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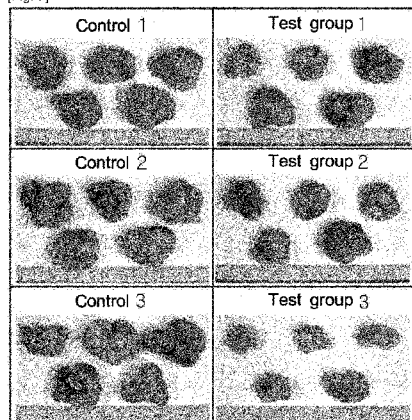
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(54) Title: ARYLOXYPHENOXYACETYL-BASED COMPOUND HAVING HIF-1 INHIBITION ACTIVITY, PREPARATION METHOD THEREOF AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME AS AN ACTIVE INGREDIENT

[Fig. 7]



(57) Abstract: Disclosed are a compound inhibitory of HIF-1 activity, a method for preparing the same, and a pharmaceutical composition comprising the same as an active ingredient. The compound has anticancer activity which is attributable to its inhibitory activity against HIF-1, but not to non-selective cellular toxicity. Thus, having inhibitory activity against HIF-1, the compound or a pharmaceutically acceptable salt thereof can be used as an ingredient in an anticancer agent for various cancers including large intestine cancer, hepatic cancer, stomach cancer and breast cancer. Further, the compound or a pharmaceutically acceptable salt thereof is applicable to the treatment of diabetic retinopathy or arthritis which is aggravated upon the activation of VEGFA by HIF-1.

Description

Title of Invention: ARYLOXYPHENOXYACETYL-BASED COMPOUND HAVING HIF-1 INHIBITION ACTIVITY, PREPARATION METHOD THEREOF AND PHARMACEUTICAL COMPOSITION CONTAINING THE SAME AS AN ACTIVE INGREDIENT

Technical Field

- [1] The present invention relates to an aryloxyphenoxyacetyl-based compound inhibitory of HIF-1, a method for preparing the same, and a pharmaceutical composition containing the same as an active ingredient.

[2]

Background Art

- [3] In spite of people making a consistent effort for decades, cancer still remains as one of refractory diseases. With the brilliant recent advances in various sciences including cancer cell biology, medicine, chemistry, etc., anticancer agents acting via novel mechanisms, such as Gleevec, have been developed. In addition, the Human Genome Project has given rise to new target molecules.
- [4] HIF-1(Hypoxia Inducible Factor-1) is a transcription factor that is induced in hypoxia. The factor is a heterodimer composed of an HIF-1 α subunit, which is degraded depending on oxygen, and an HIF-1 β subunit, which is constitutively expressed [Cancer Metastasis Rev., 17, 187-195, 1998; Trends Mol. Med., 7, 345-350, 2001]. Under normoxic conditions, HIF-1 α is selectively hydroxylated on the conserved proline residues 402 and 564 and binds to the tumor suppressor protein pVHL (Von Hippel-Lindau protein), which targets the HIF-1 α for ubiquitination and proteosomal degradation. Under hypoxic conditions, this series of reactions is inhibited so that HIF-1 α accumulates and dimerizes with preexisting HIF-1 β before translocation into the nucleus [Science 292, 468-472, 2001; Science 292, 468-472, 2001]. The stability of HIF-1 α is affected by factors involved in the oxygen sensing pathway as well as by oxygen partial pressure. Among the factors are transition metal ions, iron chelators and antioxidants. In addition, HIF-1 α protein is also accumulated by the activation of growth factors such as epidermal growth factor, heregulin, insulin-like growth factor-I and insulin-like growth factor-II or oncogenes such as Er β B2. When these growth factors bind to their corresponding receptors, the PI3K-AKT and MAPK signal transduction pathways are activated to induce the synthesis and thus accumulation of HIF-1 α protein.

- [5] Once moved into the nucleus, HIF is associated with HRE (Hypoxia Responsive Element, 5'-ACGTG-3') on the promoter of a target gene to induce the expression of the target gene. To date, approximately 60 genes including vascular endothelial growth factor A (VEGFA) have been identified as being regulated by HIF-1 (Nat. Rev. Cancer 2, 38-47, 2002; J. Biol. Chem. 278, 19575-19578, 2003; Nat. Med. 9, 677-684, 2003; Biochem. Pharmacol. 64, 993-998, 2002).
- [6] Hypoxia is generally observed in cancer, particularly in solid cancer. Solid cancer cells adapt to the hypoxic condition by means of various genetic modifications, so that cancer cells become more malignant and are resistant to anticancer agents. In fact, hypoxia is known as a major factor aggravating a tumor in at least 70% of all the human carcinoma (Nature 386, 403, 1997; Hockel M and Vaupel P, Semin. Oncol. 28, 36-41, 2001, Nature Med. 6, 1335, 2000; Bos et al. Cancer 2003, 97, 1573-1581). HIF-1 is known to play one of the most important roles in regulating cancer cells under hypoxic conditions. An HIF-1 α protein level is closely related to the prognosis of a cancer patient. When activated under hypoxic conditions or by the stimulation of above-mentioned growth factors, the activation of oncogenes, or the inactivation of a tumor suppressor gene such as pVHL, HIF-1 induces the expression of genes such as hexokinase 2, glucose transporter 1, erythropoietin, IGF-2, endoglin, VEGFA, MMP-2, uPAR, and MDR1, allowing cancer cells to acquire apoptosis resistance, angiogenesis, cell proliferation and invasion and thus to become malignant. Because HIF plays an important role in the growth, proliferation and malignancy of tumors, especially solid tumors, active research has been conducted to develop anticancer agents targeting the protein (Cancer Res. 62, 4316, 2002; Nat Rev Drug Discovery 2, 1, 2003; Semenza et al. Nature Reviews Cancer 2003, 3, 721-732). Recently, a significant number of already-known anticancer agents including taxol, rapamycin, and 17-AAG (17-allylaminogeldanamycin), and the guanylate cyclase inhibitor YC-1 (3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole) have been used as HIF-1 inhibitors in various clinical trial phases [Johnson et al Nature Reviews Drug Discovery 2003, 2, 1-9; Semenza et al. Nature Reviews Cancer 2003, 3, 721-732; JNCI 95, 516, 2003], and to develop HIF-1 inhibitors of novel structures, the cell-based reporter assays have been performed using HRE (Cancer Res 65, 4918, 2005; Cancer Cell 6, 33, 2004; Cancer Res. 62, 4316, 2002). However, this research still remains in an initial phase.
- [7] Further, HIF-1 can be the target of studies to develop therapeutics for diseases which become malignant upon angiogenesis as well as for cancer. Angiogenesis factors, such as VEGFA, activated by HIF-1 under hypoxia conditions, are involved in the progress of diabetic retinopathy and arthritis as well as cancer. Hence, a compound, if suppressive of hypoxia-inducible factors (e.g., HIF-1), can be used as a novel therapeutic agent for diabetic retinopathy or rheumatoid arthritis (Eiji Ikeda, Pathology Inter-

national, 2005, Vol 55, 603-610). However, this field is also in a nascent phase.

- [8] Leading to the present invention, intensive and thorough research into anticancer agents, resulted in the finding that a novel n aryloxyphenoxyacetyl-based compound is inhibitory of HIF-1 activity, thus showing suppressing the metastasis of cancer and angiogenesis, in addition to being safe to the body.

[9]

Disclosure of Invention

Technical Problem

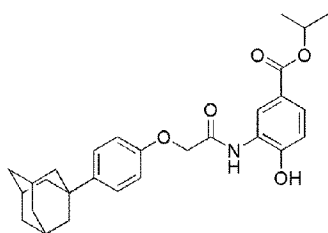
- [10] It is an object of the present invention to provide a novel compound having inhibitory activity against HIF-1.
- [11] It is another object of the present invention to provide a method for preparing the novel compound.
- [12] It is a further object of the present invention to provide a pharmaceutical composition for the treatment of cancer, comprising the novel compound as an active ingredient.
- [13] It is still a further object of the present invention to provide a pharmaceutical composition for the treatment of diabetic retinopathy, comprising the novel compound as an active ingredient.
- [14] It is still another object of the present invention to provide a pharmaceutical composition for the treatment of rheumatoid arthritis, comprising the novel compound as an active ingredient.

Solution to Problem

- [15] In accordance with an aspect thereof, the present invention provides 3-[2-(4-adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid isopropyl ester, represented by the following Chemical Formula 1, or a pharmaceutically acceptable salt thereof:

[16] [Chemical Formula 1]

[17]



[18]

- [19] In accordance with another aspect thereof, the present invention provides a method for preparing the compound of Chemical Formula 1, as illustrated in the following Reaction Scheme 1, comprising:

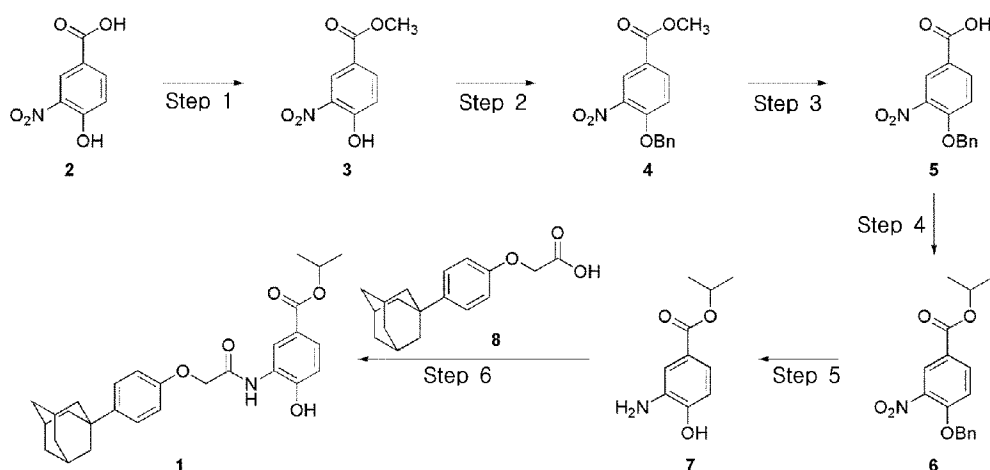
- [20] esterifying a compound of Chemical Formula 2 to a compound of Chemical Formula 3 (step 1);

- [21] alkylating the compound of Chemical Formula 3 with benzyl bromide to give a compound of Chemical Formula 4 (step 2);
- [22] hydrolyzing the compound of Chemical Formula 4, obtained in step 2, to a compound of Chemical Formula 5 (step 3);
- [23] reacting the compound of Chemical Formula 5, obtained in step 3, with alkyl halide in the presence of a Hunig's base to afford a compound of Chemical Formula 6 (step 4);
- [24] reducing the compound of Chemical Formula 6, obtained in step 4, in the presence of a catalyst in a hydrogen atmosphere to afford a compound of Chemical Formula 7 (step 5); and
- [25] esterifying the compound of Chemical Formula 7, obtained in step 5, with a compound of Chemical Formula 8 in the presence of a Hunig's base to afford the product (1) (step 6):

[26]

[27] [Reaction Scheme 1]

[28]



- [29] In accordance with a further aspect thereof, the present invention provides a pharmaceutical composition for the prevention or treatment of cancer, comprising the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.
- [30] In accordance with still a further aspect thereof, the present invention provides a pharmaceutical composition for the prevention or treatment of diabetic retinopathy that comprises the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.
- [31] In accordance with still another aspect thereof, the present invention provides a pharmaceutical composition for the prevention or treatment of rheumatoid arthritis, comprising the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.

[32]

Advantageous Effects of Invention

- [33] The compound of Chemical Formula 1 in accordance with the present invention has anticancer activity which is attributable to its inhibitory activity against HIF-1, but not to non-selective cellular toxicity. Thus, the compound decreases the transcriptional activity of HRE and shows down-regulation selective for VEGFA, EPO, NADH and CA IX, thereby suppressing the growth and metastasis of cancer. Therefore, the compound can be used as an active ingredient in an anticancer agent as well as in a therapeutic for diabetic retinopathy or arthritis.

