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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 April 2009 (16.04.2009)

PCT

(10) International Publication Number
WO 2009/048195 A1

(51) International Patent Classification:
A61K 31/05 (2006.01)

(21) International Application Number:
PCT/KR2007/005834

(22) International Filing Date:
20 November 2007 (20.11.2007)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
10-2007-0101292 9 October 2007 (09.10.2007) KR

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, 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, 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, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

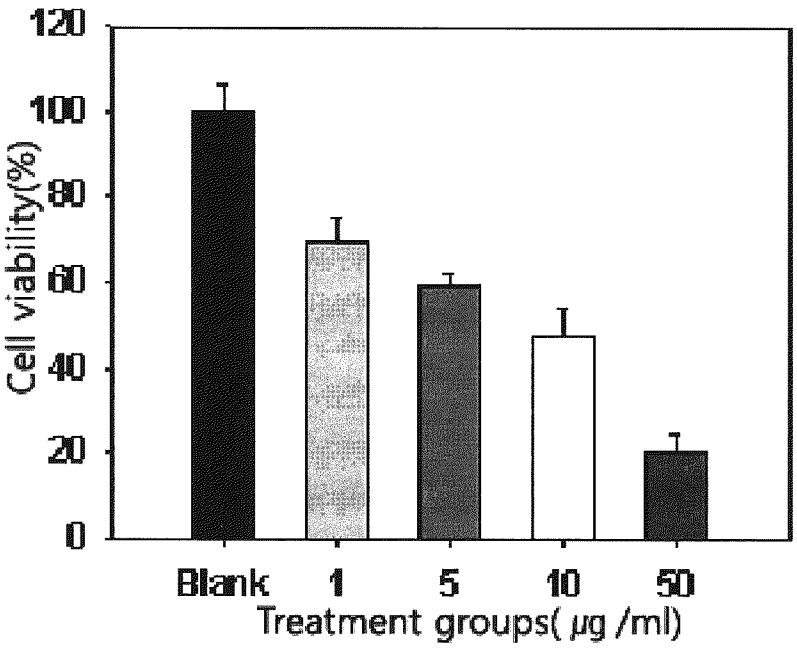
Declaration under Rule 4.17:

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[Continued on next page]

(54) **Title:** LAURINTEROL COMPOUND DERIVED FROM LAURENCIA OKAMURAI FOR THE PREVENTION AND INHIBITION OF MELANOMA

【Figure 1】



(57) **Abstract:** The present invention relates to a pharmaceutical composition for preventing and inhibiting of melanoma comprising a laurinterol compound from Laurencia okamurai as an effective constituent, in which the composition is a natural medicine containing natural effective constituent, which can effectively inhibit the growth of melanoma cells by inducing apoptosis therein without adverse effect as in synthetic medicines.

WO 2009/048195 A1

Published:
— *with international search report*

【DESCRIPTION】

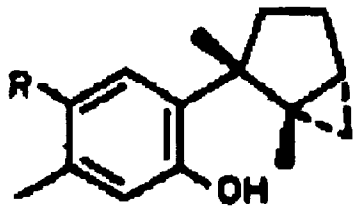
【Invention Title】

LAURINTEROL COMPOUND DERIVED FROM LAURENCIA OKAMURAI FOR THE PREVENTION AND INHIBITION OF MELANOMA

【Technical Field】

<1> The present invention relates to a laurinterol compound from *Laurencia okamurai*, more specifically, to a laurinterol compound of formula 1 from *Laurencia okamurai* having the prevention and the inhibition activity against to the growth of melanoma by inducing apoptosis in melanoma cells, and pharmaceutical composition for preventing and inhibiting of melanoma comprising the same as an effective constituent.

<2> <Formula 1>



R = Br
R = H

<3>

【Background Art】

<4> Melanoma cells can undergo self-destruction via apoptosis that occurs by apoptotic regulators such as p53, PUMA, Bax, AKT1 and Fas/FasL. These regulators in melanoma may be potential therapeutic target to develop anticancer agents. Especially, the importance for p53-induced apoptosis in tumor suppression has been increased by some human tumor-derived p53 mutants that have a selective loss-of-function only for apoptosis but not for the G1 arrest pathway. It has been known that p53 as a guardian of genomic stability has been known to play important roles in cell cycle arrest at the G1/S transition, which inhibits replication of damaged DNA and induction of apoptosis to destroy cells with defective DNA.

<5> After DNA damage, the p53 protein is separated from Mdm2, and get phosphorylated and acetylated, resulting in the activation of p53 in the cells. These event cause expression of proteins involved in the mitochondrial and the death-receptor-induced apoptotic pathways. In the first place, p53 can induce several proteins residing in the mitochondria, such as Bax, Noxa and PUMA, which induce mitochondrial release of cytochrome, resulting in activation of caspase 9 necessary for activation of caspase 3.

<6> Then, caspase 3 activates caspase-activated deoxy ribonuclease and subsequent internucleosomal DNA degradation. Caspase 3 also can activate other caspases, such as caspases 6 and 7. Otherwise p53 can trigger expression of death receptors, such as Fas, and the death-domain containing protein PIDD (pro-apoptotic interacting domain). This induction subsequently results in activation of the apoptosis machinery leading to cell death.

<7> Although the incidence of malignant melanoma as a severe skin cancer has been recently increased in human beings, any effective anticancer agent for metastatic melanoma has not been developed until now. The reason is that melanoma has resistance to chemotherapy.

