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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 August 2009 (06.08.2009)

PCT

(10) International Publication Number
WO 2009/096655 A1

(51) International Patent Classification:
A61K 31/357 (2006.01)

(21) International Application Number:
PCT/KR2008/006647

(22) International Filing Date:
11 November 2008 (11.11.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10-2008-0010128 31 January 2008 (31.01.2008) KR

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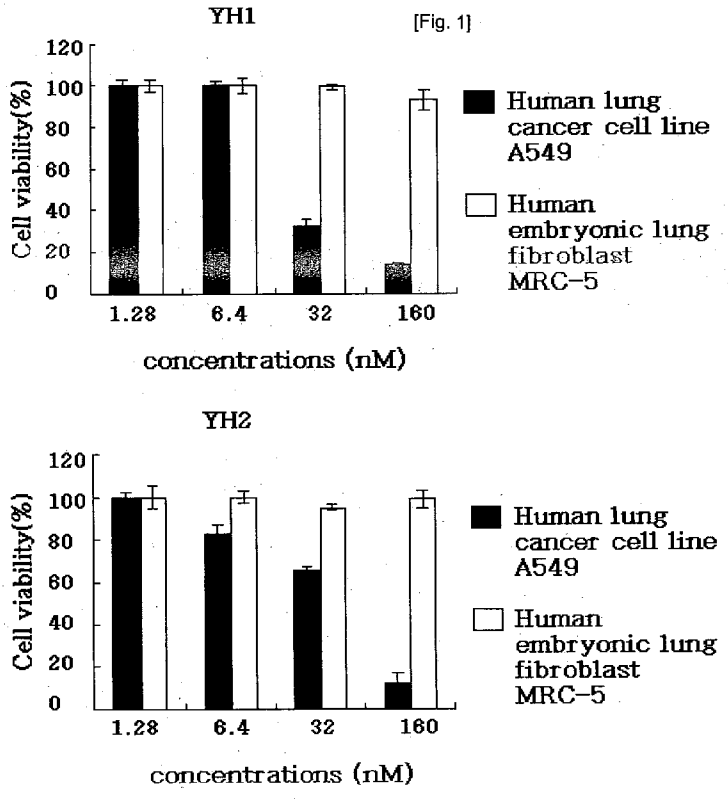
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,

[Continued on next page]

(54) Title: COMPOSITION COMPRISING THE COMPOUND ISOLATED FROM THE FLOWER EXTRACT OF DAPHNE GENKWA FOR PREVENTING AND TREATING CANCER DISEASE AND THE USE THEREOF

[Fig. 1]



(57) Abstract: The present invention relates to novel compound, a composition comprising the compounds isolated from the flower extract of *Daphne genkwa* for preventing and treating cancer disease and the use thereof. the inventive compounds strongly inhibit the growth of cancer cell lines such as lung cancer cell line, colon carcinoma cell, breast cancer cell line, prostatic carcinoma cell line, stomach cancer line, liver cancer cell line. Therefore, they can be useful in treating and preventing cancer disease as a medicament and health food composition.

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FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

Description

COMPOSITION COMPRISING THE COMPOUND ISOLATED FROM THE FLOWER EXTRACT OF DAPHNE GENKWA FOR PREVENTING AND TREATING CANCER DISEASE AND THE USE THEREOF

Technical Field

[1] The present invention relates to novel compound, a composition comprising the compounds isolated from the flower extract of *Daphne genkwa* for preventing and treating cancer disease and the use thereof.

[2]

Background Art

[3] Cancer is characterized by cell cluster called as tumor caused by abnormal and uncontrolled cell growth, which is formed, permeated into neighboring tissue and severe to be transferred to other organ, which is called as a neoplasia. Over than 20 million peoples per year are suffered with cancer in the world and among them 6 million people per year died from the disease. The origin of cancer is classified into internal factors such as genetic factor, immunological factor etc and external factor such as various chemical substances, radioactive ray, virus etc. Cancer may occur when the balance between oncogene and tumor suppressor genes is collapsed by above explained factors.

[4] Accordingly, there have been needed to develop effective anti-cancer drugs which show potent treating and preventing activity of cancer and low toxicity till now.

[5] There have been reported on the studies to develop chemo-therapeutic agents, for example, anti-metabolites such as methotrexate, 6-mercaptopurine or 6-thioguanine, 5-fluorouracil or cytarabine etc; alkylating agents such as chlorambucil or cyclophosphamide, thiotepa, busulfan, carmustine, dacarbazine etc; and antimitotic drugs such as actinomycin D, doxorubicin, bleomycin, mitomyin, vincristine, vinblastine, taxoids etc. To be used in the prevention and treatment of cancer disease, the substance should satisfy various requirements for examples, an overcoming activity of the drug resistance, a preventing or postponing activity of the metastasis pathway conversing normal cells into cancer cells by effectively blocking various carcinogenic mechanism, a recovering activity from cancer, low or no toxicity such as long-term or acute toxicity, ease use in administration, and low cost etc.

[6] Recently, it have been needed to develop more potent cancer preventive agents and those agents can be useful in preventing cancer disease as well as other diseases. Ac-

cordingly, the development of anti-cancer agents has been extensively focused on natural product material in the field of medicine as well as food chemistry field till now. There have been several reports on the development of pharmacologically active substances derived from natural resources showing potent anti-cancer activity, for example, taxol isolated from the cortex of *Taxus brevifolia*, which is effective in treating cancer, especially, breast cancer and ovarian cancer; vinblastine isolated from *Carthranthus roseus*; etoposide and teniposide, a semi-synthetic compounds from podophyllotoxin isolated from *Podophyllum peltatum*; camptothecine isolated from *Camptotheca accuminata*; homoharringtonin isolated from *Cephalotaxus harringtonia* var. drupacea; 4-ipomeanole isolated from *Fusarium solani* (Cordell et al., Bioactive Natural Products, CRS Press, pp.195-220, 1993) and so on. Additionally, the development of anti-cancer drugs showing selective anti-cancer effect on specific cancers, for example, Gleevec showing potent anti-cancer effect on chronic myelogeneous leukemia through the selective inhibition mechanism of bcr-abl, c-abl, and v-abl, a protein tyrosine kinase; Iresa (ZD1839) showing potent anti-cancer effect on lung cancer through the selective inhibition mechanism of epidermal growth factor receptor (Blume-Jensen and Hunter, *Nature*, 411, pp355-365, 2001) and so on.

