included. Injectable solutions, for example, may be prepared in which the carrier comprises saline solution, glucose solution or a mixture of saline and glucose solution. Injectable suspensions may also be prepared in which case appropriate liquid carriers, suspending agents and the like may be employed. In the compositions suitable for percutaneous administration, the carrier optionally comprises a penetration enhancing agent and/or a suitable wetting agent, optionally combined with suitable additives of any nature in minor proportions, which additives do not cause a significant deleterious effect to the skin. Said additives may facilitate the administration to the skin and/or may be helpful for preparing the desired compositions. These compositions may be administered in various ways, e.g., as a transdermal patch, as a spot-on, as an ointment. Acid addition salts of (I) due to their increased water solubility over the corresponding base form, are obviously more suitable in the preparation of aqueous compositions.

It is especially advantageous to formulate the aforementioned pharmaceutical compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used in the specification and claims herein refers to physically discrete units suitable as unitary dosages, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. Examples of such dosage unit forms are tablets (including scored or coated tablets), capsules, pills, powder packets, wafers, injectable solutions or suspensions, teaspoonfuls, tablespoonfuls and the like, and segregated multiples thereof.

The compounds of formula (I), the pharmaceutically acceptable acid addition salts and stereochemically isomeric forms thereof are useful agents in combatting fungi. For example, said compounds are found to be highly active against a wide variety of fungi such as, for example, Microsporum canis, Pityrosporum ovale, Ctenomyces mentagrophytes, Trichophyton rubrum, Phialophora verrucosa, Cryptococcus neoformans, Candida tropicalis, Candida albicans, Mucor species, Aspergillus fumigatus, Sporotrichum schenckii and Saprolegnia species. In view of their potent, topical as well as systemic, antifungal activity the compounds of this invention constitute useful tools for the destruction of fungi, or for the inhibition or prevention of the growth or development of fungi. More particularly they can effectively be used in the treatment of warm-blooded animals suffering from diseases such as, for example, tinea corporis, tinea cruris, tinea manus, tinea pedis, candidosis, pityriasis versicolor, onychomycosis, perionyxis, paracoccidioidomycosis, histoplasmosis, coccidioidomycosis, cryptococcosis, chromomycosis, mucormycosis, sporotrichosis, seborrheic dermatitis and the like.

A number of compounds of the present invention are particularly attractive due to their improved topical action against Microsporum species and are therefore particularly useful in the treatment of warm-blooded animals suffering from microsporosis, i.e. infection by Microsporum. Particular examples of said warm-blooded animals are domestic animals such as, for example, dogs, cats and horses, and humans infected by Microsporum.

Further the compounds of the present invention also show an improved activity against Candida infections. The present compounds therefor appear to be particularly useful in the topical treatment of vaginal candidosis and skin candidosis, and in the systemic treatment of skin candidosis and especially deep (or systemic) candidosis.

Those of skill in treating warm-blooded animals suffering from diseases caused by fungi could easily determine the effective amount from the test results presented here. In general it is contemplated that an effective amount would be from 0.01 mg/kg to 50 mg/kg body weight, and more preferably from 0.05 mg/kg to 20 mg/kg body weight. For topical applications it is contemplated that an effective amount would be from 0.001% to 5% (by weight) and more preferably from 0.1% to 2% (by weight).

The following examples are intended to illustrate and not to limit the scope of the present invention. Unless otherwise stated all parts therein are by weight.

55

Experimental part

A. Preparation of the intermediatesExample 1

5

a) To a refluxing and stirred solution of 457.6 parts of 1H-imidazole in 2400 parts of trichloromethane was added dropwise a solution of 320 parts of 2-chloro-1-(2,4-difluorophenyl)ethanone in 1440 parts of trichloromethane. After stirring for 1/2 hour at reflux temperature, the reaction mixture was poured into water. The organic layer was washed with water (2x), dried, filtered and evaporated. The residue was crystallized from 2-propanol, yielding 244 parts (69%) of 1-(2,4-difluorophenyl)-2-(1H-imidazol-1-yl)ethanone; mp. 125 °C (interm. 1).

b) A mixture of 100 parts of 1,2,3-propanetriol, 70 parts of intermediate (1), 450 parts of methanesulfonic acid and 108 parts of benzene was stirred for 2 hours at reflux temperature using a water separator. After cooling, the reaction mixture was added dropwise to a stirred sodium hydrogen carbonate solution. The product was extracted with chloromethane and the extract was washed with water, dried, filtered and evaporated, yielding 80 parts (100%) of (cis + trans)-2-(2,4-difluorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolane-4-methanol (interm. 2).

c) To a stirred mixture of 266.5 parts of intermediate (2), 234 parts of N,N-diethylethanamine, 8 parts of N,N-dimethyl-4-pyridinamine and 1950 parts of dichloromethane were added portionwise 227 parts of 2-naphthalenesulfonyl chloride. Stirring was continued overnight at room temperature. The reaction mixture was diluted with water and extracted with dichloromethane (3x). The combined extracts were washed with water, dried, filtered and evaporated. The residue was purified twice by column chromatography (silica gel; CHCl₃/CH₃OH 99:1; HPLC; silica gel; CH₂Cl₂/CH₃OH 99:1). The eluent of the desired fraction was evaporated and the residue was crystallized from 4-methyl-2-pentanone. The product was filtered off and dried, yielding 100 parts (22.8%) of cis-[[2-(2,4-difluorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl] 2-naphthalenesulfonate; mp. 125.0 °C (interm. 3).

Example 2

A mixture of 44.6 parts of 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone (described in GB-2,099,818), 56.0 parts of (2S)-1,2,3-propanetriol 1-(4-methylbenzenesulfonate) (ester), 296 parts of methanesulfonic acid and 200 parts of dichloromethane was stirred at reflux temperature using a water separator. After cooling, the reaction mixture was added dropwise to a mixture of ice-water, diluted potassium carbonate and dichloromethane. The organic layer was separated and the aqueous phase was re-extracted with dichloromethane. The combined dichloromethane layers were dried, filtered and evaporated. The residue was purified by column chromatography (silica gel; CHCl₃). The eluent of the desired fraction was evaporated and the residue was converted into the 4-methylbenzenesulfonate salt in 4-methyl-2-pentanone. The salt was recrystallized from 4-methyl-2-pentanone, yielding 20.5 parts (16.4%) of (-)-(2S,cis)-2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolane-4-methanol 4-methylbenzenesulfonate(ester) 4-methylbenzenesulfonate (salt) (1:1); mp. 182.5 °C; [α]_D²⁰ -13.790° (c = 1% in CH₃OH) (interm. 4).

Example 3

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A mixture of 40.0 parts of 1-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-yl)ethanone (described in GB-2,099,818), 56.0 parts of (2R)-1,2,3-propanetriol 1-(4-methylbenzenesulfonate) (ester), 370 parts of methanesulfonic acid and 133 parts of dichloromethane was stirred for 24 hours at reflux temperature using a water separator. After cooling, the reaction mixture was added dropwise to a mixture of potassium carbonate, ice-water and dichloromethane. The organic layer was separated, washed with water, dried, filtered and evaporated. The residue was purified by column chromatography (silica gel; CHCl₃). The eluent of the desired fraction was evaporated and the residue was converted into the 4-methylbenzenesulfonate salt in 4-methyl-2-pentanone. The salt was recrystallized from acetonitrile, yielding 23.1 parts (20.6%) of (+)-(2R,cis)-2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolane-4-methanol 4-methylbenzenesulfonate(ester) 4-methylbenzenesulfonate (salt, 1:1); mp. 183.5 °C; [α]_D²⁰ = +14.43° (c = 1% in CH₃OH) (interm. 5).

Example 4

a) 40 Parts of 2,2-(dimethoxy)ethanamine were reductively alkylated with 35 parts of 3-pentanone in a mixture of 4 parts of palladium-on-charcoal catalyst (10%), 2 parts of a solution of thiophene in methanol (4%) and 395 parts of methanol. The reaction mixture was filtered and the filtrate was evaporated. The residue was distilled (waterjet pump; 76 °C), yielding 55.7 parts (83.6%) of N-(2,2-dimethoxyethyl)-1-ethylpropanamine (interm. 6).

b) A mixture of 36 parts of phenyl [4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]carbamate monohydrochloride (described in example XVII of US-4,267,179), 19.2 parts of intermediate (6), 4 parts of N,N-dimethyl-4-pyridinamine, 14.6 parts of N,N-diethylethanamine and 412 parts of 1,4-dioxane was stirred for 3 hours at reflux temperature. After cooling, the reaction mixture was diluted with water and the whole was left to crystallize. The product was filtered off, washed with water, dried and stirred in 122 parts of formic acid for 3 hours at 70 °C. The whole was evaporated and the residue was boiled in 2-propanol and further purified by column chromatography (silica gel; CH₂Cl₂/CH₃OH 99:1). The eluent of the desired fraction was evaporated and the residue was triturated in 2-propanol. The product was filtered off and dried, yielding 29.3 parts (85.0%) of 1-(1-ethylpropyl)-1,3-dihydro-3-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-2H-imidazol-2-one; mp. 195.8 °C (interm. 7).

Example 5

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a) A mixture of 10 parts of phenyl [4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]carbamate monohydrochloride (described in example XVII of US-4,267,179), 3 parts of 2,2-diethoxy-ethanamine and 100 parts of 1,4-dioxane was stirred for 6 hours at reflux temperature. After cooling, the precipitate was filtered off, washed with 1,4-dioxane and purified by column chromatography (silica gel; CHCl₃/CH₃OH 99:1). The eluent of the desired fraction was evaporated and the residue was crystallized from 1,4-dioxane, yielding 3.9 parts of N-(2,2-dimethoxyethyl)-N'-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]urea; mp. 225 °C (interm. 8).

b) A mixture of 70 parts of intermediate (8), 84 parts of hydrochloric acid, 300 parts of water and 280 parts of methanol was stirred for 1/2 hour at 80 °C. After cooling, the reaction mixture was left to crystallize. The product was filtered off, washed with water and dried, yielding 24.5 parts (37%) of 1,3-dihydro-1-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-2H-imidazol-2-one monohydrochloride monohydrate; mp. 256.2 °C (interm. 9).

c) To a stirred mixture of 12 parts of intermediate (9), 6.75 parts of 1-bromopropane and 250 parts of dimethyl sulfoxide were added 3 parts of a dispersion of sodium hydride in mineral oil (50%). After stirring for 2 hours at 60 °C and subsequent cooling, the reaction mixture was poured into water. The precipitate was filtered off and purified by column chromatography (silica gel; CHCl₃). The eluent of the desired fraction was evaporated and the residue was crystallized from 1-butanol. The product was filtered off and dried, yielding 7.2 parts (61%) of 1,3-dihydro-1-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-3-propyl-2H-imidazol-2-one; mp. 214.1 °C (interm. 10). In a similar manner intermediate (9) was converted into 1,3-(dihydro-1-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-3-(1-methylpropyl)-2H-imidazol-2-one; mp. 184.0 °C (interm. 11).

Example 6

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a) To a stirred solution of 25.0 parts of 2,2,2-trifluoroethanol in 175 parts of N,N-diethylethanamine were added portionwise 62.2 parts of 2-naphthalenesulfonyl chloride and then a mixture of 1.5 parts of N,N-dimethyl-4-pyridinamine and 25 parts of ethyl acetate. After stirring overnight at room temperature, the reaction mixture was filtered and the filtrate was evaporated. The residue was stirred in water. The solid was filtered off and dissolved in dichloromethane. This solution was dried, filtered and evaporated. The residue was successively triturated with petroleum ether and crystallized from 2-propanol. The product was filtered off and dried, yielding 65.3 parts (89%) of 2,2,2-trifluoroethyl 2-naphthalenesulfonate; mp. 72.7 °C (interm. 12). b) A mixture of 17.5 parts of intermediate (9), 16.1 parts of intermediate (12), 10.6 parts of sodium carbonate, 261 parts of 1,3-dimethyl-2-imidazolidinone and 130.5 parts of methylbenzene was stirred for 48 hours at reflux temperature using a water separator. After cooling, the reaction mixture was diluted with water. Upon addition of some petroleum ether and 4.2 parts of acetic acid, the product crystallized out. It was dried and purified by column chromatography (silica gel; CH₂Cl₂/CH₃OH 99.5:0.5). The eluent of the desired fraction was evaporated and the residue was crystallized from 4-methyl-2-pentanone. The product was filtered off and dried, yielding 9.0 parts (41.6%) of 1,3-dihydro-1-[4-[4-(4-methoxyphenyl)-1-piperazinyl]-

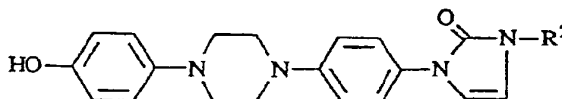
phenyl]-3-(2,2,2-trifluoroethyl)-2H-imidazol-2-one; mp. 224.1 °C (interm. 13).

Example 7

5 To a stirred and cooled (ice-bath) amount of 200 ml of a boron tribromide solution in dichloromethane 1M was added dropwise a solution of 14.6 parts of 1,3-dihydro-1-[4-[4-(4-methoxyphenyl)-1-piperazinyl]-phenyl]-3-methyl-2H-imidazol-2-one in 665 parts of dichloromethane. Stirring was continued for 5 days at room temperature and then the reaction mixture was poured into a mixture of 200 parts of water, 158 parts of methanol and 180 parts of ammonium hydroxide. After stirring for 1 hour, the precipitate was filtered off, 10 washed with dichloromethane and water and dried (= first fraction of product). The organic layer of the filtrate was separated and evaporated. The residue was triturated in dichloromethane. The solid was filtered off and dried (= second fraction). The two fractions were combined and crystallized from N,N-dimethylformamide, yielding 9.6 parts (68.5%) of 1,3-dihydro-1-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]-phenyl]-3-methyl-2H-imidazol-2-one; mp. 283.1 °C (interm. 14).

15 Following the same procedure there were also prepared the intermediates of Table 1.

Table 1



Interm. No.	R ²	Physical data (mp.)
14	CH ₃	283.1°C
15	C ₂ H ₅	241.2°C
16	n.C ₃ H ₇	246.1°C
17	i.C ₃ H ₇	249.5°C
18	n.C ₄ H ₉	195.0°C
19	CH(CH ₃)C ₂ H ₅	219.6°C
20	CH ₂ CH(CH ₃) ₂	224.5°C
21	CH(CH ₃)C ₃ H ₇	184.9°C
22	CH(C ₂ H ₅) ₂	219.5°C
23	CH(CH ₃)CH(CH ₃) ₂	238.2°C
24	c.C ₅ H ₉	230.8°C
25	c.C ₆ H ₁₁	261.8°C
26	CH ₂ CF ₃	227.7°C

Example 8

50 A mixture of 52.8 parts of 2,2,3,3-tetrafluoro-1-propanol, 117.8 parts of 2-naphthalenesulfonyl chloride, 294 parts of pyridine and 2.0 parts of N,N-dimethyl-4-pyridinamine was stirred for 48 hours at room temperature. The reaction mixture was diluted with water and the whole was left to crystallize. The product 55 was filtered off, washed with water and recrystallized from 2-propanol, yielding 98.2 parts (76.2%) of 2,2,3,3-tetrafluoropropyl 2-naphthalenesulfonate; mp. 89.6 °C (interm. 27).

Example 9

a) To a cooled (ice-bath) suspension of 41.7 parts of 2-naphthalenesulfonyl chloride in 174 parts of methylbenzene were added dropwise 20.0 parts of 1,1, 1-trifluoro-2-propanol. After stirring for 1 hour, there was added dropwise a mixture of 9.6 parts of a dispersion of sodium hydride in mineral oil (50%) and some methylbenzene while cooling on ice. Subsequently, the whole was diluted dropwise with water. The organic layer was separated, dried, filtered and evaporated, yielding 53.2 parts (99.9%) of (2,2,2-trifluoro-1-methylethyl) 2-naphthalenesulfonate (interm. 28).

b) A mixture of 17.5 parts of 2,4-dihydro-4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-3H-1,2,4-triazol-3-one (described in Example XVII of US-4,267,179), 22.0 parts of intermediate (28), 5.0 parts of lithium carbonate, 10.0 parts of sodium carbonate, 261 parts of 1,3-dimethyl-2-imidazolidinone and 130.5 parts of methylbenzene was stirred for 4 days at reflux temperature. After cooling, the reaction mixture was diluted with 1500 parts of water. Upon addition of 218 parts of 2,2'-oxybispropane, the product crystallized out. The mother liquor was filtered off and the product was dissolved in dichloromethane. The latter solution was filtered and the filtrate was evaporated, yielding a first fraction of product. The mother liquor was extracted with a mixture of 2,2'-oxybispropane and methylbenzene (1:1). The extract was dried, filtered and evaporated, yielding a second fraction of product. The combined fractions were purified by column chromatography (silica gel; CHCl₃). The eluent of the desired fraction was evaporated and the residue was crystallized from 4-methyl-2-pentanone, yielding 6 parts (26.8%) of 2,4-dihydro-4-[4-[4-(4-methoxyphenyl)-1-piperazinyl]phenyl]-2-(2,2,2-trifluoro-1-methylethyl)-3H-1,2,4-triazol-3-one; mp. 198.7 °C (interm. 29).

c) A mixture of 11.5 parts of intermediate (29), 522 parts of hydrobromic acid 48% and 3.0 parts of sodium sulfite was stirred overnight at reflux temperature. After cooling, the reaction mixture was diluted with 1000 parts of water and subsequently neutralized with ammonia. The precipitate was filtered off, dried and purified by column chromatography (silica gel; CHCl₃/CH₃COOC₂H₅/hexane/CH₃OH 498.5:300:200:1.5). The eluent of the desired fraction was evaporated and the residue was crystallized from 1-butanol. The product was filtered off and dried, yielding 8.4 parts (74.5%) of 2,4-dihydro-4-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl]-2-(2,2,2-trifluoro-1-methylethyl)-3H-1,2,4-triazol-3-one; mp. 230.4 °C (interm. 30).

In a similar manner intermediate (27) was converted into 2,4-dihydro-4-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl]-2-(2,2,3,3-tetrafluoropropyl)-3H-1,2,4-triazol-3-one; mp. 214.7 °C (interm. 31).

B. Preparation of the final compoundsExample 10

A mixture of 4.2 parts of 2,4-dihydro-4-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl]-2-(2,2,2-trifluoroethyl)-3H-1,2,4-triazol-3-one (prepared as described in example 15 of EP-0,331,232), 6.4 parts of cis-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methyl]-2-naphthalenesulfonate (prepared as described in example 2 of U.S. Pat. No. 4,791,111), 1.0 part of sodium hydroxide and 135 parts of N,N-dimethylformamide was stirred at 50 °C under a nitrogen atmosphere. After the addition of water and 1.6 parts of acetic acid, the precipitate was filtered off and taken up in trichloromethane. The organic layer was dried, filtered and evaporated. The residue was purified by column chromatography (silica gel; CHCl₃/CH₃OH 98.5:1.5). The eluent of the desired fraction was evaporated and the residue was crystallized from 4-methyl-2-pentanone, yielding 4.3 parts (62%) of cis-4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(2,2,2-trifluoroethyl)-3H-1,2,4-triazol-3-one; mp. 177.6 °C (compound 2).

In a similar manner using an equivalent amount of 2-cyclopentyl-2,4-dihydro-4-[4-[4-(4-hydroxyphenyl)-1-piperazinyl]phenyl]-3H-1,2,4-triazol-3-one (prepared as described in example 14 of EP-0,331,232) there was also prepared cis-2-cyclopentyl-4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-3H-1,2,4-triazol-3-one; 218.8 °C (compound 1).

Example 11

A mixture of 5 parts of phenyl cis-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]carbamate (prepared as described in example 3 of U.S.

Pat. No. 4,791,111), 1.8 parts of N-(2,2-dimethoxyethyl)-2-butanamine, 1 part of N,N-dimethyl-4-pyridinamine and 100 parts of 1,4-dioxane was stirred overnight at reflux temperature. The reaction mixture was evaporated and the residue was stirred for 2 hours in 120 parts of formic acid at 60 °C. After evaporation, the residue was dissolved in dichloromethane and the whole was neutralized with a sodium hydrogen carbonate solution. The organic layer was separated, dried, filtered and evaporated. The residue was purified by column chromatography (silica gel; CHCl₃/CH₃OH 99:1). The eluent of the desired fraction was evaporated and the residue was crystallized twice from 4-methyl-2-pentanone, yielding 2.8 parts (55.5%) of cis-1-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-1,3-dihydro-3-(1-methylpropyl)-2H-imidazol-2-one; mp. 159.0 °C (compound 3).

All other compounds in Table 2 were prepared following the procedure described in Example 10.

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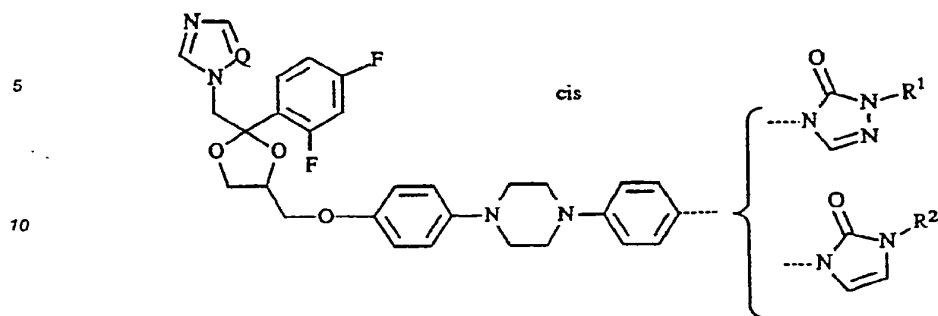
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Table 2



Comp. No.	Q	R ¹ or R ²	Physical data
1	N	R ¹ = c.C ₅ H ₉	218.8°C
2	N	R ¹ = CH ₂ CF ₃	177.6°C
3	N	R ² = CH(CH ₃)C ₂ H ₅	159.0°C
4	CH	R ² = CH(CH ₃)C ₂ H ₅	169.1°C
5	N	R ² = CH ₃	229.2°C
6	CH	R ² = CH ₃	251.1°C
7	N	R ² = CH ₂ CF ₃	197.6°C
8	N	R ² = C ₂ H ₅	222.1°C
9	CH	R ² = C ₂ H ₅	243.4°C
10	N	R ² = CH(CH ₃) ₂	204.5°C
11	CH	R ² = CH(CH ₃) ₂	200.5°C
12	N	R ² = C ₃ H ₇	188.9°C
13	CH	R ² = C ₃ H ₇	202.3°C
14	N	R ² = C ₄ H ₉	165.9°C
15	CH	R ² = C ₄ H ₉	172.1°C
16	N	R ² = CH ₂ CH(CH ₃) ₂	173.7°C
17	CH	R ² = CH ₂ CH(CH ₃) ₂	231.7°C

Comp. No.	Q	R ¹ or R ²	Physical data
18	N	R ¹ = CH ₂ CF ₃	183.9°C; [α] _D ²⁰ = -9.32° *
19	N	R ¹ = CH ₂ CF ₃	(-)-(2S, <u>cis</u>) 183.3°C; [α] _D ²⁰ = +10.03° *
20	N	R ² = c.C ₆ H ₁₁	180.7°C
21	CH	R ² = c.C ₆ H ₁₁	189.5°C
22	CH	R ¹ = CH ₂ CF ₃	178.6°C
23	N	R ¹ = CHCH ₃ CF ₃	187.7°C
24	N	R ² = c.C ₅ H ₉	180.1°C
25	CH	R ² = c.C ₅ H ₉	202.8°C
26	N	R ² = CH(CH ₃)C(CH ₃) ₂	154.0°C
27	CH	R ² = CH(CH ₃)C(CH ₃) ₂	185.1°C
28	N	R ¹ = CH ₂ -CF ₂ -CHF ₂	184.5°C
29	N	R ² = CH(C ₂ H ₅) ₂	150.1°C
30	CH	R ² = CH(C ₂ H ₅) ₂	152.6°C
31	N	R ² = CH(CH ₃)-n-C ₃ H ₇	160.2°C
32	CH	R ² = CH(CH ₃)-n-C ₃ H ₇	141.9°C
33	N	R ¹ = CH ₂ CH ₂ F	
34	N	R ¹ = CH(CH ₃)CH ₂ F	
35	N	R ¹ = CH(CH ₂ F) ₂	
36	N	R ¹ = CH(CH ₃)C ₂ F ₅	

* : conc. = 1% in CH₂Cl₂

Pharmacological examples

The antifungal activity of the compounds of formula (I) is clearly evidenced by the data obtained in the following experiments. Said data are supplemented to illustrate the useful antifungal properties of all the compounds (I) and not to limit the invention with respect to the scope of susceptible microorganisms nor with respect to the scope of formula (I).

Example 12

a) Topical and oral treatment of vaginal candidosis in rats.

Female Wistar rats of ± 100 g body weight were used. They were ovariectomized and hysterectomized and after three weeks of recovery, 100 mg of oestradiol undecylate in sesame oil was given subcutaneously once a week for 3 consecutive weeks. The thus induced pseudo-oestrus was controlled by microscopic

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examination of vaginal smears. Food and water were left available ad libitum. The rats were infected intravaginally with $8 \cdot 10^5$ cells of *Candida albicans*, grown on Sabouraud broth for 48 hours at 37° C and diluted with saline. The date of infection varied from day +25 to day +32 after surgical intervention, depending on the appearance of signs of inducing pseudo-oestrus. The drugs under investigation were administered topically in 0.2 ml PEG 200 twice a day or orally in PEG 200 once a day for three consecutive days starting from the third day after infection. For each experiment there were placebo treated controls. The results were assessed by taking vaginal smears with sterile swabs on several days after the infection. The swabs were put into Sabouraud broth in petri-dishes and incubated for 48 hours at 37° C. When the animals were negative at the end of the experiment, i.e., if no growth of *Candida albicans* occurred, this had to be due to drug administration because placebo-treated controls were always positive. Table 3 shows the lowest effective oral dose (LED) (mg/kg bodyweight) and the lowest effective topical concentration (LEG) (%) of the drugs under investigation which were found to be active up to 7 days after the last topical administration of the drug.

Table 3

Co.Nr.	Vaginal Candidosis	
	LED (mg/kg) oral	LEG (%) topical
1	2.5	0.063
2	0.63	0.016
3	2.5	0.125
5	2.5	≤ 0.125
6	2.5	≤ 0.031
8	2.5	0.125
9	1.25	< 0.125
10	2.5	0.125
12	1.25	≤ 0.125
14	1.25	< 0.125
16	2.5	< 0.125

b) Topical and oral treatment of microsporosis in guinea pigs.

Adult Albino guinea pigs were prepared by clipping their backs and infected on the scarified skin by scratching five 3 cm long transverse cuts with *Microsporium canis* (strain RV 14314). The animals were housed individually in wire mesh cages and food and water were available ad libitum. The drugs under investigation were administered topically once a day for 14 consecutive days starting the third day after infection. The oral treatment began on the day of infection and was continued once a day for 14 consecutive days. For each experiment there were placebo treated controls. The animals were evaluated 21 days after infection by microscopic examination of the skin and by cultures on Sabouraud agar comprising a suitable bacterial antibiotic and a suitable agent to eliminate contaminating fungi.

Table 4 contains the lowest effective oral dose (LED) (mg/kg bodyweight) and lowest effective topical concentration (LEG) (%) of the drugs under investigation at which no lesions were observed and at which there was no culture growth.

Table 4

Co.Nr.	Microsporium Canis	
	LED (mg/kg) oral	LEC (%) topical
1	1.25	0.063
2	1.25	0.063
3	1.25	0.063

Composition examplesExample 13 : ORAL DROPS

500 Parts of the A.I. was dissolved in 0.5 l of 2-hydroxypropanoic acid and 1.5 l of the polyethylene glycol at 60–80 °C. After cooling to 30–40 °C there were added 35 l of polyethylene glycol and the mixture was stirred well. Then there was added a solution of 1750 parts of sodium saccharin in 2.5 l of purified water and while stirring there were added 2.5 l of cocoa flavor and polyethylene glycol q.s. to a volume of 50 l, providing an oral drop solution comprising 10 mg/ml of A.I.. The resulting solution was filled into suitable containers.

Example 14: ORAL SOLUTION

9 Parts of methyl 4-hydroxybenzoate and 1 part of propyl 4-hydroxybenzoate were dissolved in 4 l of boiling purified water. In 3 l of this solution were dissolved first 10 parts of 2,3-dihydroxybutanedioic acid and thereafter 20 parts of the A.I. The latter solution was combined with the remaining part of the former solution and 12 l 1,2,3-propanetriol and 3 l of sorbitol 70% solution were added thereto. 40 Parts of sodium saccharin were dissolved in 0.5 l of water and 2 ml of raspberry and 2 ml of gooseberry essence were added. The latter solution was combined with the former, water was added q.s. to a volume of 20 l providing an oral solution comprising 5 mg of the active ingredient per teaspoonful (5 ml). The resulting solution was filled in suitable containers.

Example 15: CAPSULES

20 Parts of the A.I., 6 parts sodium lauryl sulfate, 56 parts starch, 56 parts lactose, 0.8 parts colloidal silicon dioxide, and 1.2 parts magnesium stearate were vigorously stirred together. The resulting mixture was subsequently filled into 1000 suitable hardened gelatin capsules, comprising each 20 mg of the active ingredient.

Example 16: FILM-COATED TABLETSPreparation of tablet core

A mixture of 100 parts of the A.I., 570 parts lactose and 200 parts starch was mixed well and thereafter humidified with a solution of 5 parts sodium dodecyl sulfate and 10 parts polyvinylpyrrolidone (Kollidon-K 90 ®) in about 200 ml of water. The wet powder mixture was sieved, dried and sieved again. Then there was added 100 parts microcrystalline cellulose (Avicel ®) and 15 parts hydrogenated vegetable oil (Sterotex

©). The whole was mixed well and compressed into tablets, giving 10.000 tablets, each containing 10 mg of the active ingredient.

5 Coating

To a solution of 10 parts methyl cellulose (Methocel 60 HG®) in 75 ml of denaturated ethanol there was added a solution of 5 parts of ethyl cellulose (Ethocel 22 cps ®) in 150 ml of dichloromethane. Then there were added 75 ml of dichloromethane and 2.5 ml 1,2,3-propanetriol. 10 Parts of polyethylene glycol was molten and dissolved in 75 ml of dichloromethane. The latter solution was added to the former and then there were added 2.5 parts of magnesium octadecanoate, 5 parts of polyvinylpyrrolidone and 30 ml of concentrated colour suspension (Opaspray K-1-2109®) and the whole was homogenated. The tablet cores were coated with the thus obtained mixture in a coating apparatus.

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Example 17: INJECTABLE SOLUTION

1.8 Parts methyl 4-hydroxybenzoate and 0.2 parts propyl 4-hydroxybenzoate were dissolved in about 0.5 l of boiling water for injection. After cooling to about 50 °C there were added while stirring 4 parts lactic acid, 0.05 parts propylene glycol and 4 parts of the A.I.. The solution was cooled to room temperature and supplemented with water for injection q.s. ad 1 l, giving a solution comprising 4 mg/ml of A.I.. The solution was sterilized by filtration (U.S.P. XVII p. 811) and filled in sterile containers.

25 Example 18: SUPPOSITORIES

3 Parts A.I. was dissolved in a solution of 3 parts 2,3-dihydroxybutanedioic acid in 25 ml polyethylene glycol 400. 12 Parts surfactant (SPAN®) and triglycerides (Witepsol 555 ®) q.s. ad 300 parts were molten together. The latter mixture was mixed well with the former solution. The thus obtained mixture was poured into moulds at a temperature of 37-38 °C to form 100 suppositories each containing 30 mg/ml of the A.I.

Example 19: INJECTABLE SOLUTION

35 60 Parts of A.I. and 12 parts of benzylalcohol were mixed well and sesame oil was added q.s. ad 1 l, giving a solution comprising 60 mg/ml of A.I. The solution was sterilized and filled in sterile containers.

Example 20: 2% CREAM

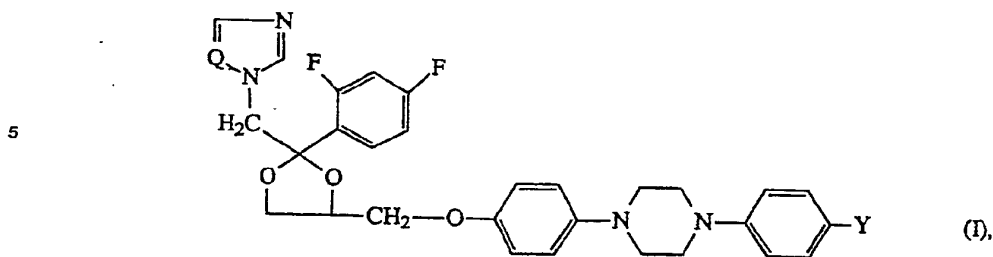
40 75 mg Stearyl alcohol, 20 mg cetyl alcohol, 20 mg sorbitan monostearate and 10 mg isopropyl myristate are introduced into a doublewall jacketed vessel and heated until the mixture has completely molten. This mixture is added to a separately prepared mixture of purified water, 200 mg propylene glycol and 15 mg polysorbate 60 having a temperature of 70 to 75 °C while using a homogenizer for liquids. The resulting emulsion is allowed to cool to below 25 °C while continuously mixing. A solution of 20 mg of A.I. of formula (I), 1 mg polysorbate 80 and 637 mg purified water and a solution of 2 mg sodium sulfite anhydrous in purified water are next added to the emulsion while continuously mixing. The cream is homogenized and filled into suitable tubes.

