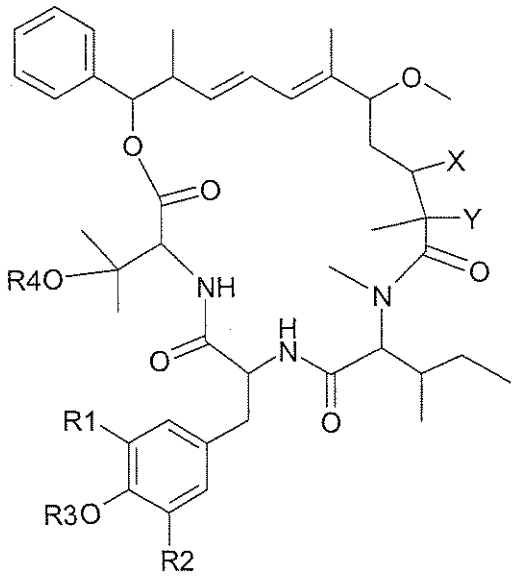
Macrolactone derivatives

The present invention relates to novel macrolactones, and processes for their preparation and use. It has now been found that the microorganism strain ST 201196 (DSM 18870) is able to form novel macrocycles which have a strongly antifungal activity against the fungus *Candida albicans*. The compounds are accordingly suitable for the treatment of local and/or systemic fungal disorders.

A large number of antiinfectives are employed therapeutically for the treatment of infectious diseases. The causative organisms, however, are becoming increasingly resistant to the medicaments used, and a great danger even impends due to "multi-resistant microorganisms", which carry resistances not only against single, but simultaneously against several, anti-infective groups. There are even causative organisms which have become resistant to all anti-infectives obtainable commercially. Infectious diseases which are caused by microorganisms of this type are no longer treatable. There is therefore a great need for novel agents which can be employed against resistant microorganisms. Although many thousands of anti-infectives have been described in the literature, most are too toxic in order to be able to be employed as medicaments.

The present invention relates to a compound of the formula (I),



(I)

wherein

X and Y independently of one another are OH, O-(C_1-C_6)-alkyl, NH_2 or NH-(C_1-C_6)-alkyl, or X and Y together form a group -O-,
R1 and R2 independently of one another are Cl or H,
R3 is H, (C_1-C_6)-alkyl, C(=O)-(C_1-C_6)-alkyl or (C_1-C_6)-alkylene-NH-(C_1-C_6)-alkyl, and
R4 is H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,
or a physiologically tolerable salt of a compound of the formula (I).

Preferably, the invention relates to a compound of the formula (I), wherein X and Y together form a group -O-, consequently X and Y in the corresponding preferred compound, together with the C atoms to which they are bonded, form an epoxide group.

More preferably, the invention relates to a compound of the formula (I), wherein R1 and R2 are Cl.

More preferably, the invention relates to a compound of the formula (I), wherein R1 is equal to Cl and R2 is equal to H.

R3 and R4 are preferably independently of one another H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl, particularly preferably both R3 and R4 are equal to H. Particularly preferably, the invention relates to a compound of the formula (I), wherein X and Y together form a group -O-, R1 and R2 independently of one another are Cl or H, and R3 and R4 independently of one another are H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

furthermore a compound of the formula (I), wherein X and Y together form a group -O-, R1 and R2 are equal to Cl and R3 and R4 independently of one another are H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

furthermore a compound of the formula (I), wherein X and Y together form a group -O-, R1 is equal to Cl, R2 is equal to H, and R3 and R4 independently of one another are H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

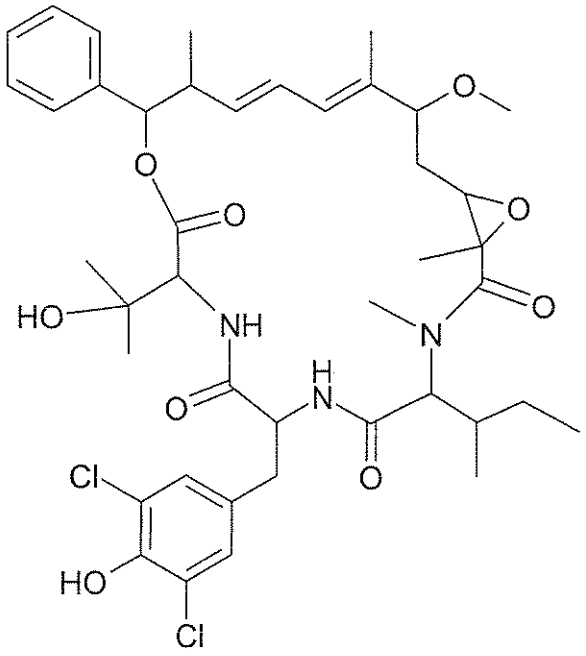
furthermore a compound of the formula (I), wherein

X and Y together form a group -O-,

R3 and R4 are equal to H, and

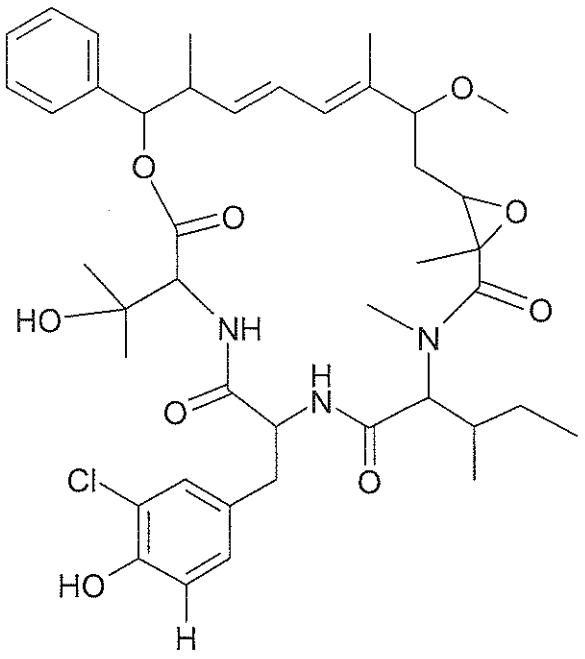
wherein R1 and R2 independently of one another are equal to Cl or H,

preferably wherein R1 and R2 are equal to Cl (also designated as the compound of the formula (II) below),



(II),

or wherein more preferably R1 is equal to Cl and R2 is equal to H (also designated as the compound of the formula (III) below),



(III),

or wherein more preferably R1 and R2 are equal to H.

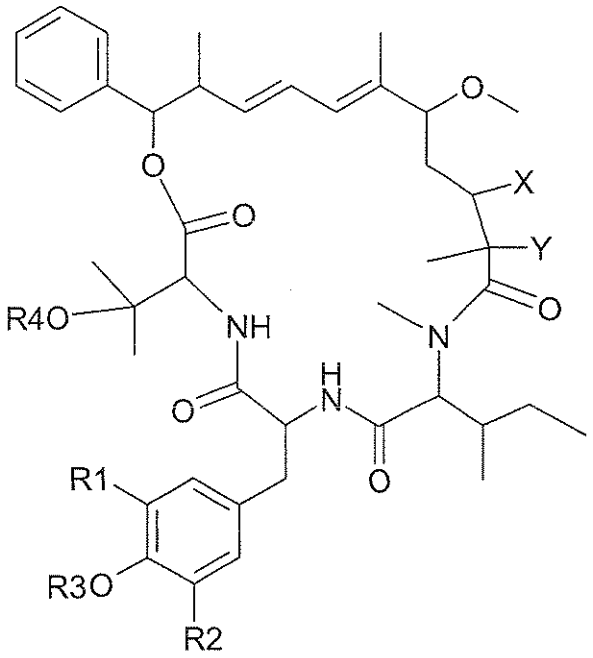
$(C_1$-$C_6)$-Alkyl is a straight-chain or branched alkyl group having 1 to 6 carbon atoms, for example methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, tert-butyl, n-pentyl or n-hexyl.

