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(54) Title: CALOPOROSIDE DERIVATIVES, METHODS FOR THE PRODUCTION THEREOF AND THEIR USE

(54) Bezeichnung: CALOPOROSID-DERIVATE, VERFAHREN ZU DEREN HERSTELLUNG UND DEREN VERWENDUNG

(57) Abstract: The invention relates to novel active substances (caloporoside derivatives), which are formed by the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784 during fermentation. The invention also relates to methods for producing said active substances, to their use as medicaments, to medicaments containing caloporoside derivatives, and to the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784 itself.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft neue Wirkstoffe (Caloporisid-Derivate), die von dem Mikroorganismus Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784. Während der Fermentation gebildet werden, Verfahren zu deren Herstellung, deren Verwendung als Arzneimittel, Caloporisid-Derivate enthaltende Arzneimittel sowie den Mikroorganismus Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784.

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Caloporoside Derivatives, Methods For The Production Thereof And Their Use.

The present invention relates to novel active substances (caloporoside derivatives) which are produced by the microorganism Gloeoporus dichrous Bres. ST001714, DSM 13784, during fermentation, to a process for their preparation, to their use as medicaments, to medicaments containing caloporoside derivatives, and to the microorganism Gloeoporus dichrous Bres. ST001714, DSM 13784.

Caloporoside was described as phospholipase C inhibitor for the first time in 1994 (W. Weber et al. J. Antibiotics, 47, 1188-1194). In the same year, two other, similar secondary metabolites were isolated (R. Shan et al. Nat. Prod. Lett., 4, 171-178). The compounds of the invention, of the formula I (below) differ in their structure from the substances described therein.

Cancer is a disease of humans and animals which usually has a fatal outcome and which is caused by the uncontrolled growth of the body's own cells. Cancer is the name given to the formation of malignant growths, of neoplasms (tumors or carcinomas) or to the malignant degeneration and disturbed maturation of white blood cells (leukemia). Cancer cells or tumor cells develop through the transformation of the body's own cells. The malignancy of the cancer cell is expressed by the autonomy of growth, that is to say its capability of uninhibited, infiltrative growth without fitting into the structural plan of the organs and with destruction of tissue. A sure sign of malignancy is the formation of metastases remote from the tumor after hematogenous or lymphogenous dissemination of tumor cells. Cancer is one of the commonest causes of death in humans and there is thus a great need for methods and means for curing or treating malignant degenerations.

Possible therapy of malignant tumors encompasses besides surgical removal of the tumor - radical where possible - radiological therapy with X-rays, α, β and γ rays, immunotherapy and chemotherapy. Immunotherapy can at present be applied only to a limited extent. The chemotherapy of tumors means the administration of cellular poisons (cytostatics) for the treatment of tumors and of tumor cells remaining after local surgical treatment or irradiation. These substances intervene specifically in particular processes of cell division so that tissues with a high

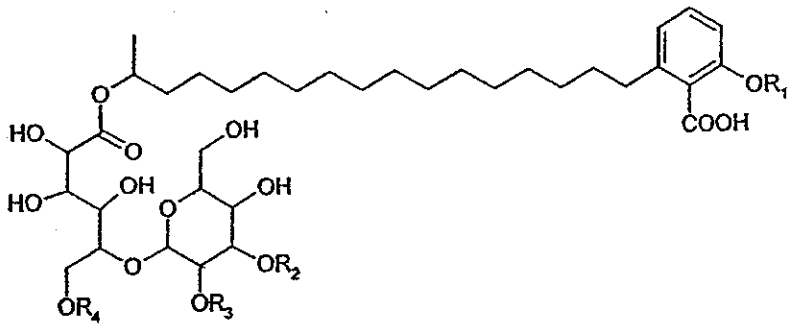
proportion of dividing cells, such as rapidly growing tumor tissue, react more sensitively. Those used are alkylating compounds such as, for example, cyclophosphamide (The Merck Index, 12th Ed. page 463), antimetabolites such as methotrexate (The Merck Index, 12th Ed. page 1025), alkaloids such as vincristine (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). However, because of extensive side effects, all these agents have great disadvantages so that the death of the patients is only delayed, but not averted. In addition, resistance to the agents used occurs in degenerate (cancerous) cells. Current medicaments then no longer have cytostatic effects but are toxic because of the side effects. In addition, it has emerged that a combined or sequential use of cytostatics exceeds the efficacy of a single cytostatic (monotherapy), and it is thus possible for there to be no addition of the considerable side effects with polychemotherapy. For all these reasons there is a pressing need and thus a world-wide search for novel chemotherapeutic agents.

Cyclin-dependent kinases (= CDKs) play a central part in regulation of the cell cycle. They catalyze phosphorylation reactions and thus start a reaction cascade which initiates a transition from the G1 phase (growth phase 1) to the S phase (synthesis phase) in the cell cycle. Cyclin-dependent kinases therefore represent a good therapeutic target for the treatment of cancer and other disorders with a pathological disturbance of cell proliferation. Low molecular weight inhibitors which regulate the cell cycle and prevent uncontrolled cell division would be helpful medicinal substances for treating cancer patients.

It has surprisingly been found that the microorganism strain Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, DSM 13784, is able to produce highly effective novel cytostatics which inhibit cyclin-dependent kinases in very low concentrations.

