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Bogotá – COLOMBIA

A12

Señor

Jefe de la División de Nuevas Creaciones

Superintendencia de Industria y Comercio

Ministerio de Desarrollo Económico

E. S. D.

Re.: Expediente No. **00.042.902**

Solicitud del examen de patentabilidad correspondiente a la Patente de Invención para:

“NUEVOS DERIVADOS DE EQUINOCANDINA, SU PROCEDIMIENTO DE PREPARACIÓN Y SU APLICACIÓN COMO ANTIFUNGICOS”

Solicitante : **HOECHST MARION ROUSSEL**

SOLICITUD EXAMEN DE PATENTABILIDAD

Yo, **MARGARITA CASTELLANOS ABONDANO**, mayor de edad, identificada y domiciliada como aparece al pié de mi firma, obrando en mi calidad de apoderada de la sociedad solicitante, atentamente me permito solicitar a Ud. que se realice el examen de patentabilidad de la invención arriba citada, para lo cual anexo el comprobante de pago **No. 03- 22.281** de **Marzo 26 de 2003**, correspondiente a la tasa establecida para dicha solicitud.

En consecuencia, atentamente solicito a Ud. se anexe este memorial junto con el comprobante de pago al expediente correspondiente a esta solicitud de Patente de Invención, y se continúe con su tramitación.

Señor Jefe,


Margarita Castellanos Abondano
C.C. No. **39.779.654** de Usaquén
T.P.A. No. **70.819** del C.S. de la J.
Carrera 9 No. 93-09 – Bogotá, D.C.
Teléfonos: 610 74 20 – 610 74 60

Bogotá, **26 MAR 2003**
MCA/gcb.- 4746



Industria y Comercio

SUBSECRETARÍA DE INDUSTRIA Y COMERCIO

SE
DE
INDUSTRIA Y COMERCIO
BOGOTÁ, D.C., COLOMBIA

WIT: 200070000-0

RECIBO OFICIAL DE CAJA : 00 - 00,001
FECHA : MARZO 31 DE 2003

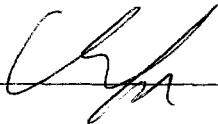
***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº. PAGO	FECHA PAGO	VAL. PAGO
MONICA VALENCIA ABONDANO CONSIGNACION		BANCO POPULAR	050-00110-6	4400075	04/02/2003	7,775,000.00

***** C O N C E P T O *****

CANT. CREDITICO	CONCEPTO	TOTAL CREDITICO
1 50005 01 AL COLIBRIEROS	110 000 000 00 EXAMEN DE PATENTABILIDAD	775,000.00
	TOTAL :	775,000.00

CON: TRESCIENTOS TREINTA Y CINCO MIL PESOS

RESPONSABLE : 

RESIDE DE CAJA APLICADO AL EXPERIENCIA N°.

Carrera 13 No. 27-00 Pisos 5, 7 y 10
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114

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

2020/
Bogotá, D.C.,

Doctor
Margarita Castellanos Abondano
Apoderado
AVENTIS PHARMA S.A.

Oficio N°

114

REFERENCIA	Radicación	00 42902
	Trámite	002
	Evento	001
	Actuación	430
	Folios	063

Como resultado del examen de patentabilidad de la solicitud de patente de invención N° 00 42902, realizado según el artículo 45 de la decisión 486 de la comisión de la Comunidad Andina, se notifica:

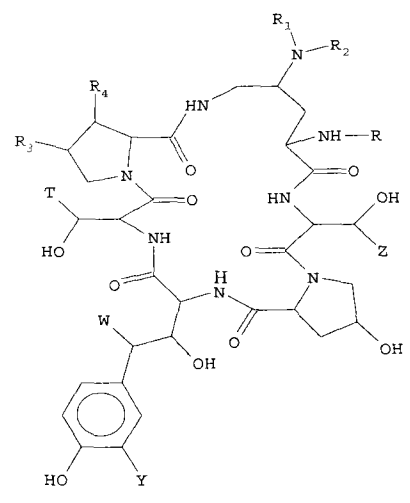
1. DOCUMENTOS ESTUDIADOS:

- La memoria descriptiva de la solicitud y el resumen del objeto de la invención en folios 11 a 27 y 38 respectivamente.
- Los ejemplos ilustrativos de la invención en los folios 20 a 26.
- El capítulo reivindicatorio de la solicitud, constituido por un pliego de 19 cláusulas y que se encuentra en los folios 28 a 37 de la solicitud.
- Primer examen jurídico y técnico de forma de los folios 53 a 56.
- Copia del documento mediante la cual se reivindica prioridad Francesa FR99 072 52 de fecha 09-06-1999, junto con su correspondiente traducción en los folios 62 a 89.

2. OBJETO DE LA INVENCION

- **Reivindicaciones 1 a 13 y 18 a 19:**

Se reivindican todas las formas de isómeros posibles de moléculas con actividad antifúngica, así como las mezclas y sus sales de adición de ácidos, que responden a la fórmula I.



Dentro de los que se destacan los compuestos:

Trifluoroacetato de 1-[4-[(2-aminoetil)-amino]-N-2-[[4-[5-[4-(pentiloxi)-fenil]-3-isoxazolil]-fenil]-carbonil]-L-ornitina]-4-[4-(4-hidroxifenil)-L-treonina-5-L-serina-equinocandina B

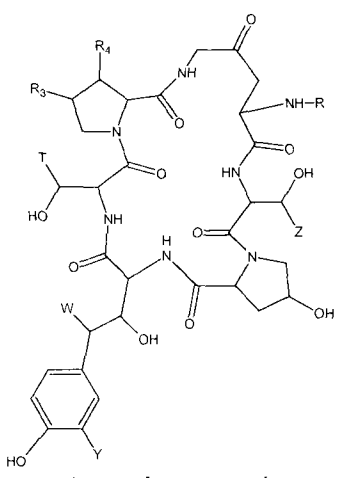
Trifluoacetato de trans -1-[4-[(2-aminociclohexil)-amino]-N-2-[[4-[5-[4-pentiloxi)-fenil]-3-isoxazolil]-fenil]-carbonil]-L-ornitina]-4-[4-(4-hidroxifenil)-L-treonina]-5-L-serina-equinocandina B

Entre otros.

De igual forma se reivindican las composiciones farmacéuticas que como medicamentos al menos un compuesto de fórmula I, así como sus sales de adición de ácidos farmacéuticamente aceptables.

• **Reivindicación 14 y 15:**

Procedimiento para preparar compuestos de fórmula I, caracterizado porque Se somete un compuesto de fórmula II



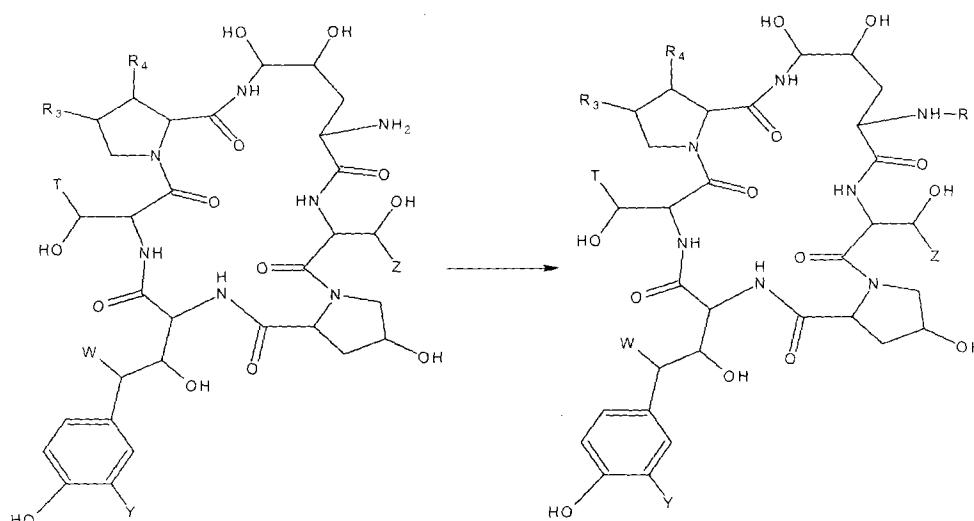
a la acción química de un agente reductor y/o un agente de funcionalización de una amina y/o un agente de separación de los diferentes isómeros obtenidos.

De igual forma se reivindican como nuevos los compuestos de fórmula II.

• **Reivindicación 16 y 17:**

Se reivindica el procedimiento según la cláusula 14, caracterizado porque se

somete un compuesto de fórmula III a la acción de un agente capaz de sustituir NH_2 con NHR , para obtener un compuesto de fórmula IV.



El compuesto de fórmula IV así obtenido se somete a la acción de Yoduro de trimetilsililo a fin de obtener el compuesto de fórmula II correspondiente. De igual forma se solicita protección sobre los compuestos definidos por las fórmulas estructurales III y IV.

Una vez catalogada la solicitud, ésta se clasificó en la sección **A 61 K³¹/195 // ³¹/33 //**
³¹/08 de la Clasificación Internacional de patentes.

3. CLARIDAD DE LA SOLICITUD

El Artículo 30 de la Decisión 486 contempla que “Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción”

Teniendo en cuenta que existen errores de transcripción en relación con la denominación de las fórmulas estructurales de los compuestos, en particular de aquellos definidos en las cláusulas 14,15, 16 y 17 de los folios 34 a 37, ésta dependencia recomienda realizar las modificaciones correspondientes a fin de hacer claridad acerca de la correspondencia de las fórmulas estructurales de las denominaciones: fórmula II, III y IV.

4. DETERMINACIÓN DEL ESTADO DE LA TÉCNICA:

Consultadas las fuentes de información con que cuenta este despacho, se encontraron los siguientes documentos, anteriores a la fecha de presentación de la solicitud 09-06-2000, relacionados con la materia técnica reivindicada:

Nº	Documento	Título	Fecha de Publicación
D1	Artículo 1*	Use of cilofungin as direct fluorescent probe for monitoring antifungal drug-membrane interactions.	Junio 1994
D2	EP0644199	Cyclic antimicrobial peptides and preparation thereof.	22/03/1995
D3	EP0736541	Cyclic hexapeptide antifungal agents.	09/10/1996
D4	CO98 73452	Nuevos derivados de Equinocandina, su preparación y su aplicación como fungicidas.	10/12/1997
D5	WO9823637	Cyclohexapeptides having antimicrobial activity.	04/06/1998

* YUAN-Tih Ko, LUDESCHER Richard, FROST David y WASSERMAN Bruce. Antimicrobial Agents and Chemotherapy. 1994. p. 1378-1385.

5. EVALUACIÓN DE LOS REQUISITOS DE PATENTABILIDAD:

El Artículo 14 de la Decisión 486 de la comisión de la comunidad andina establece que: "Los países miembros otorgarán patentes para las invenciones, sean de producto o de procedimiento, en todos los campos de la tecnología, siempre que sean nuevas, tengan nivel inventivo y sean susceptibles de aplicación industrial"

En cumplimiento del artículo 14 de la decisión 486 de la comisión de la comunidad andina, se procedió a evaluar los requisitos de novedad, nivel inventivo y aplicación industrial con el fin de establecer la patentabilidad de la invención reivindicada en la solicitud en estudio.

5.1 Evaluación de NOVEDAD

El Artículo 16 de la Decisión 486 de la comisión de la comunidad andina establece que: "Una invención se considerará nueva cuando no está comprendida en el estado de la técnica."

El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida".

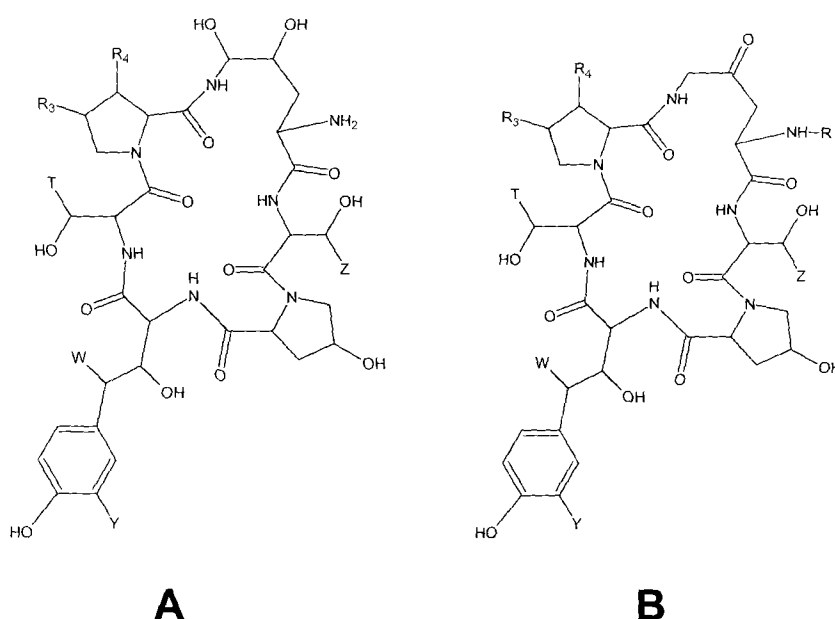
Teniendo en cuenta que se trata de compuestos derivados del núcleo de Equinocandina B, ésta dependencia evaluó la incidencia que, sobre el atributo de novedad de las moléculas, produce la modificación de los grupos sustituyentes sobre las moléculas reivindicadas en las cláusulas 1 a 13, 15, 17 y 18; así como el efecto que los cambios posicionales en determinados sustituyentes, ejercen sobre la actividad farmacológica de los compuestos citados.

Las equinocandinas son macro - lipopéptidos sintéticamente modificados con actividad antifúngica, su estructura base presenta puntos estratégicos de sustitución señalados a continuación:

- Residuo Ornitina: posiciones 4 y 5 y grupo amino a fin de formar la correspondiente amina secundaria.
- Residuo Prolina: derivados 3 hidrox, 4- metil prolina
- Residuo Treonina₁: derivados 2 alfa-hidroxi-etilo
- Residuo Tirosina: 3H, 4- hidrox, homotirosina con sustitución en el anillo de benceno en posición 3.
- Residuo Treonina₂: derivados 2 alfa-hidroxi-etilo

Anterioridad D4:

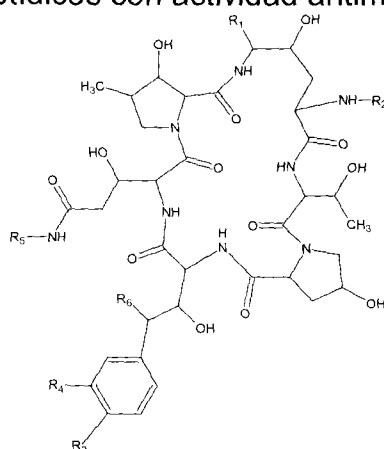
La anterioridad **D4**, solicitud de patente Colombiana CO98 73452, reivindica compuestos de equinocandina homólogos a los presentados por la solicitud en estudio, de igual forma, reivindica procedimientos para preparar derivados de equinocandina a partir de estructuras análogas de fórmulas estructurales A y B:



Procedimientos que también se describen en la solicitud objeto del presente estudio en las cláusulas 14 y 16, utilizando en cada caso como compuestos de partida, moléculas con núcleo estructural análogo a A ó B respectivamente.

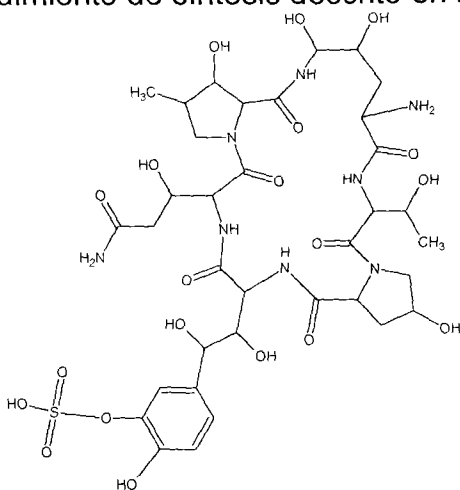
Anterioridad D2:

La anterioridad **D2**, publicación de la solicitud Europea EP0644199, revela compuestos polipeptídicos con actividad antimicrobiana de fórmula general:



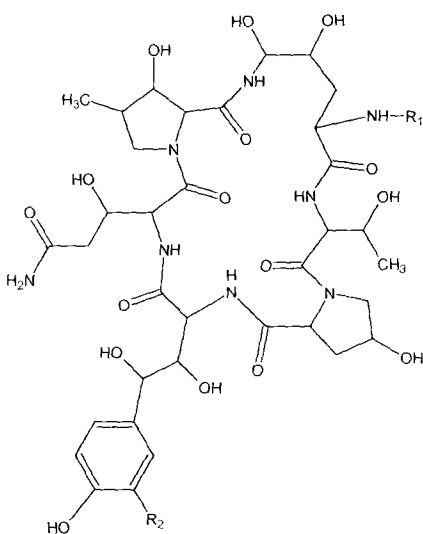
Los compuestos en mención son homólogos a aquellos reivindicados en la cláusula 17 del capítulo allegado a ésta dependencia con motivo de la solicitud de patente 00-42902. En particular el ejemplo de preparación del folio 20 - anterioridad **D2** - que corresponde a la molécula a continuación señalada y que se

encuentra comprendida dentro las modalidades preferidas de los compuestos de partida del procedimiento descrito en la cláusula 16, para los que adicionalmente se solicita explícita protección como productos nuevos en la cláusula 17. El documento afecta la novedad de la solicitud no solo en el sentido de los compuestos químicos reivindicados, sino en el de los compuestos de partida utilizados en el procedimiento de síntesis descrito en la cláusula antecedente.



Anterioridad D5:

La anterioridad **D5**, publicación de la solicitud internacional WO98 23637, comprende ciclohexapéptidos que tienen actividad antimicrobiana y que responden a la fórmula estructural:



La fórmula estructural revelada en la anterioridad comprende los compuestos químicos de partida de la cláusula 16 reivindicados como sustancias nuevas en la cláusula 17. De otro lado, el documento comprende una ruta de síntesis para la obtención de moléculas de fórmula estructural I - fórmula homóloga a la reivindicada por la solicitud en estudio - que comprende la formación de una amina secundaria en el grupo terminal amino del residuo de ornitina, proceso que se reivindica en la cláusula 16 del documento en estudio.

Teniendo en cuenta que el efecto de las combinaciones en cuanto a los grupos sustituyentes y posibles cambios posicionales no ha generado resultados insospechados que evidencien un salto significativo en la regla técnica, los compuestos reivindicados no se pueden considerar susceptibles de patentamiento por la falta evidente de novedad, afectación ejercida por las anterioridades **D4**, **D5** y **D2** estudiadas.

5.2 Evaluación del NIVEL INVENTIVO

El **Artículo 18** de la Decisión 486 dispone "*Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la Materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*"

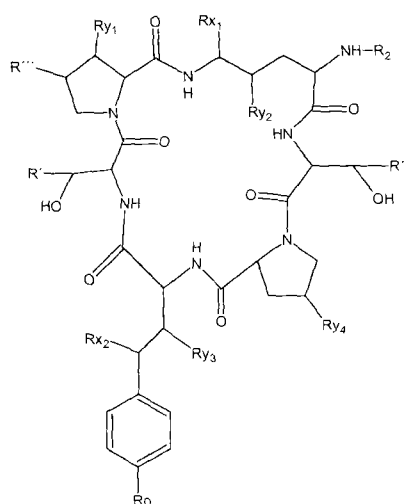
Anterioridad D1:

Alrededor de la investigación sobre el núcleo estructural de las Equinocandinas - anterioridad **D1** - se han logrado identificar diferentes compuestos que conservan el espectro antifúngico frente a diversas cepas de hongos de las especies *Candida* y *Aspergillus*. Debido a la gran capacidad de las moléculas para inhibir la síntesis de glucano, molécula indispensable para generar la pared celular del hongo, las equinocandinas se consideran de gran importancia farmacológica y terapéutica, hecho que se evidencia en el artículo: "Use of cilofungin as direct fluorescent probe for monitoring antifungal drug-membrane interactions".

En el documento mencionado se determinan sitios de la estructura en los cuales comúnmente se realizan las sustituciones, se señalan además los grupos y la conformación del anillo polipeptídico que se considera el farmacóforo de las Equinocandinas. En este sentido, es evidente que el núcleo Equinocandina se encuentra ampliamente reconocido en el estado de la técnica por su actividad antifúngica y por ende, los posibles cambios producto de la modificación en los grupos sustituyentes, generan una gran gama de compuestos que conservan la actividad farmacológica, no obstante tales cambios no pueden considerarse inventivos dado que se han derivado de manera evidente del estado del arte como bien lo demuestra la anterioridad **D1**.