Chiral centers in the compounds of the formula (I) can be present, if not stated otherwise, in the R or in the S configuration. The invention relates both to the optically pure compounds and stereoisomer mixtures like enantiomer mixtures and diastereomer mixtures.

Physiologically tolerable salts of compounds of the formula (I) are understood as meaning both their organic and inorganic salts, as are described in Remington's Pharmaceutical Sciences (17th edition, page 1418 (1985)). On account of the physical and chemical stability and the solubility, sodium, potassium, calcium and ammonium salts, inter alia, are preferred for acidic groups; salts of hydrochloric acid, sulfuric acid, phosphoric acid or of carboxylic acids or sulfonic acids, such as, for example, acetic acid, citric acid, benzoic acid, maleic acid, fumaric acid, tartaric acid and p-toluenesulfonic acid, inter alia, are preferred for basic groups.

The present invention furthermore relates to all obvious chemical equivalents of the compounds of the formula (I) according to the invention. Equivalents of this type are compounds which exhibit a slight chemical difference, thus have the same action or are converted to the compounds according to the invention under mild conditions. The equivalents mentioned also include, for example, salts, reduction products, oxidation products, esters, ethers, acetals or amides of the compounds of the formula (I) and equivalents which the person skilled in the art can prepare using standard methods.

The invention furthermore relates to a process for the preparation of a compound of the formula (I),



(I)

wherein

X and Y independently of one another are OH, O-(C_1-C_6)-alkyl, NH_2 or NH-(C_1-C_6)-alkyl, or X and Y together form a group -O-,

R1 and R2 independently of one another are Cl or H,

R3 is H, (C_1-C_6)-alkyl, C(=O)-(C_1-C_6)-alkyl or (C_1-C_6)-alkylene-NH-(C_1-C_6)-alkyl, and

R4 is H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

or of a physiologically tolerable salt of a compound of the formula (I),

which comprises

1. fermenting the strain ST 201196 (DSM 18870) or one of its variants and/or mutants under suitable conditions in a culture medium which contains a Cl source, until one or more of the compounds of the formula (I) accumulates in the culture medium and
2. isolating a compound of the formula (I) from the culture medium, and
3. optionally derivatizing the compound of the formula (I) and/or converting it into a physiologically tolerable salt.

The culture medium is a nutrient solution or a solid medium containing at least one carbon and nitrogen source, and the customary inorganic salts.

The Cl source used can be, for example, NaCl or $CaCl_2$. In this case, the strain ST 201196 (DSM 18870) preferably produces compounds of the formula (I), in which both radicals R1 and R2 are equal to Cl or in which R is equal to Cl and R2 is equal to H. Preferably, the invention relates to a process for the preparation of a compound of the formula (I), where R1 and R2 are equal to Cl. Furthermore, the invention preferably relates to a process for the preparation of a compound of the formula (I), where R1 is equal to H, R2 is equal to Cl.

Preferably, the invention relates to a process for the preparation of a compound of the formula (I), wherein X and Y form a group -O-, and furthermore R3 and R4 are as described above or preferably H.

Particularly preferably, the invention relates to a process for the preparation of a compound of the formula (II). More particularly preferably, the invention relates to a process for the preparation of a compound of the formula (III). Furthermore, the invention particularly preferably relates to a process for the preparation of a compound of the formula (I), where R1, R2, R3 and R4 are equal to H.

The process according to the invention can be employed for fermentation on the laboratory scale (milliliter to liter scale) and for the industrial scale (cubic meter scale).

Suitable carbon sources for the fermentation are assimilable carbohydrates and sugar alcohols, such as glucose, lactose, sucrose, or D-mannitol and carbohydrate-containing natural products, such as, for example, malt extract or yeast extract. Suitable nitrogen-containing nutrients are: amino acids; peptides and proteins and their degradation products, for example Probion F (Applied Microbiology and Biotechnology 1984, 19(1), 23-28), casein, peptone or tryptone; meat extracts; yeast extracts; gluten; ground seeds, for example of corn, wheat, beans, soybeans or of the cotton plant; distillation residues from alcohol production; meat meals; yeast extracts; ammonium salts; nitrates. Preferably, the nitrogen source is one or more synthetically or biosynthetically obtained peptides. Inorganic salts are, for example, chlorides, carbonates, sulfates or phosphates of the alkali metals or alkaline earth metals, iron, zinc, cobalt and manganese. Trace elements are, for example, cobalt and manganese.

Suitable conditions for the formation of the substances according to the invention are as follows: The formation of the substances according to the invention preferably proceeds in a culture medium which contains either 0.05 to 5%, preferably 0.1 to 2.5%, of Probion F; 0.02 to 1.0%, preferably 0.05 to 0.5%, of $CaCl_2 \times 2\ H_2O$; 0.02 to 1.5%, preferably 0.05 to 0.7%, of $MgSO_4 \times 7\ H_2O$, and 0.00001% to 0.001% of cyanocobalamin, 1-5% of the adsorber resin XAD-16, or 0.05 to 5%, preferably 0.1 to 2.5%, of oatmeal; 0.2 to 5.0%, preferably 0.1 to 2%, of glycerol; 0.02 to 1.0%, preferably 0.05 to 0.5%, of $CaCl_2$; 0.02 to 1.5%, preferably 0.05 to 0.7%, of $MgSO_4 \times 7\ H_2O$, and 0.00001% to 0.001% of cyanocobalamin. The data in percent are in each case based on the weight of the entire nutrient solution.

The culturing of the microorganism is carried out aerobically, that is, for example, in submerse form with shaking or stirring in shaker flasks or fermenters or on a solid medium, optionally with the introduction of air or oxygen. It can be carried out in a temperature range from approximately 18 to 35°C, preferably at approximately 20 to 32°C, in particular at 27 to 30°C. The pH range should be between 4 and 10, preferably between 6.5 and 9. The microorganism is in general cultured under these

conditions over a period of 3 to 18 days, preferably 144 to 216 hours. Advantageously, culturing is carried out in a number of stages, i.e. first one or more precultures is prepared in a liquid nutrient medium, which is then inoculated into the actual production medium, the main culture, for example in the volume ratio 1:10 to 1:100. The preculture is obtained, for example, by inoculating the strain in the form of vegetative cells or fruiting bodies into a nutrient solution and allowing it to grow for approximately 3 to 13 days, preferably 96 to 240 hours. Vegetative cells and/or fruiting bodies can be obtained, for example, by allowing the strain to grow for approximately 3 to 15 days, preferably 7 to 10 days, on a solid or liquid nutrient medium, for example yeast agar.

The isolation or purification of the substances of the formula (I) from the culture medium is carried out according to known methods taking into consideration the chemical, physical and biological properties of the natural substances. HPLC was used for testing the concentration of the respective derivatives in the culture medium or in the individual isolation stages.

For isolation, the culture broth is centrifuged and/or filtered off through a suction filter. The mycelium is lyophilized with the XAD, subsequently the natural substances are extracted from the lyophilizate using an organic solvent, for example methanol or 2-propanol. The organic solvent phase contains the natural substances according to the invention; it is optionally concentrated in vacuo and further purified.

The further purification of one or more compounds according to the invention is carried out by chromatography on suitable materials, preferably, for example, on molecular sieves, on silica gel, alumina, on ion exchangers or on adsorber resins or on reversed phases (RP). With the aid of this chromatography, the natural substance derivatives are separated. The chromatography of the compounds according to the invention is carried out using buffered aqueous solutions or mixtures of aqueous and organic solutions.
Mixtures of aqueous or organic solutions are understood as meaning all organic solvents miscible with water, preferably methanol, 2-propanol and acetonitrile, in a concentration of 0 to 100% of solvent or alternatively all buffered aqueous solutions

which are miscible with organic solvents. The buffers to be used are the same as indicated above.

