(11) Application No. AU 2003210205 B2

(19) AUSTRALIAN PATENT OFFICE

(54) Title

Phenalenone derivatives, method for the production thereof and use of the same

(51)[6] International Patent Classification(s)

C07D 307/92 (2006.01) 20060101ALI20051220BMJP
A61K 31/122 (2006.01) A61P 31/04 20060101ALI20051220BMJP
A61K 31/343 (2006.01) A61P 31/10 20060101ALI20051220BMJP
A61P 29/00 (2006.01) A61P 35/00 20060101ALI20051220BMJP
A61P 31/04 (2006.01) A61P 39/06 20060101ALI20051220BMJP
A61P 31/10 (2006.01) A61P 43/00 20060101ALI20051220BMJP
A61P 35/00 (2006.01) C07C 45/64 20060101ALI20051220BMJP
A61P 39/06 (2006.01) C07C 45/79 20060101ALI20051220BMJP
A61P 43/00 (2006.01) C07C 49/755 20060101ALI20051220BMJP
C07C 45/64 (2006.01) C07D 307/77 20060101ALI20051220BMJP
C07C 45/79 (2006.01) C09K 15/08 20060101ALI20051220BMJP
C07C 49/755 (2006.01) C12N 1/14 20060101ALI20051220BMJP
C07D 307/77 (2006.01) C12P 7/22 20060101ALI20051220BMJP
C09K 15/08 (2006.01) C12P 15/00 20060101ALI20051220BMJP
C12N 1/14 (2006.01) C12P 17/04 20060101ALI20051220BMJP
C12P 7/22 (2006.01) C12R 1/80 20060101ALI20051008BMEP
C12P 15/00 (2006.01) C07D 307/92 20060101AFI20070104BHAU
C12P 17/04 (2006.01) A61K 31/122 20060101ALI20051220BMJP
C12R 1/80 (2006.01) A61K 31/343 20060101ALI20051220BMJP
C07D 307/92 (2006.01) A61P 29/00 20060101ALI20051220BMJP
20060101AFI20070104BHAU
A61K 31/122 (2006.01) 20060101ALI20051220BMJP
A61K 31/343 (2006.01) 20060101ALI20051220BMJP
A61P 29/00 (2006.01) PCT/EP03/01060

(21) Application No: **2003210205** (22) Application Date: **2003.02.04**

(87) WIPO No: **WO03/068946**

(30) Priority Data

(31) Number	(32) Date	(33) Country
102 06 849.6	**2002.02.18**	**DE**

(43) Publication Date : **2003.09.04**

(43) Publication Journal Date : **2003.10.02**

(71) Applicant(s)

Sanofi-Aventis Deutschland GmbH

(72) Inventor(s)

Li, Ziyu; Vertesy, Laszlo; Toti, Luigi; Kurz, Michael

(74) Agent/Attorney
Watermark Patent & Trademark Attorneys, Level 2 302 Burwood Road, Hawthorn, ACT, 3122

(56) Related Art
Ayer et al., Canadian Journal of Chemistry, 1987, 65, 760-764
Buchi et al., Journal of Organic Chemistry, 1986, 51, 4813-4818
JP 03-124791

(12) NACH DEM VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS (PCT) VERÖFFENTLICHTE INTERNATIONALE ANMELDUNG

(19) Weltorganisation für geistiges Eigentum
Internationales Büro

(43) Internationales Veröffentlichungsdatum
21. August 2003 (21.08.2003)

PCT

(10) Internationale Veröffentlichungsnummer
WO 03/068946 A1

(51) Internationale Patentklassifikation[7]: C12N 1/14, C12R 1/80, C07C 49/755, C07D 307/92

(21) Internationales Aktenzeichen: PCT/EP03/01060

(22) Internationales Anmeldedatum:
4. Februar 2003 (04.02.2003)

(25) Einreichungssprache: Deutsch

(26) Veröffentlichungssprache: Deutsch

(30) Angaben zur Priorität:
102 06 849.6 18. Februar 2002 (18.02.2002) DE

(71) Anmelder: **AVENTIS PHARMA DEUTSCHLAND GMBH** [DE/DE]; Brüningstrasse 50, 65929 Frankfurt (DE).

(72) Erfinder: **VERTESY, Laszlo**; Eppenhainer Weg 6, 65817 Eppstein-Vockenhausen (DE). **KURZ, Michael**; Erlenweg 7, 65719 Hofheim (DE). **LI, Ziyu**; Pirazzistr. 41, 63067 Offenbach (DE). **TOTI, Luigi**; Frankfurter Strasse 5, 65239 Hochheim (DE).

(81) Bestimmungsstaaten *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

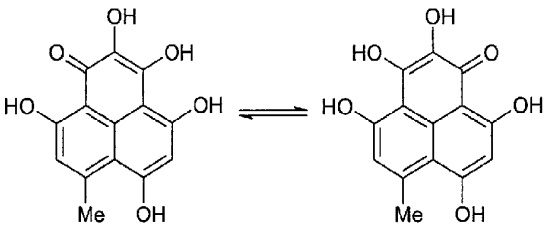
(84) Bestimmungsstaaten *(regional)*: ARIPO-Patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), eurasisches Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europäisches Patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI-Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Veröffentlicht:
— *mit internationalem Recherchenbericht*

[Fortsetzung auf der nächsten Seite]

(54) **Title:** PHENALENONE DERIVATIVES, METHOD FOR THE PRODUCTION THEREOF AND USE OF THE SAME

(54) **Bezeichnung:** PHENALENON-DERIVATIVE, VERFAHREN ZUR HERSTELLUNG UND VERWENDUNG DERSELBEN



(II-A) (II-B)

(57) **Abstract:** The invention relates to phenalenone derivatives, to the production of the same, whereby the micro-organism Penicillium herquei Bainer & Sartory, DSM 14142, is fermented and the phenalones formed during said fermentation are derivatized, and to the use of said phenalenone derivatives as medicaments, especially for fighting tumour diseases, bacterial infections and/or mycoses and rheumatic diseases.

(57) **Zusammenfassung:** Die vorliegende Erfindung betrifft Phenalenon-Derivate, deren Herstellung durch Fermentierung des Mikroorganismus Penicillium herquei Bainer & Sartory, DSM 14142, sowie durch Derivatisierung der während der Fermentation gebildeten Phenalenone, und die Verwendung der Phenalenon-Derivative als Arzneimittel, beispielsweise gegen Tumorerkrankungen, bakterielle Infektionen und/oder Mykosen und rheumatischen Erkrankungen.