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Claims

1. A compound having the formula

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a pharmaceutically acceptable acid addition salt or a stereochemically isomeric form thereof, wherein
 Q is CH or N;
 Y is a radical of formula

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R¹ is C₅₋₇-cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl; and

R² is C₁₋₆alkyl, C₅₋₇-cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl.

25 2. A compound according to claim 1 wherein Q is N; and the substituents on the dioxolane nucleus have a cis configuration.

3. A compound according to claim 2 wherein R¹ and R² are mono-, di-, tri-, tetra- or pentafluoro-C₁₋₄alkyl, cyclohexyl or cyclopentyl, or R² is C₁₋₄alkyl.

30 4. A compound according to claim 3 wherein R¹ and R² are 2,2,2-trifluoroethyl, 2,2,3,3-tetrafluoropropyl, cyclopentyl, or R² is propyl, 1-methylpropyl, 2-methylpropyl or butyl.

5. A compound according to claim 1 wherein the compound is cis-2-cyclopentyl-4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-3H-1,2,4-triazol-3-one; cis-4[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(2,2,2-trifluoroethyl)-3H-1,2,4-triazol-3-one;

35 or cis-1-[4[4-[[2-(2,4-difluorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]phenyl]-1,3-dihydro-3-(1-methylpropyl)-2H-imidazol-2-one.

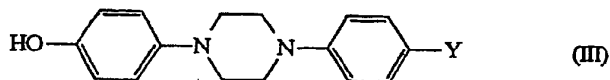
6. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and as active ingredient a therapeutically effective amount of a compound as claimed in any of claims 1 to 5.

40 7. A method of preparing a pharmaceutical composition as claimed in claim 6, characterized in that a therapeutically effective amount of a compound as claimed in any of claims 1 to 5 is intimately mixed with a pharmaceutical carrier.

8. A compound as claimed in any of claims 1 to 5 for use as a medicine.

9. A process for preparing a compound as claimed in any of claims 1 to 5, characterized by

45 a) O-alkylating a phenol of formula

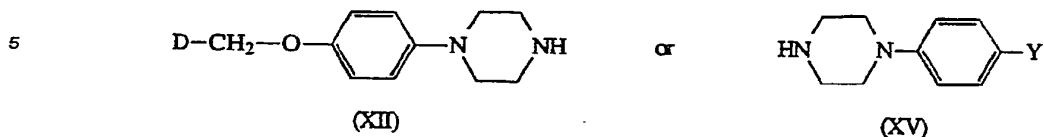


wherein Y is as defined in claim 1, with an alkylating reagent of formula D-CH₂-W (II), wherein W is a reactive leaving group, in a reaction-inert solvent in the presence of a base;

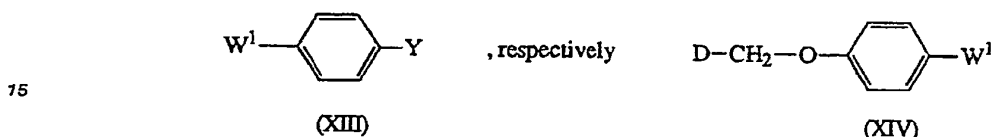
b) an acetalation reaction of a ketone of formula

55

wherein Y is as defined in claim 1, in a polar reaction-inert solvent;
 e) N-alkylating a piperazine of formula

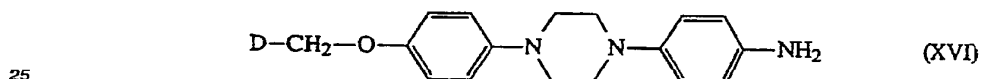


10 wherein Y is as defined in claim 1, with a benzene derivative of formula

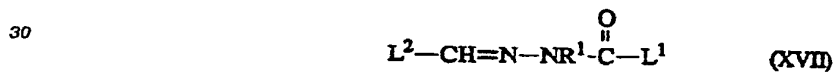


20 wherein W¹ represents a reactive leaving group and Y is as defined in claim 1, in a reaction-inert solvent in the presence of a base;

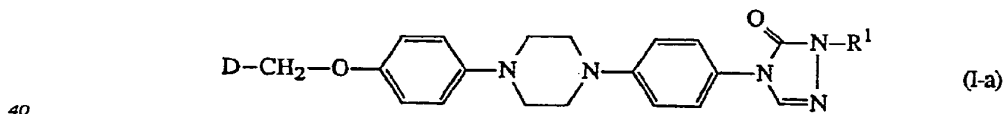
f) cyclizing a benzenamine of formula



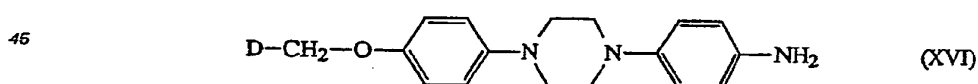
with a reagent of formula



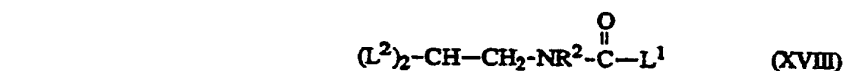
35 wherein L¹ and L² both represent a reactive leaving group and R¹ is as defined in claim 1, in a reaction-inert solvent thus yielding a compound of formula



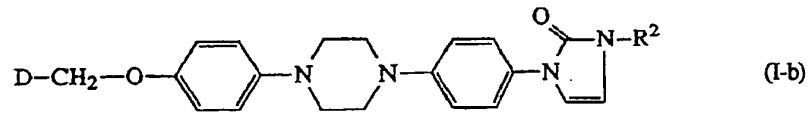
g) cyclizing a benzenamine of formula



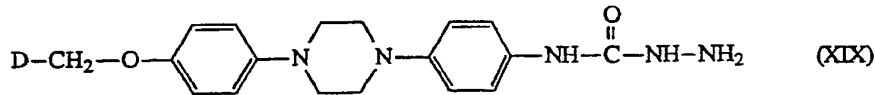
with a reagent of formula



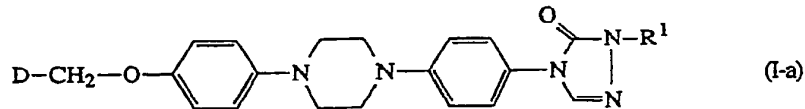
55 wherein L¹ and L² both represent a reactive leaving group and R² is as defined in claim 1, in a reaction-inert solvent thus yielding a compound of formula



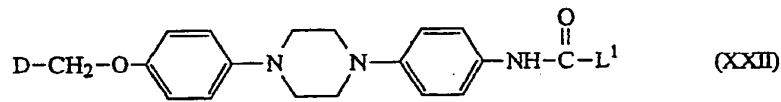
h) cyclizing an intermediate of formula



15 with an amidine of formula $HC(=NH)NH_2$ (XX) or an acid addition salt thereof, and N-alkylating the thus obtained intermediate with a reagent of formula R^1-W (XXI) wherein W is a reactive leaving group and R^1 is as defined in claim 1, in a reaction-inert solvent, thus yielding a compound of formula

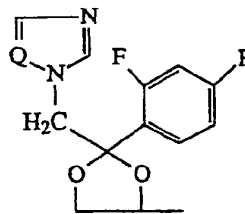


i) cyclizing an intermediate of formula



30 with a reagent of formula $(L^2)_2-CH-CH_2-NHR^2$ (XXIII) wherein L^1 and L^2 are reactive leaving groups and R^2 is as defined in claim 1, in a reaction-inert solvent in the presence of an acid; wherein

35 D is



40 and Q is as defined in claim 1; and

45 if further desired, converting the compounds of formula (I) into a salt form by treatment with a pharmaceutical acceptable acid; or conversely converting the salt into the free base with alkali; and/or preparing stereochemically isomeric forms thereof.

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(12) **EUROPEAN PATENT APPLICATION**

(21) Application number: 90201447.1

(51) Int. Cl.⁵: C07D 405/14, A61K 31/495

(22) Date of filing: 01.06.90

(30) Priority: 09.06.89 US 363795

(71) Applicant: **JANSSEN PHARMACEUTICA N.V.**
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B-2340 Beerse(BE)

(43) Date of publication of application:
 19.12.90 Bulletin 90/51

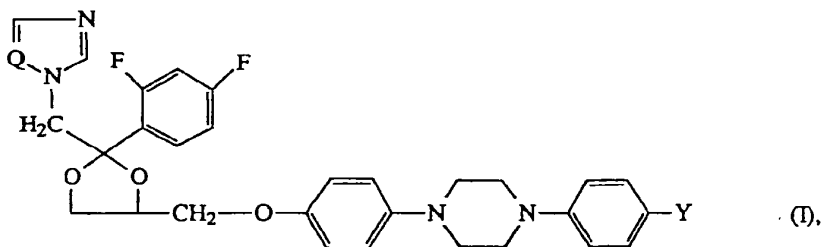
(72) Inventor: **Heeres, Jan**
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 Inventor: **Backx, Leo Jacobus Jozef**
Broekstraat 92
B-2370 Arendonk(BE)

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

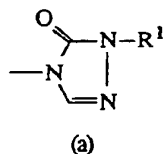
(88) Date of deferred publication of the search report:
 02.01.92 Bulletin 92/01

(54) **Antifungal**
 4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-azolylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]-phenyl]triazolones and imidazolones.

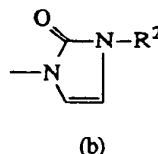
(57) 4-[4-[4-[4-[[2-(2,4-difluorophenyl)-2-(1H-azolylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-1-piperazinyl]-phenyl]triazolones and imidazolones of formula



wherein
 Q is CH or N;
 Y is a radical of formula



or



R¹ is C₅-7-cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl; and

EP 0 402 989 A3

R² is C₁₋₆alkyl, C₅₋₇cycloalkyl or mono-, di-, tri-, tetra- or pentahaloC₁₋₄alkyl, the acid addition salts and stereoisomeric forms thereof; said compounds having antifungal properties. Pharmaceutical compositions containing such compounds as an active ingredient; methods of preparing said compounds and pharmaceutical compositions.



European
Patent Office

EUROPEAN SEARCH
REPORT

Application Number

EP 90 20 1447

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
Y	CHEMICAL ABSTRACTS, vol. 106, no. 9, 2nd March 1987, page 614, abstract no. 67318k, Columbus, Ohio, US; & ES-A-539 139 (FORDONAL S.A.) 16-11-1985 * Abstract * - - - -	1,6-9	C 07 D 405/14 A 61 K 31/495
Y	EP-A-0 283 992 (JANSSEN PHARM.) * Claims * & US-A-4 916 134 (Cat. D) - - - -	1,6-9	
Y	EP-A-0 228 125 (JANSSEN PHARM.) * Claims * & US-A-4 791 111 (Cat. D) - - - -	1,6-9	
A	EP-A-0 118 138 (JANSSEN PHARM.) * Claims * & US-A-4 735 942 (Cat. D) - - - -	1,6-9	
A	EP-A-0 006 711 (JANSSEN PHARM.) * Claims * & US-A-4 267 179 (Cat. D) - - - -	1,6-9	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 D 233/00 C 07 D 249/00 C 07 D 405/00 A 61 K 31/00
The present search report has been drawn up for all claims			
Place of search		Date of completion of search	Examiner
The Hague		14 October 91	CHOULY J.
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X: particularly relevant if taken alone Y: particularly relevant if combined with another document of the same category A: technological background O: non-written disclosure P: intermediate document T: theory or principle underlying the invention</p> <p>E: earlier patent document, but published on, or after the filing date D: document cited in the application L: document cited for other reasons</p> <p>&: member of the same patent family, corresponding document</p>			

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EPW

Patentitioner's Docket No. 700938-52220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika

Application No.: 10/685,266 Group No.: 1651
Filed: 10/14/2003 Examiner: To be assigned
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

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4. Supplemental Information Disclosure Statement with PCT/SB/08a and b (4 pp);
5. COPY- References B1-B2 and C1-C2; and
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on November 17, 2004.

Linda M. Ginsberg
Signature of person mailing paper



PTO/SB/21 (09-04)

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TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	10/685,266
	Filing Date	October 14, 2003
	First Named Inventor	Yoshiyuki Tatsumi
	Art Unit	1651
	Examiner Name	To be assigned
Total Number of Pages in This Submission	Attorney Docket Number	700938-52220-DIV

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form	<input type="checkbox"/> Drawing(s)	<input type="checkbox"/> After Allowance Communication to TC
<input type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
<input type="checkbox"/> Amendment/Reply	<input type="checkbox"/> Petition	<input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
<input type="checkbox"/> After Final	<input type="checkbox"/> Petition to Convert to a Provisional Application	<input type="checkbox"/> Proprietary Information
<input type="checkbox"/> Affidavits/declaration(s)	<input type="checkbox"/> Power of Attorney, Revocation	<input type="checkbox"/> Status Letter
<input type="checkbox"/> Extension of Time Request	<input type="checkbox"/> Change of Correspondence Address	<input checked="" type="checkbox"/> Other Enclosure(s) (please identify below):
<input type="checkbox"/> Express Abandonment Request	<input type="checkbox"/> Terminal Disclaimer	Certificate of Mailing; COPY-European Search Report; COPY-References B1-B2 and C1-C2; and Return Receipt Postcard
<input checked="" type="checkbox"/> Information Disclosure Statement	<input type="checkbox"/> Request for Refund	
<input type="checkbox"/> Certified Copy of Priority Document(s)	<input type="checkbox"/> CD, Number of CD(s) _____	
<input type="checkbox"/> Reply to Missing Parts/Incomplete Application	<input type="checkbox"/> Landscape Table on CD	
<input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	Remarks The Commissioner is hereby authorized to charge any fee deficiency or credit any overpayment to the Nixon Peabody LLP Deposit Account No. 50-0850.	

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT		
Firm Name	Nixon Peabody LLP, 100 Summer Street, Boston, MA 02110	
Signature		
Printed name	Ronald I. Eisenstein/Nicole L.M. Valtz	
Date	Nov. 16, 2004	Reg. No. 30,628/47,150

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Signature		
Typed or printed name	Linda M. Ginsberg	Date 11/17/2004

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Practitioner's Docket No. 700938-52220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika

Application No.: 10/685,266 Group No.: 1651
Filed: 10/14/2003 Examiner: To be assigned
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

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Date: November 17 2004
Linda M. Ginsberg

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir/Madam:

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

In accordance with the provisions of 37 C.F.R. §1.56, 1.97, and 1.98, Applicants wish to bring to the Examiner's attention the following references, References B1-B2 and C1-C2 cited in the attached Form PTO/SB/08a and b.

REMARKS

In accordance with the provisions of 37 C.F.R. §1.97, this statement is being filed:

- (1) within three (3) months of the Filing Date or before the mailing date of the First Office Action on the merits; or
(2) within three months of the mailing date of the PCT International Search Report; or

- (3) after the period defined in (1) but before the mailing date of a **Final Rejection** or **Notice of Allowance**, and the requisite Certification or fee under Rule 1.17(p), namely **\$180.00**, is included herein; or
- (4) after the mailing date of a **Final Rejection** or **Notice of Allowance** but before the payment of the **Issue Fee**, and the requisite Certification, petition, and petition fee are included herein.

A copy of the European Search Report is enclosed herewith.

It is respectfully requested that each of the documents shown on the attached form(s) PTO/SB/08a be made of record in this application.

Copies of these documents (CHECK ONE):

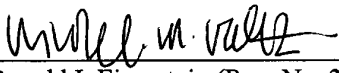
- are enclosed herewith that have not been previously submitted; or
- have been cited in the parent application, and are thus not being resubmitted herein.

FEE AUTHORIZATION

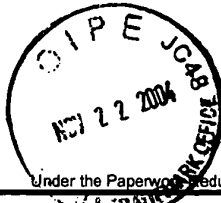
The Commissioner is authorized to charge fee deficiencies or credit overpayments associated with this submission to the NIXON PEABODY LLP Deposit Account No. 50-0850.

Date: November 16, 2004

Respectfully submitted,



Ronald I. Eisenstein (Reg. No. 30,628)
Nicole L.M. Valtz (Reg. No. 47,150)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110-2131
(617) 345-6054



PTO/SB/08a (08-03)
 Approved for use through 07/31/2006. OMB 0651-0031
 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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<p style="text-align: center;">Substitute for form 1449A/PTO</p> <h2 style="text-align: center;">INFORMATION DISCLOSURE STATEMENT BY APPLICANT</h2> <p style="text-align: center;"><i>(Use as many sheets as necessary)</i></p> <p>Sheet <u>1</u> of <u>1</u></p>	<p style="text-align: center;">Complete if Known</p> <table border="1" style="width:100%; border-collapse: collapse;"> <tr><td>Application Number</td><td>10/685,266</td></tr> <tr><td>Filing Date</td><td>10/14/2003</td></tr> <tr><td>First Named Inventor</td><td>Yoshiyuki Tatsumi</td></tr> <tr><td>Art Unit</td><td>1651</td></tr> <tr><td>Examiner Name</td><td>To be assigned</td></tr> <tr><td>Attorney Docket Number</td><td>700938-52220-DIV</td></tr> </table>	Application Number	10/685,266	Filing Date	10/14/2003	First Named Inventor	Yoshiyuki Tatsumi	Art Unit	1651	Examiner Name	To be assigned	Attorney Docket Number	700938-52220-DIV
Application Number	10/685,266												
Filing Date	10/14/2003												
First Named Inventor	Yoshiyuki Tatsumi												
Art Unit	1651												
Examiner Name	To be assigned												
Attorney Docket Number	700938-52220-DIV												

U. S. PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Document Number		Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)				
		US-				
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		US-				
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FOREIGN PATENT DOCUMENTS								
Examiner Initials*	Cite No.	Foreign Patent Document			Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
		Country Code ³	Number ⁴	Kind Code ⁵ (if known)				
	B1	WO	99/39680	A1	08/12/1999	MACROCHEM CORPORATION		
	B2	EP	0 402 989	A2	12/19/1990	JANSSEN PHARMACEUTICA N.V.		

Examiner Signature	Date Considered
--------------------	-----------------

*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. ¹ Applicant's unique citation designation number (optional). ² See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. ³ Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). ⁴ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁵ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁶ Applicant is to place a check mark here if English language translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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APPLICATION NUMBER	PATENT NUMBER	GROUP ART UNIT	FILE WRAPPER LOCATION
10/685,266		1651	

Correspondence Address / Fee Address Change

The following fields have been set to Customer Number 53143 on 05/23/2005

- Correspondence Address
- Maintenance Fee Address

The address of record for Customer Number 53143 is:

RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON,MA 02110



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UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV	4026

53143 7590 03/10/2006

RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

EXAMINER

SRIVASTAVA, KAILASH C

ART UNIT	PAPER NUMBER
1655	

1655

DATE MAILED: 03/10/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/685,266	Applicant(s) TATSUMI ET AL.	
	Examiner Dr. Kailash C. Srivastava	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) Responsive to communication(s) filed on 14 October 2003.
- 2a) This action is **FINAL**. 2b) This action is non-final.
- 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) Claim(s) 1 and 18-20 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) Claim(s) _____ is/are allowed.
- 6) Claim(s) _____ is/are rejected.
- 7) Claim(s) _____ is/are objected to.
- 8) Claim(s) 1 and 18-20 are subject to restriction and/or election requirement.

Application Papers

- 9) The specification is objected to by the Examiner.
- 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) All b) Some * c) None of:
1. Certified copies of the priority documents have been received.
2. Certified copies of the priority documents have been received in Application No. _____.
3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

1. Applicants' Preliminary amendment filed 14 October 2003 is acknowledged and entered.
2. Applicants to note that the correct Serial Number of your Application under prosecution at the United States Patent and Trademark Office (i.e., USPTO) is 10/685,266; not Divisional of 10/031,929 as recited in the Preliminary Amendment filed 14 October 2003. Please ensure that the correct U.S. Serial Number (i.e., 10/685,266) for this application is cited in all future correspondence with this Office.
3. Your application under prosecution at the USPTO is assigned to Dr. Kailash C. Srivastava in Art Unit 1655. To aid in correlating any papers for this application (i.e., USSN 10/685,266), all further correspondence regarding this application should be directed to Examiner Kailash C. Srivastava in Art Unit 1655.

Claims Status

4. Claims 2-17 have been cancelled.
5. Claims 18-20 have been added.
6. Claims 1 and 18-20 are pending.

Restriction/Election

7. Restriction to one of the following inventions is required under 35 U.S.C. 121:
 - Group I, consisting of claim 1 drawn to a method to identify a pathogenic microorganism, classified under Class 436, subclass 63, for example.
 - Group II, consisting of claims 18-20, drawn to a method to treat an individual having onychomycosis via administering an antifungal compound to said individual, classified under Class 424, subclass 404, for example.

Inventions are Independent and Distinct

8. The inventions are distinct, each from the other because of the following reasons:

Inventions in Groups I-II are unrelated to each other because each one of them is

directed to different inventions that are not connected in design, components, operation and/or effect. These inventions are independent since they are not disclosed as capable of use together. They have different modes of operation, they have different functions, and/or they have different effects. One would not have to practice the various methods at the same time to practice just one method alone (MPEP § 806.04, MPEP § 808.01). In the instant case, for example invention recited in claim encompassed in Group I is directed to a method to identify a microorganism whereas those in Group II is to treat an individual having onychomycosis. Thus, invention in Group I is a diagnostic method, while the method in Group II invention is a treatment method. Therefore, the methods claimed in inventions I-II, encompassing Claims 1 and 18-20 respectively will not be practiced together.

The inventions discussed above are independent and distinct, each from the other. They have acquired a separate status in the art as a separate subject for inventive effect and require independent searches. The search for each one of the above inventions is not coextensive particularly with regard to the literature search. Further, a reference that would anticipate the invention of one group would not necessarily anticipate or even make obvious another group. Finally, the consideration for patentability is different in each case. Thus, it would be an undue burden to examine all of the above inventions in one application.

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification (i.e., Class and subclass), and their recognized diverse subject matter, they would illicit an undue burden on the examiner to search and examine all the inventions in groups I- II in one single application. Furthermore, the criteria for patentability may not be same for each of the recited groups and what may be applicable for one group may not at all be applicable to other group. Thus, restriction for examination purposes as indicated is proper.

9. Applicants are advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement is traversed (37 CFR §1.143). An argument that a claim is allowable or that all claims are generic is considered non-responsive unless accompanied by an election.

Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which are written in dependent form or otherwise include all the limitations of an allowed generic claim as provided by 37 CFR §1.141. If claims are added after


the election, applicant must indicate which claims are readable upon the elected claims/ species [MPEP § 809.02(a)].

10. Applicants are reminded that upon the cancellation of claims to a non-elected invention and species, the inventorship must be amended in compliance with 37 CFR §1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR §1.48(b) and by the fee required under 37 CFR §1.17(I).

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Kailash C. Srivastava whose telephone number is (571) 272-0923. The examiner can normally be reached on Monday to Thursday from 7:30 A.M. to 6:00 P.M. (Eastern Standard or Daylight Savings Time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Terry McKelvey, can be reached on (571)-272-0775 Monday through Friday 8:30 A.M. to 5:00 P.M. The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding may be obtained from the Patent Application Information Retrieval (i.e., PAIR) system. Status information for the published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (i.e., EBC) at: (866)-217-9197 (toll-free). Alternatively, status inquiries should be directed to the receptionist whose telephone number is (703) 308-0196.


Kailash C. Srivastava, Ph.D.
Patent Examiner
Art Unit 1655
(571) 272-0923



RALPH GITOMER
PRIMARY EXAMINER
GROUP 1200

March 5, 2006



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
 United States Patent and Trademark Office
 Address: COMMISSIONER FOR PATENTS
 P.O. Box 1450
 Alexandria, Virginia 22313-1450
 www.uspto.gov



Bib Data Sheet

CONFIRMATION NO. 4026

SERIAL NUMBER 10/685,266	FILING OR 371(c) DATE 10/14/2003	CLASS 435	GROUP ART UNIT 1651 1655	ATTORNEY DOCKET NO. 700938-052220-DIV
APPLICANTS				
Yoshiyuki Tatsumi, Otsu-shi, JAPAN; ✓ Mamoru Yokoo, Otsu-shi, JAPAN; ✓ Kosho Nakamura, Moriyama-shi, JAPAN; ✓ Tadashi Arika, Osaka, JAPAN; ✓				
** CONTINUING DATA ***** This application is a DIV of 10/031,929 01/25/2002 ABN ✓ which is a 371 of PCT/JP00/04617 07/11/2000 ✓				
** FOREIGN APPLICATIONS ***** JAPAN 214369/1999 07/28/1999 ✓				
Foreign Priority claimed <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	35 USC 119 (a-d) conditions met <input type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> Met after Allowance	STATE OR COUNTRY JAPAN	SHEETS DRAWING 4	TOTAL CLAIMS 4
Verified and Acknowledged	Examiner's Signature _____ Initials _____	INDEPENDENT CLAIMS 2		
ADDRESS				
Ronald I. Eisenstein Nixon Peabody LLP 100 Summer Street Boston, MA 02110				
TITLE				
Method for detecting pathogenic microorganism and antimicrobial agent, method for evaluating effect of antimicrobial agent, and antimicrobial agent				
FILING FEE RECEIVED 770	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:	<input type="checkbox"/> All Fees <input type="checkbox"/> 1.16 Fees (Filing) <input type="checkbox"/> 1.17 Fees (Processing Ext. of time) <input type="checkbox"/> 1.18 Fees (Issue) <input type="checkbox"/> Other <input type="checkbox"/> Credit		

Index of Claims



Application/Control No.

10/685,266

Examiner

Dr. Kailash C. Srivastava

Applicant(s)/Patent under Reexamination

TATSUMI ET AL.

Art Unit

1655

√	Rejected
=	Allowed

-	(Through numeral) Cancelled
+	Restricted

N	Non-Elected
I	Interference

A	Appeal
O	Objected

Claim		Date	
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PATENT

Practitioner's Docket No. 700938-52220-DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika

Application No.:	10/685,266	Group No.:	1655
Filed:	10/14/2003	Examiner:	SRIVASTAVA, Kailash C.
Confirmation No.:	4026	Customer No.:	53143
For:	METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT		

RESTRICTION REQUIREMENT

MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

In response to the Restriction Requirement dated March 10, 2006, Applicants elect Group II, claims 18-20, drawn to a method to treat an individual having onychomycosis via administering an antifungal compound to said individual.

Remarks begin on page 2 of this paper.

BOS1581074.1

Application No. 10/685,266
Restriction Requirement dated March 10, 2006
Response to Restriction Requirement mailed April 6, 2006

REMARKS

Applicants elect Group II, claims 18-20, drawn to a method to treat an individual having onychomycosis via administering an antifungal compound to said individual.

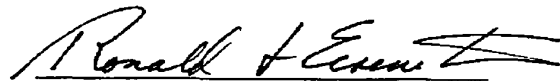
Applicants reserve the right to file a continuing application or take such other appropriate action as deemed necessary to protect the non-elected inventions. Applicants do not hereby abandon or waive any rights in the non-elected inventions.

In view of the foregoing, applicant respectfully submits that all claims are in condition for allowance. Early and favorable action is requested.

In the event that any additional fees are required, the PTO is authorized to charge our deposit account No. 50-0850.

Respectfully submitted,

Date: April 6, 2006



Ronald I. Eisenstein (Reg. No.: 30,628)
Leena H. Karttunen (Reg. No. L0207)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110
(617) 345-6054

APR 06 2006

Nixon Peabody LLP
Attorneys at Law

100 Summer Street
Boston, MA 02110-2131
(617) 345-1000

Fax: (617) 345-1300

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From: Ronald I. Eisenstein Leena H. Karttunen	Date: April 6, 2006	No. of Pages: 6 (including this page)	
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Practitioner's Docket No. 700938-52220-DIV **PATENT**
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika
 Application No.: 10/685,266 Group No.: 1655
 Filed: 10/14/2003 Examiner: SRIVASTAVA, Kailash C.
 Confirmation No.: 4026 Customer No.: 53143
 For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT,
 METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

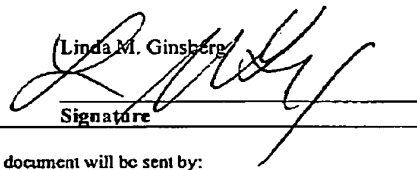
MAIL STOP AMENDMENT
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P.O. Box 1450
Alexandria, VA 22313-1450

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I hereby certify that the following papers are being facsimile transmitted to the Patent and Trademark Office at 571-273-8300 on the date shown below:

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2. COPY-Certificate of Limited Recognition (1 pg.);
3. Transmittal Form (1 pg.); and
4. Response to Restriction Requirement (2 pp.).

April 6, 2006

Linda M. Ginsberg

 Signature

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 Hartford
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 Washington (P)

BOS1583246.1

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APR 06 2006

PATENT

Practitioner's Docket No. 700938-52220-DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika

Application No.:	10/685,266	Group No.:	1655
Filed:	10/14/2003	Examiner:	SRIVASTAVA, Kailash C.
Confirmation No.:	4026	Customer No.:	53143
For:	METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT		

**MAIL STOP AMENDMENT
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450**

CERTIFICATION OF FACSIMILE TRANSMISSION

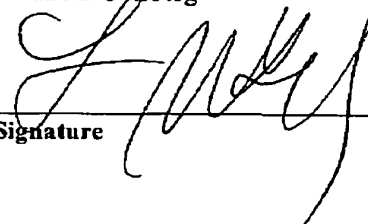
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3. Transmittal Form (1 pg.); and
4. Response to Restriction Requirement (2 pp.).

April 6, 2006
Date

Linda M. Ginsberg

Signature



**BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE
UNITED STATES PATENT AND TRADEMARK OFFICE**

LIMITED RECOGNITION UNDER 37 CFR § 11.9(b)

Leena H. Karttunen is hereby given limited recognition under 37 CFR §11.9(b) as an employee of Nixon Peabody LLP, to prepare and prosecute patent applications wherein the patent applicant is the client of Nixon Peabody LLP and an attorney or agent of record in the applications is a registered practitioner who is a member of Nixon Peabody LLP. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Leena H. Karttunen ceases to lawfully reside in the United States, (ii) Leena H. Karttunen's employment with Nixon Peabody LLP ceases or is terminated, or (iii) Leena H. Karttunen ceases to remain or reside in the United States on an H-1B visa.

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

Limited Recognition No. L0207
Expires: February 11, 2007



Harry I. Moatz
Director of Enrollment and Discipline

PTO/SB/21 (09-04)

Approved for use through 07/31/2006. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	10/685,266	RECEIVED CENTRAL FAX CENTER APR 06 2006
	Filing Date	October 14, 2003	
	First Named Inventor	Yoshiyuki Tatsumi	
	Art Unit	1655	
	Examiner Name	SRIVASTAVA, Kailash C.	
	Attorney Docket Number	700938-52220-DIV	
Total Number of Pages in This Submission		7	

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form	<input type="checkbox"/> Drawing(s)	<input type="checkbox"/> After Allowance Communication to TC
<input type="checkbox"/> Fee Attached	<input type="checkbox"/> Licensing-related Papers	<input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences
<input checked="" type="checkbox"/> Amendment/Reply	<input type="checkbox"/> Petition	<input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief)
<input type="checkbox"/> After Final	<input type="checkbox"/> Petition to Convert to a Provisional Application	<input type="checkbox"/> Proprietary Information
<input type="checkbox"/> Affidavits/declaration(s)	<input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address	<input type="checkbox"/> Status Letter
<input type="checkbox"/> Extension of Time Request	<input type="checkbox"/> Terminal Disclaimer	<input checked="" type="checkbox"/> Other Enclosure(s) (please identify below):
<input type="checkbox"/> Express Abandonment Request	<input type="checkbox"/> Request for Refund	Certificate of Facsimile; and COPY-Certificate of Limited Recognition
<input type="checkbox"/> Information Disclosure Statement	<input type="checkbox"/> CD. Number of CD(s) _____	
<input type="checkbox"/> Certified Copy of Priority Document(s)	<input type="checkbox"/> Landscape Table on CD	
<input type="checkbox"/> Reply to Missing Parts/ Incomplete Application	Remarks	
<input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	The Commissioner is hereby authorized to charge any fee deficiency or credit any overpayment to the Nixon Peabody LLP Deposit Account No. 50-0850.	

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	Nixon Peabody LLP		
Signature			
Printed name	Ronald I. Eisenstein/Leena H. Karttunen		
Date	4/6/06	Reg. No.	30,628/L0207

CERTIFICATE OF TRANSMISSION/MAILING			
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Signature			
Typed or printed name	Linda M. Ginsberg	Date	April 6, 2006

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Case Creation Option

Case "10685266us20060611" already exists. Please overwrite it or cancel the operation.