The invention accordingly relates to the active substances produced by the strain Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, DSM 13784 (caloporoside derivatives) and to the physiologically tolerated salts, esters and obvious chemical equivalents thereof.

The invention thus relates to compounds of the formula I



where

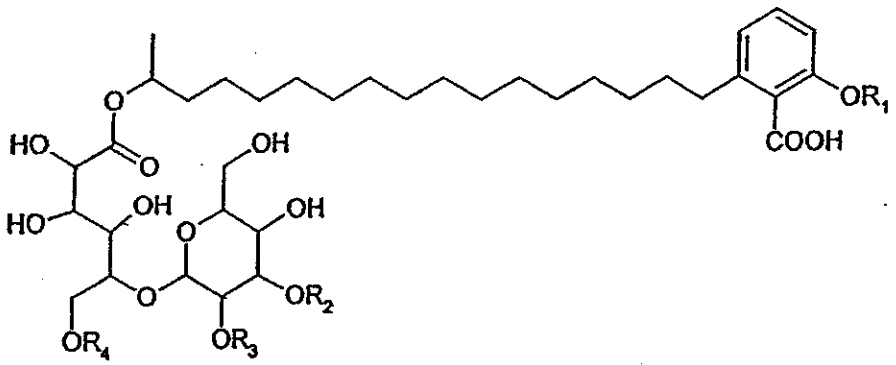
R_1, R_2 and R_3 are, independently of one another, H or an acyl radical having 2-10 carbon atoms, preferably 2 to 6 atoms, particularly preferably 2 atoms; and

R_4 is H or $-C(O)(CH_2)_nCOOH$ in which n is 1 to 7, preferably 1 to 3, particularly preferably 1 or 2;

with the exception that R_1, R_2, R_3 and R_4 are not all H;

and the physiologically tolerated salts thereof.

In an aspect of the present invention, there is provided a compound of the formula I



in which:

R_1, R_2, R_3 are, independently of one another, H or acyl radicals having 1 to 10 carbon atoms; and

R_4 is H or $-C(O)(CH_2)_nCOOH$ in which n is 1 to 7;

with the exception that R_1, R_2, R_3 and R_4 are not all H;

and the physiologically tolerated salts thereof.

The acyl radicals of the compounds of the formula I may be straight-chain or branched, saturated or mono- or diunsaturated.

An acyl radical having 2 carbon atoms means, for example, an acetyl radical.

Examples of saturated, unbranched acyl radicals are an acetic acid residue (C=2), propionic acid residue (C=3), butyric acid residue (C=4), valeric acid residue (C=5), caproic acid residue (C=6), enanthic acid residue (C=7), caprylic acid residue (C=8), pelargonic acid residue (C=9) and capric acid residue (C=10).

Examples of monounsaturated, unbranched acyl radicals are an acrylic acid residue (C=3), crotonic acid residue (C=4) or a vinylacetic acid residue (C=4).

An example of a diunsaturated unbranched acyl radical is a sorbic acid residue (C=6).

The caloporosides are antibiotics which have weak activity and are composed of a salicylic acid and a disaccharide. The two structural units are connected by an alkyl chain. The sugar moiety in the compound of the formula I may be a disaccharide composed in each case of a D-pyranose of an aldohexose (such as, for example, D-glucopyranose or D-galacto-pyranose) and the onic acid of an aldohexose (for example D-gluconic acid). The sugar moiety is preferably D-mannopyranosyl-D-mannonic acid which is unsubstituted or substituted by R_2, R_3 and/or R_4 as defined above.

The invention further relates to

a) a compound of the formula I where R_1 = acetyl; R_2 = R_3 = R_4 = H (=caloporoside B: molecular formula: $C_{38}H_{62}O_{16}$, MW 774.9) and the physiologically tolerated salts thereof;

b) a compound of the formula I where R_1 = R_2 = H; R_3 = acetyl; R_4 = malonyl (=caloporoside C: molecular formula: $C_{43}H_{66}O_{20}$, MW 902.99) and the physiologically tolerated salts thereof;

c) a compound of the formula I where R_1 = R_2 = H; R_3 = acetyl; R_4 = malonyl (=caloporoside D: molecular formula: $C_{41}H_{64}O_{19}$, MW 806.96) and the physiologically tolerated salts thereof;

d) a compound of the formula I where R_1 = R_3 = H; R_2 = acetyl; R_4 = malonyl (=caloporoside E: molecular formula: $C_{41}H_{64}O_{19}$, MW 806.96) and the physiologically tolerated salts thereof;

e) a compound of the formula I where R_1 = R_2 = R_4 = H; R_3 = acetyl; (=caloporoside F: molecular formula: $C_{38}H_{62}O_{16}$, MW 774.9) and the physiologically tolerated salts thereof.

Chirality centers in the compounds of the formula I may be in the R or S configuration unless indicated otherwise. The invention relates both to the optically pure compounds and to mixtures of stereoisomers, such as mixtures of enantiomers and mixtures of diastereomers.

The compounds of the formula I are obtainable according to the invention by fermentation of Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, or of one of its variants or mutants under suitable conditions in a

culture medium until one or more caloporoside derivatives of the formula I accumulate in the culture medium. The caloporoside derivatives are obtained by subsequent isolation of the compounds and, where appropriate, conversion into chemical equivalents, and the physiologically tolerated salts thereof.