WO 03/068946 A1

Zur Erklärung der Zweibuchstaben-Codes und der anderen Abkürzungen wird auf die Erklärungen ("Guidance Notes on Codes and Abbreviations") am Anfang jeder regulären Ausgabe der PCT-Gazette verwiesen.

Description

Phenalenone derivatives, processes for preparation and use thereof

The present invention relates to phenalenone derivatives, processes for their preparation, and their use as pharmaceuticals.

Cancer is a disease of humans and animals which usually takes a fatal course and which is caused by the uncontrolled growth of endogenous cells. Cancer is the term for the formation of malignant growths (malignomas), of neoplasms (tumors and carcinomas) or for the malignant degeneration and maturation disorder of white blood cells (leukemia, blood cancer). Cancer or tumor cells are formed by conversion of endogenous cells. The malignancy of the cancer cell is expressed in the autonomy of growth, that is its ability to grow uninhibited in an infiltrating manner and without classification in the constructional plan of the organs and with tissue destruction. A certain sign of malignancy is the formation of tumor-remote deposits (metastases) after hematogenic or lymphogenic spread of tumor cells. Cancer belongs to the most frequent causes of death of man and therefore there is a great need for methods and means for the cure or treatment of malignant degenerations.

The possibility of therapy of malignant tumors includes, in addition to the – if possible radical – surgical removal of the tumor, radiological therapy using X-rays, α-, ß-, γ-rays, immunotherapy and chemotherapy. Immunotherapy can at present only be used to a restricted extent. The chemotherapy of tumors is understood as meaning the administration of cell toxins (cytostatics) for the treatment of tumors and of remaining tumor cells after local surgical treatment or irradiation. These substances specifically intervene in certain processes of cell division, so that tissue having a high proportion of dividing cells, such as the rapidly growing tumor tissue, react more sensitively. Alkylating compounds, such as, for example, cyclophosphamide, (The Merck Index, 12th ed. page 463), antimetabolites, such as methotrexate (The Merck Index, 12th ed. page 1025), alkaloids, such as vincristine (The Merck Index, 12th ed. page 1704) and antibiotics, such as daunomycin (The Merck Index, 12th ed. page

479) and adriamycin (The Merck Index, 12th ed. pages 581-582) are used. However, all these agents have great disadvantages on account of massive side effects, so that the death of the sick person is only delayed, but not averted. Moreover, in degenerated (cancer) cells, resistance to the agents used occurs, the present medicaments then no longer act cytostatically, but toxically on account of the side effects. Moreover, it has been shown that a combined or sequential administration of cytostatics excels the activity of an individual cytostatic (monotherapy) and it is thereby possible that the considerable side effects are not additive in polychemotherapy. For all these reasons, novel chemotherapeutics are urgently needed and therefore sought worldwide.

Natural substances having a phenalenone parent structure have already been described. Phenalene is a fused, incompletely aromatic ring system, which decomposes in air. Phenalenone is its oxidation product having a carbonyl group in the 1-position.

The patent application WO 99/60992 generically describes phenalenones, which in all positions apart from C1 can be substituted by hydrogen or C_1-C_4-alkyl, preferably methyl, or C_1-C_4-alkoxy, preferably methoxy, for use as hair colorants.

Japanese patent application JP 60199849 describes the phenalenone derivative 2,7,8,9-tetrahydroxy-4-methoxy-5-methylphenalen-1-one, which is active as a PDE inhibitor and can be used for the treatment of arteriosclerosis, bronchial asthma, diabetes and cancers.

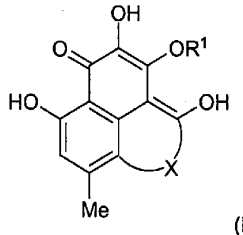
D. A. Frost & G. A. Morrison; J. Chem. Soc., Perkin Trans. 1, 20, 2388-2396, 1973 describe the isolation of norxanthoherquein (2,3,4,7,8,9-hexahydroxy-5-methyl-phenalen-1-one) from Penicillium herquei Bainer & Sartory.

D. H. R. Barton et al. (Tetrahedron, 6, 48, 1959) describe the isolation of atrovenetin from Penicillium atrovenetum and from Penicillium herquei Bainer & Sartory. Atroventin is described as an antioxidant in Y. Ishikawa et al. (J. Am. Oil Chem. Soc. 68, 666-668, 1991), and as a cytostatic having antineoplastic activity in WO 00/45165.

N. Narasimhachi et al. (J. Antibiotics, 25, 155, 1972) and J. Simpson (Chem. Soc. Perkin Trans. 1, 1979, 1233-1238) describe tautomeric forms of the compound desoxyherqueinone (2-O-methylatrovenetin), which are isolable from Penicillium herquei and exhibit antibiotic activity against gram-positive organisms.

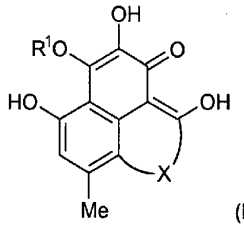
The invention is based on the object of providing alternative phenalenone derivatives which can be used in tumor therapy.

The invention relates to a compound of the formula (I-A)



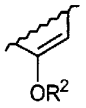
(I-A),

or a compound of the formula (I-B)

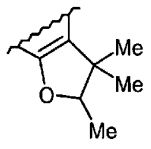


(I-B),

where X is either a group of the formula (I-C)



or of the formula (I-D),



(I-D),

and R^1 and, if present, R^2 simultaneously are

1.0 H, or

2.0 C_1-C_6-alkyl, C_2-C_6-alkenyl or C_2-C_6-alkynyl, in which alkyl, alkenyl and alkynyl are linear or branched and are optionally mono- or disubstituted by:

2.1 –OH,

2.2 =O,

2.3 –O-C_1-C_6-alkyl, in which alkyl is linear or branched,

2.4 –O-C_2-C_6-alkenyl, in which alkenyl is linear or branched,

2.5 –aryl,

2.6 –NH-C_1-C_6-alkyl, in which alkyl is linear or branched,

2.7 –NH-C_2-C_6-alkenyl, in which alkenyl is linear or branched,

2.8 –NH_2 or

2.9 halogen,

in which the substituents 2.1 to 2.9 can also be substituted still further by –CN, –amide or –oxime functions,

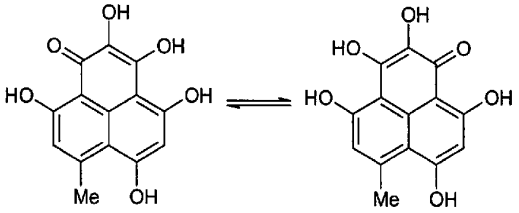
and/or a stereoisomeric form of the compound of the formula (I-A) or of the formula (I-B) and/or mixtures of this form in any ratio, and/or a physiologically tolerable salt of the compound of the formula (I-A) or (I-B).