The Contents of Case "10685266us20060611"

Qnum	Query	DB Name	Thesaurus	Operator	Plural
Q1	onychomycosis	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q2	Taenia pedia	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q3	Tenia pedis or Trichophyton	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q4	antimicrobial or antifungal or fungistasis or fungicid\$6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q5	methylen piperidine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q6	Q3 or Q5	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q7	Q4 or Q6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q8	Q1 and Q7	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q9	Q4 and Q8	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q10	Q5 and Q9	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q11	Q5 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q12	Q5 and Q4	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q13	435/32.ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q14	514/326.ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q15	514/397.ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q16	514/212.ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q17	Q13 or Q14	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
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Q20	Q5 near5 Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q21	Q5 and Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q22	Q1 near5 Q3	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q23	Q4 near5 Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q24	Q4 and Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q25	Q18 and Q24	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q26	("RN-164650-44-6")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q27	("2,4-difluorophenyl-methylenepiperidino-triazolyl-butan-2-ol")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q28	axolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q29	azolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q30	Q29 and Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q31	Q4 and Q30	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q32	Q31 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV	4026

53143 7590 06/14/2006

RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

EXAMINER

SRIVASTAVA, KAILASH C

ART UNIT	PAPER NUMBER
1655	

1655

DATE MAILED: 06/14/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 10/685,266	Applicant(s) TATSUMI ET AL.	
	Examiner Dr. Kailash C. Srivastava	Art Unit 1655	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) Responsive to communication(s) filed on 06 April 2006.
- 2a) This action is **FINAL**. 2b) This action is non-final.
- 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) Claim(s) 1 and 18-20 is/are pending in the application.
4a) Of the above claim(s) 1 is/are withdrawn from consideration.
- 5) Claim(s) _____ is/are allowed.
- 6) Claim(s) 18-20 is/are rejected.
- 7) Claim(s) _____ is/are objected to.
- 8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) The specification is objected to by the Examiner.
- 10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) All b) Some * c) None of:
1. Certified copies of the priority documents have been received.
2. Certified copies of the priority documents have been received in Application No. 10/031,929.
3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)
Paper No(s)/Mail Date <u>10/14/03&11.22.04</u> . | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

1. Applicants' response filed 06 April 2006 to Election requirement in Office Action mailed 10 March 2006 is acknowledged and entered.
2. For the record, Examiner re-iterates that the correct Serial Number of your Application under prosecution at the United States Patent and Trademark Office (i.e., USPTO) is 10/685,266; not Divisional of 10/031,929 as recited in Information Disclosure Statement filed 14 October 2003. Please ensure that the correct U.S. Serial Number (i.e., 10/685,266) for this application is cited in all future correspondence with this Office.
3. Also for the record, the Examiner re-iterates that your application under prosecution at the USPTO is assigned to Dr. Kailash C. Srivastava in Art Unit 1655 and not in 1651 as indicated in the Information Disclosure Statements filed 14 October 2003 and 22 November 2004 respectively. To aid in correlating any papers for this application (i.e., USSN 10/685,266), all further correspondence regarding this application should be directed to Examiner Kailash C. Srivastava in Art Unit 1655.

Claims Status

4. Claims 1 and 18-20 are pending.

Restriction/Election

5. Applicants' election without traverse of Group II, Claims 18-20 filed 06 April 2006 to Election requirement in Office Action mailed 10 March 2006 is acknowledged and entered. Since the election is made without traverse, the restriction requirement is deemed proper and is made FINAL.

Accordingly, Claim 1 is withdrawn from further consideration as being directed to a non-elected invention. See 37 CFR §1.142(b) and MPEP §821.03. Examiner suggests that to expedite prosecution, the non-elected claim 1 cited *supra* be canceled in response to this Office action.

6. Claims 18-20 are examined on merits.

Priority

7. Applicants' claim for foreign priority under 35 U.S.C. §119 (a-d) to PCT/JP00/04617 filed 11 July 2000 and under 35 U.S.C. §120 to U.S. Non-Provisional application 10/031, 929 filed 25 January 2002 that has since been abandoned is acknowledged.

Information Disclosure Statement

8. Applicants' Information Disclosure Statements (i.e., IDSs) filed J14 October 2003 and 22 November 2004 respectively have been made of record and considered.

Objection To Specification

9. The specification is objected to because the priority data filed at Page 1 of the amended specification in Applicants' Preliminary amendment dated 14 October 2003 is incorrect. Applicants should appropriately claim this priority as follows:

"This application is a divisional under 35 U.S.C. §120 to U.S. Non-Provisional application 10/031, 929 filed 25 January 2002 that has since been abandoned and said non-provisional U.S. Application was a 371 of PCT/JP00/04617 filed 1 July 2000, which claimed priority to Japan application Serial No.11/214369 filed 28 July 1999."

OBJECTION TO TITLE

10. The title of the invention is not descriptive. A new title is required that clearly indicates the invention to which the claims are directed, which is a method to treat onychomycosis in an individual by administering an antifungal agent. Appropriate correction is required.

Objection To Claims

11. Claim 18 is objected to because of following reasons:

- Claim 18 is objected because of the phrase, "treating subject having onychomycosis comprising administering an effective amount of an antifungal compound having a group represented". This phrase is grammatically improper. Appropriate correction stating said claim in clear and succinct language is required. Appropriate correction is required.

All other claims depend directly or indirectly from the objected claim 18, and are, therefore, also objected for the reasons set forth above.

Double Patenting

12. The non-statutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. See *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Long*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982);

In re Vogel, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and, *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR §1.321(c) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent is shown to be commonly owned with this application. See 37 C.F.R. §1.130(b).

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 C.F.R. §3.73(b).

13. Claims 18-20 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 9-12 of U.S. Patent No. 5,620,994. Although, conflicting claims are not identical, they are not patentably distinct from each other because claims 9-12 of referenced patent are drawn to a fungicidal composition comprising essentially the same composition as claimed in the cited claims of instant application. The only difference between Claims 18 and 19 of the instant application and prior patent is that in the instant application, the formula II of Claim 19 is claimed in a more generic structure of the antifungal agent as presented in Claim 18. The formula presented in instantly claimed Claim 19 is exactly the same as that in Claim of above-cited patent and Claims 9-12 of said patent claim a process to treat mycosis which again is a generic terminology for "onychomycosis" via administering the compound of formula in Claim 1 of above-referred patent.

14. Claims 18-20 directed to the same invention as that of claims 9-12 of commonly assigned U.S. Patent 5,620,994. The issue of priority under 35 U.S.C. §102(g) and possibly 35 U.S.C. §102(f) of this single invention must be resolved.

Since the U.S. Patent and Trademark Office normally will not institute an interference between applications or a patent and an application of common ownership (see MPEP Chapter 2300), the assignee is required to state which entity is the prior inventor of the conflicting subject matter. A terminal disclaimer has no effect in this situation since the basis for refusing more than one patent is priority of invention under 35 U.S.C. §§102(f) or (g) and not an extension of monopoly.

Failure to comply with this requirement will result in a holding of abandonment of this application.

15. Claims 18-20 directed to an invention not patentably distinct from claims 9-12 of commonly assigned U.S. Patent 5,620,994. The U.S. Patent and Trademark Office normally will not institute an interference between applications or a patent and an application of common ownership (see MPEP Chapter 2300). Commonly assigned U.S. Patent 5,620,994, discussed above, would form the basis for a rejection of the noted claims under 35 U.S.C. §103(a) if the commonly assigned case qualifies as prior art

under 35 U.S.C. §§102(e), (f) or (g) and the conflicting inventions were not commonly owned at the time the invention in this application was made. In order for the examiner to resolve this issue, the assignee can, under 35 U.S.C. §103(c) and 37 C.F.R. §1.78(c), either show that the conflicting inventions were commonly owned at the time the invention in this application was made, or name the prior inventor of the conflicting subject matter.

A showing that the inventions were commonly owned at the time the invention in this application was made will preclude a rejection under 35 U.S.C. §103(a) based upon the commonly assigned case as a reference under 35 U.S.C. § 102(f) or (g), or 35 U.S.C. §102(e) for applications pending on or after December 10, 2004.

Claim Rejections – 35 U.S.C. § 102

16. The following is a quotation of the appropriate paragraphs of 35 U.S.C. §102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

17. Claims 18-20 are rejected under 35 U.S.C. §102(b) as anticipated by Naito et al. (U.S. Patent 5, 620, 994 or 5,716,969).

Naito et al. teach a fungicidal composition comprising the same compounds, having the same structure (See U.S. Patent 5, 620, 994 Column 17, Line 30 to Column 18, Line19; Column 18, Lines 21-36) as compounds of formula I and II of the instantly claimed invention. Essentially, compound recited as having a group represented by the formula I in instantly claimed claim 18 is a part of formula II compound (Column 12, Lines 1-10 and Claim 1). Even though the antifungal/fungicidal composition taught in the Examiner-cited prior art do not refer to, "therapeutic agent for onychomycosis", to artisan of ordinary skill the claims remain anticipated by the examiner cited prior art because the functional intended use of a composition does not materially change a composition and is accordingly, not given any patentable weight.

Therefore, the reference deems to anticipate claims 18-20.


Conclusion

18. For aforementioned reasons, no Claims are allowed.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Kailash C. Srivastava whose telephone number is (571) 272-0923. The examiner can normally be reached on Monday to Thursday from 7:30 A.M. to 6:00 P.M. (Eastern Standard or Daylight Savings Time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Terry McKelvey, can be reached on (571)-272-0775 Monday through Friday 8:30 A.M. to 5:00 P.M. The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding may be obtained from the Patent Application Information Retrieval (i.e., PAIR) system. Status information for the published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (i.e., EBC) at: (866)-217-9197 (toll-free). Alternatively, status inquiries should be directed to the receptionist whose telephone number is (703) 308-0196.


Kailash C. Srivastava, Ph.D.
Patent Examiner
Art Unit 1655
(571) 272-0923



RALPH GITOMER
PRIMARY EXAMINER
GROUP 1200

June 11, 2006



PTO/SB08b (08-03)
 Approved for use through 06/30/2008. OMB 0651-0031
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Substitute for form 1449B/PTO		Complete if Known	
INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Application Number	10/685,266
		Filing Date	10/14/2003
		First Named Inventor	Yoshiyuki Tatsumi
		Art Unit	1051 1655
		Examiner Name	To be assigned Dr. K.C. Srivastava
		Attorney Docket Number	700938-52220-DIV
Sheet	1	of	1

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
KS	C1	KITAZAKI, TOMOYUKI ET AL., Chem. Pharm. Bull, Vol. 44 (No. 2), p. 314-327, (February, 1996).	X
KS	C2	OGURA, HIRONOBU ET AL., Chem. Pharm. Bull, Vol. 47 (No. 10), p. 1417-1425, (October, 1999).	

Examiner Signature	/Kailash Srivastava/	Date Considered	06/09/2006
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*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.
¹Applicant's unique citation designation number (optional). ²Applicant is to place a check mark here if English language Translation is attached.
 This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

FORM PTO-1449		DOCKET NO. 700938-52220-		SERIAL NO.: TBA (Divisional of 10/001,929) 10/685,266			
INFORMATION DISCLOSURE STATEMENT		DIV		APPLICANT(S): Tatsumi et al.			
		FILING DATE: Herewith 10/14/2003		GROUP NO.: TBA (1651) 1655			
UNITED STATES PATENT DOCUMENTS							
EXAM. INITIALS		DOCUMENT NUMBER	DATE	INVENTOR/ASSIGNEE	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
KS	AA	5,962,476	10/5/99	Naito et al.	-	-	
KS	AB	5,716,969	2/10/98	Naito et al.	-	-	
KS	AC	5,620,994	4/15/97	Naito et al.	-	-	
FOREIGN PATENT DOCUMENTS							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES/NO
KS	BA	8-103291	4/23/96	Japan (and English abstract attached)	-	-	Y
KS	BB	10-28597	2/3/98	Japan (and English abstract attached)	-	-	Y
KS	BC	WO 94/26734 * U.S. Patents which are National Phase Entry of above are 5,962,476 and 5,716,969	11/24/94	PCT	-	-	*Yes
OTHER DOCUMENTS (INCLUDING AUTHOR, TITLE, DATE, PERTINENT PAGES, ETC.)							
	CA						
EXAMINER: /Kailash Srivastava/					DATE: 06/09/2006		

Notice of References Cited	Application/Control No. 10/685,266	Applicant(s)/Patent Under Reexamination TATSUMI ET AL.	
	Examiner Dr. Kailash C. Srivastava	Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*	Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A US-5,620,994 A	04-1997	Naito et al.	514/326
*	B US-5,716,969 A	02-1998	Naito et al.	514/326
	C US-			
	D US-			
	E US-			
	F US-			
	G US-			
	H US-			
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	J US-			
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FOREIGN PATENT DOCUMENTS

*	Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N				
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NON-PATENT DOCUMENTS

*	Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Index of Claims



Application/Control No.

10/685,266

Examiner

Dr. Kailash C. Srivastava

Applicant(s)/Patent under Reexamination

TATSUMI ET AL.

Art Unit

1655

√	Rejected
=	Allowed

-	(Through numeral) Canceled
+	Restricted

N	Non-Elected
I	Interference

A	Appeal
O	Objected

Claim	Date			
	Final	Original	3/6/06	6/11/06
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From: Ronald I. Eiscnstein Leena H. Karttunen	Date: September 14, 2006	No. of Pages: (including this page) 12	
Practitioner's Docket No. 700938-052220-DIV		PATENT	
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE			
In re application of:	Tatsumi, et al.		
Application No.:	10/685,266	Group No.:	1655
Filed:	October 14, 2003	Examiner:	Srivastava, Kailash C.
For:	METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT		
MAIL STOP AMENDMENT			
Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450			
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I hereby certify that the following papers are being facsimile transmitted to the Patent and Trademark Office at 571-273-8300 on the date shown below:			
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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi, et al.

Application No.:	10/685,266	Group No.:	1655
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Date

Tina-Michelle Pittsley


Signature

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TRANSMITTAL FORM <small>(to be used for all correspondence after initial filing)</small>	Application Number	10/685,266
	Filing Date	October 14, 2003
	First Named Inventor	Tatsumi, et al.
	Art Unit	1655
	Examiner Name	Srivastava, Kalash C.
	Attorney Docket Number	700938-052220-DIV
Total Number of Pages in This Submission		

ENCLOSURES (Check all that apply)		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input checked="" type="checkbox"/> Amendment/Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Reply to Missing Parts/ Incomplete Application <input type="checkbox"/> Reply to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Drawing(s) <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation <input type="checkbox"/> Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____ <input type="checkbox"/> Landscape Table on CD	<input type="checkbox"/> After Allowance Communication to TC <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to TC (Appeal Notice, Brief, Reply Brief) <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input checked="" type="checkbox"/> Other Enclosure(s) (please identify below): Facsimile Cover Sheet; Certificate of Facsimile Transmission; Certificate of Limited Recognition
Remarks The Commissioner is hereby authorized to charge any fee deficiency or credit any overpayment to the Nixon Peabody LLP Deposit Account No. 19-2380.		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT			
Firm Name	Nixon Peabody LLP		
Signature			
Printed name	Ronald I. Eisenstein/Leena H. Karttunen		
Date	September 14, 2006	Reg. No.	30,628/L0207

CERTIFICATE OF TRANSMISSION/MAILING			
I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:			
Signature			
Typed or printed name	Tina-Michelle Pittsley	Date	September 14, 2006

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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SEP 14 2006

Docket No. 700938-052220-DIV

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi et al.
Application No.: 10/685,266 Group No.: 1655
Filed: October 14, 2003 Examiner: Srivastava, Kailash C.
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

CERTIFICATE OF MAILING (37 C.F.R. SECTION 1.6(d))	
I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being facsimile transmitted to the Patent and Trademark Office at (571) 273-8300 on the date shown below	
<u>September 14, 2006</u> Date	Tina-Michelle Pittsley <small>(type or print name of person mailing paper)</small>  <small>(signature of person mailing paper)</small>

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

AMENDMENT

In response to the Office Action dated August 11, 2006, please amend the above-identified application as follows:

Amendments to the Title are presented at page 2 of this paper.

Amendments to the Specification begin on page 3 of this paper.

Listing of the Claims begin on page 4 of this paper.

Remarks begin on page 6 of this paper.

10097683.3

Serial Number 10/685,266
Office Action mailed June 14, 2006
Amendment filed September 14, 2006

Amendments to the Title

Please replace the title of the invention with the following amended title:

~~METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT TREATING ONYCHOMYCOSIS~~

Serial Number 10/685,266
Office Action mailed June 14, 2006
Amendment filed September 14, 2006

Amendments to the Specification

Please replace the paragraph beginning on page 1, line 1, with the following amended paragraph:

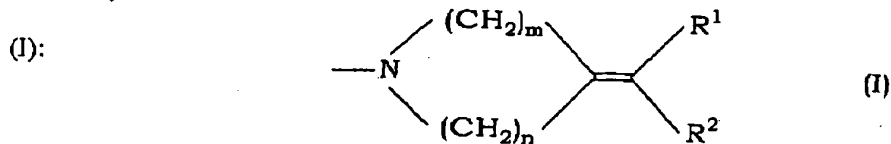
This application is a divisional under 35 U.S.C. § 120 ~~of copending application 10/031,929, filed January 25, 2002, which is the National Stage under 35 U.S.C. § 371 of International Application no. PCT/JP00/04617, filed July 11, 2000, which claims the benefit under 35 U.S.C. § 119 of Japanese Application No. 214369/1999, filed July 28, 1999.~~ of U.S. Non-Provisional application 10/031.929 filed 25 January 2002, now abandoned, which was a National Stage filing under 35 U.S.C. § 371 of PCT/JP00/04617 filed 11 July 2000, which claimed priority to Japanese patent application Serial No. 11/214369 filed 28 July 1999.

Serial Number 10/685,266
Office Action mailed June 14, 2006
Amendment filed September 14, 2006

Listing of the Claims

Claims 1-17 (CANCELLED)

Claim 18 (CURRENTLY AMENDED): A method for treating a subject having onychomycosis wherein the method comprises ~~comprising~~ topically administering to a nail of a subject having onychomycosis an effective amount of an antifungal agent compound having a group represented by the formula



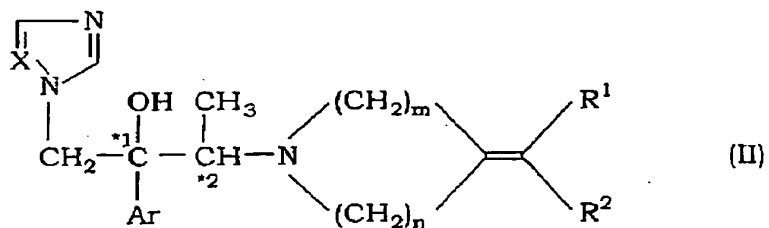
wherein, R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

or a salt thereof as an active ingredient, ~~to a subject having onychomycosis.~~

Claim 19 (PREVIOUSLY PRESENTED): The method of Claim 18, in which the compound is the compound represented by formula (II):



wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₁₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

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Office Action mailed June 14, 2006
Amendment filed September 14, 2006

m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

Claim 20 (PREVIOUSLY PRESENTED): The method of Claim 19, in which the compound represented by the formula (II) is (2R,3R)-2-(2, 4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl) butane-2-ol.

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Office Action mailed June 14, 2006
Amendment filed September 14, 2006

Remarks

Applicants have amended claim 18. Support for the amendment can be found throughout the specification, for example at page 16, lines 9 – 11, page 18, line 14, and page 19, line 5. Accordingly, no new matter is introduced by the virtue of the amendment and its entry is respectfully requested.

The Examiner objected to claim 18 because of the grammar. In light of the amendments to claim 18, Applicants respectfully submit that the objection be withdrawn.

The Examiner objected to the title. Applicants have amended the title to specifically indicate that the present application is directed to methods of treating onychomycosis. No new matter is introduced by this amendment and its entry is respectfully requested. Accordingly, Applicants respectfully request that the objection to the title be withdrawn.

The Examiner objected to the specification stating it had an imperfect priority claim. Applicants have amended the specification to perfect the priority data. No new matter is introduced by this amendment and its entry is respectfully requested. Accordingly, Applicants respectfully request that the objection to the specification be withdrawn.

Claims 18-20 were rejected under the judicially created doctrine of obviousness-type double patenting over U.S. Patent No. 5,620,994 (“994”).

Applicants respectfully submit that the rejection be withdrawn for the following reasons.

Applicants have amended claim 18 to a preferred embodiment, namely treating onychomycosis by applying the antifungal agent topically on the nail of a subject having onychomycosis.

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Office Action mailed June 14, 2006
Amendment filed September 14, 2006

Onychomycosis is a fungal infection of nail that has proven difficult to treat. The application teaches and claims topically applying at the site of onychomycosis infection, i.e., the nail, an effective amount of an azolylamine derivative. Treatment of onychomycosis significantly differs from the general treatment of mycoses claimed in '994. Onychomycosis is a condition that specifically affects the nail plate. It has been generally the rule, as discussed in the present specification (see, e.g. p. 4, lines 9-13), that onychomycosis is particularly difficult or even impossible to treat using topical administration of any agent. It is well known that anti-fungal agents typically do not permeate the nail effectively enough to attack the fungus. Further, agents are easily removed from the top of the nail. The present invention shows the unexpected ability of an azolylamine derivative to penetrate nail and be retained by the nail, and thus be able to cure onychomycosis of the nail bed by topical application directly on the nail rather than using systemic application. Accordingly, the use of the compound in topical application to treat onychomycosis has a clear and unexpected superiority.

Accordingly, in light of the amendments and the discussion above, Applicants submit that the rejection be withdrawn.

Claims 18 – 20 were also rejected under 35 U.S.C. 102(f) and (g) as unpatentable over U.S. Patent No. 5,620,994.

Claims 18 – 20 were rejected under 35 U.S.C. § 102 (b) as anticipated by either U.S. Patent No. 5,620,994 or U.S. Patent No. 5,716,969.

Applicants respectfully submit that the rejections be withdrawn for the reasons discussed in detail, *supra*. The present invention is directed to a method for treating onychomycosis.

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Office Action mailed June 14, 2006
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Applicants submit that neither '994 nor '969 teach a specific method for treating onychomycosis.


Onychomycosis is difficult to treat sub-class of mycoses. The condition is particularly difficult to treat in general, and has been almost impossible to treat using topical application of antifungals. The present claims overcome this difficulty. Accordingly, the present invention would not have been anticipated or obvious. Accordingly, the present claims are novel and the rejections should be withdrawn.

Furthermore, Applicants note that the present application and the '994 are commonly owned.

In view of the foregoing, Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is requested.

Respectfully submitted,

Date: 9/14/06



Ronald I. Eisenstein (Reg. No. 30,628)
Leena H. Karttunen (L0207)
NIXON PEABODY LLP
100 Summer Street
Boston, MA 02110
(617) 345-6054/1367 (phone)
(617) 345-1300 (fax)

BEFORE THE OFFICE OF ENROLLMENT AND DISCIPLINE
UNITED STATES PATENT AND TRADEMARK OFFICE

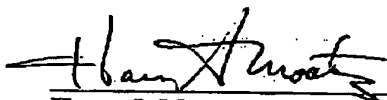
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SEP 14 2006

LIMITED RECOGNITION UNDER 37 CFR § 11.9(b)

Leena H. Karttunen is hereby given limited recognition under 37 CFR §11.9(b) as an employee of Nixon Peabody LLP, to prepare and prosecute patent applications wherein the patent applicant is the client of Nixon Peabody LLP and an attorney or agent of record in the applications is a registered practitioner who is a member of Nixon Peabody LLP. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Leena H. Karttunen ceases to lawfully reside in the United States, (ii) Leena H. Karttunen's employment with Nixon Peabody LLP ceases or is terminated, or (iii) Leena H. Karttunen ceases to remain or reside in the United States on an H-1B visa.

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

Limited Recognition No. L0207
Expires: February 11, 2007



Harry I. Moatz
Director of Enrollment and Discipline

Same

PATENT APPLICATION FEE DETERMINATION RECORD
Effective October 1, 2003

Application or Docket Number

10685266

CLAIMS AS FILED - PART I

	(Column 1)	(Column 2)
TOTAL CLAIMS	4	
FOR	NUMBER FILED	NUMBER EXTRA
TOTAL CHARGEABLE CLAIMS	0 minus 20 =	0
INDEPENDENT CLAIMS	2 minus 3 =	0
MULTIPLE DEPENDENT CLAIM PRESENT <input type="checkbox"/>		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY TYPE OR OTHER THAN SMALL ENTITY

RATE	FEE	OR	RATE	FEE
BASIC FEE	385.00		BASIC FEE	770.00
X\$ 9=			X\$18=	
X43=			X86=	
+145=			+290=	
TOTAL			TOTAL	770

CLAIMS AS AMENDED - PART II

09-14-06

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	3	** 20	=
Independent	1	*** 3	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

SMALL ENTITY OR OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=			X\$18=	
X43=			X86=	
+145=			+290=	
TOTAL ADDIT. FEE			TOTAL ADDIT. FEE	

18,

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total		**	=
Independent		***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=			X\$18=	
X43=			X86=	
+145=			+290=	
TOTAL ADDIT. FEE			TOTAL ADDIT. FEE	

	(Column 1)	(Column 2)	(Column 3)
AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total		**	=
Independent		***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM <input type="checkbox"/>			

RATE	ADDITIONAL FEE	OR	RATE	ADDITIONAL FEE
X\$ 9=			X\$18=	
X43=			X86=	
+145=			+290=	
TOTAL ADDIT. FEE			TOTAL ADDIT. FEE	

- * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
- ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
- *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
- The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Case Creation Option

Case "10685266us20061210" already exists. Please overwrite it or cancel the operation.

The Contents of Case "10685266us20061210"

Qnum	Query	DB Name	Thesaurus	Operator	Plural
Q1	onychomycosis	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q2	Taenia pedia	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q3	Tenia pedis or Trichophyton	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q4	antimicrobial or antifungal or fungistasis or fungicid\$6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q5	methylen piperidine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q6	Q3 or Q5	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q7	Q4 or Q6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q8	Q1 and Q7	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q9	Q4 and Q8	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q10	Q5 and Q9	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q11	Q5 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q12	Q5 and Q4	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q13	(435/32).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q14	(514/326).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q15	(514/397).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q16	(514/212).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q17	Q13 or Q14	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q18	Q15 or Q17	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q19	Q5 or Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
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Q23	Q4 near5 Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q24	Q4 and Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q25	Q18 and Q24	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q26	("RN-164650-44-6")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q27	("2,4-difluorophenyl-methylenepiperidino-triazolyl-butan-2-ol")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q28	axolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q29	azolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q30	Q29 and Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q31	Q4 and Q30	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q32	Q31 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q33	Q32 and @pd > 20060611	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Hit List

Search Results - Record(s) 1 through 5 of 5 returned.

1. Document ID: US 20060234981 A1

L43: Entry 1 of 5

File: PGPB

Oct 19, 2006

PGPUB-DOCUMENT-NUMBER: 20060234981
 PGPUB-FILING-TYPE:
 DOCUMENT-IDENTIFIER: US 20060234981 A1

TITLE: Boron-containing small molecules

PUBLICATION-DATE: October 19, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Baker; Stephen J.	Mountain View	CA	US
Akama; Tsutomu	Sunnyvale	CA	US
Bellinger-Kawahara; Carolyn	Redwood City	CA	US
Hernandez; Vincent S.	Watsonville	CA	US
Hold; Karin M.	Belmont	CA	US
Leyden; James J.	Malvern	PA	US
Maples; Kirk R.	San Jose	CA	US
Plattner; Jacob J.	Berkeley	CA	US
Sanders; Virginia	San Francisco	CA	US
Zhang; Yong-Kang	San Jose	CA	US

US-CL-CURRENT: [514/64](#); [558/288](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KMOC	Draw Desc	Image
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2. Document ID: US 20060003969 A1

L43: Entry 2 of 5

File: PGPB

Jan 5, 2006

PGPUB-DOCUMENT-NUMBER: 20060003969
 PGPUB-FILING-TYPE:
 DOCUMENT-IDENTIFIER: US 20060003969 A1

TITLE: Compositions and methods for treating pathological infections

PUBLICATION-DATE: January 5, 2006

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Manandhar; Madhusudan P.	Monsey	NY	US

US-CL-CURRENT: [514/125](#); [514/675](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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 3. Document ID: US 20050238672 A1

L43: Entry 3 of 5

File: PGPB

Oct 27, 2005

PGPUB-DOCUMENT-NUMBER: 20050238672
 PGPUB-FILING-TYPE: new
 DOCUMENT-IDENTIFIER: US 20050238672 A1

TITLE: Antifungal drug delivery

PUBLICATION-DATE: October 27, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Nimni, Marcel E.	Santa Monica	CA	US

US-CL-CURRENT: [424/400](#); [514/649](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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 4. Document ID: US 20050107407 A1

L43: Entry 4 of 5

File: PGPB

May 19, 2005

PGPUB-DOCUMENT-NUMBER: 20050107407
 PGPUB-FILING-TYPE: new
 DOCUMENT-IDENTIFIER: US 20050107407 A1

TITLE: Method for treating athlete's foot

PUBLICATION-DATE: May 19, 2005

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Liberman, Barnet	New York	NY	US
Lefkovits, Albert	New York	NY	US

US-CL-CURRENT: [514/263.38](#)

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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 5. Document ID: US 20040197280 A1

L43: Entry 5 of 5

File: PGPB

Oct 7, 2004

PGPUB-DOCUMENT-NUMBER: 20040197280
 PGPUB-FILING-TYPE: new
 DOCUMENT-IDENTIFIER: US 20040197280 A1

TITLE: Delivery of medicaments to the nail

Record List Display

PUBLICATION-DATE: October 7, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY
Repka, Michael A.	Oxford	MS	US

US-CL-CURRENT: 424/61

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	Claims	KWIC	Draw Desc	Image
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Clear	Generate Collection	Print	Fwd Refs	Bkwd Refs	Generate OACS
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Term	Documents
(35 AND 42) .PGPB, USPT, USOC, EPAB, JPAB, DWPI .	5
(L35 AND L42) .PGPB, USPT, USOC, EPAB, JPAB, DWPI .	5

Display Format:

[Previous Page](#) [Next Page](#) [Go to Doc#](#)

Case Creation Option

Case "10685266us20061220" already exists. Please overwrite it or cancel the operation.

The Contents of Case "10685266us20061220"

Qnum	Query	DB Name	Thesaurus	Operator	Plural
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Q2	Taenia pedia	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q3	Tenia pedis or Trichophyton	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q4	antimicrobial or antifungal or fungistasis or fungicid\$6	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q5	methylen piperidine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q6	Q3 or Q5	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
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Q12	Q5 and Q4	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q13	(435/32).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q14	(514/326).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q15	(514/397).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q16	(514/212).ccls.	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q17	Q13 or Q14	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q18	Q15 or Q17	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q19	Q5 or Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q20	Q5 near5 Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q21	Q5 and Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q22	Q1 near5 Q3	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q23	Q4 near5 Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q24	Q4 and Q22	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES

Q25	Q18 and Q24	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q26	("RN-164650-44-6")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q27	("2,4-difluorophenyl-methylenepiperidino-triazolyl-butan-2-ol")	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q28	axolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q29	azolylamine	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q30	Q29 and Q18	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q31	Q4 and Q30	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q32	Q31 and Q1	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q33	Q32 and @pd > 20060611	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q34	Q33 and @pd > 20061210	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q35	onychomycosis and (nail penetrat\$)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q36	antifungal and (nail penetrat\$6)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q37	(antifungal or mycostatin) and (nail plate)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q38	((antifungal compound) or mycostatin or fungistatic) and (nail plate)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q39	penetrat\$5 or (pass through)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q40	(nail plate) or (toe nail)	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q41	Q39 and Q40	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q42	Q38 and Q41	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES
Q43	Q35 and Q42	PGPB,USPT,USOC,EPAB,JPAB,DWPI	None	ADJ	YES



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Department of the Army
U.S. Army Patent, Copyrights & Trademark Division
Office of the Judge Advocate General
901 North Stuart St.
Arlington, VA 22203-1837

PATENT & TRADEMARK OFFICE
MAILED

DEC 23 2006

LICENSING & REVIEW

ATTN: DIANE WINTER

The application identified below was referred to you for review under 35 U.S.C. §181 on 11/14/03. The Patent and Trademark Office has not received a communication from you regarding secrecy of this application under §181.

Examination of this application has been completed and has been found to be in condition for allowance. Since the Patent and Trademark Office has no authority to withhold the issue of a patent absent a recommendation for a secrecy order under §181, this application is being prepared for issue as a patent.

The Access Acknowledgment mailed to you must be returned to this Office.

Referred Application
Serial Number


Serial Number of File Wrapper
Continuation Application (if any)

Filing Date of
Referred Appl

10/685,266

10/14/03

Sincerely,

For 
J. Samuels
Review Supervisor
Licensing & Review



UNITED STATES PATENT AND TRADEMARK OFFICE

Handwritten signature

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

53143 7590 01/04/2007

RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

EXAMINER

SRIVASTAVA, KAILASH C

ART UNIT PAPER NUMBER

1657

DATE MAILED: 01/04/2007

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV	4026

TITLE OF INVENTION: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1400	\$0	\$0	\$1400	04/04/2007

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

**Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
 Commissioner for Patents
 P.O. Box 1450
 Alexandria, Virginia 22313-1450
 or Fax (571)-273-2885**

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

53143 7590 01/04/2007

**RONALD I. EISENSTEIN
 NIXON PEABODY LLP
 100 SUMMER STREET
 BOSTON, MA 02110**

Certificate of Mailing or Transmission
 I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV	4026

TITLE OF INVENTION: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1400	\$0	\$0	\$1400	04/04/2007

EXAMINER	ART UNIT	CLASS-SUBCLASS
SRIVASTAVA, KAILASH C	1657	435-032000

<p>1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).</p> <p><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.</p> <p><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.</p>	<p>2. For printing on the patent front page, list</p> <p>(1) the names of up to 3 registered patent attorneys or agents OR, alternatively, _____ 1</p> <p>(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. _____ 2</p> <p>_____ 3</p>
--	---

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE _____ (B) RESIDENCE: (CITY and STATE OR COUNTRY) _____

Please check the appropriate assignee category or categories (will not be printed on the patent) : Individual Corporation or other private group entity Government

<p>4a. The following fee(s) are submitted:</p> <p><input type="checkbox"/> Issue Fee</p> <p><input type="checkbox"/> Publication Fee (No small entity discount permitted)</p> <p><input type="checkbox"/> Advance Order - # of Copies _____</p>	<p>4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)</p> <p><input type="checkbox"/> A check is enclosed.</p> <p><input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.</p> <p><input type="checkbox"/> The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).</p>
---	--

5. Change in Entity Status (from status indicated above)

a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.



