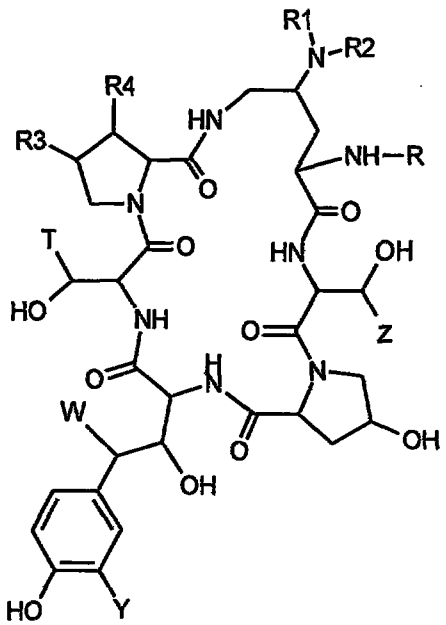
ECHINOCANDIN DERIVATIVES, THEIR METHOD OF PREPARATION AND THEIR APPLICATION AS ANTI-FUNGAL AGENTS

The present invention concerns novel echinocandin derivatives their method of preparation and their application as anti-fungal agents.

The invention has as its object, the compounds of formula (I), in any of the possible isomer forms as well as their compounds,:



(I)

in which

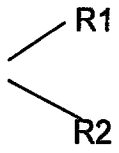
either R1 and R2 identical or different from one another, represent a hydrogen atom, a hydroxyl radical, an alkyl radical containing up to 8 linear branched or cyclic carbon atoms, optionally interrupted by an oxygen atom optionally substituted by a

halogen atom, an OH radical, an N(a)(b) radical, a and b

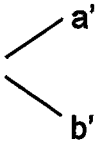
identical or different from one another,

representing a hydrogen atom or an alkyl radical containing up to 8 carbon atoms, a and b optionally able with the nitrogen atom to form a heterocycle optionally containing one or several additional heteroatoms,

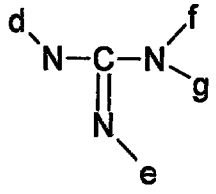
-or else R1 forms a double bond with the endocyclic carbon atom

carrying the radical N  or else R2 represents

an XR_a radical, X representing an oxygen atom or an NH or N-alkyl radical containing up to 8 carbon atoms and R_a represents a hydrogen atom, a linear, branched or cyclic alkyl radical containing up to 8 atoms of carbon optionally substituted by one or several halogen atoms, by one or several OH, CO_2H CO_2alc radicals,

by an N  radical, a' and b' representing a hydrogen atom, an alkyl

radical containing up to 8 carbon atoms, a' and b' able to form a heterocycle optionally containing one or several additional heteroatoms and/or by a heterocycle containing one or several heteroatoms or R2 represents a radical



in which d, e, f, and g represent a hydrogen atom or an alkyl radical containing up to 8 carbon atoms, f and g able moreover to represent an acyl radical containing up to 8 carbon atoms, e and f able equally to form a ring optionally containing one or several heteroatoms,
R3 represents a hydrogen atom, a methyl or hydroxyl radical
R4 represents a hydrogen atom or a hydroxyl radical R representing a linear or branched or cyclic chain containing up to 30 carbon atoms, optionally containing one or several heteroatoms, one or several heterocycles or a linear, branched or cyclic acyl radical containing up to 30 carbon atoms

optionally containing one or several heteroatoms and/or one or several heterocycles,

T represents a hydrogen atom, a methyl radical, a CH_2CONH_2, $CH_2C{\equiv}N$ radical, a $(CH_2)NH_2$ or $(CH_2)_2Nalc^+X^-$ radical, X being a halogen atom and alc an alkyl radical containing up to 8 carbon atoms,

Y represents a hydrogen atom, a hydroxyl radical or a halogen atom or an OSO3H radical or one of the salts of this radical,

W represents a hydrogen atom or an OH radical,

Z represents a hydrogen atom or a methyl radical, as well as the addition salts with the acids of the products of formula (I).

Amongst the addition salts with the acids, those formed with mineral acids, such as hydrochloric, hydrobromic, sulphuric or phosphoric acids or the organic acids like formic, acetic, trifluoroacetic, propionic, benzoic, maleic, fumaric, succinic, tartaric, citric, oxalic, glyoxylic, aspartic, alkanesulphonic, such as sulphonic methane or ethane, arylsulphonic acids like the benzene or paratoluenesulphonic acids can be cited.

In the definition of the substituents,

-the alkyl, alkenyl or alkynyl radical is preferably a methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, terbutyl, decyl or dodecyl, vinyl, allyl, ethynyl, propynyl, cyclobutyl, cyclopentyl, or cyclohexyl radical,

-the halogen is preferably fluorine or chlorine or bromine,

-the aryl radical is preferably the radical phenyl,

-the heterocyclic radical is preferably the pyrrolyle, pyrrolidinyl, pyridyl, pyrazinyl, pyrimidyl, piperidinyl, piperazinyl, quinuclidinyl, oxazoyl, isoxazoyl, morpholinyl, indolyl, imidozoyl, benzimidazoyl, triazoyle, thiazolyl, azetidinyl, aziridinyl radical.

as a salt of the SO3H radical, sodium, potassium salts or even the salts of amines can in particular be cited.

Amongst the preferred compounds of the invention:

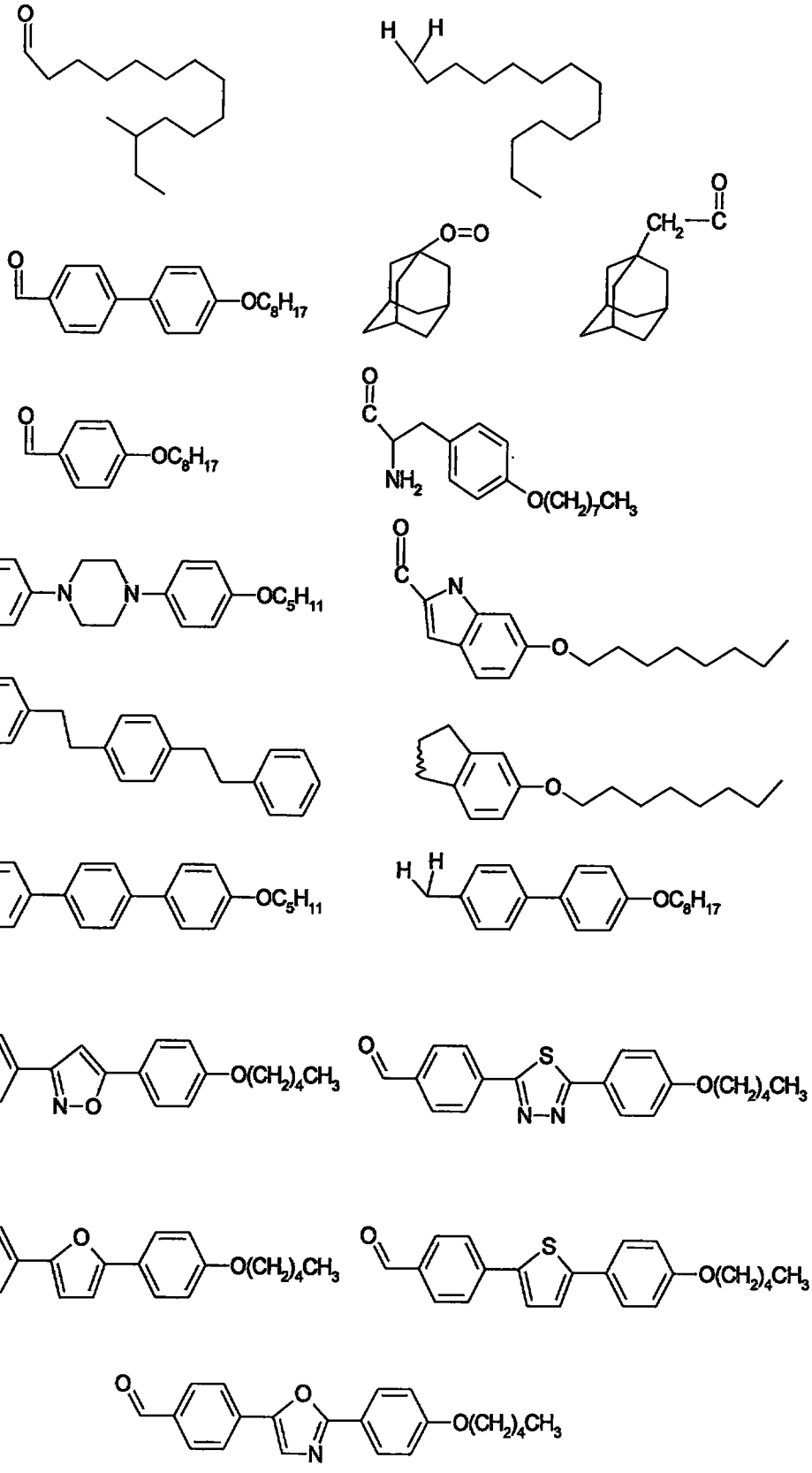
-the compounds of formula (I), in which T represents a hydrogen atom,