R^1 and R^2 are preferably H or C_1-C_6-alkyl.

Chiral centers, if not stated otherwise, are present in the R configuration or in the S configuration. The invention relates both to the optically pure compounds and to stereoisomer mixtures, such as enantiomer mixtures and diastereomer mixtures, in any ratio.

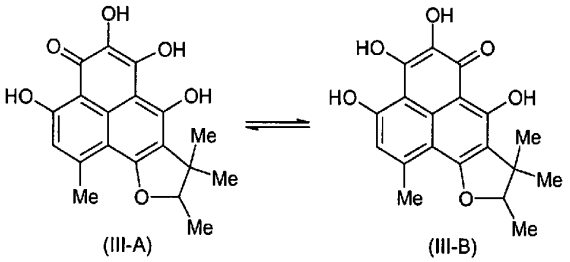
The compounds according to the invention differ from substances known from the literature by the polarity, the chemical structure, the biological activity or by further physical properties.

The strain Penicillium herquei Bainer & Sartory, DSM 14142, forms, on glucose-, starch-, oat flake- or glycerol-containing nutrient solutions, the compounds called penilenone of the formulae (II-A) and (II-B), which are designated below by way of summary as compounds of the formula (II),



(II-A) (II-B)

and atrovenetin of the formulae (III-A) and (III-B), which are designated below by way of summary as compounds of the formula (III),



(III-A) (III-B)

Compounds of the formulae (I-A) and (I-B), for which R^1 is unequal to H, are isomeric forms, which are isolable separately from another, and which can be converted into one another, for example by removing the radical R^1, for which R^1 is equal to H, and subsequently, starting from the other tautomer in each case, derivatizing to give the other isomer of the compound of the formula (I-A) or (I-B) where R^1 is unequal to H.

The compounds of the formulae (I-A) and (I-B) are designated below by way of summary as the compound of the formula (I).

The compounds of the formulae (II-A) and (II-B) are tautomers and cannot be isolated from one another separately under customary conditions (e.g. room temperature). Compounds of the formulae (II-A) and (II-B) are designated below by way of summary as the compound of the formula (II).

The compounds of the formulae (III-A) and (III-B) are likewise tautomers and cannot be isolated from one another separately under customary conditions (e.g. room temperature). Compounds of the formulae (III-A) and (III-B) are designated below by way of summary as the compound of the formula (III)

Compounds of the formulae (I-A) and (I-B), for which R^1 is equal to H, can be selectively derivatized according to the present invention by reacting the hydroxyl groups with alkylating agents in a manner known per se, such as is described, for example, by Jerry March in the book Advanced Organic Chemistry, John Wiley & Sons, 4th Edition, 1992. Alkylating agents are, for example, diazomethane derivatives, preferably trimethylsilyldiazomethane. In order to carry out reactions selectively, it may be advantageous before the reaction to introduce suitable protective groups in a manner known per se. The protective groups are removed after the reaction and the reaction product is then purified.

Until now, no selective alkylation of phenalenones to give the compounds according to the invention has been described. For example, the reaction of desoxyherqueinone with diazomethane leads, according to Suga et al. (Bull. Chem Soc. Jpn., 56, 3661-3666, 1983), to the desoxyherqueinone dimethyl ether or to the isomeric compound ent-atrovenetin trimethyl ether.
Compounds of the formulae (I-A) and (I-B), for which R^1 is equal to H, can be prepared, for example, by ether cleavage of compounds of the formulae (I-A) and (I-B), for which R^1 is unequal to H. Ether cleavages can be carried out by methods known per se, such as is described, for example, by Jerry March in the book Advanced Organic Chemistry, John Wiley & Sons, 4th Edition, 1992.

The invention therefore further relates to a compound of the formula (I), which has the formula (II), or to a pharmacologically tolerable salt of the compound of the formula (II).

The invention therefore furthermore relates to a process for the preparation of a compound of the formula (II), which comprises

1. culturing the microorganism Penicillium herquei Bainer & Sartory, DSM 14142, or one of its variants or mutants in an aqueous nutrient medium,
2. isolating and purifying a compound of the formula (II), and
3. converting the compound of the formula (II), if appropriate, into a pharmacologically tolerable salt.

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

1. culturing the microorganism Penicillium herquei Bainer & Sartory, DSM 14142, or one of its variants or mutants in an aqueous nutrient medium,
2. a) isolating and purifying a compound of the formula (II), or
   b) isolating and purifying the compound of the formula (III),
3. a) derivatizing the compound of the formula (II) to give a compound of the formula (I), or
   b) or derivatizing the compound of the formula (III) to give a compound of the formula (I), and
4. converting the compound of the formula (I), if appropriate, into a pharmacologically tolerable salt.

The strain Penicillium herquei Bainer & Sartory, DSM 14142, forms, on glucose-, starch-, oat flake- or glycerol-containing nutrient solutions, penilenone and the secondary products.

An isolate of Penicillium herquei Bainer & Sartory was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures) GmbH (DSM), Mascheroder Weg 1B, 38124 Brunswick, Germany according to the rules of the Budapest Convention on March 6, 2001 under the following number: DSM 14142.

The fungus Penicillium herquei Bainer & Sartory, DSM 14142 has a gray to luminous green substrate mycelium and very little aerial mycelium. Exudates are not formed on malt medium and dyes are not excreted into the medium. In culture, the strain forms the compact sporangia characteristic of Penicillium, 200-400 x 3.5-4.0 μm, which are rough on the surface. The "metulae" are relatively short, usually 4-6 10-12 x 3.0-5.0 μm and club-shaped. The phialida are arranged in 6-10 "verticilli", 7-10 x 3.0 μm, ampoule-shaped. The conidia are elliptical to "apiculate", 3.5-5.0 x 3.0-3.5 μm, having a smooth cell wall. The conidia are formed in parallel chains, up to 100 μm long.