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Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO.
10/685,266 10/14/2003 Yoshiyuki Tatsumi 700938-052220-DIV 4026

EXAMINER

SRIVASTAVA, KAILASH C

ART UNIT PAPER NUMBER

1657
DATE MAILED: 01/04/2007

53143 7590 01/04/2007
RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 451 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 451 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.	Applicant(s)	
	10/685,266	TATSUMI ET AL.	
	Examiner	Art Unit	
	Dr. Kailash C. Srivastava	1657	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. This communication is responsive to 09/14/2006.
2. The allowed claim(s) is/are 19 and 20.
3. Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) All b) Some* c) None of the:
 1. Certified copies of the priority documents have been received.
 2. Certified copies of the priority documents have been received in Application No. _____.
 3. Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

4. A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) hereto or 2) to Paper No./Mail Date _____.
 - (b) including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

- | | |
|---|--|
| <ol style="list-style-type: none"> 1. <input type="checkbox"/> Notice of References Cited (PTO-892) 2. <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) 3. <input type="checkbox"/> Information Disclosure Statements (PTO/SB/08),
Paper No./Mail Date _____ 4. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit of Biological Material | <ol style="list-style-type: none"> 5. <input type="checkbox"/> Notice of Informal Patent Application 6. <input checked="" type="checkbox"/> Interview Summary (PTO-413),
Paper No./Mail Date _____ 7. <input checked="" type="checkbox"/> Examiner's Amendment/Comment 8. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance 9. <input type="checkbox"/> Other _____ |
|---|--|

Examiner's Amendment/Comment

1. Applicant's responsive amendment filed 14 September 2006 in response to Office action mailed 11 August 2006 is acknowledged and entered.
2. The Art Unit Location for your application under prosecution at the United States Patent and Trademark Office (i.e., USPTO) has been changed to Art Unit 1657. To aid in correlating any papers for this application (i.e., 10/685,266), all further correspondence regarding this application should be directed to Examiner Kailash C. Srivastava in Art Unit 1657.
3. In view of Applicant's amendments and response filed 14 September 2006, the following objections and rejections in Office Action mailed 11 August 2006 are hereby withdrawn:
 - Objection to Specification for not reciting the application Priority Data at Page 1, Line 1 of the specification;
 - Objection to Title because the title of the invention as presented at that time did not conclusively summarize the elected and claimed invention;
 - Objection to Claim 18 as being improper;
 - Rejection to Claims 18-20 under Obvious Type Double Patenting in view of the teachings in Claims 9-12, U.S. Patent 5,620,994;
 - Anticipatory Rejections under 35 U.S.C. § 102 (b) to Claims 18-20 by U.S. Patent 5,820,994; and
 - Rejections under 35 U.S.C. §§ 102 (f) and (g) to Claims 18-20 over U.S. Patent 5,820,994.

Claims Status

4. Claims 1-17 have been cancelled.
5. Claim 18 has been amended
6. Claims 18-20 are pending.

Examiner's Amendment

7. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR §1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

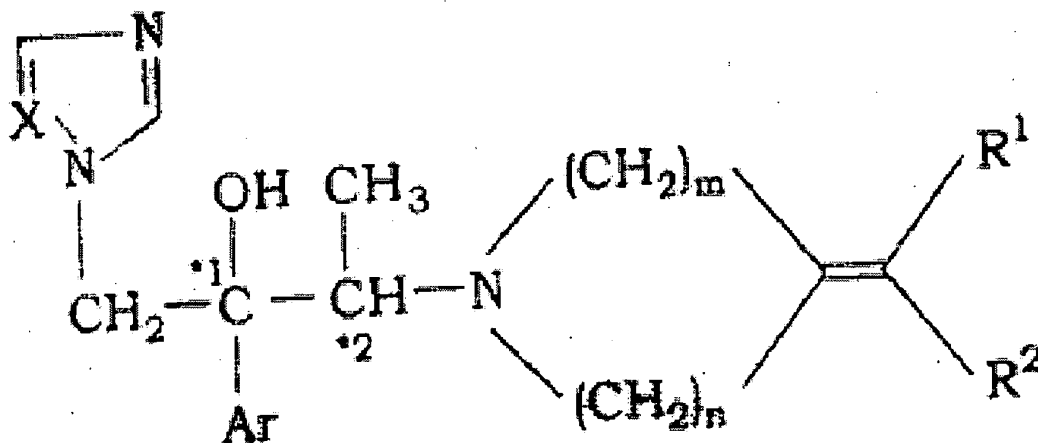
8. Authorization for this examiner's amendment was given in a telephone interview on 18 December 2006 with Mr. Ronald I. Eisenstein, Applicant's Representative.

In the Claims:

The following listings of the claims will replace all prior versions and listings of the claims in this application:

18. (Cancelled).

Claim 19 (CURRENTLY AMENDED): A method for treating a subject having onychomycosis wherein the method comprises topically administering to a nail of [a] said subject having onychomycosis [an] a therapeutically effective amount of an antifungal agent-compound having a group represented by the following formula:



wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₁₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

Claim 20 (PREVIOUSLY PRESENTED): The method of Claim 19, in which the compound represented by the formula (II) is (2R, 3R)-2-(2, 4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1, 2,4-triazole-1-yl) butane-2-ol.

Examiner's Reasons For Allowance

9. The following is an examiner's statement of reasons for allowance:
- o The closest art are:
 - U.S. Patent 5, 620, 994 issued 15 April 1997 to Naito et al., who teach a fungicidal composition comprising the same compounds, having the same structure as that claimed in the instant invention.

- US 20060003969 A1 Published 05 January 2006, Inventor: Manandhar, M.P. The invention is drawn to a method to treat pathogenic infections. The method does not teach topical administration of said composition wherein the composition penetrates the nail plate.
- US 20040197280 A1 Published 07 October 2004, Inventor: Repka, M.A. The invention is drawn to a method to deliver medicaments to nails to treat the infection. The method does not that the applied composition/medicament penetrates through he nail plate.

Thus, the cited prior or pertinent art teach treating a pathological infection, delivery of a medicament to nails, or treating the nail infection via topical and/ or systemic application of the medicament. In contrast the method claimed in instantly presented claims is a composition topically applied to the onychomycosis infection cite, wherein, unexpectedly and in contrast to previously evaluated compositions/methods, the instantly claimed method cures the onychomycosis because the medicament upon direct administration to the nail, penetrates through the nail plate and eradicates the infection at the site.


10. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

11. Claims 19-20 are allowed.

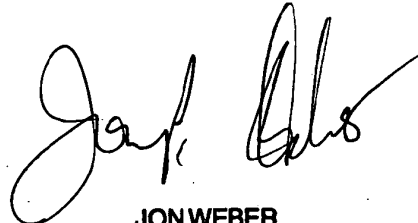
12. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Examiner Kailash C. Srivastava whose telephone number is (571) 272-0923. The examiner can normally be reached on Monday to Thursday from 7:30 A.M. to 6:00 P.M. (Eastern Standard or Daylight Savings Time).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Jon Weber can be reached at (571)-272-0925 Monday through Thursday 7:30 A.M. to 6:00 P.M. The fax phone number for the organization where this application or proceeding is assigned is (571)-273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding may be obtained from the Patent Application Information Retrieval (i.e., PAIR) system. Status information for the published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (i.e., EBC) at: (866)-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.


Kailash C. Srivastava, Ph.D.
Patent Examiner
Art Unit 1657
(571) 272-0923

December 26, 2006


JON WEBER
SUPERVISORY PATENT EXAMINER

Interview Summary	Application No.	Applicant(s)	
	10/685,266	TATSUMI ET AL.	
	Examiner	Art Unit	
	Dr. Kailash C. Srivastava	1657	

All participants (applicant, applicant's representative, PTO personnel):

- (1) Dr. Kailash C. Srivastava, Examiner. (3) _____
(2) Mr. R. I. Eisenstein, Applicant's Representative. (4) _____

Date of Interview: 18 December 2006.

Type: a) Telephonic b) Video Conference
c) Personal [copy given to: 1) applicant 2) applicant's representative]

Exhibit shown or demonstration conducted: d) Yes e) No.
If Yes, brief description: NONE.

Claim(s) discussed: Those of Record.

Identification of prior art discussed: That of Record.

Agreement with respect to the claims f) was reached. g) was not reached. h) N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Applicants' representatives authorized the Examiner to amend claims 18-20 according to Examiner's proposed amendment (See attached) to bring this application in a better condition for allowance.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Examiner's signature, if required

Summary of Record of Interview Requirements

Manual of Patent Examining Procedure (MPEP), Section 713.04, Substance of Interview Must be Made of Record

A complete written statement as to the substance of any face-to-face, video conference, or telephone interview with regard to an application must be made of record in the application whether or not an agreement with the examiner was reached at the interview.

Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews Paragraph (b)

In every instance where reconsideration is requested in view of an interview with an examiner, a complete written statement of the reasons presented at the interview as warranting favorable action must be filed by the applicant. An interview does not remove the necessity for reply to Office action as specified in §§ 1.111, 1.135. (35 U.S.C. 132)

37 CFR §1.2 Business to be transacted in writing.

All business with the Patent or Trademark Office should be transacted in writing. The personal attendance of applicants or their attorneys or agents at the Patent and Trademark Office is unnecessary. The action of the Patent and Trademark Office will be based exclusively on the written record in the Office. No attention will be paid to any alleged oral promise, stipulation, or understanding in relation to which there is disagreement or doubt.

The action of the Patent and Trademark Office cannot be based exclusively on the written record in the Office if that record is itself incomplete through the failure to record the substance of interviews.

It is the responsibility of the applicant or the attorney or agent to make the substance of an interview of record in the application file, unless the examiner indicates he or she will do so. It is the examiner's responsibility to see that such a record is made and to correct material inaccuracies which bear directly on the question of patentability.

Examiners must complete an Interview Summary Form for each interview held where a matter of substance has been discussed during the interview by checking the appropriate boxes and filling in the blanks. Discussions regarding only procedural matters, directed solely to restriction requirements for which interview recordation is otherwise provided for in Section 812.01 of the Manual of Patent Examining Procedure, or pointing out typographical errors or unreadable script in Office actions or the like, are excluded from the interview recordation procedures below. Where the substance of an interview is completely recorded in an Examiners Amendment, no separate Interview Summary Record is required.

The Interview Summary Form shall be given an appropriate Paper No., placed in the right hand portion of the file, and listed on the "Contents" section of the file wrapper. In a personal interview, a duplicate of the Form is given to the applicant (or attorney or agent) at the conclusion of the interview. In the case of a telephone or video-conference interview, the copy is mailed to the applicant's correspondence address either with or prior to the next official communication. If additional correspondence from the examiner is not likely before an allowance or if other circumstances dictate, the Form should be mailed promptly after the interview rather than with the next official communication.

The Form provides for recordation of the following information:

- Application Number (Series Code and Serial Number)
- Name of applicant
- Name of examiner
- Date of interview
- Type of interview (telephonic, video-conference, or personal)
- Name of participant(s) (applicant, attorney or agent, examiner, other PTO personnel, etc.)
- An indication whether or not an exhibit was shown or a demonstration conducted
- An identification of the specific prior art discussed
- An indication whether an agreement was reached and if so, a description of the general nature of the agreement (may be by attachment of a copy of amendments or claims agreed as being allowable). Note: Agreement as to allowability is tentative and does not restrict further action by the examiner to the contrary.
- The signature of the examiner who conducted the interview (if Form is not an attachment to a signed Office action)

It is desirable that the examiner orally remind the applicant of his or her obligation to record the substance of the interview of each case. It should be noted, however, that the Interview Summary Form will not normally be considered a complete and proper recordation of the interview unless it includes, or is supplemented by the applicant or the examiner to include, all of the applicable items required below concerning the substance of the interview.

A complete and proper recordation of the substance of any interview should include at least the following applicable items:

- 1) A brief description of the nature of any exhibit shown or any demonstration conducted,
- 2) an identification of the claims discussed,
- 3) an identification of the specific prior art discussed,
- 4) an identification of the principal proposed amendments of a substantive nature discussed, unless these are already described on the Interview Summary Form completed by the Examiner,
- 5) a brief identification of the general thrust of the principal arguments presented to the examiner,
(The identification of arguments need not be lengthy or elaborate. A verbatim or highly detailed description of the arguments is not required. The identification of the arguments is sufficient if the general nature or thrust of the principal arguments made to the examiner can be understood in the context of the application file. Of course, the applicant may desire to emphasize and fully describe those arguments which he or she feels were or might be persuasive to the examiner.)
- 6) a general indication of any other pertinent matters discussed, and
- 7) if appropriate, the general results or outcome of the interview unless already described in the Interview Summary Form completed by the examiner.

Examiners are expected to carefully review the applicant's record of the substance of an interview. If the record is not complete and accurate, the examiner will give the applicant an extendable one month time period to correct the record.

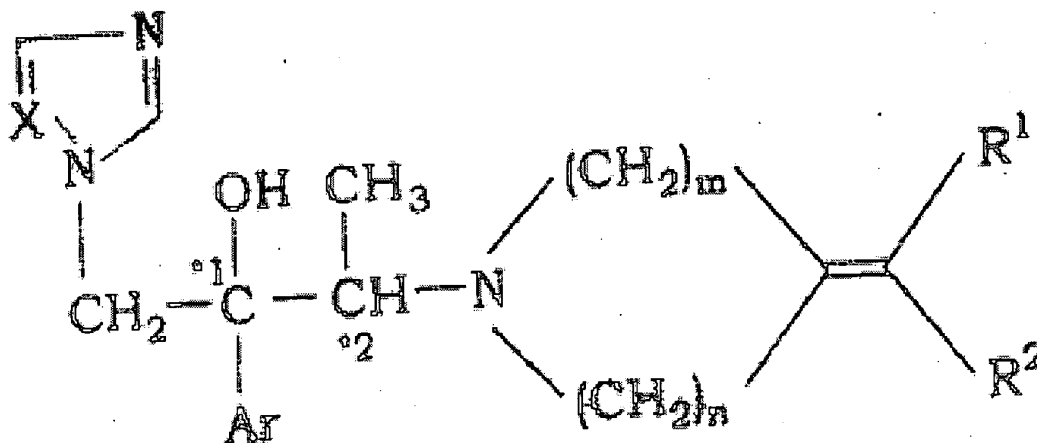
Examiner to Check for Accuracy

If the claims are allowable for other reasons of record, the examiner should send a letter setting forth the examiner's version of the statement attributed to him or her. If the record is complete and accurate, the examiner should place the indication, "Interview Record OK" on the paper recording the substance of the interview along with the date and the examiner's initials.

Listing of the Claims

Claims 1-18 (CANCELLED)

Claim 19 (CURRENTLY AMENDED): A method for treating a subject having onychomycosis wherein the method comprises topically administering to a nail of [a] said subject having onychomycosis [an] a therapeutically effective amount of an antifungal agent compound having a group represented by the following formula:



wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group,

nitro group and C₁₋₁₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,


m is 2 or 3,

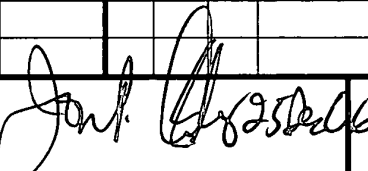
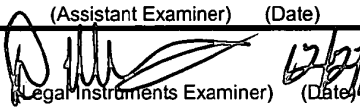
n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

Claim 20 (PREVIOUSLY PRESENTED): The method of Claim 19, in which the compound represented by the formula (II) is (2R,3R)-2-(2, 4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl) butane-2-ol.

Issue Classification 	Application/Control No. 10/685,266	Applicant(s)/Patent under Reexamination TATSUMI ET AL.
	Examiner Dr. Kailash C. Srivastava	Art Unit 1657

ISSUE CLASSIFICATION														
ORIGINAL					INTERNATIONAL CLASSIFICATION									
CLASS		SUBCLASS			CLAIMED			NON-CLAIMED						
435		32			C	12	Q	1	/18					
CROSS REFERENCES					A	01	N	43	/26					
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)				A	01	N	43	/34					
514	326				A	61	K	31	/445					
									/					
									/					
									/					
									/					
Dr. Kailash C. Srivastava, 12/26/2006 (Assistant Examiner) (Date)					 (Primary Examiner) JON WEBER SUPERVISORY PATENT EXAMINER					Total Claims Allowed: 2				
 (Legal Instruments Examiner) (Date)					O.G. Print Claim(s) 1					O.G. Print Fig. NONE				

<input checked="" type="checkbox"/> Claims renumbered in the same order as presented by applicant		<input type="checkbox"/> CPA		<input checked="" type="checkbox"/> T.D.		<input type="checkbox"/> R.1.47	
Final	Original	Final	Original	Final	Original	Final	Original
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APPLICATION NUMBER	FILING OR 371(c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV

CONFIRMATION NO. 4026

53143
RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA02110

Date Mailed. 01/04/2007

NOTICE OF NEW OR REVISED PROJECTED PUBLICATION DATE

The above-identified application has a new or revised projected publication date. The current projected publication date for this application is 04/12/2007. If this is a new projected publication date (there was no previous projected publication date), the application has been cleared by Licensing & Review or a secrecy order has been rescinded and the application is now in the publication queue.

If this is a revised projected publication date (one that is different from a previously communicated projected publication date), the publication date has been revised due to processing delays in the USPTO or the abandonment and subsequent revival of an application. The application is anticipated to be published on a date that is more than six weeks different from the originally-projected publication date.

More detailed publication information is available through the private side of Patent Application Information Retrieval (PAIR) System. The direct link to access PAIR is currently <http://pair.uspto.gov>. Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at (703) 305-3028.

Questions relating to this Notice should be directed to the Office of Patent Publication at (703) 305-8283.

PART 1 - ATTORNEY/APPLICANT COPY



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BIBDATASHEET

CONFIRMATION NO. 4026

Bib Data Sheet

SERIAL NUMBER 10/685,266	FILING OR 371(c) DATE 10/14/2003 RULE	CLASS 435	GROUP ART UNIT 1657	ATTORNEY DOCKET NO. 700938-052220-DIV
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APPLICANTS
 Yoshiyuki Tatsumi, Otsu-shi, JAPAN;
 Mamoru Yokoo, Otsu-shi, JAPAN;
 Kosho Nakamura, Moriyama-shi, JAPAN;
 Tadashi Arika, Suita-shi, JAPAN;

**** CONTINUING DATA *******
 This application is a DIV of 10/031,929 01/25/2002 ABN
 which is a 371 of PCT/JP00/04617 07/11/2000

**** FOREIGN APPLICATIONS *******
 JAPAN 214369/1999 07/28/1999

Foreign Priority claimed <input checked="" type="checkbox"/> yes <input type="checkbox"/> no	STATE OR COUNTRY JAPAN	SHEETS DRAWING 4	TOTAL CLAIMS 4	INDEPENDENT CLAIMS 2	
35 USC 119 (a-d) conditions met <input checked="" type="checkbox"/> yes <input type="checkbox"/> no <input type="checkbox"/> Met after Allowance					
Verified and Acknowledged	Examiner's Signature	Initials			

ADDRESS
 53143

TITLE
 Method for treating onychomycosis

43
11/16/07

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		<input type="checkbox"/> Other _____
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53143 7590 01/04/2007

RONALD I. EISENSTEIN NIXON PEABODY LLP 100 SUMMER STREET BOSTON, MA 02110

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03/30/2007 HDENESS2 00000022 500850 10685266

Tina-Michelle Pittsley (Depositor's name) Tina-Michelle Pittsley (Signature) March 29 2007 (Date)

01 FC:1501 1400.00 DA 02 FC:8001 30.00 DA

Table with 5 columns: APPLICATION NO., FILING DATE, FIRST NAMED INVENTOR, ATTORNEY DOCKET NO., CONFIRMATION NO. Values: 10/685,266, 10/14/2003, Yoshiyuki Tatsumi, 700938-052220-DIV, 4026

TITLE OF INVENTION: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

Table with 7 columns: APPLN. TYPE, SMALL ENTITY, ISSUE FEE DUE, PUBLICATION FEE DUE, PREV. PAID ISSUE FEE, TOTAL FEE(S) DUE, DATE DUE. Values: nonprovisional, NO, \$1400, \$0, \$0, \$1400, 04/04/2007

Table with 3 columns: EXAMINER, ART UNIT, CLASS-SUBCLASS. Values: SRIVASTAVA, KAILASH C, 1657, 435-032000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). 2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type) PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. (A) NAME OF ASSIGNEE: Kaken Pharmaceutical Co., Ltd. (B) RESIDENCE: (CITY and STATE OR COUNTRY) 28-8, Honkomagome 2-chome Bunkyo-ku Tokyo 113-8650, Japan

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4a. The following fee(s) are submitted: Issue Fee, Publication Fee, Advance Order. 4b. Payment of Fee(s): A check is enclosed, Payment by credit card, The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number 50-0850

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Authorized Signature: Ronald I. Eisenstein/Leena H. Karttunen Date: 3/29/2007 Typed or printed name: Ronald I. Eisenstein/Leena H. Karttunen Registration No. 30,628/L0207

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Nixon Peabody LLP

Attorneys at Law

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Fax: (617) 345-1300

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From: Ronald I. Eisenstein Leena H. Karttunen	Date: March 29, 2007	No. of Pages: (including this page) 7	700938-052220-DIV
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Practitioner's Docket No. 700938-052220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi, et al.
 Application No.: 10/685,266 Group No.: 1657
 Filed: October 14, 2003 Examiner: SRIVASTAVA, Kailash C.
 For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND
 ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF
 ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

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BY Leena H. Karttunen

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see doc 3/29/07

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Practitioner's Docket No. 700938-052220-DIV.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi, et al.
 Application No.: 10/685,266 Group No.: 1657
 Filed: October 14, 2003 Examiner: SRIVASTAVA, Kailash C.
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Date: March 29, 2007

Tina Michelle Pittsley
 Signature

Tina-Michelle Pittsley
 (type or print name of person certifying)

10332174.1

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UNITED STATES PATENT AND TRADEMARK OFFICE**

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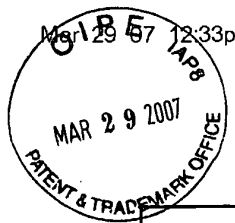
Leena H. Karttunen is hereby given limited recognition under 37 CFR §11.9(b) as an employee of Nixon Peabody LLP, to prepare and prosecute patent applications wherein the patent applicant is the client of Nixon Peabody LLP and an attorney or agent of record in the applications is a registered practitioner who is a member of Nixon Peabody LLP. This limited recognition shall expire on the date appearing below, or when whichever of the following events first occurs prior to the date appearing below: (i) Leena H. Karttunen ceases to lawfully reside in the United States, (ii) Leena H. Karttunen's employment with Nixon Peabody LLP ceases or is terminated, or (iii) Leena H. Karttunen ceases to remain authorized to be employed in accordance with an Employment Authorization Card issued pursuant to 8 CFR § 274a.12(c)(9).

This document constitutes proof of such recognition. The original of this document is on file in the Office of Enrollment and Discipline of the U.S. Patent and Trademark Office.

Limited Recognition No. L0207
Expires: January 7, 2008



Harry I. Moatz
Director of Enrollment and Discipline



Mar 29 12:33p

NIXON PEABODY

18667410075

p.4

TRANSMITTAL FORM <i>(to be used for all correspondence after initial filing)</i>	Application Number	10/685,266
	Filing Date	October 14, 2003
	First Named Inventor	TATSUMI, Yoshiyuki
	Group Art Unit	1657
	Examiner Name	SRIVASTAVA, Kailash C.
	Attorney Docket Number	700938-052220-DIV
Total Number of Pages in This Submission		

ENCLOSURES <i>(check all that apply)</i>		
<input type="checkbox"/> Fee Transmittal Form <input type="checkbox"/> Fee Attached <input type="checkbox"/> Amendment / Reply <input type="checkbox"/> After Final <input type="checkbox"/> Affidavits/declaration(s) <input type="checkbox"/> Extension of Time Request <input type="checkbox"/> Express Abandonment Request <input type="checkbox"/> Information Disclosure Statement <input type="checkbox"/> Certified Copy of Priority Document(s) <input type="checkbox"/> Response to Missing Parts/ Incomplete Application <input type="checkbox"/> Response to Missing Parts under 37 CFR 1.52 or 1.53	<input type="checkbox"/> Assignment Papers <i>(for an Application)</i> <input type="checkbox"/> Drawing(s) <input type="checkbox"/> Declaration and Power of Attorney <input type="checkbox"/> Licensing-related Papers <input type="checkbox"/> Petition <input type="checkbox"/> Petition to Convert to a Provisional Application <input type="checkbox"/> Power of Attorney, Revocation Change of Correspondence Address <input type="checkbox"/> Terminal Disclaimer <input type="checkbox"/> Request for Refund <input type="checkbox"/> CD, Number of CD(s) _____	<input type="checkbox"/> After Allowance Communication to Group <input type="checkbox"/> Appeal Communication to Board of Appeals and Interferences <input type="checkbox"/> Appeal Communication to Group <i>(Appeal Notice, Brief, Reply Brief)</i> <input type="checkbox"/> Proprietary Information <input type="checkbox"/> Status Letter <input type="checkbox"/> Application Data Sheet <input type="checkbox"/> Request for Corrected Filing Receipt with Enclosures <input type="checkbox"/> A self-addressed prepaid postcard for acknowledging receipt <input checked="" type="checkbox"/> Other Enclosure(s) <i>(please identify below):</i> Facsimile Cover Sheet/Certificate of Transmission; Statement of Limited Recognition; Notification of Filing of Continuing, Divisional or Continued Prosecution Application; Fee(s) Transmittal - Part B - PTOL-85.
Remarks		<input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees required or credit any overpayments to Deposit Account No. 50-0850 for the above identified docket number.

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT	
Firm or individual name	Nixon Peabody LLP
Signature	
Name	Ronald I. Eisenstein (Reg. No. 30,628)/Leena H. Karttunen (L0207)
Date	3/29/2007

CERTIFICATE OF TRANSMISSION [37 CFR 1.8(b)]	
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<input type="checkbox"/> deposited with the United States Postal Service on the date shown below with sufficient postage as first class mail in an envelope addressed to: MAIL STOP ISSUE FEE, Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450	
<input checked="" type="checkbox"/> transmitted by facsimile on the date shown below to the United States Patent and Trademark Office at (571) 273-2885.	
<u>March 29, 2007</u> Date	 Signature Tina-Michelle Pittsley Typed or printed name

10332192.1

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Prosecution's Docket No. 700938-052220-DIV

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Tatsumi, et al.
Application No.: 10/685,266 Group No.: 1657
Filed: October 14, 2003 Examiner: SRIVASTAVA, Kailash C.
For: METHOD FOR DETECTING PATHOGENIC MICROORGANISM AND ANTIMICROBIAL AGENT, METHOD FOR EVALUATING EFFECT OF ANTIMICROBIAL AGENT, AND ANTIMICROBIAL AGENT

MAIL STOP ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

NOTIFICATION OF FILING OF CONTINUING, DIVISIONAL OR CONTINUED PROSECUTION APPLICATION

Notification is hereby being made of the filing of a divisional application for this case on March 27, 2007.

Date: 3/29/2007

[Handwritten signature]

Ronald I. Eisenstein (Reg. No. 30,628)
Leena H. Karttunen (L0207)
Nixon Peabody LLP
100 Summer Street
Boston, MA 02110
617-345-6054

CERTIFICATION UNDER 37 C.F.R. §§ 1.8 and 1.10*

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37 C.F.R. § 1.8(a)

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37 C.F.R. § 1.10*

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TRANSMISSION

X facsimile transmitted to the Patent and Trademark Office, (571) 273-2885.

[Handwritten signature: Tina-Michelle Pittsley]
Signature

Date: March 29, 2007

Tina-Michelle Pittsley

(type or print name of person certifying)

* Only the date of filing (1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under 1.8 continues to be taken into account in determining timeliness. See 1.703(f). Consider "Express Mail Post Office to Addressee" (1.10) or facsimile transmission (1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.

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UNITED STATES DEPARTMENT OF COMMERCE
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www.uspto.gov

APPLICATION NUMBER	FILING OR 371(c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV

CONFIRMATION NO. 4026

53143
RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA02110

Title: Method for treating onychomycosis

Publication No. US-2007-0082375-A1

Publication Date: 04/12/2007

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publically available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <http://pair.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 703-305-3028.

Pre-Grant Publication Division, 703-605-4283



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/685,266	05/08/2007	7214506	700938-052220-DIV	4026

53143 7590 04/18/2007
RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 451 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Yoshiyuki Tatsumi, Otsu-shi, JAPAN;
Mamoru Yokoo, Otsu-shi, JAPAN;
Kosho Nakamura, Moriyama-shi, JAPAN;
Tadashi Arika, Suita-shi, JAPAN;

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE U.S. PATENT NO.: 7,214,506
ISSUED: May 8, 2007
INVENTORS: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura,
and Tadashi Arika
TITLE OF INVENTION: Method for Treating Onychomycosis
DOCKET NO.: P05724

Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

LETTER REGARDING REVOCATION AND POWER OF ATTORNEY

Sir:

Applicants submit the attached Revocation and Power of Attorney for the above referenced case.

Dated: July 16, 2014

Respectfully submitted,



Toan P. Vo
Attorney for Applicant
Registration No. 43,225
Telephone: 585-338-8071

Bausch & Lomb Incorporated
1400 North Goodman Street
Rochester, New York 14609
Dated: July 16, 2014

REVOCATION AND POWER OF ATTORNEY

I, Tetsuo Onuma , President & Representative Director , am a duly authorized representative of Kaken Pharmaceutical Co., Ltd., the sole assignee of U.S. Patent No. **7,214,506**, based upon U.S. Application Serial No. 10/685,266. I hereby revoke all existing authorization relating to the above referenced patent and appoint the following attorney(s) and/or agent(s) under Customer Number 23702 to prosecute this application for patent term extension under 35 U.S.C. § 156 and transact all business connected therewith:

Denis A. Polyn, Registration No. 27,152
Toan P. Vo, Registration No. 43,225

Please address all future correspondence to:

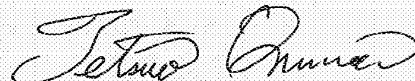
Toan P. Vo, Ph.D., Esq.
Bausch & Lomb Incorporated
1400 North Goodman Street
Rochester, New York 14609
(585) 338-8071

Dated: June 19, 2014

Signature of Applicant:

KAKEN PHARMACEUTICAL CO., LTD.

By:



Name: Tetsuo Onuma

Title: President & Representative Director

Electronic Acknowledgement Receipt

EFS ID:	19592828
Application Number:	10685266
International Application Number:	
Confirmation Number:	4026
Title of Invention:	Method for treating onychomycosis
First Named Inventor/Applicant Name:	Yoshiyuki Tatsumi
Customer Number:	53143
Filer:	Toan P. Vo/Judith Davis
Filer Authorized By:	Toan P. Vo
Attorney Docket Number:	700938-052220-DIV
Receipt Date:	16-JUL-2014
Filing Date:	14-OCT-2003
Time Stamp:	11:38:53
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	TransmittalletterandPOA.pdf	142770 <small>7eccfa238d9c9512ba3b4cf581b326248cae9605</small>	no	2

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Information:

Total Files Size (in bytes):

142770

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 04/24/2015

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01 FC : 1457 1,120.00 DA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE U.S. PATENT NO.: 7,214,506
ISSUED: May 8, 2007
INVENTORS: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and
Tadashi Arika
TITLE OF INVENTION: Method for Treating Onychomycosis

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee", mailing label ~~EK 358506655~~⁴⁵, in an envelope addressed to: Mail Stop: Hatch Waxman PTE, Director of the Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on July 16, 2014



Name: TOAN P. VO

Mail Stop: Hatch Waxman PTE
Director of the Patent and Trademark Office
P. O. Box 1450
Alexandria, VA 22313-1450

RECEIVED
JUL 16 2014
PATENT EXTENSION
OPLA

TRANSMITTAL LETTER FOR PATENT TERM EXTENSION APPLICATION

Sir:

Enclosed in triplicate is an application for the extension of U.S. Patent 7,214,506 under 35 U.S.C. § 156.

The Director is hereby authorized to charge the Application Fee of \$1,120.00 prescribed by 37 C.F.R. § 1.20(j)(1), as well as any additional fees which may be necessitated in connection with the filing of this Application for Patent Term Extension, to Applicant's Deposit Account No. 02-1425 in the name of Bausch & Lomb Incorporated. Two additional copies of

04/24/2015 CKHL0K 00000004 021425 10685266
01 FC:1457 1120.00 DA

this letter are being submitted for charging purposes.

Respectfully submitted,

A handwritten signature in black ink that reads "Toan P. Vo". The signature is written in a cursive style with a horizontal line underneath the name.

Toan P. Vo
Attorney for Applicant
Registration No. 43,225
Telephone: 585-338-8071

Bausch & Lomb Incorporated
1400 North Goodman Street
Rochester, New York 14609
Dated: July 15, 2014

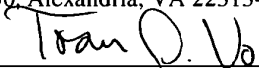
Encl: Patent Term Extension Application including Appendices A-H in triplicate
Two additional copies of this transmittal letter
Acknowledgment Receipt Card

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE U.S. PATENT NO.: 7,214,506
ISSUED: May 8, 2007
INVENTORS: Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and
Tadashi Arika
TITLE OF INVENTION: Method for Treating Onychomycosis

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee", mailing label ~~EK358506655 US~~ in an envelope addressed to: Mail Stop: Hatch Waxman PTE, Director of the Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on July 16, 2014


Name: TOAN P. VO

Mail Stop: Hatch Waxman PTE
Director of the Patent and Trademark Office
P. O. Box 1450
Alexandria, VA 22313-1450

PATENT TERM EXTENSION APPLICATION UNDER 35 U.S.C. §156

Sir:

Pursuant to 35 U.S.C. §156 and 37 C.F.R. §1.710 et seq., Kaken Pharmaceutical Co., Ltd. ("Applicant"), a corporation organized under the laws of Japan, hereby requests an extension of the patent term of U.S. Patent No. 7,214,506 ("the '506 Patent") due to regulatory review. The '506 Patent was granted on May 8, 2007.