-the compounds of formula (I), in which Y represents a hydrogen atom,

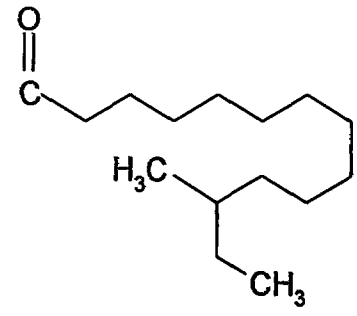
-the compounds of formula (I), in which W represents a hydrogen atom,

-the compounds of formula (I), in which Z represents a methyl radical,

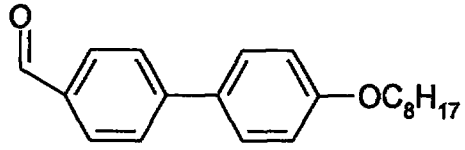
-the compounds of formula (I), in which R3 represents a methyl radical,
-the compounds of formula (I), in which R4 represents a hydroxyl radical
-the compounds of formula (I), in which R represents a radical
can be especially cited.



and more particularly those in which R represents a chain



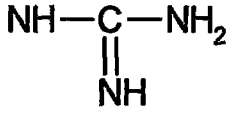
or those in which R represents a chain



-the compounds of formula (I) in which R1 forms with the endocyclic carbon atom carrying the NR1R2 radical, a double bond, and notably those in which R2 represents the radical

$$O(CH_2)_nNY'_2$$

in which n represents an integer between 1 and 8 and very particularly those in which n represents the number 2 and Y' represents a hydrogen atom or an alkyl radical containing up to 8 carbon atoms, and those in which R represents a radical



The invention has equally particularly as its object the compounds of formula (I) in which R2 represents a radical

$$(CH_2)_pNY''$$

in which Y'' represents a hydrogen atom or an alkyl radical containing up to 8 carbon atoms and p represents an integer varying from 1 to 8 and especially the compounds in which p represents the number 2.

The invention has very particularly as its object, compounds in which R1 represents a hydrogen atom.

Amongst the preferred compounds of the invention, the products of examples 8, 9, 11, 13 and 14 can be cited.

The compounds of formula (I) present significant anti-fungal properties; they are active notably on Candida albicans and other Candida like Candida glabrata, krusei, tropicalis, pseudotropicalis, parapsilosis and Aspergillus fumigatus, Aspergillus flavus, Cryptococcus neoformans.

The compounds of formula (I) can be used as medicines in man or animal, to fight against notably digestive urinary, vaginal or cutaneous candidoses, cryptococcoses, for example neuromenengeal, pulmonary or cutaneous cryptococcoses, bronchopulmonary and pulmonary aspergilloses and invasive aspergilloses of immunocompromise.

The compounds of the invention can be equally used in the prevention of mycosic ailments in people with congenital or acquired immune compromise.

The compounds of the invention are not limited to a pharmaceutical usage, they can be equally used as fungicides in domains other than pharmaceutical.

The invention thus has as its object as anti-fungal compounds, the compounds of formula (I) as well as their addition salts with the acids.

The invention equally has as its object the compounds of formula (I), as medicines.

The invention has very particularly as its object pharmaceutical compositions containing at least one compound of formula (I) or one of its addition salts with pharmaceutically acceptable acids as active ingredient.

These compounds can be administered by oral, rectal, parenteral route or by local route by topical application on the skin and the mucous membranes, but the preferred route is the oral route.

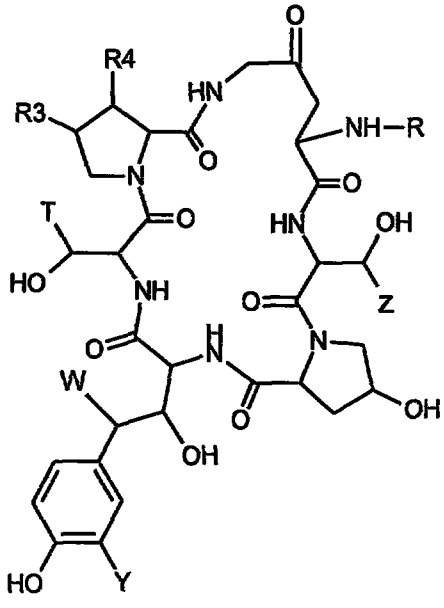
They can be solid or liquid and be presented in pharmaceutical forms currently used in human medicine, like for example, simple or sugar

coated tablets, capsules, granules, suppositories, injectable preparations, ointments, creams, gels; they are prepared following usual methods. The active ingredient(s) can be incorporated into excipients usually used in these pharmaceutical compositions, like talc, gum arabic, lactose, starch, magnesium stearate, cocoa butter, aqueous or non aqueous mediums, fatty bodies of animal or vegetable origin, paraffin derivatives, glycol, various diluting, dissolving or emulsifying agents, preservatives.

These compositions can equally be presented in the form of a powder intended to be dissolved extemporarily in an appropriate medium, for example apyrogenic sterile water.

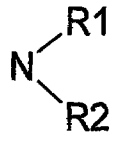
The administered dose varies according to the ailment treated, the subject concerned, the route of administration and the considered product. It can, for example, consist of between 50 and 300mg per day by oral route, in adults for the products of examples 8, 9, 11, 13 and 14.

The invention equally has as its object a method of preparation of formula (I) compounds, characterised in that a formula (II) compound is submitted:



(II)

in which R, R3, R4, T, W, Y and Z retain their prior meaning, with the action of an amine or an amine derivative likely to introduce

the radical 

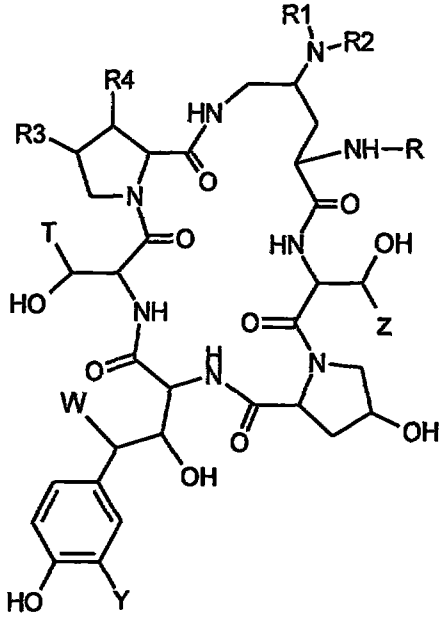
in which R1 and R2 retain their prior meaning and if desired the action of a reduction agent

and/or a functionalisation agent of the amine,

and/or an acid to form the salt of the obtained product,

and/or a separation agent of the different isomers obtained,

and thus obtains the sought formula (I) compound



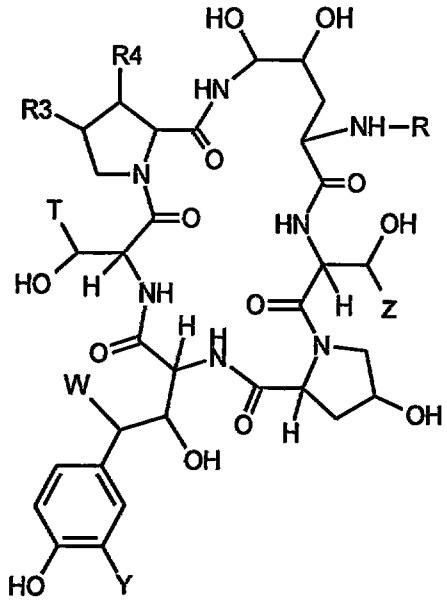
(I)

in which R1, R2, T, W, Y, R and Z retain their prior meaning in all of its possible isomer forms as well as their compounds and/or in the form of salts with the acids.