<8> Recent studies have reported that many anticancer drugs exert their anticancer effects through apoptosis and that apoptosis genes can mediate chemosensitivity. Therefore, it is required to develop new therapeutic strategies to overcome the ineffectiveness of current chemotherapeutic agents for malignant melanoma.

<9> Many natural compounds have been reported to induce apoptosis without the side effects of synthetic drugs and they could be used as potentially chemopreventive and chemotherapeutic agents.

<10> In recent years, many chemopreventive agents appear to target signaling intermediates in apoptosis-inducing pathways, and much attention has been focused on the anticancer properties of marine natural constituents that play an important role in the prevention and treatment of cancer.

<11> However, there are still vast fields to be explored in marine algae to develop anticancer agent.

<12> Among marine algae, *Laurencia okamurai* (hereinafter refer to *L. okamurai*) was known to contain various sesquiterpenes including debromolaurinterol and laurinterol which are a major halogenated sesquiterpene. Among them, some of halogenated metabolites such as Laurinterol (hereinafter refer to LOEL) was reported to have antimicrobial activity. However, any information on effect of *L. okamurai* extract containing LOEL on induction of apoptosis has not been reported until now.

<13> Therefore, in the result of a detailed study to investigate effects of laurinterol on induction of apoptosis in melanoma cells, we can achieve the present invention.

【Disclosure】

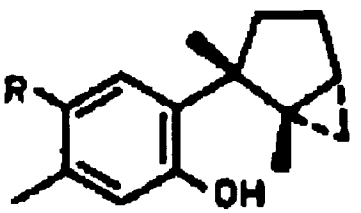
【Technical Problem】

<14> The object of the present invention can be achieved by screening marine algae having anticancer activity against melanoma cells from marine source and finding the fact that *L. okamurai* extract containing LOEL can effectively inhibit the growth of melanoma cells by inducing apoptosis therein.

【Technical Solution】

<15> The present invention provides a laurinterol compound of formula 1 from *Laurencia okamurai* having the prevention and the inhibition activity against melanoma by inducing apoptosis therein:

<16> <Formula 1>



R = Br
R = H

<17>

<18> The present invention also provides a pharmaceutical composition for preventing and inhibiting melanoma comprising the laurinterol compound of

formula 1 from *Laurencia okamurai*as as effective constituent, in which the growth of melanoma cells is inhibited by inducing apoptosis therein.

<19> In the present invention, the inhibitory effect of LOEL on cell growth was evaluated in B16F1 cells. As a consequence, LOEL clearly exhibited a dose-dependent inhibitory effect on the growth of melanoma cells. This finding is supported by the report that a halogenated compound such as LOEL plays an important role in seaweed chemical defense. Not only L. okamurai has been used an edible seaweed in Korea but also an active compound contained in this red algae exerts the anticancer effect on melanoma cells, suggesting that L. okamurai may provide a potential source to prevent or treat melanoma.

<20> In the present invention, to examine whether LOEL can induce apoptosis in melanoma cells, cell cycle analysis, DNA ladder assay and TUNEL assay as apoptosis markers were carried out in B16F1 cells. As a consequence, LOEL showed clear DNA ladder and the increase of green fluorescence intensity in nucleus reflecting DNA fragmentation, indicating that apoptotic response occurred in melanoma cells. Cell cycle analysis revealed the increase of cell numbers in sub-G1 phase by DNA fragmentation.

<21> Based on the results of thepresent invention, it is suggested that the anticancer effect of LOEL is due to apoptosis in melanoma cells. This finding was consistent with previous report that apoptosis is distinguished from necrosis by a characteristic set of features including membrane blebbing, shrinkage of the cell, chromatin condensation and nuclear fragmentation by cleaving the DNA into fragments by endonucleases.

<22> Moreover, caspase has been shown to play a key role in apoptosis process induced by many stimuli. Therefore, in order to further confirm the above findings, the activity of several caspases required for induction of apoptosis was investigated in the presence of LOEL.

<23> In the present invention, it is found that both initiator and effector caspases were activated melanoma cells treated with LOEL. Especially, activity of caspase-3, -5, -6 and -8 was increased, which was consistent with the previous reports that among caspases, caspase-3 as an effector caspase,

was the most commonly activated caspase in the apoptosis process and caspase-9 was required for p53-independent apoptosis in a human ovarian cancer cell line.

<24> In many cases, the p53 status of tumor largely influences the effectiveness of anticancer chemotherapy, since p53 gene alteration in cancercells has played an important role in drug resistance. Deprivation of p53-dependent apoptosis is a common phenomenon in solid tumor harboring a nonfunctional p53. Therefore, discovery of chemotherapeutic agents capable of inducing p53-independent apoptosis will be of great advantage.

<25> Thus, in the present invention, to investigate whether LOEL could affect transcriptional activity of p53 in B16F1 cells, reporter gene assay using a reporter construct including pRGC-luciferase was performed. It was observed that transcriptional activity of p53 in LOEL-treated cells was significantly increased in dose-dependent manner, which was consistent with the results of transcriptional activation of p21 promoter using p21 promoter-luciferase gene construct.

<26> These results suggest that the p53-mediated transactivation of the p21 promoter is achieved by LOEL which is capable of inducing apoptosis in B16F1 cells. This is in accordance with studies on human melanoma cell lines where an association between apoptosis and wild-type p53 accumulation has been observed. This observation can be further confirmed by the finding that LOEL could activate p53 transcription factor which is involved in apoptosis. The expression level of phospho-p53 was significantly increased in concentration dependent manner. These results indicate that apoptosis by LOEL was induced via the activation of p53 in B16F1 cells.