[7] As described above, there have been lots of reports on the substances showing potent preventing or treating effect on cancer disease, which had been isolated from natural resource, especially plant resource. Since the plant extract shows various advantages such as low toxicity due to long-term administration, easiness in the production of materials, relatively stable storage property against external environment etc, the development of an anti-cancer agent from plant extract has been highlighted till now.

[8] The flower of *Daphne genkwa* SIEB. Et ZUCC belonged to Thymelaeceae and distributed in Korean mountains, contains genkwadaphnin, apigenin, sitosterol, benzoic acid etc (Chung, B.S. and SHIN, M.K.; Youngrim Press, HyangyakDaeSaJeon, pp740-741, 1998) and shows various pharmacological activities such as treating effect on amnesia, nervousness, asthma, schizophrenia etc (Kai et al., *Yakugaku Zasshi*, 124, pp.349-354, 2004).

[9]

[10] However, there has been not reported or disclosed on the preventing or treating activity of the compounds isolated from the flower extract of *Daphne genkwa* showing potent anti-cancer effect in any of above cited literatures, the disclosures of which are incorporated herein by reference.

[11]

[12] To investigate the anti-cancer effect of the compounds isolated from the flower extract of *Daphne genkwa* of the present invention, the inventors of present invention have intensively carried out *in vitro* experiment concerning the inhibition effect on the

human solid cancer cell lines such as lung cancer cell line (A549), colon carcinoma cell line (HCT116), breast cancer cell line (T47D), prostatic carcinoma cell line (PC-3), stomach cancer cell line (SNU-638), and liver cancer cell line (SK-HEP-1). As a result of the investigation, the inventors finally completed the present invention by confirming that the inventive compounds of the present invention strongly inhibited the cancer cell lines, especially, in lung cancer cell line, and it can be useful as a potent anti-cancer agent.

[13]

Disclosure of Invention

Technical Problem

[14] The present invention provides novel compounds represented by following general formula (I) and the pharmaceutically acceptable salts thereof.

[15]

[16] The present invention provides a pharmaceutical composition comprising the compound represented by following general formula (I) and the pharmaceutically acceptable salts thereof or treating or preventing cancer disease as an effective ingredient, together with a pharmaceutically acceptable carrier.

[17]

[18] The present invention also provides a use of the compound represented by following general formula (I) and the pharmaceutically acceptable salts thereof for the preparation of therapeutic agent for treating or preventing cancer disease in a mammal including human in need thereof.

[19]

[20] The present invention also provides a method for treating or preventing cancer disease comprising administering to mammal an effective amount of the compound represented by following general formula (I) and the pharmaceutically acceptable salts thereof as an effective ingredient, together with a pharmaceutically acceptable carrier thereof.

[21]

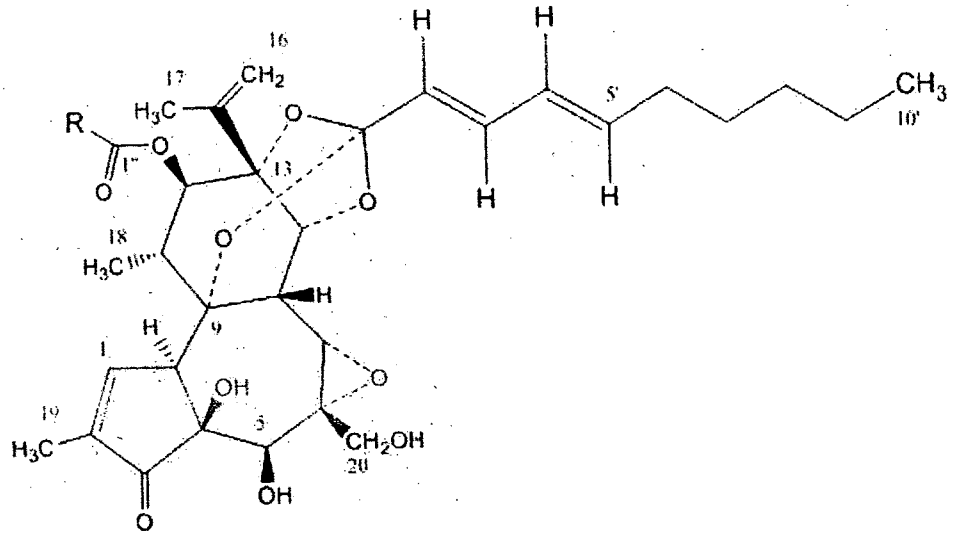
Technical Solution

[22] Accordingly, it is an object of the present invention to provide novel compound of following general formula (I) and the pharmaceutically acceptable salts thereof:

[23]

[24] [Formula 1]

[25]



[26]

[27] (I)

[28] wherein

[29] R is a hydrogen atom, C_1-C_{10} alkyl group or C_1-C_{10} alkoxyl group.

[30] Preferable compounds among the compounds of general formula (I) are the compounds wherein R group is a hydrogen atom, C_2-C_5 alkyl group or C_2-C_5 alkoxyl group and more preferably, wherein R is a hydrogen atom, ethyl group or propyl group.

[31] Most preferable compounds of the present invention are 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-propanoxyl-9,13,14-ortho-2E, 4E-dicardienoate or 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-utanoxyl-9,13,14-ortho-2E,4E-dicardienoate, which are isolated from the flower extract of *Daphne genkwa*.

[32]

[33] The present invention provides a pharmaceutical composition comprising the compound of the general formula (I) and the pharmaceutically acceptable salts thereof for treating or preventing cancer disease as an effective ingredient, together with a pharmaceutically acceptable carrier.

[34]

[35] The present invention also provides a use of the compound of general formula (I) and the pharmaceutically acceptable salts thereof for the preparation of therapeutic agent for treating or preventing cancer disease in a mammal including human in need thereof.

[36]

[37] The present invention also provides a method of treating or preventing cancer disease in human or mammal, wherein the method comprises administering a therapeutically effective amount of the compound of formula (I) or the pharmaceutically acceptable salts thereof as an effective ingredient, together with a pharmaceutically acceptable carrier thereof.

[38]

[39] The term "cancer disease" disclosed herein comprises lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer or cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors (e.g., uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina or carcinoma of the vulva), Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system (e.g., cancer of the thyroid, parathyroid or adrenal glands), sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphonas, cancer of the bladder, cancer of the kidney or ureter (e.g., renal cell carcinoma, carcinoma of the renal pelvis), or neoplasms of the central nervous system (e.g., primary CNS lymphoma, spinal axis tumors, brain stem gliomas or pituitary adenomas), preferably solid cancer such as lung cancer or blood cancer, more preferably, lung cancer, colon carcinoma, breast cancer, prostatic carcinoma, stomach cancer, or liver cancer, most preferably, lung cancer.