The invention therefore additionally relates to a process for preparing a compound of the formula I, which comprises fermenting the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, DSM 13784 or one of its variants or mutants under suitable conditions in a culture medium until one or more of the target compounds accumulate in the culture medium, and are subsequently isolated from the culture medium and, where appropriate, converted into chemical equivalents and/or physiologically tolerated salts.

In another aspect of the present invention, there is provided a process for the preparation of a compound of the formula I or a physiologically tolerated salt thereof as defined above which comprises fermentation of the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, or one of its variants or mutants under suitable conditions, isolation of one or more of the caloporoside derivatives and conversion thereof where appropriate into physiologically tolerated salts.

The strain ST001714, DSM 13784, its mutants and/or variants is preferably fermented in a nutrient solution or a solid medium (also referred to as culture medium) with source of carbon and nitrogen and with conventional inorganic salts until the compounds of the invention accumulate in the culture medium, and subsequently the compounds are isolated from the culture medium and, where appropriate, fractionated into the individual active components.

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

The strain Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714 was grown in a preculture. An isolate was deposited at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1B, 3300 Brunswick, Germany, in accordance with the rules of the Budapest treaty on December 14, 1999, under the following number: DSM13784.

Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, DSM 13784, has a white mycelium and purple spores. It occurs preferentially on Betula, but may also infest other hosts, for example Alnus, Salix, Populus, Ulmus, Prunus.

In place of strain Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, DSM 13784, it is also possible to employ mutants and variants thereof which synthesize one or more of the compounds of the invention. Such mutants

can be generated 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).

Screening for mutants and variants which synthesize one or more of the compounds of the invention takes place in accordance with the following scheme:

- lyophilization of the plate cultures;
- extraction of the lyophilizate with an organic solvent;
- extraction of the compound from the culture filtrate with solid phases
- analysis by HPLC, TLC or by assaying the biological activity.

The fermentation conditions described below apply to Gloeoporus dichrous (Fr.:Fr.) Bres. ST 001714, the deposited isolate DSM 13784 and mutants and variants of these.

In a further aspect of the present invention, there is provided the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784.

In a nutrient solution containing a carbon source and a nitrogen source and the usual inorganic salts, Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, produces the caloporoside derivatives.

Suitable and 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. Suitable nitrogen-containing nutrients are: amino acids, peptides and proteins and their degradation products such as casein, peptones or tryptones, also meat extracts, yeast extracts, ground seeds, for example of corn, wheat, beans, soybean or the cotton plant, distillation residues from the production of alcohol, meat meals or yeast extracts, but also ammonium salts and nitrates, but especially also peptides obtained synthetically or biosynthetically. Inorganic salts which the nutrient solution may contain are, for example, chlorides, carbonates, sulfates or phosphates of the alkali metals or alkaline earth metals, iron, zinc, cobalt and manganese.

The compounds of the invention are produced particularly well in a nutrient solution which contain about 0.05 to 5%, preferably 1 to 2%, of malt extract, 0.05 to 3%, preferably 0.05 to 1%, of yeast extract and 0.2 to 5%,

preferably 0.5 to 2%, of glucose, 0.5 to 3%, preferably 0.5 to 3%, of cellulose powder and traces of ammonium sulfate. The percentage data are in each case based on the weight of the complete nutrient solution.

In this nutrient solution, Gloeoporus dichrous (Fr.:Fr.) Bres. *ST001714*, DSM 13784, produces a mixture of caloporoside derivatives. The amount in terms of quantity of one or more of the caloporoside derivatives of the invention may vary depending on the composition of the nutrient solution. In addition, it is possible to control the synthesis of individual caloporoside derivatives through the composition of the medium, so that a caloporoside derivative is not produced at all or is produced in an amount below the limit of detection by the microorganism.

The microorganism is cultivated aerobically, that is to say, for example, submerged with shaking or stirring in shaken flasks or fermenters, where appropriate with introduction of air or oxygen, or on solid medium. It can be carried out in a temperature range of about 18 to 35°C, preferably at about 20 to 30°C, in particular at 25 to 30°C. The pH range should be between 5 and 8, preferably between 5.5 and 6.5. The microorganism is generally cultivated under these conditions for a period of from 24 to 720 hours, preferably 288 to 576 hours.

Cultivation is advantageously carried out in a plurality of stages, i.e. firstly one or more precultures are produced in a liquid medium and are then transferred into the actual production medium, the main culture, for example in the ratio 1:10 by volume. The preculture is obtained, for example, by transferring a mycelium into a nutrient solution and allowing it to grow for about 36 to 120 hours, preferably 48 to 72 hours. The mycelium can be obtained, for example, by allowing the strain to grow for about 3 to 40 days, preferably 10 to 30 days, on a solid or liquid nutrient medium, for example malt/yeast agar or potato/dextrose agar.

In still another aspect of the present invention, there is provided a compound of the formula I or a physiologically tolerated salt thereof as defined above, which can be produced by fermentation of the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, or one of its variants or mutants under suitable conditions, isolation of one or more of the caloporoside derivatives and conversion thereof where appropriate into physiologically tolerated salts.

The progress of the fermentation can be monitored on the basis of the pH of the cultures or of the mycelium volume and by chromatographic methods such as, for example, high performance liquid chromatography (HPLC), or testing the biological activity.