The separation of the compounds according to the invention on the basis of their differing polarity is carried out with the aid of reversed phase chromatography, for example on MCI® (adsorber resin, Mitsubishi, Japan) or Amberlite XAD® (TOSOHAAS), or on another hydrophobic material, such as, for example, on RP-8 or RP-18 phases. Moreover, the separation can be carried out with the aid of normal phase chromatography, for example on silica gel, alumina and the like.

The chromatography of the natural substance derivatives was carried out according to methods known to the person skilled in the art, preferably using buffered, basic or acidified aqueous solutions or mixtures of aqueous solutions with alcohols or other, water-miscible organic solvents. Acetonitrile and/or methanol is preferably used as an organic solvent.

Buffered, basic or acidified aqueous solutions are understood as meaning, for example, water, phosphate buffer, ammonium acetate, ammonium formate, citrate buffer in a concentration of up to 0.5 M, and formic acid, acetic acid, trifluoroacetic acid, ammonia, triethylamine or all commercially available acids and bases known to the person skilled in the art, preferably in a concentration of up to 1%. In the case of buffered aqueous solutions, 0.1% ammonium acetate is particularly preferred.

Chromatography was carried out, for example, using a gradient which began with 100% water and ended with 100% solvent; a linear gradient of 5 to 95% acetonitrile was preferably operated.

Alternatively, gel chromatography or chromatography on hydrophobic phases can also be carried out. Gel chromatography is carried out on polyacrylamide or mixed polymer gels, such as, for example, Biogel-P 2® (Biorad) or Fractogel TSK HW 40® (Merck, Germany). The sequence of the aforementioned chromatographies is reversible.

If the compound of the formula (I) is present as a stereoisomer mixture, the stereoisomers can be separated by means of known methods, for example by separation by means of a chiral column.

The derivatization of the OH groups on the 3,5-dichlorotyrosine amino acid of the compound of the formula (I) [R3 equal to H] to an acyl group [R3 equal to C(=O)-(C_1-C_6)-alkyl)] and/or of the OH group on the 3-hydroxyvaline amino acid of the compound of the formula (I) [R4 equal to H] to an acyl group [R4 equal to C(=O)-(C_1-C_6)-alkyl)] is carried out according to methods known per se (J. March, Advanced Organic Chemistry, John Wiley & Sons, 4th Edition, 1992), for example by reaction with an acid chloride in the presence of a base or with an acid anhydride.

The alkylation of the OH group on the 3,5-dichlorotyrosine amino acid of the compound of the formula (I) [R3 equal to H] with an alkyl group [R3 equal to (C_1-C_6)-alkyl] and/or the OH group on the 3-hydroxyvaline amino acid of the compound of the formula (I) [R4 equal to H] with an alkyl group [R4 equal to (C_1-C_6)-alkyl] is carried out by means of methods known per se to the person skilled in the art (J. March, Advanced Organic Chemistry, John Wiley & Sons, 4th Edition, 1992), for example by reaction with a (C_1-C_6)-alkyl bromide in the presence of a base or in the case of a methylation by reaction with methyl iodide or Me_2SO_4.

A selective differentiation between the phenolic OH group (R3=H) and the aliphatic OH group (R4=H) is carried out by means of methods known per se to the person skilled in the art for the introduction of protective groups (T. W. Greene, P. G. M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, 3rd Edition, 1999). For example, Pettus et al. (J. Am. Chem. Soc. 2000, 122, 6160-6168) describes a selective alkylation of a phenolic OH group in the presence of a tertiary aliphatic alcohol by reaction with (C_1-C_6)-alkyl bromide in the presence of K_2CO_3 in acetone or by reaction with (C_1-C_6)-alkyl-OH in the presence of $(CF_3CO)_2O$ and $CuCl_2$ hydrate in DBU. A further possibility for differentiation between the phenolic OH group and the aliphatic OH group is carried out by means of the methods known

per se to the person skilled in the art for the selective deprotection of a bis-alkylated [R3 equal to R4 equal to (C_1-C_6)-alkyl] or of a bis-acylated [R3 equal to R4 equal to C(=O)-(C_1-C_6)-alkyl)] compound of the formula (I) (T. W. Greene, P. G. M. Wuts, Protective Groups in Organic Synthesis, John Wiley & Sons, 3rd Edition, 1999). For example, Jones et al. (J. Org. Chem. 2001, 66, 3688-3695) describe the selective deprotection of a tert-butylsilyl (TBS)-protected phenol in the presence of a TBS-protected tertiary alcohol by tert-butylammonium fluoride (TBAF) at -20°C. The phenolic OH group (R3 = H) can furthermore be derivatized to a group -(C_1-C_6)-alkylene-NH-(C_1-C_6)-alkyl by reaction with H_2N-(C_1-C_6)-alkyl in the presence of Cl-[(C_1-C_6)-alkyl]-Cl or Br-[(C_1-C_6)-alkyl]-Br.

The derivatization of compounds of the formula (I), in which X and Y form a group -O- to a compound of the formula (I), in which X and Y independently of one another are OH, O-(C_1-C_6)-alkyl, NH_2 or NH-(C_1-C_6)-alkyl, is carried out by means of methods known per se to the person skilled in the art (J. March, Advanced Organic Chemistry, John Wiley & Sons, 4th Edition, 1992), for example by reaction of the epoxide group with a (C_1-C_6)-alcoholate [X equal to OH if Y equal to O-(C_1-C_6)-alkyl, or Y equal to OH if X equal to O-(C_1-C_6)-alkyl], NH_3 [X equal to OH if Y equal to NH_2, or Y equal to OH if X equal to NH_2], or H_2N-(C_1-C_6)-alkyl [X equal to OH if Y equal to NH-(C_1-C_6)-alkyl, or Y equal to OH if X equal to NH-(C_1-C_6)-alkyl].

An isolate of the microorganism strain ST 201196 was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen [German Collection of Microorganisms and Cell Cultures] GmbH (DSMZ), Mascheroder Weg 1B, 38124 Brunswick, Germany, according to the rules of the Budapest Convention on the 09.11.2003 under the following number. The following number was assigned as the deposit number: DSM 18870.

The vegetative cells of the strain ST 201196 (DSM 18870) have a characteristic rod shape. On solid nutrient media, ST 201196 (DSM 18870) forms orange-yellow fruiting bodies, which contain round myxospores. The taxonomy of the strain ST 201196 can therefore be described as Myxobacterium sp..

Instead of the strain ST 201196 (DSM 18870), it is also possible to employ its mutants and/or variants which synthesize one or more of the compounds according to the invention.

A mutant is a microorganism in which one or more genes of the genome have been modified, where the gene or the genes which are responsible for the ability of the organism to produce the inventive compound remain functional and inheritable.

Mutants of this type can be produced in a manner known per se by physical means, for example irradiation, such as with ultraviolet or X-ray beams, or chemical mutagens, such as, for example, ethyl methanesulfonate (EMS); 2-hydroxy-4-methoxybenzophenone (MOB) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), or as described by Brock et al. in "Biology of Microorganisms", Prentice Hall, page 238-247 (1984).

A variant is a phenotype of the microorganism. Microorganisms have the ability to adapt to their environment and therefore show marked physiological flexibility. In the case of phenotypic adaptation, all cells of the microorganism are involved, where the nature of the modification is not genetically conditioned and is reversible under modified conditions (H. Stolp, Microbial ecology: organismus, habitats, activities. Cambridge University Press, Cambridge, GB, page 180, 1988).

Screening for mutants and/or variants which synthesize one or more of the compounds according to the invention is carried out according to the following scheme:

- lyophilization of the fermentation medium;
- extraction of the lyophilizate with an organic solvent
- extraction of the compound from the culture filtrate using solid phases
- analysis by means of HPLC, TLC or by testing of the biological activity.