Said process comprises the culturing of Penicillium herquei Bainer & Sartory, DSM 14142, its mutants and/or variants under aerobic conditions in a culture medium containing at least in each case one carbon and nitrogen source, inorganic salts and optionally trace elements.

The culturing is preferably carried out at a temperature between 20° and 35°C and at a pH between 2 and 9.

Instead of the strain DSM 14142, its mutants and variants can also be employed, insofar as they produce the compounds according to the invention. Such mutants can be produced in a manner known per se by physical means, for example irradiation, such as with ultraviolet or X-rays, or chemical mutagens, such as, for example, ethyl methanesulfonate (EMS); 2-hydroxy-4-methoxybenzophenone (MOB) or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Suitable preferred 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, such as casein, peptones or tryptones, furthermore meat extracts, yeast extracts, ground seeds, for example of corn, wheat, beans, soy, rice or the cotton plant, distillation residues of alcohol production, meat meals or yeast extracts, but also ammonium salts and nitrates, but in particular also synthetically or biosynthetically obtained peptides. Inorganic salts which the nutrient

solution can contain are, for example, chlorides, carbonates, sulfates or phosphates of the alkali metals or alkaline earth metals, iron, zinc, cobalt and manganese.

The formation of the compounds according to the invention proceeds particularly well, for example, in a nutrient solution which contains approximately 0.05 to 5%, preferably 1 to 2%, of malt extract; and 0.05 to 3%, preferably 0.05 to 1%, of yeast extract; 0.2 to 5%, preferably 0.5 to 2%, of glucose; and 0.5 to 3%, preferably 1.5% to 3%, of oat flakes. The details in % are in each case based on the weight of the entire nutrient solution.

In this nutrient solution, Penicillium herquei Bainer & Sartory, DSM 14142, forms a mixture of the compounds according to the invention. Depending on the composition of the nutrient solution, the quantitative amount of one or more of the compounds according to the invention can vary. Moreover, the synthesis of individual compounds can be controlled by the media composition, so that a compound is not produced at all or is produced in an amount below the detection limit of the microorganism.

The culturing of the microorganism is carried out aerobically, that is, for example, submerse with shaking or stirring in shaker flasks or fermenters or on solid medium, optionally with introduction of air or oxygen. It can be carried out in a temperature range from approximately 15 to 30°C, preferably at approximately 20 to 30°C, in particular at 25 to 30°C. The pH range should be between 4 and 10, preferably between 6.5 and 7.5. The microorganism is in general cultured under these conditions over a period of 48 to 720 hours, preferably 72 to 350 hours. The culturing is advantageously carried out in a number of stages, i.e. one or more precultures are first prepared in a liquid nutrient medium, which is then inoculated into the actual production medium, the main culture, for example in the volume ratio 1:10-1:100. The preculture is obtained, for example, by inoculating the mycelium into a nutrient solution and allowing it to grow for approximately 20 to 120 hours, preferably 48 to 72 hours. The mycelium can be obtained, for example, by allowing the strain to grow for approximately 1 to 42 days, preferably 21 to 35 days, on a solid or liquid nutrient medium, for example yeast malt agar, oat flake agar or potato dextrose agar.

The course of fermentation and the screening for mutants and variants which produce the compounds according to the invention can be monitored according to methods known to the person skilled in the art, such as, for example, by testing the biological activity in bioassays or by chromatographic methods such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

The fungus Penicillium herquei Bainer & Sartory, DSM 14142, can form the compounds according to the invention by means of a surface or stand culture on solid nutrient media. Solid nutrient media are prepared by addition of, for example, agar or gelatine to aqueous nutrient media. It is moreover possible to obtain the compounds according to the invention by fermentation of the fungus Penicillium herquei Bainer & Sartory, DSM 14142, in the submerse process, i.e. in aqueous suspension. The compounds according to the invention can occur both in the mycelium and in the culture filtrate, usually the main amount is found in the cell mass. It is therefore expedient to separate the fermentation solution by filtration or centrifugation. The filtrate is extracted using an adsorption resin as a solid phase. The mycelium, but also the surface culture, is expediently extracted with an organic solvent, for example methanol or propan-2-ol.

The extractions can be carried out in a wide pH range, but it is expedient to work in a neutral or weakly acidic medium, preferably between pH 3 and pH 7. The extracts can be concentrated and dried, for example, in vacuo.

The compounds of the formula (II) and of the formula (III) are substances which are unstable, if particular measures are not taken during the isolation and purification process. It has been found that the penilenones can be obtained in very good yields from the cultures of the strain DSM 14142 if 1) work is carried out under reducing conditions during the isolation and purification process, e.g. always in the presence of ascorbic acid, 2) the isolation is carried out in acidic medium at a pH of less than 7, preferably in the pH range from 2 to 5, 3) during the purification step only mild agents are used, such as, for example, adsorption resins as chromatographic supports, and 4) the presence of amines is excluded during the entire process.

A suitable method for the isolation of the compounds according to the invention is solution distribution in a manner known per se. Another method of purification is chromatography on adsorption resins such as, for example, on Diaion® HP-20 (Mitsubishi Casei Corp., Tokyo), on Amberlite® XAD 7 (Rohm and Haas, USA), on Amberchrom® CG, (Toso Haas, Philadelphia, USA) or on the like. Moreover suitable under the circumstances indicated are numerous reverse-phase supports, e.g. RP_8 and RP_{18}, such as have generally become known, for example, in the context of high-pressure liquid chromatography (HPLC). A further purification possibility under the circumstances indicated is the use of "normal-phase" chromatographic supports, such as, for example, silica gel or Al_2O_3 or others in a manner known per se. An alternative isolation process is the use of molecular sieves, such as, for example, Fractogel® TSK HW-40, Sephadex® G-25 and others, in a manner known per se.

It is moreover possible to obtain the compounds of the formula (I) according to the invention after enrichment by crystallization, where, for example, organic solvents and their mixtures, anhydrous or with addition of water, can be used.

An additional process for the isolation and purification of the compounds according to the invention consists in the use of anion exchangers, preferably in the pH range from 4 to 7, and cation exchangers, preferably in the pH range from 2 to 5. Particularly suitable for this is the use of buffer solutions to which portions of organic solvents have been added.

However, it is also possible to isolate and/or to purify the compounds according to the invention by sublimation.

A particularly advantageous purification method for the isolation of the compounds according to the invention is crystallization, which is carried out in a manner known per se.