Anterioridad D3:

La anterioridad **D3**, publicación de la solicitud Europea EP0736541, enseña composiciones farmacéuticas y un método para inhibir el crecimiento y la actividad de hongos mediante el uso de compuestos de fórmula:



Esta dependencia encuentra que los compuestos reivindicados presentan analogía estructural con respecto a la fórmula señalada por el documento, analogía derivada de los grupos sustituyentes y las posiciones en las cuales se hace evidente la modificación de la estructura química en los residuos mencionados en el estudio de novedad realizado al documento.

De otro lado, también se hace mención al procedimiento de síntesis de la reivindicación 16, el cual parte, como en el caso de la solicitud, del derivado hidroxilado en el residuo de ornitina, que sufre una N-sustitución a fin de formar la correspondiente amina secundaria.

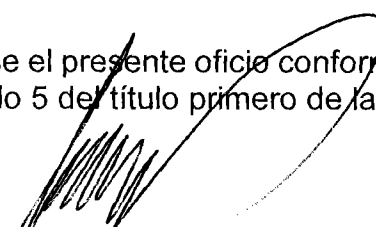
Así pues, a partir del conocimiento revelado por el documento **D1** y la publicación **D3** que advierten la actividad farmacológica del núcleo ciclopeptídico e identifican compuestos y sitios específicos de sustitución, resulta derivado del arte la obtención de los compuestos reivindicados, más aún si se tiene en cuenta que no se prueba un adelanto sorprendente con respecto a la regla técnica ó un avance inesperado en la terapéutica de infecciones causadas por hongos.

En conclusión los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

Nº	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	Artículo *	1 a 13, 18 y 19	Nivel Inventivo
D2	EP0644199	16 y 17	Novedad
D3	EP0736541	1 a 13 y 16 ,18 y 19	Nivel Inventivo
D4	CO98 73452	1 a 19	Novedad
D5	WO9823637	16 y 17	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de la notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2,literal e), del capítulo 5 del título primero de la Circular Única Nº 10 de 2001


Álix Carmenza Céspedes de Vergel
Jefe de la División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 08 JUL 2005

CONCEPTO TÉCNICO Número 0599	EXAMINADOR TÉCNICO Código 202044
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Use of Cilofungin as Direct Fluorescent Probe for Monitoring Antifungal Drug-Membrane Interactions†

YUAN-TIH KO,¹ RICHARD D. LUDESCHER,¹ DAVID J. FROST,² AND BRUCE P. WASSERMAN^{1*}

¹Department of Food Science, Cook College, New Jersey Agricultural Experiment Station, Rutgers University, New Brunswick, New Jersey 08903-0231, and ²Anti-Infective Research Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500²

Received 27 December 1993/Returned for modification 8 February 1994/Accepted 21 March 1994

Cilofungin is an antifungal cyclopeptide which inhibits cell wall (1,3)- β -glucan biosynthesis in fungal organisms, and its action against *Candida albicans* (1,3)- β -glucan synthase has been widely studied. Since glucan synthase inactivation is thought to partially result from perturbations of the membrane lipid environment, the interaction of cilofungin with fungal membranes and phosphatidylcholine membrane vesicles was studied. Cilofungin, which contains two independent aromatic groups, has an excitation maximum of 270 nm and an emission maximum of 317 nm in aqueous solution. Comparison of the fluorescence properties of cilofungin with those of the analogs pneumocandin B₀, *N*-acetyl-tyrosinamide, and 4-hydroxybenzamide indicated that the emission of cilofungin largely derived from the *p*-octyloxybenzamide side chain. Microsomal membranes from *Saccharomyces cerevisiae*, *C. albicans*, and phosphatidylcholine membrane vesicles induced a blue shift in the cilofungin emission spectrum and increased the cilofungin steady-state emission anisotropy, providing direct evidence for a cilofungin-membrane interaction. Cilofungin interacted more strongly with membranes of *C. albicans* than with those of *S. cerevisiae*, correlating with previous findings that *C. albicans* is far more susceptible than *S. cerevisiae* to the action of cilofungin. These findings support the hypothesis that drug-induced inhibition of the (1,3)- β -glucan synthesis results from the perturbation of the membrane environment and the interaction with the glucan synthase complex combined. The study demonstrated ways in which the fluorescence properties of drugs can be used to directly evaluate drug-membrane interactions and structure-activity relationships.

New antifungal drugs that are directed against specific fungal targets and that cause minimal toxicity to the host have attracted considerable interest in recent years. One such target is the plasma membrane-localized (1,3)- β -glucan synthase (GS; EC 2.4.1.34), which catalyzes the biosynthesis of (1,3)- β -glucan, an essential structural polymer of the cell wall (17, 36). A number of antifungal drugs have been targeted against GS from various fungi including the lipopeptide echinocandin-like class of inhibitors (12, 30, 31, 35), aculeacin A (23, 24), cilofungin (8, 10, 11), pneumocandins (12, 31), and the papulacandins (2, 16, 26, 37), which are lipid-like saccharides. Each of these compounds contains fatty acid side chains, which have been shown to be essential for the actions of cilofungin (34, 38) and papulacandin B (37). Although various lipophilic compounds are known to rapidly inactivate fungal glucan (18, 25) and chitin synthases (25), the mechanism by which these compounds interfere with GS has only recently begun to attract considerable attention (17, 18, 36).

Drug-membrane interactions can be probed directly by fluorescence spectroscopic techniques, provided that the drug contains a structural moiety exhibiting fluorescence properties (6, 7). In the present study, we demonstrated that cilofungin, which contains two aromatic moieties, exhibits an excitation maximum at 270 nm and an emission maximum of 317 nm in aqueous solution. These fluorescence properties served as the basis for studying cilofungin-membrane interactions by fluo-

rescence titration and steady-state anisotropy techniques. Fluorescence measurements provide a direct means for characterizing the interaction of the fatty acid side chain group of cilofungin with fungal membranes or vesicles of defined compositions.

MATERIALS AND METHODS

Materials. *Saccharomyces cerevisiae* GS-1-36, a glycogen synthase-defective mutant, was obtained from E. Cabib, National Institutes of Health, and was used in all experiments (29). *Candida albicans* CCH 442 was obtained as a clinical isolate from Cook County Hospital (Cook County, Ill.) and is a standard culture at Abbott Laboratories (Abbott Park, Ill.). [¹⁴C]uridine diphosphate glucose (UDP-[¹⁴C]Glc; 256 mCi mmol⁻¹) was obtained from ICN Radiochemicals (Irvine, Calif.). Brij 35 (polyoxyethylene ether, 23 lauryl ether) was purchased from Pierce (Rockford, Ill.). Cilofungin and pneumocandin B₀ were gifts from Eli Lilly (Indianapolis, Ind.) and Merck Research Laboratories (Rahway, N.J.), respectively. Guanosine-5'- α -(3-thiotriphosphate) (GTP- γ -S) was obtained from Boehringer Mannheim (Indianapolis, Ind.). Egg 1- α -phosphatidylcholine (PC, purity, >99%) and *N*-acetyl tyrosinamide (NATA) were purchased from Sigma (St. Louis, Mo.). 4-Hydroxybenzamide (HBA) and spectroscopic solvents were obtained from Aldrich (Milwaukee, Wis.). Growth media were obtained from Difco (Detroit, Mich.).

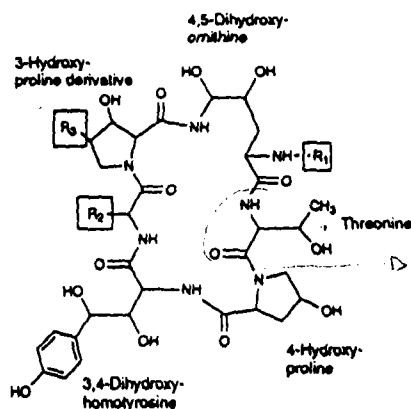
Growth conditions. Cultures of *S. cerevisiae* GS-1-36 were maintained on YEPD (1% yeast extract, 2% peptone, 2% glucose) medium plates containing 2% agar. Cells were grown at 30°C in 200 ml of YEPD medium for 12 h at 200 rpm. Cells (200 ml) were then transferred to 2 liters of YEPD medium and were grown to the mid-log phase (4 to 6 h) at 30°C and 200

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†This is publication D-10558-3-93 of the New Jersey Agricultural Experiment Station.

123

A



Compound	R ₁	R ₂	R ₃
Echinocandin B	 (linoleic acid)	 (Threonine)	CH ₃
Cilofungin	 (p-octyloxybenzoic acid)	 (Threonine)	CH ₃
Pneumocandin B ₀	 (dimethylmyristic acid)	 (Hydroxyglutamine)	H

B

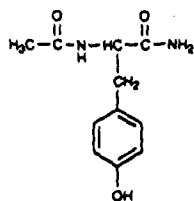
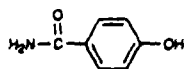
N-Acetyl-tyrosinamide
(NATA)4-Hydroxybenzamide
(HBA)

FIG. 1. Lipopeptide and analog structures. (A) Structures of echinocandin B, cilofungin, and pneumocandin B₀. (B) Structures of the tyrosine analog NATA and the cilofungin side chain analog HBA.

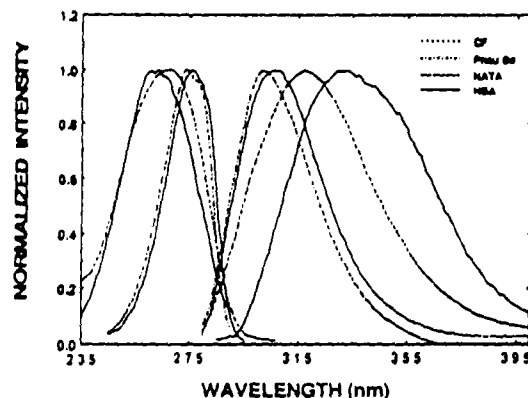


FIG. 2. Normalized fluorescence excitation and emission spectra of cilofungin (CF), pneumocandin B₁ (Pneu B₁), HBA, and NATA. Each compound was present at a level of 10 μ M in TBS buffer. Emission spectra were scanned by using excitation and emission bandwidths of 7.6 and 3.8 nm, respectively. Excitation spectra were scanned by using excitation and emission bandwidths of 3.8 and 7.6 nm, respectively. Each spectrum was normalized to unity by dividing by its peak intensity.

rpm. *C. albicans* was inoculated into 375 ml of YEPD medium, and the mixture was shaken at 30°C for 24 h. Cells were then transferred to 15 liters of YEPD medium, grown to the mid-log phase (optical density at 600 nm, 3.0), and harvested by centrifugation at $3,000 \times g$ for 10 min.

Membrane isolation. All membrane isolation steps were conducted as described previously (9) on ice or at 4°C. Both *S. cerevisiae* and *C. albicans* were disrupted with a Bead Beater (Biospec Products, Inc., Bartlesville, Okla.). Wet paste (50 g) was suspended in 250 ml of 250 mM sucrose–3 mM EDTA–3 mM EGTA [ethylene glycol-bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid]–2 mM dithiothreitol (DTT)–1 mM phenylmethylsulfonyl fluoride–70 mM Tris-HCl (pH 7.5) in a 340-ml vessel. Prechilled glass beads (diameter, 0.5 mm; total weight, 160 g) were added and the vessel was then fully filled. The Bead Beater was operated for five 20 s bursts, with 5-min cooling intervals. The crude extract was recovered, and the beads were washed with an equal volume of homogenization buffer (minus sucrose). The fractions were combined and centrifuged at $1,500 \times g$ for 10 min to remove the cell debris.

Microsomal membrane fractions were prepared by differential centrifugation (9). Mitochondria were removed by centrifugation of the extract at $23,000 \times g$ for 10 min. Microsomes were recovered by centrifugation of the supernatant at $100,000 \times g$ for 1 h. The microsomes were resuspended to concentrations of 12 to 48 mg ml⁻¹ in 1 mM EDTA–1 mM DTT–33% (vol/vol) glycerol–50 mM Tris-HCl (pH 7.5) and were stored at -80°C. The protein content was determined by the Coomassie brilliant blue dye-binding method (4), with bovine serum albumin used as a standard.

GS assays. *S. cerevisiae* and *C. albicans* were assayed in mixtures of 100 μ l containing 20 μ M GTP- γ -S, 1 mM EDTA, 8% (vol/vol) glycerol, 0.5% (vol/vol) Brij 35, 80 mM Tris-HCl (pH 7.75), microsomal membranes (100 μ g of protein), and 2 mM UDP-[¹⁴C]Glc (0.625 mCi mmol⁻¹) (9). Assay mixtures were incubated for 40 min at 30°C, and the assays were stopped by the addition of 100 μ l of 10% trichloroacetic acid. Samples were transferred to a 96-well Millipore apparatus (Millipore, Bedford, Mass.). The filters were removed, air-dried, and counted on a direct beta counter (Matrix-9600; Packard In-

struments, Downers Grove, Ill.). The 50% inhibitory concentrations were defined as the concentrations that inhibited 50% of the GS activity in assay mixtures containing standard components.

Liposome preparation. Small unilamellar vesicles (SUVs) were prepared by sonication as described previously (15). One milliliter of PC stock solution (100 mg ml⁻¹) stored at -20°C in chloroform was withdrawn into a tube and was dried under nitrogen gas. Traces of solvent were further removed by vacuum centrifugation for 1 h. One milliliter of TBS buffer (40 mM Tris-HCl, 100 mM NaCl [pH 7.4]) was then added to the dried lipid, and the combination was mixed vigorously for 5 min. Vesicles were formed by sonicating lipid dispersions in a probe-type (1/2-in. [1.27-cm]) sonicator (model W-225; Heat System-Ultrasonics, Inc., Farmingdale, N.Y.) at 70 W for 2 h. Tubes were sonicated for 10-min bursts with 5-min intervals on ice until the vesicle solutions became clear. Vesicles were then centrifuged at $13,600 \times g$ for 2 min to remove the debris. Half of the vesicle suspensions was used for fluorescence titration, and half was used to prepare SUVs. SUVs were obtained by centrifugation at $105,000 \times g$ for 45 min at 4°C. PC vesicles and SUVs were prepared fresh for each use and were maintained at 4°C.

PL assay. Membrane phospholipid (PL) was quantified by determining P_i levels as described previously (1).

Fluorescence spectra. Fluorescence excitation and emission spectra were measured on a SPEX FIT11 spectrofluorimeter (SPEX Industries, Metuchen, N.J.). Solutions of cilofungin, pneumocandin B₁, NATA, and HBA were prepared at 10 μ M in TBS buffer by diluting stock solutions prepared at 40 mM in dimethyl sulfoxide (DMSO). The final concentration of DMSO in the samples was 0.025%. Excitation wavelengths were determined from examination of the UV absorption spectrum of each compound on a UV-160A model UV-VIS recording spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, Md.). Emission spectra were collected by using various excitation wavelengths spanning the absorption band of each compound. The wavelength maximum of an emission spectrum was determined by evaluating the wavelength at which the first derivative of the spectrum had a value of zero. Excitation and emission spectra were plotted either as normalized intensity or as relative intensity, in which the intensity at each value of a series of spectra was normalized to the peak value of one of the spectra in the series (usually the cilofungin spectrum).

The quantum yield (*Q*) of cilofungin in various solvent systems was measured by standard techniques (5). Cilofungin was dissolved to 10 mg ml⁻¹ in ethanol, and aliquots containing 200 μ g were removed and dried under speed vacuum for 2 h. The dried material was then redissolved to 5% in organic alcohols (see Table 2). Each sample was excited at 270 nm (7.6-nm bandwidth), and the emission spectra (3.8-nm bandwidth) were collected. Emission maxima were obtained as described above. The quantum yield of cilofungin in solvent (*Q*₂) relative to that in aqueous buffer (*Q*₁) was calculated according to the following relation (5): $Q_2/Q_1 = (I_2/I_1)(A_1/A_2)$, where *I* is the integrated emission intensity of cilofungin in aqueous buffer (*I*₁) or in the solvent (*I*₂), and *A* is the absorbance of cilofungin in buffer (*A*₁) or in solvent (*A*₂) at an excitation wavelength of 270 nm.

Fluorescence titration and anisotropy measurements. Cilofungin fluorescence titrations were conducted in 2-ml mixtures, the cilofungin concentration was held constant at 40 μ M, and microsomal membranes from *S. cerevisiae* (1.23 mg of PL mg⁻¹ of protein; 20 μ g of PL μ l⁻¹), *C. albicans* (1.52 mg of PL mg⁻¹ of protein; 20 μ g of PL μ l⁻¹), or PC vesicles (30 μ g of PL μ l⁻¹)

TABLE 1. Excitation and emission maxima of the tested compounds

Compound (10 μ M)	Excitation maximum (nm)	Emission maximum (nm)
HBA	265	334
Cilofungin	270	317
HBA + NATA	274	310
Pneumocandin B ₀	275	303
NATA	276	306

were used. These PL levels are in agreement with previously published values (14, 39). The cilofungin fluorescence spectra measured for each sample were corrected for background fluorescence from the buffer and/or membrane samples by subtracting an emission spectrum obtained from a control sample containing membranes in buffer at the same concentration but without cilofungin. Relative intensities were calculated relative to the intensity of the cilofungin control, which consisted of cilofungin in TBS with no added PL. Fluorescence titrations with pneumocandin B₀ were conducted similarly.

Steady-state cilofungin fluorescence anisotropy experiments were measured at ambient temperature ($22 \pm 2^\circ\text{C}$) (33). Cilofungin at 40 μ M in TBS (0.1% DMSO) was directly titrated with increasing amounts of fungal membranes (the concentrations were given above) or PC SUVs (19 μ g of PL μ L⁻¹). DMSO at 0.1% did not affect the anisotropy values. Fluorescence measurements were made on the SPEX FIT11 spectrofluorimeter equipped with Glan-Thompson polarization optics by using an excitation wavelength of 270 nm (23-nm bandwidth) and an emission wavelength of 318 nm (15-nm bandwidth).

Fluorescence anisotropy (r) was calculated by using the following equation: $r = (I_{VV} - G I_{VH}) / (I_{VV} + 2 G I_{VH})$, where I_{VV} and I_{VH} are the fluorescence intensities determined at vertical and horizontal orientations of the emission polarizer, respectively, when excitation is set in the vertical position, and I_{HV} and I_{HH} are the fluorescence intensities determined at the vertical and horizontal orientations of the emission polarizer, respectively, when the excitation polarizer is set horizontally. $G (= I_{HV}/I_{HH})$ is a factor that corrects for the differential responses of the emission monochromator and detector to vertically and horizontally polarized light. Individual fluorescence polarization intensities were corrected for background fluorescence and light scattering from the membranes without probes (33) by the method described above.

RESULTS

Spectral properties of cilofungin. Drugs that possess aromatic or conjugated structures often exhibit fluorescence properties and can therefore be used as fluorescent probes (6, 19). The cyclic peptide cilofungin, which contains two aromatic groups, a 3,4-dihydroxyhomotyrosine moiety and a *p*-octyloxybenzoic acid side chain amide bonded to the ring (Fig. 1), had an excitation maximum of 270 nm and an emission maximum of 317 nm in aqueous TBS buffer (Fig. 2 and Table 1). Since the observed spectral properties of cilofungin may be due to either of the two aromatic groups (3, 19), the fluorescence properties of the cilofungin analog pneumocandin B₀ (32) and analogs of the two aromatic chromophores found in cilofungin were also studied in order to determine the relative contributions of the two aromatic groups to the observed cilofungin spectra.

Pneumocandin B₀ is a close structural analog of cilofungin in

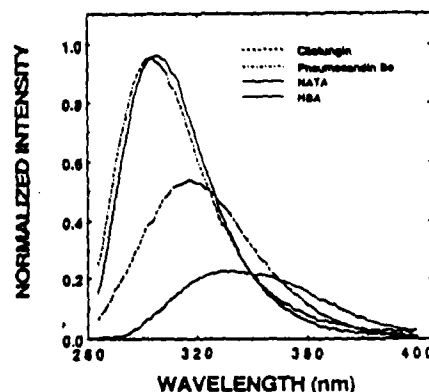


FIG. 3. Relative fluorescence emission intensities of cilofungin, pneumocandin B₀, NATA, and HBA. Each compound was present at a level of 10 μ M in TBS buffer. An excitation wavelength of 270 nm (7.6-nm bandwidth) was used for each compound, and emission spectra (3.8-nm bandwidth) were monitored. All spectra were normalized to the peak intensity value of a spectrum of a 1:1 mixture of NATA and HBA in TBS buffer at a total drug concentration of 10 μ M.

which the *p*-octyloxybenzoic acid side chain is replaced by a 10,12-dimethylmyristate group at the R1 position (Fig. 1); two additional structural changes at positions R2 and R3 should not significantly influence the spectral properties of the molecule. The fluorescence from pneumocandin B₀ should thus reflect the spectral properties of the 3,4-dihydroxyhomotyrosine moiety of cilofungin. Pneumocandin B₀ had an excitation maximum of 275 nm and an emission maximum of 303 nm (Table 1). The observed spectra were nearly identical in shape (Fig. 2), spectral maxima (Table 1), and relative intensity (Fig. 3) to those of the tyrosine analog NATA; the slight differences in excitation and emission maxima between pneumocandin B₀ and NATA may reflect the electronic influence of the hydroxyl group adjacent to the phenol ring in pneumocandin B₀ (Fig. 1). These spectral similarities indicated that the dihydroxyhomotyrosine moiety of pneumocandin B₀, and thus, that of cilofungin, has spectroscopic properties typical of the phenol side chain of tyrosine.