[40] The inventive compounds represented by general formula (I) can be transformed into their pharmaceutically acceptable salt and solvates by the conventional method well known in the art. For the salts, acid-addition salt thereof formed by a pharmaceutically acceptable free acid thereof is useful and can be prepared by the conventional method. For example, after dissolving the compound in the excess amount of acid solution, the salts are precipitated by the water-miscible organic solvent such as methanol, ethanol, acetone or acetonitrile to prepare acid addition salt thereof and further the mixture of equivalent amount of compound and diluted acid with water or alcohol such as glycol monomethylether, can be heated and subsequently dried by evaporation or filtrated under reduced pressure to obtain dried salt form thereof.

[41]

[42] As a free acid of above-described method, organic acid or inorganic acid can be used. For example, organic acid such as methansulfonic acid, *p*-toluensulfonic acid, acetic acid, trifluoroacetic acid, citric acid, maleic acid, succinic acid, oxalic acid, benzoic acid, lactic acid, glycolic acid, gluconic acid, galacturonic acid, glutamic acid, glutaric

acid, glucuronic acid, aspartic acid, ascorbic acid, carbonylic acid, vanillic acid, hydroiodic acid and the like, and inorganic acid such as hydrochloric acid, phosphoric acid, sulfuric acid, nitric acid, tartaric acid and the like can be used herein.

[43]

[44] Further, the pharmaceutically acceptable metal salt form of inventive compounds may be prepared by using base. The alkali metal or alkali-earth metal salt thereof can be prepared by the conventional method, for example, after dissolving the compound in the excess amount of alkali metal hydroxide or alkali-earth metal hydroxide solution, the insoluble salts are filtered and remaining filtrate is subjected to evaporation and drying to obtain the metal salt thereof. As a metal salt of the present invention, sodium, potassium or calcium salt are pharmaceutically suitable and the corresponding silver salt can be prepared by reacting alkali metal salt or alkali-earth metal salt with suitable silver salt such as silver nitrate.

[45]

[46] The pharmaceutically acceptable salt of the compound represented by general formula (I) comprise all the acidic or basic salt which may be present at the compounds, if it does not indicated specifically herein. For example, the pharmaceutically acceptable salt of the present invention comprise the salt of hydroxyl group such as the sodium, calcium and potassium salt thereof; the salt of amino group such as the hydrogen bromide salt, sulfuric acid salt, hydrogen sulfuric acid salt, phosphate salt, hydrogen phosphate salt, dihydrophosphate salt, acetate salt, succinate salt, citrate salt, tartarate salt, lactate salt, mandelate salt, methanesulfonate(mesylate) salt and *p*-toluenesulfonate (tosylate) salt etc, which can be prepared by the conventional method well known in the art.

[47]

[48] The inventive compounds isolated from the flower extract of *Daphne genkwa* can be prepared by following procedures.

[49] For example, the flower extract of *Daphne genkwa* is dried, cut, crushed and mixed with 1 to 50-fold, preferably, about 8-fold volume of distilled water, lower alcohols having 1 to 4 carbon atoms such as methanol, ethanol, butanol and the like, acetone, chloroform or the mixtures thereof, preferably, or the mixture solvent mixed with chloroform, acetone and methanol, more preferably, mixture solvent chloroform: acetone: methanol (1:1:1), and then is subjected to the extraction, reflux extraction, or ultra-sonication extraction, preferably, at the temperature ranging from room temperature to 100°C for the period ranging from 5 hours to 7 days and repeated 2 to 7 times, preferably 5 times, consecutively. The residue is filtered to obtain the supernatant to be concentrated with rotary evaporator, and then concentrated under reduced pressure to obtain crude extract of the present invention.

[50]

[51] Also, inventive compounds of the present invention can be prepared by following procedure, for example, the crude extract prepared by the above described step, is suspended in water or mixture solvent (methanol: chloroform=1:1, v/v%), to divide into the precipitate phase and supernatant phase and then the collected supernatant phase is concentrated and subjected to repeated Silica gel column chromatography and reverse phase-ODS flash column chromatography to isolate the inventive compounds of the present invention.

[52] Also, the above described procedures may be modified or subjected to further step to fractionate or isolate more potent fractions or compounds by conventional procedure well-known in the art, for example, the procedure disclosed in the literature (Harborne J. B. Phytochemical methods: *A guide to modern techniques of plant analysis*, 3rd Ed. pp6-7, 1998).

[53]

[54] Alternatively, the inventive compounds of the present invention may be also synthesized by the conventional synthetic method in accordance with a using method well known in the art (Herbert O. House, *Modern Synthetic Reactions*, 2nd Ed., The Benjamin/Cummings Publishing Co., 1972).

[55] Moreover, the ester, ether or salt of inventive compounds may be prepared by conventional method for synthesizing ester or ether well known in the art.

[56]

[57] To investigate the effect of the inventive compounds on the cancer disease, *in vitro* experiments concerning the inhibition effect on the human solid cancer cell lines such as lung cancer cell line (A549), colon carcinoma cell line (HCT116), breast cancer cell line (T47D), prostatic carcinoma cell line (PC-3), stomach cancer line (SNU-638) and liver cancer cell line (SK-HEP-1) were performed by the present inventors. As a result of the investigation, it has been confirmed that the inventive compounds of the present invention strongly inhibited the cancer cell lines, especially, in lung cancer cell line, and it can be useful as a potent anti-cancer agent.

[58]

[59] The present invention also provides a pharmaceutical composition comprising an efficient amount of the compound represented by general formula (I) or the pharmaceutically acceptable salt thereof as an active ingredient in amount effective to treat or prevent cancer diseases together with pharmaceutically acceptable carriers or diluents.

[60]

[61] The pharmaceutical composition of the present invention can contain about 0.01 ~ 50 % by weight of the above extract based on the total weight of the composition.

[62]

[63] The compound of formula (I) according to the present invention can be provided as a pharmaceutical composition containing pharmaceutically acceptable carriers, adjuvants or diluents. For example, the compounds of the present invention can be dissolved in oils, propylene glycol or other solvents which are commonly used to produce an injection. Suitable examples of the carriers include physiological saline, polyethylene glycol, ethanol, vegetable oils, isopropyl myristate, etc., but are not limited to them. For topical administration, the compounds of the present invention can be formulated in the form of ointments and creams.