The described fermentation conditions apply for ST 201196 (DSM 18870) and for mutants and/or variants thereof.

For the detection of the antifungal activity of rapidly growing, aerobic causative organisms, the bouillon dilution method (microdilution) according to a procedure of the CLSI (Clinical and Laboratory Standards Institute, M7-A7, Vol. 26, No. 2) is used. The IC_{50} value was determined. This is the concentration of an active substance which is necessary in order to inhibit the growth of the test organism *Candida albicans* by 50%.

The compound of the formula (II) has an IC_{50} value of 0.06 µg/ml against Candida albicans. The compound of the formula (IV) has an IC_{50} value of 0.41 µg/ml against *Candida albicans.*

The invention furthermore relates to the use of the compound of the formula (I) or of a physiologically tolerable salt thereof as a medicament in human or veterinary medicine, in particular for the treatment and/or prophylaxis of fungal disorders. Preferably, the invention relates to the use of a compound of the formula (I) or of a physiologically tolerable salt for the treatment of local and/or systemic fungal disorders.

In addition, the present invention relates to a medicament containing at least one compound of the formula (I), where the compound or the compounds of the formula (I) can be administered as such in substance or preferably as a mixture with one or more of the customary pharmacologically suitable vehicles or excipients.

The compounds according to the invention are stable in the solid state and in solutions in the pH range between 2 and 9, in particular 5 and 7, and can thus be incorporated into customary galenical preparations.

The medicaments according to the invention can be administered orally or parenterally, but rectal administration is also possible in principle. Suitable solid or liquid galenical preparation forms are, for example, granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, aerosols, drops or injectable solutions in ampoule form, and preparations with protracted release of active substance, in whose preparation pharmacologically

suitable vehicles or excipients such as disintegrants, binders, coating agents, swelling agents, glidants or lubricants, flavor additives, sweeteners or solubilizers are customarily used, for example magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatine, starch, vitamins, cellulose and its derivatives, animal or vegetable oils, polyethylene glycols and solvents, such as, for example, sterile water, alcohols, glycerol and polyhydric alcohols.

Optionally, the dose units for oral administration can be microencapsulated in order to delay release or to extend it over a longer period, such as, for example, by coating or embedding the active substance in particle form in suitable polymers, waxes or the like.

Preferably, the pharmaceutical preparations are prepared and administered in dose units, where each unit contains as active constituent a certain dose of one or more compounds of the natural substance derivatives according to the invention. In the case of solid dose units such as tablets, capsules and suppositories, this dose can be up to approximately 500 mg, but preferably approximately 0.1 to 200 mg, and in the case of injection solutions in ampoule form up to approximately 200 mg, but preferably approximately 0.5 to 100 mg, per day.

The daily dose to be administered is dependent on the body weight, age, sex and condition of the mammal. Under certain circumstances, however, higher or lower daily doses can also be appropriate. The administration of the daily dose can be carried out both by single administration in the form of an individual dose unit or else in a number of smaller dose units and by multiple administration of subdivided doses at certain intervals.

The medicaments according to the invention are prepared by optionally mixing one or more of the compounds of the formula (I) according to the invention with one or more of the customary vehicles or excipients, and bringing into a suitable administration form.

The following examples are intended to serve for the more detailed illustration of the

invention, without restricting the breadth of the invention in any way.

Percentages relate to the weight. Mixing ratios in the case of liquids relate to the volume, if no other particulars have been given.

Example 1: Storage of ST 201196 (DSM 18870) at -135°C

An agar plate (1% fresh baker's yeast; 1% $CaCl_2$ x 2 H_2O; 0.477% HEPES (20 mM); 0.00005% cyanocobalamin; 1.8% agar; pH 7.2) was inoculated with the strain ST 201196 (DSM 18870) and incubated for about 7-10 days at 30°C. The cells of this surface culture were scraped from the agar surface using a sterile spatula, suspended in 1 ml of Casitone medium (1% Casitone (Difco); 0.15% $MgSO_4$ x $7H_2O$; pH 7.0) in cryotubes and stored at -135°C.

Example 2: Storage of ST 201196 (DSM 18870) at -196°C

An agar plate (1% fresh baker's yeast; 1% $CaCl_2$ x 2 H_2O; 0.477% HEPES (20 mM); 0.00005% cyanocobalamin; 1.8% agar; pH 7.2) was inoculated with the strain ST 201196 (DSM 18870) and incubated for about 7-10 days at 30°C. The cells of this surface culture were scraped from the agar surface using a sterile spatula, suspended in 1 ml of Casitone medium (1% Casitone (Difco); 0.15% $MgSO_4$ x $7H_2O$; pH 7.0) in cryotubes and stored at -196°C.

Example 3: Preparation of a preculture of ST 201196 (DSM 18870) in an Erlenmeyer flask

100 ml of nutrient solution (1% fresh baker's yeast; 1% $CaCl_2$ x 2 H_2O; 0.477% HEPES (20 mM); 0.00005% cyanocobalamin; 1.8% agar; pH 7.2) in a sterile 300 ml Erlenmeyer flask were inoculated with the strain ST 201196 (DSM 18870) and incubated for 7 days at 30°C and 180 rpm on a rotary shaker. 10 ml (10%) in each case of this preculture were subsequently used for the preparation of the main cultures.

Example 4: Preparation of a liquid main culture ST 201196 (DSM 18870) using Medium 1

A sterile 300 ml Erlenmeyer flask containing 100 ml of the following nutrient solution (1% Probion F; 0.1% $CaCl_2$ x 2 H_2O; 0.2% $MgSO_4$ x 7 H_2O; 0.00005% cyanocobalamin; 2% of the adsorber resin XAD-16, pH 8.4) was inoculated with 10 ml (10%) of a preculture (see Example 3) or a culture washed on a fresh agar plate (1% fresh baker's yeast; 1% $CaCl_2$ x 2 H_2O; 0.477% HEPES (20 mM); 0.00005% cyanocobalamin; 1.8% agar; pH 7.2) and incubated on a shaker at 180 rpm and 30°C. The maximum production of the substances according to the invention was reached after 144 to 216 hours. A 144-196 hour-old submerse culture (inoculum 10%) of the same nutrient solution as described in Example 3 sufficed for the inoculation of 10 to 200 l fermenters.

Example 5: Preparation of a liquid main culture ST 201196 (DSM 18870) using Medium 2

A sterile 300 ml Erlenmeyer flask containing 100 ml of the following nutrient solution (1% oatmeal; 0.5% glycerol; 0.1% $CaCl_2$ x 2 H_2O; 0.2% $MgSO_4$ x 7 H_2O; 0.00005% cyanocobalamin; 2% of the adsorber resin XAD-16, pH 9.0) was inoculated with 10 ml (10%) of a preculture (see Example 3) or a culture grown on a fresh agar plate (1% fresh baker's yeast; 1% $CaCl_2$ x 2 H_2O; 0.477% HEPES (20 mM); 0.00005% cyanocobalamin; 1.8% agar; pH 7.2) and incubated on a shaker at 180 rpm and 30°C. The maximum production of the substances according to the invention was reached after 144 to 216 hours. A 144-196 hour-old submerse culture (inoculum 10%) of the same nutrient solution as described in Example 3 sufficed for the inoculation of 10 to 200 l fermenters.

Example 6: Preparation of the substances according to the invention in the fermenter

The 1 l and 50 l fermenters were operated under the following conditions :

Inoculum:	20%
Nutrient medium:	1% oatmeal; 0.5% glycerol; 0.1% yeast extract; 0.1% $CaCl_2 \times 2\ H_2O$; 0.2% $MgSO_4 \times 7\ H_2O$; 0.00005% cyanocobalamin, 2% of the adsorber resin XAD-16
Incubation temperature:	30°C
Stirrer speed:	200 rpm
Aeration:	0.6 m^3/h
pH regulation :	none, before sterilization pH 7.6 ± 0.3 by means of KOH
pO_2 regulation :	none
Antifoam additive:	0.05% Desmophen (Bayer)
Run time:	155 h

The pH regulation was carried out using 10% KOH, or 10% H_2SO_4.