[34]

Brief Description of Drawings

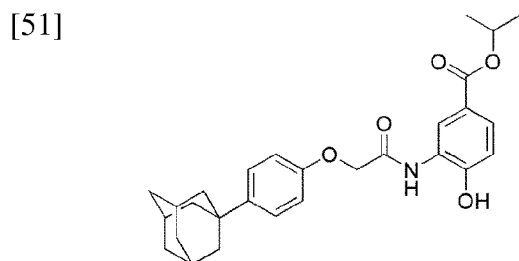
- [35] FIG. 1 is a graph showing results of the assay of the compound of the present invention for inhibition action against HIF-1-mediated transcriptional activity of HRE.
- [36] FIG. 2 shows the inhibitory activity of the compound of the present invention against HIF-1 α accumulation.
- [37] FIG. 3 shows effects of the compound of the present invention on the expression of VEGFA and EPO.
- [38] FIG. 4 is a graph showing the effect of the compound on the expression of LDHA.
- [39] FIG. 5 is a graph showing the effect of the compound on the expression of CA IX.
- [40] FIG. 6 is of optical images showing the down-regulation of HIF-1 α expression by the compound of the present invention.
- [41] FIG. 7 shows changes in tumor volume upon the intravenous injection of the compound of the present invention and the oral administration of the preexisting anticancer agent
- [42] FIG. 8 shows changes in tumor volume upon oral co-administration of the compound of the present invention and the conventional anticancer agent.
- [43] FIG. 9 is of optical photographs showing the effect of the compound of the present invention on tube formation.
- [44] FIG. 10 is a graph showing the effect of the compound of the present invention on angiogenesis.
- [45] FIG. 11 is of photographs showing the effect of the compound of the present invention on angiogenesis.
- [46] FIG. 12 is a graph showing the number of cancer clusters metastasized to the lung upon treatment with the compound of Chemical Formula 1.
- [47] FIG. 13 is of photographs the number of cancer clusters metastasized to the lung upon treatment with the compound of Chemical Formula 1.

[48]

Best Mode for Carrying out the Invention

[49] The present invention addresses 3-[2-(4-adamantan-1-yl-phenoxy)-acetylamino]-4-hydroxy-benzoic acid isopropyl ester, represented by the following Chemical Formula 1, or a pharmaceutically acceptable salt thereof. In detail, the compound shows potent anticancer activity by suppressing the growth and metastasis of tumor cells, with minimal side effects. In addition, having inhibitory activity against HIF-1, the compound is applicable to the treatment of diabetic retinopathy or arthritis.

[50] [Chemical Formula 1]



[52]

[53] The compound of Chemical Formula 1 in accordance with the present invention is a novel compound which has a molecular weight of 463.6 and melts at 203.4~204.5°C, with the following ¹H-NMR spectrum.

[54] ¹H-NMR (CDCl₃, 300 MHz) δ 9.72 (1H, s, OH), 8.62 (1H, s, NH), 7.82-7.86 (1H, m, aromatic-H), 7.73 (1H, m, aromatic-H), 7.36 (2H, d, J = 9.0 Hz, aromatic-H), 7.05 (1H, d, J = 8.1 Hz, aromatic-H), 6.96 (2H, d, J = 8.7 Hz, aromatic-H), 5.23 (1H, m, CH), 4.68 (2H, s, CH₂), 2.10 (3H, m, adamantly-H), 1.90 (6H, m, adamantly-H), 1.77 (6H, m, adamantly-H), 1.37 (3H, s, CH₃), 1.35 (3H, s, CH₃)

[55] Also, the present invention addresses a method for preparing the compound of Chemical Formula 1, as illustrated in the following Reaction Scheme 1, comprising:

[56] esterifying a compound of Chemical Formula 2 to a compound of Chemical Formula 3 (step 1);

[57] alkylating the compound of Chemical Formula 3 with benzyl bromide to give a compound of Chemical Formula 4 (step 2);

[58] hydrolyzing the compound of Chemical Formula 4, obtained in step 2, to a compound of Chemical Formula 5 (step 3);

[59] reacting the compound of Chemical Formula 5, obtained in step 3, with alkyl halide in the presence of a Hunig's base to afford a compound of Chemical Formula 6 (step 4);

[60] reducing the compound of Chemical Formula 6, obtained in step 4, in the presence of a catalyst in a hydrogen atmosphere to afford a compound of Chemical Formula 7 (step 5); and

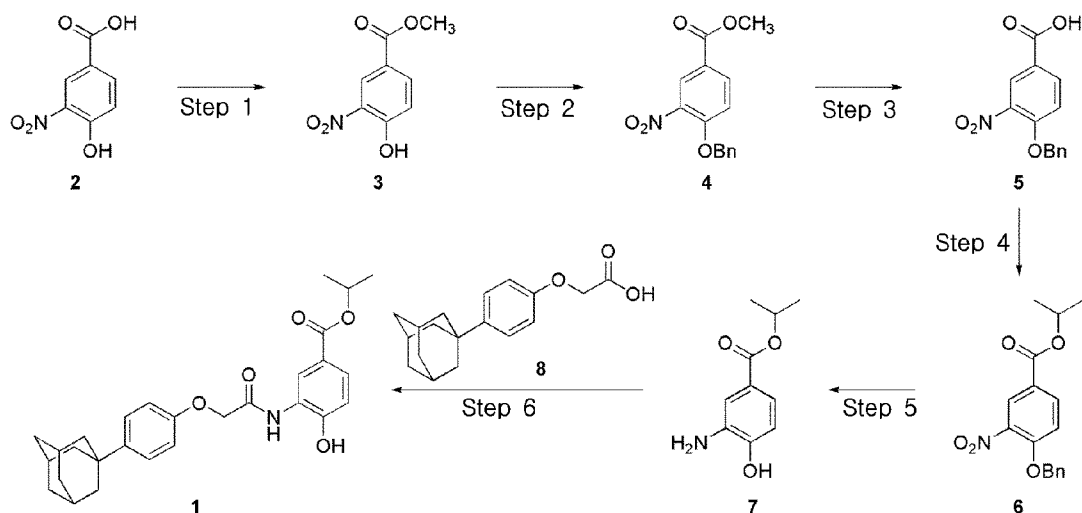
[61] esterifying the compound of Chemical Formula 7, obtained in step 5, with a

compound of Chemical Formula 8 in the presence of a Hunig's base to afford the product (1) (step 6):

[62]

[63] [Reaction Scheme 1]

[64]



[65]

[66] Below, a stepwise detailed description will be given of the present invention.

[67] In step 1 of the method of the present invention, the compound of Chemical Formula 2, used as a starting material, is esterified to the compound of Chemical Formula 3 via a chloride intermediate using a chlorination reagent.

[68] In detail, 4-hydroxy-3-nitro-benzoic acid of Chemical Formula 2 is dissolved in an organic solvent such as methanol and reacted with a chlorination reagent such as oxalyl chloride or thionyl chloride at -10~10°C to form a chloride intermediate which is then converted into the 4-hydroxy-3-nitro-benzoic acid methyl ester of Chemical Formula 3.

[69] Step 2 of the method of the present invention is a process for alkylating the compound of Chemical Formula 3 under an alkaline condition to give the compound of Chemical Formula 4.

[70] In this regard, a solution of 4-hydroxy-3-nitro-benzoic acid methyl ester of Chemical Formula 3 and benzyl bromide (BnBr) in an organic solvent such as DMF is alkalified with an inorganic base such as potassium carbonate, sodium hydroxide, potassium hydroxide, etc., followed by the alkylation of the ester to 4-benzyloxy-3-nitro-benzoic acid methyl ester of Chemical Formula 4.

[71] Step 3 of the method of the present invention is to hydrolyze the compound of Chemical Formula 4 to the compound of Chemical Formula 5.

[72] For this, 4-benzyloxy-3-nitro-benzoic acid methyl ester of Chemical Formula 4 in a mixture of an organic solvent such as THF and water is treated with an inorganic base such as lithium hydroxide, sodium hydroxide, potassium hydroxide, etc., or an

inorganic acid such as chloric acid, sulfuric acid, etc., in the presence of lithium hydroxide monohydrate to give the 4-benzyloxy-3-nitro-benzoic acid of Chemical Formula 5.

[73] Step 4 of the method of the present invention is designed to alkylate the compound of Chemical Formula 5 with alkyl halide in the presence of a Hunig's base to afford the compound of Chemical Formula 6.

[74] In detail, 4-benzyloxy-3-nitro-benzoic acid of Chemical Formula 5 is dissolved in (trihexyl)tetradecyl-phosphonium bis-triflamide and alkylated with 2-bromopropane in the presence of a Hunig's base such as diisopropylethylamine (DIPEA), triethylamine (TEA), etc. to afford the 4-benzyloxy-3-nitro-benzoic acid isopropyl ester of Chemical Formula 6.

[75] In step 5 of the method of the present invention, the compound of Chemical Formula 6 is reduced to the compound of Chemical Formula 7 in the presence of a catalyst in a hydrogen atmosphere.

[76] For this, 4-benzyloxy-3-nitro-benzoic acid isopropyl ester (the compound of Chemical Formula 6) in an organic solvent such as methanol or ethanol is reduced with hydrogen gas with the aid of a palladium catalyst to form the 3-amino-4-hydroxy-benzoic acid isopropyl ester of Chemical Formula 7.

[77] Step 6 in the method of the present invention is to carry out the condensation reaction of the compound of Chemical Formula 7 with the compound of Chemical Formula 8 to produce the compound of Chemical Formula 1.

[78] In this context, 3-amino-4-hydroxy-benzoic acid isopropyl ester of Chemical Formula 7 is condensed with (4-adamantan-1-yl-phenoxy)-acetic acid (compound of Chemical Formula 8) in the presence of a Hunig's base such as DIPEA, TEA etc., with the aid of a condensation reagent such as 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBt) or benzotriazol-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) in an organic solvent such as DMF to afford the final product, 3-[2-(4-adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid isopropyl ester of Chemical Formula 1.

[79] The aryloxyphenoxy acetyl compound, after being prepared according to the method of the present invention, was purified using high-performance liquid chromatography, followed by identifying its molecular structure with nuclear magnetic resonance analysis.

[80] Further, the present invention provides a pharmaceutical composition for the prevention or treatment of cancer, comprising the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient.

- [81] The compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof in accordance with the present invention has anticancer activity which is attributable to its inhibitory activity against HIF-1, a transcription factor playing an important role in the growth and metastasis of tumor cells, but not to non-selective cellular toxicity.
- [82] As used herein, the term "inhibitory activity against HIF-1" is intended to encompass all of the activities of suppressing the transcription of HRE (Hypoxia Responsive Element, 5'-ACGTG-3'), the accumulation of HIF-1 α protein and the expression of HIF-1 target genes.
- [83] The effect of the compound of Chemical Formula 1 according to the present invention on the activation of HRE transcription by HIF-1 under an hypoxic condition was examined. As a result, the compound of Chemical Formula 1 was found to potently inhibit HIF-1 activity, compared to conventional compounds (Comparative Example 1) that are structurally similar to the compound of Chemical Formula 1 (FIG. 1). That is, the compound of Chemical Formula 1 according to the present invention inhibits the transcriptional activity of HRE activated by HIF-1 and so the expression of target genes implicated in the malignancy of cancer. Thus, capable of suppressing the growth and metastasis of cancer, the compound of Chemical Formula 1 may be used as the active ingredient of an anticancer agent.
- [84] Also, the compound of Chemical Formula 1 was observed to inhibit the production of HIF-1 α in a dose-dependent manner under an hypoxic condition, without any effect on the production of topoisomerase-1 (TOPO-1) (FIG. 2), indicating that the anticancer activity of the compound according to the present invention is not attributed to non-selective cellular toxicity, but to the selective inhibition of the accumulation of HIF-1 α . Therefore, the compound of the present invention can be used to suppress the growth and metastasis of cancer, with the generation of minimal side effects.
- [85] Further, the compound of Chemical Formula 1 according to the present invention shows dose-dependent inhibitory activity against the expression of the HIF-1 target genes involved in the growth and metastasis of cancer, including VEGFA (Vascular endothelial growth factor A) that plays an important role in the growth and metastasis of cancer, EPO (erythropoietin) that promotes the generation of erythrocytes, LDHA (lactate dehydrogenase A) that increases glyconeogenesis and the uptake of glucose into tumor cells, and CA IX (carbonic anhydrase 9) that converts carbon dioxide into carbonic acid to induce tumor acidification (FIGS. 3, 4 and 5). That is, functioning to inhibit the expression of the HIF-1 targets contributing to the growth and metastasis of cancer, including VEGFA, EPO, LDHA and CA IX, the compound of the present invention can be used as an active ingredient of an anticancer agent.
- [86] Hence, a pharmaceutical composition comprising the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient can ef-

fectively inhibit the activity of HIF-1 and is applicable to the treatment of various cancers including large intestine cancer, hepatic cancer, stomach cancer, breast cancer, colon cancer, bone cancer, pancreatic cancer, head or neck cancer, cervical cancer, ovarian cancer, rectal cancer, esophageal cancer, small intestine cancer, perianal cancer, fallopian tube cancer, endometrial cancer, uterine cervical cancer, vaginal cancer, vulvar cancer, Hodgkin's disease, prostatic cancer, bladder cancer, kidney cancer, ureter cancer, renal cell carcinoma, renal pelvis cancer, and central nervous system tumors.