<27> Moreover, previous research has shown that the p53 wild-type over expression results in apoptosis of human melanoma cell but appears to have no detrimental effect on non-tumor cells.

<28> In conclusion, our findings provide the first experimental evidence that LOEL can induce apoptosis via the activation of p53 transcription factor in melanoma cells.

<29> These results suggest that LOEL have atherapeutic potential for the prevention and treatment of melanoma in skin.

【Advantageous Effects】

<30> The laurinterol compound from *Laurencia okamurai* according to the present invention has therapeutic effects in preventing and treating melanoma, because it can induce apoptosis in melanoma cells by activating caspase and causing p53-mediated transactivation of the p21 promoter to activate p53 and therefore can effectively inhibit the growth of melanoma cells.

【Description of Drawings】

<31> Figure 1 shows effect of LOEL on viability of melanoma cells (B16F1) in the presence of FBS.

<32> Figure 2 shows effect of LOEL on induction of apoptosis in melanoma cells (B16F1).

<33> Figure 3 shows effect of LOEL on activity of several caspases activity in melanoma cells (B16F1).

<34> Figure 4 shows effects of laurinterol from LOEL on the transcriptional activity of p53 and p21 promoter activation in melanoma cells (B16F1).

<35> Figure 5 shows western blot analysis of protein expression of phospho-p53 in melanoma cells (B16F1) after treatment with LOEL.

<36>

【Best Mode】

<37> In the followings, the present invention is more detailed described with reference to the attached drawings. However, a range of the present invention should not be restricted by those examples.

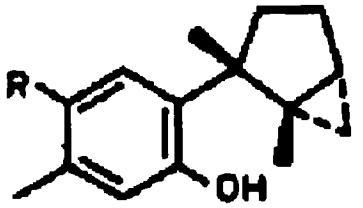
<38>

<39> <Example 1>

<40> Extraction and isolation of LOEL

<41> *L. okamurai* was directly collected in the costal area of Jejudo, Korea, and then the extraction of LOEL from *L. okamurai* and purification and structure determination of it was performed according to the method of Suzuki

M. et al.(Suzuki M. and Kurosawa E., Halogenated and nonhalogenated aromatic sesquiterpenes from the red algae Laurencis okamurai Yamada. Bull chem., Soc. Japan 52: 3352-3354, 1979).



R = Br
R = H

<42>

<43> The obtained LOEL was dissolved in dimethyl sulfoxide and used in the subsequent example.

<44>

<45> <Example 2>

<46> Cell culture

<47> Cell lines were separately grown as monolayers in T-75 tissue culture flasks (Nunc, Denmark) at 5% CO_2 and 37℃ humidified atmosphere using appropriate media supplemented with 10% fetal bovine serum, 2 mM glutamine and 100 μg/mL penicillin-streptomycin. DMEM was used as the culture medium for B16F1 cells. Cells were passaged 3 times a week by treating with trypsin-EDTA and used for experiments after 5 passages.

<48>

<49> <Example 3>

<50> MTT assay

<51> Anticancer effect of LOEL on B16F1 cells were measured using MTT method as described by Hansen et al. (Hansen M.B., Nielsen S.E. and Berg K., Re-examination and further development of a precise and rapid dye method for measuring cell growth/cell kill. J Immunol. Meth. 11: 203-210, 1989.).

<52>

<53> <Example 4>

<54> Flow cytometry analysis for cell cycle distribution

<55> B16F1 cells (2×10^5 in 60 mm^2 culture dishes) were trypsinized and washed with phosphate buffered saline (PBS) after treatment with LOEL for 24h. Approximately 1×10^6 cells were fixed with 75% ethanol at -20℃ overnight. Cells were stained with 50㎍/mL propidium iodide and 100㎍/mL RNase A at 4℃. The analysis of samples was performed by using flow cytometry (FACS Canto™, Beckton Dickinson Biosciences, San Jose, CA). Phase distributions were calculated from the resultant DNA histogram using the ModFit LT version 3.0 software, and expressed as a percentage of cells in the respective phases.

<56>

<57> <Example 5>

<58> DNA ladder assay

<59> Following treatment with LOEL for 24 h, Cells (1×10^6 in 100 mm^2 culture dishes) were lyzed in a buffer containing Tris-HCl, and Triton X-100. Lysates were then incubated with RNase A and proteinase K. DNA was obtained with an equal volume of neutral phenol:chloroform:isomyl alcohol mixture (25:24:1) and then precipitated with absolute ethanol and sodium acetate at -20℃. Equal amount (6㎍) of the extracted DNA was resolved over 1.8% agarose gel containing 0.5㎍/mL of ethidium bromide. Images were visualized under UV light using a gel documentation system (AlphaImager™ 2200; Alpha InnotechCorp., San Leandro, CA) and captured using the software AlphaEaseFC.

<60>

<61> <Example 6>

<62> TUNEL Assay

<63> The percentage of apoptotic cells in the excised tumor tissues was determined by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP in situ nick-end labeling (TUNEL) technique. Paraffin-embedded tumor sections were deparaffinized and treated with 20㎍/mL proteinase K to strip proteins from the nuclei. The sections were incubated with TdT and biotin-dUTP in TdT

buffer at 37℃. Following termination of the reaction and blockade of non-specific binding, sections were incubated with streptavidin-biotin-peroxidase complex and having the sites of peroxidase binding being detected using DAB. Negative control was prepared using similar procedures but without adding TdT. Positive control was prepared by prior incubation of the section with DNase I before hybridization with TdT and dUTP. Ten microscopic fields from three different sections were observed, and TUNEL positive apoptotic cells were counted at 400 × magnification.