We also examined the spectral properties of HBA, an analog of the amide-linked *p*-octyloxybenzoic acid side chain of cilofungin (Fig. 1). While the shape of the HBA excitation and emission spectra were similar to those of cilofungin (Fig. 2), the HBA fluorescence had an excitation maximum of 265 nm and an emission maximum of 334 nm; these spectra were blueshifted and redshifted, respectively, from the comparable spectra seen in cilofungin. The relative intensity of the HBA emission spectrum was about half that seen in cilofungin (Fig. 3).

Since the excitation and emission spectral maxima (Fig. 2 and Table 1) and the relative emission intensity (Fig. 3) of cilofungin were intermediate between those of NATA and HBA, the analogs of the two chromophores present in cilofungin, it appeared that the cilofungin fluorescence was a sum of the fluorescence properties of both aromatic chromophores in the molecule. We tested this possibility by comparing the cilofungin emission spectrum to the emission spectra of samples containing NATA and HBA mixed at different mole ratios of HBA/NATA but at identical total chromophore concentrations (Fig. 4). The emission spectrum of the 1:1 mixture of HBA and NATA, which reflects the actual ratio of chro-

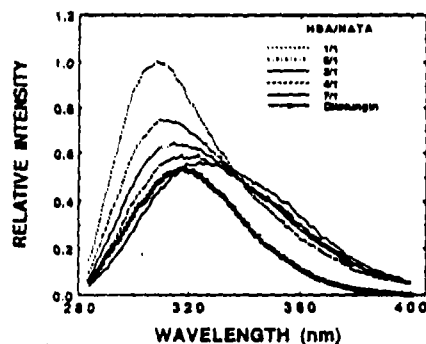


FIG. 4. Estimation of energy transfer efficiency. The ratios of HBA:NATA were varied (as indicated), while the total fluorophore or cilofungin concentration was held constant at a level of 10 μ M in TBS buffer. Samples were excited at 270 nm, and emission spectra were monitored by using a bandwidth of 3.8 nm.

mophores in cilofungin, was clearly different from that of cilofungin in both relative intensity and spectral position (Fig. 4). However, the spectra of mixtures containing mole ratios of 4:1 or 7:1 HBA to NATA did resemble the cilofungin spectrum in both intensity and spectral position. At least to the extent that these analogs model the two aromatic chromophores of cilofungin, this result indicated that the fluorescence emission of cilofungin is dominated by the emission from the *p*-octyloxybenzoic acid side chain. This suggests that the fluorescence from the dihydroxyhomotyrosine moiety of cilofungin is quenched and that the fluorescence from the *p*-octyloxybenzamide side chain is enhanced; a likely possibility for this quenching is intramolecular resonance energy transfer from the dihydroxyhomotyrosine to the *p*-octyloxybenzamide chromophore (19). Since there is significant spectral overlap between the excitation spectrum of HBA and the emission spectrum of NATA (Fig. 2), but not between the excitation spectrum of NATA and the emission spectrum of HBA, this explanation is reasonable. We estimate that the energy transfer efficiency between homotyrosine and *p*-octyloxybenzamide was in the range of from 60 to 80%.

The quantum yields and emission maxima of cilofungin in various organic solvents are given in Table 2. The emission maxima of cilofungin were shifted to shorter wavelengths in organic solvents in comparison with the values in organic buffer. This shift is in accordance with the spectral theory which predicts a blueshift of the emission spectra in more hydrophobic environments (19). The quantum yields of cilofungin were also lower in organic solvents. The effects of

TABLE 2. Spectral characteristics of cilofungin in various solvents

Solvent	Emission maximum (nm)	Quantum yield ^a
TBS	317	1.00
Methanol	304	0.54
Ethanol	305	0.49
2-Propanol	306	0.66
1-Butanol	305	0.55

^a Quantum yield values are expressed relative to that of the drug in TBS buffer.

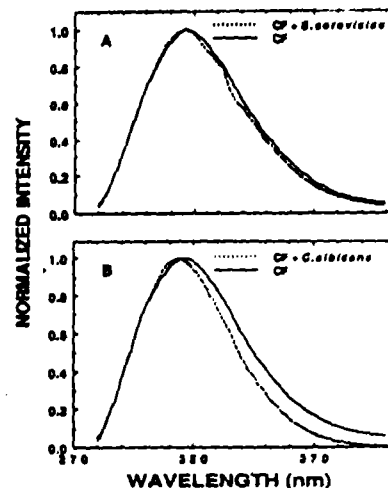


FIG. 5. Cilofungin (CF) emission spectral shift induced by interaction with fungal membranes. Cilofungin at 40 μ M was incubated in the absence and presence of microsomal membranes from *S. cerevisiae* (A) and *C. albicans* (B). Both membranes were added to a level of 66.8 μ M membrane PL.

various solvents on the emission spectrum of HBA were also tested. The spectrum of HBA was blueshifted in methanol, ethanol, and 2-propanol (from 334 to 312 to 316 nm; data not shown). Thus, HBA emission was also affected by exposure to hydrophobic solvents.

Interaction of cilofungin with yeast membranes. Cilofungin is an inhibitor of (1,3)- β -glucan synthesis in *C. albicans* (35), but it is not an inhibitor of the GS from *S. cerevisiae*. We obtained 50% inhibitory concentrations of 37 μ M and >1 mM for *C. albicans* and *S. cerevisiae*, respectively, using standard assay conditions. The MIC of cilofungin for *C. albicans* was 3.0 μ g ml⁻¹, whereas it was 50 μ g ml⁻¹ for *S. cerevisiae*. The MICs were determined by the microtiter dilution method with yeast nitrogen broth medium (28). The fatty acid side chain of cilofungin has been shown to be essential for cilofungin's inhibitory effects (34). This differential inhibition led us to investigate the ability of cilofungin to bind to membranes from both organisms. Cilofungin's fluorescence properties enabled a direct observation of the interactions between cilofungin and the fungal membranes (Fig. 5 and 6).

Upon incubation of cilofungin with equal concentrations of membranes from either *C. albicans* or *S. cerevisiae*, the emission of the probe blueshifted to shorter wavelengths (Fig. 5). Such a shift is direct evidence for cilofungin-membrane interactions (6). The magnitude of the shift, however, was significantly greater with *C. albicans* membranes (4-nm shift of the emission maximum) than for *S. cerevisiae* membranes (1-nm shift of the emission maximum). A blueshift in the emission spectrum is typically observed upon transfer of a fluorophore from an aqueous environment to a more hydrophobic environment (6, 19); as noted above (Table 2), both cilofungin and the side chain analog of the *p*-octyloxybenzamide side chain of cilofungin also displayed a significant blueshift upon transfer to a nonpolar medium. These results indicated that there is a

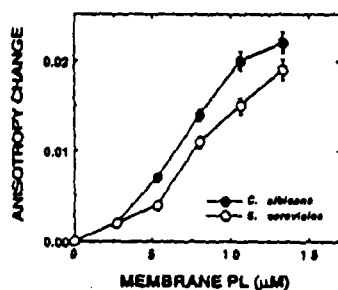


FIG. 6. Anisotropy titration of cilofungin with yeast membranes. Cilofungin at a level of 40 μM in TBS buffer was titrated with increasing amounts of yeast membranes, as indicated. The change in anisotropy was calculated as described in the text.

direct interaction between cilofungin and the fungal membranes. Moreover, the larger blueshift seen in the presence of *C. albicans* membranes indicated that cilofungin interacts more strongly with these membranes than with *S. cerevisiae* membranes.

The influence of fungal membranes on the cilofungin emission spectra was not uniform across the emission band. Interaction with the membranes produced a significant blueshift on the red edge of the spectrum and but had no effect on the blue edge of the spectrum (Fig. 5). Since the cilofungin emission spectrum is a combination of emission from the dihydroxyhomotyrosine moiety, which has a peak position at about 303 nm, and emission from the *p*-octyloxybenzamide side chain, which has a peak position at about 334 nm (using emission maxima values from Table 1), the blue edge of the cilofungin emission reflects emission from the dihydroxyhomotyrosine moiety and the red edge reflects emission from the *p*-octyloxybenzamide side chain (Fig. 2). Binding to membranes thus produced a large perturbation in the emission of the *p*-octyloxybenzamide side chain of cilofungin and little or no perturbation in the emission of the dihydroxyhomotyrosine moiety in the peptide ring; this suggests that the former group, but not the latter group, is involved in physical interactions with the membrane.

The emission anisotropy of cilofungin was also used to monitor the interaction of the drug with fungal membranes. The cilofungin anisotropy was 0.242 in aqueous TBS buffer; titration of cilofungin with increasing concentrations of fungal membranes produced a dose-dependent increase in the cilofungin anisotropy (Fig. 6), predicting further evidence that the drug interacted with the membranes. The increase in anisotropy was larger with *C. albicans* membranes than with *S. cerevisiae* membranes, providing further indications that *C. albicans* membranes bind cilofungin more strongly than membranes from *S. cerevisiae*.

Interaction of cilofungin and pneumocandin B₀ with membrane vesicles. The data in Fig. 5 suggest that the interaction of cilofungin with fungal membranes is directly mediated by the *p*-octyloxybenzamide side chain of the drug. We tested this possibility by comparing the spectral responses of cilofungin and pneumocandin B₀, which does not contain the *p*-octyloxybenzamide side chain, to titration with PC SUVs. PC was chosen because it is a major membrane phospholipid of both *S. cerevisiae* (39) and *C. albicans* (14). Fluorescence titrations were performed by holding the drug levels constant and varying the level of PC vesicles (Figs. 7 and 8). Interaction with the PC vesicles produced a large blueshift, from 317 to 307 nm (Fig. 7A), in the emission maximum of cilofungin as well as a

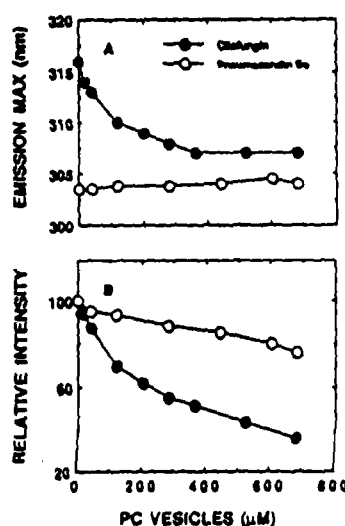


FIG. 7. Interactions of cilofungin and pneumocandin B₀ with PC membrane vesicles. Cilofungin and pneumocandin B₀ were held constant at a level of 10 μM in TBS buffer and increasing amounts of PC vesicles were added, as indicated. (A) Emission wavelength maxima; (B) relative emission intensities.

decrease of over 70% in the relative intensity of the emission (Fig. 7B); as noted above (Table 2) these are the spectral responses expected for the transfer of cilofungin from an aqueous to a nonpolar environment. Similar titrations of pneumocandin B₀ with PC vesicles, however, had no effect on the emission maximum of the drug (Fig. 7A) and little effect on the relative emission intensity (Fig. 7B), indicating that, although pneumocandin B₀ appears to interact with these membranes, the interaction has little influence on the spectral properties of the dihydroxyhomotyrosine chromophore.

The lack of any significant fluorescence perturbation associated with interactions of pneumocandin B₀ with PC vesicles provides a strong indication that the tyrosine group, which is located on the core peptide ring of both cilofungin and pneumocandin B₀ (Fig. 1), does not interact with the membranes. The significant fluorescence perturbations associated with interactions of cilofungin with PC vesicles, however, provides a strong indication that the *p*-octyloxybenzamide side chain does interact with the hydrophobic region of the mem-

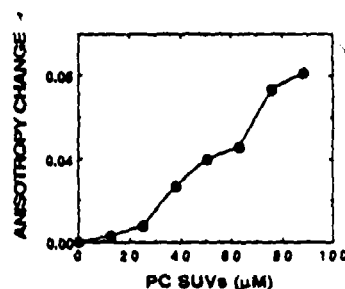


FIG. 8. Anisotropy titration of cilofungin with PC SUVs. Cilofungin at a level of 40 μM in TBS buffer was titrated with PC SUVs, as indicated. The change in anisotropy was obtained as described in the text.

branes. Further evidence for the strong interaction of cilofungin with PC vesicles was provided by the dose-dependent increase in the cilofungin anisotropy caused by titration with PC vesicles (Fig. 8). This increase provided direct evidence for a physical interaction between the drug and the vesicles.

DISCUSSION

In the study described here, we demonstrated that cilofungin possesses spectral properties which enable it to be used as a direct fluorescent probe of cilofungin-membrane interactions. Cilofungin exhibits an excitation wavelength maximum of 270 nm and an emission maximum of 317 nm (Fig. 2). This excitation wavelength suggests that cilofungin's fluorescence derives from either the homotyrosine group of the peptide or the oxybenzamide group on the amide-linked *p*-octyloxybenzoic acid moiety (19). To clarify the relative contribution of each of these structural moieties to cilofungin's emission properties, various structural analogs, which included pneumocandin B₁, the tyrosine analog NATA, and the side chain analog HBA, were characterized. On the basis of cilofungin's spectral properties (Fig. 2 and 3) and measurements of energy transfer efficiency (Fig. 4), it was shown that cilofungin's fluorescence properties derive largely from the oxybenzamide side chain group. Thus, echinocandin-like antibiotics and their structural analogs can be added to the growing list of drugs that possess fluorescence properties which can be used as direct probes for monitoring drug-membrane binding interactions (6, 7, 13).

When cilofungin was incubated with fungal membranes (Fig. 5) or lipid vesicles (Fig. 7), spectral blueshifts were observed. Blueshifts are indicative of the transfer of fluorescent groups to more hydrophobic environments (6, 7, 27) and occur as the result of the interactions of cilofungin with the lipid bilayer. Consistent with this interpretation, the drug anisotropy increased in a dose-dependent manner (Fig. 6 and 8) and the quantum yield decreased in a manner expected for transfer to a nonpolar solvent (Table 2 and Fig. 7A). Binding of the calcium channel and β -adrenergic blockers depend on the drug interactions with the receptor and membrane bilayer (21). It should be noted that with membranes from *C. albicans*, higher anisotropy increases were obtained when compared with those from equivalent levels of membranes from *S. cerevisiae* (Fig. 6); this implies that membranes from *C. albicans* have a greater affinity for cilofungin. These results are interesting in light of the differential susceptibilities of the two GAs to inhibition by cilofungin, but they do not prove that variations in membrane structure are solely responsible for the differential effects that cilofungin has on the two types of membranes. The significance of these results should be viewed in a proper context because of the many inherent structural variabilities present when comparing mixed membrane preparations derived from different sources. However, resistance to echinocandin-like antibiotics in *C. albicans* has been attributed to changes in membrane composition (22). Since PC is the major phospholipid of *S. cerevisiae* and *C. albicans* (20), we used PC SUVs as a model system to show the interaction of cilofungin with membrane components. Minor changes in membrane composition between *S. cerevisiae* and *C. albicans* could potentiate the mode of action of echinocandin-like antibiotics.

In conclusion, the results of the present study demonstrate that echinocandin-like antibiotics and semisynthetic analogs such as cilofungin possess fluorescence properties which can be used to directly study the interactions of membrane vesicles with antifungal drugs. Fluorescence spectroscopic techniques can be used as a means of examining structure-activity rela-

tionships and interactions of the fatty acid side chain moieties with lipid bilayers preclinically. The strong interaction of cilofungin with synthetic liposomes of defined composition suggests that the inhibition of (1,3)- β -glucan synthesis could result from the combined effects of perturbation of the membrane environment as well as the interaction of cilofungin with the (1,3)- β -GS complex.

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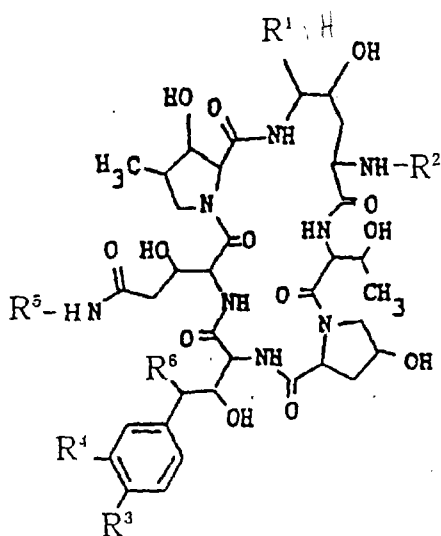
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EUROPEAN PATENT APPLICATION

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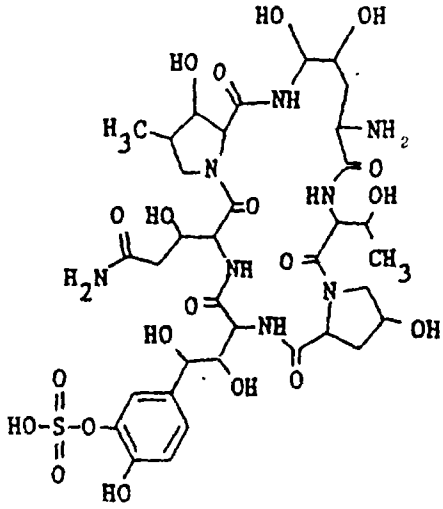
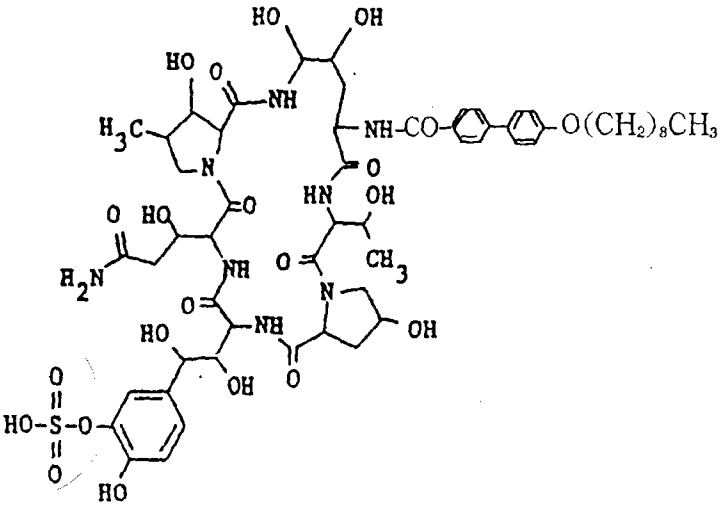
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D-40593 Düsseldorf (DE)**(54) **Cyclic antimicrobial peptides and preparation thereof.**

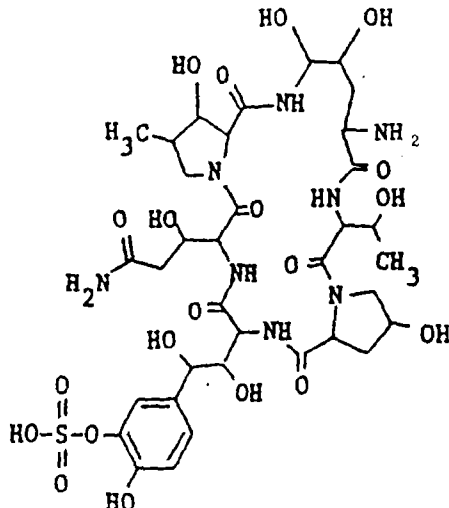
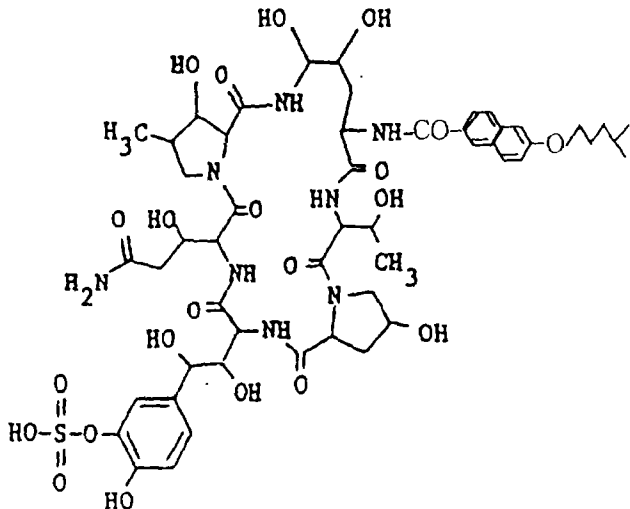
(57) A polypeptide compound of the following general formula :