The formula (II) compounds used as initial compounds of the process of the invention are novel products and are themselves an object of the

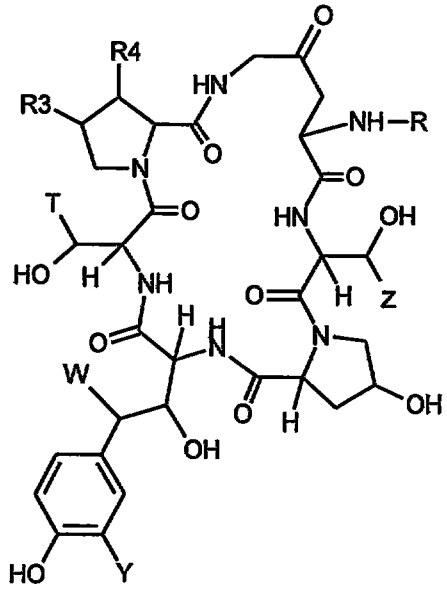
present invention, their preparation given in the experiment section can be schematised as follows:

(IV)



NaI/CH3CN/ClSi(CH3)3 ou ISi(CH3)3/CH3CN

(II)



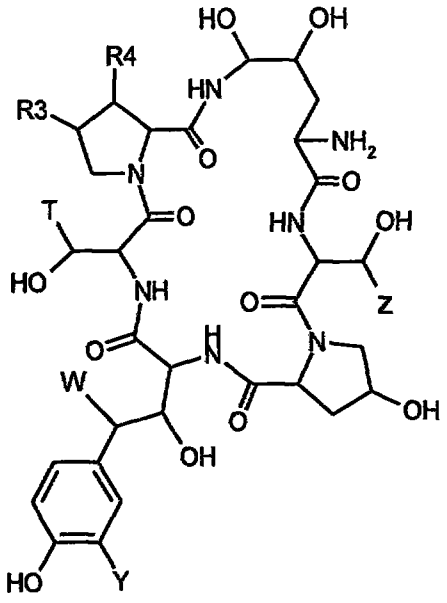
Isi-$(CH_3)_3$ or any other Lewis acid can be used.

A detailed example of the preparation of the compound of formula (II) is given in the experiment section, and the invention has more particularly as its object as novel chemical product 1-[4-oxo-N2-(12-methyl-1-oxotetradecyl) L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine}-5-L-serine-echinocandin B.

The product (IV) corresponding to the initial product of preparation 1 is a known product described and claimed in the European patent 438813.

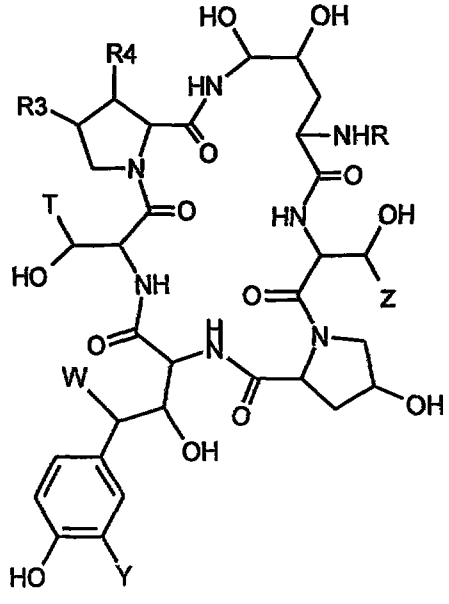
The following examples illustrate the invention without at the same time limiting it.

The invention equally has as its object a preparation process characterised in that a formula (III) compound is submitted



(III)

in which the different substituents retain their prior meaning with the action of an agent capable of replacing NH_2 with NHR, R retaining its prior meaning to obtain the formula (IV) compound



(IV)

in which the different substituents retain their prior meaning and are submitted to the action of silyl trimethyl iodide to obtain the compound of formula (II).

The compounds of formula (III) used as initial products are novel products and are themselves an object of the present invention. An example of preparation of the formula (III) compound is given hereafter in the experiment section.

The invention has more particularly as its object the deoxymulundocandin nucleus, compound of formula (III) the preparation of which is given hereafter in the experiment section.

The formula (IV) compounds as described above, with the exception of mulundocandin and deoxymulundocandin are novel products and are in themselves an object of the present invention.

The invention has more particularly as its object the compounds of formula (IV) whose preparation is given in the experiment section.

These following examples illustrate the invention without at the same time limiting it.

PREPARATION 1 : 1-[N2-(12-methyl-1-oxotetradecyl)-4-oxo-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B.

1g of 1-[(4R,5R)-4,5-dihydroxy-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B is introduced under magnetic stirring and under nitrogen atmosphere into 25ml of acetonitrile. 455µl of trimethylsilyl iodide is added. It is heated at 55°C for 40 minutes. It is hydrolysed with a solution of sodium thiosulphate at 3%. After 10 minutes of stirring, it is dried under reduced pressure and purified by chromatography on silica. 62% of sought product is obtained.

CCM: rf = 0.25 (eluent: CH_2Cl_2-MeOH-H_2O 86-13-1).

EXAMPLE 1 : Trifluoroacetate of 1-[4-amino-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B (isomer B).

50mg of the product of preparation 1 is introduced into 2.5ml of methanol in the presence of 4A activated siliporite. 158mg of ammonium acetate at 20°C is added. The obtained solution is heated at 50°C and 5.5mg of $NaBH_3CN$ is added. It is stirred for 3 hours 15 minutes. 1ml of distilled water is added and the solution is concentrated dry. 166mg of product is obtained that is purified by HPLC (C_{18}) by eluting with the compound CH_3CN-H_2O-TFA (50-50-0.02). 17mg of sought product is obtained.

MH^+ = 975.

EXAMPLE 2 : Trifluoroacetate of 1-[4-[[2-dimethylaminoethyl-amino-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B (isomers A and B).

80mg of the product of preparation 1, is introduced at 20°C into a solution containing 1ml of methanol, 160µl of 2-dimethyl-aminoethylamine, 8ml of a solution 1M of hydrochloric acid in methanol in the presence of 4A siliporite. 35mg of sodium cyanoborohydrure is introduced and stirred for 20 hours at 20°C. It is filtered, washed in methanol and concentrated dry. 325mg of product are obtained that is purified by HPLC (C_{18}) (eluent : CH_3CN-H_2O-TFA 45-55-0.02 then CH_3CN-H_2O- TFA 42-58-0.02). 8.1gmg of sought isomer A product and 9.4mg of isomer B sought product are obtained.

Mass Spectrometry:

MH^+ = 1046

MNa^+ = 1068

EXAMPLE 3 : Trifluoroacetate of 1-[4-[(3-aminopropyl)amino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxy-phenyl)-L-threonine]-5-L-serine echinocandin B (A and B isomers).

30cm3 of a 1M solution of hydrochloric acid is added at 0°C in methanol into a solution containing 200mg of the product of preparation 1, 2 ml of methanol and 300µl of diaminopropane. It is stirred for 15 minutes at 0°C and 84mg of sodium cyanoborohydrure at 95% is added. It is stirred for 6

hours at ambient temperature and dried under reduced pressure. The obtained residue is made into a paste in acetonitrile, spun and dried under reduced pressure. 312mg of product that is purified by HPLC (C_{18}) (eluent : CH_3CN-H_2O-TFA 45-55-0.02) and 15mg of isomer A and 10mg of isomer B is obtained.