The compounds of the formula (I) according to the invention can be converted into the corresponding pharmacologically tolerable salts according to methods known to the person skilled in the art. Pharmacologically tolerable salts of the compounds according to the invention are understood as meaning both inorganic and organic

salts, such as are described in Remingtons Pharmaceutical Sciences (17th edition, page 1418 [1985]). Possible salts are in particular alkali metal salts, ammonium salts, alkaline earth metal salts, salts with physiologically tolerable amines and salts with inorganic or organic acids such as, for example, HCl, HBr, H_2SO_4, maleic acid, fumaric acid. Possible salts, however, are also complexes with metal ions, such as, for example, with calcium, magnesium, zinc, iron or others. The compounds of the formula (I) have a marked tendency to bind ions, preferably cations, in complex form.

It has surprisingly been found that as the compounds of the formula (I) according to the invention have strong cytostatic effects, they are therefore suitable for the therapy and/or prophylaxis of diseases which are caused by uncontrolled growth of tissue or cells, or oncoses. It is particularly worthy of note that the compounds according to the invention have no cross-resistance at all with conventional cytostatics.

It has been found that the compounds of the formula (I) inhibit protein kinases. The kinases belong to the transferases, which transfer phosphate radicals from adenosine triphosphate to other substrates. Proteins and enzymes are phosphorylated and modified in their activity by the protein kinases, usually on serine, threonine or tyrosine side chains, which has been recognized as a widespread regulation principle in metabolism and signal transduction. In the case of the cancers, the diseased tissue proliferates in an uncontrolled manner; an intervention into the regulation of the kinase-controlled proliferation is therefore desirable. A number of kinases are involved in the cascade of cell proliferation. Several of these kinases are inhibited by the compounds according to the invention.

Moreover to be emphasized is an antimicrobial inhibitory action of the compounds of the formula (I) according to the invention on bacteria, such as, for example, Staphylococcus aureus, Streptomyces murinus and against fungi, such as Aspergillus niger, which can cause stubborn, life-threatening infectious diseases. The antimicrobial activity can be demonstrated, for example, by "agar diffusion tests". Thus penilenone on an agar plate containing Streptomyces murinus culture in a solution of 1 mg per ml causes an inhibition halo of 11 mm and in a solution of 0.1 mg per ml an inhibition halo of 8 mm. The compounds of the formula (I)

according to the invention are therefore likewise suitable for the therapy and/or prophylaxis of bacterial infections and/or fungal diseases.

The compounds of the formula (I) can also be used as antioxidants. Antioxidants (oxidation inhibitors) are organic compounds which inhibit or prevent undesired changes in the substances to be protected caused by the effects of oxygen. Antioxidants are needed, for example, in plastics for protection against ageing, in fats for protection against rancidity, in oils against resinification, in aromatic substances against odor impairment, in foodstuffs, and in pharmaceuticals. The action of the antioxidants usually consists in acting as radical scavengers for the free radicals occurring in the oxidation. The antioxidative action of atrovenetin (compound of the formula (III)) has already been described by Y. Ishikawa et al. J. Am. Oil Chem. Soc. 68, 666-668, 1991. Microbial antioxidants, however, are often too weak or not highly tolerable in their action. There is therefore a great need for novel, efficacious and tolerable antioxidants. The compounds of the formula (I) are highly active antioxidants, which considerably excel atrovenetin in its action: While atrovenetin in solution and in solid form reacts only slowly with atmospheric oxygen (for example in hours), penilenone of the formula (II), for example, combines with oxygen within seconds or in a few minutes. This increased affinity of penilenone for oxygen, however, is decidedly advantageous for very oxidation-sensitive substances.

Another chemical peculiarity of the compounds according to the invention is the ability for complex formation with polyvalent, preferably 2- and 3-valent cations, such as, for example, with Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}. The complex formation ability can be advantageous for the production of pharmaceuticals, thus, for example, inhibitors of matrix metalloproteases (MMPs) have become known which are able to bind the zinc of these enzymes. However, other possibilities of use are also conceivable in diseases whose expression is manifested in an abnormal metal ion concentration in the body. It is also possible to make the complex formation ability of the compounds according to the invention utilizable outside medicine, for example in water technology, in bodycare compositions, and in polymerization technology (Ullmans Enzyklopädie der Technischen Chemie [Ullman's encyclopedia of industrial chemistry], 5th edition, A 10, 95-100, 1985-1995).

The compounds of the formula (I) according to the invention can likewise act in the treatment of rheumatic diseases, for example rheumatoid arthritis. The active principle in the reduction of oxidative stress in rheumatoid arthritis by free radical scavengers or antioxidants has been described, for example, by Ostrakhovitch and Afanas (Biochemical Pharmacology, 2001, 743-746).

The present invention accordingly also relates to the use of the compounds of the formula (I) according to the invention as pharmaceuticals, in particular for the treatment and/or prophylaxis of oncoses, bacterial infections, mycoses, rheumatic diseases and of diseases which can be treated by the inhibition of matrix metalloproteases.

In addition, the present invention relates to a pharmaceutical containing at least one of the compounds according to the invention.

Said pharmaceutical is produced by mixing at least one compound of the formula (I) with one or more physiologically acceptable excipient and bringing it into a suitable administration form.

The pharmaceuticals according to the invention can be administered enterally (orally), parenterally (intramuscularly or intravenously), rectally or locally (topically). They can be administered in the form of solutions, powders (tablets, capsules including microcapsules), ointments (creams or gel), or suppositories. Possible physiologically acceptable excipients for formulations of this type are the pharmaceutically customary liquid or solid fillers and extenders, solvents, emulsifiers, lubricants, flavor corrigents, colorants and/or buffer substances. As an expedient dose, 0.1 - 1000, preferably 0.2 - 100, mg/kg of body weight are administered. They are expediently administered in dose units which contain at least the efficacious daily amount of the compounds according to the invention, e.g. 30 - 3000, preferably 50 – 1000, mg.

The following examples are intended to serve to illustrate the invention in greater detail, without wishing to restrict the breadth of the invention in any manner.

Example 1 Preparation of a glycerol culture of Penicillium herquei Bainer & Sartory, DSM 14142

30 ml of nutrient solution (malt extract 2.0%, yeast extract 0.2%, glucose 1.0%, $(NH_4)_2 HPO_4$ 0.05%, pH 6.0) in a sterile 100 ml Erlenmeyer flask are inoculated with the strain Penicillium herquei Bainer & Sartory, DSM 14142, and incubated on a rotating shaker for 6 days at 25°C and 140 rpm. 1.5 ml of this culture are then diluted with 2.5 ml of 80% strength glycerol and stored at -135°C.