Applicant asserts that it is the owner of the entire right, title and interest in the '506 Patent by virtue of an assignment from the inventors, Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika to the current assignee. This assignment was recorded in the United States Patent and Trademark Office on October 14, 2003, and is at Reel 014614, Frame 0495.

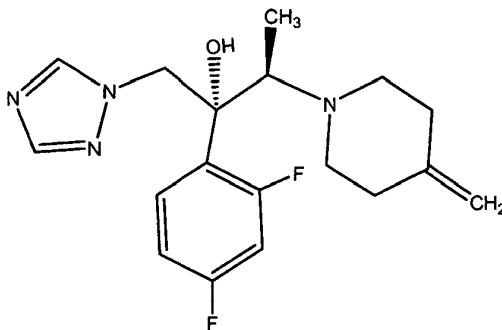
A copy of the assignment is attached hereto as Appendix A.

A copy of the Power of Attorney evidencing that Kaken Pharmaceutical Co., Ltd., the owner of the entire right, title and interest in the '506 Patent, has appointed Toan P. Vo as its agent with respect to this Application for Patent Term Extension, is attached hereto as Appendix B.

In accordance with 35 U.S.C. §156 and 37 C.F.R. §1.740, Applicant provides the following information in support of its request for a patent term extension. The following sections are numbered analogously to 37 C.F.R. § 1.740.

1. Identification of the Approved Product

The approved product is Jublia[®] Topical Solution, 10%, which contains the active ingredient efinaconazole having the chemical name (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylenepiperidin-1-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol, and having the chemical structure



2. Identification of the Federal Statute Under Which Regulatory Review Occurred

The approved product was subject to regulatory review under the Federal Food, Drug and Cosmetic Act, Section 505(b)(1) (21 U.S.C. §355(b)(1)).

3. The Date of Permission for Commercial Marketing

The approved product received permission for commercial marketing under Section 505(c) of the Federal Food, Drug and Cosmetic Act (21 U.S.C. §355(c)) on June 6, 2014. A copy of the United States Food and Drug Administration (FDA) approval letter is attached hereto as Appendix C.

4. Active Ingredient Statement

The sole active ingredient in Jublia[®] Topical Solution, 10%, is efinaconazole, which has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum Toxin Act prior to the approval of NDA 203567 by the FDA on June 6, 2014.

5. Statement of Timely Filing

The last day on which this application could be submitted is August 4, 2014, which is 60 days after the approval of NDA 203567 on June 6, 2014. This application is timely filed on or prior to August 4, 2014.

6. Identification of Patent for which Extension is Sought

This application seeks to extend the term of U.S. Patent No. 7,214,506, which (i) issued May 8, 2007 to Kaken Pharmaceutical Co., Ltd., (ii) has as inventors, Yoshiyuki Tatsumi, Mamoru Yokoo, Kosho Nakamura, and Tadashi Arika, and (iii) has a term which would otherwise expire on October 5, 2021.

7. Patent Copy

A complete copy of U.S. Patent No. 7,214,506, identified in paragraph 6 above, is attached as Appendix D.

8. Post-Issuance Activity Statement

No Reexamination certificate or Reissue has been issued or requested with respect to U.S. Patent No. 7,214,506. There are no Certificates of Correction or Terminal Disclaimers associated with the '506 Patent.

All prior maintenance fees due for the '506 Patent were paid. A copy of the USPTO's Patent Bibliographic Data Statement for the '506 Patent is attached hereto as Appendix E and shows that the eighth-year maintenance fee for the '506 Patent is payable, without a surcharge, between May 8, 2014 and November 11, 2014.

9. Statement Showing How the Claims of the Patent for Which Extension is Sought Cover the Approved Product

Claims 1-2 of the '506 Patent cover the approved method of use of the approved product, Jublia[®] Topical Solution, 10%, which is a topical solution having efinaconazole as its sole active ingredient. Efinaconazole has the chemical name (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylenepiperidin-1-yl)-1-(1H-1,2,4-triazol-1-yl)butan-2-ol.

Claim 1 claims a method of treating a subject having onychomycosis, wherein the method comprises topically administering to a nail of said subject a therapeutically effective amount of a compound belonging to a family of compounds including efinaconazole, which is the sole active ingredient in the approved product.

Claim 2 claims a method of treating a subject having onychomycosis, wherein the method comprises topically administering to a nail of said subject a therapeutically effective amount of efinaconazole, which is the sole active ingredient of the approved product.

10. Statement of the Relevant Dates to Determine the Regulatory Review Period

The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(A) The patent for which extension of the term thereof is sought claims an approved method of use of a human drug product. The human drug product is a composition containing efinaconazole.

(B) An Investigational New Drug Application for luliconazole was submitted on June 14, 2007, was received by the Department of Health and Human Services on June 15, 2007, was assigned IND No. 77,732 and became effective July 14, 2007. The original IND was filed for IDP-108 (or KP-103), which was the Applicant's internal identification of the drug product at the time of the IND submission. A copy of the IND letter from the FDA is attached as Appendix F.

(C) A New Drug Application for Clenafin™ Topical Solution, 10%, was received by the Department of Health and Human Services on July 26, 2012 and granted NDA No. 203567. A copy of the NDA letter from the FDA is attached as Appendix G. The FDA conditionally accepted the change of the drug product name from "Clenafin™" to "Jublia®" on April 15, 2013.

(D) NDA No. 203567 was approved on June 6, 2014.

11. Brief Description of Activities Undertaken During the Regulatory Review Period

As a brief description of the activities undertaken during the applicable regulatory review period, attached hereto as Appendix H is a chronology of the major communications between the U.S. Food and Drug Administration and the Applicant in IND No. 77,732 and NDA No. 203567.

12. Opinion of Eligibility for Extension and Length of Extension Claimed Under 37 C.F.R. §1.740(a)(12)

Applicant is of the opinion that U.S. Patent No. 7,214,506 is eligible for extension under 35 U.S.C. §156, 37 C.F.R. §1.710 and 37 C.F.R. §1.720 because U.S. Patent No. 7,214,506 satisfies all of the requirements for such extension as follows:

(a) 35 U.S.C. §156(a) and 37 C.F.R. §1.710(a)

U.S. Patent No. 7,214,506 claims a method of using efinaconazole, the active ingredient of a human drug product that contains the active ingredient, efinaconazole. MPEP 2751 states:

“A patent is eligible for extension of the patent term if the patent claims a product as defined in paragraph (b) of this section, either alone or in combination with other ingredients that read on a composition that received permission for commercial marketing or use, or a method of using such a product, or a method of manufacturing such a product, and meets all other conditions and requirements of this subpart.”

(b) 35 U.S.C. §156(a)(1) and 37 C.F.R. §1.720(g)

The term of U.S. Patent No. 7,214,506 (expiring October 5, 2021) has not expired before the submission of this application.

(c) 35 U.S.C. §156(a)(2) and 37 C.F.R. §1.720(b)

The term of the U.S. Patent No. 7,214,506 has never been extended under 35 U.S.C. §156(e)(1).

(d) 35 U.S.C. §156(a)(3) and 37 C.F.R. §1.720(c)

The application for extension of the term of U.S. Patent No. 7,214,506 is submitted by the authorized attorney of the owner of record thereof in accordance with the requirements of 35 U.S.C. §156(d) and 37 C.F.R. § 1.740.

(e) 35 U.S.C. §156(a)(4) and 37 C.F.R. §1.720(d)

The approved product, Jublia[®] Topical Solution, 10%, has been subjected to a regulatory review period before its commercial marketing or use.

(f) 37 C.F.R. §1.720(h)

No other patent has been extended for the same regulatory review period for the approved product, Jublia[®] Topical Solution, 10%.

(g) 35 U.S.C. §156(a)(5)(A) and 37 C.F.R. §1.720(e)(1)

The permission for the commercial marketing or use of the approved product, Jublia[®] Topical Solution, 10% is the first received permission for commercial marketing or use of Jublia[®] Topical Solution, 10% under the provision of law under which applicable regulatory review occurred.

The length of extension of the patent term of U.S. Patent No. 7,214,506 that is presently claimed by Applicant is 1601 days, which length was calculated in accordance with 37 C.F.R. §1.775 as follows:

(A) The regulatory review period under 35 U.S.C. §156(g)(1)(B) began on July 14, 2007 (the effective date of the IND) and ended on June 6, 2014, amounting to a total of 2520 days which is the sum of (i) and (ii) below:

(i) The period of review under 35 U.S.C. § 156(g)(1)(B)(i), the “Testing Period,” began on July 14, 2007 and ended on July 26, 2012, amounting to 1840 days.

(ii) The period of review under 35 U.S.C. §156(g)(1)(B)(ii), the “Application Period,” began on July 26, 2012 and ended on June 6, 2014, amounting to 681 days.

(B) The regulatory review period upon which the period for extension is calculated is the entire regulatory review period as determined in subparagraph (12)(g)(A) above (2520) less:

(i) The number of days in the regulatory review period which were on or before the date on which the patent issued (May 8, 2007), i.e., zero days, and

(ii) The number of days which the Applicant did not act with due diligence, i.e., zero days, and

(iii) One-half of the number of days remaining in the period in subparagraph (12)(g)(A)(i) after subtracting the number of days in subparagraphs (12)(g)(B)(i) and (12)(g)(B)(ii), which is one-half of 1840 or 920 days;

(iv) The sum of the periods determined in subparagraphs (12)(g)(B)(iii) and (12)(g)(A)(ii) is 1601 days.

(C) The number of days as determined in subparagraph (12)(g)(B), when added to the original term (October 5, 2021), would result in the date of February 22, 2026.

(D) Fourteen (14) years when added to the date of the NDA Approval Letter (June 6, 2014) would result in the date of June 6, 2028.

(E) The earlier date as determined by subparagraphs (12)(g)(C) and (12)(g)(D) is February 22, 2026.

(F) Since the original patent was issued after September 24, 1984, the extension otherwise obtainable is limited to not more than five (5) years. Five years, when added to the original expiration of U.S. Patent No. 7,214,560 (October 5, 2021) results in the date of October 5, 2026.

(G) The earlier date as determined 10 subparagraphs (12)(g)(E) and (12)(g)(F) is February 22, 2026.

13. Duty of Disclosure Acknowledgement Under 37 C.F.R. §1.740(a)(13)

Applicant acknowledges a duty to disclose to the Director of the Patent and Trademark Office and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought.

14. Fee Charge

The prescribed fee for receiving and acting upon this application is to be charged to Applicant's Deposit Account No. 02-1425 as authorized in the attached transmittal letter, submitted in triplicate.

15. Correspondence Address Required 37 C.F.R. §1.740(a)(15)

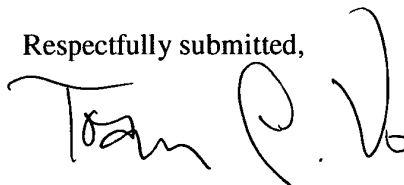
All correspondence relating to this application for patent term extension should be addressed to:

Toan P. Vo, Esq.
Bausch & Lomb Incorporated
1400 N. Goodman Street
Rochester, New York 14609

16. Certification Under 37 C.F.R. §1.740(A)(13)

The undersigned hereby certifies that the instant application, including its attachments and supporting papers, is being submitted as one original and two copies thereof in accordance with 37 C.F.R. §1.740(b).

Respectfully submitted,



Toan P. Vo, Esq.
Attorney for Applicant
Registration No. 43,225
Telephone: 585-338-8071

Bausch & Lomb Incorporated
1400 N. Goodman Street
Rochester, New York 14609
Dated: July 15, 2014

APPENDIX A

Copy of Assignment

APPENDIX B

Power of Attorney

REVOCATION AND POWER OF ATTORNEY

I, Tetsuo Onuma , President & Representative Director , am a duly authorized representative of Kaken Pharmaceutical Co., Ltd., the sole assignee of U.S. Patent No. **7,214,506**, based upon U.S. Application Serial No. 10/685,266. I hereby revoke all existing authorization relating to the above referenced patent and appoint the following attorney(s) and/or agent(s) under Customer Number 23702 to prosecute this application for patent term extension under 35 U.S.C. § 156 and transact all business connected therewith:

Denis A. Polyn, Registration No. 27,152
Toan P. Vo, Registration No. 43,225

Please address all future correspondence to:

Toan P. Vo, Ph.D., Esq.
Bausch & Lomb Incorporated
1400 North Goodman Street
Rochester, New York 14609
(585) 338-8071

Dated: June 19, 2014

Signature of Applicant:

KAKEN PHARMACEUTICAL CO., LTD.

By:



Name: Tetsuo Onuma

Title: President & Representative Director

APPENDIX C

FDA Approval Letter



NDA 203567

NDA APPROVAL

Dow Pharmaceutical Sciences, Inc.
Attention: Sean Humphrey
Manager, Regulatory Affairs
1330 Redwood Way
Petaluma, CA 94954

Dear Mr. Humphrey:

Please refer to your New Drug Application (NDA) dated and received on July 25, 2012, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act (FDCA) for Jublia (efinaconazole) topical solution, 10%.

We acknowledge receipt of your amendments dated August 6, 10, and 20, September 26, October 17 and 22, December 6, 7, 14, 19 and 20, 2012; January 9 and 17, March 18 and 29, December 20, 2013; January 16, February 4, May 16, 23 and 27, June 4, 2014.

The December 20, 2013, submission constituted a complete response to our May 13, 2013, action letter.

This new drug application provides for the use of Jublia (efinaconazole) topical solution, 10% for the topical treatment of onychomycosis of the toenails due to *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

We have completed our review of this application, as amended. It is approved, effective on the date of this letter, for use as recommended in the enclosed agreed-upon labeling text.

CONTENT OF LABELING

As soon as possible, but no later than 14 days from the date of this letter, submit the content of labeling [21 CFR 314.50(l)] in structured product labeling (SPL) format using the FDA automated drug registration and listing system (eLIST), as described at <http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm>. Content of labeling must be identical to the enclosed labeling (text for the package insert, text for the patient package insert, and text for the instructions for use). Information on submitting SPL files using eLIST may be found in the guidance for industry *SPL Standard for Content of Labeling Technical Qs and As*, available at

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072392.pdf>.

The SPL will be accessible via publicly available labeling repositories.

CARTON AND IMMEDIATE CONTAINER LABELS

Submit final printed carton and immediate container labels that are identical to the enclosed carton and immediate container labels as soon as they are available, but no more than 30 days after they are printed. Please submit these labels electronically according to the guidance for industry *Providing Regulatory Submissions in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (June 2008)*. Alternatively, you may submit 12 paper copies, with 6 of the copies individually mounted on heavy-weight paper or similar material. For administrative purposes, designate this submission “**Final Printed Carton and Container Labels for approved NDA 203567.**” Approval of this submission by FDA is not required before the labeling is used.

Marketing the product(s) with FPL that is not identical to the approved labeling text may render the product misbranded and an unapproved new drug.

ADVISORY COMMITTEE

Your application for Jublia (efinaconazole) topical solution, 10% was not referred to an FDA advisory committee because the application did not raise significant public health questions on the role of the drug in the diagnosis, cure, mitigation, treatment, or prevention of a disease.

REQUIRED PEDIATRIC ASSESSMENTS

Under the Pediatric Research Equity Act (PREA) (21 U.S.C. 355c), all applications for new active ingredients, new indications, new dosage forms, new dosing regimens, or new routes of administration are required to contain an assessment of the safety and effectiveness of the product for the claimed indication(s) in pediatric patients unless this requirement is waived, deferred, or inapplicable.

We are waiving the pediatric study requirement for ages 0 years to 11 years, 11 months because necessary studies are impossible or highly impracticable. There appears to be few culture positive cases of onychomycosis in subjects less than 12 years of age in the general population.

We are deferring submission of your pediatric study for ages 12 to less than 17 years for this application because this product is ready for approval for use in adults and the pediatric study has not been completed.

Your deferred pediatric study required by section 505B(a) of the FDCA is a required postmarketing study. The status of this postmarketing study must be reported annually according to 21 CFR 314.81 and section 505B(a)(3)(B) of the FDCA. This required study is listed below.

- 2156-1 A multicenter, randomized, double-blind study evaluating the safety, efficacy and pharmacokinetics of Jublia (efinaconazole) topical solution, 10% versus vehicle in

pediatric subjects ages 12 to less than 17 years with onychomycosis of the toenails

Final Protocol Submission: 09/14

Study Completion: 03/18

Final Report Submission: 09/18

Submit the protocol to your IND 077732, with a cross-reference letter to this NDA. Reports of this required pediatric postmarketing study must be submitted as a new drug application (NDA) or as a supplement to your approved NDA with the proposed labeling changes you believe are warranted based on the data derived from these studies. When submitting the reports, please clearly mark your submission "**SUBMISSION OF REQUIRED PEDIATRIC ASSESSMENTS**" in large font, bolded type at the beginning of the cover letter of the submission.

PROMOTIONAL MATERIALS

You may request advisory comments on proposed introductory advertising and promotional labeling. To do so, submit, in triplicate, a cover letter requesting advisory comments, the proposed materials in draft or mock-up form with annotated references, and the package insert to:

Food and Drug Administration
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion
5901-B Ammendale Road
Beltsville, MD 20705-1266

As required under 21 CFR 314.81(b)(3)(i), you must submit final promotional materials, and the package insert, at the time of initial dissemination or publication, accompanied by a Form FDA 2253. Form FDA 2253 is available at

<http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM083570.pdf>.

Information and Instructions for completing the form can be found at

<http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM375154.pdf>. For

more information about submission of promotional materials to the Office of Prescription Drug Promotion (OPDP), see <http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm090142.htm>.

REPORTING REQUIREMENTS

We remind you that you must comply with reporting requirements for an approved NDA (21 CFR 314.80 and 314.81).

MEDWATCH-TO-MANUFACTURER PROGRAM

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at <http://www.fda.gov/Safety/MedWatch/HowToReport/ucm166910.htm>.

POST APPROVAL FEEDBACK MEETING

New molecular entities and new biologics qualify for a post approval feedback meeting. Such meetings are used to discuss the quality of the application and to evaluate the communication process during drug development and marketing application review. The purpose is to learn from successful aspects of the review process and to identify areas that could benefit from improvement. If you would like to have such a meeting with us, call the Regulatory Project Manager for this application.

If you have any questions, call Strother D. Dixon, Regulatory Project Manager, at (301) 795-1015.

Sincerely,

{See appended electronic signature page}

Julie Beitz, MD
Director
Office of Drug Evaluation III
Center for Drug Evaluation and Research

Enclosures:

Content of Labeling
Carton and Container Labeling

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use JUBLIA safely and effectively. See full prescribing information for JUBLIA.

JUBLIA® (efinaconazole) topical solution, 10%

For topical use

Initial U.S. Approval: 2014

INDICATIONS AND USAGE

JUBLIA is an azole antifungal indicated for the topical treatment of onychomycosis of the toenails due to *Trichophyton rubrum* and *Trichophyton mentagrophytes*. (1)

DOSAGE AND ADMINISTRATION

- Apply JUBLIA to affected toenails once daily for 48 weeks using the integrated flow-through brush applicator. (2)
- When applying JUBLIA, ensure the toenail, the toenail folds, toenail bed, hyponychium, and the undersurface of the toenail plate, are completely covered. (2)
- For topical use only. (2)

- Not for oral, ophthalmic, or intravaginal use. (2)

DOSAGE FORMS AND STRENGTHS

Solution: 10%. (3)

CONTRAINDICATIONS

None. (4)

ADVERSE REACTIONS

The most common adverse reactions (incidence >1%) were ingrown toenails, application site dermatitis, application site vesicles, and application site pain. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Valeant Pharmaceuticals North America LLC at 1-800-321-4576 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See 17 for PATIENT COUNSELING INFORMATION and FDA-Approved Patient Labeling

Revised: 06/2014

FULL PRESCRIBING INFORMATION: CONTENTS*

- 1 INDICATIONS AND USAGE
- 2 DOSAGE AND ADMINISTRATION
- 3 DOSAGE FORMS AND STRENGTHS
- 4 CONTRAINDICATIONS
- 6 ADVERSE REACTIONS
 - 6.1 Clinical Trials Experience
- 7 DRUG INTERACTIONS
- 8 USE IN SPECIFIC POPULATIONS
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- 17 PATIENT COUNSELING INFORMATION

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

JUBLIA (efinaconazole) topical solution, 10% is an azole antifungal indicated for the topical treatment of onychomycosis of the toenail(s) due to *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

2 DOSAGE AND ADMINISTRATION

Apply JUBLIA to affected toenails once daily for 48 weeks, using the integrated flow-through brush applicator. When applying JUBLIA, ensure the toenail, the toenail folds, toenail bed, hyponychium, and the undersurface of the toenail plate, are completely covered.

JUBLIA is for topical use only and not for oral, ophthalmic, or intravaginal use.

3 DOSAGE FORMS AND STRENGTHS

JUBLIA (efinaconazole) topical solution, 10% contains 100 mg of efinaconazole in each gram of clear, colorless to pale yellow solution.

4 CONTRAINDICATIONS

None.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

In two clinical trials, 1227 subjects were treated with JUBLIA, 1161 for at least 24 weeks and 780 for 48 weeks. Adverse reactions reported within 48 weeks of treatment and in at least 1% of subjects treated with JUBLIA and those reported in subjects treated with the vehicle are presented in Table 1.

Table 1: Adverse Reactions Reported by at Least 1% of Subjects Treated for up to 48 Weeks

Adverse Event, n (%)	JUBLIA N = 1227	Vehicle N = 413
Ingrown toenail	28 (2.3%)	3 (0.7%)
Application site dermatitis	27 (2.2%)	1 (0.2%)
Application site vesicles	20 (1.6%)	0 (0.0%)
Application site pain	13 (1.1%)	1 (0.2%)

7 DRUG INTERACTIONS

In vitro studies have shown that JUBLIA, at therapeutic concentrations, neither inhibits nor induces cytochrome P450 (CYP450) enzymes.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C

There are no adequate and well-controlled studies with JUBLIA in pregnant women. JUBLIA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Systemic embryofetal development studies were conducted in rats and rabbits. Subcutaneous doses of 2, 10 and 50 mg/kg/day efinaconazole were administered during the period of organogenesis (gestational days 6-16) to pregnant female rats. In the presence of maternal toxicity, embryofetal toxicity (increased embryofetal deaths, decreased number of live fetuses, and placental effects) was noted at 50 mg/kg/day [559 times the Maximum Recommended Human Dose (MRHD) based on Area Under the Curve (AUC) comparisons]. No embryofetal toxicity was noted at 10 mg/kg/day (112 times the MRHD based on AUC comparisons). No malformations were observed at 50 mg/kg/day (559 times the MRHD based on AUC comparisons).

Subcutaneous doses of 1, 5, and 10 mg/kg/day efinaconazole were administered during the period of organogenesis (gestational days 6-19) to pregnant female rabbits. In the presence of maternal toxicity, there was no embryofetal toxicity or malformations at 10 mg/kg/day (154 times the MRHD based on AUC comparisons).

In a pre- and post-natal development study in rats, subcutaneous doses of 1, 5 and 25 mg/kg/day efinaconazole were administered from the beginning of organogenesis (gestation day 6) through the end of lactation (lactation day 20). In the presence of maternal toxicity, embryofetal toxicity (increased prenatal pup mortality, reduced live litter sizes and increased postnatal pup mortality) was noted at 25 mg/kg/day. No embryofetal toxicity was noted at 5 mg/kg/day (17 times the MRHD based on AUC comparisons). No effects on postnatal development were noted at 25 mg/kg/day (89 times the MRHD based on AUC comparisons).

8.3 Nursing Mothers

It is not known whether efinaconazole is excreted in human milk. After repeated subcutaneous administration, efinaconazole was detected in milk of nursing rats. Because many drugs are excreted in human milk, caution should be exercised when JUBLIA is administered to nursing women.

8.4 Pediatric Use

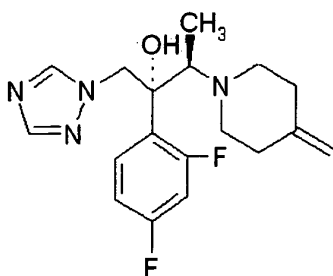
Safety and effectiveness of JUBLIA in pediatric subjects have not been established.

8.5 Geriatric Use

Of the total number of subjects in clinical trials of JUBLIA, 11.3% were 65 and over, while none were 75 and over. No overall differences in safety and effectiveness were observed between these subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and the younger subjects, but greater sensitivity of some older individuals cannot be ruled out.

11 DESCRIPTION

JUBLIA (efinaconazole) topical solution, 10% is a clear colorless to pale yellow solution for topical use. Each gram of JUBLIA contains 100 mg of efinaconazole. Efinaconazole is an azole antifungal with a chemical name of ((2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylenepiperidin-1-yl)-1-(1H-1,2,4-triazol-1-yl) butan-2-ol). The structural formula for efinaconazole is represented below:



Molecular Formula: C₁₈H₂₂F₂N₄O Molecular Weight: 348.39

JUBLIA contains the following inactive ingredients: alcohol, anhydrous citric acid, butylated hydroxytoluene, C12-15 alkyl lactate, cyclomethicone, diisopropyl adipate, disodium edetate, and purified water.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

JUBLIA topical solution is an azole antifungal [see *Clinical Pharmacology (12.4)*].

12.2 Pharmacodynamics

The pharmacodynamics of JUBLIA is unknown.

12.3 Pharmacokinetics

Systemic absorption of efinaconazole in 18 adult subjects with severe onychomycosis was determined after application of JUBLIA once daily for 28 days to patients 10 toenails and 0.5 cm adjacent skin. The concentration of efinaconazole in plasma was determined at multiple time points over the course of 24-hour periods on days 1, 14, and 28. Efinaconazole mean ± SD plasma C_{max} on Day 28 was 0.67 ± 0.37 ng/mL and the mean ± SD AUC was 12.15 ± 6.91 ng*h/mL. The plasma concentration versus time profile at steady state was generally flat over a 24-hour dosing interval. In a separate study of healthy volunteers, the plasma half-life of efinaconazole following daily applications when applied to all 10 toenails for 7 days was 29.9 hours.

Drug Interactions

JUBLIA is considered a non-inhibitor of the CYP450 enzyme family. In in vitro studies using human liver microsomes, efinaconazole did not inhibit CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2PE1 and CYP3A4 enzyme activities at expected clinical systemic

concentrations. In vitro studies in human primary hepatocytes showed that efinaconazole did not induce CYP1A2 or CYP3A4 activities.

12.4 Microbiology

Mechanism of Action

Efinaconazole is an azole antifungal. Efinaconazole inhibits fungal lanosterol 14 α -demethylase involved in the biosynthesis of ergosterol, a constituent of fungal cell membranes.

Activity In Vitro and In Vivo

Efinaconazole has been shown to be active against isolates of the following microorganisms, both in vitro and in clinical infections. Efinaconazole exhibits in vitro minimum inhibitory concentrations (MICs) of 0.06 μ g/mL or less against most (\geq 90%) isolates of the following microorganisms:

Trichophyton rubrum

Trichophyton mentagrophytes

Mechanism of Resistance

Efinaconazole drug resistance development was studied in vitro against *T. mentagrophytes*, *T. rubrum* and *C. albicans*. Serial passage of fungal cultures in the presence of sub-growth inhibitory concentrations of efinaconazole increased the MIC by up to 4-fold. The clinical significance of these in vitro results is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

A 2-year dermal carcinogenicity study in mice was conducted with daily topical administration of 3%, 10% and 30% efinaconazole solution. Severe irritation was noted at the treatment site in all dose groups, which was attributed to the vehicle and confounded the interpretation of skin effects by efinaconazole. The high dose group was terminated at week 34 due to severe skin reactions. No drug-related neoplasms were noted at doses up to 10% efinaconazole solution (248 times the MRHD based on AUC comparisons).

Efinaconazole revealed no evidence of mutagenic or clastogenic potential based on the results of two in vitro genotoxicity tests (Ames assay and Chinese hamster lung cell chromosome aberration assay) and one in vivo genotoxicity test (mouse peripheral reticulocyte micronucleus assay).

No effects on fertility were observed in male and female rats that were administered subcutaneous doses up to 25 mg/kg/day efinaconazole (279 times the MRHD based on AUC comparisons) prior to and during early pregnancy. Efinaconazole delayed the estrous cycle in females at 25 mg/kg/day but not at 5 mg/kg/day (56 times MRHD based on AUC comparisons).

14 CLINICAL STUDIES

The safety and efficacy of once daily use of JUBLIA for the treatment of onychomycosis of the toenail were assessed in two 52-week prospective, multi-center, randomized, double-blind clinical trials in patients 18 years and older (18 to 70 years of age) with 20% to 50% clinical involvement of the target toenail, without dermatophytomas or lunula (matrix) involvement. The trials compared 48-weeks of treatment with JUBLIA to the vehicle solution. The Complete Cure rate was assessed at Week 52 (4-weeks after completion of therapy). Complete cure was defined as 0% involvement of the target toenail (no clinical evidence of onychomycosis of the target toenail) in addition to Mycologic Cure, defined as both negative fungal culture and negative KOH. Table 2 lists the efficacy results for trials 1 and 2.

Table 2: Efficacy Endpoints

	Trial 1		Trial 2	
	JUBLIA	Vehicle	JUBLIA	Vehicle
	N = 656	N = 214	N = 580	N = 201
Complete Cure ^a	117 17.8%	7 3.3%	88 15.2%	11 5.5%
Complete or Almost Complete Cure ^b	173 26.4%	15 7.0%	136 23.4%	15 7.5%
Mycologic Cure ^c	362 55.2%	36 16.8%	310 53.4%	34 16.9%

^a Complete cure defined as 0% clinical involvement of the target toenail plus negative KOH and negative culture.

^b Complete or almost complete cure defined as ≤5% affected target toenail area involved and negative KOH and culture.

^c Mycologic cure defined as negative KOH and negative culture.

16 HOW SUPPLIED/STORAGE AND HANDLING

JUBLIA (efinaconazole) topical solution, 10% is a clear, colorless to pale yellow solution supplied in a white plastic bottle with an integrated flow-through brush applicator as follows:

- 4 mL (NDC 0187-5400-04)
- 8 mL (NDC 0187-5400-08)

Storage and Handling Conditions:

Store at 20°C - 25°C (68°F - 77°F); excursions permitted to 15°C - 30°C (59°F - 86°F) [*see USP Controlled Room Temperature*].

- Solution is flammable; keep away from heat or flame
- Protect from freezing
- Keep out of the reach of children
- Keep bottle tightly closed
- Store in upright position

17 PATIENT COUNSELING INFORMATION

See FDA-Approved Patient Labeling (Patient Information)

- JUBLIA is for external use only and is not for ophthalmic, oral, or intravaginal use. It is for use on toenails and immediately adjacent skin only.
- Apply JUBLIA once daily to clean dry toenails. Wait for at least 10 minutes after showering, bathing, or washing before applying.
- Use JUBLIA only on the affected toenails, as directed by your healthcare provider.
- Inform a health care professional if the area of application shows signs of persistent irritation (for example, redness, itching, swelling).
- Avoid pedicures, the use of nail polish, and cosmetic nail products while using JUBLIA.
- Flammable, avoid use near heat or open flame.

Manufactured for: Valeant Pharmaceuticals North America LLC, Bridgewater, NJ 08807 USA

Manufactured by: Kaken Pharmaceutical Co. Ltd, Shizuoka, Japan

Product of Japan

U.S. Patents 8,039,494; 7,214,506

9391900

Issued: 06/2014

PATIENT INFORMATION
JUBLIA (joo-blee-uh)
(efinaconazole) topical solution, 10%

Important information: JUBLIA is for use on toenails and surrounding skin only. Do not use JUBLIA in your mouth, eyes, or vagina.

What is JUBLIA?

JUBLIA is a prescription medicine used to treat fungal infections of the toenails. It is not known if JUBLIA is safe and effective in children.

What should I tell my healthcare provider before using JUBLIA?

Before you use JUBLIA, tell your healthcare provider about all your medical conditions, including if you:

- are pregnant or plan to become pregnant. It is not known if JUBLIA can harm your unborn baby.
- are breastfeeding or plan to breastfeed. It is not known if JUBLIA passes into your breast milk.

Tell your healthcare provider about all the medicines you take, including prescription and over-the-counter medicines, vitamins, and herbal supplements.

How should I use JUBLIA?

See the "Instructions for Use" at the end of this Patient Information leaflet for detailed information about the right way to use JUBLIA.

- Use JUBLIA exactly as your healthcare provider tells you to use it. Apply JUBLIA to your affected toenails 1 time each day. Wait for at least 10 minutes after showering, bathing, or washing before applying JUBLIA. JUBLIA is used for 48 weeks.

What should I avoid while using JUBLIA?

- JUBLIA is flammable. Avoid heat and flame while applying JUBLIA to your toenail.
- Avoid pedicures, use of nail polish, or cosmetic nail products, while using JUBLIA.

What are the possible side effects of JUBLIA?

JUBLIA may cause irritation at the treated site. The most common side effects include: ingrown toenail, redness, itching, swelling, burning or stinging, blisters, and pain. Tell your healthcare provider if you have any side effects that bother you or that does not go away.