The isolation process described below is used to purify the caloporoside derivatives of the invention.

Isolation or purification of a caloporoside derivative of the invention from the culture medium takes place by known methods, taking account of the chemical, physical and biological properties of the natural substances. HPLC can be used to assay the concentration of the respective caloporoside derivatives in the culture medium or in the individual isolation stages, expediently comparing the amount of substance produced with a calibration solution.

To isolate the compounds of the invention, the culture broth or the culture together with solid medium are lyophilized, and then the caloporoside derivatives are extracted from the lyophilizate with an organic solvent which is optionally miscible with water. The organic solvent phase contains the natural substances of the invention, and it is concentrated, where appropriate in vacuo, and further purified.

Further purification of one or more compounds of the invention takes place by chromatography on suitable materials, preferably, for example, on molecular sieves, on silica gel, alumina, on ion exchangers or on adsorber resins or on reverse phases (reversed phases, RP). The caloporoside derivatives are separated by means of this chromatography. Chromatography of the caloporoside derivatives takes place with buffered aqueous solutions or mixtures of aqueous and organic solutions.

Mixtures of aqueous or organic solutions means all water-miscible organic solvents, preferably methanol, propanol and acetonitrile, in a concentration of from 5 to 80% of solvent, preferably 20 to 50% of solvent, or else all buffered aqueous solutions which are miscible with organic solvents. The buffers to be used are the same as indicated above.

Separation of the caloporoside derivatives on the basis of their polarity differences takes place with the aid of reversed phase chromatography, for example on MCI® (adsorber resin from Mitsubishi, Japan) or Amberlite XAD® (TOSOHAAS), on other hydrophobic materials such as, for example, on RP-8 or RP-18 phases. The separation can additionally take place with the aid of normal phase chromatography, for example on silica gel, alumina and the like.

Chromatography of the caloporoside derivatives takes place with buffered or acidified aqueous solutions or mixtures of aqueous solutions with alcohols or other water-miscible organic solvents. Propanol and acetonitrile is preferably used as organic solvent.

Buffered or acidified aqueous solutions mean, for example, water, phosphate buffer, ammonium acetate, citrate buffer in a concentration of 0 to 0.5 M, and formic acid, acetic acid, trifluoroacetic acid or all commercially available acids known to the skilled worker, preferably in a concentration of from 0 to 1%. In the case of buffered aqueous solutions, 0.1% ammonium acetate is particularly preferred.

Chromatography is done with a gradient which starts with 100% water and ends with 100% solvent, preferably running a linear gradient from 20 to 100% propanol or acetonitrile.

An alternative possibility is also to carry out gel chromatography or chromatography on hydrophobic phases.

Gel chromatography is carried out on polyacrylamide or copolymer gels, such as, for example, Biogel-P 2® (from Biorad) or Fractogel TSK HW 40® (from Merck, Germany or Toso Haas, USA).

The sequence of the aforementioned chromatographies can be reversed.

The compounds of the invention are stable in the solid state and in solutions in the pH range between 3 and 8, in particular 5 and 7, and can thus be incorporated into conventional pharmaceutical preparations.

One or more compounds of the compounds of the invention are suitable, because of their valuable pharmacological properties, for use in human or veterinary medicine as medicament.

The present invention thus relates to the use of the compound of the formula I or a physiologically tolerated salt thereof for producing a cytostatic for the treatment of oncoses.

In yet another aspect of the present invention, there is provided the use of a compound of the formula I or a physiologically tolerated salt thereof as defined above for producing medicaments for the treatment of cancer or other disorders with a pathological disturbance of cellular proliferation.

The present invention further relates to all obvious chemical equivalents of the compounds of the formula I of the invention. Such equivalents are

compounds which display a slight chemical difference, that is to say have the same effect, or are converted under mild conditions into the compounds of the invention. Said equivalents also include, for example, salts, reduction products, esters, ethers, acetals or amides of the compounds of the invention, and equivalents which the skilled worker can prepare by standard methods, and in addition all optical antipodes, diastereomers and all stereomeric forms.

Physiologically tolerated salts of compounds of the formula I mean both organic and inorganic salts thereof, as described in Remington's Pharmaceutical Sciences (17th edition, page 1418 (1985)). Because of the physical and chemical stability and the solubility, sodium, potassium, calcium and ammonium salts inter alia are preferred for acidic groups; preferred for basic groups are, inter alia, 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.

Esters, ethers and acetals can be prepared by methods described in the literature, for example in Advanced Organic Synthesis, 4th Edition, J. March, John Wiley & Sons, 1992 or Protective Groups in Organic Synthesis 3rd Edition, T.W. Greene & P.G.M. Wuts, John Wiley & Sons, 1999.

The carboxyl group can be reduced, for example, to the alcohol with $LiAlH_4$.

It is possible initially to eliminate the glycoside moiety of compounds of the formula I by alkaline hydrolysis (W. Weber et al. J. Antibiotics, 47, 1188-1194). It is then possible to introduce any desired sugar residues by glycosylation (for example Königs-Knorr reaction). Corresponding methods are described in the literature, for example in Carbohydrate Chemistry, J.F. Kennedy, Oxford University Press, 1988.