Example 2 Preparation of a preculture in an Erlenmeyer flask of Penicillium herquei Bainer & Sartory, DSM 14142

100 ml of nutrient solution (malt extract 2.0%, yeast extract 0.2%, glucose 1.0%, $(NH_4)_2 HPO_4$ 0.05%, pH 6.0) in a sterile 300 ml Erlenmeyer flask are inoculated with the strain Penicillium herquei Bainer & Sartory, DSM 14142, and incubated on a rotating shaker for 4 days at 25°C and 140 rpm. 2 ml of this preculture are then inoculated for the preparation of the main cultures.

Example 3 Preparation of a main culture of Penicillium herquei Bainer & Sartory, DSM 14142.

A sterile 300 ml Erlenmeyer flask containing 100 ml of the following nutrient solution malt extract 2.0%, yeast extract 0.2%, glucose 1.0% $(NH_4)_2 HPO_4$ 0.05%, pH 6 is inoculated with a culture grown in a slant tube (same nutrient solution, but with 2% agar) or with 2 ml of a preculture (see example 2) and incubated at 140 rpm and 25°C on a shaker. The maximum production of one or more compounds of the penilenone according to the invention is achieved after about 144 hours. For the inoculation of 10 to 200 l fermenters, a 96 to 144 hour-old submerse culture (inoculation amount about 10%) of the same nutrient solution suffices. The conditions for this fermenter are:

Temperature 25°C

Stirrer speed: 200 rpm

Aeration 15 l min^{-1}

Foam formation can be suppressed by repeated addition of ethanolic polyol solution. The production maximum is achieved after about 96 to 144 hours.

Example 4: Isolation of the compounds (II) and (III)

5 liters of culture solution, obtained according to example 3, were centrifuged and the cell mass (0.5 liter) was extracted with 2 liters of methanol, to which 0.1% ascorbic acid has been added. The clear-filtered methanolic phase was concentrated to 1 l in vacuo and applied to a column of 1 liter capacity, packed with adsorption resin MCI Gel® CHP20P. Column dimensions: width x height: 7 cm x 27 cm. Elution was carried out using a solvent gradient of 10% propan-2-ol to 90% propan-2-ol in 0.1% aqueous ascorbic acid solution. The column effluent (140 ml/minute) has been collected in fractions of 250 ml each. The penilenone-containing fractions 23 to 26 (mixture of the compounds of the formulae (II-A) and (II-B), in summary called compounds of the formulae (II)) and the atrovenetin-containing fractions 43 to 51 (mixture of the compounds of the formulae (III-A) and (III-B), in summary called compounds of the formulae (III)), which were checked by HPLC analyses, have been collected and concentrated in vacuo. The combined fractions were in each case concentrated in vacuo and stored cold. Penilenone (260 mg of compound of the formula (II)) crystallized from fractions 23 to 26, fractions 43 to 51 afforded 1.2 g of atrovenetin (compound of the formula (III)). The crystallizates were filtered off under an argon protective gas atmosphere and stored cold with exclusion of oxygen.

Example 5: Isolation and purification by HPLC.

Column:	Superspher 100 RP-18e®, 250-4, with precolumn,
Mobile phase:	2 minutes: 5% acetonitrile in 0.1% phosphoric acid,
	18 minutes: gradient of 5% to 100% acetonitrile in 0.1% phosphoric acid, then
	100% acetonitrile constant.
Flow rate:	1 ml per minute,

Detection by UV absorption at 210 nm.

A retention time of 13.5 minutes was found for the compound of the formula (II), and 20.5 minutes for the compound of the formula (III).

Example 6: Characterization of the compound of the formula (II).

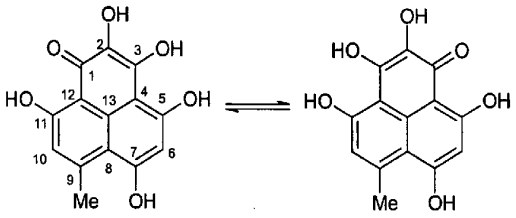
The physicochemical and spectroscopic properties of penilenone can be summarized as follows:

Appearance:
Yellow crystalline substance, soluble in medium polar and polar organic solvents, not very soluble in water. The melting point is not determinable because of decomposition. Stable in mildly acidic medium under reducing conditions. Under the influence of oxygen, penilenone turns green in neutral medium or in the presence of amines.

Empirical formula: $C_{14}H_{10}O_6$
Molecular weight: 274.23
By means of ESI+ mass spectrometry, a molecular ion 275.2 $[M + H]^+$ was found, and under ESI (negative) conditions 273 $[M - H]^-$ or 271 $[M - 3H]^-$ was measured.

UV maxima: 215 nm, 248 (sh) nm, 275 (sh), 389 nm.

Table 1: NMR data - ^{1}H and ^{13}C chemical shifts of penilenone of the formula (II) in DMSO-d_6 at 300K (in ppm; numbering for the purpose of the NMR analysis does not correspond to the IUPAC nomenclature).



(IIA) (IIB)

	^{1}H	^{13}C
1	-	a)
2	-	131.12
3	-	a)
4	-	102.22
5	-	~ 170.2 (broad)
6	6.44	99.72
7	-	165.89
7-OH	11.63	-
8	-	110.71
9	-	145.42
9-Me	2.81	25.13
10	6.81	116.48
11	-	~ 163.0 (broad)
12	-	105.04
13	-	124.86

a) For C3 and C5, no signal is observed in the ^{13}C spectrum.

Example 7: Obtainment of the penilenone monomethyl ether derivatives of the formulae (IV-A) and (IV-B).

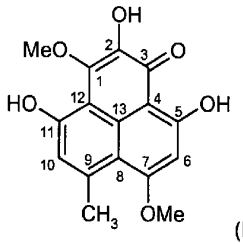
40 mg of compound of the formula (II) (penilenone, isolated corresponding to Example 4) were dissolved in 30 ml of tetrahydrofuran and treated with 0.5 ml of 2.0 M (trimethylsilyl)diazomethane, dissolved in hexane [Aldrich, cat. no. 36,283-2]. After one hour, the reaction was ended by addition of water and the solvent was distilled off in vacuo. The reaction product is then separated on a Nucleosil HD® column (21 mm x 250 mm). The eluent used was a gradient of 10% to 99% acetonitrile in 0.1% acetic acid. The column flow, 20 ml per minute, was collected in fractions. The fractions which contained the methylation product were in each case combined, concentrated in vacuo and crystallized.