<64>

<65> <Example 7>

<66> Apoptotic caspase activation

<67> B16F1 cells were cultured in the presence or absence of LOEL at 50 μg/mL in serum free DMEM medium. Caspase activity was assayed using several kinds of caspase substrates conjugated with pNA such as Ac-YVAD-pNA (caspase-1), Ac-VDVAD-pNA (caspase-2), Ac-DEVD-pNA (caspase-3), Ac-WEHD-pNA(caspase-5), Ac-VEID-pNA (caspase-6), Ac-VEIDpNA (caspase-8), Ac-VEID-pNA (caspase-9) according to the method described in manual of caspase-family colorimetric substrate set (Medical & Biological Laboratories CO.. LTD, USA).

<68>

<69> <Example 8>

<70> Western Blot Analysis

<71> Western blotting was performed according to standard procedures. Briefly, B16F1 cells were lysed in lysis buffer containing 50mM Tris-HCl, pH 7.5, 0.4% Nonidet P-40, 120mM NaCl, 1.5mM $MgCl_2$, 2mM phenylmethylsulfonyl fluoride, 80μg/mL leupeptin, 3mM NaF and 1mM DTT at 4 ℃ for 30 min. Cell lysates (10μg) were resolved on a 4-20% Novexgradient gel (Invitrogen, USA), electrotransferred onto a microcellulose membrane, and blocked with 10% skim milk. Phospho-p53 monoclonal antibody (Cat. No. 9286S, Cell Signaling Technology, USA) were used to detect phospho-p53 using chemiluminescent ECL assay kit (Amersham Pharmacia Biosciences, NJ, USA) according to the manufacturer instructions. Western blot bands were quantified using

ImageMaster software (Amersham Pharmacia Biosciences, NJ, USA).

<72>

<73> <Example 9>

<74> Transfection and Reporter Gene Assay

<75> B16F1 cells were seeded in 24-well plates and incubated at 37℃. At about 70-80% of confluency, cells were washed with DMEM and incubated with DMEM without serum and antibiotics for 5 h. Then, the cells were transfected with DNA mixture containing 1 μg of a reporter construct including pRGC (p53-binding fragments found in the human ribosomal gene cluster region) or p21 promoter containing pGL3 reporter vector and 30ng of pcDNA 3.1 which adjusts a total amount of DNA per well by Lipofectamine™reagent (Invitrogen, USA) as instructed by the manufacturer. After 72 h of incubation, cells were lysed and luciferase activity was measured using a luminometer (Tecan Austria GmbH, Austria). The luciferase activity was normalized to transfection efficiency by β-galactosidase activity using o-nitrophenyl β-galactopyranoside as a substrate. β-galactosidase staining was carried out by method as described by Lin et al. (Lin M.T.., Wang F., Uitto J., and Yoon K., Differential expression of tissue-specific promoters by gene gun. Bri. J. Dermato.14: 34 39, 2001.). Data were expressed as mean ± standard error of the mean (n = 3). Values were analyzed statistically relative to the control using Student's t-test.

<76>

<77> <Example 10>

<78> Statistical Analysis

<79> Comparisons of all data were performed using two-tailed, unpaired Student's t-test. A P value less than 0.05 was considered statistically significant. Data are expressed as means ± S.E.

<80>

<81> <Example 11>

<82> Effect of LOEL on viability of melanoma cells (B16F1)

<83> The anticancer effect of LOEL on melanoma cells (B16F1) was determined

by MTT assay and the result was shown in Fig. 1.

<84> Fig. 1 showed effect of LOEL on viability of melanoma cells (B16F1) in the presence of FBS in which cells were treated with LOEL at indicated concentration and cell viability was determined by MTT assay after 24h. Data are given as means of values ± SD from three independent experiments.

<85> As shown in Fig.1, LOEL exhibited a dose-dependent inhibitory effect on the growth of melanoma cells. It was observed that treatment of cells with LOEL at 10㎍/mL inhibited the growth of melanoma cells by 50%. Addition of 1 ㎍/mL of LOEL exerted 30% inhibition on growth of melanoma cells in the presence of FBS.

<86>

<87> <Example 12>

<88> LOEL induces apoptosis in melanoma cells (B16F1)

<89> In order to determine the effect of LOEL on induction of apoptosis in melanoma cells, B16F1 cells were treated with various concentrations of LOEL.

<90> Fig. 2 showed effect of LOEL on induction of apoptosis in melanoma cells (B16F1), in which cells were treated with LOEL at indicated concentration for 24 h. (A) DNA fragmentation in apoptic cells was analyzed using ethdium bromide staining, (B) apoptic cells were detected by TUNEL assay and fluorescence microscopy, and (C) after labeling cells with propidium iodide, cell cycle was monitored by flow cytometry and the percentage of cells at the subG1 phase was determined.

<91> The collected cells after treatment for 24h were examined for internucleosomal cleavage of DNA as an apoptosis marker using DNA fragmentation assay.

<92> As shown in Fig. 2A, the cells treated with LOEL clearly exhibited DNA fragmentation with increment of 180 base pairs above 1㎍/mL. In addition, TUNEL assay used to evaluate the apoptotic response remarkably caused green fluorescence to the cells treated with LOEL (Fig. 2B). Cells incubated with LOEL at 10㎍/mL for 24h showed approximately 50% apoptotic cells. Phenotypes of apoptosis characterized by cell shrinkage, chromatin compaction, nuclear

blebbing and apoptotic bodies were evidently observed in fluorescent images of LOEL - treated cells.