Mass Spectrometry:

MH^+ = 1032.

EXAMPLE 4 : (Z+E) Trifluoroacetate of 1-[4-[(4.5-dihydro-1H-imidazol-2-yl)hydrazono]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B.

350mg of the product of preparation 1, 12ml of methanol and 130mg of of 2-hydazino 2-imidazoline hydrobromide is kept at reflux for 2 hours whilst stirring. After evaporating dry, 510mg of product is obtained that is purified by chromatography on silica by eluting with the compound CH_2-Cl_2-MeOH-H_2O (86-13-1) then by semi-preparative (C_{18}) HPLC by eluting with the compound CH_3CN-H_2O-TFA (55-45-0.02). 133mg of sought product is thus obtained.

Mass spectrometry:

MH^+ = 1056

MNa^+ = 1078

EXAMPLE 5: (Z) 1-[4-[(2-Hydroxyethoxy) imino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding E isomer.

a mixture of 36mg of O-(2-hydroxyethyl) hydroxylamine, 5ml of ethanol, 12µl of pyridine, 4µl of pure acetic acid and 150mg of the product of preparation 1 is kept at reflux for 4 hours. 205mg of product that is purified by chromatography on silica by eluting with the methylene chloride-methanol-water (86-13-1) mixture. 2 products of rf=0.2 and 0.25 (isomer Z and isomer E) are isolated.

Mass spectrometry:

MH^+ = 1033

MNa^+ = 1055

EXAMPLE 6: (E) 1-[4-(hydroxyimino)-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding Z isomer.

A mixture containing 200mg of the product of preparation 1, 8ml of ethanol, 36mg of hydroxylamine hydrochloride is left for 1 hour at reflux whilst stirring. It is dried and purified by chromatography HPLC (C_{18}) (eluent CH_3CN-H_2O 60-40). 72mg of Z isomer and 60mg of E isomer is obtained.

Mass spectrometry:

$MH^+ = 989$

$MNa^+ = 1011$

EXAMPLE 7 : Trifluoroacetate of 1-[4-(hydroxyamino)-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B (isomer A and isomer B).

70mg of E+Z oxime mixture obtained in the previous example, 1cm^3 of trifluoroacetic acid, 12mg of sodium cyanoborohydrure at 95% mixture is stirred for 3 hours. It is dried under reduced pressure. It is purified by HPLC (C_{18}). The products sought are obtained.

Mass spectrometry:

$MH^+ = 991$

$MNa^+ = 1013$

EXAMPLE 8 : (Z) Chlorohydrate of 1-[(S)-N2-(12-methyl-1-oxotetradecyl)-4-[[(3-piperidinyl)oxy] imino]-L-ornithine] 4-]4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B.

Stage A:

146mg of the product of preparation 1 and 60µlof acetic acid is added to a solution containing 45mg of R-3-(aminooxy)-1-piperidine phenylmethyl carboxylate and 2 ml of methanol. It is stirred for 2 hours at ambient temperature. It is concentrated, purified by chromatography on silica by eluting with the 98-2 methylene chloride-methanol compound. The sought product is thus obtained.

Mass spectrometry:

MH^+ = 1206

MNa^+ = 1228

Stage B:

A compound containing 61mg of the product prepared in stage A, 20mg of palladium on carbon and 1 ml of acetic acid is placed under hydrogen atmosphere and stirred vigorously for 5 hours. It is filtered and concentrated. 65% of sought product is obtained.

Mass spectrometry:

MH^+ = 1072

EXAMPLE 9 : Trifluoroacetate of 1-[4-[(2-aminoethyl) amino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B (isomer A and isomer B).

To the solution of 300mg of preparation 1 in 6ml of methanol in the presence of 375µl of ethylenediamine is added 63ml of a solution of 1M of hydrochloric acid in methanol. After 15 minutes of agitation, 126mg of sodium cyanoborohydrure ($NaBH_3CN$) is added. The reaction medium is stirred for 5 hours. It is filtered and dried, the products purified by HPLC (C_{18}) by eluting with the CH_3CN - H_2o - TFA (40-60-0.02) mixture. The sought products are thus obtained.

Mass spectrometry:

MH^+ = 1018

MNa^+ = 1040

EXAMPLE 10: (E) 1-[4-[(2-bromoethoxy) imino)-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding Z isomer.

402mg of bromo-2-ethoxyamine bromhydrate is added to a solution containing 710mg of the product of preparation 1 and 28ml of absolute methanol. The mixture is brought to reflux for 55 minutes. It is concentrated under reduced pressure. The obtained product is purified by flash chromatography on silica by eluting with the (9-1) methylene chloride-methanol compound. The sought products isomer A: Rf=0.54, isomer B: Rf = 0.47 are obtained.

Mass spectrometry:

MH^+ = 1095

MNa^+ = 1117

EXAMPLE 11 : (+) Trifluoroacetate of 1-[4-[(aminoiminomethyl) hydrazono]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B.

162mg of aminoguanidine hydrochloride is added to a solution containing 260mg of the product of preparation 1 and 10ml of n-butanol. The reaction medium is brought to reflux for 2 hours 30 minutes. It is concentrated under reduced pressure. The obtained product is purified by semi-preparative HPLC. 225mg of product in a 50/50 mixture of isomers is obtained.

Mass spectrometry:

MH^+ = 1030

MNa^+ = 1052

EXAMPLE 12 : (Z) Trifluoroacetate of 1-[4-[[2 (dimethylamino)ethoxyimino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding E isomer.

80.5mg of the product of example 10 are introduced into 32ml of an ethanolic solution of dimethylamine. The reaction medium is brought to reflux for 45 minutes. It is concentrated. The obtained product is purified by HPLC (C_{18}) (CH_3CN-H_2O - TFA 60-40-0.02). The sought products are thus obtained.

Mass spectrometry:

MH^+ = 1060

EXAMPLE 13 : (E) Trifluoroacetate of 1-[4-[[2-aminoethoxy)-imino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding Z isomer.

50mg of the product of example 10 is introduced into ammonia. It is stirred under pressure for 16 hours whilst allowing it to come to ambient temperature. The reaction medium is again placed in the (45-55) CH_3CN-H_2O compound to be purified by HLPC (C_{18}). The sought products are obtained.

Mass spectrometry:

MH^+ = 1032.

Preparation 2: deoxymulundocandin "nucleus"

2g of deoxymulundocandin are dissolved in 20ml of DMSO. This solution is poured into a suspension containing 120g of FH2264 Utahensis actinoplanes in 870ml of a KH2PO4, K2HPO4 (pH: 6.8) buffer. The reaction medium is stirred for 70 hours at 30°C. It is filtered. The mycelium is washed with the phosphate buffer (pH: 6.8). The washing liquids and the filtrate are joined. The obtained product is chromatographed on a DIAION HP 20, resin and a product is obtained that is used as hereafter.

EXAMPLE 14 : Trifluoroacetate of 1-[4-[(2-aminoethyl) amino] N2-[[4'-(octyloxy)[1.1'-biphenyl]-4-yl]carbonyl]-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B (isomer A)

Stage A: 1-[(4R,5R)-4.5-dihydroxy-N2-[[4'-(octyloxy)[1.1'-biphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B

1-Preparation of the ester

632g of 2.3.4.5.6 pentafluorophenol is added in 695mg of N, N'-dicyclohexylcarbodiimide to 1g of 4'-octyloxy-[1.1'-biphenyl]4-carboxylic acid in 22ml of tetrahydrofurane, stirred for 22 hours at ambient temperature, filtered, the solvents are eliminated under reduced pressure, the residue is placed into ether, stirred at about 35°C, filtered, the solvent is evaporated, it is dried and 1.46g of expected product is obtained, used as it is.