[64]

[65] Hereinafter, the following formulation methods and excipients are merely exemplary and in no way limit the invention.

[66]

[67] The compounds of the present invention in pharmaceutical dosage forms may be used in the form of their pharmaceutically acceptable salts, and also may be used alone or in appropriate association, as well as in combination with other pharmaceutically active compounds.

[68]

[69] The compounds of the present invention may be formulated into preparations for injections by dissolving, suspending, or emulsifying them in aqueous solvents such as normal saline, 5% Dextrose, or non-aqueous solvent such as vegetable oil, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol. The formulation may include conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

[70]

[71] The desirable dose of the inventive compounds varies depending on the condition and the weight of the subject, severity, drug form, route and period of administration, and may be chosen by those skilled in the art. However, in order to obtain desirable effects, it is generally recommended to administer at the amount ranging 0.0001 - 100 mg/kg, preferably 0.001 - 100 mg/kg by weight/day of the inventive compounds of the present invention. The dose may be administered in single or divided into several times per day. In terms of composition, the compounds should be present between 0.0001 to 10% by weight, preferably 0.0001 to 1% by weight based on the total weight of the composition.

[72]

[73] The pharmaceutical composition of present invention can be administered to a subject animal such as mammals (rat, mouse, domestic animals or human) *via* various routes. All modes of administration are contemplated, for example, administration can be made orally, rectally or by intravenous, intramuscular, subcutaneous, intrathecal,

epidural or intracerebroventricular injection.

[74]

[75] The inventive compounds of the present invention also can be used as a main component or additive and aiding agent in the preparation of various functional health food and health care food.

[76]

[77] Accordingly, it is the other object of the present invention to provide a functional health food comprising the compounds of general formula (I), or the pharmacologically acceptable salt thereof for aiding cancer chemotherapy.

[78]

[79] The term "a functional health food" defined herein the functional food having enhanced functionality such as physical functionality or physiological functionality by adding the compound of the present invention to conventional food to prevent or improve cancer disease in human or mammal.

[80]

[81] It is the other object of the present invention to provide a health care food comprising the compounds of general formula (I), or the pharmacologically acceptable salt thereof, together with a sitologically acceptable additive for the prevention and alleviation of cancer disease.

[82]

[83] The term "a health care food" defined herein the food containing the compound of the present invention showing no specific intended effect but general intended effect in a small amount of quantity as a form of additive or in a whole amount of quantity as a form of capsule, pill, tablet etc.

[84]

[85] The term "a sitologically acceptable additive" defined herein any substance the intended use which results or may reasonably be expected to result-directly or in-directly-in its becoming a component or otherwise affecting the characteristics of any food for example, thickening agent, maturing agent, bleaching agent, sequesterants, humectant, anticaking agent, clarifying agents, curing agent, emulsifier, stabilizer, thickner, bases and acid, foaming agents, nutrients, coloring agent, flavoring agent, sweetner, preservative agent, antioxidant, etc, which had been well-known in the art.

[86] If a substance is added to a food for a specific purpose in that food, it is referred to as a direct additive and indirect food additives are those that become part of the food in trace amounts due to its packaging, storage or other handling.

[87]

[88] The term "special nutritional food" defined herein the food containing the compound of the present invention showing specific intended effect by supplementing a substitute

food such as a baby food or nutrient meal used in the baby or patient in need of special nutrient or ingredients as a form of food.

[89]

[90] Above described health foods can be contained in food, health beverage, dietary therapy etc, and may be used as a form of powder, granule, tablet, chewing tablet, capsule, beverage etc for preventing or improving cancer disease.

[91]

[92] Also, above described compounds can be added to food or beverage for prevention and improvement of cancer disease. The amount of above described compound in food or beverage as a functional health food or health care food may generally range from about 0.01 to 100 w/w % of total weight of food for functional health food composition. In particular, although the preferable amount of the compound of the present invention in the functional health food, health care food or special nutrient food may be varied in accordance to the intended purpose of each food, it is preferably used in general to use as a additive in the amount of the compound of the present invention ranging from about 0.01 to 5% in food such as noodles and the like, from 40 to 100% in health care food on the ratio of 100% of the food composition.

[93]

[94] Providing that the health beverage composition of present invention contains above described compound as an essential component in the indicated ratio, there is no particular limitation on the other liquid component, wherein the other component can be various deodorant or natural carbohydrate etc such as conventional beverage. Examples of aforementioned natural carbohydrate are monosaccharide such as glucose, fructose etc; disaccharide such as maltose, sucrose etc; conventional sugar such as dextrin, cyclodextrin; and sugar alcohol such as xylitol, and erythritol etc. As the other deodorant than aforementioned ones, natural deodorant such as taumatin, stevia extract such as levaudioside A, glycyrrhizin et al., and synthetic deodorant such as saccharin, aspartam et al., may be useful favorably. The amount of above described natural carbohydrate is generally ranges from about 1 to 20 g, preferably 5 to 12 g in the ratio of 100ml of present beverage composition.

[95]

[96] The other components than aforementioned composition are various nutrients, a vitamin, a mineral or an electrolyte, synthetic flavoring agent, a coloring agent and improving agent in case of cheese, chocolate et al., pectic acid and the salt thereof, alginic acid and the salt thereof, organic acid, protective colloidal adhesive, pH controlling agent, stabilizer, a preservative, glycerin, alcohol, carbonizing agent used in carbonate beverage et al. The other component than aforementioned ones may be fruit juice for preparing natural fruit juice, fruit juice beverage and vegetable beverage,

wherein the component can be used independently or in combination. The ratio of the components is not so important but is generally range from about 0 to 20 w/w % per 100 w/w % present composition. Examples of addable food comprising aforementioned extract therein are various food, beverage, gum, vitamin complex, health improving food and the like.

[97]

[98] It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, use and preparations of the present invention without departing from the spirit or scope of the invention.

[99]

[100] The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

[101]

Advantageous Effects

[102] To investigate the anti-cancer effect of the compounds isolated from the flower extract of *Daphne genkwa* of the present invention, the inventors of present invention have intensively carried out *in vitro* experiment concerning the inhibition effect on the human solid cancer cell lines such as lung cancer cell line (A549), colon carcinoma cell line (HCT116), breast cancer cell line (T47D), prostatic carcinoma cell line (PC-3), stomach cancer cell line (SNU-638), and liver cancer cell line (SK-HEP-1). As a result of the investigation, the inventors finally completed the present invention by confirming that the inventive compounds of the present invention strongly inhibited the cancer cell lines, especially, in lung cancer cell line, and it can be useful as a potent anti-cancer agent.