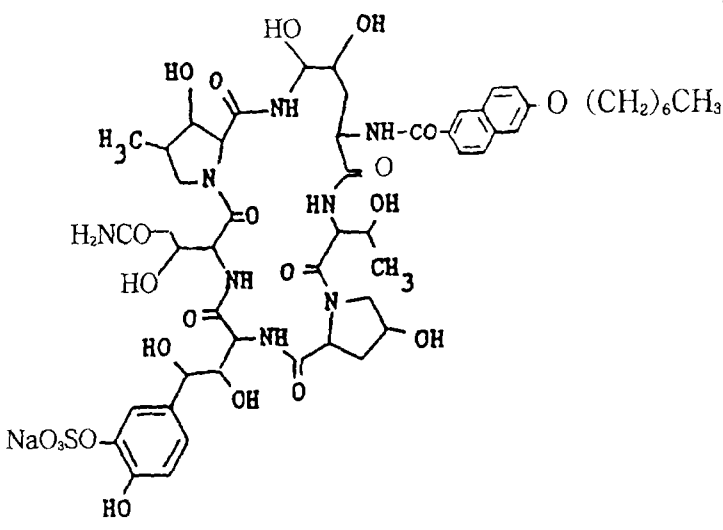
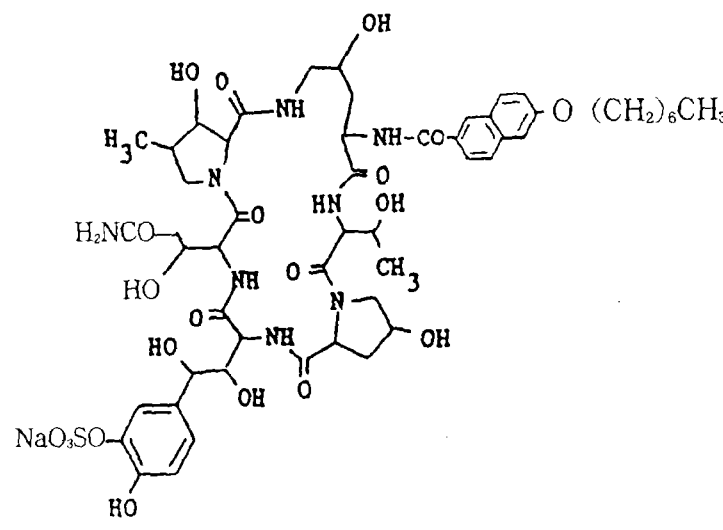


[1]

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Preparation No.	Formula
12	
	

Preparation No.	Formula
13	 The chemical structure is a complex molecule featuring a central core with multiple hydroxyl groups and amide bonds. It includes a sulfonate group (HO-SO₂-O-) and a methyl group (H₃C). The structure is highly branched and contains several nitrogen atoms.
	 This chemical structure is similar to the one above, but it features a different side chain on the right-hand side, which includes a sulfonate group (HO-SO₂-O-) and a methyl group (H₃C). The overall structure is highly branched and contains several nitrogen atoms.

Example No.	Formula
3	 The chemical structure is a complex molecule featuring several interconnected rings and functional groups. It includes a central amide bond, multiple hydroxyl groups, and a sodium sulfonate group (NaO₃SO-) attached to a benzene ring. The structure is highly branched and contains various stereocenters.
3	 This chemical structure is identical to the one in the first row, showing a complex molecule with multiple hydroxyl groups, amide bonds, and a sodium sulfonate group (NaO₃SO-) attached to a benzene ring.

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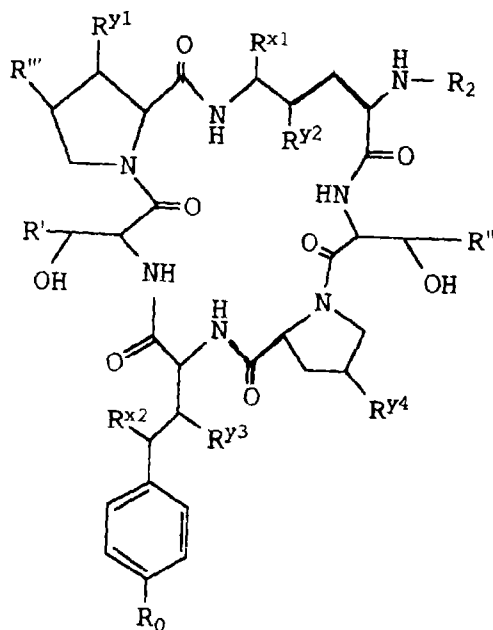
European Patent Operations

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(54) Cyclic hexapeptide antifungal agents

(57) Provided are pharmaceutical formulations, and methods of inhibiting fungal and parasitic activity using a compound of formula I:



wherein:

R' is hydrogen, methyl or NH₂C(O)CH₂-;

R'' and R''' are independently methyl or hydrogen; R^{x1}, R^{x2}, R^{y1}, R^{y2}, R^{y3}, and R^{y4} are independently hydroxy or hydrogen;

Description

This invention relates to semi-synthetic cyclic peptide compounds which are useful as antifungal and antiparasitic agents and which have improved stability and water solubility. In particular, it relates to derivatives of the echinocandin class of cyclic peptides; to methods for treating fungal and parasitic infections, and to formulations useful in the methods.

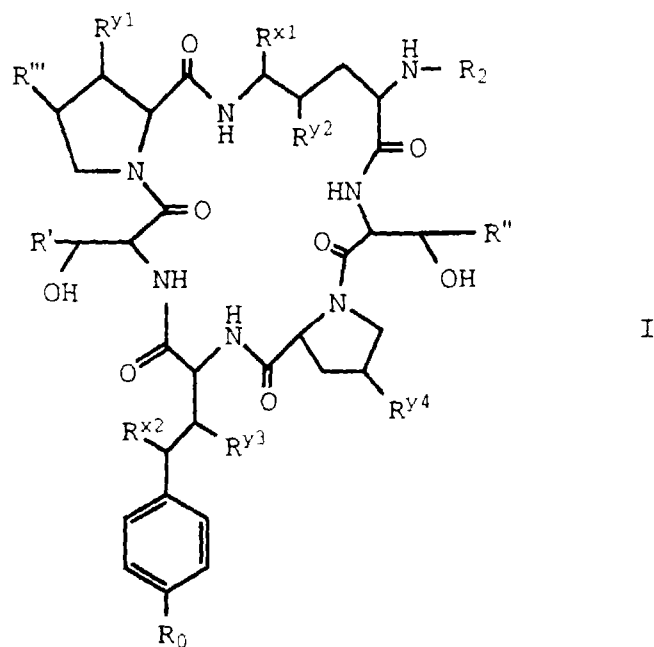
The compounds provided by this invention are semi-synthetic compounds derived from cyclic peptides which are produced by culturing various microorganisms. A number of cyclic peptides are known in the art including echinocandin B (A30912A), aculeacin, mulundocandin, sporiofungin, L-671,329, and S31794/F1.

In general, these cyclic peptides may be structurally characterized as a cyclic hexapeptide core (or nucleus) with an acylated amino group on one of the core amino acids. The amino group is typically acylated with a fatty acid group forming a side chain off the nucleus. For example, echinocandin B has a linoleoyl side chain while aculeacin has a palmitoyl side chain.

The fatty acid side chains may be removed from the cyclic peptide core to provide an amino nucleus (for example, a compound of formula I, below, where R_2 is hydrogen). The amino group may then be re-acylated to provide semi-synthetic compounds such as those claimed in the present application.

The echinocandin B nucleus has been re-acylated with certain non-naturally occurring side chain moieties to provide a number of antifungal agents (see, Debono, U.S. Pat. No. 4,293,489). Among such antifungal agents is cilofungin which is represented by a compound of formula I where R' , R'' , and R''' are methyl; R^{X1} and R^{X2} are hydroxy; R^{Y1} , R^{Y2} , R^{Y3} , R^{Y4} are hydroxy, R_0 is hydroxy, and R_2 is p-(octyloxy)benzoyl.

The present invention provides a compound of formula I:



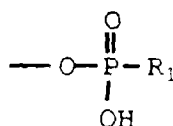
wherein:

R' is hydrogen, methyl or $NH_2C(O)CH_2-$;

R'' and R''' are independently methyl or hydrogen;

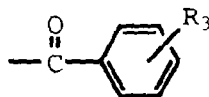
R^{X1} , R^{X2} , R^{Y1} , R^{Y2} , R^{Y3} , and R^{Y4} are independently hydroxy or hydrogen;

R_0 is a group of the formula



R_1 is C_1 - C_6 alkyl, C_1 - C_6 alkoxy, phenyl, p-halophenyl, p-nitrophenyl, phenoxy, benzyl, p-halo-benzyl, or p-nitro-benzyl;

I) R_2 is a group of the formula



where:

A) R_3 is C_1 - C_{12} alkyl, C_1 - C_6 alkoxy or quinolyl;

B) R_3 is $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$;

m and n are independently 2, 3 or 4;

p is 0 or 1; or

C) R_3 is $-Y-(C_1-C_{12} \text{ alkyl})$;

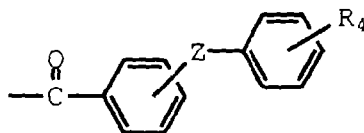
Y is $-C\equiv C-$ or $-CH=CH-$; or

D) R_3 is $-O-(CH_2)_q-G$;

q is 2, 3 or 4;

G is C_7 - C_{10} bicycloalkyl or C_7 - C_{14} tricycloalkyl; or

II) R_2 is a group of the formula



where:

Z is $-O-$, $-C\equiv C-$, $-CH=CH-$, $-CH_2-CH_2-$, $-CH_2-$, or a bond;

A) R_4 is hydrogen, C_1 - C_{12} alkyl, C_1 - C_{12} substituted alkyl, C_2 - C_{12} alkenyl, C_2 - C_{12} substituted alkenyl, C_2 - C_{12} alkynyl, C_2 - C_{12} substituted alkynyl, C_1 - C_{12} alkoxy, C_3 - C_{12} cycloalkyl, C_7 - C_{10} bicycloalkyl, C_7 - C_{14} tricycloalkyl, C_3 - C_{12} cycloalkoxy, naphthyl, pyridyl, thienyl, benzothienyl, quinolyl or phenyl; or

B) R_4 is phenyl substituted by amino, C_1 - C_{12} alkylthio, halo, C_1 - C_{12} alkyl, C_2 - C_{12} alkenyl, C_2 - C_{12} alkynyl, C_1 - C_{12} substituted alkyl, C_2 - C_{12} substituted alkenyl, C_2 - C_{12} substituted alkynyl, C_1 - C_{12} alkoxy, trifluoromethyl, phenyl, substituted phenyl, or phenyl substituted with a group of the formula $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$ where m , n and p are as defined above; or

C) R_4 is C_1 - C_{12} alkoxy substituted with halo, C_3 - C_{12} cycloalkyl, C_7 - C_{10} bicycloalkyl, C_7 - C_{14} tricycloalkyl, C_1 - C_6 alkoxy, C_2 - C_{12} alkynyl, amino, C_1 - C_4 alkylamino, di(C_1 - C_4 alkyl)amino, formamido, C_2 - C_{12} alkanoylamino, or phenyl substituted with a group of the formula $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$ where m , n and p are as defined above; or

D) R_4 is $-O-(CH_2)_r-W-R_5$;

r is 2, 3 or 4;

W is pyrrolidino, piperidino or piperazino;

R_5 is hydrogen, C_1 - C_{12} alkyl, C_3 - C_{12} cycloalkyl, benzyl or C_3 - C_{12} cycloalkylmethyl; or

E) R_4 is $-Y^1-R_6$;

Y^1 is $-C\equiv C-$ or $-CH=CH-$;

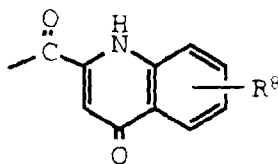
R_6 is C_3-C_{12} cycloalkyl, C_7-C_{10} bicycloalkyl, C_7-C_{14} tricycloalkyl, C_3-C_{12} cycloalkenyl, naphthyl, benzo-thiazolyl, thienyl, indanyl, fluorenyl, or phenyl substituted with C_1-C_{12} alkylthio, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, halo(C_1-C_6 alkoxy) or a group of the formula $-O-(CH_2)_r-W-R_5$ where r , W and R_5 are as defined above; or

R_6 is phenyl substituted with a group of the formula $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$ where m , n and p are as defined above; or

F) R_4 is C_1-C_{12} alkoxy substituted with a group of the formula $-NHC(O)R_7$;

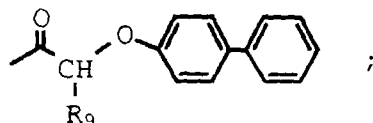
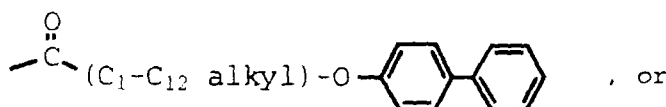
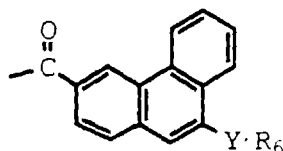
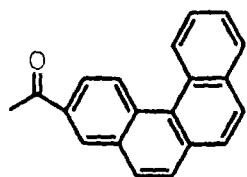
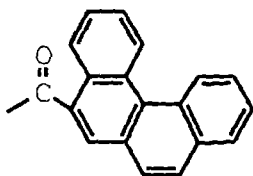
R_7 is C_1-C_6 alkoxy, or phenyl(C_1-C_6 alkoxy); or

III) R_2 is a group of the formula



where R^8 is C_1-C_{12} alkoxy or a group of the formula $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$ where m , n and p are as defined above; or

IV) R_2 is a group of the formula



where:

Y and R₆ are as defined above;

R₉ is phenyl, C₁-C₁₂ alkyl, or C₁-C₁₂ alkoxy; or

V) R₂ is naphthoyl substituted with R₄ where R₄ is as defined above;

or a pharmaceutically acceptable salt thereof.

Also provided are pharmaceutical formulations, methods for inhibiting parasitic or fungal activity and methods of treating fungal or parasitic infections which employ the compounds of the invention.

As used herein, the term "C₁-C₁₂ alkyl" refers to a straight or branched alkyl chain having from one to twelve carbon atoms. Typical C₁-C₁₂ alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl, pentyl, 5-methylpentyl, hexyl, heptyl, 3,3-dimethylheptyl, octyl, 2-methyl-octyl, nonyl, decyl, undecyl, dodecyl and the like. The term "C₁-C₁₂ alkyl" includes within its definition the terms "C₁-C₆ alkyl" and "C₁-C₄ alkyl."

The term "halo" refers to chloro, fluoro, bromo or iodo.

The term "C₂-C₁₂ alkenyl" refers to a straight or branched alkenyl chain having from two to twelve carbon atoms. Typical C₂-C₁₂ alkenyl groups include ethenyl, 1-propen-2-yl, 3-buten-1-yl, 1-buten-2-yl, 1-buten-1-yl, 1-penten-3-yl, 2-hexen-3-yl, 1-decen-2-yl, 2-decen-5-yl and the like.

The term "C₂-C₁₂ alkynyl" refers to a straight or branched alkynyl chain having from two to twelve carbon atoms. Typical C₂-C₁₂ alkynyl groups include ethynyl, 1-propyn-1-yl, 1-propyn-2-yl, 1-butyne-1-yl, 1-butyne-3-yl, 1-pentyne-3-yl, 4-pentyne-2-yl, 1-hexyn-3-yl, 3-hexyn-1-yl, 5-methyl-3-hexyn-1-yl, 5-octyn-1-yl, 7-octyn-1-yl, 4-decyn-1-yl, 6-decyn-1-yl and the like.

The term "C₁-C₁₂ alkylthio" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to a sulfur atom. Typical C₁-C₁₂ alkylthio groups include methylthio, ethylthio, propylthio, isopropylthio, butylthio, 3-methyl-heptylthio, octylthio, 5,5-dimethyl-hexylthio and the like.

The term "C₁-C₁₂ alkoxy" refers to a straight or branched alkyl chain having from one to twelve carbon atoms attached to an oxygen atom. Typical C₁-C₁₂ alkoxy groups include methoxy, ethoxy, propoxy, butoxy, sec-butoxy, pen-

compounds and their inherent antiparasitic and antifungal properties, or in other words, the compounds, used in the claimed methods are the causative agent for such inhibition.

The term "pharmaceutically acceptable salt" as used herein, refers to salts of the compounds of the above formula which are substantially non-toxic to living organisms. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a mineral or organic acid or an inorganic base. Such salts are known as acid addition and base addition salts.

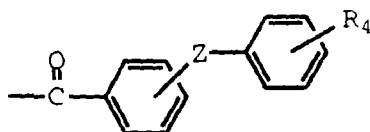
Acids commonly employed to form acid addition salts are mineral acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid and the like, and organic acids such as *p*-toluenesulfonic, methanesulfonic acid, oxalic acid, *p*-bromophenylsulfonic acid, carbonic acid, succinic acid, citric acid, benzoic acid, acetic acid, and the like. Examples of such pharmaceutically acceptable salts are the sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propionate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, butyne-1,4-dioate, hexyne-1,6-dioate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, sulfonate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, γ -hydroxybutyrate, glycolate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Preferred pharmaceutically acceptable acid addition salts are those formed with mineral acids such as hydrochloric acid and hydrobromic acid, and those formed with organic acids such as maleic acid and methanesulfonic acid.

Base addition salts include those derived from inorganic bases, such as ammonium or alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like. Such bases useful in preparing the salts of this invention thus include sodium hydroxide, potassium hydroxide, ammonium hydroxide, potassium carbonate, sodium carbonate, sodium bicarbonate, potassium bicarbonate, calcium hydroxide, calcium carbonate, and the like. The potassium and sodium salt forms are particularly preferred.

It should be recognized that the particular counterion forming a part of any salt of this invention is not of a critical nature, so long as the salt as a whole is pharmacologically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

Typical examples of acyl groups at R_2 in formula I include benzoyl substituted by a polyoxa-alkyl group such as 2-methoxyethoxy (*p* is 0, *m* is 1), 2-ethoxyethoxy (*p* is 0, *m* is 2), 2-(2-ethoxyethoxy)ethoxy (*m* is 2, *p* is 1, *n* is 2), 3-(2-ethoxyethoxy)propoxy, 4-(2-methoxyethoxy)butoxy, and the like, or benzoyl substituted by alkynyl groups ($-C\equiv C-$ (C_1-C_{12} alkyl)) such as propynyl, butynyl, hexynyl, undecynyl, and the like, or *cis* or *trans* alkenyl groups ($-CH_2=CH_2-$ (C_1-C_{12} alkyl)) such as propenyl, butenyl, hexenyl, decenyl, and the like.

Examples of acyl groups where R_2 is a group of the formula



include diphenyl ethers (*Z* is $-O-$), diphenyl acetylenes (*Z* is $-C\equiv C-$), stilbenes (*Z* is $-CH=CH-$) and biphenyls (*Z* is a bond).

Examples of diphenyl ether groups include 4-(4-butoxyphenoxy)benzoyl, 4-(4-hexoxyphenoxy)benzoyl, 4-(4-ethoxyphenoxy)benzoyl, 4-(4-phenyloxyphenoxy)benzoyl, 4-[4-(4-dodecyloxyphenoxy)benzoyl], 4-[4-(3-dimethylaminopropoxy)phenoxy]benzoyl and the like.

Examples of diphenyl acetylene and stilbene groups include 4-styrylbenzoyl, 4-(4-methoxystyryl)benzoyl, 4-(4-butoxystyryl)benzoyl, 4-(phenylethynyl)benzoyl, 4-(4-ethoxyphenylethynyl)benzoyl, 4-(4-cyclohexyloxyphenylethynyl)benzoyl and the like.

Examples of biphenyl groups include 4-[4-(butoxy)phenyl]benzoyl, 4-[4-(cyclobutylmethoxy)phenyl]benzoyl, 4-[4-(cyclopentylmethoxy)phenyl]benzoyl, 4-[4-(cyclohexylethoxy)phenyl]benzoyl, 4-[4-(hexoxy)phenyl]benzoyl, 4-phenylbenzoyl, 4-[4-(11-amino-undecyloxy)phenyl]benzoyl, 4-[4-(11-formamidoundecyloxy)phenyl]benzoyl, 4-[4-(isopentoxy)phenyl]benzoyl and the like.