2-Coupling

677mg of deoxymulundocandin <<nucleus>> obtained in preparation 2 is introduced, into 16ml of DMF. The obtained solution is stirred for 5 minutes and 793g of 4'-(octyloxy)-[1.1'-biphenyl-4-pentafluorophenyl carboxylate obtained above is added.

The reaction compound is stirred and kept under nitrogen atmosphere for 24 hours. It is filtered and concentrated. The residue is placed into ether, triturated, stirred for 25 minutes, spun, washed with ethylic ether, chromatographed on silica by eluting with the (86/13/1) then (80/20/1)

methylene chloride, methanol, water mixture. The sought product is thus obtained. Yield 73%.

Stage B: 1-[N2-[[4'-(octyloxy)-[1.1'-biphenyl]-4-yl]carbonyl]-4-oxo-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B

311µl of trimethylsilyl iodide is added to a suspension containing 809mg of the product of stage A and 19ml of acetonitrile. The reaction medium is stirred for 15 minutes at 60°C under nitrogen atmosphere. The compound is poured into a sodium thiosulphate saturated solution. The residue obtained is evaporated and chromatographed on silica, by eluting with the 86/13/1 methylene-chloride methanol water compound. The sought product is obtained. Yield 55%.

Stage C: 1-[4-[(2-aminoethyl) amino]-N2-[[4'-(octyloxy)[1.1'-biphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L serine echinocandin B (isomer A) trifluoroacetate

560µl of acetic acid are added to a solution containing 900mg of the product of the preceding stage, 16ml of methanol and 250µl of diamine ethylene. It is stirred for 15 minutes and 64mg of sodium cyanoborohydrure is added. It is stirred for 18 hours. It is filtered and concentrated. The residue is placed in a minimum of water, triturated, spun and purified by preparative HPLC by eluting with the compound CH_3CN/H_2O/TFA/ (55-45-0.2). The sought product is obtained. Yield 26%.

Spectrum RMN $CDCl_3$

9.07 (m wide) 1H; 8.48 (dl, J=8) 1H ; 8.00 (dl, J=8) 2H ; 7.96 (dl, J=8.5) 2H ; 7.71 (dl, J=8.5) 2H ; 7.64 (dl, J=8.5) 2H ; 7.60 (dl, J=9) 1H ; 7.37 (dl, J=9.5) 1H ; 7.02 (dl, J=8.5) 2H ; 6.97 (dl, J=8.5) 2H ; 6.65 (dl, J=8.5) 2H ; 4.90 (m) 1H ; 4.77 (m) 1H ; 4.66 (m) 1H ; 4.45 (m) 1H ; 4.42 (m) 1H ; 4.39 (m) 1H ; 4.34 (sl) 1H ; 4.26 (m) 1H ; 4.22 (m) 1H ; 4.08 (m) 1H ; 4.01 (t,J=6.5) 2H ; 3.88 (m) 3H ; 3.70 (m) 2H ; 3.51 (m) 2H ; 3.48 (m) 1H ; 3.31 (m) 2H ; 3.28 (m) 1H ; 3.16 (m) 2H ; 2.53 (dd, J=6 et 13.5) 1H ; 2.44 (dd, J=7.5 et 13.5) 1H ; 2.27 (m) 1H ; 2.25 (m) 1H ; 2.15 (m) 2H ; 1.94 (m) 1H ; 1.74 (m) 2H ; 1.44 (m) 2H ; 1.22 to 1.40 (m) 8H ; 1.13 (d, J=6) 3H ; 0.99 (d, J=6.5) 3H ; 0.88 (t, J=7) 3H.

EXAMPLE 15: 1-[4-[(aminoiminomethyl)hydranol]-n2-[[4-[4-[4-(pentyloxy)-phenyl]-1-piperazinyl]phenyl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B

Stage A: 1-[(4R, 5R)-4.5-dihydroxy-N2-[[4-[4-[4-(pentyloxy)phenyl]-1-piperazinyl]phenyl]carbonyl]-L-ornithine]-4-[4-(4-hydroxy-phenyl)-L-threonine]-5-L-serine echinocandin B

1-Preparation of the ester

55mg of pentafluorophenol and 61mg of N, N' dicyclohexyl carbodiimide is added to a mixture of 100mg of [4-[4-[4-(pentyloxy)phenyl]-1-piperazinyl]phenyl]carboxylic acid and 3ml of tetrahydrofurane. The reaction compound is stirred at 20°C for 16 hours, filtered, washed with THF and concentrated dry. It is placed in diethylic ether, filtered, washed and concentrated. 71mg of product is obtained.

2-Coupling

A suspension containing 71mg of the ester above, 70mg of deoxymulundocandin <<nucleus>> obtained as in preparation 2, 2.5ml of DMF in the presence of 4A activated siliporite is stirred at 20°C for one night. It is concentrated, the product obtained is placed in ether and filtered. A product is obtained that is chromatographed on silica by eluting with the mixture acetonitrile/water/trifluoroacetic acid (60-40-0.02). 30mg of sought product is thus obtained.

Stage B: 1-[N2-[[4-[4-[4-(pentyloxy)phenyl]-1-piperazinyl]-phenyl]-carbonyl]4-oxo-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B

1-Preparation of the ester

a mixture of 1g of the product of stage A, 25 ml of acetonitrile, in the presence of 4A activated siliporite is heated to 55°C. 430ml of trimethylsilane iodide is added. It is stirred for 45 minutes then 150µl of an aqueous solution of sodium thiosulphate at 30% is added. It is stirred for 40 minutes at 20°C and concentrated. The dry extract is placed in water, stirred for 1 hour at 20°C spun and washed. A product is obtained that is chromatographed on silica by eluting with the compound methylene chloride-methanol-water (86/13/1). 497mg of sought product are obtained. Yield 42%.

Stage C: 1-[4-[(aminoiminomethyl)hydrazono]-N2-[[4-[4-[4-(pentyloxy)-phenyl]-1-piperazinyl]phenyl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B

A suspension containing 400mg of the product of stage B, 4.8ml of n-butanol and 221 mg of aminoguanidine hydrochloride is heated at 130°C for 3 hours. It is concentrated and 705mg of a product is obtained that is chromatographed on silica by eluting with the methylene chloride methanol

compound 85/15, then by semi-preparative HPLC (kromasil C18) with a 40.60.0.02) acetonitrile/water/trifluoroacetic acid compound. 64mg of sought product is thus obtained.

Spectrum RMN $CDCl_3$

10.75 (s) 0.66H ; 10.45 (s) 0.33H ; 8.39 (d, J=8) 0.33H ; 8.34 (m) 1H ; 8.10 (d, J=7.5) 0.66H ; 8.08 (d, J=8) 0.33H ; 7.99 (d, J=8.5) 0.66H ; 7.74 (d, J=8.5) 1.33H ; 7.71 (d, J=8.5) 0.66H ; 7.60 (d, J=8.5) 0.66H ; 7.50 (m) 1.33H ; 7.00 (m) 6H ;6.86 (d, J=8.5) 2H ; 6.65 (d, J=8) 2H ; 5.08 (dt, J=2 et 11.5) 0.66H ; 4.94 (m) 1H ; 4.88 (m) 0.33H ; 4.75 (dm, J=8) 0.33H ;4.67 (dd, J=3 et 7.5) 0.66H ;4.43 (m) 1H ; 4.38 (m) 1.66H ;4.33 (m) 0.66H ;4.26 to 4.20 (heavy) 2.33H ; 4.12 (d, J=9) 0.66H ;4.00 to 3.68 (heavy) 7.33H ; 3.90 (t,J=7) 2H ;3.62 (d,J=12) 0.33H ;3.43 (swide) 2H ;3.30 to 3.20 (m) 1H ; 3.20 (swide) 2H ;2.91 (d,J=14)0.66H ;2.86 (m) 0.33H ; 2.76 (m) 0.33H ; 2.63 (dd, J=14 et 12.5) 0.66H ;2.52 (dt, J=6 et 13) 1H ; 2.44 (dd, J=8 and 13) 1H ;2.35 (m) 0.33H ; 2.25 (m) 1.66H ;1.93 (twide,J=13) 1H ; 1.69 (m) 2H ; 1.42 to 1.30 (heavy) 4H ;1.15 (d, J=6) 1.98H ;1.10 (,J=6) 0.99H ; 0.98 (d, J=6.5) 3H ; 0.90 (t, J=7) 3H.