[87] Moreover, the present invention provides a pharmaceutical composition, comprising the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient, which is therapeutically effective for diabetic retinopathy or arthritis on the basis of inhibitory activity against HIF-1.

[88] Herein, the inhibitory activity against HIF-1 is intended to encompass all of the activities of suppressing the transcriptional activity of HRE, the accumulation of HIF-1 α protein and the expression of HIF-1 target genes.

[89] HIF-1 can be a target of the study to develop therapeutics for diseases which become aggravated in association with angiogenesis. Angiogenesis factors, such as VEGFA, activated by HIF-1 under hypoxia conditions, are involved in the progress of diabetic retinopathy and arthritis. Diabetic retinopathy or rheumatoid arthritis may be aggravated when the expression of VEGFA is increased by HIF-1 in an hypoxic condition. Hence, a compound, if suppressive of hypoxia-inducible factors (e.g., HIF-1), can be used as a therapeutic agent for diabetic retinopathy or rheumatoid arthritis (Eiji Ikeda, Pathology International, 2005, Vol 55, 603-610) (Eiji Ikeda, Pathology International, 2005, Vol 55, 603-610).

[90] As stated above, the compound of Chemical Formula 1 according to the present invention can selectively suppress the expression of the angiogenesis factor VEGFA (vascular endothelial growth factor A) in a hypoxic condition, without affecting the expression of the control gene GAPDH, so that it can be employed as an active ingredient of a therapeutic for diabetic retinopathy or arthritis, which is aggravated upon the activation of VEGFA by HIF-1.

[91] The pharmaceutical composition of the present invention may be formulated into various dosage forms, whether oral or non-oral. Examples of the oral dosage form include tablets, pills, hard/soft capsules, liquids, suspensions, emulsions, syrups, granules, elixirs, etc. These formulations may contain diluents (e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine), and a lubricant (e.g., silica, talc, stearic acid and magnesium or calcium salt thereof, and/or polyethylene glycol), in addition to the active ingredient. A tablet formulation may further contain a binder such as magnesium aluminum silicate, starch paste, gelatin, methyl cellulose, sodium

carboxymethylcellulose and/or polyvinylpyrrolidone and optionally a disintegrant such as starch, agar, or alginic acid or sodium salt thereof, an effervescent agent and/or an absorbent, a colorant, a fragrant, and a sweetener.

[92] For use in parenteral administration, the pharmaceutical composition comprising the compound of Chemical Formula 1 in accordance with the present invention may be formulated into injections via subcutaneous, intravenous, intramuscular, and intra-pulmonary routes. Injections may be prepared by mixing the compound of Chemical Formula 1 or a pharmaceutically acceptable salt thereof with a stabilizer or buffer in water to give solutions or suspensions which are packaged in unit dosages such as ampules or vials. Further, the composition may be sterilized and/or may contain subsidiary agents such as preservatives, stabilizers, water dispersible powders or emulsifiers, salts for regulating osmotic pressure, and/or buffers, and other therapeutically useful materials. The composition may be formulated using typical methods such as by mixing, granulation or coating. The compound of Chemical Formula 1, as an active ingredient, may be orally or parenterally administered at a dose of 0.1 to 5000 mg/kg of weight to mammalian animals including humans in a single dose or multiple doses a day.

[93] The composition of the present invention may be used alone or in combination with surgery, radiotherapy, hormone treatment, chemotherapy or a biological response regulator.

[94]

Mode for the Invention

[95] A better understanding of the present invention may be obtained through the following examples which are set forth to illustrate, but are not to be construed as limiting the present invention.

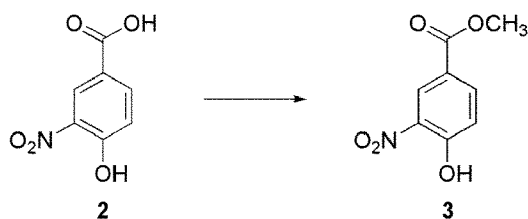
[96]

[97] <EXAMPLE 1> Preparation of
3-[2-(4-Adamantan-1-yl-phenoxy)-acetylamino]-4-hydroxy-benzoic acid isopropyl ester (3-[2-(4-Adamantan-1-yl-phenoxy)-acetylamino]-4-hydroxy-benzoic acid isopropyl ester)

[98]

[99] Step 1: Preparation of 4-hydroxy-3-nitro-benzoic acid methyl ester
(4-Hydroxy-3-nitro-benzoic acid methyl ester)

[100]



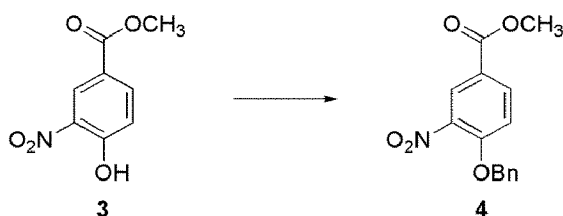
[101] To a solution of 4-hydroxy-3-nitro-benzoic acid(1.0 g, 5.46 mmol) in methanol(10 ml) was slowly added thionyl chloride (SOCl₂, 1.30 g, 0.8 ml, 10.92 mmol) at 0°C, followed by refluxing for 4 hours to give a reaction solution. This reaction solution was concentrated, and separated with ethylacetate (EtOAc) and an aqueous sodium chloride solution. The organic layer was dried over anhydrous magnesium sulfate and concentrated to afford the title compound as a yellowish solid (992.3 mg, yield: 92.2%).

[102] ¹H-NMR (CDCl₃, 300 MHz) δ 10.89 (1H, s, OH), 8.82 (1H, d, J = 1.5 Hz, aromatic-H), 8.23 (1H, dd, J = 2.1 , 8.9 Hz, aromatic-H), 7.22 (1H, d, J = 8.7 Hz, aromatic-H), 3.95 (3H, s, OCH₃)

[103]

[104] Step 2: Preparation of 4-benzyloxy-3-nitro-benzoic acid methyl ester
(4-Benzyloxy-3-nitro-benzoic acid methyl ester)

[105]



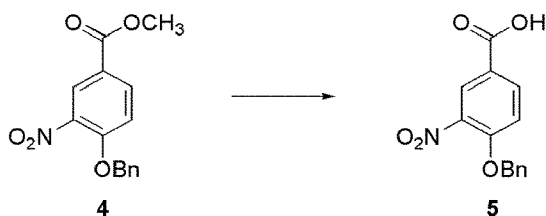
[106] To a solution of 4-hydroxy-3-nitro-benzoic acid methyl ester (992 mg, 5.03 mmol) and benzyl bromide (BnBr, 1.033 g, 6.04 mmol) in dimethylformamide (DMF, 8 ml) was added sodium carbonate (K₂CO₃, 1.74 g, 12.6 mmol), followed by stirring the mixture overnight at room temperature. The reaction solution thus formed was separated with ethyl acetate and an aqueous sodium chloride solution, and the organic layer was dried over anhydrous magnesium sulfate and concentrated. The residue was purified using silica gel column chromatography (n-Hexane:EtOAc = 3:1) to afford the title compound as a yellowish solid (1.3 g, yield: 90.0%).

[107] ¹H-NMR (CDCl₃, 300 MHz) δ 10.89 (1H, s, OH), 8.82 (1H, d, J = 1.5 Hz, aromatic-H), 8.23 (1H, dd, J = 2.1 , 8.9 Hz, aromatic-H), 7.22 (1H, d, J = 8.7 Hz, aromatic-H), 3.95 (3H, s, OCH₃)

[108]

[109] Step 3: Preparation of 4-benzyloxy-3-nitro-benzoic acid
(4-Benzyloxy-3-nitro-benzoic acid)

[110]



[111] To a solution of 4-benzyloxy-3-nitro-benzoic acid methyl ester(5.41 g, 18.83 mmol)

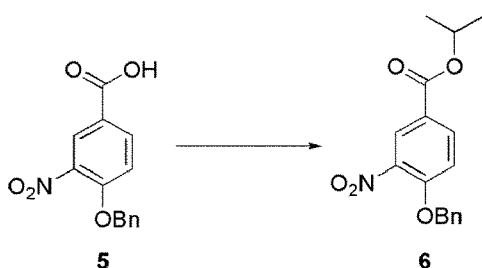
in THF/H₂O (3:1, 280 ml) was added lithium hydroxide monohydrate (153 mg, 3.65 mmol), followed by stirring the solution overnight. The reaction solution thus formed was treated with 10% HCl and separated with ethyl acetate and an aqueous NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and concentrated to form the title compound as a yellowish solid (4.55 g, yield: 88.4%).

[112] ¹H-NMR (CDCl₃, 300 MHz) δ 8.58 (1H, d, J = 2.4 Hz, aromatic-H), 8.23 (1H, dd, J = 2.4, 8.7 Hz, aromatic-H), 7.36-7.48 (5H, m, aromatic-H), 7.20 (1H, d, J = 9.0 Hz, aromatic-H), 5.34 (2H, s, CH₂)

[113]

[114] Step 4: Preparation of 4-benzyloxy-3-nitro-benzoic acid isopropyl ester
(4-Benzyloxy-3-nitro-benzoic acid isopropyl ester)

[115]



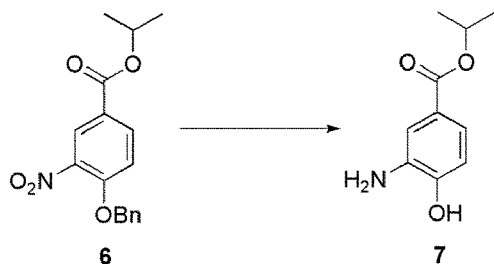
[116] To a solution of 4-benzyloxy-3-nitro-benzoic acid (400.0 mg, 1.46 mmol) in (trihexyl)tetradecyl-phosphonium bis-triflamide (2.0 ml) were added 2-bromopropane (630.0 mg, 0.48 ml, 5.12 mmol) and diisopropylethylamine (DIPEA, 661.8 mg, 0.89 ml, 5.12 mmol). The resulting reaction solution was stirred at 40 C for 12 hours and separated with ethyl acetate and an aqueous NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and concentrated. The concentrate was purified using silica gel column chromatography (n-Hexane:EtOAc = 20:1) to afford the title compound as a yellowish solid (200.2 mg, yield: 43.5%).

[117] ¹H-NMR (CDCl₃, 300 MHz) δ 8.49 (1H, m, aromatic-H), 8.17 (1H, d, J = 9.0 Hz, aromatic-H), 7.32-7.46 (5H, m, aromatic-H), 7.15 (1H, d, J = 8.7 Hz, aromatic-H), 5.30 (2H, s, CH₂), 5.24 (1H, m, CH), 1.38 (3H, s, CH₃), 1.36 (3H, s, CH₃)

[118]

[119] Step 5: Preparation of 3-amino-4-hydroxy-benzoic acid isopropyl ester
(3-Amino-4-hydroxy-benzoic acid isopropyl ester)

[120]



[121] A vessel containing 4-benzyloxy-3-nitro-benzoic acid isopropyl ester (370 mg) was

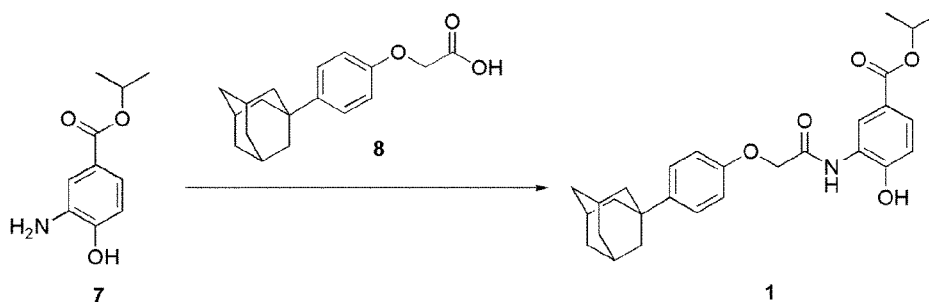
purged with argon, followed by adding 25 mL of methanol to the container. To the resulting reaction solution was slowly added 5 wt% of palladium-carbon. The solution was stirred overnight at room temperature in a hydrogen atmosphere. After completion of the reaction, the palladium catalyst was removed using a Celite bed. The filtrate was concentrated and purified using silica gel column chromatography (n-Hexane:EtOAc = 3:1) to afford the title compound as a yellowish solid (211.7 mg, yield: 92.4%).