Example 7: Isolation of the compounds (II) and (III) from the shaker cultures of the microorganism strain ST201196 (DSM 18870)

After completion of the fermentation of the microorganism strain ST201196 (DSM 18870), the culture broth from Example 4 (60 l of culture broth) was filtered. The biomass containing the XAD was lyophilized and subsequently extracted with methanol (4 x 5 l). The methanol extract was reduced to 8 l in vacuo and subsequently applied to a prepared column, which was filled with about 3.0 liters of CHP-20P material (MCI® Gel, 75-150µ, Mitsubishi Chemical Corporation). Elution was carried out using a methanol gradient of from 10% to 95%. The column flow (120 ml/min) was collected in fractions (1 l fractions). Fractions 11 to 14 were combined, the solvent was removed on a Rotavapor and the fraction pool was subsequently lyophilized (yield ~ 0.9 g).

Example 8: Pre-separation of the compounds (II) and (III) by RP-18 chromatography

The fraction pool 11-14 from Example 7 was dissolved in 100 ml of methanol and applied to a Phenomenex Luna® 10μ C18 (2) column (dimensions: 250 mm x 50 mm) having a Luna® 10μ C18 (2) pre-column (dimensions: 60 mm x 21.2 mm) and eluted over the course of 40 min using a gradient of from 5% to 95% acetonitrile in water (0.1% ammonium acetate, pH 4.6 set using acetic acid). The flow was 190 ml/min, the fraction size 190 ml. Fractions 28-29 and 31 were subsequently worked up further.

Example 9: Purification of compound (II)

Fraction 31 from Example 8 was first lyophilized (yield ~ 98 mg), subsequently dissolved in 50 ml of methanol and again purified by means of HPLC on a Phenomenex Luna® Axia 5 μm C18 (2) column (dimensions: 100 mm x 30 mm) having an XTerra® Prep MS C18 10 μm pre-column (Waters, dimensions: 19 x 10 mm). Elution was carried out over the course of 40 min using a gradient of from 5% to 95% acetonitrile in water (with addition of 0.1% ammonium acetate, pH 4.6 set using acetic acid). The column flow (50 ml/min) was collected in fractions according to UV. Fractions 4 to 14 contained the compound of the formula (II) and afforded, after lyophilization, 38 mg (purity > 95%).

Example 10: Characterization of the compound of the formula (II)

Colorless solid, crystals from acetonitrile/water
UV: 208, 232, 286 nm
ESI-MS: MW = 815.3312
Empirical formula: $C_{42}H_{55}Cl_2N_3O_9$
Specific rotation (MeOH): -0.19°, α_D= -38°

Table 1: NMR chemical shifts of the macrolactone (II); c = 3 mg/ml in d_6-DMSO at 300 K.

Position	δ (^{13}C)	δ (^{1}H)
1	126.87	7.251
2 (2C)	127.71	7.322
3	125.89	7.566
4	139.90	-
5	78.78	5.898
6	41.82	2.635
6-Me	9.50	0.961
7	138.29	6.175
8	124.58	6.379
9	128.83	6.116
10	133.27	-
10-Me	10.62	1.696
11	83.90	3.619
11-OMe	55.08	3.094
12	30.50	2.115 1.456
13	57.97	2.633
14	61.26	-
14-Me	14.81	1.427
15	169.23	-
16 N-Me	29.59	2.979
17 *α*	59.11	4.474
β	30.94	1.704
β-Me	14.14	0.348 d
γ	24.15	1.218 0.863
δ	9.99	0.770 t
18 CO	168.59	-
19 NH		8.014
20 *α*	52.67	4.686

	β	2.806 37.02 2.483
	γ	(136.8) -
	δ	129.55 7.345
	ε	121.50 -
	φ	147.65 -
	φ-OH	9.88 br
21	CO	170.43 -
22	NH	8.596
23	α	59.11 4.677
	β	71.84 -
	β-OH	5.110
	γ	24.36 1.037
	γ´	28.14 1.142
24	CO	170.55 -

Example 11: Purification of compound (III)

Fractions 28-29 (380 ml) from Example 8 were again purified by means of HPLC on a Waters XTerra® 10 µm C18 column (dimensions: 100 mm x 30 mm) having an XTerra® Prep MS C18 10 µm pre-column (Waters, dimensions: 19 x 10 mm). Elution was carried out over the course of 40 min using a gradient of from 10% to 95% acetonitrile in water (with addition of 10% formic acid, pH = 2.0). The column flow (70 ml/min) was collected in fractions according to UV. Fractions 45 to 47 contained the compound of the formula (III) and afforded, after lyophilization, ~ 5.4 mg (purity > 50%).

Example 12: Characterization of the compound (III)

Colorless solid

UV: 204, 232, 286 nm

ESI-MS: MW = 799.3002

Empirical formula: $C_{42}H_{56}ClN_3O_9$

Table 2: NMR chemical shifts of the macrolactone (III); c = 5 mg/ml in d_6-DMSO at 300 K.

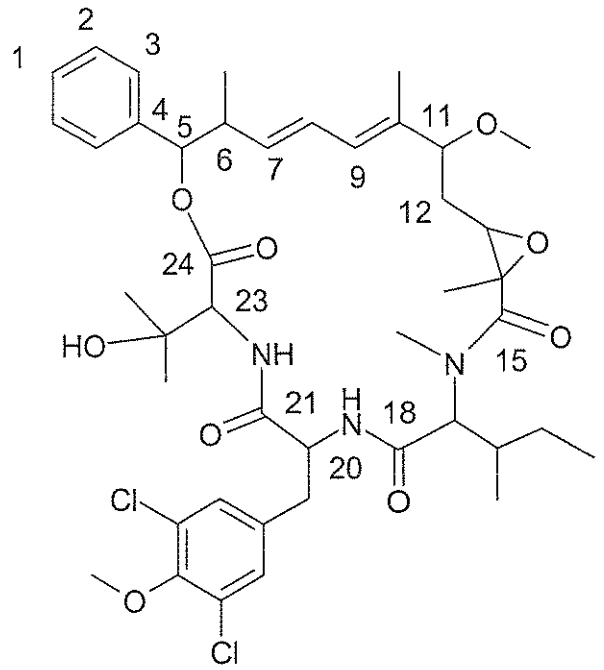
Position	δ (^{13}C)	δ (^{1}H)
1	126.88	7.252
2 (2C)	127.71	7.331
3	125.93	7.566
4	139.91	-
5	78.76	5.896
6	41.84	2.659 br.
6-Me	9.59	0.958
7	138.25	6.171
8	124.64	6.375
9	128.79	6.114
10	133.29	-
10-Me	10.68	1.692
11	83.86	3.620
11-OMe	55.09	3.090
12	30.52	2.113 1.470
13	57.98	2.642
14	61.23	-
14-Me	14.81	1.431
15	169.27	-
16 N-Me	29.63	2.981
17 *α*	59.08	4.471
β	31.04	1.693
β-Me	14.80	0.315 d
γ	24.16	1.221 0.862
δ	9.99	0.773 t
18 CO	168.57	-

19 NH		7.995
20 α	52.68	4.716
β	37.26	2.824 2.488
γ	129.25	-
δ	129.12	7.069
δ'	130.67	7.335
ε	115.83	6.767
ε'	118.85	-
φ	151.40	-
φ-OH		n.d.
21 CO	170.73	-
22 NH		8.636
23 α	59.20	4.674
β	71.85	-
β-OH		n.d
γ	24.41	1.035
γ'	28.15	1.142
24 CO	170.54	-

Example 13: Synthesis of compound (IV)

Compound (II) (80 mg, 0.098 mmol) was dissolved in 5 ml of acetonitrile and the solution was treated at room temperature with potassium carbonate (27 mg, 0.196 mmol) and methyl iodide (70 mg, 0.490 mmol). The mixture was subsequently stirred for 12 h at 50°C. The solution was filtered and purified by means of HPLC on a Phenomenex Luna® Axia 5 μm C18 (2) column (dimensions: 100 mm x 30 mm) having an XTerra® Prep MS C18 10 μm pre-column (Waters, dimensions: 19 x 10 mm). Elution was carried out over the course of 40 min using a gradient of from 5% to 95% acetonitrile in water (with addition of 0.1% ammonium acetate, pH 4.6, set using acetic acid). The column flow (50 ml/min) was collected in fractions according to UV. Fractions 4 and 5 contained the desired compound of the formula (IV) and afforded, after lyophilization, 50 mg (yield: 61%, purity > 95%).

