(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International publication date
12 February 2004 (12.02.2004)

PCT

(10) International publication number
WO 2004/012744 A1

(51) International patent classification[7]: A61K 31/685, A61P 35/00

(21) International application number: PCT/EP2003/008346

(22) International filing date: 29 July 2003 (29.07.2003)

(25) Language of filing: German

(26) Language of publication: German

(30) Data relating to the priority:
60/399,615 30 July 2002 (30.07.2002) US

(71) Applicant: ZENTARIS GMBH [DE/DE]; Weismüllerstrasse 45, 60314 Frankfurt/Main.

(72) Inventors: ENGEL, Jürgen; Erlenweg 3, 63755 Alzenau (DE). GÜNTHER, Eckhard; Wingertstr. 176, 63477 Maintal (DE). SINDERMANN, Herbert; Leipziger Ring 73, 63110 Rodgau (DE).

(81) Designated states *(national)*: AU, BR, BY, CA, CN, CO, GE, HR, ID, IL, IN, IS, JP, KR, LT, LV, MK, MX, NO, NZ, PH, PL, RU, SG, UA, UZ, YU, ZA.

(84) Designated states *(regional)*: Eurasian Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European 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).

Declaration under Rule 4.17:
- *As to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii)) for all designations.*

Published:
- *With the International Search Report.*

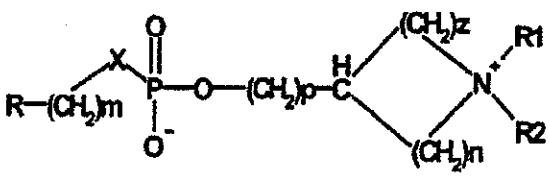
For an explanation of the two-letter codes and the other abbreviations, reference is made to the explanations ("Guidance Notes on Codes and Abbreviations") at the beginning of each regular edition of the PCT Gazette.

As printed

(54) Title: USE OF ALKYL PHOSPHOCHOLINES IN COMBINATION WITH ANTITUMOR MEDICAMENTS

(54) Bezeichnung: ANWENDUNG VON ALKYLPHOSPHOCHOLINEN IN KOMBINATION MIT ANTITUMORMEDIKAMENTEN

WO 2004/012744 A1



(II)

(57) Abstract: The invention relates to the use of alkyl phosphocholines in combination with antitumor medicaments for treating benign and malignant tumor diseases in humans and mammals. The alkyl phosphocholines can be used in an inventive combination with one or a combination of several approved cytostatics. Preferred alkyl phosphocholines are represented in formula II.

(57) Zusammenfassung: Die Erfindung betrifft die Verwendung von Alkylphosphocholinen in Kombination mit Antitumormedikamenten zur Behandlung gutartiger und bösartiger Tumorerkrankungen am Menschen und Säugetier. Dabei können die Alkylphosphocholine in einer erfindungsgemässen Kombination mit einem oder einer Kombination von verschiedenen zugelassenen Zytostatika eingesetzt werden. Bevorzugte Alkylphosphocholine werden durch die Formel II dargestellt.

Use of alkylphosphocholines in combination with antitumor medicaments

Alkylphosphocholines are a new class of organic compounds which show diverse antineoplastic activities (M. Lohmeyer and R. Bittman; Antitumor ether lipids and alkylphosphocholines, DOF, **19** (11), 1021-1037 (1994)). The effect of the alkylphosphocholines in this connection may be based on various molecular and biochemical mechanisms, some of which take place at the level of the plasma membrane of the cells. It is well known that alkylphosphocholines influence inositol metabolism, the interaction with phospholipases or inhibition of protein kinase C and thus that this class of substances has a general influence on cellular signal transduction (K. Maly, F. Überall, C. Schubert, E. Kindler, J. Stekar, H. Brachwitz and H. H. Grunicke, Interference of new alkylphospholipid analogues with mitogenic signal transduction, Anti-Cancer Drug Design, **10**, 411-425 (1995)). Thus, the alkylphosphocholine perifosine shows growth-inhibitory properties in relation to various melanoma, CNS, lung, colon, prostate and breast cancer cell lines with an IC_{50} in the region of 0.2 – 20 μM (P. Hilgard, T. Klenner, J. Stekar, G. Nössner, B. Kutscher and J. Engel; D-21266, a New Heterocyclic Alkylphospholipid with Antitumor Activity, Eur. J. Cancer, **33** (3), 442-446 (1997)). It is further known that perifosine blocks tumor cells in the G_1-S and G_2-M phase of the cell cycle (V. Patel, T. Lahusen, T. Sy, E. A. Sausville, J. S. Gutkind and A. M. Senderowicz; Perifosine, a Novel Alkylphospholipid, Induces $p21^{Waf1}$ Expression in Squamous Carcinoma Cells through a p53-independent Pathway, Leading to Loss in Cyclin-dependent Kinase Activity and Cell Cycle Arrest, Cancer Research **62**, 1401-1409 (2002)).