EXAMPLE 16: 1-[4-[(2-aminoethyl)amino]-N2-[4-[4''-(pentyloxy)[1.1':4'1''-terphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(hydroxyphenyl)-L-threonine]5-L-serine-echinocandin B (isomer A and isomer B).

Operating as previously, from deoxy-mulundocandin <<nucleus>> prepared as indicated in preparation 2 by obtaining 1-[(4R, 5R)-4.5-dihydroxy-N2-[[4''-(pentyloxy)[1.1': 4'.1''-terphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl-L-threonine]-5-L-serine-echinocandin B and the corresponding 4-oxo derivative as intermediate product, the sought product is obtained.

Spectrum RMN $CDCl_3$

9.00 (wide) 1H ; 8.37 (dl, J=8.5) 1H ; 8.28 (m) 1H ; 8.10 (dl, J=6) 1H ; 8.02 (dl, J=8) 2H ; 7.82 (m) 4H ; 7.73 (dl, J=8) 2H ; 7.66 (dl, J=8) 2H ; 7.38 (dl, J=9) 1H ; 7.32 (dl, J=9) 1H ; 7.03 (dl, J=8.5) 2H ; 6.96 (dl, J=8) 2H ; 6.66 (dl, J=8) 2H ; 5.03 (m) 1H ; 4.84 (m) 1H ; 4.67 (m) 1H ; 4.45 (m) 2H ; 4.36 (dd, J=7.5 and 10.5) 1H ; 4.23 (m) 2H ; 4.18 (sl) 1H ; 4.04 (m) 1H ; 4.02 (t, J=6.5) 2H ; 4.00 (m) 1H ; 3.87 (dl, J=9.5) 1H ; 3.76 (m) 1H ; 3.72 (m) 2H ; 3.55 (m) 1H ; 3.44 (m) 1H ; 3.35 (m) 2H ; 3.30 (m) 1H ; 3.19 (m) 2H ; 3.12 (m) 1H ; 2.53 (m) 1H ; 2.45 (m) 1H ; 2.12 to 2.30 (m) 3H ; 1.90 to 2.05 (m) 2H ; 1.74 (m) 2H ; 1.30 to 1.55 (m) 4H ; 1.20 (d, J=5.5) 3H ; 0.96 (d, J=6.5) 3H ; 0.91 (t, J=7) 3H.

EXAMPLE: Pharmaceutical composition:

Tablets have been prepared containing:

-The product of example 14.....150mg

-Excipient q.s.p.....1g

(Detail of the excipient: starch, talc, magnesium stearate).

PHARMACOLOGICAL STUDY

A – Inhibition of the glucane synthesis of Candida albicans.

The membranes of Candida albicans are purified according to the process described by Tang and al Antimicrob. Agents Chemother 35, 99-103, 1991. 22.5µg of membrane proteins are incubated in a mixture of 2Mm of 14C-UDP glucose (specific activity = 0.34mCi./mmol, 50µg of α-amylase, 1Mm of dithiotreitol (DTT), 1Mm EDTA, 100Mm NaF, 7µM of GTP-γ-S, 1M of sucrose and 5oMm OF Ttris-HCL (pH 7.8) in a volume of 100µl. The medium is incubated at 25°C for 1 hour and the reaction terminated by addition of TCA to a final concentration of 5%. The reaction medium is transferred onto a pre-humidified glass fibre filter. The filter is washed, dried and its radioactivity is counted.

Mulundocandin is used as positive control.

The control of the medium is carried out with the same quantity of DMSO 1%.

The obtained results show that the products of the invention present a good activity in this test, particularly the products of examples 9, 11, and 14.

B – activity on the enzyme Aspergillus fumigatus.

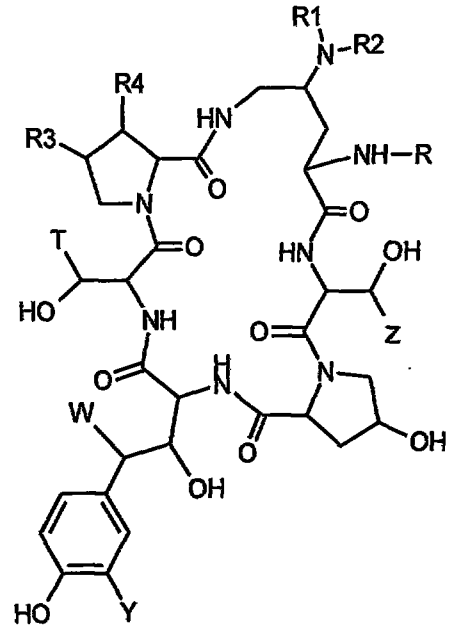
The enzyme is prepared according to the Beaulieu et al. (Antimicrob. Agents Chenother 38, 937-944, 1994) process.

The protocol used is identical to the protocol described above for the enzyme Candida albicans except that dithiotreitol is not used in the reaction medium.

The products present a good activity in this test.

CLAIMS

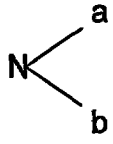
1) The compounds of formula (I) in all the possible isomer forms as well as their compounds:



(I)

in which

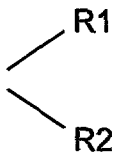
either R1 and R2 identical or different from one another, represent a hydrogen atom, a hydroxyl radical, an alkyl radical containing up to 8 atoms of linear, branched or cyclic carbon atoms, optionally interrupted by an oxygen atom optionally substituted by a

hydrogen atom, an OH radical, an  radical, a and b identical or

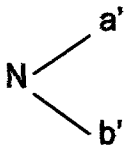
different from one another,

representing a hydrogen atom or an alkyl radical containing up to 8 carbon atoms, a and b able optionally to form a heterocycle with the nitrogen atom optionally containing one or several additional heteroatoms,

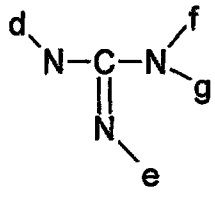
-or else R1 forms a double bond with the endocyclic carbon atom

carrying the radical  or else R2 represents an XR_a radical,

X representing an oxygen atom or an NH or N-alkyl radical containing up to 8 carbon atoms and R_a represents a hydrogen atom, a linear, branched or cyclic alkyl radical containing up to 8 carbon atoms optionally substituted by one or several hydrogen atoms, by one or several OH, CO_2H, CO_2alc radicals,

an  radical, a' and b' representing a

hydrogen atom, an alkyl radical containing up to 8 carbon atoms, a' and b' able to form a heterocycle optionally containing one or several additional heteroatoms and/or by a heterocycle containing one or several

heteroatoms where R2 represents a 

radical in which d, e, f and g represent a hydrogen atom or an alkyl radical containing up to 8 carbon atoms, f and g able moreover to represent an acyl radical containing up to 8 carbon atoms, e and f equally able to form a ring optionally containing one or several heteroatoms,

R3 represents a hydrogen atom, a methyl or hydroxyl radical

R4 represents a hydrogen atom or a hydroxyl radical

R represents a linear, branched or cyclic chain containing up to 30 carbon atoms, optionally containing one or several heteroatoms, one or several heterocycles or a linear, branched or cyclic acyl radical containing up to 30 carbon atoms optionally containing one or several heteroatoms and/or one or several heterocycles,

T represents a hydrogen atom, a methyl radical, a CH_2CONH_2, $CH_2C{\equiv}N$ radical, a $(CH_2)_2NH_2$ or $(CH_2)_2Nalc^+X^-$ radical,

X being a halogen atom and alc an alkyl radical containing up to 8 carbon atoms,

Y represents a hydrogen atom, a hydroxyl radical or a halogen atom or an OSO3H radical or one of the salts of this radical,



W represents a hydrogen atom or an OH radical,

Z represents a hydrogen atom or a methyl radical,

as well as the addition salts with the acids of the products of formula (I).