[122] ¹H-NMR (CDCl₃, 300 MHz) δ 7.43 (1H, s, aromatic-H), 7.38 (1H, d, J = 8.7 Hz, aromatic-H), 6.74 (1H, d, J = 8.1 Hz, aromatic-H), 5.19 (1H, m, CH), 1.34 (3H, s, CH₃), 1.32 (3H, s, CH₃)

[123]

[124] Step 6: Preparation of
3-[2-(4-adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid isopropyl ester (3-[2-(4-Adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid isopropyl ester)

[125]



[126] To DMF(14 ml) were added (4-adamantan-1-yl-phenoxy)-acetic acid (143.2 mg, 0.5 mmol), 3-amino-4-hydroxy-benzoic acid isopropyl ester (211.7 mg, 1.08 mmol), EDC(207.8 mg, 1.08 mmol), HOBt(146.5 mg, 1.08 mmol) and DIPEA(0.19 ml, 1.08 mmol). The resulting reaction solution was stirred and separated with ethyl acetate and 10% HCl. The organic layer was washed with an aqueous NaCl solution, dried over anhydrous magnesium sulfate and purified using silica gel column chromatography (n-Hexane:EtOAc = 3:1) to afford the compound of Chemical Formula 1 as a white solid (195.6 mg, yield: 81.92%).

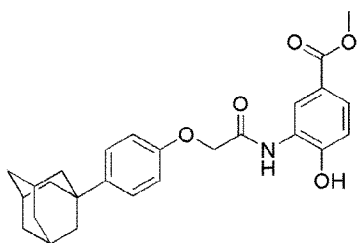
[127] ¹H-NMR (CDCl₃, 300 MHz) δ 9.72 (1H, s, OH), 8.62 (1H, s, NH), 7.82-7.86 (1H, m, aromatic-H), 7.73 (1H, m, aromatic-H), 7.36 (2H, d, J = 9.0 Hz, aromatic-H), 7.05 (1H, d, J = 8.1 Hz, aromatic-H), 6.96 (2H, d, J = 8.7 Hz, aromatic-H), 5.23 (1H, m, CH), 4.68 (2H, s, CH₂), 2.10 (3H, m, adamantyl-H), 1.90 (6H, m, adamantyl-H), 1.77 (6H, m, adamantyl-H), 1.37 (3H, s, CH₃), 1.35 (3H, s, CH₃)

[128]

[129] <COMPARATIVE EXAMPLE 1> Preparation of
 3-[2-(4-Adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid methyl ester
 (3-[2-(4-Adamantan-1-yl-phenoxy)-acetyl-amino]-4-hydroxy-benzoic acid methyl

ester)

[130]



[131] To a solution of 4-adamantan-1-yl-phenoxy acetic acid (143.2 mg, 0.5 mmol) in 5 mL of THF and 0.11 mL of oxalyl chloride (178.5 mg, 1.5 mmol) was dropwise added DMF. After being allowed to react at room temperature for one hour, the reaction mixture was mixed with 3-amino-4-hydroxy-benzoic acid methyl ester (125.4 mg, 0.75 mmol) and 0.05 mL of pyridine. Again, the reaction mixture was allowed to react at room temperature. After completion of the reaction, the mixture was separated with ethyl acetate and an aqueous NaCl solution. The organic layer was dried over anhydrous magnesium sulfate and concentrated. The concentrate was purified using silica gel column chromatography (N-Hexane: EtOAc:MeOH=6:3:1) to afford the title compound as a white solid (183.2 mg, yield:84.1%).

[132] ¹H-NMR (DMSO-d₆, 300 Hz) 11.10(1H, s, OH), 9.24(1H, s, NH), 8.69(1H, m, aromatic-H), 7.60-7.64 (1H, m, aromatic-H), 7.30(2H, d, J=8.4 Hz, aromatic-H), 6.94-6.99 (3H, m, aromatic-H), 4.74(2H, s, CH₂), 3.79(3H, s, CH₃), 2.04(3H, m, adamantly-H), 1.83(6H, m, adamantly-H), 1.72(6H, m, adamantly-H)

[133]

[134] <TEST EXAMPLE 1> Assay for Inhibitory Activity against HIF-1-Mediated HRE Transcription

[135]

[136] For use in evaluating the inhibitory activity of the compound of Chemical Formula 1, prepared in Example 1, against the transcriptional activity of HIF-1, the pGL3-basic vector (Promega) comprising luciferase as a reporter was manipulated to have six repeats of HRE (Hypoxia Responsive Element, 5'-ACGTG-3') of the human VEGFA promoter at the multi-cloning site to prepare a recombinant vector pGL3-HRE-luciferase.

[137] The human rectal cell line HCT116 cells (ATCC) were seeded into 48-well plates and incubated overnight. Using the Polyfect transfection reagent (Qiagen), 25 ng of the pGL3-HRE-luciferase vector was transfected into the cells. For comparison, 2.5 ng of a Renilla vector was used as a control. After the cells were cultured for 24 hrs, the medium was replaced with a fresh one. The cells were incubated for an additional 4 hours and treated with the compound of Chemical Formula 1 prepared in Example 1 or the compound of Comparative Example 1 at a concentration of 0, 1, 3, 5, 10, and 20

μm , followed by incubation for 12 hours in a hypoxic condition (oxygen 1%, nitrogen 94%, carbon dioxide 5%). The cells were treated with a passive lysis buffer and the cell lysates thus obtained were used to determine the activity of the luciferase induced in the hypoxic condition by means of the Dual-luciferase assay system (Promega) to evaluate the inhibitory activities of the compounds prepared in Example 1 and the Comparative Example against HIF-1. The results are summarized in Table 1 and shown in FIG. 1.

[138] Table 1

[Table 1]

Comparison of HRE Transcription Activity (%)		
	Cpd. of Ex. 1	Cpd. of C. Ex. 1
0 μM	100	100
1 μM	128.7	119.4
3 μM	103.7	121.5
5 μM	52.3	98.8
10 μM	18.9	75.6
20 μM	12	43.1

[139]

[140] FIG. 1 is a graph showing results of the assay of the compound of the present invention for inhibition action against HIF-1-mediated transcriptional activity of HRE.

[141]

[142] As shown in Table 1 and FIG. 1, inhibitory activity of the compound of Chemical Formula 1 according to the present invention against HIF-1-mediated HRE transcription activation in the cell line in a hypoxic condition was four times as strong as that of the compound of Comparative Example 1.

[143]

[144] The compound of the present invention inhibits the HIF-1-mediated activation of HRE, thus suppressing the growth and metastasis of cancer. Therefore, the compound of the present invention can be used as an active ingredient in an anticancer agent.

[145] <TEST EXAMPLE 2> Assay for Inhibitory Activity against HIF-1 α Accumulation in Hypoxia

[146] The HIF-1 α protein is a HIF-1 component that plays an important role in the expression of HIF-1 target genes.

[147] The compound of Chemical Formula 1 that potently inhibited the transcriptional activity of HIF-1 as proven in Test Example 1 was assayed for inhibitory activity against HIF-1 α accumulation in the rectal cancer cell line HCT116. In this regard, the inhibitory effect of the compound of Chemical Formula 1 prepared in Example 1 on

the production of HIF-1 α was measured using Western blot analysis.

- [148] First, the rectal cancer cell line HCT-116 cells (ATCC) were seeded at a density of 2×10^5 cells/ml into cell culture dishes, incubated for 24 hours and subjected to hypoxia (oxygen 1%, nitrogen 94%, carbon dioxide 5%, represented by 1% O₂ in FIG. 1) for 4 hours to induce the accumulation of HIF-1 α . Then, the cells were treated with 0, 2, 5, and 10 μ M solutions of the compound of Chemical Formula 1 in DMSO and incubated for 12 hours under the hypoxic condition, followed by preparing nuclear extracts using RIPA buffer. For comparison with the expression of HIF-1 target genes under the hypoxic condition, a normoxic condition containing 20% oxygen was used as a control. Approximately 30 μ g of each of the nuclear extracts was separated by SDS PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred onto a polyvinylidene fluoride membrane, followed by determining the amount of HIF-1 α with an anti-HIF-1 α antibody (R,D System) and an HRP (horseradish peroxidase)-conjugated secondary antibody (Amersham-Pharmacia). In addition, to confirm the presence of the same amount of the nuclear extract on each polyvinylidene fluoride, the anti-HIF-1 α antibody was removed from the membrane used for HIF-1 α detection using a buffer containing 2-mercaptoethanol and then the amount of topoisomerase-1 (expressed as TOPO-1 in FIG. 2) was analyzed using an antibody (Santa Cruz). The results are shown in FIG. 2.
- [149] FIG. 2 shows the inhibitory activity of the compound of the present invention against HIF-1 α accumulation.
- [150] As can be seen in FIG. 2, the compound of Chemical Formula 1, prepared in Example 1, inhibited the production of HIF-1 α in a dose-dependent manner under a hypoxic condition without affecting the expression of topoisomerase-1 (TOPO-1).
- [151] Therefore, the compound according to the present invention inhibits the accumulation of HIF-1 α which leads to the malignancy of cancer and thus can be used as an active ingredient in an anticancer agent.
- [152] <TEST EXAMPLE 3> Effect of Compound of Chemical Formula 1 on Expression of HIF-1 Target Genes VEGFA, EPO, LDHA and CA IX (RT-PCR (reverse transcriptase polymerase chain reaction) Assay)
- [153] Among HIF-1 target genes are VEGFA (vascular endothelial growth factor A) that plays an important role in the growth and metastasis of cancer, EPO (erythropoietin) that promotes the generation of erythrocytes, LDHA (lactate dehydrogenase A) that increases glyconeogenesis and the uptake of glucose into tumor cells, and CA IX (carbonic anhydrase 9) that converts carbon dioxide into carbonic acid to induce tumor acidification. These target genes are known to contribute to the growth and metastasis of cancer.
- [154] The inhibitory activity of the compound of the present invention, prepared in

Example 1, against HIF-1 was examined by measuring its effect on the expression of the representative HIF-1 target genes VEGFA, EPO, LDHA and CA IX. For this, the rectal cell line HCT116 was used.

- [155] HCT116 cells were seeded at a density of 2×10^5 cells/ml into cell culture dishes, incubated for 24 hours and subjected to hypoxia (oxygen 1%, nitrogen 94%, carbon dioxide 5%, expressed as 1% O₂ in FIG. 1) for 4 hours to induce the accumulation of HIF-1 α . Then, the cells were treated with 0, 5, 10, 15 and 20 μ M solutions of the compound of Chemical Formula 1 and incubated for 12 hours in the hypoxic condition, followed by isolating mRNA with Trizol. For comparison with the expression of HIF-1 target genes according to the hypoxic condition, a normoxic condition containing 20% oxygen was used as a control. cDNA was synthesized from the isolated mRNA using an RT-PCR kit (Invitrogen) and used to quantitatively determine mRNA levels of the HIF-1 α target genes VEGFA (Vascular endothelial growth factor A), EPO (erythropoietin), LDHA (Lactate dehydrogenase A) and CA IX (carbonic anhydrase 9) using RT-PCR and real-time PCR. GAPDH was simultaneously amplified as an internal control gene to examine the selectivity of the compound of Chemical Formula for VEGFA, EPO, LDHA, and CA IX. The results are shown in Tables 2 and 3 and FIGS. 3, 4 and 5.

- [156] Table 2

[Table 2]

Inhibitory Activity of Cpd of Chemical Formula 1 against LDHA Expression in Hypoxia				
Oxygen Condition	20% O ₂	1% O ₂		
Cpd. Of Chemical Formula 1 (μ M)	0	0	10	20
mRNA Level of LDHA	1	1.7	0.6	0.7

- [157]

- [158] Table 3

[Table 3]

Inhibitory Activity of Cpd of Chemical Formula 1 against CA IX Expression in Hypoxia				
Oxygen Condition	20% O ₂	1% O ₂		
Cpd. Of Chemical Formula 1 (μ M)	0	0	10	20
mRNA Level of CA IX	1	48	5	4

- [159]

- [160] FIG. 3 shows effects of the compound of the present invention on the expression of

VEGFA and EPO.

[161] FIG. 4 is a graph showing the effect of the compound on the expression of LDHA.