The mechanism of action of the caloporoside derivatives is unknown, but a significant effect was detectable.

Inhibitors of CDKs are detected by using an assay in which the rate of phosphorylation of a specific peptide substrate by the cyclin-dependent kinases is measured. The cyclin-dependent kinases are activated by binding to the particular cyclin. [γ-P]-phosphate is transferred from [γ-P]-

ATP to the peptide substrate by the enzyme. The assay is carried out in 96-well microtiter plates: the radioactivity of the [γ-P]-phosphate transferred to the substrate is measured.

IC_{50} values for the caloporoside derivatives are indicated in Table 1; it is the concentration which 50% inactivates CDK-4.

Caloporoside B	1.5 μM
Caloporoside C	3.1 μM
Caloporoside D	1.8 μM
Caloporoside E	1.8 μM
Caloporoside F	1.5 μM

The present invention further relates to medicaments with a content of at least one compound of the invention.

One or more compounds of the caloporoside derivatives of the invention can in principle be administered as such without diluent. The preferred use is mixed with suitable excipients or carrier material. The carrier material which can be used is the carrier materials and/or excipients which are pharmacologically suitable and usual in medicaments.

In a further aspect of the present invention, there is provided a medicament with a content of at least one compound of the formula I or a physiologically tolerated salt thereof as defined above.

The medicaments of the invention are generally administered orally or parenterally, but rectal use is also possible in principle. Suitable solid or liquid pharmaceutical formulations are, for example, granules, powders, (coated) tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, aerosols, drops or injectable solutions in ampoule form and products with protracted release of active substance, in the production of which normally carriers and additives and/or adjuvants such as

disintegrants or binders, coating agents, swelling agents, glidants or lubricants, flavorings, sweeteners or solubilizers are used. Frequently used carriers or excipients which may be mentioned are, for example, magnesium carbonate, titanium dioxide, lactose, mannitol and other sugars, talc, milk protein, gelatin, starch, vitamins, cellulose and their derivatives, animal or vegetable oils, polyethylene glycols and solvents such as, for example, sterile water, alcohols, glycerol and polyhydric alcohols.

The dose units for oral administration may be microencapsulated where appropriate in order to delay release or 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.

The pharmaceutical products are preferably produced and administered in dose units, each unit containing as active ingredient a particular dose of one or more compounds of the caloporoside derivatives of the invention. In the case of solid dose units such as tablets, capsules and suppositories, this dose may be up to about 500 mg, but preferably about 0.1 to 200 mg, and in the case of injection solutions in ampoule form up to about 200 mg, but preferably about 0.5 to 100 mg, per day.

In still another aspect of the present invention, there is provided a process for producing medicaments as defined above, which comprises converting at least one compound of the formula I or a physiologically tolerated salt thereof as defined above with suitable excipients and/or carriers into a suitable dosage form.

The daily dose to be administered depends on the body weight, age, sex and condition of the mammal. However, in some circumstances, higher or lower daily doses may also be appropriate. Administration of the daily dose can take place both through a single administration in the form of a single dose unit or else in a plurality of smaller dose units and through multiple administration of divided doses at particular intervals.

The medicaments of the invention are produced by converting one or more of the compounds of the invention with conventional carriers and, where appropriate, additives and/or excipients into the or a suitable dosage form.

The invention is explained further in the examples which follow. Percentage data are based on weight. Mixing ratios of liquids are based on volume unless indicated otherwise.

Examples

Example 1: Preparation of a glycerol culture of Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784

100 ml of nutrient solution (malt extract 2.0%, yeast extract 0.2%, glucose 1.0% $(NH_4)_2HPO_4$ 0.05%, pH 6.0) in a sterile 300 ml Erlenmeyer flask are incubated with the strain Gloeoporus dichrous (Fr.:Fr.) Bres. *ST001714*, DSM 13784 at 25°C and 140 rpm on a rotating shaker and 7 days. 1.5 ml of this culture are then diluted with 2.5 ml of 80% glycerol and stored at -135°C.

Example 2: Preparation of a preculture in an Erlenmeyer flask of Gloeoporus dichrous (Fr.:Fr.) Bres. *ST001714*, DSM 13784

30 ml of nutrient solution (malt extract 2.0%, yeast extract 0.2%, glucose 1.0% $(NH_4)_2HPO_4$ 0.05%, pH 6.0) in a sterile 100 ml Erlenmeyer flask are inoculated with the strain Gloeoporus dichrous (Fr.:Fr.) Bres. *ST001714*, DSM 13784, and incubated at 25°C and 140 rpm on a rotating shaker for 4 days. 2 ml portions of this preculture are then used to inoculate the plates for preparing the main cultures.

Example 3: Preparation of a main culture of Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784 on solid medium plates.

Sterile 25×25 cm plates (from Nunc) are cast with 200 ml of the following nutrient solution 20 g/l malt extract, 2 g/l yeast extract, 10 g/l glucose, and 0.5 g/l $(NH_4)_2HPO_4$ pH 6.0. These plates were each inoculated with 2 ml of a preculture. Maximum production of one or more of the compounds of the invention is reached after about 480 hours.