[103]

Brief Description of the Drawings

[104] The above and other objects, features and other advantages of the present invention will more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which;

[105]

[106] Fig. 1 shows the inhibitory activities of inventive compound on the growth of human lung cancer cell and normal lung cell;

[107] Fig. 2 depicts the observed pictures showing that inventive compounds strongly inhibited the growth of human lung cancer cell.

[108]

Best Mode for Carrying Out the Invention

[109] It will be apparent to those skilled in the art that various modifications and variations can be made in the compositions, use and preparations of the present invention without departing from the spirit or scope of the invention.

[110]

[111] The present invention is more specifically explained by the following examples. However, it should be understood that the present invention is not limited to these examples in any manner.

[112]

Mode for the Invention

[113] The following Reference Example, Examples and Experimental Examples are intended to further illustrate the present invention without limiting its scope.

[114]

[115] **Example 1. Preparation of inventive compounds**

[116]

[117] 1-1. Preparation of crude extract

[118] 10kg of dried flower of *Daphne genkwa* distributed in Korea was mixed with 40 L of mixture solvent (chloroform: acetone: methanol= 1:1:1 [v/v/v]) and extracted for 3 hours at room temperature with ultra-sonificator. The above extraction steps were repeated three times to collect supernatant, and the collected supernatants were concentrated under reduced pressure to obtain 386g of crude extract.

[119]

[120] 1-2. Preparation of WH extract

[121] The crude extract prepared in Example 1-1 was suspended in the solvent mixture solvent (methanol: chloroform= 2:8 [v/v]) and performed to fractionation. The resulting precipitate was removed and the remaining supernatant was collected to obtain 216g of purified fraction soluble in mixture solvent mixed with methanol and chloroform (designated as "WH extract" hereinafter).

[122]

[123] 1-3. Preparation of inventive compounds

[124] WH extract prepared in Example 1-2 was subjected to Silica gel column chromatography eluted with a stepwise application of solvent mixture containing linear gradient of chloroform: methylene chloride (1:1, v/v) and chloroform:methanol (1:1, v/v) to give 3 sub-fractions. Among the 3 fractions, the 2nd fraction showing strongest anti-cancer activity, was further loaded onto the ODS flash chromatography using 50-100% methanol to give 4 sub-fractions. Among the 4 fractions, the 3rd fraction was performed to Silica gel column chromatography using by linear gradient of acetone: methylene chloride (5:100 → 30:100, v/v) to give 6 sub-fractions. Among the

fractions, the 3rd fraction was further subjected to ODS HPLC using by 93% methanol to afford inventive compounds showing following physicochemical properties, i.e. 40mg of 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-propanoxyl-9,13,14-ortho-2E, 4E-dicardienoate (**1**; designated as "YH1" hereinafter) and 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-utanoxyl-9,13,14-ortho-2E,4E-dicardienoate (**2**, designated as "YH2" hereinafter).

[125]

[126] 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-propanoxyl-9,13,14-ortho-2E, 4E-dicardienoate (1)

[127]

[128] White amorphous form: $C_{33}H_{44}O_{10}$

[129] HRESI-MS: m/z-601 $[M+H]^+$, 623 $[M+Na]^+$,

[130]

[131] ^{1}H-NMR (300MHz, $CDCL_3$ solvent): *See* Table 1

[132]

[133] ^{13}C-NMR (300MHz, $CDCL_3$ solvent): *See* Table 1

[134]

[135] 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-utanoxyl-9,13,14-ortho-2E,4E-dicardienoate (2)

[136]

[137] White amorphous form: $C_{34}H_{46}O_{10}$

[138] HRESI-MS: m/z-615 $[M+H]^+$, 637 $[M+Na]^+$,

[139]

[140] ^{1}H-NMR (300MHz, $CDCL_3$ solvent): *See* Table 2

[141]

[142] ^{13}C-NMR (300MHz, $CDCL_3$ solvent): *See* Table 2

[143]

[144] Table 1

[Table 1]

[Table]

Assignment	^{13}C (ppm)	^{1}H (ppm)
1	160.7	7.57 (1H, br. s)
2	137.1	
3	209.7	
4	72.7	
5	72.4	4.26 (1H,s)
6	60.7	
7	64.3	3.56 (1H,s)
8	35.6	3.51 (1H, d, 2.7)
9	78.3	
10	47.7	3.92 (1H, br.s)
11	44.3	2.36 (1H, g, 7.2)
12	78.3	4.99 (1H, s)
13	83.9	
14	80.7	4.76 (1H, d, 2.7)
15	143.3	
16	113.6	5.01 & 4.95 (2H, br. s)
17	18.9	1.84 (3H, s)
18	18.5	1.31 (3H, d, 7.2)
19	10.1	1.80 (3H, dd, 2.7, 1.2)
20	65.2	3.94 & 3.81 (2H, d, 11.7)
1'	117.2	
2'	122.5	5.65 (1H, d, 16.5)
3'	135.5	6.67 (1H, dd, 16.5, 10.5)
4'	128.8	6.05 (1H, 15.0, 10.5)
5'	139.6	5.87 (1H, m)
6'	32.9	2.10 (2H, q, 7.2)
7'	28.9	1.39 (2H, m)
8'	31.5	1.26 (2H, m)

9'	22.7	1.31 (2H, overlapped)
10'	14.2	0.89 (3H, t, 6.9)
1"	173.4	
2"	28.0	2.25 (2H, q, 7.5)
3"	9.2	1.09 (3H, t, 7.5)

[145]

[146] Table 2

[Table 2]

[Table]

Assignment	^{13}C (ppm)	^{1}H (ppm)
1	159.4	7.36 (1H, br. s)
2	136.8	
3	208.9	
4	72.7	
5	70.2	4.00 (1H,d. 2.1)
6	61.5	
7	63.8	3.33 (1H,d. 2.1)
8	35.2	3.32 (1H, 3.9)
9	78.3	
10	47.3	3.70 (1H, br. s)
11	43.8	2.24 (1H, m)
12	78.1	4.08 (1H, s)
13	83.6	
14	80.3	4.61 (1H, d, 2.7)
15	143.0	
16	113.2	4.85 & 4.79 (2H, br. s)
17	18.4	1.67 (3H, s)
18	18.0	1.12 (3H, d, 7.2)
19	9.6	1.61 (3H, br. d. 12.3)
20	64.7	3.56 & 3.76 (2H, br. d, 12.3)
1'	116.9	
2'	122.2	5.46 (1H, d, 15.3)
3'	135.0	6.49 (1H, dd, 15.3, 10.5)
4'	128.4	5.88 (1H, m)
5'	139.3	5.70 (1H, m)
6'	32.5	1.94 (2H, q, 6.9)
7'	28.5	1.21 (2H, m)
8'	31.2	1.14 (2H, m)