[162] FIG. 5 is a graph showing the effect of the compound on the expression of CA IX.

[163] As is apparent from the data of Tables 2 and 3 and FIGS. 3, 4 and 5, the compound of Chemical Formula 1, prepared in Example 1, had no influence on the expression of the internal control gene GAPDH in hypoxia, but inhibited the expression of the HIF-1 target genes VEGFA, EPO, LDHA and CA IX in a dose-dependent manner.

[164] Thus, having selective inhibitory activity against the expression of VEGFA, EPO, LDHA and CA IX, which play important roles in the growth and metastasis of cancer, the compound of Chemical Formula 1 can be usefully used as an active ingredient of an anticancer agent. In addition, the compound is also applicable to the treatment of diabetic retinopathy or arthritis both of which are aggravated when the expression of VEGFA is increased in hypoxia.

[165]

[166] <TEST EXAMPLE 4> In vivo Assay of Compound of Chemical Formula 1 for Anticancer Activity upon Oral Administration

[167]

[168] To assay the in vivo anticancer activity thereof, the compound of Chemical Formula 1, prepared in Example 1, was orally administered to mice and its inhibition of tumor growth was analyzed. In detail, mice were divided into many test groups and control groups, each consisting of five mice, and then their body weights, tumor volumes and tumor weights were measured to determine the anticancer activity of the compound.

[169] During the experiment, 6-week-old nude mice (BALB/c nu/nu, Charles River) were bred in a sterilized condition at constant temperature and humidity. After the nude mice were anesthetized, the rectal cancer cell line HCT116 was implanted at a density of 4×10^7 cells/mouse into a rectal tissue of each of the nude mice, followed by closure with surgical clips. After the transplantation of the rectal cancer line, the volumes of cancers thus formed were measured using calipers. When the cancers grew to 54.5 mm³, the compound of Chemical Formula 1, prepared in Example 1, was administered. In this regard, the compound of Chemical Formula 1 was dissolved at a concentration of 30 mg/kg in a solvent containing saline 80%, DMAC 10% and Tween 80 10% (hereinafter, referred to as "solvent A" and the solution was orally administered at a dose of 15 ml/kg once a day. Solvent A itself was used as a control and orally administered at a dose of 15 ml/kg once a day. Thereafter, cancer volumes and body weights were measured on day 0, 2, 4, 6, 8, 11, 13, and 14.

[170] The tumor volume was calculated according to the following Math Formula 1. Changes in body weight and tumor weight and size were measured to evaluate the toxicity of the compound of Chemical Formula 1 upon repeated administration and the

body weight results are summarized in Table 4 and the

[171] tumor volume and size in FIG. 5. In Table 5, % inhibition, calculated according to the following Math Formula 2, was expressed as % of the reduction of tumor volume by the compound.

[172]

[173] MathFigure 1

[Math.1]

$$\text{Tumor Volume (mm}^3\text{)} = \text{Length of Long Axis of Tumor (mm)} \times (\text{Length of Short Axis of Tumor (mm)})^2 \times 0.5$$

[174] MathFigure 2

[Math.2]

$$\text{Inhibition (\%)} = \left[\frac{\text{Tumor Volume of Control} - \text{Tumor Volume of Test Group}}{\text{Tumor Volume of Control}} \right] \times 100$$

[175]

[176] Table 4

[Table 4]

Weight Change in Mice upon Repeated Oral Administration of Cpd. of Chemical Formula 1 (%)									
Group (n=5)	Dose (mg/kg)	No of Administration (Day)							
		0	2	4	6	8	11	13	14
Control	0	100.0	104.2	104.6	103.9	103.2	101.7	101.6	99.9
		±0.0	±1.6	±2.6	±2.7	±3.8	±2.2	±2.5	±3.0
Test	30	100.0	104.0	102.8	102.2	102.4	102.7	101.6	99.9
		±0.0	±2.0	±3.5	±5.3	±4.8	±8.1	±8.4	±8.5

[177]

[178] As can be seen in Table 4, no particular symptoms were observed in the nude mice during the administration of the compound of Chemical Formula 1 at a dose of 30 mg/kg. On the final day (Day 14), the mice in the test groups did not show a statistically significant loss in weight, compared to the control. Hence, the compound of Chemical Formula 1 can be used as an active ingredient of an anticancer agent with little toxicity.

[179] Table 5

[Table 5]

Change in Tumor Volume upon Repeated Oral Administration of Cpd. of Chemical Formula 1 and Tumor Weight on Final Day										
Group (n=5)	Dose (mg/kg)	Tumor Volume (mm ³)								Tumor Weight (mg)
		No. of Administration (Day)								
Control		0	2	4	6	8	11	13	14	14
Test	0	0.0 ±0.0	29.8 ±4.8	85.4 ±16.6	171.3 ±29.9	246.0 ±51.9	455.0 ±88.8	604.8 ±108.9	745.3 ±132.5	1799.3 ±308.9
Group (n=5)	30	0.0 ±0.0	23.8 ±6.1	65.1 ±7.3	129.6 ±19.9	184.0 ±16.4	329.3 ±49.8	435.6 ±68.6	505.6 ±77.2	1207.3 ±140.9
Inhibition(%)	-		19.9	23.8	24.3	25.2	27.6	28	32.2	32.9

[180]

[181] As can be seen in Table 5, the tumors in the test group administered with the compound of Chemical Formula 1 at a dose of 30 mg/kg grew by 32.9% less, compared to those in the control.

[182] Therefore, the compound of Chemical Formula 1 shows in vivo anticancer activity without toxicity upon oral administration and can be used as an active ingredient of an anticancer agent.

[183]

[184] <TEST EXAMPLE 5> In vivo Assay of Compound of Chemical Formula 1 for Anticancer Activity upon Intravenous Injection

[185]

[186] To assay the in vivo anticancer activity thereof, the compound of Chemical Formula 1, prepared in Example 1, was intravenously injected into mice and its inhibition of tumor growth was analyzed. In this context, body weights, tumor volumes and tumor weights in mice were measured in the same manner as in Example 1, with the exception that the compound of Chemical Formula 1 was intravenously injected at a dose of 10, 20 and 30 mg/kg.

[187] The tumor volume was calculated according to the following Math Formula 1 while % inhibition was calculated according to the following Math Formula 2. Changes in body weight and tumor weight and size were measured to evaluate the toxicity of the compound of Chemical Formula 1 upon repeated injection and the body weight results are summarized in Table 6 and the tumor volume and size in FIG. 7.

[188] Table 6

[Table 6]

Weight Change in Mice upon Repeated Intravenous Injection of Cpd. of Chemical Formula 1 (%)									
Group (n=5)	Dose (mg/kg)	No. of Administration (Day)							
		0	2	4	6	8	11	13	14
Control	0	100.0 ±0.0	102.8 ±1.1	104.5 ±4.5	102.9 ±5.5	102.2 ±4.3	99.5 ±4.8	100.4 ±2.9	98.6 ±2.7
Test Group 1	10	100.0 ±0.0	102.9 ±3.0	102.3 ±3.6	102.1 ±5.6	101.3 ±4.5	98.5 ±3.8	99.8 ±2.1	98.7 ±2.8
Test Group 2	20	100.0 ±0.0	102.3± 2.1	102.1± 3.8	102.0 ±1.7	101.3 ±2.2	99.6 ±2.6	99.5 ±2.3	99.4 ±2.8
Test Group 3	30	100.0 ±0.0	101.6 ±5.6	101.8± 4.0	101.4 ±3.2	101.2 ±3.4	100.7 ±7.6	100.3 ±7.1	99.6 ±6.8

[189]

[190] As can be seen in Table 6, no particular symptoms were observed in the nude mice during the administration of the compound of Chemical Formula 1 at a dose of 10, 20 and 30 mg/kg. On the final day (Day 14), the mice in test groups did not show statistically significant loss in weight, compared to the control. Hence, the compound of Chemical Formula 1 can be used as an active ingredient in an anticancer agent with little toxicity.

[191] Table 7

[Table 7]

Change in Tumor Volume upon Repeated Intravenous Injection of Cpd. of Chemical Formula 1 and Tumor Weight on Final Day										
Group (n=5)	Dose (mg/kg)	Tumor Volume (mm ³)								Tumor Wt. (mg)
		No. of Administration (Day)								
		0	2	4	6	8	11	13	14	
Control	0	0.0 ±0.0	28.4 ±3.6	86.6 ±20.0	156.5 ±40.8	261.7 ±58.5	457.9 ±129.5	634.2 ±121.1	825.1 ±116.2	2037.4 ±249.2
Test Group 1	10	0.0 ±0.0	22.7 ±3.5	69.6 ±13.3	127.2 ±18.0	223.9 ±34.4	412.7 ±66.6	595.8 ±87.4	765.0 ±192.8	1855.9 ±343.6
Inhibition (%)		-	20.2	19.6	18.7	14.4	9.9	6.0	7.3	8.9
Test Group 2	20	0.0 ±0.0	21.1 ±2.1	63.3 ±7.9	113.0 ±9.3	184.1 ±9.1	315.8 ±32.4	461.7 ±79.0	586.4 ±80.3	1450.7 ±258.0
Inhibition (%)		-	25.7	26.9	27.8	29.6	31.0	27.2	28.9	28.8
Test Group 3	30	0.0 ±0.0	19.5 ±2.2	58.5 ±4.4	100.3 ±11.8	166.5 ±24.7	266.3 ±32.7	367.8 ±91.1	453.7 ±91.2	1136.3 ±202.7
Inhibition (%)		-	31.3	32.4	36.0	36.4	41.8	42.0	45.0	44.2

[192]

[193] As can be seen in Table 7, the compound of Chemical Formula 1, prepared in Example 1, inhibited the growth of tumor 7.3%, 28.9% and 45.0% more upon administration at doses of 10, 20 and 30 mg/kg, respectively, compared to the control. These results indicate that the inhibitory activity of the compound of Chemical Formula 1 against tumor growth increases with an increase in the dose of the compound.

[194] In addition, to examine whether the anticancer activity of the compound of Chemical Formula 1 results from the down-regulation of HIF-1 α expression, tumor tissues were tested for immune response. For this, tumor tissues were treated with topotecan as a comparative group and with the compound of Chemical Formula 1 as a test group. The results are shown in FIG. 6.

[195] FIG. 6 is of optical images showing the down-regulation of HIF-1 α expression by the compound of the present invention.

[196] As can be seen in FIG. 6, the expression of HIF-1 α was distinctly observed in the control, but certainly decreased in the tumor tissue of the mice administered with the compound of Chemical Formula 1.

[197] Accordingly, the compound of Chemical Formula 1 down-regulates the expression of HIF-1 α upon intravenous injection, showing shows anticancer activity without toxicity and thus can be used as an active ingredient in an anticancer agent.

[198]

[199] <TEST EXAMPLE 6> In vivo Assay for Anticancer Activity upon Intravenous Injection of Compound of Chemical Formula 1 and Oral Administration of Conventional Anticancer Agent

[200]

[201] The compound of Chemical Formula 1 was assayed for in vivo anticancer activity when administered alone or in combination with a pre-existing anticancer agent. In this regard, mice were injected with the compound of Chemical Formula 1 at a dose of 30 mg/kg through the tail vein (Test Group 1), orally administered with a preexisting anticancer agent (sunitinib) at a dose of 30 mg/kg (Test Group 2), and injected with the compound of Chemical Formula 1 at a dose of 30 mg/kg through the tail vein and orally administered with a preexisting anticancer agent (sunitinib) at a dose of 30 mg/kg (Test Group 3). For control, mice were injected with Solution B containing carbonate buffer 80%, dimethylacetamide 10% and cremophor EL 10% through the tail vein (control 1), orally administered with distilled water (control 2), and injected with Solution B through the tail vein and orally administered with distilled water (control 3). The initial tumor volume was 54.0 mm³ and tumor volumes and weights of the mice were measured on Day 0, 3, 5, 7, 10, 12, and 14. Other procedures were carried out in the same manner as in Test Example 4 to determine the anticancer

activity.

[202] The tumor volume was calculated according to the following Math Formula 1 while % inhibition was calculated according to the following Math Formula 2. Changes in body weight and tumor weight and size of Test Groups 1 and 3 were measured to evaluate the toxicity of the compound of Chemical Formula 1 upon repeated injection and the body weight results are summarized in Table 8 and the tumor volume and size in Table 9 and FIG. 7.