These are not all the possible side effects of JUBLIA.

Call your doctor for medical advice about side effects. You may report side effects to the FDA at 1-800-FDA-1088.

How should I store JUBLIA?

- Store JUBLIA at room temperature, between 68°F to 77°F (20°C to 25°C). Do not freeze JUBLIA.
- Keep the bottle tightly closed and store in an upright position.
- JUBLIA is flammable. Keep away from heat and flame.

Keep JUBLIA and all medicines out of the reach of children.

General information about the safe and effective use of JUBLIA

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet. You can ask your pharmacist or healthcare provider for information about JUBLIA that is written for health professionals. Do not use JUBLIA for a condition for which it was not prescribed. Do not give JUBLIA to other people, even if they have the same condition you have. It may harm them.

What are the ingredients in JUBLIA?

Active ingredients: efinaconazole

Inactive ingredients: alcohol, anhydrous citric acid, butylated hydroxytoluene, C12-15 alkyl lactate, cyclomethicone, diisopropyl adipate, disodium edetate, and purified water.

Manufactured for: Valeant Pharmaceuticals North America LLC, Bridgewater, NJ 08807

Manufactured by: Kaken Pharmaceutical Co. Ltd, Shizuoka, Japan. Product of Japan

For more information, call 1-800-321-4576.

This Patient Information has been approved by the U.S. Food and Drug Administration.

Issued: 06/2014

Instructions for Use
JUBLIA® (joo-blee-uh)
(efinaconazole) topical solution, 10%

Important information: JUBLIA is for use on toenails and surrounding skin only. Do not use JUBLIA in your mouth, eyes or vagina.

Read the Instructions for Use that comes with JUBLIA before you start using it. Talk to your healthcare provider if you have any questions.

How to apply JUBLIA:

Your toenails should be clean and dry before you apply JUBLIA.

Step 1: Before you apply JUBLIA to your affected toenail, remove the cap from the JUBLIA bottle (**See Figure A**).



Figure A

Step 2: Hold the bottle directly over the affected toenail and gently squeeze the bottle to apply one drop of JUBLIA onto the toenail (**See Figure B**).



Figure B

Step 3: For the big toenail, also apply a second drop to the end of the toenail (**See Figure C**).

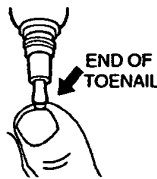


Figure C

Step 4: Use the brush attached to the bottle to gently spread JUBLIA around the entire toenail including: the cuticle, folds of the skin next to the sides of the toenail, and underneath the nail (**See Figure D**). Do not squeeze the bottle while spreading JUBLIA with the brush.



Figure D

Step 5: Repeat Steps 2 to 4 to apply JUBLIA to each affected toenail.

Step 6: Let JUBLIA dry completely.

Step 7: After applying JUBLIA to your affected toenails, place the cap on the bottle and screw it on tightly.

Step 8: Wash your hands with soap and water after applying JUBLIA.

This Patient Information and Instructions for Use has been approved by the U.S. Food and Drug Administration.

Manufactured for: Valeant Pharmaceuticals North America LLC, Bridgewater, NJ 08807 USA
Manufactured by: Kaken Pharmaceutical Co. Ltd, Shizuoka, Japan.

Product of Japan

Issued: 06/2014



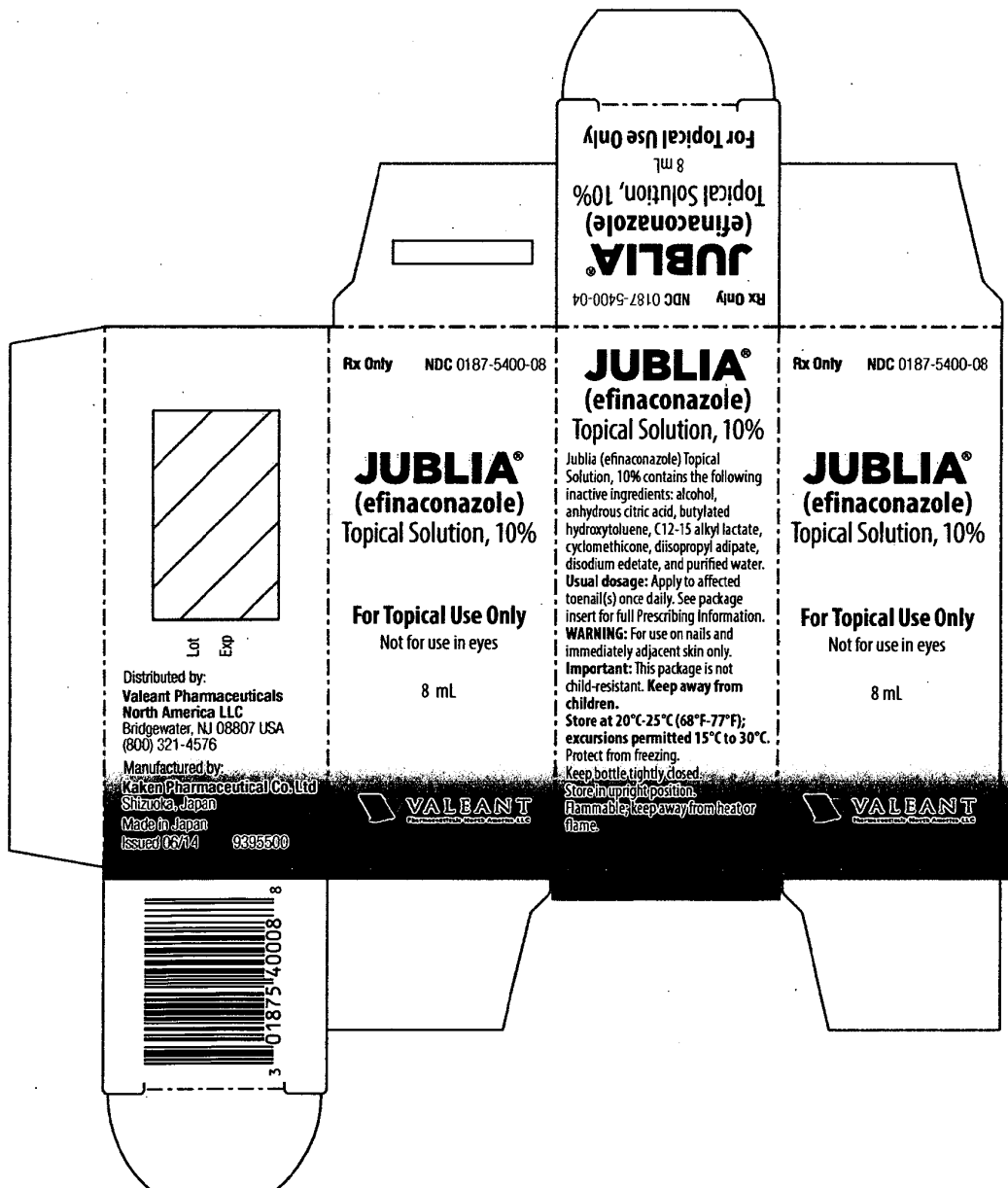
VALEANT - US Pharmaceutical Labeling Group - 1400 North Goodman Street - Rochester, NY 14609 - USA
 GRAPHICS CONTACT: Renee Condon - e: renee.condon@bausch.com - t: 585.338-8252

DESCRIPTION: 8 mL Jublia Topical Solution 10% Carton, US (PPS)
PART No.: 9395500
SPECIAL INSTRUCTIONS / PLACED IMAGES: n/a

DIELINE DOES NOT PRINT

ARTWORK SET AT 100%

BLACK 1	PMS 2597 2	PMS 2613 3					
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VALEANT - US Pharmaceutical Labeling Group - 1400 North Goodman Street - Rochester, NY 14609 - USA
 GRAPHICS CONTACT: Renee Condon - e: renee.condon@bausch.com - t: 585.338.8252

DESCRIPTION: 8 mL Jublia Topical Solution 10% Label, US (PPS)
 PART No.: 9395400
 SPECIAL INSTRUCTIONS / PLACED IMAGES: n/a

DIELINE DOES NOT PRINT

ARTWORK SET AT 100%

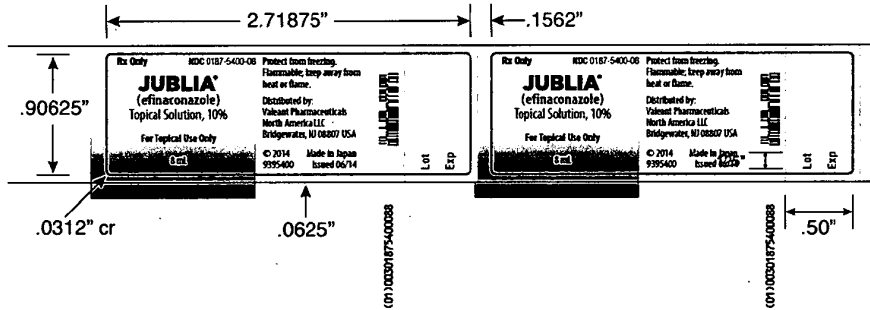


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DESCRIPTION: 4 mL Jublia Topical Solution 10% Carton, US (PPS)

PART No.: 9391100

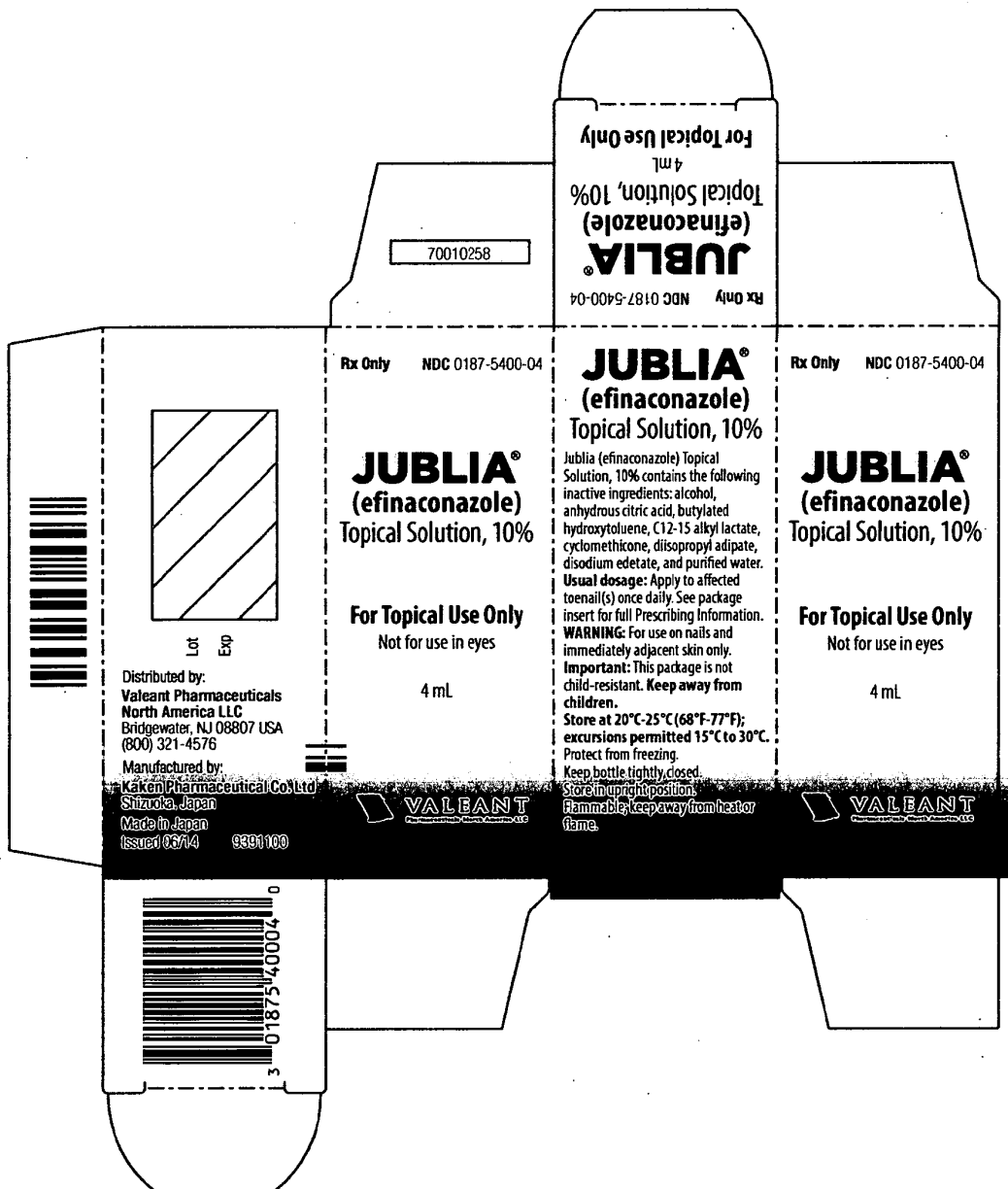
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DESCRIPTION: 4 mL Jublia Topical Solution 10% Label, US (PPS)
 PART No.: 9391200
 SPECIAL INSTRUCTIONS / PLACED IMAGES: n/a

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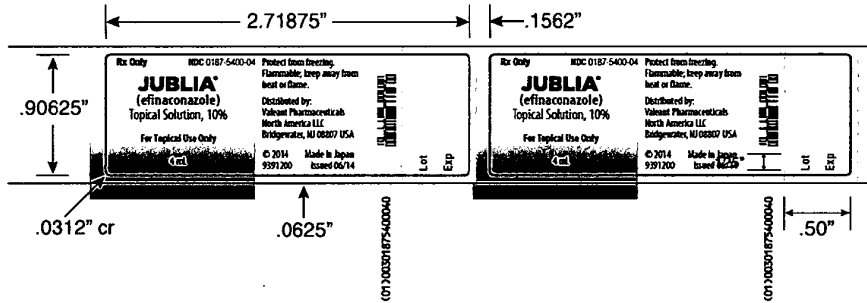


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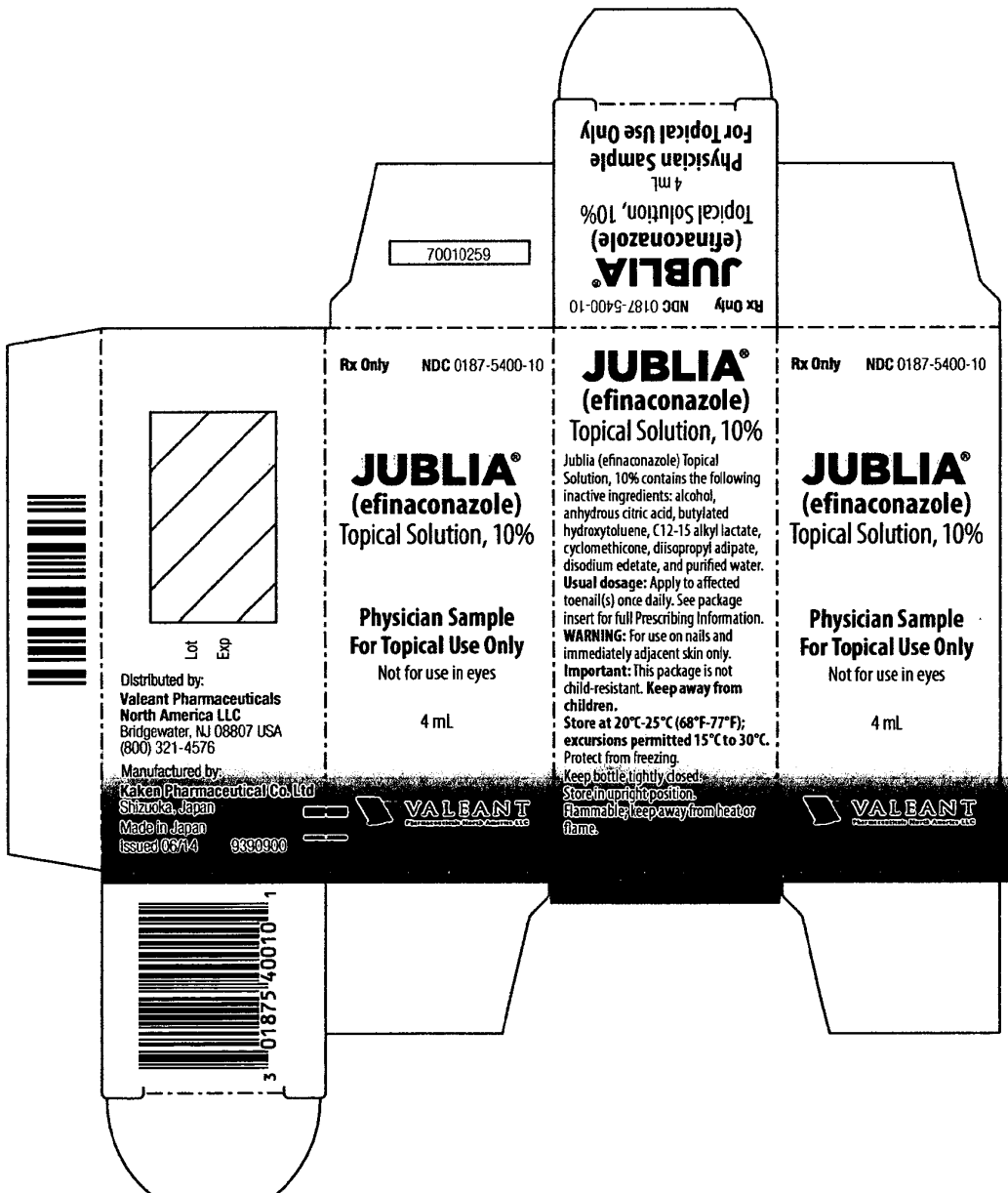
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DESCRIPTION: 4 mL Jublia Topical Solution 10% Sample Carton, US (PPS)
PART No.: 9390900
SPECIAL INSTRUCTIONS / PLACED IMAGES: n/a

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DESCRIPTION: 4 mL Jublia Topical Solution 10% Sample Label, US (PPS)
 PART No.: 9391000
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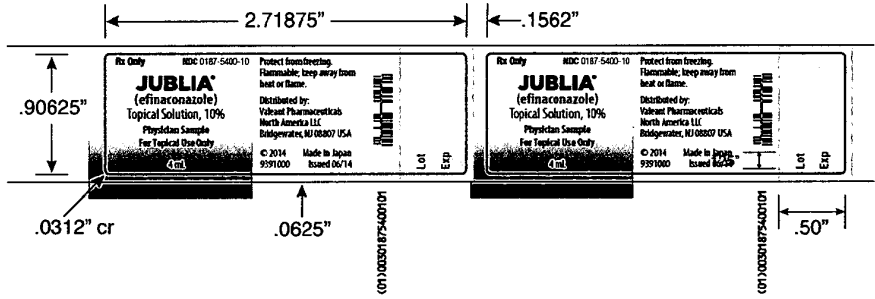


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JULIE G BEITZ
06/06/2014

APPENDIX D

Copy of U.S. Patent 7,214,506



US007214506B2

(12) **United States Patent**
Tatsumi et al.

(10) **Patent No.:** **US 7,214,506 B2**
(45) **Date of Patent:** **May 8, 2007**

(54) **METHOD FOR TREATING ONYCHOMYCOSIS**
(75) **Inventors:** **Yoshiyuki Tatsumi, Otsu (JP); Mamoru Yokoo, Otsu (JP); Koshi Nakamura, Moriyama (JP); Tadashi Arika, Suita (JP)**
(73) **Assignee:** **Kaken Pharmaceutical Co., Ltd., Bunkyo-ku, Tokyo (JP)**
(*) **Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 451 days.

(21) **Appl. No.:** **10/685,266**

(22) **Filed:** **Oct. 14, 2003**

(65) **Prior Publication Data**
US 2007/0082375 A1 Apr. 12, 2007

Related U.S. Application Data
(62) Division of application No. 10/031,929, filed as application No. PCT/JP00/04617 on Jul. 11, 2000, now abandoned.

(30) **Foreign Application Priority Data**
Jul. 28, 1999 (JP) 11/214369

(51) **Int. Cl.**
C12Q 1/18 (2006.01)
A01N 43/26 (2006.01)
A01N 43/34 (2006.01)
A61K 31/445 (2006.01)

(52) **U.S. Cl.** **435/32; 514/326**
(58) **Field of Classification Search** **435/32; 514/326**

See application file for complete search history.

(56) **References Cited**
U.S. PATENT DOCUMENTS
5,620,994 A * 4/1997 Naito et al. 514/326
5,716,969 A * 2/1998 Naito et al. 514/326
5,962,476 A 10/1999 Naito et al.

FOREIGN PATENT DOCUMENTS
EP 0 402 989 A2 12/1990
JP 8-103291 4/1996
JP 10-28597 2/1998
WO WO 94/26734 11/1994
WO WO 99/39680 A1 8/1999

OTHER PUBLICATIONS
Kitazaki Tomoyuki et al., Chem. Pharm. Bull, vol. 44 (No. 2), p. 314-327, (Feb. 1996).
Ogura, Hironobu et al., Chem. Pharm. Bull, vol. 47 (No. 10), p. 1417-1425, (Oct. 1999).

* cited by examiner
Primary Examiner—Jon Weber
Assistant Examiner—Kailash C. Srivastava
(74) *Attorney, Agent, or Firm*—Nixon Peabody LLP

(57) **ABSTRACT**
A novel method for evaluating an effect of an antimicrobial agent which comprises removing the antimicrobial agent remaining in a biological sample or the like to thereby accurately evaluate the effect of the antimicrobial agent without being affected by the remaining antimicrobial agent. A therapeutic agent for onychomycosis which can be obtained according to the evaluation method of the drug effect.

2 Claims, 4 Drawing Sheets

FIG. 1(a)

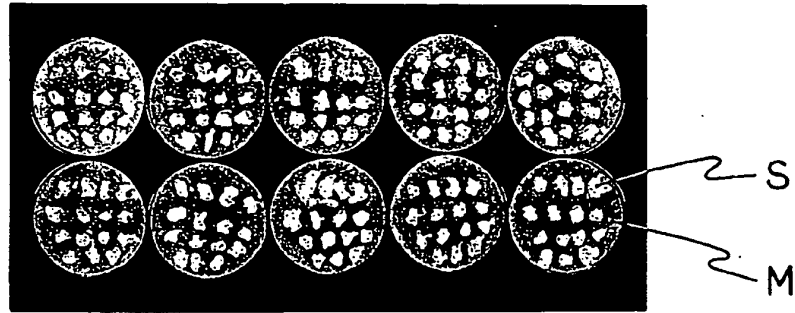


FIG. 1(b)

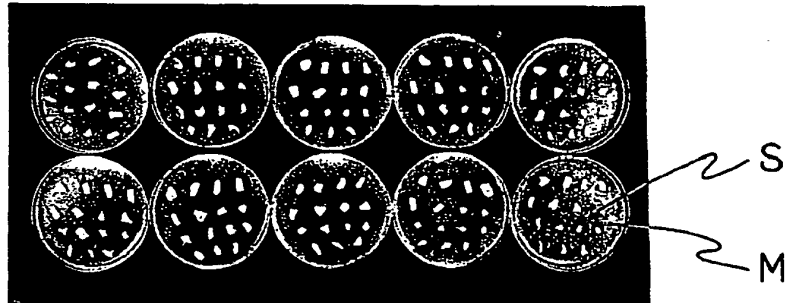


FIG. 1(c)

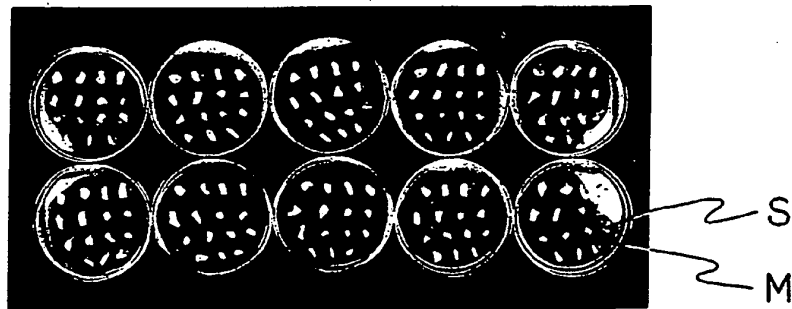


FIG. 2(a)

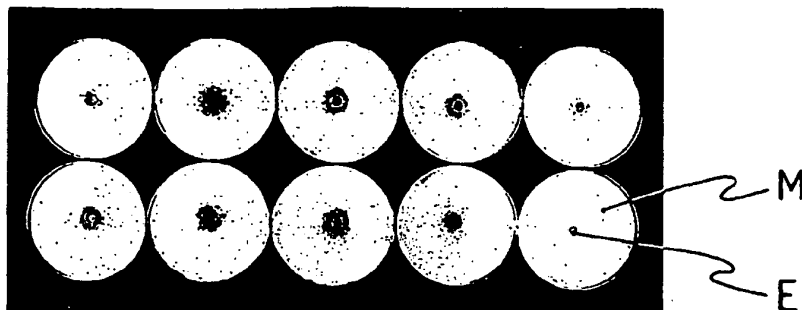


FIG. 2(b)

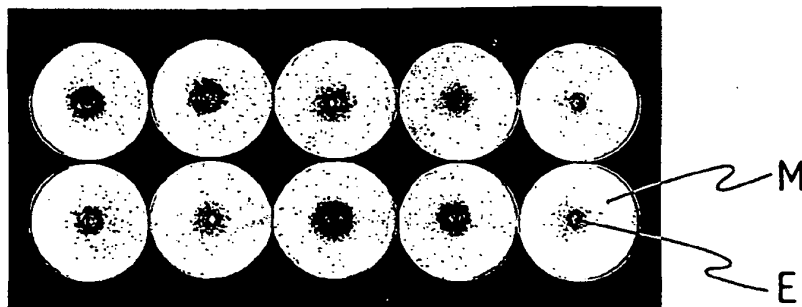


FIG. 2(c)

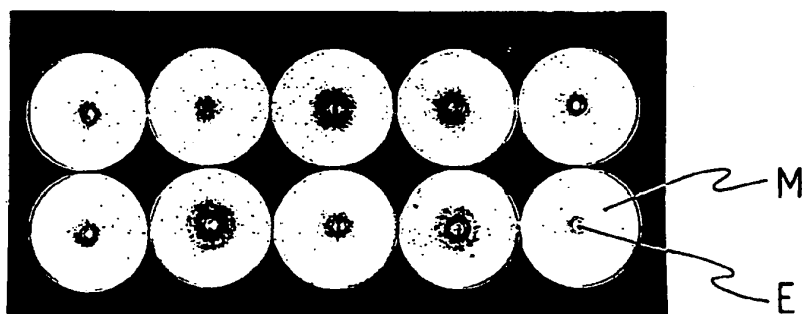


FIG. 3

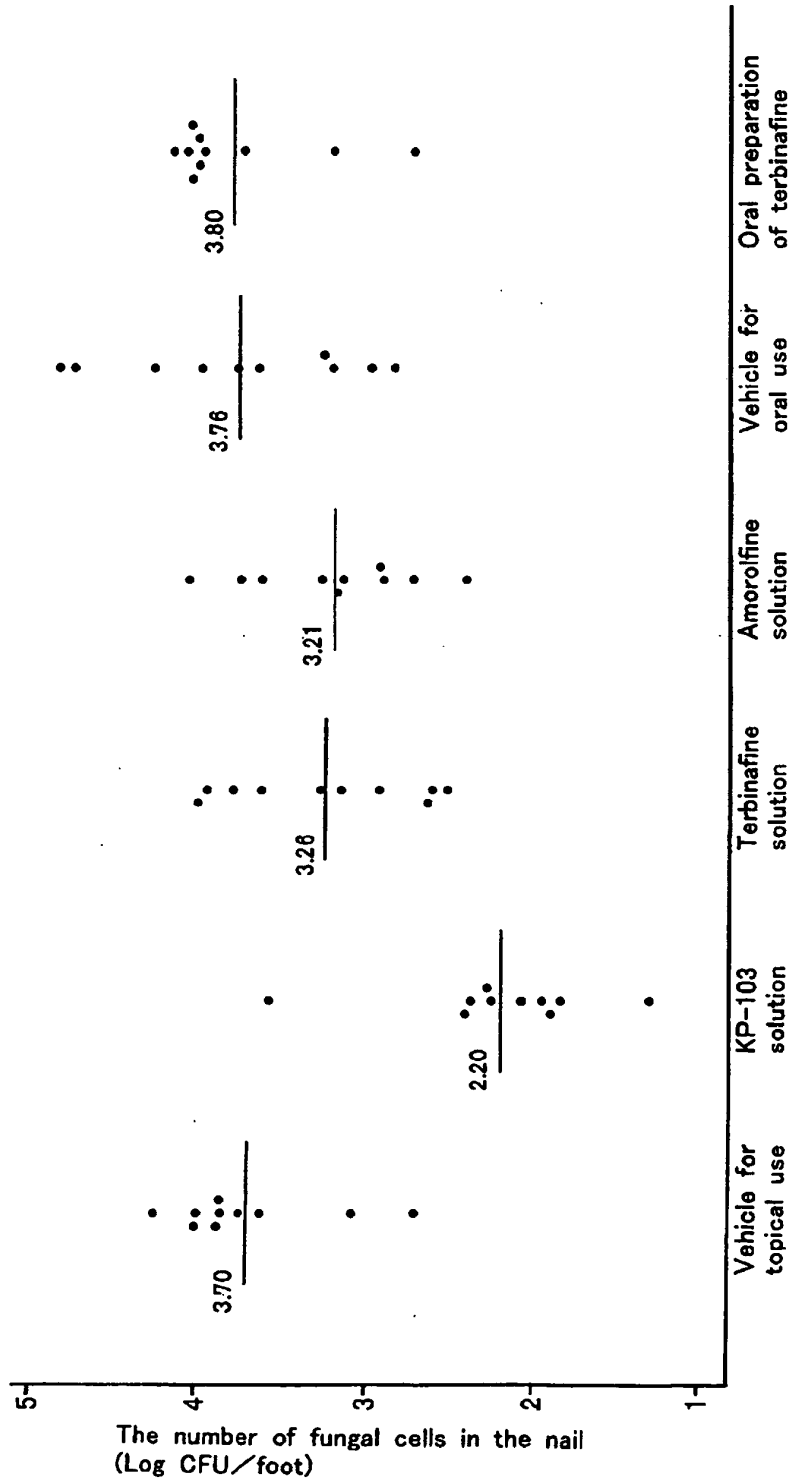
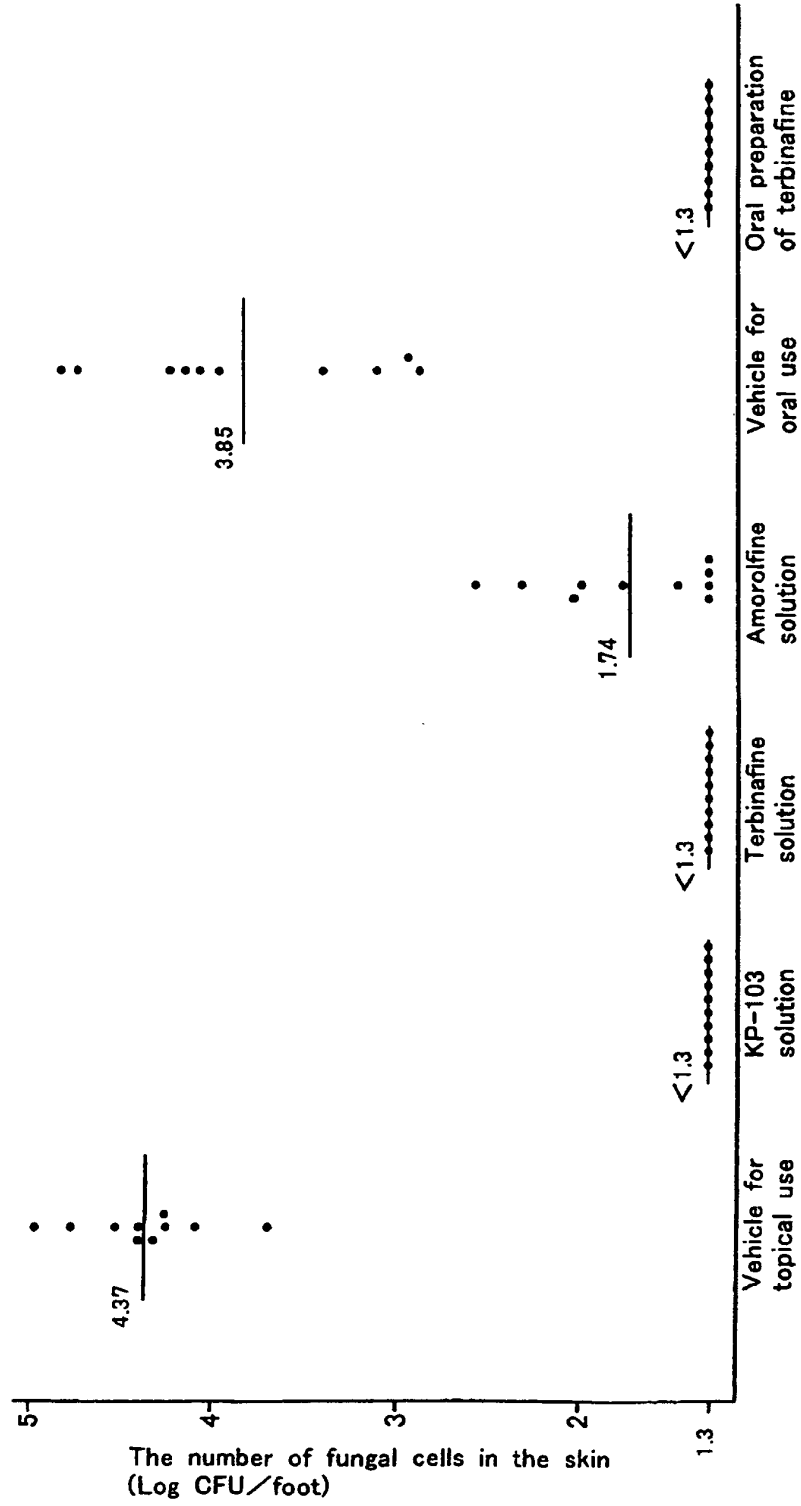


FIG. 4



1

METHOD FOR TREATING ONYCHOMYCOSIS

This application is a divisional under 35 U.S.C. § 120 of U.S. Non-Provisional application Ser. No. 10/031,929 filed 25 Jan. 2002, now abandoned, which was a National Stage filing under 35 U.S.C. § 371 of PCT/JP00/04617 filed 11 Jul. 2000, which claimed priority to Japanese patent application Ser. No. 11/214,369 filed 28 Jul. 1999.