Example 4: Production of the caloporoside derivatives

50 plates 25×25 cm were prepared and inoculated with the preculture:

Nutrient medium:
20 g/l malt extract
2 g/l yeast extract
10 g/l glucose
0.5 g/l $(NH_4)_2HPO_4$
pH 6 (before sterilization)
Incubation time: 480 hours
Incubation temperature: 25°C

Example 5: Isolation of the caloporoside mixture from the plate cultures of Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784.

After completion of the fermentation of Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, the plate cultures obtained as in Example 3 are lyophilized, and the lyophilizate is extracted with 5 liters of methanol. The

active substance-containing methanolic solution is filtered to remove residue and concentrated in vacuo. The concentrate is diluted with water and loaded onto a prepared 1.0 liter MCI GEL, CHP20P column. Elution is with a gradient from water to 100% acetonitrile. The column flow-through (25 ml per minute) is collected in fractions (25 ml each), and the caloporoside derivative-containing fractions (from 40% to 100% acetonitrile) are combined. Concentration in vacuo and subsequent lyophilization afford 8.5 g of a yellow-brown powder.

Example 6: Preliminary separation of the caloporoside derivatives by RP18 chromatography.

0.5 g of the product obtained as in Example 4 are loaded onto a Nucleosil® 100-7 C18 HD column (size: 40 mm × 250 mm). Elution is with a gradient from 20% acetonitrile (+ water with addition of 0.1% ammonium acetate) to 100% acetonitrile with a flow rate of 35 ml per minute. The column outflow is collected in fractions (35 ml). The caloporoside derivatives are present mainly in fractions 39 to 68. They are combined, freed of solvent in vacuo and then lyophilized. This resulted in caloporoside B (22.4 mg) and F (10.5 mg) already >95% pure. Caloporoside C (fraction 41; 43.4 mg), D (fraction 43-45; 76.2 mg) and E (fraction 43-45; 76.2 mg) were obtained about 70% pure and therefore were purified further by chromatography.

Example 7: Purification of caloporosides C, D and E.

20 mg of the caloporoside C isolated and concentrated as in Example 5 are loaded onto a LUNA® 5μ C18(2) column (size: 10 mm × 250 mm) and chromatographed with a gradient from 25 to 35% acetonitrile in 0.1% ammonium acetate/water. The flow-through of the eluent is 6.5 ml per minute, and the fraction size is 6.5 ml. Caloporoside C is present in fractions 35 to 42. Lyophilization of said fractions affords >95% pure caloporoside C (7.5 mg).

25 mg of the mixture of caloporoside D and E isolated and concentrated as in Example 5 are loaded onto a LUNA® 5μ C18(2) column (size: 10 mm × 250 mm) and chromatographed with a gradient from 30 to 40% acetonitrile in 0.1% ammonium acetate/water. The flow-through of the eluent is 6.5 ml per minute, and the fraction size is 6.5 ml. Caloporoside D is present in fractions 18 and 19, and caloporoside E in fractions 20 to 21. Lyophilization

of said fractions affords >95% pure caloporoside D (7.0 mg) and caloporoside E (6.0 mg).

The physicochemical and spectroscopic properties of the substances of the invention can be summarized as follows:

Caloporoside B:

Molecular formula: $C_{38}H_{62}O_{16}$
Molecular weight: 774.9
UV maxima: 208, 244, 310
^{1}H- and ^{13}C-NMR: see Table 2

The high resolution FAB mass spectrum shows an intense MH^+ at m/z 775.4120 Da, in good agreement with the calculated mass (for $C_{38}H_{63}O_{16}$, monoisotopic) of 775.4116 Da.

Caloporoside C:

Molecular formula: $C_{43}H_{66}O_{20}$
Molecular weight: 902.99
UV maxima: 208, 244, 310
^{1}H- and ^{13}C-NMR: see Table 3

The high resolution FAB mass spectrum shows an intense $M+H^+$ at m/z 903.4264 Da, in good agreement with the calculated mass (for $C_{36}H_{71}O_{25}$, monoisotopic) of 903.4284 Da.

Caloporoside D:

Molecular formula: $C_{41}H_{64}O_{19}$
Molecular weight: 860.96
UV maxima: 208, 244, 310
^{1}H- and ^{13}C-NMR: see Table 4

The high resolution FAB mass spectrum shows an intense $M+Na^+$ at m/z 883.3942 Da, in good agreement with the calculated mass (for $C_{41}H_{64}O_{19}Na$, monoisotopic) of 883.3939 Da.

Caloporoside E:

Molecular formula: $C_{41}H_{64}O_{19}$
Molecular weight: 860.96
UV maxima: 208, 244, 310
^{1}H- and ^{13}C-NMR: see Table 4

The high resolution FAB mass spectrum shows an intense $M+Na^{+}$ at m/z 833.3942 Da, in good agreement with the calculated mass (for $C_{41}H_{64}O_{19}Na$, monoisotopic) of 883.3939 Da.

Caloporoside F:

Molecular formula: $C_{38}H_{62}O_{16}$
Molecular weight: 774.9
UV maxima: 208, 244, 310
^{1}H- and ^{13}C-NMR: see Table 5

The high resolution FAB mass spectrum shows an intense $M+H^{+}$ at m/z 775.4128 Da, in good agreement with the calculated mass (for $C_{38}H_{63}O_{16}$, monoisotopic) of 775.4116 Da.