6 mg of penilenone dimethyl ether of the formula (IV-A), empirical formula: $C_{16}H_{14}O_6$, molecular weight: 302.29, and 1 mg of penilenone dimethyl ether of the formula (IV-B), empirical formula: $C_{16}H_{14}O_6$, molecular weight: 302.29, were obtained.

Penilenone dimethyl ether of the formula (IV-A):

UV maxima: 216 nm, 242 nm, 280 nm (sh), 387 nm.

Table 2: NMR data - ^1H- and ^{13}C-chemical shifts δ of penilenone dimethyl ether of the formula (IV-A) in DMSO-d_6 at 300K (in ppm; numbering for the purpose of the NMR analysis does not correspond to the IUPAC nomenclature).



(IV-A)

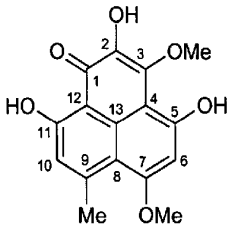
	^{1}H	^{13}C
1	-	148.25
1-Ome	4.14	60.97
2	-	137.12
2-OH	~ 9.3 (broad)	-
3	-	174.13[a)]
4	-	104.79
5	-	174.28[a)]
5-OH	17.29	-
6	6.57	96.82
7	-	168.03
7-Ome	4.04	56.45
8	-	111.30
9	-	143.18
9-Me	2.74	25.12
10	6.88	117.65
11	-	159.10
11-OH	~ 10.5 (broad)	-
12	-	105.72
13	-	124.58

a) C3 and C5 cannot be clearly differentiated.

Penilenone dimethyl ether of the formula (IV-B):

UV maxima: 213 nm, 241 nm and 390 nm.

Table 2: NMR data - ^{1}H and ^{13}C chemical shifts δ of penilenone dimethyl ether of the formula (IV-B) in DMSO-d_6 at 300K (in ppm; numbering for the purpose of the NMR analysis does not correspond to the IUPAC nomenclature).



(IV-B)

	^{1}H	^{13}C
1	-	176.16
2	-	136.10
2-OH	broad	-
3	-	149.54
3-OMe	4.18	61.07
4	-	101.12
5	-	162.49
5-OH	broad	-
6	6.66	97.44
7	-	163.72
7-OMe	4.00	56.17
8	-	110.63
9	-	149.31
9-Me	2.78	25.86
10	6.87	117.65
11	-	169.56
11-OH	16.74	-
12	-	108.22
13	-	124.51

Example 8: Obtainment of the atrovenetin derivatives (V-A) and (V-B).

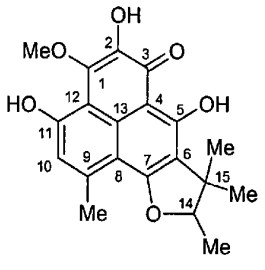
100 mg of compound of the formula (III) (atrovenetin, prepared according to example 4) were dissolved in 5 ml of tetrahydrofuran and treated with 1 ml of 2.0 M (trimethylsilyl)diazomethane, dissolved in hexane [Aldrich, cat. no. 36,283-2]. After 15 minutes, the reaction was ended by addition of water and the solvent was distilled off in vacuo. The reaction product is then separated on a Nucleosil AB® column (21 mm x 250 mm). The eluent used was a gradient of 10% to 99% acetonitrile in 0.02% trifluoroacetic acid, which has been adjusted to pH 4.5 with ammonium hydroxide. The column flow, 15 ml per minute, was collected in fractions. The fractions which contained the methylation products were in each case combined, concentrated in vacuo and crystallized.

24 mg of atrovenetin monomethyl ether (V-A), empirical formula: $C_{20}H_{20}O_6$, molecular weight: 356.38, and 10 mg of atrovenetin monomethyl ether (V-B), empirical formula $C_{20}H_{20}O_6$, molecular weight: 356.38 were obtained.

Atrovenetin monomethyl ether (V-A):

UV maxima: 218 nm, 260 nm (sh), 394 nm.

Table 3: NMR data - ^1H and ^{13}C chemical shifts δ of atrovenetin monomethyl ether (V-A) in DMSO-d_6 at 300K (in ppm; numbering for the purpose of the NMR analysis does not correspond to the IUPAC nomenclature).



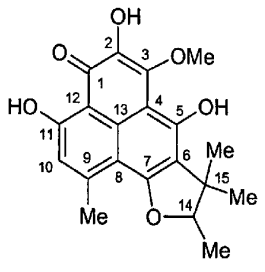
(V-A)

	^{1}H	^{13}C
1	-	147.64
1-OMe	4.16	60.94
2	-	137.35
2-OH	9.26	-
3	-	174.04
4	-	105.81
5	-	170.14
5-OH	17.45	-
6	-	117.94
7	-	166.28
8	-	107.35
9	-	142.91
9-Me	2.76	22.90
10	6.91	116.71
11	-	159.95
11-OH	10.63	-
12	-	105.63
13	-	124.10
14	4.75	91.03
14-Me	1.46	14.39
15	-	42.57
15-Me	1.51	25.31
15-Me'	1.27	20.42

Atrovenetin monomethyl ether (V-B):

UV maxima: 222 nm, 282 nm, 385 nm.

Table 4: NMR data - ^{1}H and ^{13}C chemical shifts δ of atrovenetin monomethyl ether (V-B) in DMSO-d_6 at 300K (in ppm; numbering for the purpose of the NMR analysis does not correspond to the IUPAC nomenclature).



(V-B)

	^{1}H	^{13}C
1	-	173.97
2	-	135.31
2-OH	9.21	-
3	-	149.90
3-OMe	4.27	61.50
4	-	101.74
5	-	158.77
5-OH	10.63	-
6	-	118.96
7	-	162.55
8	-	106.20
9	-	149.30
9-Me	2.78	23.69
10	6.88	117.21
11	-	171.98
11-OH	17.18	-
12	-	108.14
13	-	123.22
14	4.68	90.41
14-Me	1.45	14.25
15	-	43.21
15-Me	1.51	25.13
15-Me'	1.26	20.42

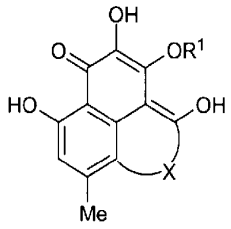
2003210205 01 Jun 2007

Comprises/comprising and grammatical variations thereof when used in this specification are to be taken to specify the presence of stated features, integers, steps or components or groups thereof, but do not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof.