9'	22.3	1.10 (2H, m)
10'	13.7	0.72 (3H, t, 6.6)
1"	172.9	
2"	36.2	2.06 (2H, t, 6.6)
3"	18.0	1.42 (2H, m)
	13.3	0.75 (3H, t, 7.5)

[147]

[148] **Experimental Example 1. *In vitro* inhibitory test on the growth of human cancer cell**

[149]

[150] The inhibitory effect of the inventive compounds prepared in Example 1 (test sample) on the growth of cancer cell line was determined by following sulforhodamine B test disclosed in the literature (Lee et al., Chemico-Biol. Interact., pp215-228, 1998).

[151]

[152] The human solid cancer cell lines procured by Korean Cell Line Bank (Seoul, Korea), i.e., lung cancer cell line (A549), colon carcinoma cell line (HCT116), breast cancer cell line (T47D), prostatic carcinoma cell line (PC-3), stomach cancer line (SNU-638), and liver cancer cell line (SK-HEP-1), were performed to sub-culture with RPMI/MEME/DMEM medium supplemented with 10% FBS, 1% PSF etc. 10 microliter of test sample solution dissolved in 10% DMSO and 190 microliter of cell suspension (5x 10^4 cells/ml) were added to 96-well plates and incubated for 3 days. The 16 well plates containing the cell suspension were incubated for 30 mins to use as a zero-day control. The incubated cells were fixed with 10% TCA (trichloroacetic acid) and stained with SRB solution. The staining solution was dissolved in 10mM Tris-base and the absorbance of the cells was determined at 515 nm. The group treated with 10% DMSO was used as a negative control and the cell survival rate according to the concentrations of test samples was calculated by following math formula 1.

[153]

[154] [Math Formula 1]

[155]

[156] Cell survival rate (%)= [OD(sample) - OD(0-day)] / [OD(10% DMSO) - OD(0-day)] x 100

[157]

[158] The value of test sample group was expressed as the percentage for control group where the value of negative control group was set to 100%, and the treatment of each test sample was calculated by mean value ± SEM of triple tests.

[159]

[160] The inhibitory effect of test samples on human cancer cell lines and normal human cell line was shown in Tables 3, 4 and Fig. 1.

[161]

[162] At the result, as can be seen in Table 3, the inventive compounds selectively inhibited the cancer cell lines in a dose dependent manner and IC_{50} for human lung cancer cell line and other cancer cell lines showed 0.7-53 nM and about 10 μM, respectively, which confirmed that the inventive compounds have more than 10,000 fold selectivity for lung cancer cell line compared with other cancer cell lines.

[163]

[164] As can be seen in Table 4, the inventive compounds showed more decreased inhibitory effect on the normal human lung cell compared with human cancer cell line, which showed the reduced toxicity of inventive compounds.

[165]

[166] Table 3

[Table 3]

[Table]

Origin	Cell line	IC_{50} (μM)	
		YH1	YH2
lung cancer	A549	5.3×10^{-2}(53 nM)	2.3×10^{-2}(23 nM)
colon carcinoma	HCT116	13.2	15.8
breast cancer	T47D	11.9	10.3
prostatic carcinoma	PC-3	8.2	9.3
stomach cancer	SNU-638	19.1	18.3
liver cancer	SK-HEP-1	10.9	10.5

[167]

[168] Table 4

[Table 4]

[Table]

Origin	Cell line	IC_{50} (μM)	
		YH1	YH2
lung cancer	A549	5.3×10^{-3}	2.3×10^{-3}
Normal lung	MRC-5	12.5	14

[169]

[170] **Experimental Example 2. Observation of cell morphology**

[171]

[172] Human lung cancer cell line (A549) was suspended in 10% FBS-MEME (2x 10^5 cells/ml) and 10ml of the cell suspension was inoculated into cell culture dish (10cm). Various concentrations of YH 1 prepared in Example 1 were added thereto and incubated for 48 hours at 37 ℃ in 50% CO_2 condition. After incubating, the culture medium was removed, and washed with PBS twice. The cell morphology was observed by microscope (x100) and the result was shown in Fig. 2.

[173]

[174] As can be seen in Fig. 2, the cell number of test sample group treated with YH1 was more decreased compared with that of negative control group.

[175]

[176] Hereinafter, the formulating methods and kinds of excipients will be described, but the present invention is not limited to them. The representative preparation examples were described as follows.

[177]

[178] Preparation of injection

[179] YH1 100mg

[180] Sodium metabisulfite 3.0mg

[181] Methyl paraben 0.8mg

[182] Propyl paraben 0.1mg

[183] Distilled water for injection optimum amount

[184] Injection preparation was prepared by dissolving active component, controlling pH to about 7.5 and then filling all the components in 2ml ample and sterilizing by conventional injection preparation method.

[185]

[186] Preparation of powder

[187] YH2 500mg

[188] Corn Starch 100mg

[189] Lactose 100mg

[190] Talc 10mg

[191] Powder preparation was prepared by mixing above components and filling sealed package.

[192]

[193] Preparation of tablet

[194] YH1 200mg

[195] Corn Starch 100mg

[196] Lactose 100mg

[197] Magnesium stearate optimum amount

[198] Tablet preparation was prepared by mixing above components and entabletting.

[199]

[200] Preparation of capsule

[201] YH 2 100mg

[202] Lactose 50mg

[203] Corn starch 50mg

[204] Talc 2mg

[205] Magnesium stearate optimum amount

[206] Tablet preparation was prepared by mixing above components and filling gelatin capsule by conventional gelatin preparation method.

[207]

[208] Preparation of liquid

[209] YH 1 1000mg

[210] Sugar 20g

[211] Polysaccharide 20g

[212] Lemon flavor 20g

[213] Liquid preparation was prepared by dissolving active component, and then filling all the components in 1000ml ample and sterilizing by conventional liquid preparation method.