TECHNICAL FIELD

The present invention relates to a method for detecting pathogenic microorganism, method for evaluating an effect of an antimicrobial agent on pathogenic microorganism and a method for detecting an antimicrobial agent. The present invention also relates to an antimicrobial agent and a therapeutic agent for onychomycosis, which are obtained according to the above-mentioned method for evaluating the drug effect.

BACKGROUND ART

A method for evaluating a drug effect with an animal model is needed in order to explore a novel antimicrobial agent (also hereinafter referred to "drug"). Further, a method enabling a drug effect to be evaluated with accuracy is needed because of grate importance in view of predicting a clinical therapeutic efficiency thereof.

Historically, an experimental dermatophytosis model that back, planta and interdigital of a guinea pig have been infected with *Trichophyton mentagrophytes* has been used in order to evaluate an effect of an antifungal agent on dermatophytosis. Such animal models have been already employed to develop some antifungal agent. The evaluation of the effect of such antifungal agent carried out by applying the antifungal agent to the infected animal, by excising the skin after the certain period of time to cut into plural small pieces, by cultivating the skin pieces on the medium, and by counting the number of pieces wherein no growth of fungus is seen or the number of animals or feet wherein no growth of fungus is seen in all skin pieces, as an indicator (Antimicrobial Agents and Chemotherapy, 36: 2523-2525, 1992, 39: 2353-2355, 1995). Hereinafter, the conventional method for evaluating the drug effect is referred to as "the conventional method".

Although the drug having a potent activity against *Trichophyton* in vitro such as itraconazole or amorolfine has been marketed in these days, an improvement of cure rate in a clinical use is hardly seen. As a main reason thereof, a relapse that since fungus in the skin is not completely killed after a treatment, the fungus grow again is pointed.

In also animal experiments, when an effect of itraconazole on guinea pig models of tinea pedis was evaluated using the conventional method, though "fungus-negative" was observed in all feet out of 20 feet 2 days after the last treatment, a relapse was observed in 11 out of 20 feet 30 days after the last treatment, and no correlation was seen between the effect 2 days after the last treatment and the effect 30 days after the last treatment (36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 1996, Abstr. F80).

As a reason thereof, there were followings. Since itraconazole have very potent antitrichophyton activity in vitro, itraconazole persisted in the skin 2 days after the last treatment in the concentration wherein the sterilization effect was shown. Therefore, when the skin is excised and culti-

2

ated on the medium to detect fungus, the itraconazole remaining in the skin is diffused in the medium, and therefore, no fungus was detected due to prevention of the growth regardless of the presence of viable fungus in the excised skin. On the other hand, since the concentration of the drug remained in the skin is reduced 30 days after the last treatment, fungus in the skin can grow again and can be detected by culture study.

According to this hypothesis, it is ascertained that the drug remain in the skin through the inhibition of the growth of fungus around the skin blocks completely, when the itraconazole-treated skin blocks were located and cultivated on the medium which contains dermatophytes.

Therefore, it became to clear that the conventional method has the problem that the drug effect can not be accurately evaluated, because the apparent therapeutic effect need to be evaluated after removing the drug remaining in the skin.

Meanwhile, a kind of mycosis, dermatophytosis, is the superficial dermatosis which is caused by dermatophyte parasitizing the keratin such as skin (stratum corneum), the nail and the hair. In particular, tinea unguium formed in the nail is known as the intractable disease among dermatomycoses based on dermatophytoses, and is accompanied by symptom such as opacity, tylosis, destruction and deformation of nail plate. Now the oral preparation (such as griseofulvin or terbinafine) is used for the treatment of such tinea unguium. However, there are many cases where the patient stops taking the drug or that takes the drug irregularly, since they have to take the drug for a long period, for example at least a half a year in order to completely cure tinea unguium. It is thought that this is a main cause of difficulty of curing tinea unguium completely. Furthermore, by taking the drug for a long period, griseofulvin has the problem of side effects on internal organ (gastrointestinal disorder, hepatotoxicity) and hepatotoxicity is reported as the side effect in terbinafine. Therefore, in order to improve the compliance of the patient it is desired to develop a topical preparation which cure tinea unguium for a short period and has less the systemic side effect than the oral preparation.

However, in case of the simple application on nail plate with the current antifungal agent for topical use, the antifungal effect on fungus in the nail was not seen, because the drug could not sufficiently permeate the thick keratin in nail plate (Markus Niewerth and Hans C. Korting, Management of Onychomycoses, Drugs, 58: 283-296, 1999).

In addition, the therapeutic effect of a topical preparation of antifungal agent on the experiment model of trichophytosis can not be evaluated using the conventional method as mentioned above. This may be a reason why the drug effect on the guinea pig model of tinea unguium has been hardly reported.

DISCLOSURE OF INVENTION

The present invention has been accomplished based on findings that it is desirable that an effect of antimicrobial agent such as particularly antifungal agent is evaluated after removing a drug remaining in the infected site after treatment of an animal or a biosample such as skin with the pathogenic microorganism. An object of the present invention is to provide a novel method for evaluating the effect of the antimicrobial agent and the antimicrobial agent obtained according to the method for evaluating the drug effect. In detail, the present invention provides the method for detecting the viable pathogenic microorganism in the above-mentioned infected site of the animal or the biosample with the pathogenic microorganism after removing the antimi-

In the present invention, the term "pathogenic microorganism" means a microorganism which causes human and animal disease in one way or another. An example of the pathogenic microorganism (hereinafter referred to "microorganism") is bacteria including aerobic Gram-negative bacillus and coccus such as *Pseudomonas* and *Neisseriaceae* species; facultative anaerobic Gram-negative bacillus such as *Escherichia*, *Salmonella* and *Enterobacter* species; Gram-positive coccus such as *Staphylococcus* and *Streptococcus* species. The other examples of microorganism are fungi including Hyphomycetes such as *Trichophyton*, *Microsporium* and *Epidermophyton* species; Blastomycetes such as *Candida* and *Malassezia*; Ascomycetes such as *Aspergillus* species; Zygomycetes such as *Mucor* species; and variants thereof. Examples of such variants are resistant strain which naturally obtains drug resistance; auxotrophic mutation strain which comes to have nutritious dependency; artificial mutation strain which is artificially mutated by treatment with mutagenic agent; and the like.

Mycosis means a disease which is caused by invading and proliferating in the tissue of human or animal. Usually, mycosis is broadly divided into superficial mycosis and deep mycosis. A seat of the disease lie in the skin or visible mucosa in case of the former; in viscus, central nervous system, subcutaneous tissue, muscle, bone or articulation in case of the latter. Chief example of superficial mycosis is dermatophytosis which is caused by infecting with dermatophyte such as *Trichophyton*, *Microsporium* and *Epidermophyton* species, including three disease, tinea, tinea favosa and tinea imbricata. Tinea may be conventionally employed a synonymous with dermatophytosis. In addition, dermatophyte belonging to *Trichophyton* species is referred usually to as trichophytosis.

In the present invention, an antimicrobial agent means a compound having an antimicrobial effect or a composition containing the compound. The composition includes a preparation form being artificial composition and a natural composition such as a natural product.

A method for administration of the antimicrobial agent in the present invention depends on the kind thereof and includes topical application, subcutaneous administration, oral administration, intravenous administration or the like.

When the method for detecting the pathogenic microorganism, the method for evaluating the drug effect and the method for detecting the antimicrobial agent according to the present invention is carried out, either an infection with microorganism or an administration of the antimicrobial agent may be carried out first. Especially, in the method for evaluating the drug effect of the present invention (hereinafter referred to "the present evaluation method"), a therapeutic effect of the antimicrobial agent can be evaluated in case where the antimicrobial agent is administered after the infection with microorganism, meanwhile, a effect of the antimicrobial agent protecting from the infection and the retention capacity thereof can be evaluated in case where the infection with microorganism is carried out after the administration of the antimicrobial agent. In order to evaluating the retention capacity of the antimicrobial agent, the evaluation can be carried out with varying the period until infection with microorganism from the administration of the antimicrobial agent.

In the present invention, it is preferable to use dialysis or ultra filtration for removing the antimicrobial agent in view point of the usefulness, but not limited thereto as long as a microorganism to be a detecting target or a microorganism used in the present evaluation method and the like is not affected by it.

In dialysis, a marketed dialysis membrane made of cellulose is convenient. A membrane made of the other material can be used without problem, as long as the microorganism to be the detecting target or the microorganism used in the present evaluation method and the like can not be passed, and the antimicrobial agent can be passed through it. Since sizes of most fungi and bacteria are at least 0.2 μm , it is preferable to use the membrane having less than 0.2 μm of the pore size, particularly it is suitable to use dialysis membrane having fractional molecular weight of 1,000 to 50,000.

As out side solutions used in dialysis, there include physiological saline, distilled water, phosphate buffered physiological saline, the other buffer and the like.

In removing the antimicrobial agent according to the present invention, even though the infected site with the microorganism is the nail, organ or the like as well as the skin, the antimicrobial agent can be efficiently removed. Usually, since there is the case where it takes longer time dialysis to remove the antimicrobial agent from nail than skin, the following treatment with digestive enzyme may be carried out before removing it in order to enhance the removal effect.

Dialysis conditions depend on variety, dose concentration, dose term and the drug holidays (the term until evaluation from last day of treatment) of an antimicrobial agent. Therefore, it is preferable to previously investigate the dialysis conditions enabling the antimicrobial agent to be removed from the treated skin about individual cases using the following detecting method of the existing antimicrobial agent in the infected site with a microorganism in the present invention (hereinafter referred to "the present method for detecting an agent") to adjust the conditions appropriately.

Whether an antimicrobial agent has been removed can be easily determined using the following method.

The present method for detecting an agent is carried out by placing and cultivating the infected site with a microorganism which is subjected to the removing method of the antimicrobial agent (e.g. an skin piece) or a suspension obtained according to the following extraction procedure of the microorganism from the above skin piece on an agar medium containing the microorganism, and observing a growth inhibition of the microorganism found around it. When there is the remaining antimicrobial agent, the growth inhibition of the microorganism is observed.

The present evaluation method can be carried out by locating and cultivating, on a medium, the skin piece in which a removal of an antimicrobial agent has been determined using the above-mentioned present method for detecting the agent after carrying out the appropriate removal of the antimicrobial agent and observing whether there is a growth of microorganism or not, or by smearing and cultivating a suspension obtained according to the extraction procedure of the microorganism from the skin piece on an agar medium and observing whether there is the growth of microorganism or not or counting colonies emerging on those medium.

A treatment with trypsin can be carried out in order to extract a microorganism efficiently from a biosample such as a skin or a nail. Other digestive enzyme than trypsin such as pronase or keratinase, or a keratin resolvent such as urea also can be used without limitation to trypsin as long as they have an extraction effect. It is necessary to adjust concentrations of the digestive enzyme such as trypsin and keratin resolvent in a treating solution, and reaction time to no affect range to a microorganism. The treatment with digestive enzyme such as trypsin may be carried out either before or after dialysis.

When the treatment with trypsin is carried out before dialysis, it is necessary to remove the digestive enzyme sufficiently so that the microorganism is not affected on dialysis.

As a medium used for a cultivation of a microorganism in the present invention, any medium can be used as long as it can be conventionally used for the cultivation and a separation of the microorganism. In case of fungi, example of the medium is Sabouraud medium, modified Sabouraud medium, Czapek agar medium, Potato dextrose agar medium or the like. On the other hand, in case of bacteria, example of the medium is Mueller Hinton medium, modified Mueller Hinton medium, Heart Infusion agar medium, Brain Heart Infusion agar medium, normal agar medium or the like.

A reacting temperature is 10 to 40° C., preferably 20 to 40° C. A microorganism may be cultivated with standing during a sufficient time when the microorganism can be growth, for example, 1 to 20 days in case of fungi, 1 to 5 days in bacteria.

The present evaluation method be utilizable as a evaluation method of a drug effect in exo vivo which comprises infecting a skin, a nail excised from an animal body with a microorganism, thereafter administering an antimicrobial agent as a test compound, then removing the antimicrobial agent and detecting and determining quantity of the microorganism in the sample.

The present evaluation method also can be applied to an evaluation of an antimicrobial agent such as a therapeutic agent for deep mycosis or an antibacterial agent as well as an evaluation of an effect of a therapeutic agent for superficial mycosis. That is to say, it is possible to evaluate an effect of a therapeutic agent for deep mycosis or an antibacterial agent by means of administering an antimicrobial agent to an animal infected with a microorganism such as a fungus or a bacterium by inoculating percutaneously, orally, intravenously, transbronchially, transnasally, intraperitoneally, then obtaining biosample such as skin, kidney, lung or brain, and detecting the viable microorganism in the biosample in which removed the remaining antimicrobial agent has been removed.

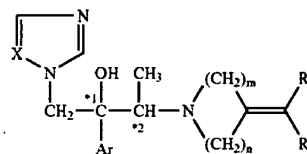
In addition, the present evaluation method enables a quantitative comparison of antimicrobial effects by means of determining the number of viable microorganisms in the treated biosample.

That is to say, a significant deference test is carried out about the number of microorganisms in the infected site with the microorganism for the treated group with drug and for the reference infected group using a statistical method such as Kruskal-Wallis Test, and thereby a quantitative comparison between the groups can be done by using a multiple test such as Tukey method.

The present invention is useful as either a method for evaluating a drug effect or a method for detecting the antimicrobics in keratin substance or nail, after administering the antifungus to the patient infected with fungus. For example, according to the present invention, an effect of an antifungal agent can be evaluated by administering it to the patient whose skin or nail is infected with fungus, obtaining the keratin substance or nail, then removing the above-mentioned antifungal agent, and thereafter detecting the viable fungus in the keratin substance or nail. Additionally, according to the present invention, a detection of an antifungal agent can be carried out by administering it to the patient whose skin or nail is infected with fungus, then obtaining the keratin substance or nail, cultivating it on agar medium containing fungus, and thereafter detecting the

existing antifungal agent in the keratin substance or nail through a growth inhibition of fungus observed around the keratin substance or nail. Such evaluation of an antifungal agent administered to a patient with fungus and detection of the antifungal agent from the keratin substance or nail can be carried out in the same manner as in the above-mentioned evaluation method of a drug effect and detecting method of the antimicrobial agent administered to an animal or a biosample.

Furthermore, the present invention provides various useful antimicrobial agents according to the present evaluation method. As the antimicrobial agent obtained by the present evaluation method, there is an antimicrobial agent comprising a compound having an eradication effect for microorganism in vivo or a composition for therapy of the superficial mycosis, deep mycosis or bacterial infection containing the compound; an antimicrobial agent having the true effect selected by means of showing a statistically significant effect; furthermore, an antimicrobial agent having an excellent eradication effect for microorganism in vivo, which is selected by means of appearing the pure antimicrobial activity thereof; or an antimicrobial agent of the complete cure type without relapse. A concrete example is a therapeutic agent for onychomycosis comprising a compound having the group represented by the above-mentioned formula (I). Among them, more preferable concrete example is a therapeutic agent for onychomycosis comprising the compound represented by the formula (II):



(II)

wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or

C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

In the above-mentioned formula (I) or (II), the substituted phenyl group is a phenyl group having 1 to 3 substituents selected from a halogen atom and trifluoromethyl, and includes, for instance, 2,4-difluorophenyl, 2,4-dichlorophenyl, 4-fluorophenyl, 4-chlorophenyl, 2-chlorophenyl, 4-trifluoromethylphenyl, 2-chloro-4-fluorophenyl, 4-bromophenyl or the like. C₁₋₆ alkyl group includes, for example, a straight chain, branched chain or cyclic alkyl group having 1 to 6 carbon atoms such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, tert-pentyl and cyclohexyl. The non-substituted aryl group includes, for example, phenyl,

naphthyl, biphenyl or the like. The substituted aryl group includes, for example, 2,4-difluorophenyl, 2,4-dichlorophenyl, 4-fluorophenyl, 4-chlorophenyl, 2-chlorophenyl, 4-trifluoromethylphenyl, 2-chloro-4-fluorophenyl, 4-bromophenyl, 4-tert-butylphenyl, 4-nitrophenyl or the like. C₂₋₈ alkenyl group includes, for example, vinyl, 1-propenyl, styryl or the like. C₂₋₆ alkynyl group includes, for example, ethynyl or the like. C₇₋₁₂ aralkyl group includes, for example, benzyl, naphthylmethyl, 4-nitrobenzyl or the like.

In addition, the most preferable compound among the above-mentioned antimicrobial agent includes the compound which shows the therapeutic efficiency like the following KP-103.

The above-mentioned KP-103 means an antifungal indicated by (2R,3R)-2-(2,4-difluorophenyl)-3-(4-methylenepiperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl)butane-2-ol. The compound can be prepared by allowing (2R,3S)-2-(2,4-difluorophenyl)-3-methyl-2-[(1H-1,2,4-triazole-1-yl)methyl]oxirane to react with 4-methylenepiperidine based on Example 1 in WO94/26734.

An effectiveness of the KP-103 used as an antifungal in the present invention for onychomycosis has not been confirmed, but its antifungal activity has been already known (WO94/26734).

The antimicrobial agent obtained in such manner can be used as a drug composition, the drug composition in order to sterilize a microorganism. In other words, it comes to be a drug composition which cures disease such as mycosis completely, and prevents a relapse.

Onychomycosis means a kind of the above-mentioned superficial mycosis, in the other word a disease which is caused by invading and proliferating in the nail of human or an animal. *Trichophyton rubrum* and *Trichophyton mentagrophytes* mainly cause onychomycosis in human. In rare case, *Microsporium*, *Epidermophyton*, *Candida*, *Aspergillus* or *Fusarium* causes it.

As a disease which is susceptible to treat with a therapeutic agents for onychomycosis of the present invention, there is included tinea unguium caused by *Trichophyton* species, Onychocandidiasis caused by *Candida* species or onychomycosis (sensu stricto) caused by the other fungus.

When a therapeutic agent for onychomycosis being a kind of antimicrobial agent in the present invention is given as topical preparation, there is liquid preparation, cream, ointment or manicure preparation as dosage form. In this case, it can be prepared using oil vehicle, emulsion vehicle or the like. The preferable amount of active ingredient is in 0.1 to 10% by weight. A dose amount may be appropriately aligned depending on the width of affected area and condition of disease.

In case of an oral preparation, it is used as powder, tablet, granule, capsule or syrup. In addition, it is used in form of injection such as subcutaneous injection, intramuscular injection or intravenous injection.

In the present invention, although the dosage amount of a therapeutic agent for onychomycosis depends on age, weight and individual conditions of a patient, it is about 10 mg to about 10 g per day, preferably about 50 mg to about 5 g as amount of the active ingredient. The agent was given in the above-mentioned daily dose at once or several divided portions.

The present invention is further explained in details based on the Examples hereinafter, but is not limited thereto.

PRETREATMENT OF COMPARATIVE EXAMPLE 1 AND EXAMPLES 1 TO 3

[1] Preparation of Fungal Solution and Production of a Guinea Pig Model of Interdigital Type of Tinea Pedis.

Millipore Filter (made by Millipore Corporation, HA, diameter 47 mm, 0.45 μ m) was placed on Brain-Heart-infusion agar medium (available from Nissui Pharmaceutical Co., LTD.), and 10⁶ cells of microconidium of *Trichophyton mentagrophytes* KD-04 strain were applied thereon. The cultivation was carried out at 30° C. under 17% of CO₂ for 7 days. After the cultivation, just amount of physiological saline containing 0.05% of Tween 80 was dropped on the filter and arthroconidia were collected using a platinum loop. After a hyphal mass was removed by a filtration with a sterile gauze, the number of arthroconidia in the filtrate was calculated by hemocytometer to adjust in concentration of 1×10⁸ arthroconidia/ml to obtain a fungal inocula.

A guinea pig model of interdigital type of tinea pedis was prepared according to the method of Arika et al (Antimicrobial Agents and Chemotherapy, 36: 2523-2525, 1992). Concretely, in two hind foos of male Hartley strain guinea pigs of 7 weeks age, the interdigital skin was lightly abraded with sandpaper. A paper disc (A disc available from Whatmen International Ltd cut in 8×4 mm) moisten with the above-mentioned solution of the inoculated organism was applied onto the region between the interdigital toes of the hind feet and fixed using Self-adhering-Foam Pad (Restone 1560M; available from 3M) and adhesive stretch bandage (ELASTPORE; available from Nichiban Co., Ltd). The paper disc and the bandage were removed seven days after of the infection.

[2] Preparation of Drug-Solution and Topical Treatment for the Guinea Pig Model of Interdigital Type of Tinea Pedis

A marketed 1% lanoconazole solution (commercial name: Astat (trade name) solution) and a solution in which KP-103 was solved in a concentration of 1% in polyethylene glycole #400: ethanol (75:25 v/v) mixture were used as test substance. Each solution in amount of 0.1 ml was applied to the plantar skin once a day from 10 days after the infection for 10 days.

COMPARATIVE EXAMPLE 1

Conventional Method for Evaluating Drug Effect

The conventional method was described as follows. For the infected control group without an application of the drug, the KP-103-treated group and the lanoconazole-treated group, 10 guinea pigs (hereinafter referred to "animal") were employed, respectively. Animals of each group were sacrificed two days after and 30 days after the last treatment. Their two hind feet were excised and wiped with the cotton swab containing alcohol sufficiently. A skin of whole sole was excised and cut into 15 skin pieces in total including 12 skin pieces from plantar parts and 3 skin pieces from an interdigital part. Each skin pieces were placed on 20 ml of Sabouraud dextrose agar medium (available from Difco laboratories) containing 50 μ g of chloramphenicol (available from Wako Pure Chemical Industries, Ltd.), 100 μ g of gentamicin (available from Schering-Plough Corporation), 50 μ g of 5-fluorocytosine (available from Wako Pure Chemical Industries, Ltd.) and 1 mg of cycloheximide (available from nacalai tesque, Inc.) per ml. An antibiotic substance added to the medium was set to a condition which enable bacteria not to grow and which enable fungi to grow without

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problem. After 10 days cultivation at 30° C., the result is described as "fungus-negative" when no growth of fungus was observed in all skin pieces, and the number of fungus-negative feet was determined. In the evaluation of the effect 30 days after last treatment, two days after the last treatment the treated feet were wiped with a cotton swab containing alcohol and fixed with the bandage in order to prevent a reinfection. The bandage was changed once a week. The therapeutic effects of KP-103 and lanoconazole two days after and 30 days after the last treatment are shown in Table 1.

TABLE 1

Test substance	The number of fungus-negative feet/ Total number of infected feet	
	Two days after the last treatment	30 days after the last treatment
Infected control	0/20	0/20
KP-103	20/20	16/20
Lanoconazole	20/20	9/20

As shown in the Table 1, in the KP-103-treated group, fungus-negative was observed in all feet two days after the last treatment, and also fungus-negative was observed in 16 out of 20 feet 30 days after the last treatment. On the other hand, in the lanoconazole-treated group, fungus-negative was observed in all feet two days after the last treatment, but fungus-negative was observed in only 9 feet 30 days after the last treatment, and there is no correlation between the therapeutic effects two days after and 30 days after the last treatment. The number of fungus-negative feet decreased 30 days after the last treatment. It was thought that the therapeutic effect of lanoconazole observed two days after the last treatment resulted from the inhibition of the growth of fungus caused by an interfusion of the drug remaining in the treated skin into culture system, because lanoconazole had a potent in vitro antifungal activity against dermatophytes, it was eight-fold more active than KP-103 against *Trichophyton* with a growth inhibitory concentration of 15.6 ng/ml. The determination test of the remaining agent was carried out.

EXAMPLE 1

Determination of drug remaining in skin which has been already evaluated five days after the last treatment according to conventional method.

A model was prepared according to Comparative Example 1. Lanoconazole being a test compound was used for a therapeutic experience as 1% solution with the same vehicle as KP-103. For the infected control group without an application of a drug, the KP-103-treated group and the lanoconazole-treated group, 20 animals were employed, respectively. The two hind feet were excised from each animal five days after the last treatment in the same manner as in Comparative Example 1. A total of 20 light feet were used for an evaluation by the conventional method, and a total of 20 right feet were used in the present evaluation method.

The skin pieces of light foot were placed on 20 ml of Sabouraud dextrose agar medium containing *Trichophyton mentagrophytes* KD-04 strain (2×10^4 cells/ml) and the antibiotic substance described in Comparative Example 1. After the cultivation was carried out at 30° C. for 3 days, a growth inhibitory-zone of fungus appeared around the skin was

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observed and was photographed for 10 out of 20 feet. FIG. 1 is an electronic date of the photograph of the skin after the cultivation in the above-mentioned condition. (a) indicates the infected control group without the drug application, (b) the KP-103-treated group and (c) the lanoconazole-treated group. One plate was explained as a representative of ten plates corresponding to each animal in the infected control group (a). In FIG. 1, S indicates one of 15 skin pieces of planta derived from the animal and M the above-mentioned medium. S and M described in both the KP-103-treated group (b) and the lanoconazole-treated group (c) are also the same. In the medium, the white zone shows the growth of fungus, on the other hand, the black zone shows the inhibition of the growth of fungus.

As shown FIG. 1, a good growth of the fungus was observed around the skin piece of the infected control group without any drug. In the group treated with KP-103, the growth of fungus was observed in all skin pieces, although in around the skin pieces the growth of fungus was slightly inhibited as compared with the infected control group. On the other hand, the growth of fungus was completely inhibited in around the skin pieces treated with lanoconazole. As these results, the therapeutic effect of lanoconazole in the conventional method shown in Table 1 was considered as an apparent therapeutic effect such that the agent remaining in the skin come to be mixed in culture system to inhibit the growth of fungus.

Therefore, it came to appear that the drug effect could not be evaluated by the conventional method precisely.

EXAMPLE 2

Determination of Remaining Drug after Removing Drug from Skin.

As Example 1, 20 right feet were excised from each animal five days after the last treatment, and sufficiently wiped with the cotton swab containing alcohol. The planta was cut off from each foot. The skin minced by a scissors was put into dialysis membrane (fractional molecular weight: 12,000-14,000, made of cellulose, available from VISKASE SALES Corporation) together with 4 ml of distilled water. Dialysis was carried out under 3 L of distilled water at 4° C. for 2 days. The dialysis water was changed twice a day 4 times in total. The content was transfer into a glass homogenizer. Thereto 4 ml double-concentration phosphate buffered saline containing 4% of trypsin derived from pig pancreas (available from BIOZYME Laboratories Limited) was added and the resulting mixture was homogenized. It was left at 37° C. for one hour and was filtrated with the two-ply gauze. The resulting filtrate was centrifuged. To a precipitate obtained by removing the supernatant were added 8 ml of phosphate buffered saline containing 2% of trypsin and further it allow to react with shaking at 37° C. for one hour. After a centrifugation, the precipitate obtained by removing the supernatant was washed three times by centrifuging with phosphate buffered saline in order to remove trypsin. To the precipitate 2 ml of the same saline were added to prepare a suspension thereof.

When dialysis and the treatment with trypsin were carried out using the same fungus used in this Example, an affect of these procedures on a survival rate of fungus could not be observed. Previously, a well was prepared in the center of Sabouraud dextrose agar medium (20 ml) containing *Trichophyton mentagrophytes* KD-04 strain (2×10^4 cells/ml) and the antibiotic substance described in Comparative Example 1. Into the well 100 μ l of the above-mentioned

suspension were added to cultivate at 30° C. for three days. After the cultivation, a growth inhibitory-circle of fungus appeared was observed and was photographed for 10 out of 20 feet. FIG. 2 is an electronic date of the photograph of the skin after the cultivation in the above-mentioned condition. (a) indicates the infected control group without the drug application, (b) the KP-103-treated group and (c) the lanoconazole-treated group. One plate was explained as a representative often plates corresponding to each animal in the infected control group (a). In FIG. 2, E indicates the skin suspension prepared from planta of the animal and M the above-mentioned medium. E and M described in both the KP-103-treated group (b) and the lanoconazole-treated group (c) are also the same. In the whole medium, the white zone shows the growth of fungus, on the other hand, the black zone around the well shows the inhibition of the growth of fungus.

In FIG. 1 showing the conventional method, no growth of fungus was observed in around the skin of the lanoconazole-treated group taken five days after the last treatment and the remaining drug was determined in the skin. By contrast, in FIG. 2, although few growth-inhibitory circle was observed in 2 out 10 feet suspension obtained by removing the drug using dialysis treatment of the present invention for the skin of the lanoconazole-treated group taken five days after the last treatment, the growth-inhibitory circle was never observed in residual 8 feet.

Since it came to appear that the drug remaining in treated skin could be sufficiently removed using dialysis according to the present invention, it was confirmed that the evaluation of the drug effect was not affect by the remaining drug.

EXAMPLE 3

Detection of Viable Fungus in Skin and Evaluation of Drug Effect

To two mediums of Sabouraud dextrose agar medium (20 ml) containing the antibiotic substance described in Comparative Example 1 were applied 100 µl of the suspension from one right feet of each animals obtained in Example 2. After the cultivation was carried out at 30° C. for 10 days, the result is described as "fungus-negative" when a colony of fungus was not observed in two agar plates (detection limit: 10 CFU (colony forming unit)/feet). The number of fungus-negative feet was counted. On the other hand, 20 left feet were evaluated in the same manner as in Comparative Example 1. Table 2 shows the result of comparing the therapeutic effect evaluated by the conventional method with that by the present evaluation method.

TABLE 2

Test substance	The number of fungus-negative feet/Total number of infected feet	
	Conventional Method	Present evaluation method
Infected control	0/20	0/20
KP-103	19/20	17/20
Lanoconazole	20/20	3/20

In case of the group treated with KP-103, no significant difference was observed in the number of fungus-negative feet, even if the number was evaluated by either the conventional method or the present evaluation method, as shown in Table 2. The rate of a fungus-negative foot

evaluated by the present evaluation method is 85% in case of KP-103. On the other hand, in the group treated with lanoconazole, although "fungus-negative" was observed in all feet by the conventional method, but "fungus-negative" was just observed in only three feet by the present evaluation method.

As mentioned above, it came to appear that using the present evaluation method, a true drug effect can be substantially evaluated without an affect by the remaining drug after the treatment therewith.

Furthermore, a result in the present evaluation method correlates with a result obtained by evaluation in the conventional method described in Comparative Example 1 in 30 days after the last treatment. Thereby, by using the present evaluation method, an effect of an antimicrobial agent to prevent a relapse can be estimated by the evaluation at early time after a treatment. Therefore, a complete cure type of the antimicrobial agent without the relapse can be obtained by using the present evaluation method.

PRETREATMENT OF EXAMPLES 4 AND 5

[1] Preparation of Fungal Solution and Production of Guinea Pig Model of Tinea Unguium and Tinea Pedis.

A fungal solution was prepared in the same manner as in the pretreatment of Comparative Example 1 except for changing *Trichophyton mentagrophytes* KD-04 strain to *Trichophyton mentagrophytes* SM-110 strain.

A guinea pig model of tinea unguium and tinea pedis was prepared in the same manner as in the above-mentioned preparation of the guinea pig model in interdigital tinea pedis except for changing male Hartley strain guinea pigs of 7 weeks age to male Hartley strain guinea pigs of 5 weeks age and except that the paper disc and the bandage was removed 21 days after the infection changing from seven days after the infection. The invasion of dermatophytes in plantar skin and nail plate was observed 60 days after the infection.

[2] Preparation of Drug Solution and Treatment of Guinea Pig of Tinea Unguium and Tinea Pedis

As test compounds, solutions were prepared by dissolving raw powders of KP-103, amorolfine and terbinafine in a concentration of 1% thereof to mixture solution of polyethylene glycole #400: ethanol (75:25 v/v), respectively. Capsule of terbinafine was prepared by crushing the marketed tablet, suspending in the concentration of 100 mg/ml into Miglyol 812 (available from Mitsuba trade Co., Ltd)with glass homogenizer uniformity, and injecting the resulting suspension into each capsule in the concentration of 40 mg/kg depending on body weight measured on administration day. A solution of KP-103, amorolfine or terbinafine in the amount of 0.1 ml was applied a plantar skin and nail of one foot once a day for 30 consecutive days. In case of terbinafine capsule, one capsule (40 mg/kg) was administered orally.

EXAMPLE 4

Evaluation of Drug Effect on Tinea Unguium

The effect on tinea unguium was evaluated by the following method.