Example 14: Characterization of the compound of the formula (IV)



Colorless solid, crystals from acetonitrile/water

UV: 235, 286 nm

MW = 830.85

Empirical formula: $C_{43}H_{57}Cl_2N_3O_9$

Table 3: NMR chemical shifts of the compound of the formula (IV); c = 3 mg/ml in d_6-DMSO at 300 K.

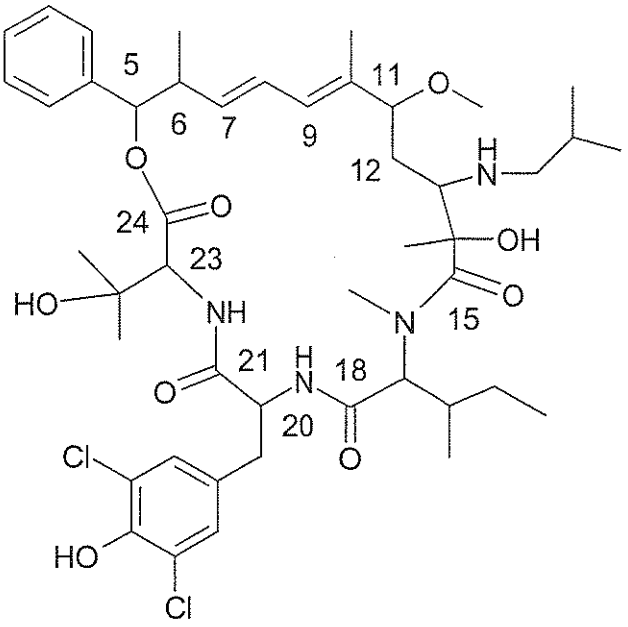
Position	δ (^{13}C)	δ (^{1}H)
1	126.88	7.267
2 (2C)	127.72	7.332
3 (2C)	125.88	7.568
4	139.87	-
5	78.78	5.903
6	41.81	2.660
6-Me	9.593	0.969
7	138.23	6.192
8	124.60	6.385
9	128.78	6.124
10	133.27	-
10-Me	10.65	1.700
11	83.88	3.624
11-OMe	55.08	3.103
12	30.46	2.130 1.470
13	57.94	2.637
14	61.26	-
14-Me	14.82	1.437
15	169.23	-
16 N-Me	29.55	2.984
17 *α*	59.04	4.471
β	30.87	1.715
β-Me	14.20	0.284
γ	24.10	1.216 0.884
δ	9.95	0.775
18 CO	168.53	-
19 NH		8.103

20	α	52.37	4.771
	β		2.889
		37.19	2.569
	γ	136.23	-
	δ	130.29	7.511
	ε	127.33	-
	φ	149.79	-
	φ-OMe	60.29	3.742
21	CO	170.19	-
22	NH		8.620
23	α	59.13	4.691
	β	71.80	-
	β-OH		5.161
	γ	24.38	1.047
	γ´	28.12	1.153
24	CO	170.55	-

Example 15: Synthesis of the compounds (V) and (VI)

Compound (II) (30 mg, 0.037 mmol) was dissolved in 10 ml of 1,2-dichloroethane and the solution was treated at room temperature with isobutylamine (500 µl, 5.03 mmol). The mixture was stirred for 48 h at 70°C, subsequently filtered and purified by means of HPLC on a Phenomenex Luna® Axia 5 µm C18 (2) column (dimensions: 100 mm x 30 mm) having an XTerra® Prep MS C18 10 µm pre-column (Waters, dimensions: 19 x 10 mm). Elution was carried out over the course of 40 min using a gradient of from 5% to 95% acetonitrile in water (with addition of 0.1% ammonium acetate, pH 4.6 set using acetic acid). The column flow (50 ml/min) was collected in fractions according to UV. Fraction 70 contained the two compounds of the formulae (V) and (VI) and afforded, after lyophilization, 3 mg of the two compounds in a ratio of 55:45.

Example 16: Characterization of the compound of the formula (V)



MW = 889.97

Empirical formula: $C_{46}H_{66}Cl_2N_4O_9$

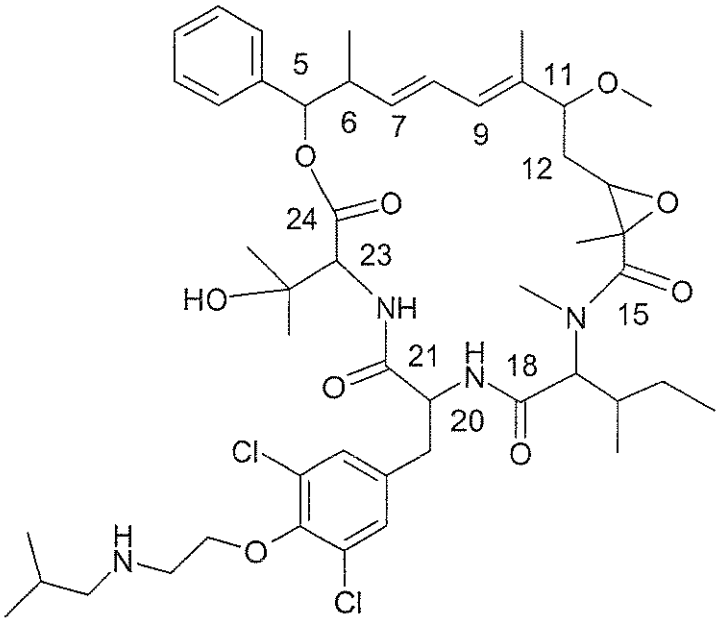
Table 4: NMR chemical shifts of the compound of the formula (V); c = 3 mg/ml in d_6-DMSO at 300 K.

Position	δ (^{13}C)	δ (^{1}H)
1	126.94	7.244
2 (2C)	127.86	7.340
3 (2C)	125.82	7.507
4	139.29	-
5	78.48	5.768
6	41.63	2.679
6-Me	12.06	0.880
7	134.90	5.776
8	126.58	6.483
9	124.04	5.901
10	137.71	-
10-Me	13.72	1.690
11	82.17	3.679

11-OMe	55.06	3.083
12		1.379
	37.38	1.265
13	61.04	2.927
13-NH		n.d.
13-iBu-1		2.435
	58.81	2.460
13-iBu-2	28.98	1.624
13-iBu-3	20.66	0.903
13-iBu-3	20.66	0.896
14	61.28	-
14-OH		n.d.
14-Me	26.44	1.302
15	174.64	-
16	-	-
16 N-Me	28.98	3.190
17 *α*	59.71	4.750
β	32.15	1.678
β-Me	14.15	0.208
γ		0.814
	23.70	1.207
δ	10.17	0.724
18 CO	168.46	-
19 NH		8.242
20 *α*	52.74	4.927
β		2.646
	37.96	2.843
γ	122.4	-
δ	129.59	7.358
ε	121.57	-
φ	147.54	-
φ-OH		n.d. br.