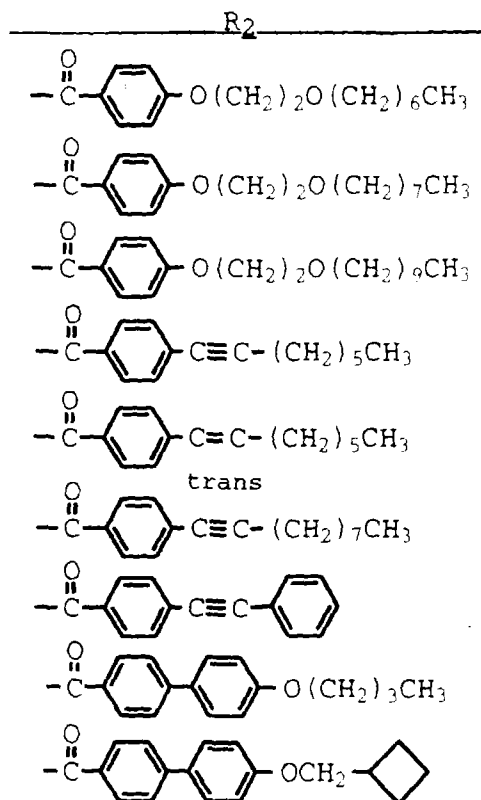
Examples of biphenyl groups where R_4 is $-O-(CH_2)_r-W-R_5$ include 4-[4-[2-(*N*-cyclohexylpiperidino-4-yl)ethoxy]phenyl]benzoyl, 4-[4-[2-(*N*-hexylpiperidino-4-yl)ethoxy]phenyl]benzoyl, 4-[4-[2-(4-benzylpiperidino)-ethoxy]phenyl]benzoyl, 4-[4-[2-(4-cyclohexylpiperidino)-ethoxy]phenyl]benzoyl and the like.

Examples of biphenyl and diphenyl ether groups where R_4 is $-Y^1-R_6$ include 4-[4-(phenylethynyl)phenyl]benzoyl, 4-[4-(phenylethynyl)phenoxy]benzoyl, 4-[4-(hexynyl)phenyl]benzoyl, 4-[4-(styryl)phenoxy]benzoyl, 4-[4-[4-methylpiperidino]ethoxy]phenylethynyl]benzoyl, and the like.

Acyl groups where R_4 is $-O-(CH_2)_r-W-R_5$ may form acid addition salts of the basic amino groups of the piperidine and piperazine heterocyclic groups with organic or mineral acids such as hydrochloric acid, hydrobromic acid, sulfuric acid and phosphoric acid and with organic acids such as the sulfonic acids, benzenesulfonic acid, toluenesulfonic acid, methanesulfonic acid, acetic acid, chloroacetic acid, trifluoroacetic acid, benzoic acid, isophthalic acid, salicylic acid, citric acid, malic acid, succinic acid, malonic acid and the like.

Table 1, below, provides further examples of acyl groups, R_2 , found on cyclic peptides of formula I.

Table 1



or a pharmaceutically acceptable salt thereof.

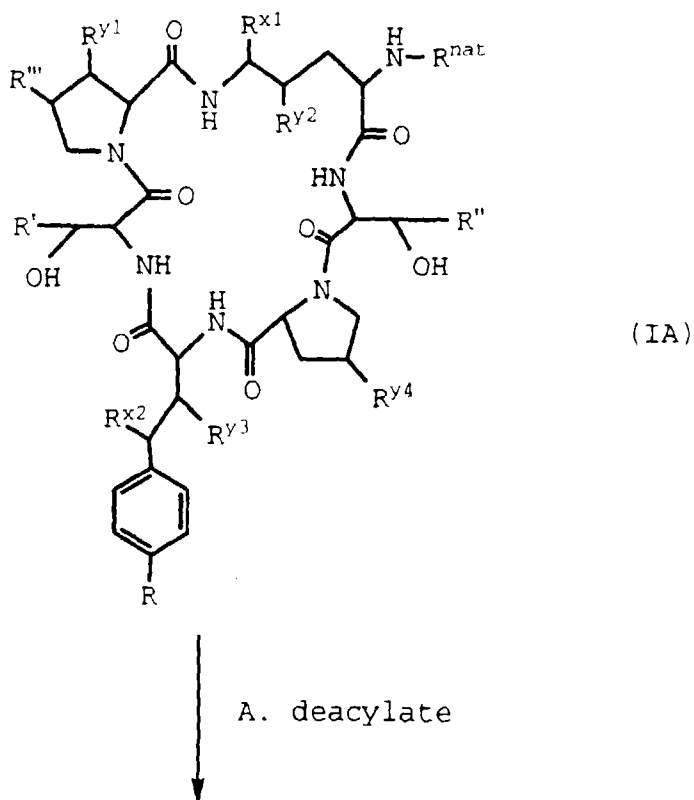
Of these compounds, more preferred are those compounds of formula I where:

R^{x1} and R^{x2} are hydrogen;

or a pharmaceutically acceptable salt thereof.

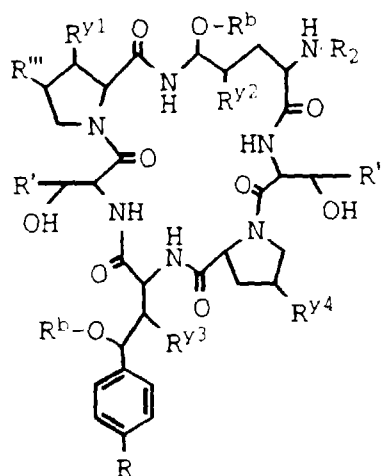
5 The compounds of formula I may be prepared according to Reaction Scheme I, as follows.

Reaction Scheme I



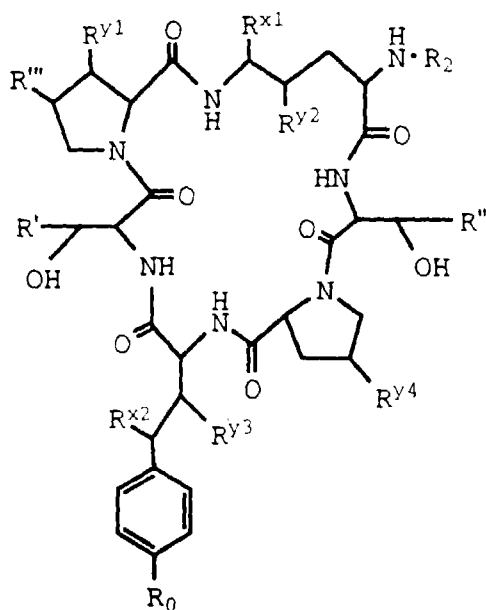
C'. Protect amination
hydroxy groups
[optional]

C. Phosphorylate



C''. Phosphorylate

C'''. Deprotect



wherein:

R^{nat} is a naturally occurring cyclic peptide sidechain;

R is hydroxy;

R^b is an hydroxy protecting group; and

R' , R'' , R''' , R^{x1} , R^{x2} , R^{y1} , R^{y2} , R^{y3} , R^{y4} , R, R_0 and R_2 are as defined above.

Reaction scheme I, above, is accomplished by carrying out reactions A-C (or A-C'''), in order. Once a reaction is complete, the intermediate compound may be isolated by procedures well-known in the art, for example, the compound may be crystallized and then collected by filtration, or the reaction solvent may be removed by extraction, evaporation or decantation. The intermediate compound may be further purified, if desired, by common techniques such as crystallization or chromatography over solid supports such as silica gel, alumina and the like, before carrying out the next step of the reaction scheme.

In reaction IA, a naturally occurring cyclic peptide of the formula (IA) is deacylated using procedures known in the art to provide an amino nucleus of formula (IB). This reaction is typically carried out using enzymatic acylation by exposing the naturally occurring cyclic peptide to a deacylase enzyme. The deacylase enzyme may be obtained from the microorganism Actinoplanes utahensis and used substantially as described in U.S. Patent Nos. 4,293,482 and 4,304,716, herein incorporated by reference. The deacylase enzyme may also be obtained from the Pseudomonas species. Deacylation may be accomplished using whole cells of Actinoplanes utahensis or Pseudomonas or the crude or purified enzyme thereof or using an immobilized form of the enzyme. See European Patent Application No. 0 460 882 (December 11, 1991). Examples of naturally occurring cyclic peptides which may be used as starting materials include aculeacin (palmitoyl side chain), tetrahydroechinocandin B (stearoyl side chain), mulundocandin (branched C_{15} side chain), L-671,329 (C_{16} branched side chain), S 31794/F1 (tetradecanoyl side chain), sporiofungin (C_{15} branched side chain), FR901379 (palmitoyl side chain) and the like. A preferred naturally occurring cyclic peptide is echinocandin B (a compound of formula (IA) where R' , R'' , and R''' are each methyl, R^{x1} and R^{x2} are each hydroxy, R^{y1} , R^{y2} , R^{y3} , R^{y4} are each hydroxy, R is hydroxy and R_2 is linoleoyl).

In Reaction IB, the resulting amino nucleus is then re-acylated using procedures known in the art to provide a compound of formula I where R_2 is an acyl group as defined hereinabove.

For example, the amino nucleus may be acylated by reaction with an appropriately substituted acyl halide, preferably in the presence of an acid scavenger such as a tertiary amine, such as triethylamine. The reaction is typically carried out at a temperature of from about -20°C to about 25°C . Typical solvents for this reaction include polar aprotic solvents such as dioxane or dimethylformamide. Solvent choice is not critical so long as the solvent employed is inert to the ongoing reaction and the reactants are sufficiently solubilized to effect the desired reaction.

The amino nucleus may also be acylated by reaction with an appropriately substituted carboxylic acid, in the presence of a coupling agent. Typical coupling agents include dicyclohexylcarbodiimide (DCC), N,N'-carbonyldiimidazole, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl), N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and the like.

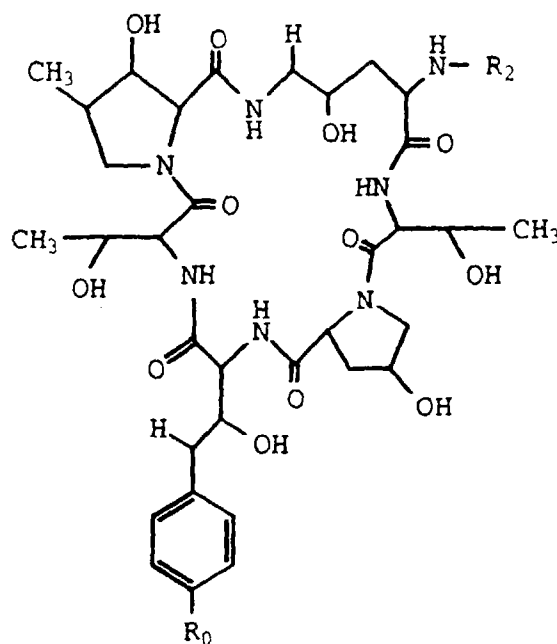
In addition, the amino nucleus may be acylated with an activated ester of a carboxylic acid (RCOOH) such as an ester of a carboxylic acid of the formula $R_2\text{-COOH}$ and p-nitrophenyl, 2,4,5-trichlorophenyl, hydroxybenzotriazole hydrate (HOBT- H_2O), pentafluorophenol, N-hydroxysuccinimide and the like. Preferred acylating moieties are the active esters of the carboxylic acid $R_2\text{-COOH}$ such as 2,4,5-trichlorophenyl ester and HOBT ester. The reaction is typically carried out for one to sixty five hours at a temperature from about 0°C to about 30°C in an aprotic solvent. The reaction is generally complete after about twenty four to forty eight hours when carried out a temperature of from about 15°C to about 30°C . Typical solvents for this reaction are tetrahydrofuran and dimethylformamide or a mixture of such solvents. The amino nucleus is generally employed in equimolar proportions relative to the activated ester or with a slight excess of the amino nucleus.

In Reaction IC, the compound of formula (IC) is phosphorylated by reaction with an appropriately substituted alkyl or phenyl phosphate to provide a compound of formula I where R_0 is $\text{P}(\text{O})_2\text{OH-R}_1$ where R_1 is $\text{C}_1\text{-C}_6$ alkoxy or phenoxy, or by reaction with an appropriately substituted alkyl or phenyl phosphonic acid to provide a compound of formula I where R_0 is $\text{-P}(\text{O})_2\text{OH-R}_1$ where R_1 is $\text{C}_1\text{-C}_6$ alkyl, an appropriately substituted phenyl or benzyl moiety. The reaction is carried out in the presence of a base such as lithium trimethyl-silanolate (LiOTMS), lithium bis(trimethylsilyl)amide (LHMDS), pyridine and the like. The reaction is typically carried out for up to one hour at a temperature from about -30°C to about 0°C in an aprotic solvent such as tetrahydrofuran and dimethylformamide. The reaction is generally complete in about fifteen minutes when carried out under these conditions. The phosphate or phosphonate reactant is generally employed in equimolar proportions to about a one mole excess relative to the amino nucleus in the presence of an equimolar or slight excess of the base. Phosphorylation of an amino nucleus with unprotected aminal hydroxy groups (R^{x1} and R^{x2}) is typically carried out at a temperature from about -30°C to about -15°C .

Alternatively, in Reaction IC' the acylated nucleus of formula (IC), where R^{x1} and/or R^{x2} are hydroxy, may be optionally protected with an hydroxy protecting group using procedures known in the art. For example, the reaction is typically carried out by combining the compound of formula (IC) with a suitable hydroxy protecting group in the presence

Claims

1. A compound of formula I:



I

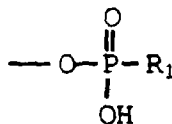
wherein:

R' is hydrogen, methyl or $\text{NH}_2\text{C}(\text{O})\text{CH}_2$;

R'' and R''' are independently methyl or hydrogen;

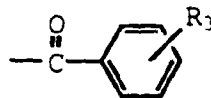
R^{x1}, R^{x2}, R^{y1}, R^{y2}, R^{y3}, and R^{y4} are independently hydroxy or hydrogen;

R₀ is a group of the formula



R₁ is C₁-C₆ alkyl, C₁-C₆ alkoxy, phenyl, p-halophenyl, p-nitrophenyl, phenoxy, benzyl, p-halo-benzyl, or p-nitro-benzyl;

I) R₂ is a group of the formula



where:

A) R₃ is C₁-C₁₂ alkyl, C₁-C₆ alkoxy or quinolyl;

B) R₃ is $-\text{O}-(\text{CH}_2)_m-[\text{O}-(\text{CH}_2)_n]_p-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl})$;

m and n are independently 2, 3 or 4;
p is 0 or 1; or

C) R₃ is $-\text{Y}-(\text{C}_1-\text{C}_{12} \text{ alkyl})$;

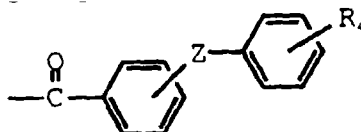
Y is $-\text{C}\equiv\text{C}-$ or $-\text{CH}=\text{CH}-$; or

D) R_3 is $-\text{O}-(\text{CH}_2)_q-\text{G}$:

q is 2, 3 or 4;

G is C_7-C_{10} bicycloalkyl or C_7-C_{14} tricycloalkyl; or

II) R_2 is a group of the formula



where:

Z is $-\text{O}-$, $-\text{C}\equiv\text{C}-$, $-\text{CH}=\text{CH}-$, $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}_2-$, or a bond;

A) R_4 is hydrogen, C_1-C_{12} alkyl, C_1-C_{12} substituted alkyl, C_2-C_{12} alkenyl, C_2-C_{12} substituted alkenyl, C_2-C_{12} alkynyl, C_2-C_{12} substituted alkynyl, C_1-C_{12} alkoxy, C_3-C_{12} cycloalkyl, C_7-C_{10} bicycloalkyl, C_7-C_{14} tricycloalkyl, C_3-C_{12} cycloalkoxy, naphthyl, pyridyl, thienyl, benzothienyl, quinolyl or phenyl; or

B) R_4 is phenyl substituted by amino, C_1-C_{12} alkylthio, halo, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, C_1-C_{12} substituted alkyl, C_2-C_{12} substituted alkenyl, C_2-C_{12} substituted alkynyl, C_1-C_{12} alkoxy, trifluoromethyl, phenyl, substituted phenyl, or phenyl substituted with a group of the formula $-\text{O}-(\text{CH}_2)_m-[\text{O}-(\text{CH}_2)_n]_p-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl})$ where m, n and p are as defined above; or

C) R_4 is C_1-C_{12} alkoxy substituted with halo, C_3-C_{12} cycloalkyl, C_7-C_{10} bicycloalkyl, C_7-C_{14} tricycloalkyl, C_1-C_6 alkoxy, C_2-C_{12} alkynyl, amino, C_1-C_4 alkylamino, di(C_1-C_4 alkyl)amino, formamido, C_2-C_{12} alkanoylamino, or phenyl substituted with a group of the formula $-\text{O}-(\text{CH}_2)_m-[\text{O}-(\text{CH}_2)_n]_p-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl})$ where m, n and p are as defined above; or

D) R_4 is $-\text{O}-(\text{CH}_2)_r-\text{W}-\text{R}_5$;

r is 2, 3 or 4;

W is pyrrolidino, piperidino or piperazino;

R_5 is hydrogen, C_1-C_{12} alkyl, C_3-C_{12} cycloalkyl, benzyl or C_3-C_{12} cycloalkylmethyl; or

E) R_4 is $-\text{Y}^1-\text{R}_6$;

Y^1 is $-\text{C}\equiv\text{C}-$ or $-\text{CH}=\text{CH}-$;

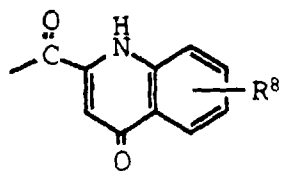
R_6 is C_3-C_{12} cycloalkyl, C_7-C_{10} bicycloalkyl, C_7-C_{14} tricycloalkyl, C_3-C_{12} cycloalkenyl, naphthyl, benzothiazolyl, thienyl, indanyl, fluorenyl, or phenyl substituted with C_1-C_{12} alkylthio, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, halo(C_1-C_6 alkoxy) or a group of the formula $-\text{O}-(\text{CH}_2)_r-\text{W}-\text{R}_5$ where r, W and R_5 are as defined above; or

R_6 is phenyl substituted with a group of the formula $-\text{O}-(\text{CH}_2)_m-[\text{O}-(\text{CH}_2)_n]_p-\text{O}-(\text{C}_1-\text{C}_{12} \text{ alkyl})$ where m, n and p are as defined above; or

F) R_4 is C_1-C_{12} alkoxy substituted with a group of the formula $-\text{NHC}(\text{O})\text{R}_7$;

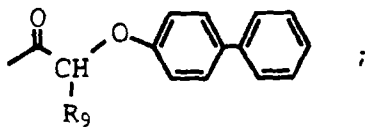
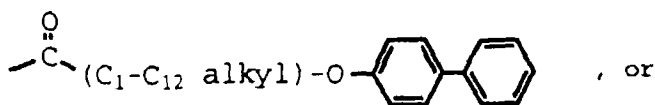
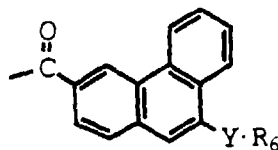
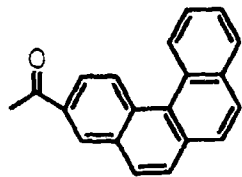
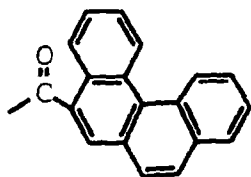
R_7 is C_1-C_6 alkoxy, or phenyl(C_1-C_6 alkoxy); or

III) R_2 is a group of the formula



where R^8 is C_1 - C_{12} alkoxy or a group of the formula $-O-(CH_2)_m-[O-(CH_2)_n]_p-O-(C_1-C_{12} \text{ alkyl})$ where m , n and p are as defined above; or

IV) R_2 is a group of the formula



where:

Y and R_6 are as defined above;

R_9 is phenyl, C_1 - C_{12} alkyl, or C_1 - C_{12} alkoxy; or

V) R_2 is naphthoyl substituted with R_4 where R_4 is as defined above;

or a pharmaceutically acceptable salt thereof.

2. A compound according to claim 1 where:

R^1 , R^2 , and R^3 are each methyl;

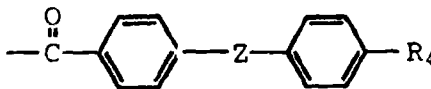
R^{y1} , R^{y2} , R^{y3} , R^{y4} are each hydroxy;

R^{x1} and R^{x2} are hydrogen;

or a pharmaceutically acceptable salt thereof.