<93> Next, the effect of LOEL on the cell cycle phase distribution was analyzed. As shown in Fig. 2C, treatment with LOEL at 10μg/mL for 24h resulted in arrest of cell growth. It was observed that the percentage of cells in sub-G1 phases increased in cells treated with LOEL compared to blank group. These results suggest that LOEL can induce apoptosis in melanoma cells.

<94>

<95> <Example 13>

<96> Effect of LOEL on activity of several caspases activity in melanoma cells (B16F1)

<97> We determined the effect LOEL on the activation of caspases required to induce apoptosis to confirm the above findings based on the above results.

<98> Cytosolic extracts from B16F1 cells were subjected to caspase activity assay using several kinds of substrates conjugated with pNA.

<99> Fig. 3 showed effect of LOEL on activity of several caspases activity in melanoma cells (B16F1), in which the activity of several caspases was determined using caspase-family colorimetric substrate set at 405nm after treatment with LOEL at 50μg/mL to cells for 24h. Data are given as means of values ± SD from three independent experiments.

<100> As shown in Fig. 3, there was a significant increase in the activities of the caspase-3, -5, -6, -8 and -9 tested in the LOEL-treated cells. Especially, it was observed clearly that LOEL caused a marked increase in activity of caspase-6, an effector caspase. These results indicated that both imitator caspases and effector caspases were activated in apoptosis of melanoma cells induced by LOEL.

<101>

<102> <Example 14>

<103> Effects of LOEL on the transcriptional activity of p53 and p21 promoter activation in melanoma cells (B16F1)

<104> Furthermore, it was examined whether transcriptional activity of p53 is affected by LOEL in B16F1 cells using a reporter construct including pRGC (p53-binding fragments found in the human ribosomal gene cluster region)-luciferase gene.

<105> Fig. 4 showed effects of laurinterol from LOEL on the transcriptional activity of p53 and p21 promoter activation in melanoma cells (B16F1) in which (A) pcDNA 3.1 vector was cotransfected to normalize the luciferase activity for all treatment groups and the transfected cells were stained for beta-galactosidase, and (B) transcriptional activity of p53 and (C) p21 promoter activation was measured by luciferase assay after transfection with p53 and p21 reporter vector respectively. Data represent the mean ± S.E. of at least three independent experiments. Level of significance was identified statistically ($P < 0.01$) using Student't test.

<106> As shown in Fig. 4A, the B16F1 cells cotransfected with vectors of pRGC or p21 promoter with pcDNA 3.1 vector showed above 50% of SA-beta gal positive cells. Transcriptional activity of p53 in LOEL-treated cells was significantly ($P < 0.01$) increased indose-dependent manner compared to the untreated blank group as shown Fig. 4B. In particular, the transcriptional activity of p53 induced by LOEL at 50 μg/mL was similar to that by treatment of control, etoposide which is known as a p53 activating topoisomerase II inhibitor. Treatment with LOEL at 50 μg/mL exhibited about 3-fold increase in the transcriptional activity of p53 compared to the untreated blank group.

<107> It was next examined whether LOEL enhances transcriptional activation of p21 promoter which is mediated by p53 transcription factor. Transcriptional activation of p21 promoter was remarkably increased by LOEL, compared with the transcriptional activity of p53 (Fig. 4C). It was observed that LOEL treatment exerted a dose-dependent promoting effect on transcriptional activation of p21 promoter. Furthermore, treatment with 50 μg/mL of LOEL showed about 5-fold increase on p21 promoter activation compared to the untreated blank group. These results suggest that the p53- mediated transactivation of the p21 promoter is achieved by LOEL capable of inducing

apoptosis in B16F1 cells.

<108>

<109> <Example 15>

<110> Effect of LOEL on protein expression of phospho-p53 in melanoma cells (B16F1)

<111> We determined the expression level of phospho-p53 using western blot analysis to confirm the effect of LOEL on activation of p53 which is involved in apoptosis, .

<112> Fig. 5 showed Western blot analysis of protein expression of phospho-p53 in melanoma cells (B16F1) after treatment with LOEL, in which western blot analysis of cell lysates was performed using phospho-p53 monoclonal antibody as indicated. Lower panel represents respective relative protein expression as percentage. Values are expressed as relative protein expression using the following equation. Relative protein expression (%) = (Intensity of a band / intensity of blank bands) 100. Expression of β-lactin protein was used as the control for normalization of phospho-p53 protein.

<113> As shown in Fig. 5, there was no significant difference between blank group and treatment group with 1μg/mL of LOEL. However, treatment with LOEL above 5μg/mL significantly increased the expression level of phospho-p53 compared with blank group ($P < 0.01$). Moreover, the expression level of phospho-p53 by LOEL was increased in concentration dependent manner. These results indicate that apoptosis by LOEL may be resulted from the activation of p53 in B16F1 cells.

<114>

【Industrial Applicability】

<115> The pharmaceutical composition for preventing and inhibiting of melanoma comprising a laurinterol compound from *Laurencia okamurai*as an effective constituent according to the present invention is a natural medicine containing a natural effective constituent, which can effectively inhibit the growth of melanoma cells by inducing apoptosis therein without adverse effect as in synthetic medicines such that the present invention is

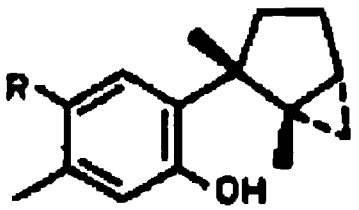
very useful in the pharmaceutical industry.