21 CO	170.72	-
22 NH		8.451
23 α	59.99	4.598
β	71.16	-
β-OH		5.120
γ	25.13	1.092
γ'	28.09	1.191
24 CO	170.72	-

Example 17: Characterization of the compound of the formula (VI)



MW = 916.00

Empirical formula: $C_{48}H_{68}Cl_2N_4O_9$

Table 5: NMR chemical shifts of the compound of the formula (VI); c = 3 mg/ml in d_6-DMSO at 300 K.

Position	δ (^{13}C)	δ (^{1}H)
1	127.00	7.267
2 (2C)	127.72	7.330
3 (2C)	125.89	7.564
4	139.88	-
5	78.79	5.900

6	41.82	2.660
6-Me	9.53	0.968
7	138.25	6.186
8	124.61	6.383
9	128.82	6.123
10	133.28	-
10-Me	10.67	1.700
11	83.89	3.626
11-OMe	54.09	3.097
12		2.125
	30.47	1.463
13	57.95	2.631
14	61.27	-
14-Me	14.83	1.436
15	169.25	-
16	-	-
16 N-Me	29.36	2.979
17 *α*	59.06	4.467
β	30.89	1.701
β-Me	14.26	0.274
γ		0.864
	24.13	1.217
δ	9.97	0.773
18 CO	168.55	-
19 NH		8.104
20 *α*	52.37	4.758
β		2.555
	37.10	2.887
γ	136.04	-
δ	130.32	7.506
ε	127.34	-
φ	148.96	-

φ-1	72.94	3.952
φ-2	48.79	2.864
NH		n.d. br.
iBu-1	57.13	2.379
iBu-2	27.94	1.662
iBu-3	20.52	0.872
iBu-3	20.52	0.852
21 CO	170.21	-
22 NH		8.612
23 α	59.14	4.680
β	71.81	-
β-OH		5.161
γ	24.38	1.042
γ´	28.13	1.149
24 CO	170.56	-

Example 18: Determination of the antifungal activity against *Candida albicans*

A stock solution of 1000 µg/ml of active substance [for example the compound of the formula (II) or the compound of the formula (IV)] in methanol was prepared. The test strain (*Candida albicans* FH 2173) was stored at -80°C. The inoculum was prepared from a fresh, liquid preculture. The preculture was prepared from a bead of the material stored at -80°C and 30 ml of nutrient medium (Sabourad dextrose broth, Difco) and incubated at 37°C and 180 revolutions per minute for 24 hours. The inoculum is to be adjusted such that after the inoculation of the test container the necessary number of colony-forming units is achieved. For this, the inoculum was adjusted to a value of 10^7 CFU/ml (CFU: colony forming units) by means of a photometer at a wavelength of 590 nm. After the adjustment of the inoculum, the suspension was diluted with nutrient solution (Mueller Hinton broth, Difco) 1:100. The microtiter plate was inoculated within 15 minutes of preparation of the inoculum. The exact colony count was determined by means of surface culture. Using the stock solution of the active substance and the nutrient medium (Mueller Hinton broth, Difco), a dilution series was prepared beforehand on the microtiter

plate. The active substance was present in a volume of 20 µl and was treated with 20 µl of inoculum such that a total test volume of 40 µl was obtained. The inoculated microtiter plates were subsequently sealed with a lid and incubated at 37°C in 5% CO_2 and 95% atmospheric humidity for 20 hours. For each test, an active substance-free control, a sterile control and, as reference substances, ciprofloxacin and nystatin were co-tested on a 384 well microtiter plate. The microtiter plates were read with the aid of a photometer at a wavelength of 590 nm by measurement of the absorption. The IC_{50} values were subsequently calculated from the values of the dilution series according to a standard process as the concentration of the active substance which is necessary in order to inhibit the growth of the test organism *Candida albicans* by 50%.

BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONALES FORMBLATT

Sanofi-Aventis
Natural Products Science
Industriepark Höchst H 811
65926 Frankfurt/Main

EMPFANGSBESTÄTIGUNG BEI ERSTHINTERLEGUNG, ausgestellt gemäß Regel 7.1 von der unten angegebenen INTERNATIONALEN HINTERLEGUNGSSTELLE

I. KENNZEICHNUNG DES MIKROORGANISMUS	
Vom HINTERLEGER zugeteiltes Bezugszeichen: ST 201196	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM 18870

II. WISSENSCHAFTLICHE BESCHREIBUNG UND/ODER VORGESCHLAGENE TAXONOMISCHE BEZEICHNUNG

Mit dem unter I. bezeichneten Mikroorganismus wurde

( ) eine wissenschaftliche Beschreibung
(x) eine vorgeschlagene taxonomische Bezeichnung

eingereicht.
(Zutreffendes ankreuzen).

III. EINGANG UND ANNAHME

Diese internationale Hinterlegungsstelle nimmt den unter I bezeichneten Mikroorganismus an, der bei ihr am 2006-12-08 (Datum der Erst-hinterlegung)[1] eingegangen ist.

IV. EINGANG DES ANTRAGS AUF UMWANDLUNG

Der unter I bezeichnete Mikroorganismus ist bei dieser Internationalen Hinterlegungsstelle am eingegangen (Datum der Erst-hinterlegung) und ein Antrag auf Umwandlung dieser Ersthinterlegung in eine Hinterlegung gemäß Budapester Vertrag ist am eingegangen (Datum des Eingangs des Antrags auf Umwandlung).

V. INTERNATIONALE HINTERLEGUNGSSTELLE

Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Inhoffenstr. 7 B D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: V. Wecks Datum: 2006-12-21

[1] Falls Regel 6.4 Buchstabe d zutrifft, ist dies der Zeitpunkt, zu dem der Status einer internationalen Hinterlegungsstelle erworben worden ist.

Formblatt DSMZ-BP/4 (einzige Seite) 08/2006

BUDAPESTER VERTRAG ÜBER DIE INTERNATIONALE ANERKENNUNG DER HINTERLEGUNG VON MIKROORGANISMEN FÜR DIE ZWECKE VON PATENTVERFAHREN

DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONALES FORMBLATT

Sanofi-Aventis
Natural Products Science
Industriepark Höchst H 811
65926 Frankfurt/Main

LEBENSFÄHIGKEITSBESCHEINIGUNG
ausgestellt gemäß Regel 10.2 von der unten angegebenen INTERNATIONALEN HINTERLEGUNGSSTELLE

I. HINTERLEGER	II. KENNZEICHNUNG DES MIKROORGANISMUS
Name: Sanofi-Aventis Natural Products Science Anschrift: Industriepark Höchst H 811 65926 Frankfurt/Main	Von der INTERNATIONALEN HINTERLEGUNGSSTELLE zugeteilte EINGANGSNUMMER: DSM 18870 Datum der Hinterlegung oder Weiterleitung[1]: 2006-12-08
III. LEBENSFÄHIGKEITSBESCHEINIGUNG	
Die Lebensfähigkeit des unter II genannten Mikroorganismus ist am 2006-12-08 [2] geprüft worden. Zu diesem Zeitpunkt war der Mikroorganismus (x)[3] lebensfähig ( )[3] nicht mehr lebensfähig	
IV. BEDINGUNGEN, UNTER DENEN DIE LEBENSFÄHIGKEITSPRÜFUNG DURCHGEFÜHRT WORDEN IST[4]	
V. INTERNATIONALE HINTERLEGUNGSSTELLE	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Anschrift: Inhoffenstr. 7 B D-38124 Braunschweig	Unterschrift(en) der zur Vertretung der internationalen Hinterlegungsstelle befugten Person(en) oder des (der) von ihr ermächtigten Bediensteten: V. Weiko Datum: 2006-12-21

[1] Angabe des Datums der Ersthinterlegung. Wenn eine erneute Hinterlegung oder eine Weiterleitung vorgenommen worden ist, Angabe des Datums der jeweils letzten erneuten Hinterlegung oder Weiterleitung.
[2] In den in Regel 10.2 Buchstabe a Ziffer ii und iii vorgesehenen Fällen Angabe der letzten Lebensfähigkeitsprüfung.
[3] Zutreffendes ankreuzen.
[4] Ausfüllen, wenn die Angaben beantragt worden sind und wenn die Ergebnisse der Prüfung negativ waren.