2) The compounds of formula (I) described in claim 1 in which T represents a hydrogen atom.

3) The compounds of formula (I) described in claim 1 or 2 in which W represents a hydrogen atom.

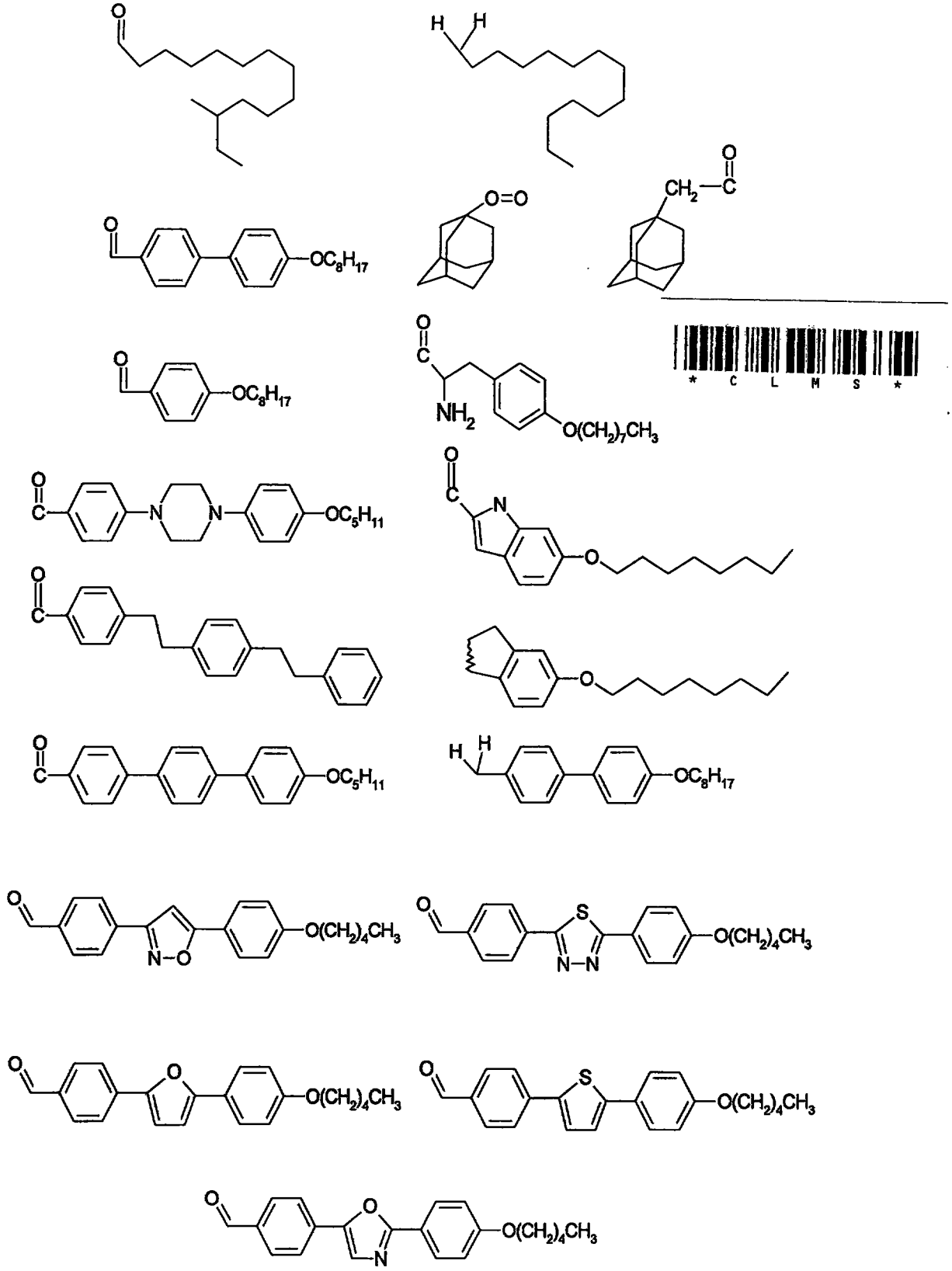
4) The compounds of formula (I) described in any of the claims 1 to 3, in which Z represents a methyl radical.

5) The compounds of formula (I) described in any of claims 1 to 4 in which Y represents a hydrogen atom.

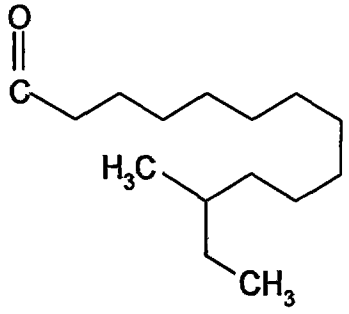
6) The compounds of formula (I) described in any of claims 1 to 5 in which R3 represents a methyl radical.

7) The compounds of formula (I) described in any of claims 1 to 6 in which R4 represents a hydroxyl radical.

8) The compounds of formula (I) described in any of the claims 1 to 7 in which R represents a radical

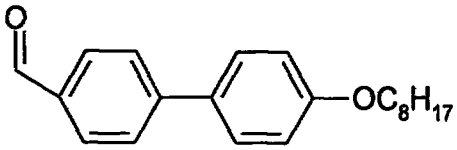


9) The compounds of formula (I) described in claim 8, in which R represents a chain





10) The compounds of formula (I) described in claim 8, in which R represents a chain



11) The compounds of formula (I) described in one of the claims 1 to 10, in which R1 forms a double bond with the endocyclic carbon atom carrying the NR1R2 radical.

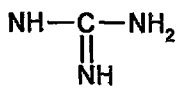
12) The compounds of formula (I) described in claim 11, in which R2 represents the radical

$$O(CH_2)_nNY'_2$$

in which n represents an integer between 1 and 8 and Y' represents a hydrogen atom or an alkyl radical containing up to 8 carbon atoms.

13) The compounds of formula (I) described in claim 12, in which n represents the number 2.

14) The compounds of formula (I) described in claim 8, in which R2 represents a radical



15). The compounds of formula (I) described in one of claims 1 to 10, in which R2

represents a radical

$(CH_2)_pNY''$

in which Y'' represents a hydrogen atom or an alkyl radical containing up to 8 carbon atoms and p represents an integer varying from 1 to 8.

16)The compounds of formula (I) described in any of the claims 1 to 10 and 15, in which R1 represents a hydrogen atom.

17) The compounds of formula (I) described in claim 15, in which p represents the number 2.

18)The compounds of formula (I) described in claim 1, the names of which follow:

-1-[(S)-N2-(12-methyl-1-oxotetradecyl) 4-[[(3-piperidinyl)oxy]imino]-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B.

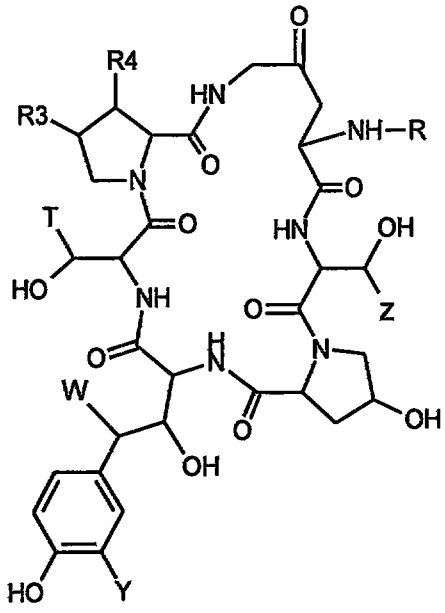
-1-[4-[(2-aminoethyl) amino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B (isomer A and isomer B).

-1-[4-[(aminoiminomethyl) hydrazono]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B.