[203] Table 8

[Table 8]

Change in Body Weight upon Intravenous Injection of Cpd. of Chemical Formula 1 and Oral Administration of Preexisting Anticancer Agent (%)								
Group (n=5)	Dose (mg/kg)	No. of Administration (Day)						
		0	3	5	7	10	12	14
Control 1	0	100.0 ±0.0	101.2 ±3.0	101.2 ±2.1	101.7 ±2.8	99.8 ±2.1	98.7 ±3.6	96.4 ±4.5
Test Group 1	30	100.0 ±0.0	99.6 ±2.3	99.9 ±2.2	99.5 ±1.9	98.9 ±1.7	98.5 ±4.0	97.2 ±3.7
Control 2	0	100.0 ±0.0	101.7 ±2.8	101.5 ±2.4	101.8 ±2.4	100.7 ±4.1	98.5 ±3.9	96.1 ±3.8
Test Group 2	30	100.0 ±0.0	101.6 ±2.6	101.3 ±3.0	100.2 ±2.6	101.0 ±4.5	100.1 ±4.0	99.2 ±5.1
Control 3	0	100.0 ±0.0	100.3 ±2.0	100.4 ±2.8	102.0 ±3.2	99.6 ±3.5	98.0 ±3.3	95.4 ±3.6
Test Group 3	30+30	100.0 ±0.0	99.9 ±1.6	100.1 ±2.1	99.3 ±0.9	98.1 ±2.6	97.1 ±2.9	96.8 ±4.0

[204]

[205] As can be seen in Table 8, no particular symptoms were observed in the nude mice of each test group and each control during the intravenous injection of the compound of Chemical Formula 1 and the preexisting anticancer agent. On the final day (Day 14), the mice in each test group did not show statistically significant loss in weight, compared to the controls. Hence, the compound of Chemical Formula 1 can be used as an active ingredient in an anticancer agent with little toxicity.

[206] Table 9

[Table 9]

Change in Tumor Volume upon Intravenous Injection of Cpd. of Chemical Formula 1 and Oral Administration of Preexisting Anticancer Agent (mm ³)								
Group (n=5)	Dose (mg/kg)	No. of Administration (Day)						
		0	3	5	7	10	12	14
Control 1	0	0.0 ±0.0	26.7 ±4.7	59.8 ±8.0	120.2 ±12.9	238.0 ±19.1	410.2 ±67.6	558.9 ±108.1
Test Group 1	30	0.0 ±0.0	17.8 ±3.1	39.1 ±4.9	78.6 ±10.7	153.6 ±18.7	260.4 ±25.5	356.8 ±49.9
Inhibition (%)			33.1	34.5	34.6	35.4	36.5	36.2
Control 2	0	0.0 ±0.0	28.8 ±3.6	72.3 ±14.8	142.3 ±31.8	269.7 ±51.0	432.6 ±96.7	588.9 ±56.4
Test Group 2	30	0.0 ±0.0	20.5 ±3.8	50.0 ±6.4	96.1 ±13.8	174.7 ±28.9	280.5 ±54.8	380.5 ±67.8
Inhibition (%)			29.0	30.8	32.4	35.2	35.1	35.4
Control 3	0	0.0 ±0.0	37.9 ±7.9	92.4 ±11.9	172.5 ±27.7	317.1 ±64.8	502.0 ±99.3	672.9 ±152.5
Test Group 3	30+30	0.0 ±0.0	16.6 ±3.5	36.7 ±5.6	61.2 ±12.7	104.9 ±25.7	152.0 ±35.7	177.4 ±47.1
Inhibition (%)			56.3	60.2	64.5	66.9	69.7	73.6

[207]

[208] FIG. 7 shows changes in tumor volume upon the intravenous injection of the compound of the present invention and the oral administration of the preexisting anticancer agent

[209] As can be seen in Table 9 and FIG. 7, the intravenous injection of the compound of Chemical Formula 1 (Test Group 1) and the oral administration of the conventional anticancer agent (Test Group 2) inhibited the growth of tumor 36.2% and 35.4% more, respectively, compared to the control, while the intravenous injection of the compound of Chemical Formula 1 in combination with the oral administration of the conventional anticancer agent (Test Group 3) increased the inhibition rate of tumor growth to 73.6%, which is more than twice that obtained upon the individual administration.

[210] Therefore, the compound of Chemical Formula 1 according to the present invention enhance the anticancer activity of conventional anticancer agents, when used in combination, without toxicity, and can be used as an active ingredient in an anticancer agent.

[211]

[212] <TEST EXAMPLE 7> In vivo Assay for Anticancer Activity upon Oral Co-

Administration of Compound of Chemical Formula 1 and Conventional Anticancer Agent

[213]

[214] The compound of Chemical Formula 1 was assayed for in vivo anticancer activity when orally administered in combination with a pre-existing anticancer agent. In this regard, mice were orally administered with the compound of Chemical Formula 1 alone at a dose of 30 mg/kg (Test Group 1), a preexisting anticancer agent (sunitinib) alone at a dose of 30 mg/kg (Test Group 2), and both the compound of Chemical Formula 1 and the preexisting anticancer agent (sunitinib), each at a dose of 30 mg/kg (Test Group 3). For control, mice were orally administered with Solution B containing carbonate buffer 80%, dimethylacetamide 10% and cremophor EL 10%. Other procedures were carried out in the same manner as in Test Example 6 to determine the anticancer activity.

[215] The tumor volume was calculated according to the following Math Formula 1 while % inhibition was calculated according to the following Math Formula 2. Changes in body weight and tumor weight and size of Test Groups 1 and 3 were measured to evaluate the toxicity of the compound of Chemical Formula 1 upon repeated injection and the body weight results are summarized in Table 10 and the tumor volume and size in Table 11 and FIG. 8.

[216] Table 10

[Table 10]

Change in Body Weight upon Oral Co-Administration of Cpd. of Chemical Formula 1 and Preexisting Anticancer Agent (%)									
Group (n=5)	Dose (mg/kg)	No. of Administration (Day)							
		0	2	4	6	8	10	13	14
Control	0	100.0 ±0.0	104.0 ±1.3	103.4 ±2.1	103.9 ±2.5	104.1 ±4.0	103.5 ±3.4	103.2 ±2.3	103.3 ±2.0
Test Group 1	30	100.0 ±0.0	105.2 ±1.5	104.3 ±1.3	102.8 ±1.9	104.8 ±1.7	102.5 ±2.5	102.9 ±2.8	103.1 ±3.9
Test Group 2	30	100.0 ±0.0	103.9 ±1.5	103.0 ±0.8	105.3 ±1.4	107.4 ±3.2	106.0 ±2.9	104.2 ±3.2	103.4 ±4.0
Test Group 3	30+30	100.0 ±0.0	106.3 ±1.5	103.6 ±2.7	105.5 ±4.4	110.1 ±1.3	107.9 ±1.6	107.0 ±4.0	106.5 ±4.0

[217]

[218] As can be seen in Table 10, no particular symptoms were observed in the mice of each test group and each control during the repeated oral injection of the compound of Chemical Formula 1 and the preexisting anticancer agent. On the final day (Day 14), the mice in each test group did not show any statistically significant loss in weight,

compared to the controls. Hence, the compound of Chemical Formula 1 can be used as an active ingredient in an anticancer agent with little toxicity.

[219] Table 11

[Table 11]

Change in Tumor Volume upon Oral Co-Administration of Cpd. of Chemical Formula 1 and Preexisting Anticancer Agent										
Group (n=5)	Dose (mg/kg)	Tumor Volume (mm ³)								Tumor Wt. (mg)
		No. of Administration (Day)								
	0	2	4	6	8	10	13	14	14	
Control	0	0.0	27.2	59.9	116.0	177.5	243.9	386.2	559.3	1659.5
		±0.0	±2.7	±2.7	±8.5	±21.7	±35.9	±53.8	±95.2	±251.0
Test Group 1	30	0.0	20.8	46.2	88.7	133.9	182.3	273.1	388.5	1140.0
		±0.0	±6.5	±9.4	±12.4	±15.5	±18.3	±24.8	±47.9	±92.6
Inhibition (%)		-	23.5	22.8	23.5	24.6	25.3	29.3	30.5	31.3
Test Group 2	30	0.0	20.5	45.1	86.0	125.8	169.0	244.7	346.9	1028.2
		±0.0	±3.2	±6.5	±10.5	±17.5	±21.3	±20.0	±41.6	±126.1
Inhibition (%)		-	24.7	24.7	25.9	29.1	30.7	36.6	38.0	38.0
Test Group 3	30+30	0.0	13.2	27.7	49.8	71.3	97.3	137.6	171.3	510.5
		±0.0	±2.0	±4.4	±6.8	±11.0	±14.6	±32.1	±39.0	±50.6
Inhibition (%)		-	51.7	53.7	57.1	59.8	60.1	64.4	69.4	69.2

[220]

[221] FIG. 8 shows changes in tumor volume upon oral co-administration of the compound of the present invention and the conventional anticancer agent.

[222] As can be seen in Table 11 and FIG.8, the oral administration of the compound of Chemical Formula 1 (Test Group 1) and the oral administration of the conventional anticancer agent (Test Group 2) inhibited the growth of tumor 30.5% and 38.0% more, respectively, compared to the control, while the oral co-administration of the compound of Chemical Formula 1 and the conventional anticancer agent (Test Group 3) increased the inhibition rate of tumor growth to 69.4%, which is more than twice that obtained upon the individual administration.

[223] Therefore, the compound of Chemical Formula 1 according to the present invention enhance the anticancer activity of conventional anticancer agents, when orally administered in combination, without toxicity, and can be used as an active ingredient in an anticancer agent.

[224]

[225] <TEST EXAMPLE 8> Assay for Inhibitory Activity against Tube Formation

[226]

[227] To evaluate the effect of the compound of Chemical Formula 1 on angiogenesis, an experiment for tube formation was carried out.

[228] When HUVEC (Human Umbilical Vein Endothelial Cell) is placed on the basement membrane matrix matrigel, capillary-like tube formation occurs, which is just like to an angiogenesis process in which endothelial cells move and differentiate. In a HUVEC tube formation assay, which is highly similar to an in vivo assay, the compound of Chemical Formula 1 was in vitro tested for ability to induce tube formation, whereby the effect of the compound on angiogenesis could be indirectly analyzed.

[229]

[230] A HUVEC cell line, obtained from the Korea Research Institute of Bioscience & Biotechnology, was maintained in nutrient-supplemented EBM-2 (Cambrex Bio Science Walkersville, Inc.) and treated with the compound of Chemical Formula 1 at a concentration of 1, 3 and 10 μM . A control was treated with DMSO and a comparative group was treated with suramin at a concentration of 10 μM before optical observation. The results are shown in FIG. 9.

[231] FIG. 9 is of optical photographs showing the effect of the compound of the present invention on tube formation.

[232] As seen in FIG. 9, tube formation was suppressed in all of the cells treated with the compound of Chemical Formula 1 at a concentration of 1, 3 and 10 μM , indicating that the compound of Chemical Formula 1 has inhibitory activity against angiogenesis.

[233] Accordingly, having inhibitory activity against angiogenesis, the compound of Chemical Formula 1 according to the present invention can be used as an active ingredient in an anticancer agent.

[234]

[235] <TEST EXAMPLE 9> Assay of Compound of Chemical Formula 1 against Angiogenesis

[236]

[237] To examine the inhibition of angiogenesis by the compound of Chemical Formula 1, the following experiment was performed.

[238] In detail, six-week-old female mice (C537BL) were divided of many groups of five and implanted with B16F10 melanoma cells. After the cancer cells were stabilized, the compound of Chemical Formula 1 was intraperitoneally injected at a dose of 20 mg/kg every day for five days while tumor size and angiogenesis were monitored for the mice. As a control, a solution containing 10% DMAC, 10% cremophor and 80% buffer (pH 10) was administered every day while topotecan was injected at a dose of 2 mg/kg to a comparative group every two days. Body weights and number of the newly formed

blood vessels were measured and the results are shown in Table 12 and FIGS. 10 and 11.

[239] Table 12

[Table 12]

Change in Body Weight of Mice Intraperitoneally Injected with Compound of Chemical Formula (%)				
Group (n=5)	Dose (mg/kg)	No. of Administration (Day)		
		0	2	5
Control	0/day	100.0±0.0	102.9±0.7	107.5±1.7
Comparative Group	2/2day	100.0±0.0	101.2±0.6	103.7±1.4
Test Group	20/day	100.0±0.0	101.6±2.1	106.7±3.7

[240]

[241] As can be seen in Table 12, no particular symptoms were observed in the mice of each test group and each control during the repeated intraperitoneal injection of the compound of Chemical Formula 1 and the preexisting anticancer agent. On the final day (Day 14), the mice in each test group did not show any statistically significant loss in weight, compared to the controls. Hence, the compound of Chemical Formula 1 can be used as an active ingredient in an anticancer agent with little toxicity.