Table 2: ^{1}H and ^{13}C chemical shifts of caloporoside B in methanol-d_4 and DMSO-d_6 at 300K

	DMSO	MeOD	DMSO	MeOD
1	-	-	171.24	a)
2	-	-	a)	a)
3	-	-	162.22	163.57
4	6.68	6.77	115.28	116.81
5	7.17	7.25	~131.6	b
6	6.57	6.67	121.02	~123.0
7	-	-	137.90	139.57
8	a)	a)	a)	a)
9	a)	a)	a)	a)
10-20	1.30-1.20	1.32-1.27	29.01-28.69	30.76-30.47
21	1.30	1.38/1.32	24.81	26.52
22	1.54/1.45	1.65/1.52	35.31	37.00
23	4.81	4.95	70.04	73.09
24	1.15	1.24	19.69	20.22
1'	-	-	173.43	175.35
2'	3.95	4.13	70.89	72.97
3'	3.95	4.17	70.04	71.59
4'	3.54	3.82	67.96	69.80
5'	3.65	3.86	78.94	80.12
6'	3.74/3.44	3.90/3.69	62.11	63.25
1"	4.53	4.71	100.27	101.34
2"	3.74	3.95	70.37	72.46
3"	3.22	3.44	73.67	75.30
4"	3.21	3.49	67.54	69.00
5"	3.04	3.22	77.15	78.23
6"	3.72/3.31	3.88/3.63	61.80	63.25
Ac-C'	-	a)	-	a)
Ac-Me	a)	a)	a)	a)

a) no signal is observed for these protons/carbon atoms in MeOD or DMSO (aggregation).

Table 3: ^{1}H and ^{13}C chemical shifts of caloporoside C in methanol-d_4 at 300K

	^{1}H	^{13}C
1	-	175.90
2	-	116.73
3	-	163.67
3-Ac-Me	a)	a)
3-Ac-C'	-	a)
4	6.73	116.73
5	7.16	~132.6[b)]
6	6.55	123.53
7	-	139.51
8	2.49	43.06
9	1.53	24.75
10-20	1.36-1.26	30.78-30.62
21	1.38/1.33	26.54
22	1.65/1.52	37.02
23	4.95	73.04
24	1.24	20.22
1'	-	175.17
2'	4.17	72.82
3'	4.08	71.60
4'	3.71	69.79
5'	4.07	76.38
6'	4.60/4.14	66.43
7'	-	170.55
8'	3.27[(c	~44.8
9'	-	173.12
1"	4.93	99.40
2"	5.33	73.40
3"	3.72	73.40
4"	3.42	69.34
5"	3.34	78.19
6"	3.89/3.62	63.11
2"-Ac-Me	2.11	21.17
2"-Ac-C'	-	172.56

a) No signal observed for these nuclei (signal greatly broadened).

b) Signal broadened

c) The signal for the protons in position 8' is observed only in the freshly prepared solution (rapid exchange with deuterium).

Table 4: ^{1}H and ^{13}C chemical shifts of caloporoside D and E in methanol-d_4 at 300K

	^{1}H caloporoside D	^{13}C caloporoside D	^{1}H caloporoside E	^{13}C caloporoside E
1	-	-	176.28	176.28
2	-	-	120.37	120.37
3	-	-	162.12	162.12
4	6.61	6.61	114.91	114.91
5	7.06	7.06	131.53	131.53
6	6.58	6.58	122.23	122.23
7	-	-	147.22	147.22
8	3.04	3.04	36.31	36.31
9	1.55	1.55	33.35	33.35
10-20	1.36-1.25	1.36-1.25	31.07/30.61	31.07/30.61
21	1.38/1.32	1.38/1.32	26.53	26.53
22	1.65/1.53	1.65/1.53	37.01	37.01
23	4.95	4.95	73.13[a)]	73.07[a)]
24	1.24	1.24	20.23	20.23
1'	-	-	175.18	175.40
2'	4.17	4.15	72.80	72.80
3'	4.08	4.15	71.61	71.53
4'	3.71	3.85	69.78	69.27
5'	4.07	4.08	76.40	77.07
6'	4.58/4.14	4.59/4.24	66.35	65.43
7'	-	-	~170.4	~170.4
8'	3.28	3.28	~45.0	~45.0
9'	-	-	~173.0	~173.0
1"	4.93	4.84	99.40	100.20
2"	5.33	4.03	73.41	70.27
3"	3.72	4.78	73.39	77.44
4"	3.42	3.71	69.32	66.35

5"	3.34	3.38	78.35	78.00
6"	3.91/3.62	3.89/3.65	63.09	62.96
2"/3"-Ac-Me	2.11	2.00	21.18	20.98
2"/3"-Ac-C'	-	-	172.59	172.36

a) These signals cannot be unambiguously assigned.