-1-[4-[(2-aminoethoxy) imino]-N2-(12-methyl-1-oxotetradecyl)-L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B and corresponding isomer Z,

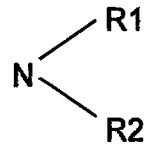
-1-[4-[(2-aminoethyl)amino]-N2-[[4'-(octyloxy)[1.1'-biphenyl]-4-yl]carbonyl]-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B (isomer A) as well as their addition salts with the acids.

19)Preparation process of the compounds of formula (I) described in any of the claims 1 to 18, characterised in that a compound of formula (II) is submitted



(II)

in which R, R3, R4, T, Y, W and Z retain their prior signification, with the action of an amine or an amine derivative likely to introduce

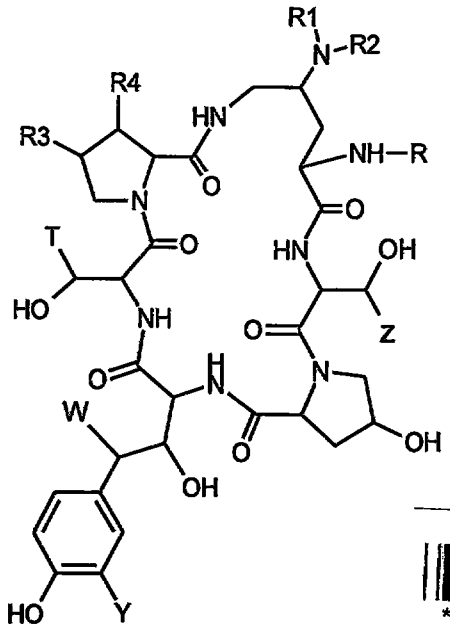
the radical  in which R1 and R2

retain their prior meaning and if desired with the action of a reduction agent and/or agent for the functionalisation of the amine,

and/or an acid to form the salt of the product obtained,

and/or a separation agent of the different isomers obtained,

The sought compounds of formula (I) are thus obtained



(I)

in which R1, R2, R3, R4, T, Y, W, R and Z retain their prior meaning then if desired the compound of formula (I) is submitted to the action of an acid to form the salt and if desired the different isomers obtained are separated.

20)As novel chemical products, the compounds of formula (II) described in claim 19.

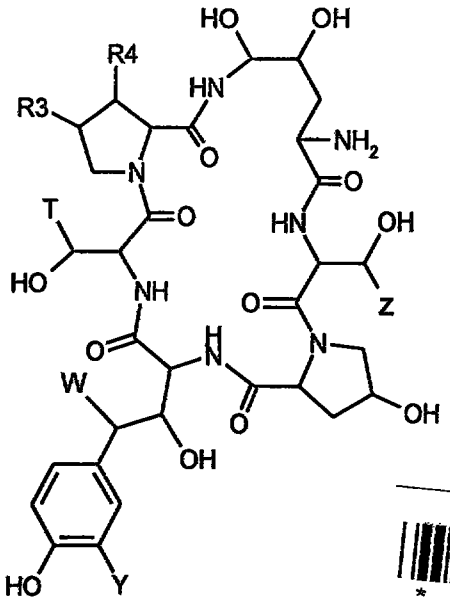
21) As novel chemical product described in claim 20, the compound of formula (II) the names of which follow:

1([-4-oxo-N2-(12-methyl-1-oxotetradecyl) L-ornithine] 4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B.

1-[N2-[[4'-octyloxy)-[1.1'-biphenyl]-4-yl]carbonyl]-4-oxo-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine-echinocandin B

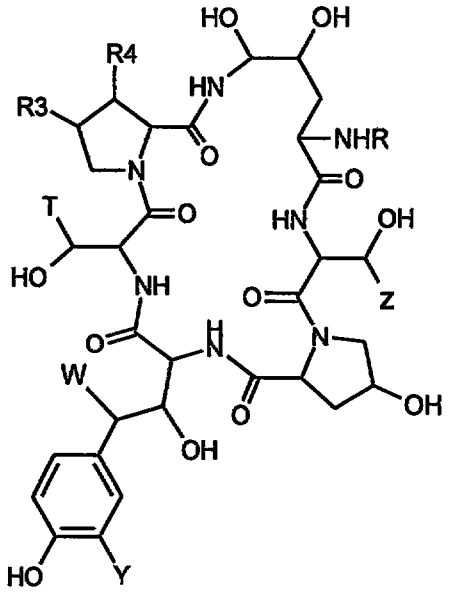
1-[N2-[[4-[4-[4-(pentyloxy)phenyl]-1-piperazinyl]-phenyl]-carbonyl]4-oxo-L-ornithine]-4-[4-(4-hydroxyphenyl)-L-threonine]-5-L-serine echinocandin B

22)Process according to claim 19 characterised in that a compound of formula (III) is submitted



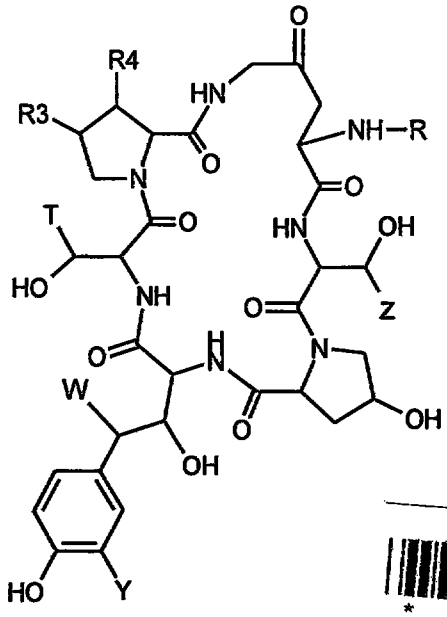
(III)

in which the different substituents retain their prior meaning with the action of an agent capable of replacing NH_2 by NHR, R retaining its prior meaning to obtain the compound of formula (IV)



(IV)

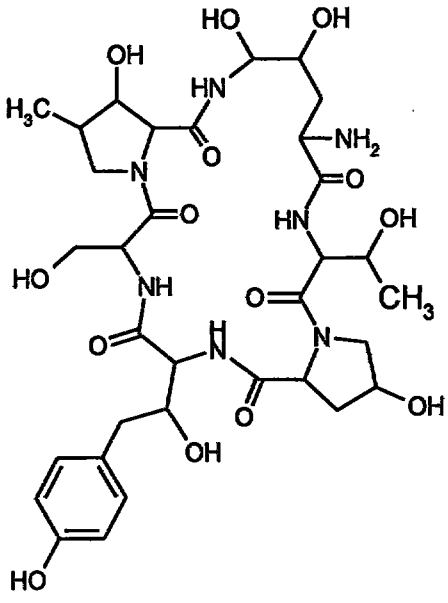
that is submitted to the action of trimethylsilyl iodide to obtain the corresponding compound of formula (II)



(II)

23)As novel chemical products the compounds of formula (III) described in claim 22.

24)As a novel chemical product described in claim 23 the following deoxymulundocandin nucleus of formula (III):



25)As novel chemical products the compounds of formula (IV) described in claim 22 with the exception of mulundocandin and deoxymulundocandin.

26)As antifungal compounds, the compounds of formula (I) described in any of the claims 1 to 18, as well as their addition salts with the acids.

27) The pharmaceutical compositions containing as a medicine at least one compound of formula (I) described in any of claims 1 to 18, as well as their addition salts with pharmaceutically acceptable acids.



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(54) Title: ECHINOCANDIN DERIVATIVES, PREPARATION METHOD AND APPLICATION AS ANTI-FUNGAL AGENTS

(57) Abstract:
The invention concerns compounds of formula (I) in which: either R1 and R2 = H, OH, alkyl optionally substituted, or NR1 forms with the carbon bearing NR1R2 a double bond and R2 is Xra, X being O, NH or N-alkyl and Ra being H, alkyl optionally substituted; R = a chain containing up to 10 carbon atoms, optionally comprising one or several heteroatoms, one or several heterocycles; T=H, CH_2, CH_2CONH_2, $CH_2C{=}N$, $(CH_2)2NH_2$; Y=H, OH, halogen; W=H, OH; Z=H OR CH_3. Said products have antifungal properties.