[242] FIG. 10 is a graph showing the effect of the compound of the present invention on angiogenesis.

[243] FIG. 11 is of photographs showing the effect of the compound of the present invention on angiogenesis.

[244] As seen in FIGS. 10 and 11, the number of the newly formed vessels in the mice treated with the compound of Chemical Formula 1 was reduced to approximately 50% of that in the mice treated with the control.

[245] Accordingly, the compound of Chemical Formula 1 according to the present invention has inhibitory activity against angiogenesis and can be used as an active ingredient in an anticancer agent.

[246]

[247] <TEST EXAMPLE 10> Assay for Inhibitory Activity against Metastasis of Cancer

[248]

[249] To evaluate the ability of the compound of Chemical Formula 1 to inhibit the metastasis of cancer, the following experiment was performed.

[250] In this context, 6-week-old female mice (C57BL) were divided into groups of six, and implanted with B16F10 melanoma cells. After the cancer cells were stabilized, the compound of Chemical Formula 1 was intraperitoneally injected at a dose of 20 mg/kg

every day for 12 days while tumor size and angiogenesis were monitored for the mice. As a control, distilled water was used while topotecan was injected at a dose of 2 mg/kg to a comparative group every two days. Body weights and the number of the newly formed blood vessels were measured and the results are shown in Tables 13 and 14 and FIG. 13.

[251] Table 13

[Table 13]

Change in Body Weight upon Intraperitoneal Injection of Cpd. of Chemical Formula 1 (%)							
Group (n=6)	Dose (mg/kg)	No. of Administration (Day)					
		0	2	5	7	9	12
Control	0/day	100.0 ±0.0	101.4 ±2.5	105.5 ±2.9	105.0 ±2.5	106.8 ±2.6	107.1 ±2.7
Comparative Group	2/2day	100.0 ±0.0	101.0 ±2.9	103.5 ±6.1	104.0 ±4.6	104.3 ±6.2	103.7 ±5.5
Test Group	20/day	100.0 ±0.0	100.9 ±1.9	102.6 ±4.0	103.5 ±1.1	107.5 ±1.6	108.7 ±3.8

[252]

[253] As can be seen in Table 13, no particular symptoms were observed in the mice of each test group and each control during the repeated intraperitoneal injection of the compound of Chemical Formula 1 at a dose of 20 mg/kg to the nude mice. On the final day (Day 12), the mice in each test group did not show any statistically significant loss in weight, compared to the controls. Hence, the compound of Chemical Formula 1 can be used as an active ingredient in an anticancer agent with little toxicity.

[254]

[255] Table 14

[Table 14]

Change in No. of Metastasized Tumor Clusters upon Intraperitoneal Injection of Cpd. of Chemical Formula 1		
Group (n=6)	Dose (mg/kg)	No. of Metastasized Tumor Cluster
Control	0	306.8±23.1
Comparative Group	2	148.3±49.4
Metastasis Inhibition (%)	-	51.7
Test Group	20	179.2±54.0
Metastasis Inhibition (%)	-	41.6

[256]

[257] FIG. 12 is a graph showing the number of cancer clusters metastasized to the lung upon treatment with the compound of Chemical Formula 1.

[258] FIG. 13 is of photographs the number of cancer clusters metastasized to the lung upon treatment with the compound of Chemical Formula 1.

[259] On the final day (day 12), the mice were sacrificed and the number of tumor clusters in the lung were counted with the naked eye. In the control, the number of metastasized tumor clusters was 306.8 on average. On the other hand, the tumor clusters in the test group counted 179.2 on average, which corresponded to 41.6% of the number in the control, with statistical significance.

[260] Accordingly, the compound of Chemical Formula 1 according to the present invention exhibited inhibitory activity against cancer metastasis and thus can be used as an active ingredient in an anticancer agent.

[261]

[262] Below, a description is given of the formulations of the composition according to the present invention.

[263]

[264] <FORMULATION EXAMPLE 1> Preparation of Powder

[265]

[266] Cpd. of Chemical Formula 2 g

[267] Lactose 1 g

[268] These ingredients were mixed and loaded into an airtight sac to afford powder

[269]

[270] <FORMULATION EXAMPLE 2> Preparation of Tablet

[271]

[272] Cpd. of Chemical Formula 100 mg

[273] Corn Starch 100 mg

[274] Lactose 100 mg

[275] Mg Stearate 2 mg

[276] These ingredients were mixed together and tableted according to a typical method to afford a tablet.

[277]

[278] <FORMULATION EXAMPLE 3> Preparation of Capsule

[279]

[280] Cpd. of Chemical Formula 100 mg

[281] Corn Starch 100 mg

[282] Lactose 100 mg

[283] Mg Stearate 2 mg

[284] These ingredients were mixed together and loaded into gelatin capsules according to a typical method to afford capsules.

[285]

[286] <FORMULATION EXAMPLE 4> Preparation of Injection

[287]

[288] Cpd. of Chemical Formula 10 $\mu\text{g}/\text{mL}$

[289] Dil. HCl BP added to form pH 3.5

[290] NaCl BP for injection up to 1 mL

[291]

[292] The compound of Chemical Formula 1 was dissolved in a suitable volume of injectable sodium chloride BP and the pH of the solution was adjusted to 3.5 with diluted HCl BP. Injectable NaCl BP was added to the solution to achieve a suitable volume, followed by sufficiently mixing the solution. The mixture solution was then put into a 5-ml type I ampule made of transparent glass. The glass was melted to seal the ampule, which was subsequently autoclaved at 120°C for 15 min, thereby giving an injectable solution

[293]

[294] Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

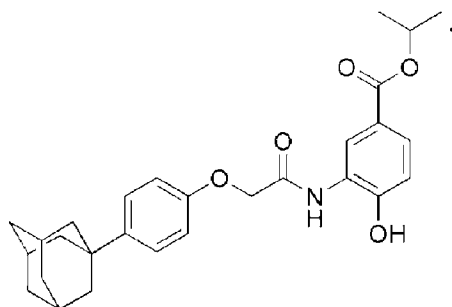
[295]

Claims

[Claim 1]

A novel compound, represented by the following Chemical Formula 1, or a pharmaceutically acceptable salt thereof:

[Chemical Formula 1]

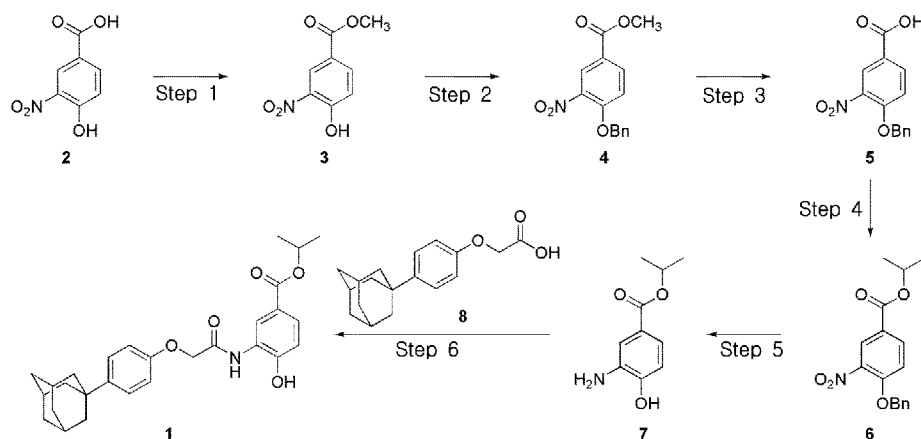


[Claim 2]

A method for preparing the compound of Chemical Formula 1, as illustrated in the following Reaction Scheme 1, comprising:

- esterifying a compound of Chemical Formula 2 to a compound of Chemical Formula 3 (step 1);
- alkylating the compound of Chemical Formula 3 with benzyl bromide to give a compound of Chemical Formula 4 (step 2);
- hydrolyzing the compound of Chemical Formula 4, obtained in step 2, to a compound of Chemical Formula 5 (step 3);
- reacting the compound of Chemical Formula 5, obtained in step 3, with alkyl halide in the presence of a Hunig's base to afford a compound of Chemical Formula 6 (step 4);
- reducing the compound of Chemical Formula 6, obtained in step 4, in the presence of a catalyst in a hydrogen atmosphere to afford a compound of Chemical Formula 7 (step 5); and
- esterifying the compound of Chemical Formula 7, obtained in step 5, with a compound of Chemical Formula 8 in the presence of a Hunig's base to afford the product (1) (step 6):

[Reaction Scheme 1]



- [Claim 3] According to claim 2, wherein the Hunig base of step 4 or 6 is diisopropylethylamine (DIPEA) or triethylamine (TEA).
- [Claim 4] According to claim 2, wherein the alkyl halide of step 4 is 2-bromopropane.
- [Claim 5] According to claim 2, wherein the catalyst of step 5 is a palladium catalyst.
- [Claim 6] A pharmaceutical composition for preventing or treating cancer, comprising the compound of claim 1 as an active ingredient.
- [Claim 7] According to claim 6, wherein the cancer is characterized by accumulation of HIF-1 α and is selected from the group consisting of large intestine cancer, hepatic cancer, stomach cancer, breast cancer, colon cancer, bone cancer, pancreatic cancer, head or neck cancer, cervical cancer, ovarian cancer, rectal cancer, esophageal cancer, small intestine cancer, perianal cancer, fallopian tube cancer, endometrial cancer, uterine cervical cancer, vaginal cancer, vulvar cancer, Hodgkin's disease, prostatic cancer, bladder cancer, kidney cancer, ureter cancer, renal cell carcinoma, renal pelvis cancer, and central nervous system tumors.
- [Claim 8] According to claim 6, wherein the composition shows anticancer activity by inhibiting HIF-1 activity.
- [Claim 9] According to claim 6, wherein the composition shows anticancer activity by inhibiting HIF-1 α accumulation.
- [Claim 10] According to claim 6, wherein the composition suppresses cancer metastasis by inhibiting HIF-1 α accumulation.
- [Claim 11] A pharmaceutical composition for preventing or treating diabetic retinopathy, comprising the compound of claim 1 as an active ingredient.
- [Claim 12] According to claim 11, wherein the composition suppresses an-

giogenesis by inhibiting HIF-1 activity.

[Claim 13] According to claim 11, wherein the composition suppresses angiogenesis by inhibiting HIF-1 α accumulation.

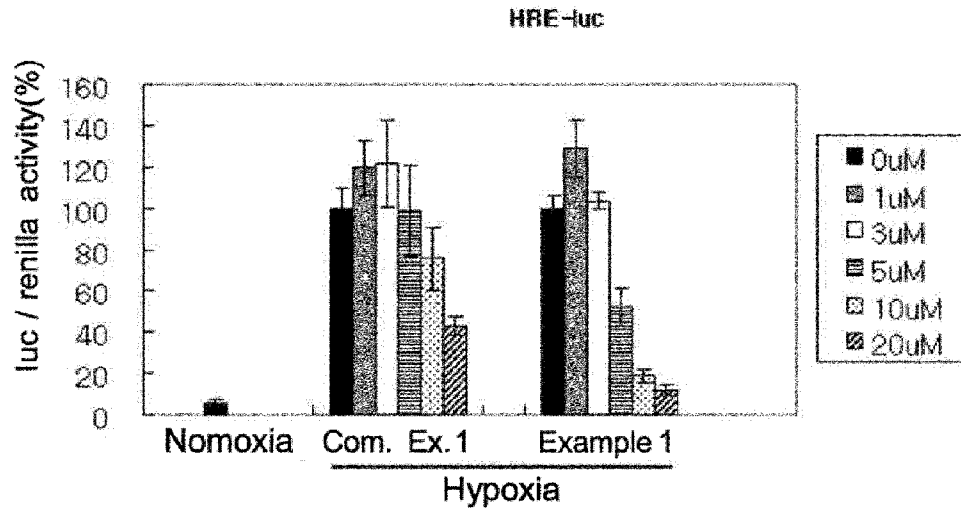
[Claim 14] According to claim 11, wherein the composition suppresses angiogenesis by inhibiting expression of VEGFA.

[Claim 15] A pharmaceutical composition for preventing or treating rheumatoid arthritis, comprising the compound of claim 1 as an active ingredient.