3. A compound according to claim 2 where:

R_2 is a group of the formula



where:

Z is $-C\equiv C-$ or a bond;

A) R_4 is hydrogen, C_2-C_{12} alkynyl, C_2-C_{12} substituted alkynyl, C_1-C_{12} alkoxy, C_3-C_{12} cycloalkoxy, or phenyl; or

B) R_4 is phenyl substituted by C_1-C_{12} alkoxy, or a group of the formula $-O-(CH_2)_2-O-(C_1-C_6 \text{ alkyl})$; or

C) R_4 is C_1-C_{12} alkoxy substituted with C_3-C_{12} cycloalkyl, C_7-C_{14} tricycloalkyl, C_1-C_6 alkoxy, amino, di(C_1-C_4 alkyl)amino, formamido, or phenyl substituted with a group of the formula $-O-(CH_2)_2-O-(C_1-C_6 \text{ alkyl})$; or

D) R_4 is $-O-(CH_2)_r-W-R_5$;

r is 2 or 3;

W is piperidino;

R_5 is hydrogen, C_1-C_{12} alkyl, C_3-C_{12} cycloalkyl, benzyl or C_3-C_{12} cycloalkylmethyl; or

E) R_4 is $-Y^1-R_6$;

Y^1 is $-C\equiv C-$;

R_6 is phenyl substituted with C_1-C_{12} alkylthio, C_2-C_{12} alkenyl, C_2-C_{12} alkynyl, halo(C_1-C_6 alkoxy);

or R_6 is phenyl substituted with a group of the formula $-O-(CH_2)_r-W-R_5$ where r , W and R_5 are as defined above;

or R_6 is phenyl substituted with a group of the formula $-O-(CH_2)_2-O-(C_1-C_6 \text{ alkyl})$;

F) R_4 is C_1-C_{12} alkoxy substituted with a group of the formula $-NHC(O)R_7$;

R_7 is C_1-C_6 alkoxy, or phenyl (C_1-C_6 alkoxy); or a pharmaceutically acceptable salt thereof.

4. A compound according to claim 3 where R_1 is C_1-C_4 alkyl, C_1-C_4 alkoxy, phenyl, p-chloro-phenyl, p-bromo-phenyl, or p-nitro-phenyl, benzyl, p-chloro-benzyl, p-bromo-benzyl, or p-nitro-benzyl; or a pharmaceutically acceptable salt thereof.

5. A compound according to claim 4 where R_1 is methyl, ethyl, methoxy, ethoxy, phenyl, benzyl; or a pharmaceutically acceptable salt thereof.

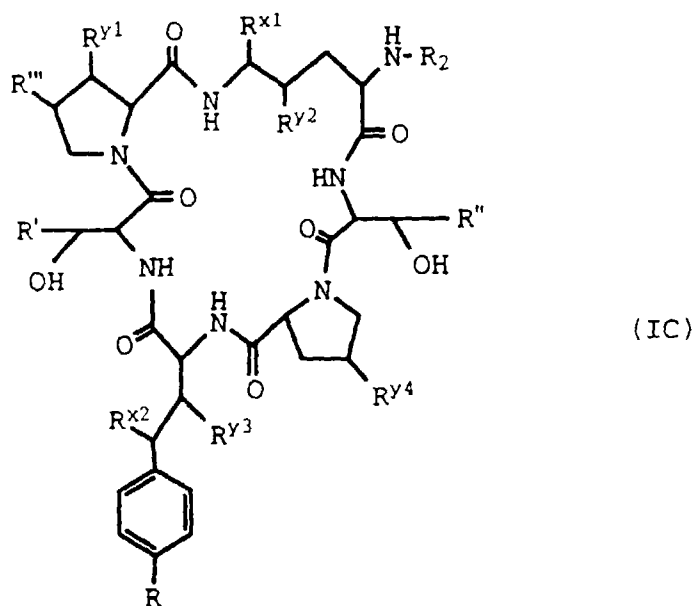
6. The compound according to claim 5 where Z is a bond; R_4 is phenyl substituted by n-pentoxy; and R_1 is methyl; or a pharmaceutically acceptable salt thereof.

7. A pharmaceutical formulation comprising a compound of formula I or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 6, associated with one or more pharmaceutically acceptable carriers, diluents or excipients therefor.

8. A compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any of claims 1 to 6 for use as a pharmaceutical.

9. A method of treating a fungal infection in a mammal which comprises administering to such a mammal a compound of formula I, or a pharmaceutically acceptable salt thereof, as claimed in any one of claims 1 to 6.

10. A process for preparing a compound of formula I, or a pharmaceutically acceptable salt thereof as claimed in any of claims 1 to 6, comprising reacting a compound of formula IC





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SIC SUPERINTENDENCIA
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Y COMERCIO

DIVISION DE NUEVAS CREACIONES



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DATOS DEL SOLICITANTE

Pres y Apellidos o Razón Social

e Identificación

representante Legal (Persona Jurídica)

e Domicilio

tamento o Estado

lón

FRANCIA

F 92800 PUTEAUX

1, Terrasse Bellini

(74) DATOS DEL APODERADO

e

ón

ABONDANO

CARRERA 9 No. 93 - 09, SANTA FE DE BOGOTA, D.C.

DATOS DEL INVENTOR

del Inventor (es)

a y Domicilio

ación

(VER ANEXO 1)

DATOS DE LA PATENTE DE INVENCION

e la Invención

" NUEVOS ~~...~~ DE LA BOHINOCANDINA, SU PROCEDIMIENTO
DE PREPARACION Y SU APLICACION COMO FUNGICIDAS "

AD REIVINDICADA

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FRANCIA

(32) Fecha

10/ 12/ 1997

26/ 10/ 1998

(31) No. Solicitud

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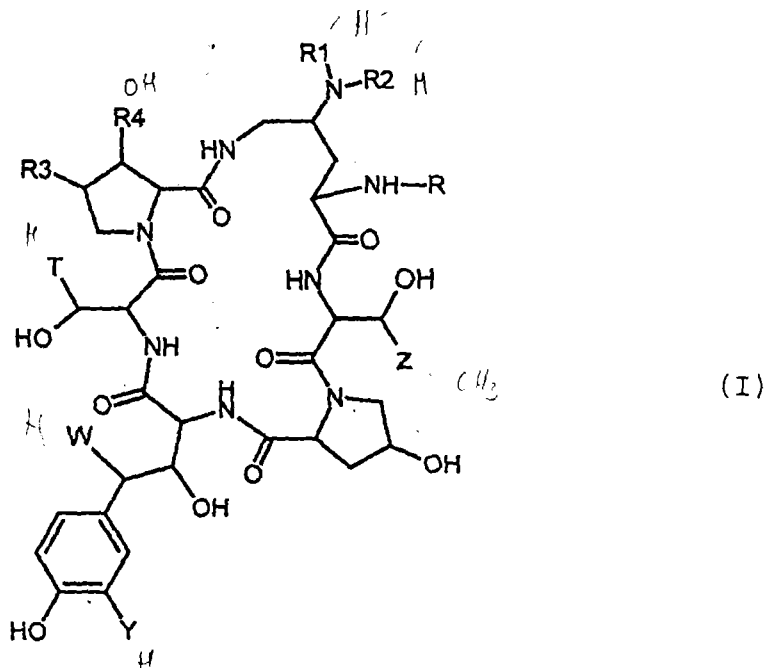
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SE REIVINDICA EXPRESAMENTE LA PRIORIDAD DERIVADA DE ESTA SOLICITUD, DE CONFORMIDAD CON EL ART.
DEL CONVENIO DE PAIS, VIGENTE EN LA LEY 178 DEL 28 DE DICIEMBRE DE 1.994.

ACION INTERNACIONAL

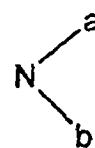
REIVINDICACIONES iniciales.

1.- En todas las formas de isómeros posibles, así como sus mezclas, los compuestos de fórmula (I):



los cuales

donde R1 y R2, idénticos o diferentes uno de otro, representan un átomo de hidrógeno, un radical hidroxilo, un radical alquilo que contiene hasta 8 átomos de carbono, lineal, ramificado o cíclico, eventualmente interrumpido por un átomo de oxígeno eventualmente sustituido con un átomo de

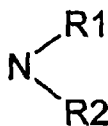


hidrógeno, un radical OH, un radical

representando a y b un átomo de hidrógeno o un radical alquilo que contiene hasta 8 átomos de carbono, pudiendo a eventualmente formar con el átomo de nitrógeno un ciclo, que contiene eventualmente uno o varios

heteroátomos suplementarios,

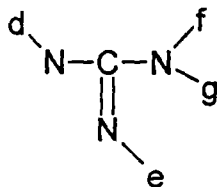
o bien NR1 forma con el átomo de carbono endocíclico que



lleva el radical , un doble enlace y o bien R2 representa un radical XR_a , representando X un átomo de oxígeno o un radical NH, o N-alquilo que contiene hasta 8 átomos de carbono, y R_a representa un átomo de hidrógeno, un radical alquilo lineal, ramificado o cíclico, que contiene hasta 8 átomos de carbono, eventualmente sustituido con uno o varios átomos de halógeno, con uno o varios radicales OH, CO_2H , CO_2alc ,

con un radical $\begin{array}{c} \text{a'} \\ \diagup \\ \text{N} \\ \diagdown \\ \text{b'} \end{array}$, representando a' y b'

un átomo de hidrógeno, un radical alquilo que contiene hasta 8 átomos de carbono, pudiendo a' y b' formar un heterociclo que contiene eventualmente uno o varios heteroátomos suplementarios y/o con un heterociclo que contiene uno o varios heteroátomos, o representado R2 un radical



en el que d, e, f y g representan cada uno un átomo de hidrógeno o un radical alquilo que contiene hasta 8 átomos de carbono, pudiendo f y g además representar un radical acilo que contiene hasta 8 átomos de carbono, pudiendo e y f igualmente formar un anillo que contiene eventualmente uno o varios heteroátomos,

R3 representa un átomo de hidrógeno, un radical metilo o hidroxilo,

191
35
153

R₄ representa un átomo de hidrógeno o un radical hidroxilo,
R representa una cadena lineal, ramificada o cíclica, que contiene hasta 30 átomos de carbono, la cual contiene eventualmente uno o varios heteroátomos, uno o varios heterociclos, o un radical acilo lineal, ramificado o cíclico, que contiene hasta 30 átomos de carbono, el cual contiene eventualmente uno o varios heteroátomos y/o uno o varios heterociclos,

T representa un átomo de hidrógeno, un radical metilo, un radical CH_2CONH_2 , $\text{CH}_2\text{C}\equiv\text{N}$, un radical $(\text{CH}_2)_2\text{NH}_2$ o $(\text{CH}_2)_2\text{Nalc}^+\text{X}^-$, siendo X un átomo de halógeno y alc un radical alquilo que contiene hasta 8 átomos de carbono,

Y representa un átomo de hidrógeno, un radical hidroxilo o un átomo de halógeno, o un radical OSO_3H o una de las sales de este radical

W representa un átomo de hidrógeno o un radical OH,

- Z representa un átomo de hidrógeno o un radical metilo, así como las sales por adición con ácidos de los productos de fórmula (I).

20 2.- Los compuestos de fórmula (I) definidos en la reivindicación 1, en los cuales T representa un átomo de hidrógeno.

25 3.- Los compuestos de fórmula (I) definidos en la reivindicación 1 ó 2, en las cuales W representa un átomo de hidrógeno.

4.- Los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 3, en los cuales Z representa un radical metilo.

30 5.- Los compuestos en los cuales Y representa un átomo de hidrógeno.

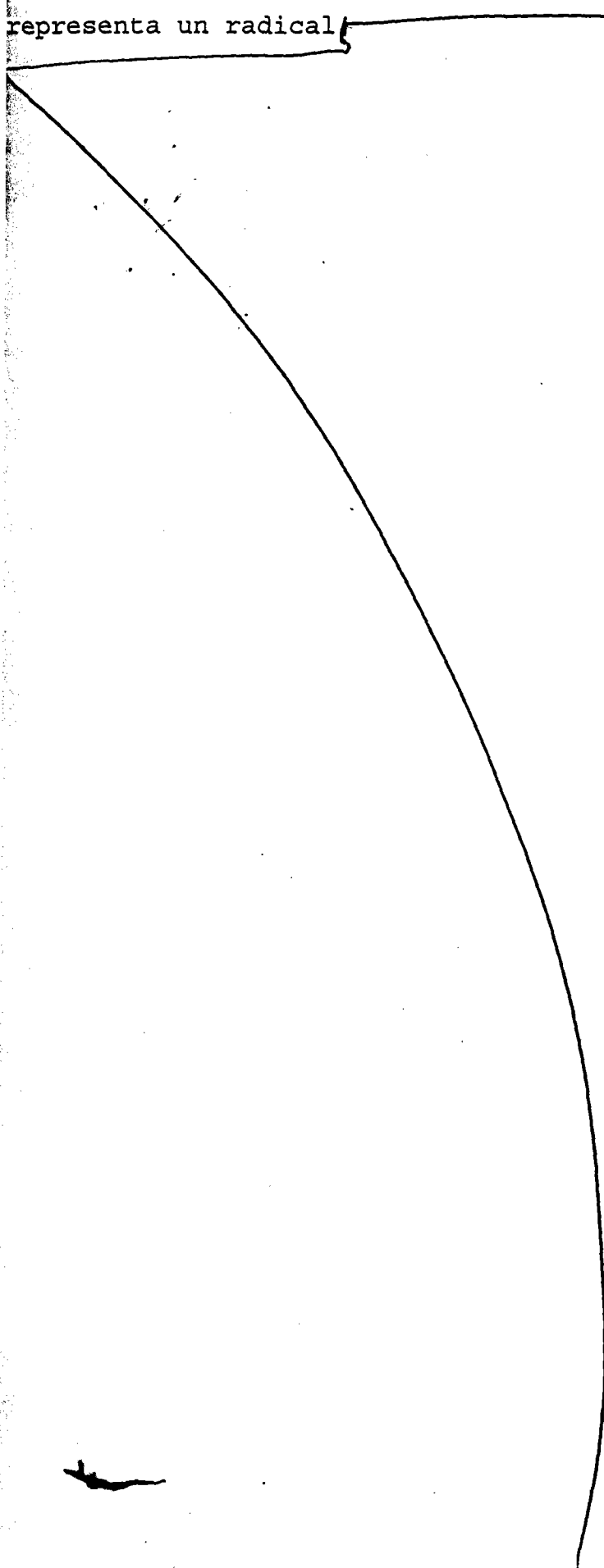
6.- Los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 5, en los cuales R₃ representa un radical metilo.

7.- Los compuestos de fórmula (I) definidos en una

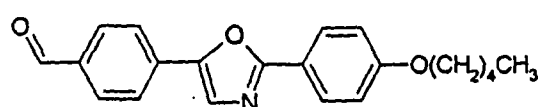
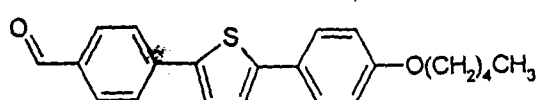
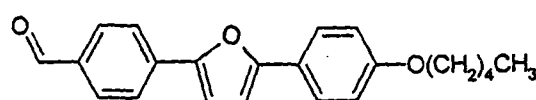
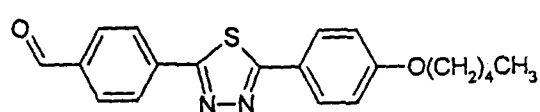
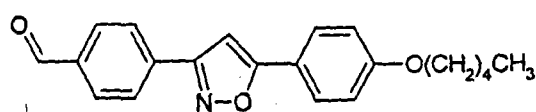
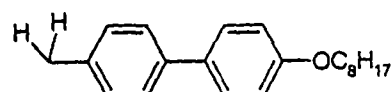
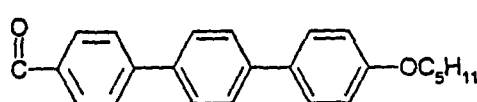
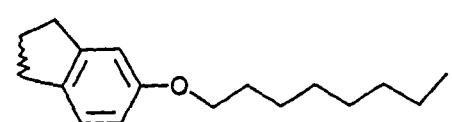
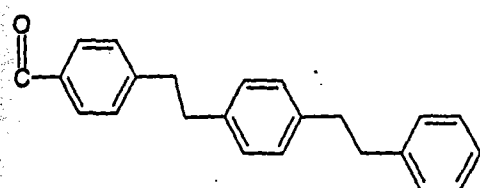
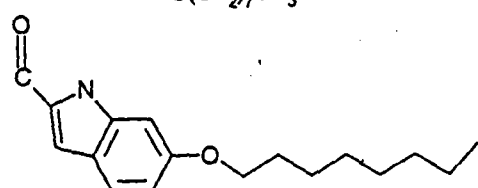
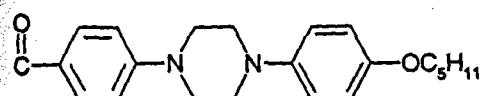
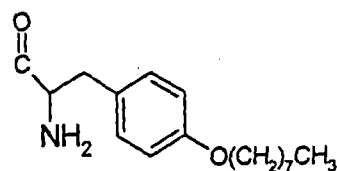
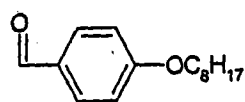
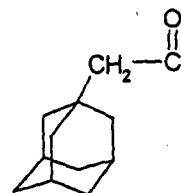
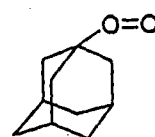
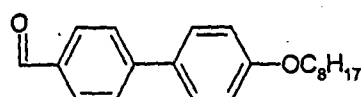
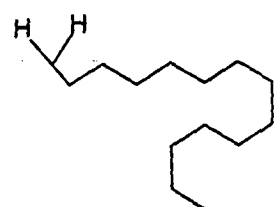
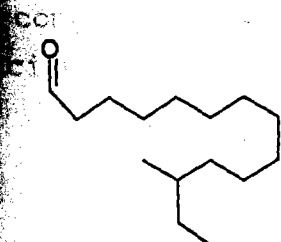
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cualquiera de las reivindicaciones 1 a 6, en los cuales R4 representa un radical hidroxilo.

8.- Los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 7, en los cuales R representa un radical

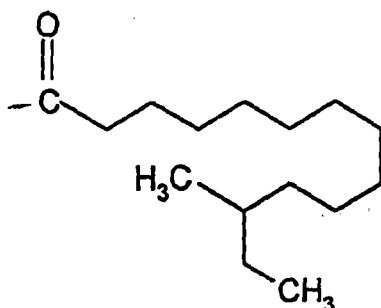


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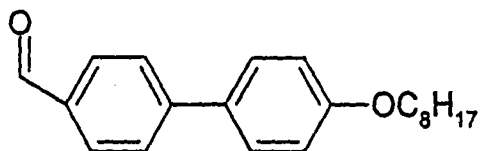


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Los compuestos de fórmula (I) definidos en la reivindicación 8, en los cuales R representa una cadena

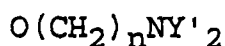


10.- Los compuestos de fórmula (I) definidos en la reivindicación 8 en los cuales R representa una cadena



11.- Los compuestos de fórmula (I) definidos en una de las reivindicaciones 1 a 10 en los cuales R1 forma, con el átomo de carbono endocíclico que lleva el radical NR1R2, un doble enlace.

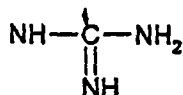
12.- Los compuestos de fórmula (I) definidos en la reivindicación 11, en los cuales R2 representa el radical



en el cual n representa un número entero comprendido entre 1 y 8 e Y' representa un átomo de hidrógeno o un radical alquilo que contiene hasta 8 átomos de carbono.

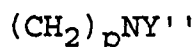
13.- Los compuestos de fórmula (I) definidos en la reivindicación 11, en los cuales n representa el número 2.

14.- Los compuestos de fórmula (I) definidos en la reivindicación 8, en los cuales R2 representa un radical



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15.- Los compuestos de fórmula (I) definidos en una de las reivindicaciones 1 a 10, en los cuales R2 representa un radical



en el cual Y' representa un átomo de hidrógeno o un radical alquilo que contiene hasta 8 átomos de carbono y p representa un número entero que varía entre 1 y 8.

16.- Los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 9 y 15, en los cuales R1 representa un átomo de hidrógeno.

17.- Los compuestos de fórmula (I) definidos en la reivindicación 15, en los cuales p representa el número 2.