[214]

[215] The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the present invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

[216]

Industrial Applicability

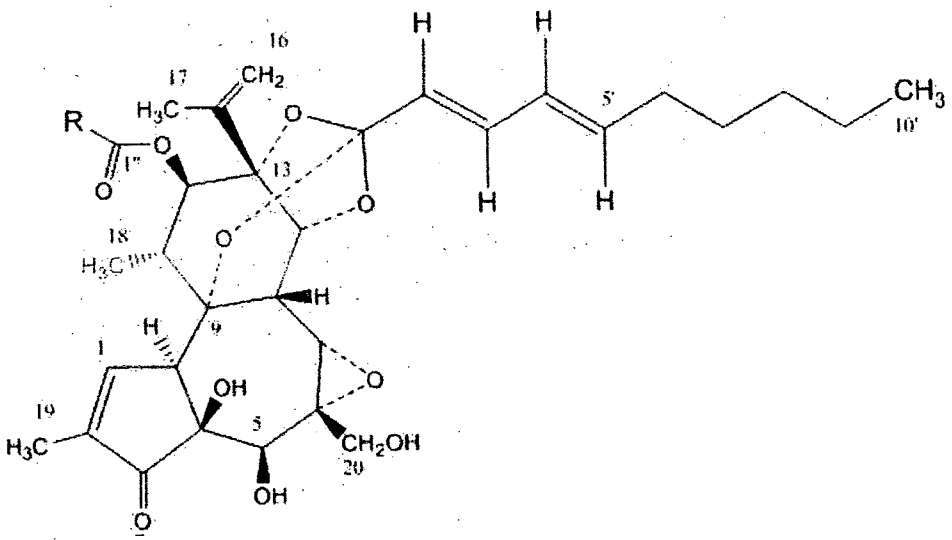
[217] As described in the present invention, the inventive compounds strongly inhibit the growth of cancer cell lines such as lung cancer cell line, colon carcinoma cell, breast cancer cell line, prostatic carcinoma cell line, stomach cancer line, and liver cancer cell line. Therefore, they can be useful in treating and preventing cancer disease as a medicament and health food composition.

[218]

Claims

[1] A novel compounds of following general formula (I) and the pharmaceutically acceptable salts thereof:

[Formulae 1]



(I)

wherein

R is a hydrogen atom, C_1-C_{10} alkyl group or C_1-C_{10} alkoxyl group.

[2] The compound of according to claim 1, wherein R group of said compounds of general formula (I) is a hydrogen atom, C_2-C_5 alkyl group or C_2-C_5 alkoxyl group.

[3] The compound of according to claim 2, wherein R group of said compounds of general formula (I) is a hydrogen atom, ethyl group or propyl group.

[4] The compound of according to claim 3, wherein said compound is 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-propanoxyl-9,13,14-ortho-2E, 4E-dicardienoate or 5-beta-hydroxyresinifegonol-6-alpha, 7-alpha-epoxy-12-beta-utanoxyl-9,13,14-ortho-2E,4E-dicardienoate, which are isolated from the flower extract of *Daphne genkwa*.

[5] A pharmaceutical composition comprising the compound of general formula (I) as set forth in claim 1 and the pharmaceutically acceptable salts thereof for treating or preventing cancer disease as an effective ingredient, together with a pharmaceutically acceptable carrier.

[6] The pharmaceutical composition according to claim 5, wherein said cancer disease is selected from the group of lung cancer, bone cancer, pancreatic cancer, skin cancer, cancer of the head and neck, cutaneous or intraocular melanoma,

uterine cancer, ovarian cancer, rectal cancer or cancer of the anal region, stomach cancer, colon cancer, breast cancer, gynecologic tumors such as uterine sarcomas, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina and carcinoma of the vulva, Hodgkin's disease, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system such as cancer of the thyroid, parathyroid or adrenal glands, sarcomas of soft tissues, cancer of the urethra, cancer of the penis, prostate cancer, chronic or acute leukemia, solid tumors of childhood, lymphocytic lymphomas, cancer of the bladder, cancer of the kidney or ureter such as renal cell carcinoma, and carcinoma of the renal pelvis, or neoplasms of the central nervous system such as primary CNS lymphoma, spinal axis tumors, and brain stem gliomas and pituitary adenomas.

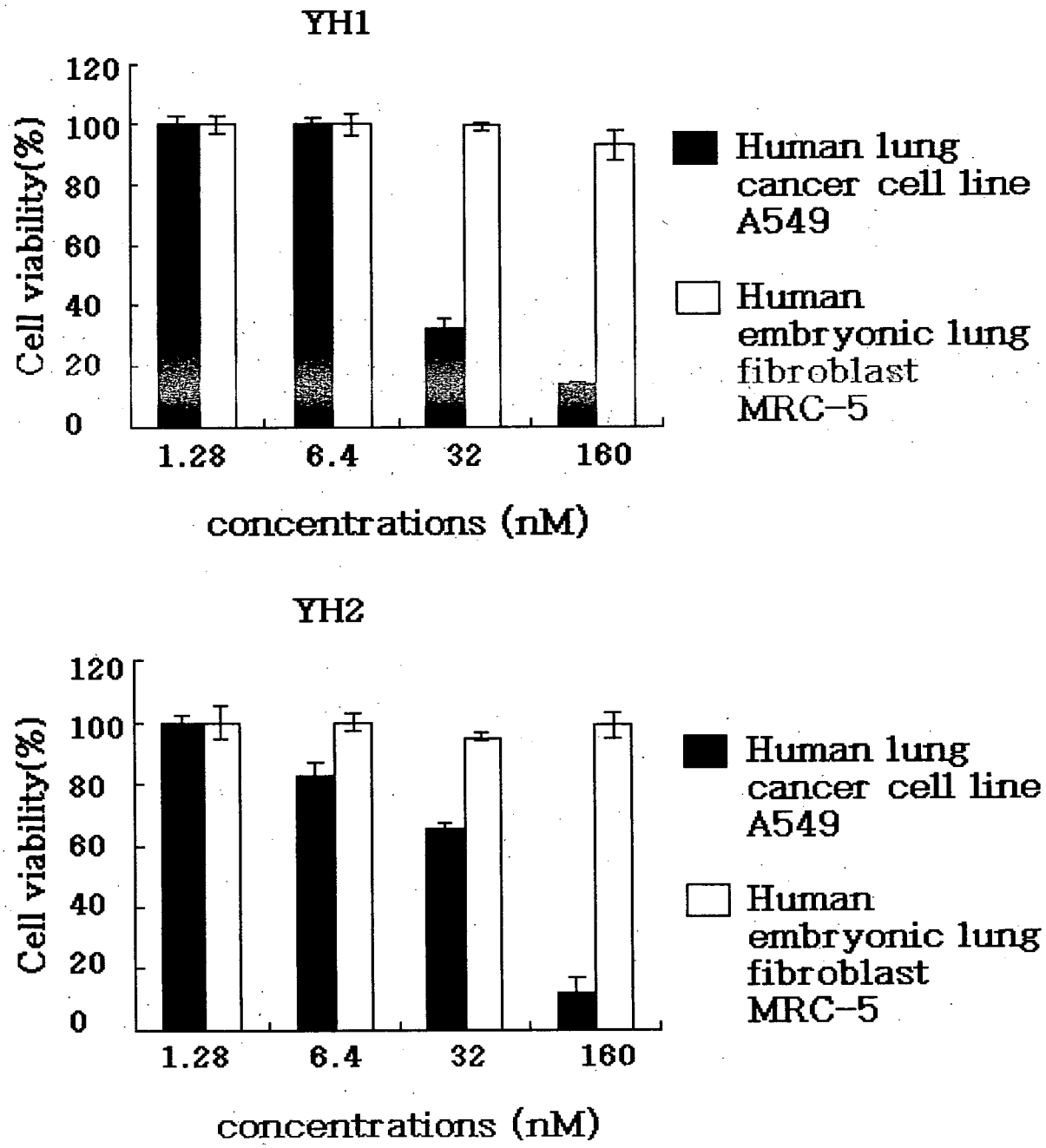
[7] The pharmaceutical composition according to claim 6, wherein said cancer disease is selected from lung cancer, colon carcinoma, breast cancer, prostatic carcinoma, stomach cancer, or liver cancer.