Formblatt DSMZ-BP/9 (einzige Seite) 08/2006

BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH

INTERNATIONAL FORM

Sanofi-Aventis
Natural Products Science
Industriepark Höchst H 811
65926 Frankfurt/Main

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: ST 201196	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 18870
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: ( ) a scientific description (x) a proposed taxonomic designation (Mark with a cross where applicable).	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2006-12-08 (Date of the original deposit)[1].	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B, D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2006-12-21

[1] Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

Form DSMZ-BP/4 (sole page) 08/2006

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

DSMZ
Deutsche
Sammlung von
Mikroorganismen
und Zellkulturen GmbH

INTERNATIONAL FORM

Sanofi-Aventis
Natural Products Science
Industriepark Höchst H 811
65926 Frankfurt/Main

VIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Sanofi-Aventis Natural Products Science Address: Industriepark Höchst H 811 65926 Frankfurt/Main	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 18870 Date of the deposit or the transfer[1]: 2006-12-08

III. VIABILITY STATEMENT

The viability of the microorganism identified under II above was tested on 2006-12-08 [2].
On that date, the said microorganism was

(x)[3] viable

( )[3] no longer viable

IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED[4]

V. INTERNATIONAL DEPOSITARY AUTHORITY

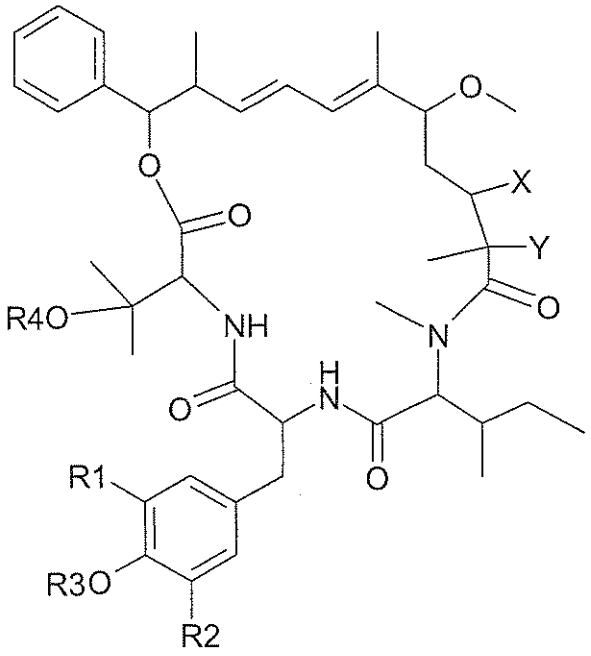
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Inhoffenstr. 7 B D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weiss Date: 2006-12-21

1 Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
2 In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
3 Mark with a cross the applicable box.
4 Fill in if the information has been requested and if the results of the test were negative.

Form DSMZ-BP/9 (sole page) 08/2006

Patent claims

1. A compound of the formula (I),



(I)

wherein

X and Y independently of one another are OH, O-(C_1-C_6)-alkyl, NH_2 or NH-(C_1-C_6)-alkyl, or X and Y together form a group -O-,

R1 and R2 independently of one another are Cl or H,

R3 is H, (C_1-C_6)-alkyl, C(=O)-(C_1-C_6)-alkyl or (C_1-C_6)-alkylene-NH-(C_1-C_6)-alkyl, and

R4 is H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

or a physiologically tolerable salt of a compound of the formula (I).

2. A compound of the formula (I) as claimed in claim 1, wherein X and Y together form a group -O-.

3. A compound of the formula (I) as claimed in one of claims 1 to 2, where R3 and R4 independently of one another are H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl.

4. A compound of the formula (I) as claimed in one of claims 1 to 3, wherein X and Y together form a group -O-, R1 and R2 independently of one another are Cl or H, and R3 and R4 independently of one another are H, $(C_1$-$C_6)$-alkyl or $C(=O)$-$(C_1$-$C_6)$-alkyl.

5. A compound of the formula (I) as claimed in one of claims 1 to 4, where R1 and R2 are equal to Cl.

6. A compound of the formula (I) as claimed in one of claims 1 to 4, where R1 is equal to Cl and R2 is equal to H.

7. A compound of the formula (I) as claimed in one of claims 1 to 6, where R3 and R4 are equal to H.

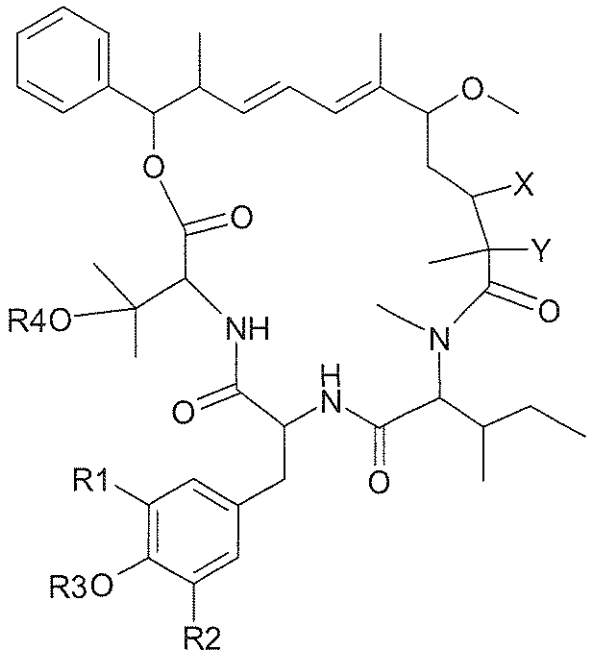
8. A compound of the formula (I) as claimed in one of claims 1 to 7, where R1, R2, R3 and R4 are equal to H.

9. The use of a compound of the formula (I) or of a physiologically tolerable salt thereof as claimed in one of claims 1 to 8 for the preparation of a medicament.

10. The use of a compound of the formula (I) or of a physiologically tolerable salt thereof as claimed in one of claims 1 to 8 for the preparation of a medicament for the treatment and/or prophylaxis of fungal disorders.

11. A medicament containing at least one compound of the formula (I) as claimed in one of claims 1 to 8.

12. A process for the preparation of a compound of the formula (I),



(I),

wherein

X and Y independently of one another are OH, O-(C_1-C_6)-alkyl, NH_2 or NH-(C_1-C_6)-alkyl, or X and Y together form a group -O-,

R1 and R2 independently of one another are Cl or H,

R3 is H, (C_1-C_6)-alkyl, C(=O)-(C_1-C_6)-alkyl or (C_1-C_6)-alkylene-NH-(C_1-C_6)-alkyl, and

R4 is H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl,

or a physiologically tolerable salt of a compound of the formula (I),

which comprises

1. fermenting the strain ST 201196 (DSM 18870) or one of its variants and/or mutants under suitable conditions in a culture medium which contains a Cl source, until one or more of the compounds of the formula (I) accumulates in the culture medium and
2. isolating a compound of the formula (I) from the culture medium, and
3. optionally derivatizing the compound of the formula (I) and/or converting it into a physiologically tolerable salt.

13. The process as claimed in claim 12, where in the compound of the formula (I) X and Y together form a group -O-, R1 and R2 independently of one another are Cl

or H, and R3 and R4 independently of one another are H, (C_1-C_6)-alkyl or C(=O)-(C_1-C_6)-alkyl.

14. The microorganism strain ST 201196 (DSM 18870).