18.- Los compuestos de fórmula (I) definidos en la reivindicación 1, cuyos nombres se dan seguidamente:

- la 1-[(S)-N2-(12-metil-1-oxo-tetradecil)-4-[(3-piperidinil)-oxi]imino]-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B;

- la 1-[4-[(2-aminoetil)-amino]-N2-(12-metil-1-oxo-tetradecil)-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B (isómero A e isómero B);

- la 1-[4-[(amino-iminometil)-hidrazono]-N2-(12-metil-1-oxo-tetradecil)-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B;

- la 1-[4-[(2-aminoetoxi)-imino]-N2-(12-metil-1-oxo-tetradecil)-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B y el isómero Z correspondiente;

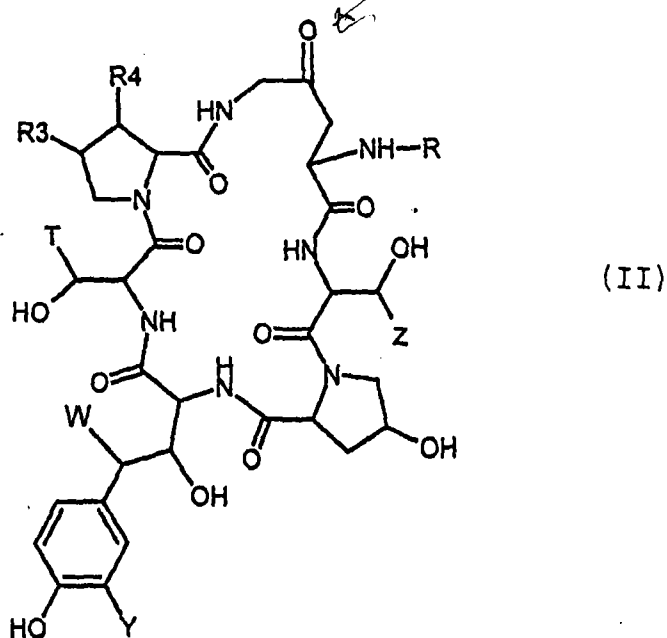
- trifluoroacetato de 1-[4-[(2-amino-etil)amino]-N2-[[4'-(octiloxi)-[1,1'-bifenil]-4-il]carbonil]-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B (isómero A),

así como sus sales por adición con ácidos.

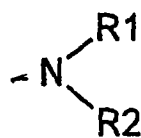
19.- Procedimiento de preparación de los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 18, caracterizado porque se somete un compuesto

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de fórmula (II)

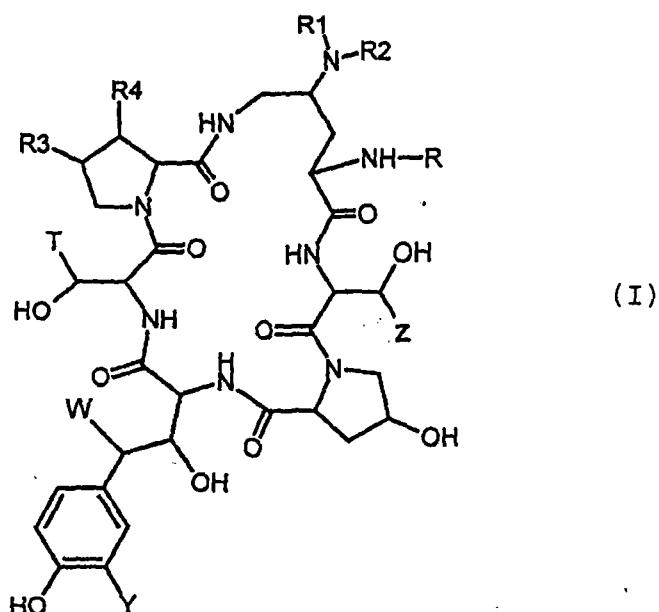


en la que R, R3, R4, T, W, Y y Z conservan sus significados precedentes, a la acción de una amina o de un derivado de



amina susceptible de introducir el radical en el que R1 y R2 conservan sus significados precedentes, y, si se desea, a la acción de un agente de reducción y/o de un agente de funcionalización de la amina, y/o de un ácido para formar la sal del producto obtenido, y/o de un agente de separación de los diferentes isómeros obtenidos, y se obtiene de esta manera el compuesto de fórmula (I)

buscado



en la que R1, R2, T, W, Y, R y Z conservan sus significados precedentes y luego se somete, si se desea, el compuesto de fórmula (I) a la acción de un ácido para formar la sal, y se separan, si se desea, los diferentes isómeros obtenidos.

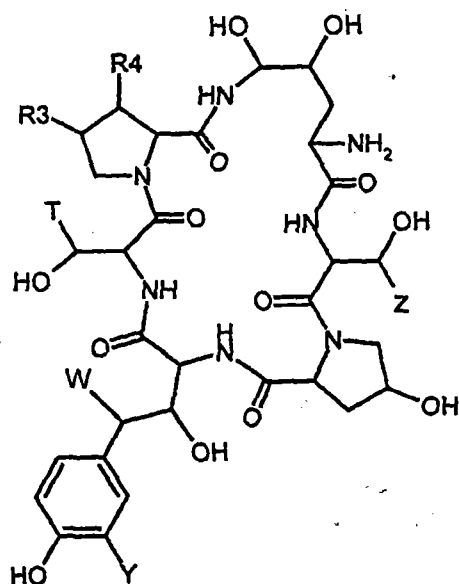
20.- A título de productos químicos nuevos, los compuestos de fórmula (II) definidos en la reivindicación 19.

21.- A título de producto químico nuevo definido en la reivindicación 20, el compuesto de fórmula (II) cuyo nombre se da seguidamente:

- la 1-([(4R,5R)-4-oxo-N2-(12-metil-1-oxo-tetradecil)-L-ornitina]-4-[4-(4-hidroxi-fenil)-L-treonina]-5-L-serina-equinocandina B,
- la 1-([-4-oxo-N2-(12-méthyl-1-oxotétradécyl) L-ornithine] 4-[4-(4-hydroxyphényl)-L-thréonine]-5-L-sérine-échinocandine B.
- 1-[N2-[[4'-(octiloxi)-[1,1'-bifenil]-4-il] carbonil]-4-oxo-L-ornitina]-4-[4-(4-hidroxifenil)-L-treonina]-5-L-serina-equinocandina B
- 1-[N2-[[4-[4-[4-(pentiloxi) fenil]-1-piperazinil]-fenil]-carbonil]4-oxo-L-ornitina]-4-[4-(4-hidroxifenil)-L-treonina]-5-L-serina equinocandina B.

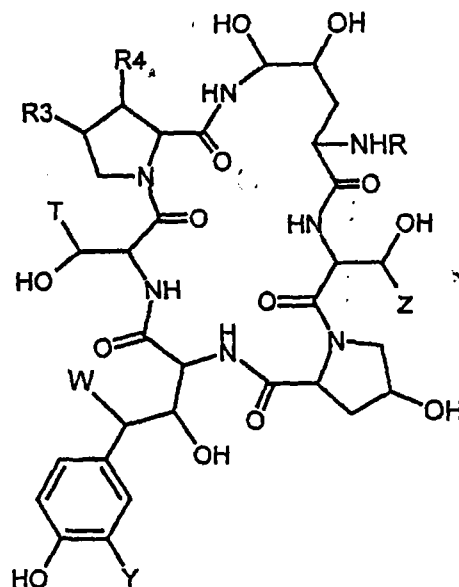
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#2
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(24) Procedimiento según la reivindicación 18, caracterizado porque se somete un compuesto de fórmula (III)



(III)

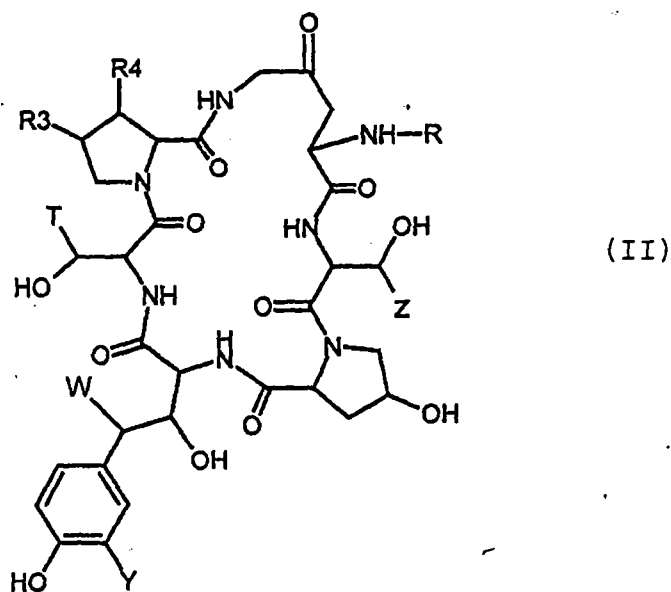
la que los diferentes sustituyentes conservan su significado precedente, a la acción de un agente capaz de reemplazar por NHR, conservando R su significado precedente, para obtener el compuesto de fórmula (IV)



(IV)

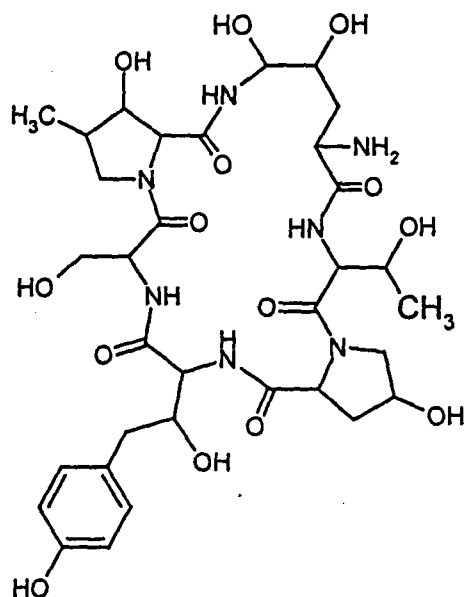
43 149
161

que se somete a la acción del yoduro de trimetil-sililo para obtener el compuesto de fórmula (II) correspondiente



23.- A título de productos químicos nuevos, los compuestos de las fórmulas (III), definidos en la reivindicación 22.

24.- A título de producto químico nuevo definido en la reivindicación 23, el producto de fórmula (III) siguiente:



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25.- A título de productos químicos nuevos los compuestos de la fórmula (IV) definidos en la reivindicación 22 con excepción de mulundocandina y deoximulundocandina.

26.- A título de compuestos antifúngicos, los compuestos de fórmula (I) definidos en una cualquiera de las reivindicaciones 1 a 18, así como sus sales por adición con ácidos.

27.- Las composiciones farmacéuticas que contienen en calidad de medicamento al menos un compuesto de fórmula (I) definida en una cualquiera de las reivindicaciones 1 a 18, así como sus sales por adición con ácidos farmacéuticamente aceptables.

HOECHST MARION ROUSSEL

Santa Fe de Bogotá, D.C.

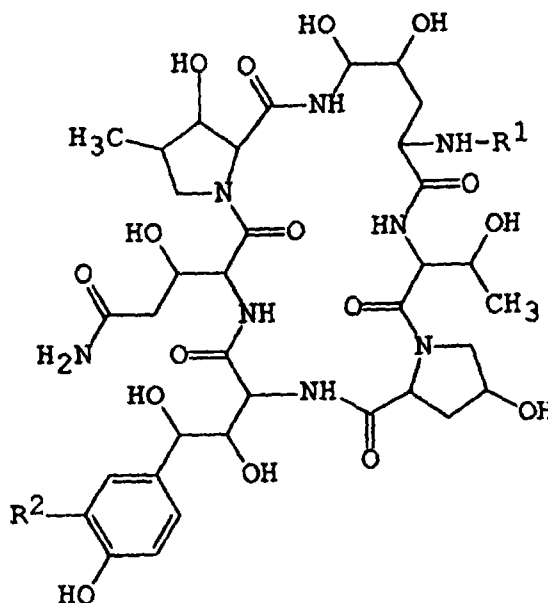
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ : C07K 7/56, A61K 38/12	A1	(11) International Publication Number: WO 98/23637
(21) International Application Number: PCT/JP97/04193		(43) International Publication Date: 4 June 1998 (04.06.98)
(22) International Filing Date: 18 November 1997 (18.11.97)		(81) Designated States: CA, CN, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).
(30) Priority Data: PO 3814 25 November 1996 (25.11.96) AU		Published <i>With international search report.</i>
(71) Applicant (for all designated States except US): FUJISAWA PHARMACEUTICAL CO., LTD. [JP/JP]; 4-7, Doshomachi 3-chome, Chuo-ku, Osaka-shi, Osaka 541 (JP).		
(72) Inventors; and		
(75) Inventors/Applicants (for US only): OHKI, Hidenori [JP/JP]; 4-4-13-107, Nakasuji, Takarazuka-shi, Hyogo 665 (JP). TOMISHIMA, Masaki [JP/JP]; 3-33-5, Gein, Minoo-shi, Osaka 562 (JP). YAMADA, Akira [JP/JP]; 4-8-30, Sawada, Fujiidera-shi, Osaka 583 (JP). TAKASUGI, Hisashi [JP/JP]; 3-116-10, Mozu Umekita, Sakai-shi, Osaka 591 (JP).		
(74) Agent: SEKI, Hideo; Fujisawa Pharmaceutical Co., Ltd., Osaka Factory, 1-6, Kashima 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532 (JP).		

(54) Title: CYCLOHEXAPEPTIDES HAVING ANTIMICROBIAL ACTIVITY

(57) Abstract

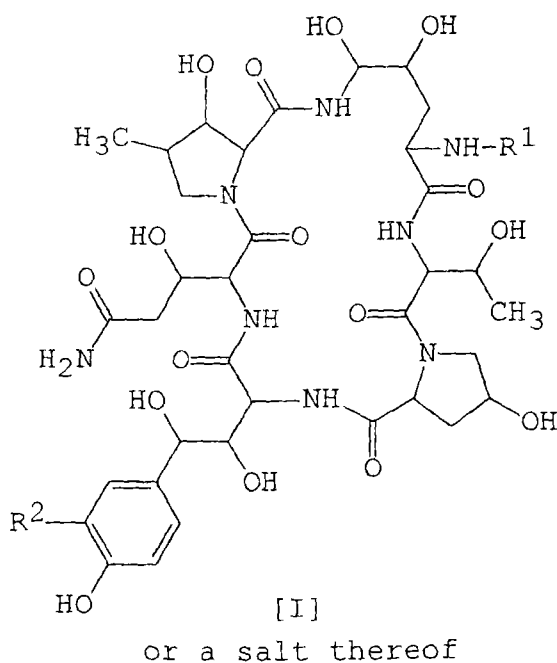
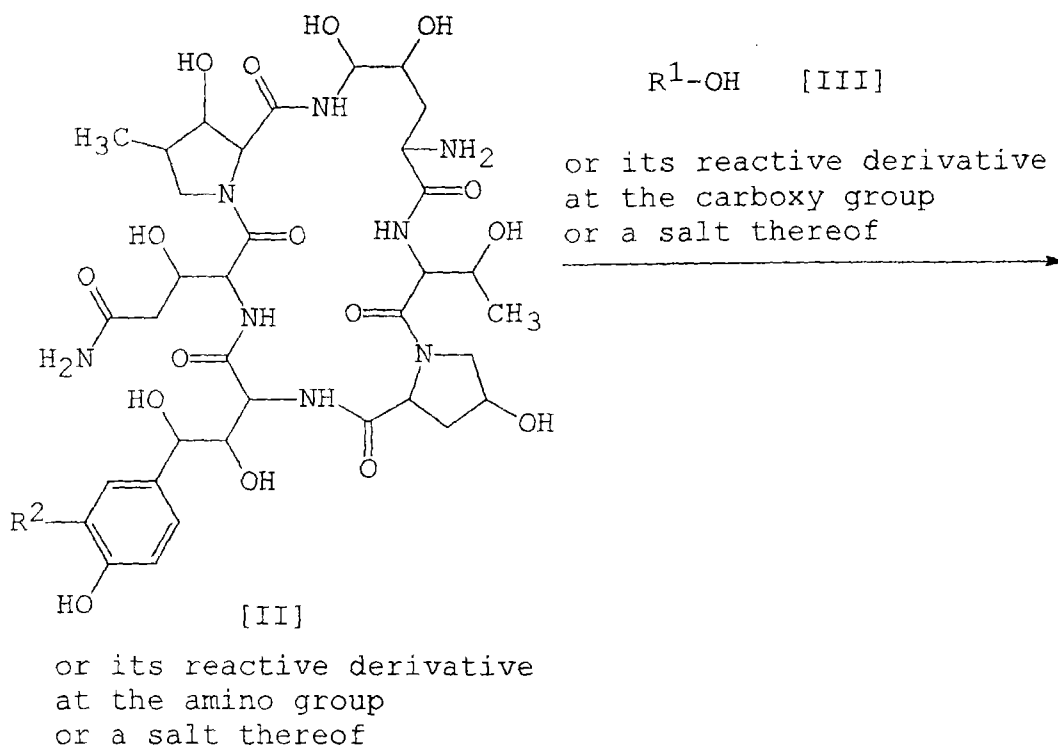
This invention relates to new polypeptide compounds represented by general formula (I), wherein R^1 and R^2 are as defined in the description and pharmaceutically acceptable salt thereof which have antimicrobial activities (especially antifungal activities), inhibitory activity on β -1,3-glucan synthase, to process for preparation thereof, to a pharmaceutical composition comprising the same, and to a method for the prophylactic and/or therapeutic treatment of infectious disease including *Pneumocystis carinii* infection (e.g. *Pneumocystis carinii* pneumonia in a human being or an animal).

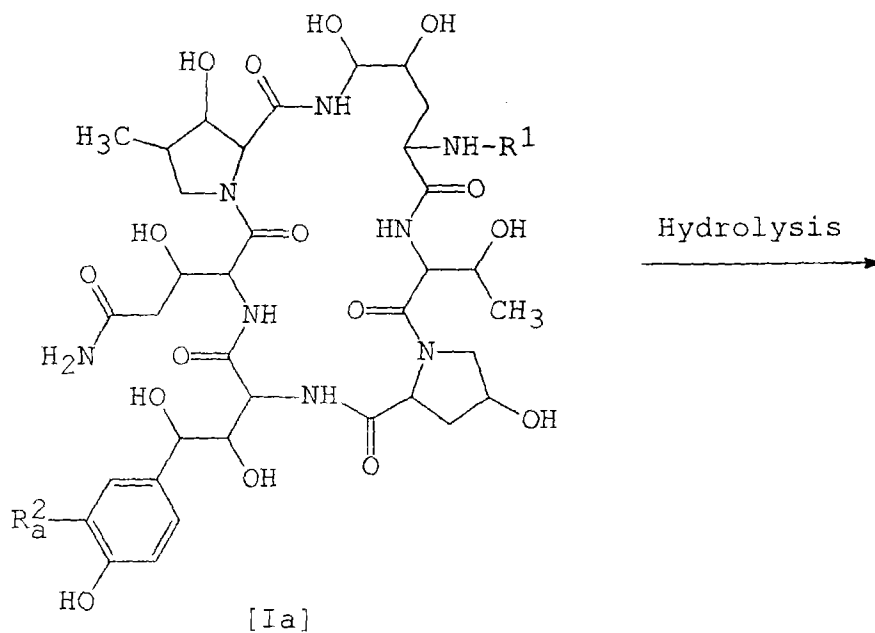


(1)

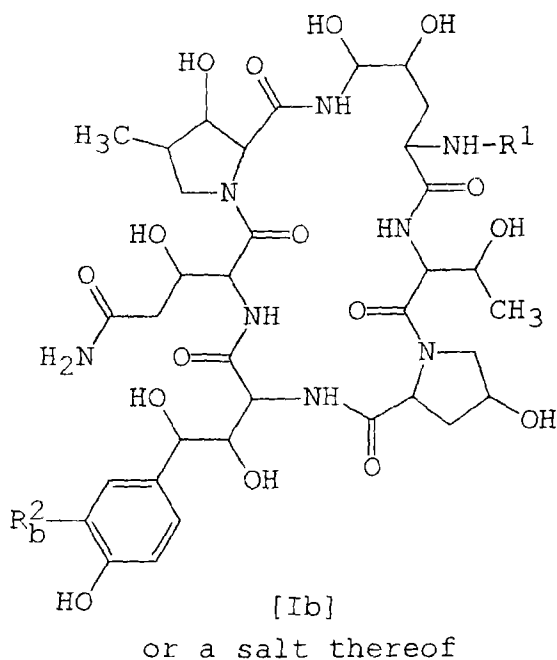
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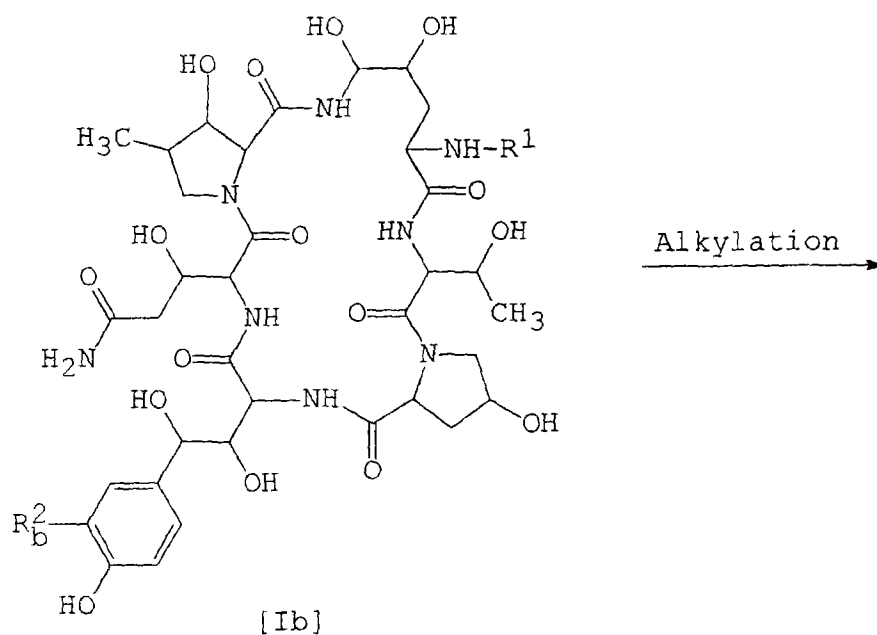
The new polypeptide compound [I] and a salt thereof can be prepared by the process as illustrated in the following reaction scheme.