Table 5: ^{1}H and ^{13}C chemical shifts of caloporoside F in methanol-d_4 at 300K

	^{1}H	^{13}C
1	-	174.95
2	-	117.32
3	-	162.18
4	6.68	115.42
5	7.17	133.17
6	6.67	122.65
7	-	146.91
8	2.93	36.57
9	1.56	33.27
10-20	1.36-1.25	30.93-30.62
21	1.35	26.53
22	1.65/1.53	37.02
23	4.95	73.05
24	1.24	20.23
1'	-	175.15
2'	4.17	72.98
3'	4.09	71.73
4'	3.71	69.88
5'	3.85	79.59
6'	3.90/3.61	63.57
1"	4.88	99.76
2"	5.37	73.52
3"	3.63	73.56
4"	3.46	69.27
5"	3.28	78.33
6"	3.91/3.63	63.06

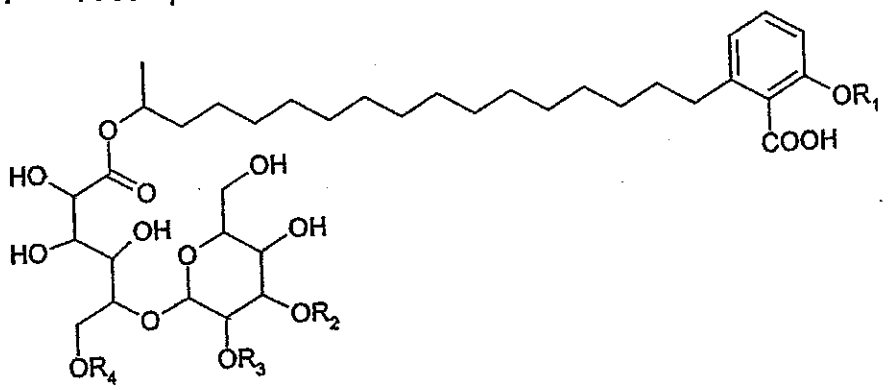
Ac-C[1]	-	173.03
Ac-Me	2.13	21.21

Example 8: Bioassay for CDK-4 inhibitors

To determine the IC_{50}, stock solutions of the natural substances of the invention are prepared in a concentration of 10 mM. 384-well flash plates are coated with 50 µl (50 µg/well) of biotinylated peptide substrate at room temperature for 2 hours and then washed 3 × with PBS buffer. For the reaction, 30 µl of a buffer-diluted solution of the caloporoside derivatives and 20 µl of a premixed ATP/cyclinD1/CDK4 solution (final concentration: 1 µCi 33P-γ-ATP, 2 µM ATP and 1 µg enzyme mixture) are pipetted onto the plates. After reaction at 37°C for 2 hours, the plates are washed 3 × with 80 µl of 3% phosphoric acid each time and then measured with a MicroBeta counter for 30 seconds. Determination of the percentage inhibition takes place with the assistance of mathematical equations. For the determination of IC_{50} values, 10 concentrations of a freshly diluted DMSO solution of the substances of the invention are assayed.

Patent Claims:

1. A compound of the formula I



in which:
R_1, R_2, R_3 are, independently of one another, H or acyl radicals having 1 to 10 carbon atoms; and
R_4 is H or $-C(O)(CH_2)_nCOOH$ in which n is 1 to 7;
with the exception that R_1, R_2, R_3 and R_4 are not all H;
and the physiologically tolerated salts thereof.

2. A compound of the formula I as claimed in claim 1, in which

R_1, R_2, R_3 are, independently of one another, H or acetyl; and
R_4 is H or malonyl (n = 1);
and the physiologically tolerated salts thereof.

3. A compound of the formula I as claimed in claim 1 or 2, in which

R_1 is acetyl; and
R_2, R_3 and R_4 are H;
and the physiologically tolerated salts thereof.

4. A compound of the formula I as claimed in claim 1 or 2, in which

R_1 and R_3 are acetyl;
R_2 is H; and
R_4 is malonyl;
and the physiologically tolerated salts thereof.

5. A compound of the formula I as claimed in claim 1 or 2, in which

R_1 and R_2 are H;
R_3 is acetyl; and
R_4 is malonyl;
and the physiologically tolerated salts thereof.

6. A compound of the formula I as claimed in claim 1 or 2, in which

R_1 and R_3 are H;
R_2 is acetyl; and
R_4 is malonyl;
and the physiologically tolerated salts thereof.

7. A compound of the formula I as claimed in claim 1 or 2, in which

R_1, R_2 and R_4 are H; and
R_3 is acetyl;
and the physiologically tolerated salts thereof.

8. A compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-7, which can be produced by fermentation of the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, or one of its variants or mutants under suitable conditions, isolation of one or more of the caloporoside derivatives and conversion thereof where appropriate into physiologically tolerated salts.

9. A process for the preparation of a compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1 - 7, which comprises fermentation of the microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784, or one of its variants or mutants under suitable conditions, isolation of one or more of the caloporoside derivatives and conversion thereof where appropriate into physiologically tolerated salts.

10. The process as claimed in claim 9, wherein the fermentation is carried out under aerobic conditions at a temperature between 18 and 35°C and at a pH between 5 and 8.

11. A compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-8 for use as medicament.

12. A compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-8 for use as CDK inhibitor.

13. The use of a compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-8 for producing medicaments for the treatment of cancer or other disorders with a pathological disturbance of cellular proliferation.

14. A medicament with a content of at least one compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-8.

15. A process for producing medicaments as claimed in claim 14, which comprises converting at least one compound of the formula I or a physiologically tolerated salt thereof as claimed in one or more of claims 1-8 the with suitable excipients and/or carriers into a suitable dosage form.

16. The microorganism Gloeoporus dichrous (Fr.:Fr.) Bres. ST001714, DSM 13784.