It is known that the use of alkylphosphocholines before or together with radiotherapy leads to synergistic effects in the treatment of tumors (G.A. Ruitter, M. Verheijl, S.F. Zerp and W.J. van Blitterswijk; Alkyl-Lysophospholipids as Anticancer Agents and Enhancers of Radiation-Induced Apoptosis, Int. J. Radiation Oncology Biol. Phys., **49** (2), 415-420, 2001). It has also been reported that various glycero-3-phospholipids, e.g. ET-18-OCH_3, in combination with various DNA-interacting substances or tubulin binders increase the antitumor activity in vitro on various tumor cell lines (A. Noseda, M.E. Berens, J.G. White and E.J. Modest; In vitro antiproliferative activity of combinations of ether lipid analogs and DNA-Interactive

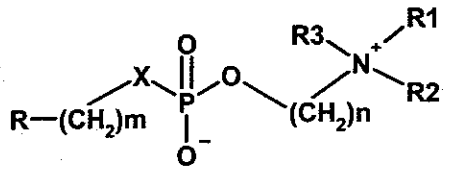
agents against human tumor cells, Cancer Res., **48** (7), 1788-1791 (1988); P. Principe, H. Coulomb, C. Broquet and P. Braquet; Evaluation of combinations of antineoplastic ether phospholipids and chemotherapeutic drugs, Ant-Cancer Drugs, **3** (6), 577-587 (1992); P. Principe, H. Coulomb, J.-M. Mencia-Huerta, C. Broquet and P. Braquet; Synergistic cytotoxic effect of aza-alkylphospholipids in association with chemotherapeutic drugs, J. Lipid Mediators Cell Signalling, **10** (1-2), 171-173 (1994)).

It has now been possible, surprisingly, to show that linear alkylphosphocholines of the general formula I and II are suitable for use in a combination according to the invention with other drug products for the treatment of benign and malignant oncoses in humans and mammals. It is possible in this connection for the compounds of the general formula I and II to be employed in a combination according to the invention with antitumor substances. Antitumor substances may be alkylating agents, antimetabolites, plant alkaloids, platinum compounds, tumor antibiotics and agonists or antagonists of natural hormones. The antitumor substances may be selected from but not restricted to: cisplatin, carboplatin, oxaliplatin, bleomycin, doxorubicin, methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, cyclophosphamide, 5-fluorouracil, fludarabine, gemcitabine and cytarabine.

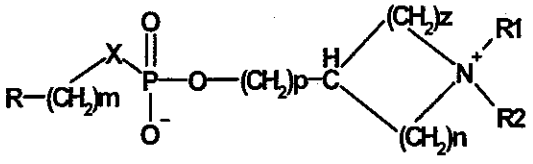
It is moreover possible for the alkylphosphocholines of the general formula I and II to be employed in a claimed combination with inhibitors of signal transduction in the form of high and low molecular weight inhibitors of receptor and/or cytosolic kinases. These inhibitors may be selected from but not restricted to monoclonal antibodies and heterocyclic compounds.

The alkylphosphocholines of the general formula I and II on which the invention is based can be used in the form of finished drug products.

The compounds on which the invention is based are described by the general formulae I and II:



formula I



formula II

where, independently of one another:

n, m, p, z are an integer between 0 and 4;

X is O, S, NH;

R is H, a straight-chain or branched (C_1-C_{20})-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals.

R_1, R_2, R_3 is, independently of one another, H, a straight-chain or branched (C_1-C_6)-alkyl radical, preferably methyl and ethyl, a (C_3-C_7)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals.

According to a further aspect of the invention, a method for controlling tumors in humans and in mammals is provided and comprises administering at least one of the compounds of the general formula I and II on which the invention is based to the

human or a mammal in an amount effective for tumor treatment before or during a treatment with approved antitumor substances.

The therapeutically effective dose, to be administered for the treatment, of the particular compound of the general formula I and II on which the invention is based depends inter alia on the nature and the stage of the oncosis, the age and sex of the patient, the mode of administration and the duration of treatment.

The compounds on which the invention are based can be administered in a drug product as liquid, semisolid and solid drug forms. This takes place in the manner suitable in each case in the form of aerosols, oral powders, dusting powders and epipastics, uncoated tablets, coated tablets, emulsions, foams, solutions, suspensions, gels, ointments, pastes, pills, pastilles, capsules or suppositories.

Exemplary embodiments:

1. Administration of perifosine (D-21 266) in combination with cisplatin

   In vivo test: DMBA-induced rat mammary carcinoma model

   Experimental animal: Sprague-Dawley rat, female

   Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

   Administration: Perifosine 14 x 6.81 mg/kg p.o.
   Cisplatin 4 x 1 mg/kg i.p.

   Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

Treatment	Tumor Initial weight [g]	Day 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Cisplatin	0.9	410	0.120
Perifosine (D-21266) + Cisplatin	0.8	-75	<0.001

2. Administration of perifosine in combination with cyclophosphamide

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.
Cyclophosphamide 100 mg/kg, VZ 0, i.v.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

Treatment	Tumor Initial weight [g]	Day 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Cyclophosphamide	0.9	500	0.011
Perifosine (D-21266) + Cyclophosphamide	0.8	-83.3	<0.001

3. Administration of perifosine in combination with Adriamycin

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor was mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

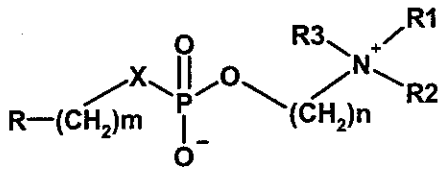
Administration: Perifosine 14 x 6.81 mg/kg p.o.
Adriamycin 4 x 2.15 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

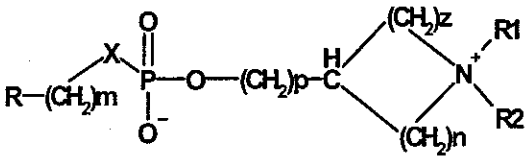
Treatment	Tumor Initial weight [g]	Tag 21 Change in [%]	p test vs. Control
Control	1.0	875	-
Perifosine (D-21266)	0.9	-25	<0.001
Adriamycin	1.0	781.3	0.197
Perifosine (D-21266) + Adriamycin	01.0	-70	<0.001

Claims

1. The use of alkylphosphocholines of the general formula I and II



formula I



formula II

where, independently of one another:

n, m, p, z are an integer between 0 and 4;

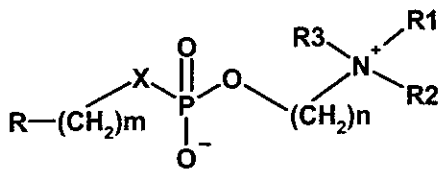
X are O, S, NH;

R is H, a straight-chain or branched (C_1-C_{20})-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals;

R_1, R_2, R_3 is, independently of one another, H, a straight-chain or branched (C_1-C_6)-alkyl radical, preferably methyl and ethyl, a (C_3-C_7)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, (C_1-C_6)-alkoxy, amino, mono-(C_1-C_4)-alkylamino or di-(C_1-C_4)-alkylamino radicals,

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an approved antitumor medicament.

2. The use of alkylphosphocholines of the general formula I as claimed in claim 1



formula I

where, independently of one another:

n is the integer 1 or 2;

m is the integer 1;

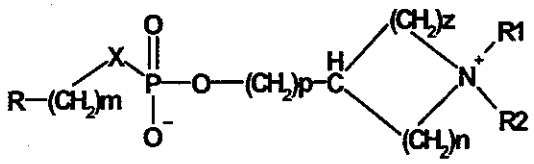
X is O;

R is H or a straight-chain or branched (C_1-C_{17})-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds;

R_1, R_2, R_3 is, independently of one another, H, a straight-chain or branched (C_1-C_6)-alkyl radical, preferably methyl and ethyl, a (C_3-C_7)-cycloalkyl radical may be,

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an approved antitumor medicament.

3. The use of alkylphosphocholines of the general formula II as claimed in claim 1



formula II

where, independently of one another:

m, p is the integer 1;

n, z is the integer 2;

X is O;

R is H, a straight-chain or branched (C_1-C_{17})-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds;

R_1, R_2, R_3 is, independently of one another, H, a straight-chain or branched (C_1-C_6)-alkyl radical, preferably methyl and ethyl, a (C_3-C_7)-cycloalkyl radical may be,

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an approved antitumor medicament.

4. The use of octadecyl 1,1-dimethylpiperidinio-4-yl phosphate as claimed in claim 1 for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an approved antitumor medicament.

5. The use of alkylphosphocholines of the general formula I and II as claimed in claims 1 to 4, where the approved antitumor medicaments may be alkylating agents, antimetabolites, plant alkaloids, platinum compounds, tumor antibiotics and agonists or antagonists of natural hormones.

6. The use as claimed in claim 5, where the antitumor medicaments may be cisplatin, cyclophosphamide or Adriamycin.

7. The use of alkylphosphocholines of the general formula I and II as claimed in claims 1 to 4, where the approved antitumor medicaments may be inhibitors of signal transduction in the form of high and low molecular weight inhibitors of receptor kinases and/or cytosolic kinases.

8. The use as claimed in claim 7, where the inhibitors may be monoclonal antibodies or heterocyclic compounds.

9. The use of alkylphosphocholines of the general formula I and II as claimed in claims 1 to 8 in a therapeutic dose which is effective for the treatment before and/or during the treatment with an approved antitumor medicament.

10. The use of alkylphosphocholines of the general formula I and II as claimed in claims 1 to 9, where the approved antitumor medicament is a combination of various cytostatics.

11. The use of alkylphosphocholines of the formula I and II as claimed in claims 1 to 4 for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during the treatment with an approved antitumor medicament, wherein the drug product comprises the customary pharmaceutical carriers, excipients and/or diluents in addition to the alkylphosphocholine of the formula I and II.

12. A drug product comprising at least one alkylphosphocholine of the general formula I and II and, where appropriate, carriers and/or excipients for use in the treatment of benign and malignant oncoses before and/or during the treatment with an approved antitumor medicament.