5 Process 1

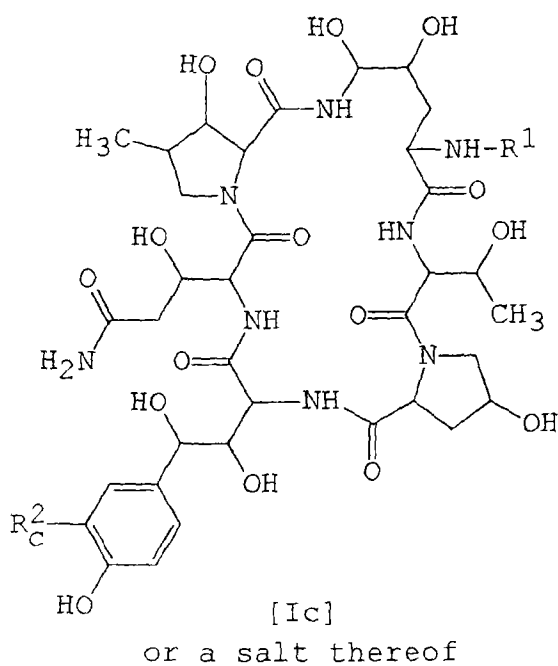
Process 2

or its reactive derivative
at the sulfonic acid group
or a salt thereof



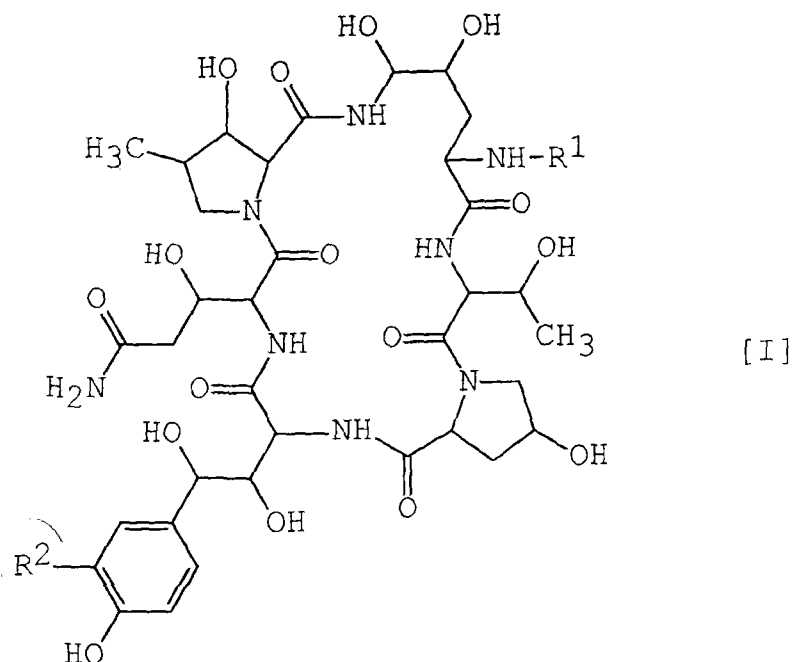
Process 3

or its reactive derivative
at the hydroxy group
or a salt thereof



C L A I M S

1. A polypeptide compound of the following general formula
[I] :



wherein

R¹ is aryl substituted with heterocyclic group which has
a suitable substituent selected from the group
consisting of

aryl having cyclo(lower)alkyloxy,
aryl having morpholinyl,
aryl having aryloxy(lower)alkoxy,
heterocyclic group having cyclo(lower)alkyl,
heterocyclic group having higher alkyl,
ar(lower)alkyl having lower alkoxy, and
cyclo(lower)alkyl which may have one or more
suitable substituent(s);

aryl substituted with heterocyclic group which has
hydroxy and may have additional one or more suitable
substituent(s);

aroyl substituted with piperidyl which has aryl
having lower alkoxy;

aroyl substituted with thiadiazolyl which has a
suitable substituent selected from the group consisting

5 of aryl having pentyl,
aryl having hexyl,
aryl having methoxy,
aryl having butoxy, and
aryl having higher alkoxy;

10 aroyl substituted with aryl which has aryl having
pentyloxy;

aroyl substituted with piperazinyl which has 3-
hexyloxyphenyl;

15 aroyl substituted with 1,2,3,6-tetrahydropyridyl
which may have one or more suitable substituent(s);

aroyl substituted with thienyl which may have one
or more suitable substituent(s);

aroyl substituted with furyl which may have one or
more suitable substituent(s);

20 aroyl substituted with heterocyclic(lower)alkyl
which may have one or more suitable substituent(s);

aroyl substituted with ar(lower)alkynyl which may
have one or more suitable substituent(s);

25 lower alkanoyl substituted with thiazolyl which may
have one or more suitable substituent(s);

aroyl substituted with imidazothiazolyl which may
have one or more suitable substituent(s);

aroyl substituted with isoxazolyl having halogen
which may have one or more suitable substituent(s);

30 or

4-[5-(4-pentyloxyphenyl)isoxazol-3-yl]benzoyl;

and

R^2 is hydroxy, hydroxysulfonyloxy or lower alkoxy,
with proviso that

35 R^2 is not hydroxysulfonyloxy,

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when R¹ is 4-[5-(4-pentyloxyphenyl)isoxazol-3-yl]benzoyl,
and a salt thereof.

2. A compound of claim 1, wherein

- 5 R¹ is benzoyl substituted with piperazinyl which has
cyclo(lower)alkyl having cyclo(lower)alkyl,
benzoyl substituted with piperazinyl which has
phenyl having cyclo(lower)alkyloxy,
10 benzoyl substituted with piperazinyl which has
phenyl having morpholinyl, or
benzoyl substituted with piperazinyl which has
phenyl having 3-hexyloxy.

3. A compound of claim 1, wherein

- 15 R¹ is benzoyl substituted with piperidyl which has
hydroxy and phenyl having lower alkoxy,
benzoyl substituted with piperidyl which has phenyl
having lower alkoxy, or
20 benzoyl substituted with piperidyl which has
piperidyl having cyclo(lower)alkyl.

4. A compound of claim 1, wherein

- 25 R¹ is benzoyl substituted with 1,2,3,6-tetrahydropyridyl
which has phenyl having lower alkoxy.

5. A compound of claim 1, wherein

- 30 R¹ is benzoyl substituted with phenyl which has phenyl
having pentyloxy.

6. A compound of claim 1, wherein

- 35 R¹ is benzoyl substituted with thiadiazolyl which has
phenyl having methoxy,
benzoyl substituted with thiadiazolyl which has
phenyl having butoxy,
benzoyl substituted with thiadiazolyl which has

phenyl having pentyl,
benzoyl, substituted with thiadiazolyl which has
phenyl having hexyl,
benzoyl substituted with thiadiazolyl which has
5 phenyl having higher alkoxy,
benzoyl substituted with thiadiazolyl which has
phenyl having phenoxy(lower)alkoxy,
benzoyl substituted with thiadiazolyl which has
piperidyl having higher alkyl, and
10 benzoyl substituted with thiadiazolyl which has
phenyl(lower)alkyl having lower alkoxy.

7. A compound of claim 1, wherein

15 R¹ is benzoyl substituted with thienyl which has phenyl
having lower alkoxy, or
benzoyl substituted with furyl which has phenyl
having lower alkoxy.

8. A compound of claim 1, wherein

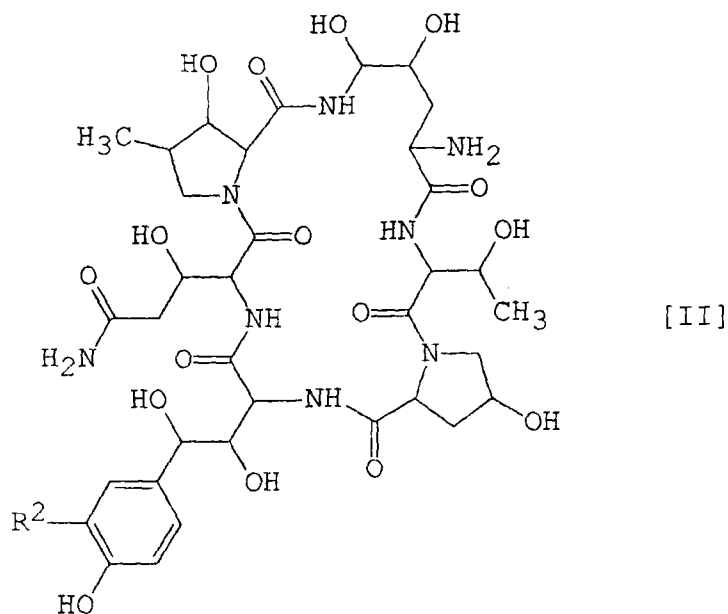
20 R¹ is benzoyl substituted with piperazinyl(lower)alkyl
which has phenyl having cyclo(lower)alkyl, or
benzoyl substituted with phenyl(lower)alkynyl which
has lower alkoxy.

25 9. A compound of claim 1, wherein

R¹ is thiazolyl-carbonyl substituted with phenyl which
has phenyl having lower alkoxy.

30 10. A process for the preparing a polypeptide compound [I]
of claim 1,
which comprises

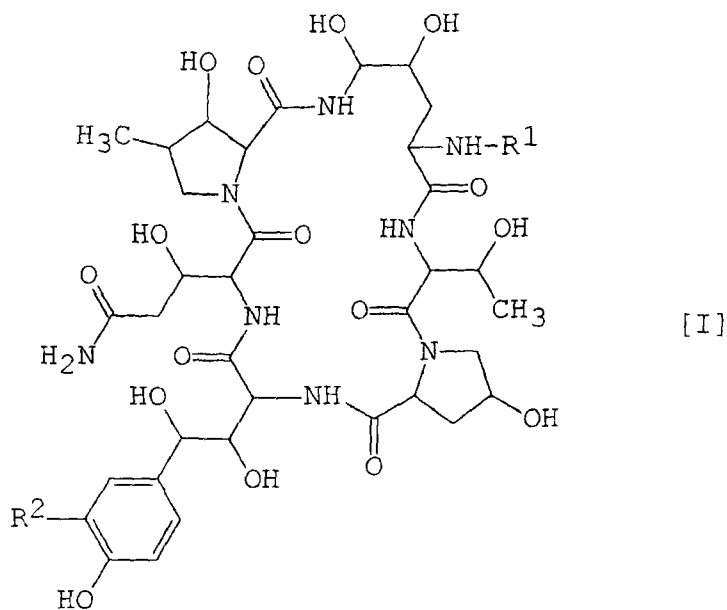
1) reacting a compound of the formula :

159
471

or its reactive derivative at the amino group or a salt thereof, with a compound of the formula :



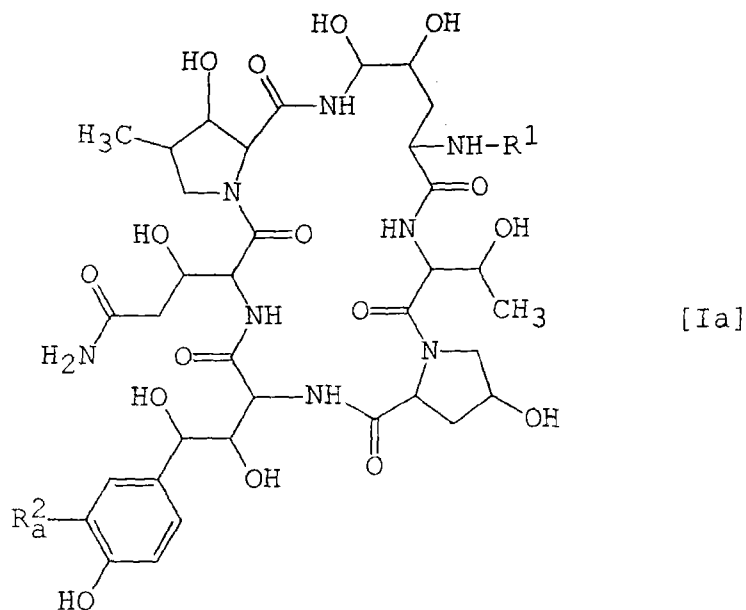
wherein R¹ and R² are defined in claim 1,
or its reactive derivative at the carboxy group or a salt thereof, to give a compound [I] or the formula :



160
172

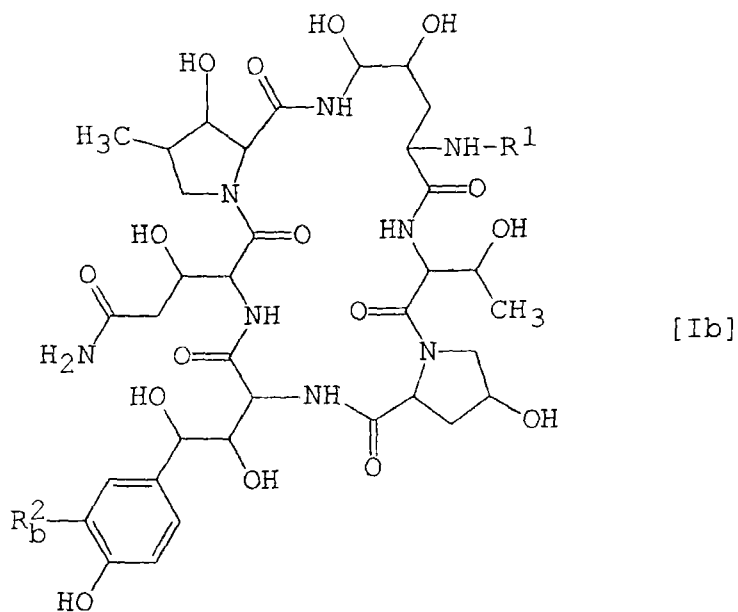
wherein R^1 and R^2 are defined in claim 1,
or a salt thereof, or

2) subjecting a compound [Ia] of the formula :



wherein R^1 is defined in claim 1,

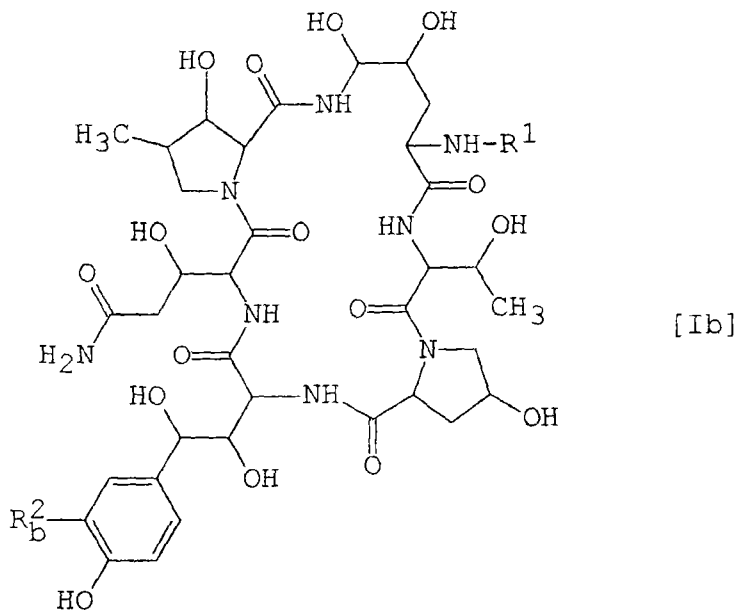
R_a^2 is hydroxysulfonyloxy or
a salt thereof, to hydrolysis reaction of the sulfonic
acid group, to give a compound [Ib] of the formula :



wherein R^1 is defined in claim 1,

R_b^2 is hydroxy or
a salt thereof, or

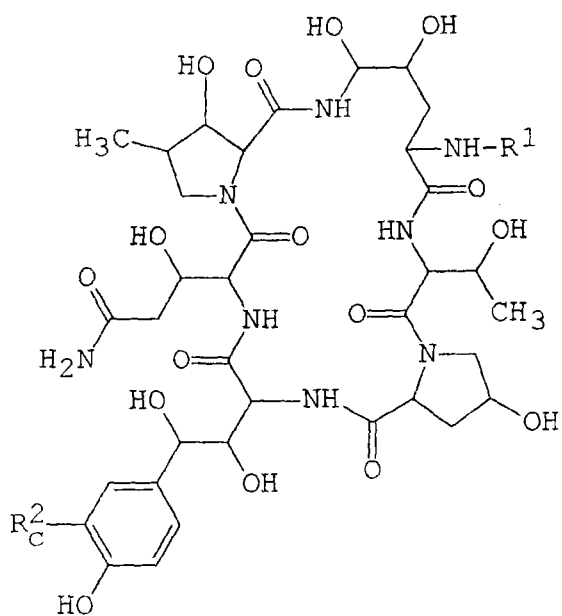
3) subjecting a compound [Ib] of the formula :



wherein R^1 is defined in claim 1,

R_b^2 is hydroxy or
its reactive derivative at the hydroxy group
or a salt thereof, to alkylation reaction of the hydroxy
group, to give a compound [Ic] of the formula :

174 162



[Ic]

wherein R^1 is defined in claim 1,
 R_C^2 is lower alkoxy or
a salt thereof.

11. A pharmaceutical composition which comprises, as an active ingredient, a compound of claim 1 or a pharmaceutically acceptable salt thereof in admixture with pharmaceutically acceptable carriers or excipients.

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12. Use of a compound of claim 1 or a pharmaceutically acceptable salt thereof as a medicament.

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13. A compound of claim 1 or a pharmaceutically acceptable salt thereof for use as a medicament.

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14. A method for the prophylactic and/or the therapeutic treatment of infectious diseases caused by pathogenic microorganisms which comprises administering a compound of claim 1 or a pharmaceutically acceptable salt thereof to a human being or an animal.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN: 0-42902

EDICTO No. 11610

**LA SECRETARÍA GENERAL AD-HOC
HACE SABER:**

Que esta Superintendencia profirió **Resolución No. 21503 de 30-AGO-2005**. Que el contenido de la parte resolutive es como sigue: El Superintendente de Industria y Comercio.

RESUELVE

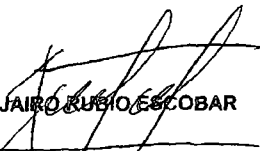
ARTICULO PRIMERO: Negar el privilegio de patente de invención para la solicitud correspondiente a "NUEVOS DERIVADOS DE EQUINOCANDINA, SU PROCEDIMIENTO DE PREPARACION Y SU APLICACIÓN COMO ANTIFUNGICOS".

ARTICULO SEGUNDO: Notificar personalmente el contenido de la presente resolución a la doctora Margarita Castellanos Abondano, o a quien haga sus veces, en su calidad de apoderada de Hoechst Marion Roussel., entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio, al momento de la notificación o dentro de los 5 días hábiles siguientes a ella.

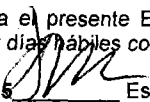
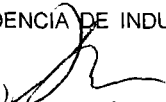
NOTIFÍQUESE Y CUMPLASE

Dada en Bogotá, D.C., a los **30 AGO. 2005**

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO,


JAIRO RUBIO ESCOBAR

Para notificar a los interesados, se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO, por el término de (10) diez días hábiles contados a partir de las 8:30 a.m. de hoy.

Secretaría General Ad-Hoc **13-SEP-2005**  Este edicto se desfija hoy **26-SEP-2005** a las cinco (5:00 p.m.) 
Secretaría General Ad-Hoc
EDICTO No. 11610