Animals were sacrificed two days after the last treatment. One hind foot was excised and wiped sufficiently with the cotton swab containing alcohol. Nails (three in total) of one hind foot was excised and miced by a scissors. It was

transferred into glass homogenizer and was homogenized adding 4 ml double-concentration phosphate buffered saline (Phosphate Buffered Salts, available from Takara Shuzo Co., Ltd.) containing 4% of trypsin derived from pig pancreas (available from BIOZYME Laboratories Limited). The reaction was carried out with shaking at 37° C. for one hour. After a centrifugation, the obtaining precipitate was washed three times by centrifuging with phosphate buffered saline in order to remove trypsin. The precipitate was suspended with 4 ml of distilled water and put into dialysis membrane (fractional molecular weight: 12,000-14,000, made of cellulose, available from VISKASE SALES Corporation). Dialysis was carried out into 3 L of distilled water at 4° C. for 14 days. Dialysis water was replaced twice a day 28 times in total. After a centrifugation, 1 ml of phosphate buffered saline was added to the precipitate obtained by removing the supernatant to prepare a suspension. This suspension was defined as stock solution and was diluted by tenfold. To Sabouraud dextrose agar medium (20 ml) containing the antibiotic substance described in Comparative Example 1 were added 100 µl of the stock solution or the dilution. After the cultivation was carried out for 10 days, the result was described as "fungus-negative" when no colony of fungus was observed in all medium (detection limit: 10 CFU/feet). The number of fungus-negative feet in the nail was counted. When the colony was appeared on the medium, the number of colonies (CFU) was counted to calculate the number of colonies in the nail of one foot by the dilution rate. After Kruskal Wallis Test was carried out for the number of fungi in the nail, the multiple comparison was carried out based on Tukey method to analysis the significant difference between groups. Those results were shown in FIG. 3 and Table 3 thereof was made. In FIG. 3, the number of CFU in nails in each treated group was plotted and the mean number of CFU was shown by horizontal line and numerical value.

Using the above-mentioned suspension, sufficient removal of the remaining drug was determined by the present evaluation method in the same manner as in Example 2.

EXAMPLE 5

Evaluation of Drug Effect on Tinea Pedis

Skin pieces of hind feet were excised from each animal described in Example 4. A removal of the drug and a determination of the remaining drug were carried out in the same as in Example 2 except that dialysis for removing the drug carried out for 3 days and that dialysis water was changed six times in total. The sufficient removal of the remaining drug was confirmed.

Then the drug effect was evaluated in the same manner as in Example 4 (detection limit: 20 CFU/feet). Those results were shown in FIG. 4 and Table 4 thereof was made. In FIG. 4, the number of CFU in the skin in each treated group was plotted and the mean number of CFU was shown by horizontal line and numerical value.

TABLE 3

Test substance	The number of feet with fungus-negative nail/ Total number of infected feet	Mean number of fungal cells in the nail (Log CFU ± SD)
	Vehicle for topical use	0/10
KP-103 solution	0/10	2.20 ± 0.56**
Amorolfine solution	0/10	3.26 ± 0.54

TABLE 3-continued

Test substance	The number of feet with fungus-negative nail/ Total number of infected feet	Mean number of fungal cells in the nail (Log CFU ± SD)
	Terbinafine solution	0/10
Vehicle for oral use	0/10	3.76 ± 0.67
Oral preparation of terbinafine	0/10	3.80 ± 0.44

**significant difference versus the vehicle for topical use, the vehicle for oral use and the oral preparation of terbinafine in 0.01% of significant level is shown.

As shown in FIG. 3 and Table 3, no foot with fungus-negative nail was observed in all groups treated with substance tested for 30 days. But, KP-103 significantly reduced the number of fungal cells in the nail as compared with the vehicle for topical use. The therapeutic effect thereof was significantly superior to the oral preparation of terbinafine. On the other hand, no significant fungicidal effect was seen in amorolfine and terbinafine (for external use, oral use) as compared with the vehicle. The therapeutic effect thereof was not seen. As mentioned above, it was suggested that KP-103 exhibited the therapeutic effect on tinea unguium by topical application and that KP-103 could cure tinea unguium earlier than the oral preparation of terbinafine.

TABLE 4

Test substance	The number of feet with fungus-negative skin/ Total number of infected feet	Mean number of fungal cells in the skin (Log CFU ± SD)
	Vehicle for topical use	0/10
KP-103 solution	10/10**	<1.3**
Amorolfine solution	4/10	1.74 ± 0.45*
Terbinafine solution	10/10**	<1.3**
Vehicle for oral use	0/10	3.85 ± 0.68
Oral preparation of terbinafine	10/10**	<1.3**

*significant difference versus the vehicle for topical use in 0.05% of significant level is shown.

**significant difference versus the vehicle for topical use and the vehicle for oral use in 0.01% of significant level is shown.

As shown in FIG. 4 and Table 4, the excellent therapeutic effect on tinea pedis was seen in all drugs, KP-103, terbinafine and amorolfine in either case where it was evaluated by the rate of fungus-negative foot or where by the number of fungal cells in the skin. On the other hand, it became clear that KP-103 exhibited the excellent fungicidal effect on tinea unguium, although terbinafine and amorolfine did not exhibited the therapeutic effect on tinea unguium as shown in FIG. 3 and Table 3.

INDUSTRIAL APPLICABILITY

As mentioned above, recently developed drugs having an extremely potent activity against *Trichophyton* in vitro such as lanconazole brings about the judgement of fungus-negative according to the conventional method regardless of the existence of the no-treated fungus in the skin, since the drug remaining in the treated skin inhibits a growth of the fungus in the skin.

On the contrary, according to the present invention, an effect of an antimicrobial agent can be evaluated accurately, since a remaining drug can be removed by dialyzing the

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infected site with a microorganism of animal or biosample such as the treated skin using a dialysis membrane. Furthermore, although it is difficult to quantitatively compare of an antimicrobial effect such as an antifungal effect in conventional method, the present evaluation method enables the antimicrobial effects to compare quantitatively, since the number of viable fungi in the infected site of an animal or a biosample such as a skin can be determined precisely. In addition, the therapeutic effect based on the present evaluation method reflect a result as to relapse in the conventional method and therefore an effect to prevent relapse can be estimated by evaluating at earlier time after the treatment according to the present evaluation method. Therefore, in the present evaluation method, a true effect of an antimicrobial agent can be evaluated and it is possible to select an antimicrobial agent having an excellent sterilization effect against fungi in vivo or an antimicrobial agent of complete cure type which does not bring about relapse. As mentioned above, the present evaluation method is very useful as a method for evaluating the antimicrobial agent.

Additionally, in onychomycosis it is the first time that it is possible to evaluate a therapeutic effect against onychomycosis on a model of tinea unguium by the present evaluation method.

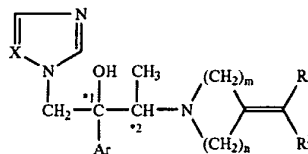
As a result of the evaluation of the therapeutic effect against onychomycosis according to the present evaluation method, it comes to clear that KP-103 exhibits the excellent therapeutic effect against onychomycosis with a simple application on which the effect is not exhibited using the conventional topical antifungal agent. Therefore, KP-103 is a beneficial agent for treating onychomycosis, industrially.

The invention claimed is:

1. A method for treating a subject having onychomycosis wherein the method comprises topically administering to a

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nail of said subject having onychomycosis a therapeutically effective amount of an antifungal compound represented by the following formula:



(II)

wherein, Ar is a non-substituted phenyl group or a phenyl group substituted with 1 to 3 substituents selected from a halogen atom and trifluoromethyl group,

R¹ and R² are the same or different and are hydrogen atom, C₁₋₆ alkyl group, a non-substituted aryl group, an aryl group substituted with 1 to 3 substituents selected from a halogen atom, trifluoromethyl group, nitro group and C₁₋₁₆ alkyl group, C₂₋₈ alkenyl group, C₂₋₆ alkynyl group, or C₇₋₁₂ aralkyl group,

m is 2 or 3,

n is 1 or 2,

X is nitrogen atom or CH, and

*1 and *2 mean an asymmetric carbon atom.

2. The method of claim 1, in which the compound represented by the formula (II) is (2R, 3R)-2-(2,4-difluorophenyl)-3-(4-methylen piperidine-1-yl)-1-(1H-1,2,4-triazole-1-yl)butane-2-ol.

* * * * *

APPENDIX E

Patent Bibliographic Data Statement

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**United States
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Page

Patent Bibliographic Data		07/15/2014 09:07 AM	
Patent Number:	7214506	Application Number:	10685266
Issue Date:	05/08/2007	Filing Date:	10/14/2003
Title:	Method for treating onychomycosis		
Status:	8th year fee window opens: 05/08/2014	Entity:	LARGE
Window Opens:	05/08/2014	Surcharge Date:	11/11/2014
Fee Amt Due:	\$3,600.00	Surchg Amt Due:	\$0.00
Fee Code:	1552	Total Amt Due:	\$3,600.00
Surcharge Fee Code:	MAINTENANCE FEE DUE AT 7.5 YEARS		
Most recent events (up to 7):	10/20/2010 Payment of Maintenance Fee, 4th Year, Large Entity. --- End of Maintenance History ---		
Address for fee purposes:	RONALD I. EISENSTEIN NIXON PEABODY LLP 100 SUMMER STREET BOSTON MA 02110		
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APPENDIX F

IND Letter from the FDA



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20857

IND 77,732

received
Miller 7-19-07

Dow Pharmaceutical Sciences, Inc.
Attention: Barry M. Calvarese, MS
Vice President, Regulatory and Clinical Affairs
1330 Redwood Way
Petaluma, CA 94954

Dear Mr. Calvarese:

We acknowledge receipt of your Investigational New Drug Application (IND), submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act. Please note the following identifying data:

IND Number Assigned: 77,732

Sponsor: Dow Pharmaceutical Sciences, Inc.

Name of Drug: IDP-108 (KP-103)

Date of Submission: June 14, 2007

Date of Receipt: June 15, 2007

Studies in humans may not be initiated until 30 days after the date of receipt shown above. If, on or before July 13, 2007, we identify deficiencies in the IND that require correction before human studies begin or that require restriction of human studies, we will notify you immediately that (1) clinical studies may not be initiated under this IND ("clinical hold") or that (2) certain restrictions apply to clinical studies under this IND ("partial clinical hold"). In the event of such notification, you must not initiate or you must restrict such studies until you have submitted information to correct the deficiencies, and we have notified you that the information you submitted is satisfactory.

It has not been our policy to object to a sponsor, upon receipt of this acknowledgement letter, either obtaining supplies of the investigational drug or shipping it to investigators listed in the IND. However, if the drug is shipped to investigators, they should be reminded that studies may not begin under the IND until 30 days after the IND receipt date or later if the IND is placed on clinical hold.

IND 77,732
Page 2

As sponsor of this IND, you are responsible for compliance with the Federal Food, Drug, and Cosmetic Act and the implementing regulations (Title 21 of the Code of Federal Regulations). Those responsibilities include (1) reporting any unexpected fatal or life-threatening adverse experience associated with use of the drug by telephone or fax no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]; (2) reporting any adverse experience associated with use of the drug that is both serious and unexpected in writing no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]; and (3) submitting annual progress reports [21 CFR 312.33].

Please forward all future communications concerning this IND in triplicate, identified by the above IND number, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Dermatology and Dental Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Kalyani Bhatt, Regulatory Project Manager, at (301) 796-2110.

Sincerely,

{See appended electronic signature page}

Margaret Kober, R.Ph., M.P.A.
Acting Supervisor, Project Management Staff
Division of Dermatology & Dental Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

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/s/

Margaret Kober
7/13/2007 02:19:04 PM

APPENDIX G

NDA Acknowledgement Letter from the FDA



NDA 203567

NDA ACKNOWLEDGMENT

Dow Pharmaceutical Sciences
Attention: Charity Abelardo, RAC
Acting Sr. Director, Regulatory Affairs
1330 Redwood Way
Petaluma, CA 94954

Dear Ms. Abelardo:

We have received your New Drug Application (NDA) submitted under section 505(b)(1) of the Federal Food, Drug, and Cosmetic Act (FDCA) for the following:

Name of Drug Product: Clenafin™ (efinaconazole) Topical Solution, 10%

Date of Application: July 26, 2012

Date of Receipt: July 26, 2012

Our Reference Number: NDA 203567

Unless we notify you within 60 days of the receipt date that the application is not sufficiently complete to permit a substantive review, we will file the application on September 24, 2012, in accordance with 21 CFR 314.101(a).

If you have not already done so, promptly submit the content of labeling [21 CFR 314.50(l)(1)(i)] in structured product labeling (SPL) format as described at <http://www.fda.gov/ForIndustry/DataStandards/StructuredProductLabeling/default.htm>. Failure to submit the content of labeling in SPL format may result in a refusal-to-file action under 21 CFR 314.101(d)(3). The content of labeling must conform to the content and format requirements of revised 21 CFR 201.56-57.

You are also responsible for complying with the applicable provisions of sections 402(i) and 402(j) of the Public Health Service Act (PHS Act) [42 USC §§ 282 (i) and (j)], which was amended by Title VIII of the Food and Drug Administration Amendments Act of 2007 (FDAAA) (Public Law No. 110-85, 121 Stat. 904).

The NDA number provided above should be cited at the top of the first page of all submissions to this application. Send all submissions, electronic or paper, including those sent by overnight mail or courier, to the following address:

Food and Drug Administration
Center for Drug Evaluation and Research
Division of Dermatology and Dental Products
5901-B Ammendale Road
Beltsville, MD 20705-1266

All regulatory documents submitted in paper should be three-hole punched on the left side of the page and bound. The left margin should be at least three-fourths of an inch to assure text is not obscured in the fastened area. Standard paper size (8-1/2 by 11 inches) should be used; however, it may occasionally be necessary to use individual pages larger than standard paper size.

Non-standard, large pages should be folded and mounted to allow the page to be opened for review without disassembling the jacket and refolded without damage when the volume is shelved. Shipping unbound documents may result in the loss of portions of the submission or an unnecessary delay in processing which could have an adverse impact on the review of the submission. For additional information, please see

<http://www.fda.gov/Drugs/DevelopmentApprovalProcess/FormsSubmissionRequirements/DrugMasterFilesDMFs/ucm073080.htm>.

Secure email between CDER and applicants is useful for informal communications when confidential information may be included in the message (for example, trade secrets or patient information). If you have not already established secure email with the FDA and would like to set it up, send an email request to SecureEmail@fda.hhs.gov. Please note that secure email may not be used for formal regulatory submissions to applications.

If you have any questions, call me at (301) 796-1015.

Sincerely,

{See appended electronic signature page}

Strother D. Dixon
Regulatory Health Project Manager
Division of Dermatology and Dental Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

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/s/

STROTHER D DIXON
07/27/2012



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration
Silver Spring MD 20993

NDA 203567

**ACKNOWLEDGE –
CLASS 2 RESUBMISSION**

Dow Pharmaceutical Sciences, Inc.
Attention: Sean Humphrey
Manager, Regulatory Affairs
1330 Redwood Way
Petaluma, CA 94954

Dear Mr. Humphrey:

We acknowledge receipt on December 20, 2013, of your December 19, 2013, resubmission to your supplemental new drug application submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for (efinaconazole) topical solution, 10%.

We consider this a complete, class 2 response to our May 13, 2013 action letter. Therefore, the user fee goal date is June 20, 2014.

If you have any questions, call me at (301) 796-1015.

Sincerely,

{See appended electronic signature page}

Strother D. Dixon
Regulatory Project Manager
Division of Dermatology and Dental Products
Office of Drug Evaluation III
Center for Drug Evaluation and Research

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/s/

STROTHER D DIXON
12/30/2013

APPENDIX H

Activities Undertaken During the Applicable Regulatory Review Periods

Efinaconazole Solution 10%

IND 77,732

FDA Correspondence Log

Date	Description
6/14/2007	Original Submission
7/13/2007	FDA request for IRB, CV and 1571 information
7/13/2007	IND acknowledgement letter
8/1/2007	SN0001 – Response to FDA for clinical information
9/7/2007	SN0002 – Protocol Amendment
1/14/2008	SN0003 – Final Nonclinical Reports
9/10/2008	SN0004 – intent to request a SPA (carc studies)
9/17/2008	SN0005 – IND annual report
9/18/2008	SN0006 – Updated IB
9/29/2008	SN0007 – Final phase 1 clinical study report (DPSI-IDP-108-PI-01)
10/1/2008	SN0008 – Study reports
10/31/2008	SN0009 – SPA for 2 year carcinogenicity by subcu in rats
10/31/2008	Fax of SPA cover letter to OND
11/3/2008	SN0010 – SPA for 2 year carcinogenicity by dermal application in mice
11/3/2008	Fax of SPA cover letter to OND
11/3/2008	Fax of SPA cover letter to project manager
12/11/2008	SN0011 – Updated IB
12/15/2008	ECAC meeting minutes
1/29/2009	Courtesy copy of SN0012
1/30/2009	SN0012 – Request for teleconference (ECAC)
3/3/2009	ECAC meeting request follow up
3/4/2009	ECAC meeting request denied
4/17/2009	SN0013 – CMC update plus PK protocol and investigator info
4/24/2009	SN0014 – EOP2 meeting request
5/19/2009	SN0015 – Request for waiver of TQT Study
5/21/2009	FDA request for Phase 2 results
6/15/2009	Meeting request granted for EOP2
6/18/2009	SN0016 – Highlights of clinical pharmacology form

Efinaconazole Solution 10%

IND 77,732

FDA Correspondence Log

Date	Description
7/7/2009	SN0017 – EOP2 BB
7/16/2009	FDA request for updated IB to be submitted to IND
8/3/2009	Draft comments for the EOP2 meeting
8/4/2009	EOP2 Meeting
8/7/2009	EOP2 meeting telephone attendees
8/11/2009	SN0018 – Additional TQT supplement
8/17/2009	EOP2 meeting minutes
8/20/2009	SN0019 – Nonclinical Studies
10/5/2009	TQT waiver request follow up – email
10/21/2009	SN0020 – annual report
10/12/2009	TQT waiver request follow up – left voicemail
10/29/2009	TQT waiver request follow up – email
10/29/2009	TQT waiver request follow up – telephone conversation
11/3/2009	SN0021 – Final Phase 2 study report (DPSI-IDP-108-P2-01)
11/5/2009	SN0022 – Protocol amendment with Phase 3 protocols
11/4/2009	TQT waiver request follow up
11/6/2009	TQT waiver request follow up – Voicemail from FDA
11/11/2009	SN0023 – Information amendment – Carc study progress report
11/24/2009	FDA response to carc study progress report
11/25/2009	SN0024 – Waiver for in vivo studies – rat carc waiver request
12/8/2010	TQT waiver request follow up
12/15/2009	FDA acknowledging receipt of samples
12/23/2009	SN0025 – Protocol amendment for Phase 3 protocols
1/15/2010	SN0026 – Final PK study report
1/22/2010	TQT waiver request follow up
2/5/2010	TQT waiver request follow up
2/25/2010	TQT and Carc waiver request follow up
3/1/2010	SN0027 – Protocol amendment phase 3 protocols

Efinaconazole Solution 10%

IND 77,732

FDA Correspondence Log

Date	Description
3/5/2010	SN0028 – Information amendment
3/9/2010	SN0029 – Request for PK waiver
3/24/2010	TQT and Carc waiver request follow up
3/31/2010	FDA requesting status update for Phase 3 trials
4/1/2010	TQT and Carc waiver request follow up
4/9/2010	TQT and Carc waiver request follow up
4/13/2010	SN0030 – protocol amendment phase 3 protocols
4/14/2010	FDA comments/advice letter
4/14/2010	Carc waiver granted (received via mail on 4/19/2010)
4/14/2010	TQT waiver granted (received via mail on 4/19/2010)
4/19/2010	TQT and Carc waiver request follow up
4/23/2010	Email to FDA regarding clarification on ECG collection
5/17/2010	SN0031 – protocol amendment phase 3 protocols
5/21/2010	SN0032 – Request for waiver of photoallergy and photoirritation
5/27/2010	SN0033 – Information amendment – nonclinical reports
5/27/2010	Request for status update of PK waiver request
8/5/2010	Request for status update of PK waiver request
8/20/2010	Request for status update of PK waiver request
8/30/2010	FDA letter regarding ECG collection in Phase 3 studies
9/5/2010	SN0034 – annual report
10/15/2010	SN0035 – withdraw request for PK waiver
11/5/2010	Status update for PM on the PK waiver withdrawal
11/30/2010	SN0036 – Request for comment on PK protocol
12/23/2010	USAN submission
1/18/2011	SN0037 – stability bracketing request
1/21/2011	SN0038 – protocol amendment
2/4/2011	SN0039 – Response to FDA comment on ECG collection during phase 3 studies
2/11/2011	USAN acknowledgement

Efinaconazole Solution 10%

IND 77,732

FDA Correspondence Log

Date	Description
2/14/2011	FDA phone call requesting Michelle Carpenter's email address
2/14/2011	FDA advice letter – CMC and Clinical
2/17/2011	Telephone conversation to Inform FDA of nonclinical submission for dermal carc study
2/17/2011	Email to FDA – Courtesy copy of SN0040
2/18/2011	SN0040 – information amendment
2/24/2011	Request for Status update regarding SN0032
2/25/2011	FDA guidance on early termination of dermal carc study
3/16/2011	Advice and information from FDA
4/1/2011	SN0041 – information amendment
4/11/2011	Courtesy Copy of SN0042
4/12/2011	SN0042 – information amendment
4/13/2011	Status request for termination criteria in the dermal carc study
4/13/2011	Email with instructions for termination of animals in the dermal carc study
4/15/2011	SN0043 – nonclinical studies
4/18/2011	FDA letter – early termination of mice in carc study
4/22/2011	SN0044 – investigator update
4/27/2011	Efinaconazole approved as USAN name
5/11/2011	Signed adoption statement – no changes to USAN information
8/12/2011	SN0045 – PK protocol
8/23/2011	SN0046 – RIPT protocol
9/12/2011	SN0047 – investigator update
9/12/2011	SN0048 – annual report
10/19/2011	SN0049 – nonclinical studies
11/19/2011	Contact report – Previous PM notified us that she is no longer in the derm division
11/19/2011	Contact report – Asked Barbara Gould who the new PM is for this IND
11/19/2011	Contact report for new FDA PM
11/11/2011	SN0050 – SAP for phase 3 studies
12/23/2011	SN0051 – protocol amendment

Efinaconazole Solution 10%

IND 77,732

FDA Correspondence Log

Date	Description
12/23/2011	SN0052 – Pre-NDA meeting request
1/12/2012	Pre-NDA meeting acknowledgement
1/13/2012	Voicemail to FDA – meeting request question
1/24/2012	Confirmation of meeting background materials submission date
2/21/2012	SN0053 – Pre-NDA meeting background material
2/24/2012	FDA voicemail
2/24/2012	Email follow up to voicemail
2/27/2012	Information Request
2/29/2012	FDA confirming materials were received
3/1/2012	Pre-NDA meeting room location
3/5/2012	SN0054 – Request for proprietary name
3/22/2012	SN0055 – Response to request for information
4/2/2012	Foreign visitor data forms
4/3/2012	FDA voicemail
4/6/2012	List of attendees for Pre-NDA meeting
4/10/2012	Contact report
4/11/2012	FDA preliminary comments to Pre-NDA briefing package
4/15/2012	Response to FDA preliminary comments – no Pre-NDA meeting necessary
4/16/2012	Contact report
4/18/2012	FDA preliminary response
5/14/2012	Meeting Minutes
6/25/2012	Proprietary Name Unacceptable
9/17/2012	SN0056 – annual report
3/29/2013	SN0057 – change in regulatory contact
8/30/2013	SN0058 – annual report

Efinaconazole Solution 10%

NDA 203567

FDA Correspondence Log

Date	Description
7/26/2012	NDA - Original Submission
7/27/2012	NDA acknowledgement letter
8/1/2012	FDA request for clarification on FEI numbers
8/2/2012	FDA request for dermal carc datasets
8/3/2012	Email to FDA: courtesy copy of SN0001
8/3/2012	Follow up phone with FDA nonclinical request for dermal carc datasets
8/3/2012	FDA phone call confirming contact information
8/6/2012	FDA phone call: request for clarification on drug substance manufacturing facilities
8/7/2012	FDA email: request for clarification on drug substance manufacturing facilities
8/7/2012	Email to FDA providing contact information requested on 8/3/2012
8/7/2012	FDA voicemail request for information
8/6/2012	SN0001 – FEI number clarification
8-9/2012	SN0002 – clarification on drug substance manufacturing responsibilities
8/20/2012	SN0003 – separate amendment containing the proprietary name request submitted in the NDA
9/4/2012	Email to FDA confirming shipment of samples
9/5/2012	Shipment of samples to FDA RPM
9/6/2012	FDA email acknowledgment for receipt of samples
9/26/2012	SN0004 – BMIO requests parts 2 and 3
9/27/2012	FDA email: courtesy copy of the filing communication letter
9/28/2012	FDA request for methods validation and material
10/1/2012	FDA email request for 6 additional samples
10/1/2012	Email to FDA that 6 additional samples have been sent
10/4/2012	FDA email confirming receipt of 6 additional samples
10/4/2012	Email to FDA with sponsor attendees for the CMC teleconference
10/8/2012	Email to FDA confirming receipt of the request for method validation and materials
10/12/2012	FDA email requesting contact information for sites 118 and 123
10/16/2012	FDA email requesting contact information for site 244
10/17/2012	Email to FDA confirming shipment of methods validation and material to FDA

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NDA 203567

FDA Correspondence Log

Date	Description
10/17/2012	FDA letter confirming receipt of methods validation and material
10/17/2012	SN0005 – response to filing communication letter dated 9/27/2012 (CMC and labeling)
10/22/2012	SN0006 – response to filing communication letter dated 9/27/2012 (CMC)
11/9/2012	FDA letter: Proprietary name CLENAFIN found unacceptable
11/20/2012	FDA letter: CMC information request
11/30/2012	FDA letter : CAC final report
12/4/2012	Email to FDA outlining our plans for the 120-day safety update
12/6/2012	SN0007 – Additional stability data for NDA (30 mo trade size and 12 mo sample)
12/7/2012	SN0008 – response to filing communication letter dated 9/27/2012 (CMC)
12/11/2012	FDA email providing a response to an email clarification
12/12/2012	Email to FDA providing clarification on request for clinical information
12/14/2012	SN0009 – response to information request dated 11/20/2012 (CMC)
12/17/2012	SN0010 – response to information request (clinical). Provided photos for pivotal studies
12/19/2012	SN0011 – response to information request (extrapolation of foreign clinical data)
12/26/2012	FDA letter: CMC information request
1/9/2013	SN0012 – response to information request dated 12/21/2012 (CMC)
1/9/2013	Email response to information request dated 12/21/2012 (CMC)
1/17/2013	SN0013 – proprietary name review request (JUBLIA)
1/29/2013	FDA phone call regarding proprietary name review request
2/14/2013	FDA Form 482 – Notice of Inspection
2/27/2013	Email to FDA regarding inspection closeout
3/8/2013	FDA letter: Discipline Review letter
3/8/2013	Email to FDA asking for an agenda to the 3/12/2013 teleconference
3/18/2012	Phone call to FDA between Sue Hall and the CMC Branch Chief
3/18/2013	SN0014 – response to CMC discipline review letter dated 3/8/2013
3/29/2013	SN0015 – amendment to SN0014
4/3/2013	Email to FDA asking for status update on the DRL
4/15/2013	FDA letter: JUBLIA conditionally acceptable

Efinazazole Solution 10%

NDA 203567

FDA Correspondence Log

Date	Description
5/13/2013	FDA letter: Complete Response Letter (CLR)
6/5/2013	Email from FDA: FDA meeting attendees at the 3/12/2013 and 3/20/2013 teleconferences
6/17/2013	SN0016 – Type A meeting request
6/21/2013	FDA letter: Meeting Granted
7/2/2013	FDA letter regarding February inspection
8/2/2013	SN0017 – Sponsor meeting minutes for the 7/17/2013 meeting
8/5/2013	FDA Meeting minutes for 7/17/2013 meeting
8/15/2013	SN0018 – clarification on the meeting minutes
9/4/2013	Phone call from FDA providing their clarification to the meeting minutes
10/15/2013	SN0019 - notifying FDA of our change in strategy for our response to the CLR
12/20/2013	SN0020 – resubmission (in response to CLR)
12/20/2013	Notification to FDA of Resubmission
12/30/2013	Resubmission acknowledgement letter
1/14/2014	FDA request for track changes package insert that was included in the resubmission
1/14/2014	Response to FDA request for track changes package insert
1/16/2014	SN0021 – response to FDA request for information
1/30/2014	FDA request for proprietary name submission (Jublia)
2/5/2014	SN0022 – proprietary name submission (Jublia for re-review)
3/29/2014	Jublia proprietary name conditionally acceptable
4/11/2014	Email to FDA with the requested MS Word Versions of Sections 1.9.1 and 1.9.2
5/9/2014	FDA proposed labeling edits
5/15/2014	Courtesy Copy of response to FDA proposed labeling
5/16/2014	SN0023 – response to FDA proposed labeling
5/21/2014	FDA proposed labeling edits
5/23/2014	SN0024 – response to FDA proposed labeling
5/23/2014	FDA proposed language and milestone dates for PMR
5/23/2014	Courtesy Copy of response to the FDA proposed language and milestone dates for PMR
5/27/2014	SN0025 – response to the FDA proposed language and milestone dates for PMR

Efinaconazole Solution 10%

NDA 203567

FDA Correspondence Log

Date	Description
6/4/2014	FDA proposed edits for the package insert
6/4/2014	Courtesy Copy of response to FDA proposed edits for the package insert
6/4/2014	SN0026 -- response to FDA proposed edits for the package insert
6/6/2014	NDA Approval Letter

ARTIFACT SHEET

Enter artifact number below. Artifact number is application number + artifact type code (see list below) + sequential letter (A, B, C ...). The first artifact folder for an artifact type receives the letter A, the second B, etc..

Examples: 59123456PA, 59123456PB, 59123456ZA, 59123456ZB

10685266ZA

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 - Doc Code: Computer
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 - and/or table
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 - content unspecified or combined
 - Doc Code: Artifact Artifact Type Code: U
- Stapled Set(s) Color Documents or B/W Photographs
Doc Code: Artifact Artifact Type Code: C
- Microfilm(s)
Doc Code: Artifact Artifact Type Code: F
- Video tape(s)
Doc Code: Artifact Artifact Type Code: V
- Model(s)
Doc Code: Artifact Artifact Type Code: M
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Doc Code: Artifact Artifact Type Code: B
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Doc Code: Artifact Artifact Type Code X
- 1** Other, description: **PATENT GRANT**
Doc Code: Artifact Artifact Type Code: Z

March 8, 2004



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United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV

CONFIRMATION NO. 4026

POA ACCEPTANCE LETTER

23702
Bausch & Lomb Incorporated
1400 North Goodman Street
Rochester, NY 14609



OC000000070082762

Date Mailed: 08/08/2014

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/16/2014.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/dtvernon/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
10/685,266	10/14/2003	Yoshiyuki Tatsumi	700938-052220-DIV

CONFIRMATION NO. 4026

POWER OF ATTORNEY NOTICE



OC000000070082755

53143
RONALD I. EISENSTEIN
NIXON PEABODY LLP
100 SUMMER STREET
BOSTON, MA 02110

Date Mailed: 08/08/2014

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 07/16/2014.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/dtvernon/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101



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Office of Management
Food and Drug Administration
10001 New Hampshire Ave.,
Hillandale Campus RM 3180
Silver Spring, MD 20993

APR 24 2015

Attention: Beverly Friedman

The attached application for patent term extension of U.S. Patent No. 7,214,506 was filed on July 16, 2014, under 35 U.S.C. § 156.

The assistance of your Office is requested in confirming that the product identified in the application, JUBLIA® Topical Solution, 10% (efinaconazole), has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was filed within the sixty-day period beginning on the date the product was approved. Since a determination has not been made whether the patent in question claims a product which has been subject to the Federal Food, Drug and Cosmetic Act, or a method of manufacturing or use of such a product, this communication is NOT to be considered as notice which may be made in the future pursuant to 35 U.S.C. § 156(d)(2)(A).

Our review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156.

Inquiries regarding this communication should be directed to the undersigned at (571) 272-7755 (telephone) or (571) 273-7755 (facsimile).

Mary C. Till
Senior Legal Advisor
Office of Patent Legal Administration
Office of the Associate Commissioner
for Patent Examination Policy

cc: Toan P. Vo, Esq.
Bausch & Lomb Inc.
1400 N. Goodman St.
Rochester, NY 14609



NOV 14 2015

Food and Drug Administration
10903 New Hampshire Avenue
WO Building 51, Room 6250
Silver Spring, MD 20993-0002

Re: JUBLIA
Docket No. FDA-2015-E-1664

The Honorable Michelle K. Lee
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Lee:

This is concerning the application for patent term extension for U.S. Patent No. 7,214,506 filed by Kaken Pharmaceutical Co., Ltd., under 35 U.S.C. 156. The human drug product claimed by the patent is JUBLIA (efinaconazole), which was assigned new drug application (NDA) No. 203567.


A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. 156(a)(4). Our records also indicate that it represents the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. 156(f)(1).

The NDA was approved on June 6, 2014, which makes the submission of the patent term extension application on July 16, 2014, timely within the meaning of 35 U.S.C. 156(d)(1).

Should you conclude that the subject patent is eligible for patent term extension, please advise us accordingly. As required by 35 U.S.C. 156(d)(2)(A) we will then determine the applicable regulatory review period, publish the determination in the *Federal Register*, and notify you of our determination.

Please let me know if we can be of further assistance.

Sincerely yours,


for Jane A. Axelrad

Associate Director for Policy
Center for Drug Evaluation and Research

cc: Toan P. Vo, Esq.
Bausch & Lomb Incorporated
1400 N. Goodman Street
Rochester, NY 14609