[8] A use of the compound of general formula (I) as set forth in claim 1 and the pharmaceutically acceptable salts thereof for the preparation of therapeutic agent for treating or preventing cancer disease in a mammal including human in need thereof.

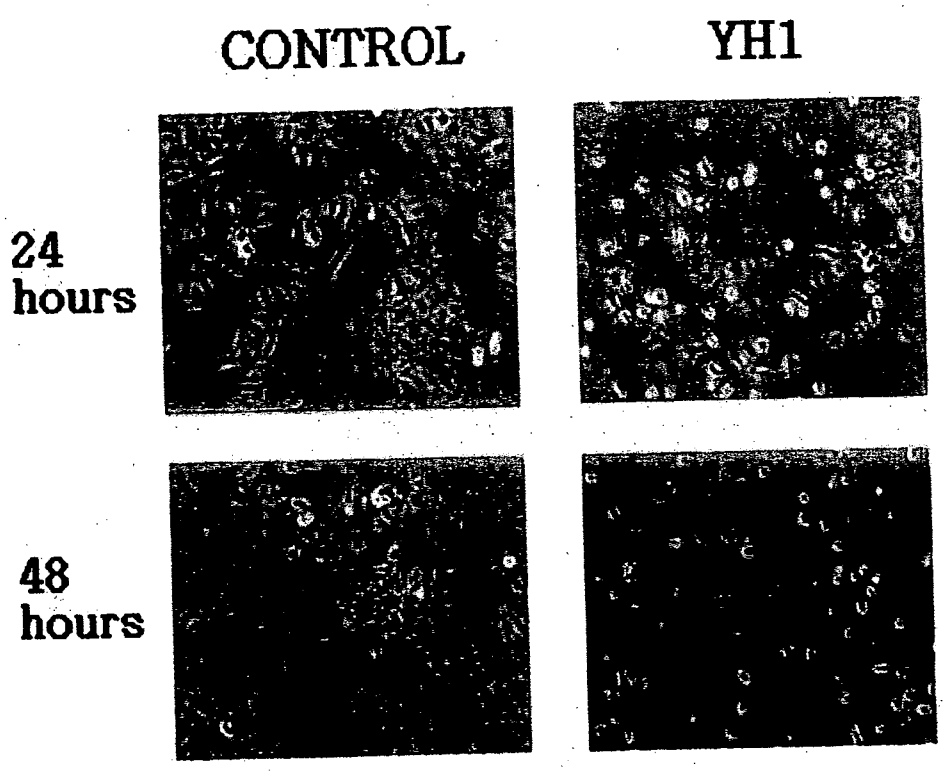
[9] A method of treating or preventing cancer disease in human or mammal, wherein the method comprises administering a therapeutically effective amount of the compound of general formula (I) as set forth in claim 1 or the pharmaceutically acceptable salts thereof as an effective ingredient, together with a pharmaceutically acceptable carrier thereof.

[10] A health care food comprising the compound of general formula (I) as set forth in claim 1 and the pharmaceutically acceptable salts thereof as an effective ingredient, together with a sitologically acceptable additive for the prevention and alleviation of cancer disease.

[Fig. 1]


YH1
120
100
80
60
40
20
0
Cell viability(%)
1.28
6.4
32
160
concentrations (nM)
Human lung cancer cell line A549
Human embryonic lung fibroblast MRC-5
YH2
120
100
80
60
40
20
0
Cell viability(%)
1.28
6.4
32
160
concentrations (nM)
Human lung cancer cell line A549
Human embryonic lung fibroblast MRC-5

[Fig. 2]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/006647

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/357(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKIPASS(KIPO internal), STN(REG, CAplus), PubMed, JPO, USPTO, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEIDONG HE, et al., Neurotrophic and antileukemic daphnane diterpenoids from Synaptolepis kirkii. Bioorganic & Medicinal Chemistry. October 2002, 10(10), pp.3245-3255 See the abstract, page 3245~3246, 3249, Table 5	1-8, 10
X	SHIXUAN ZHANG, et al., Preparation of yuanhuacine and relative daphne diterpene esters from Daphne genkwa and structure-activity relationship of potent inhibitory activity against DNA topoisomerase I. Bioorganic & Medicinal Chemistry. 1 June 2006, 14(11), pp.3888-3895 See the abstract, page 3892~3894, Figure 1, Table 3	1-8, 10
X	ZHA-JUN ZHAN, et al., Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant Daphne genkwa. Bioorganic & Medicinal Chemistry. 1 February 2005, 13(3), pp.645-655 See the abstract, page 652~653, Figure 1, Table 6	1-8, 10

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 APRIL 2009 (22.04.2009)	**22 APRIL 2009 (22.04.2009)**

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seonsa-ro, Seo-gu, Daejeon 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

Kim, Bum Soo

Telephone No. 82-42-481-5412

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/006647

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 9
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 9 perains to methods for treatment of the human or animal body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.