【CLAIMS】

【Claim 1】

<117> A laurinterol compound of formula 1 from *Laurencia okamurai* having the prevention and the inhibition activity against melanoma by inducing apoptosis therein:

<118> <Formula 1>



R = Br
R = H

<119>

<120>

【Claim 2】

<121> A pharmaceutical composition for preventing and inhibiting melanoma comprising the laurinterol compound from *Laurencia okamurai* according to claim 1 as effective constituent, in which the growth of melanoma cells is inhibited by inducing apoptosis therein.

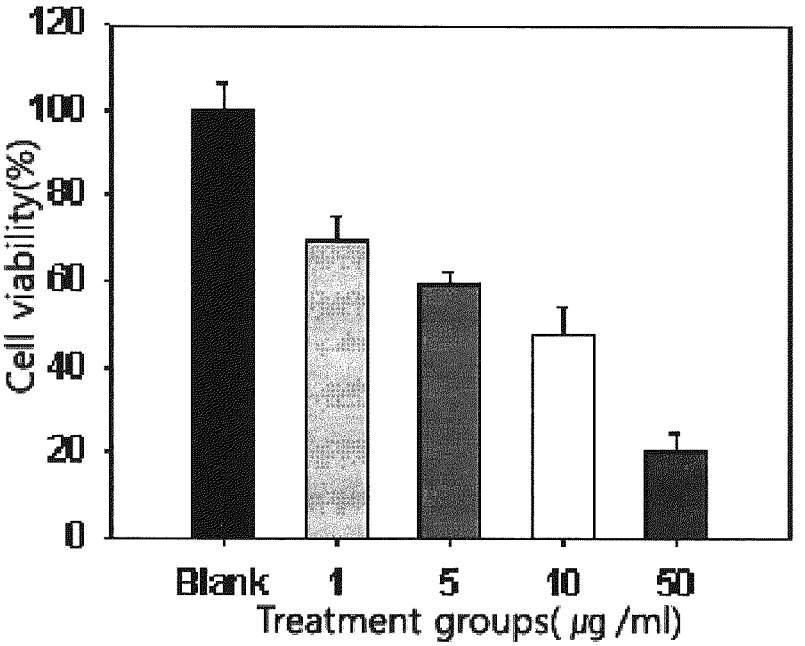
<122>

【Claim 3】

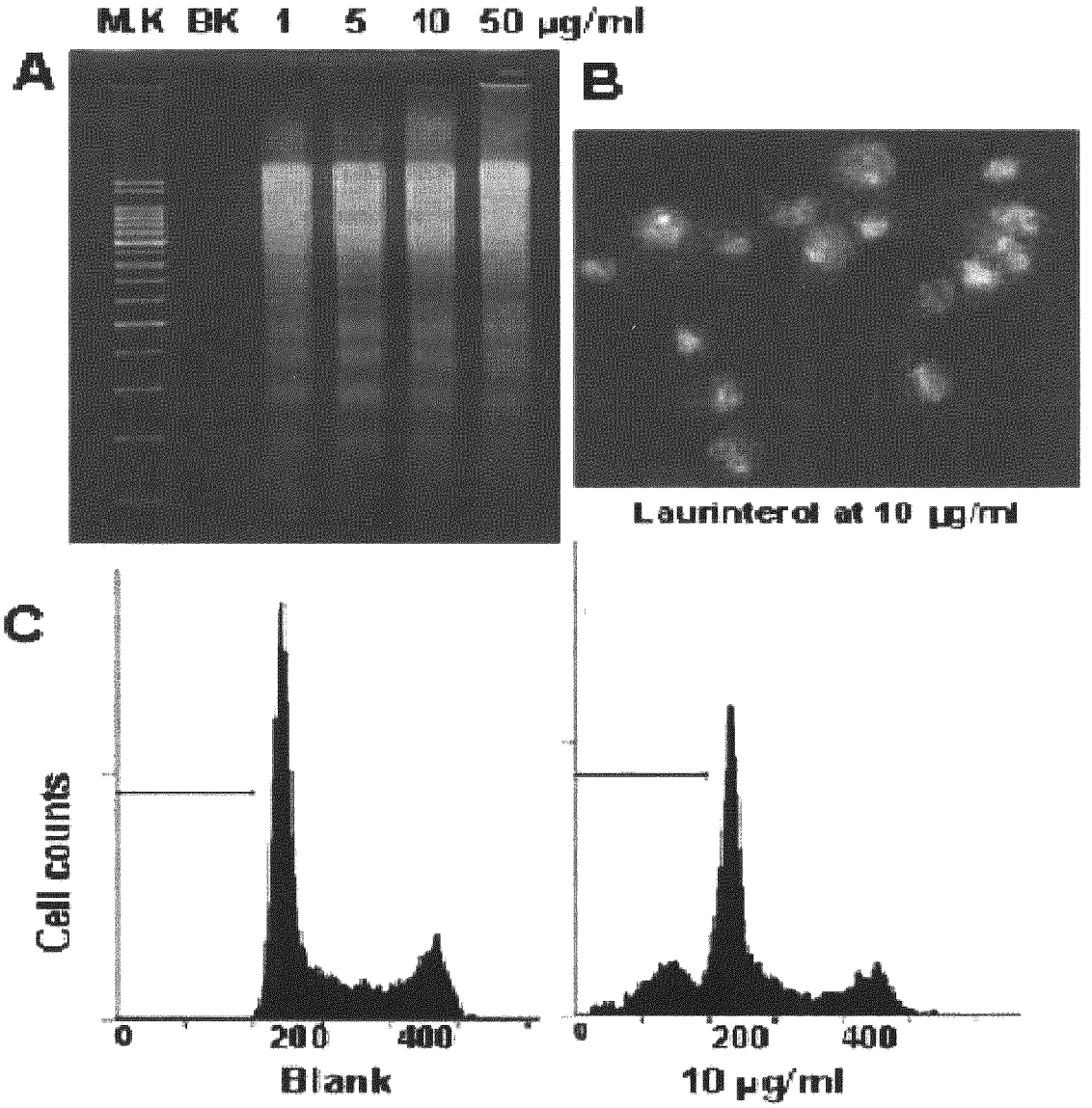
<123> The pharmaceutical composition according to claim 2, in which the composition comprises the laurinterol compound in amount of 0.1 to 1000 μg/mL.