2003210205 01 Jun 2007

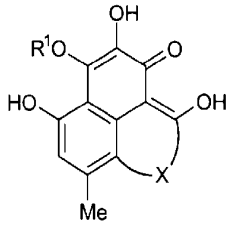
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. An isolated compound of the formula (I-A)



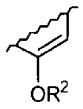
(I-A),

or a compound of the formula (I-B)



(I-B),

where X is a group of the formula (I-C)



and R^1 and, if present, R^2 simultaneously are

1.0 H, or

2.0 C_1-C_6-alkyl, C_2-C_6-alkenyl or C_2-C_6-alkynyl, in which alkyl, alkenyl and alkynyl are linear or branched and are optionally mono- or disubstituted by:

2.1 –OH,

2.2 =O,

2.3 –O-C_1-C_6-alkyl, in which alkyl is linear or branched,

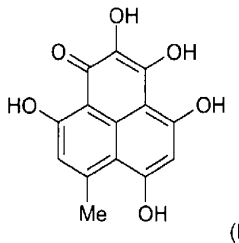
2003210205 01 Jun 2007

2.4–O-C_2-C_6-alkenyl, in which alkenyl is linear or branched,
2.5–aryl,
2.6–NH-C_1-C_6-alkyl, in which alkyl is linear or branched,
2.7–NH-C_2-C_6-alkenyl, in which alkenyl is linear or branched,
2.8–NH_2 or
2.9 halogen,
in which the substituents 2.1 to 2.9 can also be substituted still further by –CN, –amide or –oxime functions,

and/or a stereoisomeric form of the compound of the formula (I-A) or of the formula (I-B) and/or mixtures of this form in any ratio, and/or a physiologically tolerable salt of the compound of the formula (I-A) or (I-B).

2. An isolated compound of the formula (I-A) or (I-B) as claimed in claim 1, where R^1 and R^2 are H or C_1-C_6-alkyl.

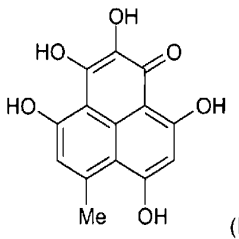
3. An isolated compound of the formula (I-A) or (I-B) as claimed in one or more of claims 1 and 2, characterized in that it has the formula (II-A)



(II-A)

or the formula (II-B)

2003210205 01 Jun 2007



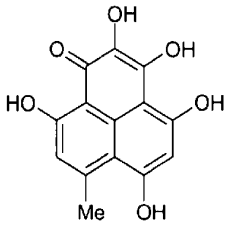
(II-B),

or its physiologically tolerable salts.

4. A process for the preparation of a compound of the formula (I-A) or (I-B) as claimed in one of claims 1 or 2, characterized in that it comprises

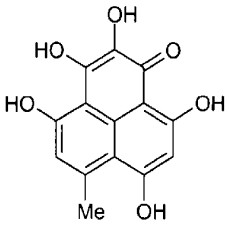
1. culturing the microorganism Penicillium herquei Bainer & Sartory, DSM 14142, or one of its variants or mutants in an aqueous nutrient medium,

2. a) isolating and purifying a compound of the formula (II-A)



(II-A)

or of the formula (II-B)



(II-B),

2003210205 01 Jun 2007

3. derivatizing the compound of the formula (II-A) or (II-B) to give a compound of the formula (I-A) or (I-B),

4. converting the compound of the formula (I-A) or (I-B), if appropriate, into a pharmacologically tolerable salt.

5. A process for the preparation of a compound of the formula (II-A) or (II-B) as claimed in claim 3, characterized in that it comprises

1. culturing the microorganism Penicillium herquei Bainer & Sartory, DSM 14142, or one of its variants or mutants in an aqueous nutrient medium,

2. isolating and purifying a compound of the formula (II-A) or (II-B), and

3. converting the compound of the formula (II-A) or (II-B), if appropriate, into a pharmacologically tolerable salt.

6. The process as claimed in claim 4, the derivatization being carried out by means of an alkylating agent.

7. The process as claimed in claim 6, the alkylating agent being a diazomethane derivative.

8. The use of a compound as claimed in of claims 1 to 3 for the production of a pharmaceutical.

9. The use of a compound as claimed in claim 8 for the production of a pharmaceutical for the treatment and prophylaxis of oncoses.

10. The use of a compound as claimed in claim 8 for the production of a pharmaceutical for the treatment and prophylaxis of bacterial infectious diseases and/or mycoses.

11. The use of a compound as claimed in claim 8 for the production of a pharmaceutical for the treatment and prophylaxis of rheumatoid diseases.

2003210205 01 Jun 2007

12. The use of a compound as claimed in claim 8 for the production of a pharmaceutical against diseases which can be treated by inhibition of matrix metalloproteinases.

13. The use of a compound as claimed in one of claims 1 to 3 as an antioxidant.

14. A pharmaceutical containing at least one compound as claimed in one of claims 1 to 3 and one or more physiologically acceptable excipients.

15. The isolated strain Penicillium herquei Bainer & Sartory, DSM 14142.

16. A method of treatment or prophylaxis of oncoses by the administration to a patient requiring such treatment of an effective amount of a compound according to any one of claims 1 to 3 or a pharmaceutically acceptable derivative thereof.

17. A method of treatment or prophylaxis of bacterial infectious diseases or mycoses by the administration to a patient requiring such treatment of an effective amount of a compound according to any one of claims 1 to 3 or a pharmaceutically acceptable derivative thereof.

18. A method of treatment or prophylaxis of rheumatoid diseases by the administration to a patient requiring such treatment of an effective amount of a compound according to any one of claims 1 to 3 or a pharmaceutically acceptable derivative thereof.

19. A method of treatment or prophylaxis of diseases which can be treated by inhibition of matrix metalloproteinases by the administration to a patient requiring such treatment of an effective amount of a compound according to any one of claims 1 to 3 or a pharmaceutically acceptable derivative thereof.

2003210205 01 Jun 2007

20. Compounds of the formulae (II) substantially as hereinbefore described according to Example 4.

SANOFI-AVENTIS DEUTSCHLAND GMBH

WATERMARK PATENT & TRADE MARK ATTORNEYS

P24402AU00