【DRAWINGS】

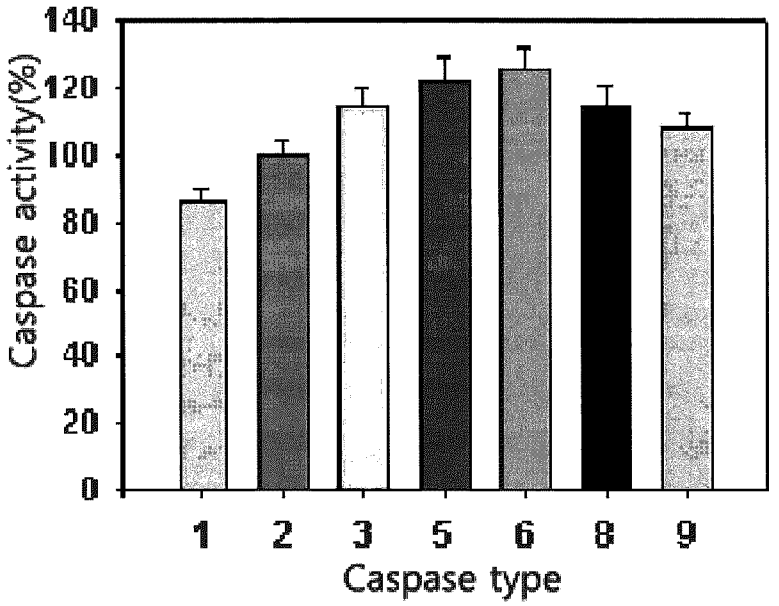
【Figure 1】



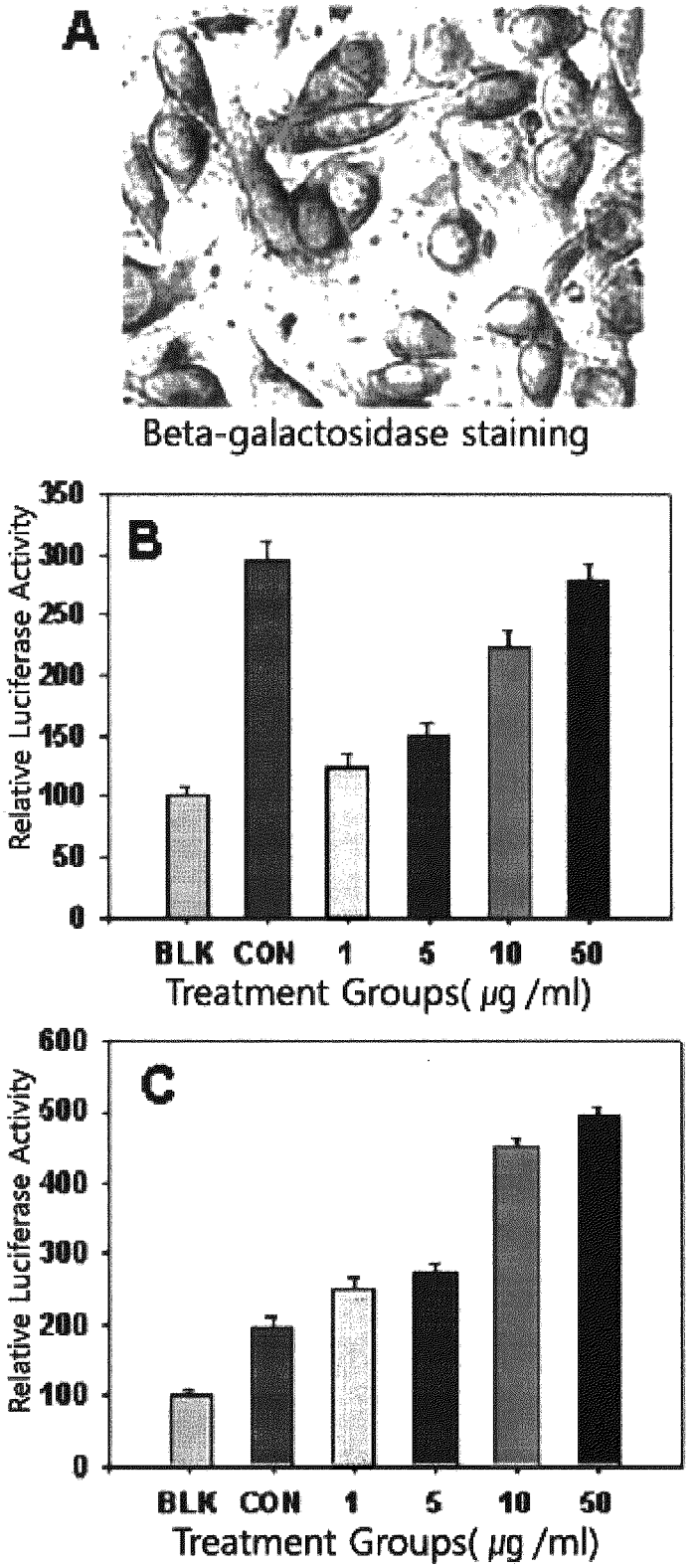
【Figure 2】


MK BK 1 5 10 50 μg/ml
A
B
Laurinterol at 10 μg/ml
C
Cell counts
0
200
400
Blank
0
200
400
10 μg/ml

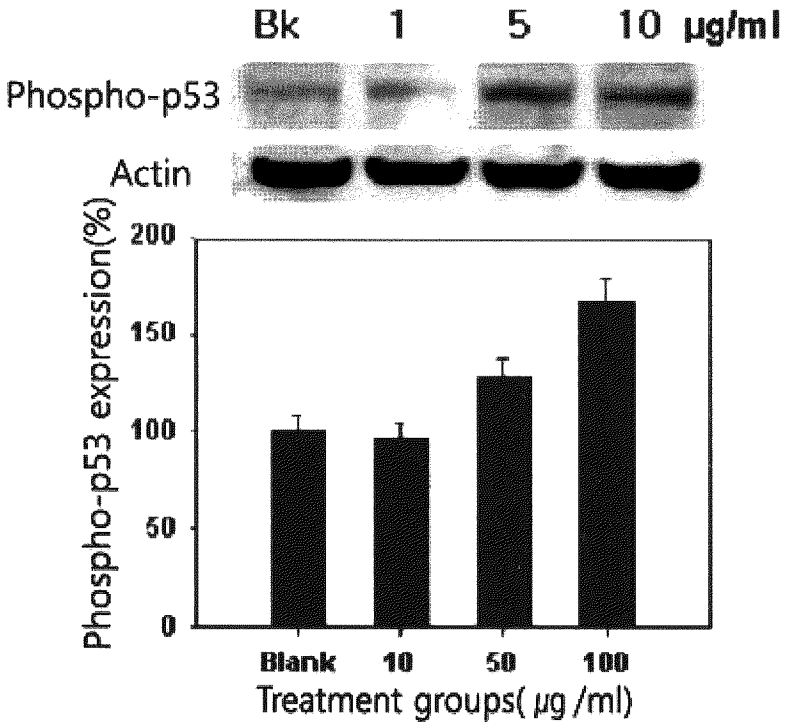
【Figure 3】


140
120
100
80
60
40
20
0
Caspase activity(%)
1
2
3
5
6
8
9
Caspase type

【Figure 4】


A
Beta-galactosidase staining
B
Relative Luciferase Activity
350
300
250
200
150
100
50
0
BLK CON 1 5 10 50
Treatment Groups(μg /ml)
C
Relative Luciferase Activity
600
500
400
300
200
100
0
BLK CON 1 5 10 50
Treatment Groups(μg /ml)

【Figure 5】


Bk
1
5
10 μg/ml
Phospho-p53
Actin
Phospho-p53 expression(%)
200
150
100
50
0
Blank
10
50
100
Treatment groups(μg /ml)

VIII-5-1	**Declaration: Non-prejudicial disclosure or exceptions to lack of novelty** Declaration as to non-prejudicial disclosures or exceptions to lack of novelty (Rules 4.17(v) and 51bis.1(a)(v)): Name (LAST, First)	in relation to this international application KIM, Moon-Moo declares that the subject matter claimed in this international application was disclosed as follows:
VIII-5-1(i)	Kind of disclosure:	publication
VIII-5-1(ii)	Date of disclosure:	07 July 2007 (07.07.2007)
VIII-5-1(iii)	Title of disclosure:	Laurinterol from Laurencia okamurai induces apoptosis via p53 activation
VIII-5-1(iv)	Place of disclosure:	Vienna, Austria

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2007/005834

A. CLASSIFICATION OF SUBJECT MATTER

A61K 31/05(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 A61K

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 in KIPO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GEONSEEK RYU et al., "Cytotoxic Activities of Brominated Sesquiterpenes from the Red Alga Laurencia okamurae", Natural Product Sciences, 2002, vol. 103, no. 3, pp 103-107 See abstract and page 105, 106	1-3
X A	JP 08-245359 A (KAISOU SHIGEN KENKYUSHO:KK) 24 September 1996 See [0008]	1 2,3
X A	JP 07-082554 A (KAISOU SHIGEN KENKYUSHO:KK) 28 March 1995 See [0009]	1 2,3
A	JP 61-103835 A (TEIJIN LTD) 22 May 1986 See claims and examples	1-3
A	JP 10-330219 A (NOEVIR CO LTD) 15 December 1998 See claims, [0006], [0012]	1-3

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

☒ See patent family annex.

* Special categories of cited documents:
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"E" earlier application or patent but published on or after the international filing date
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"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
03 JULY 2008 (03.07.2008)	**03 JULY 2008 (03.07.2008)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2007/005834

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JP 1998-330219	15.12.1998	NONE	