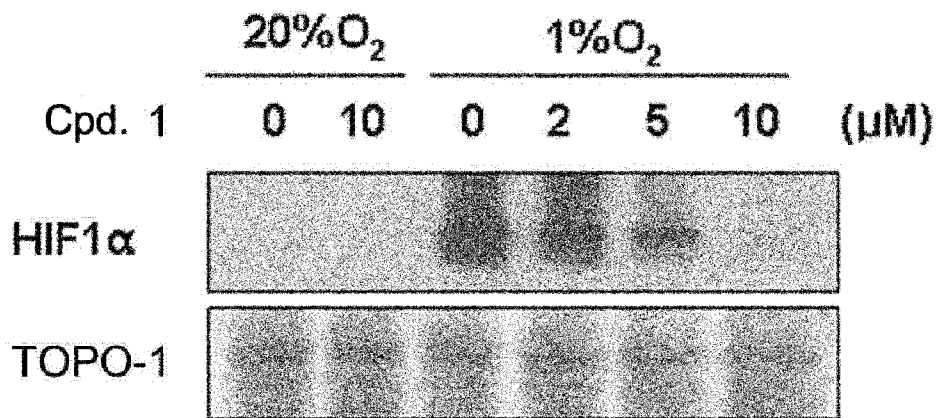
[Claim 16] According to claim 15, wherein the composition suppresses angiogenesis by inhibiting HIF-1 activity.

[Claim 17] According to claim 15, wherein the composition suppresses angiogenesis by inhibiting HIF-1 α accumulation.

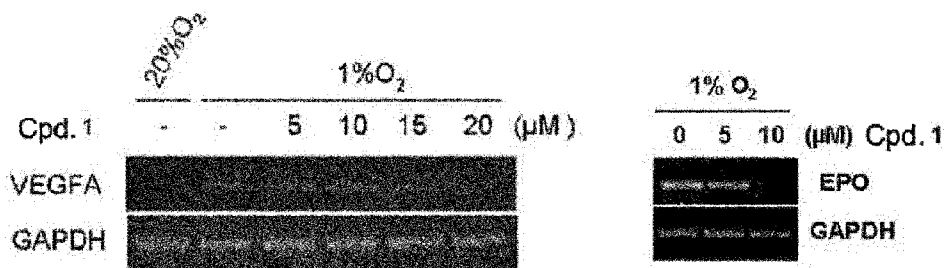
[Fig. 1]



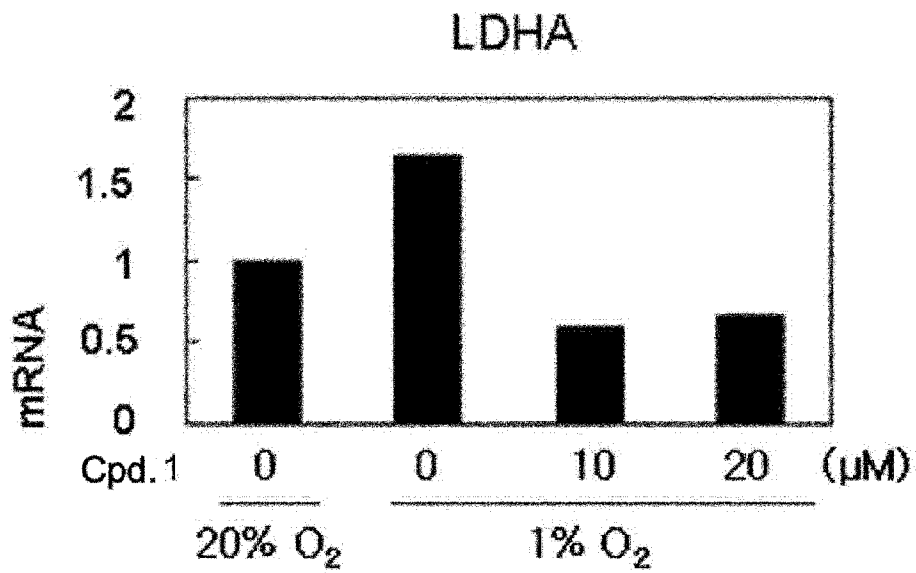
[Fig. 2]



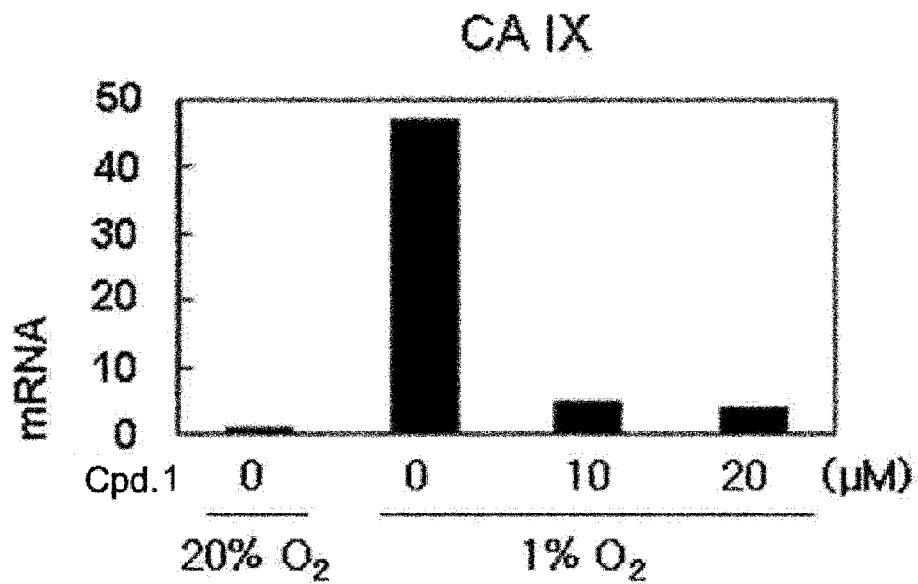
[Fig. 3]



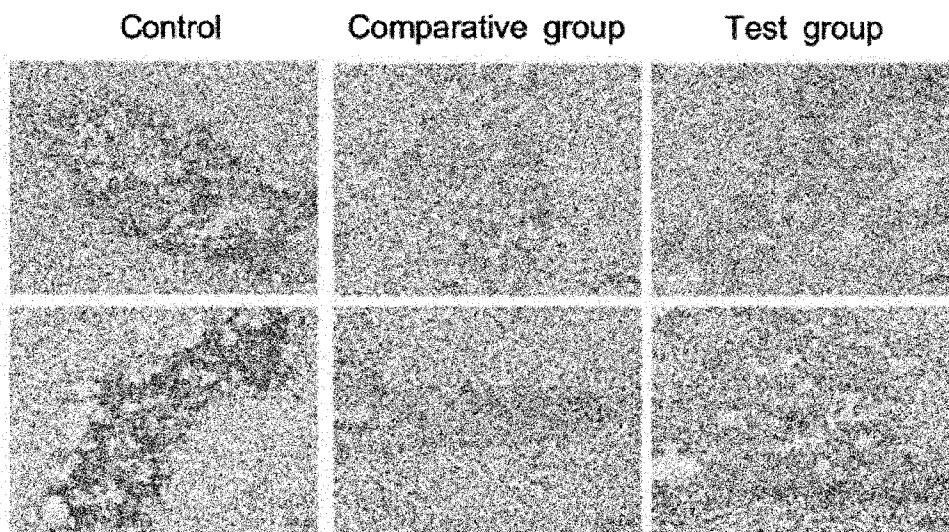
[Fig. 4]



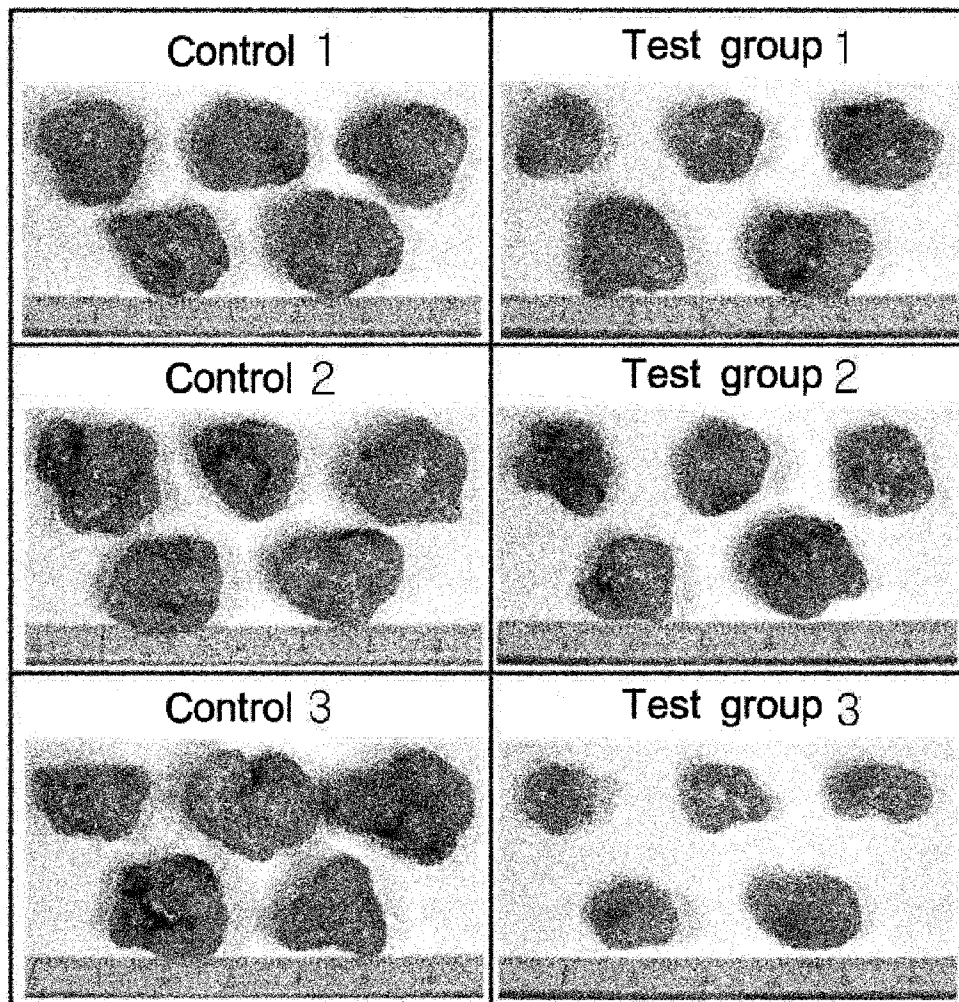
[Fig. 5]



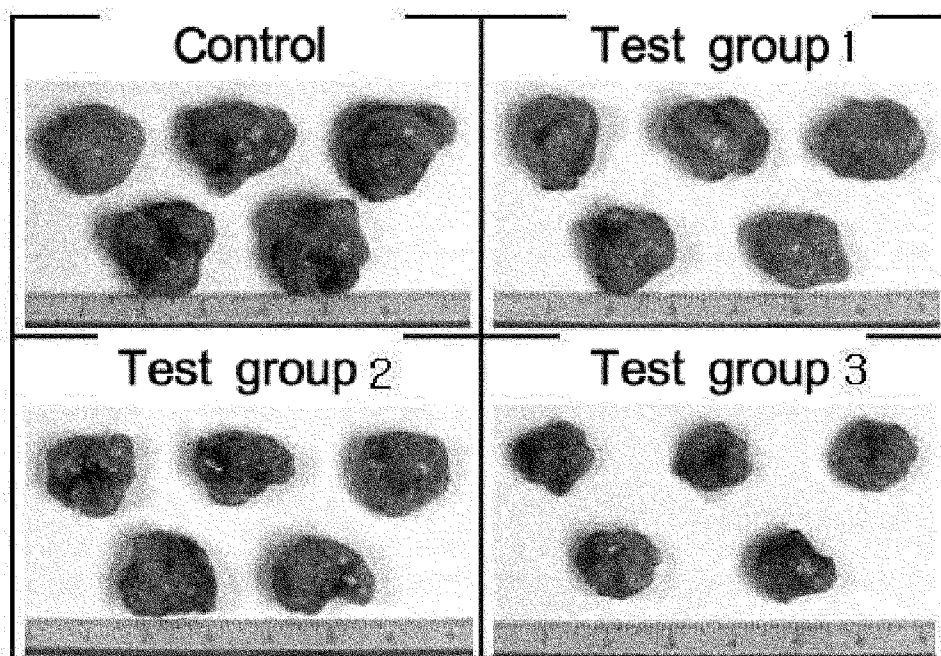
[Fig. 6]



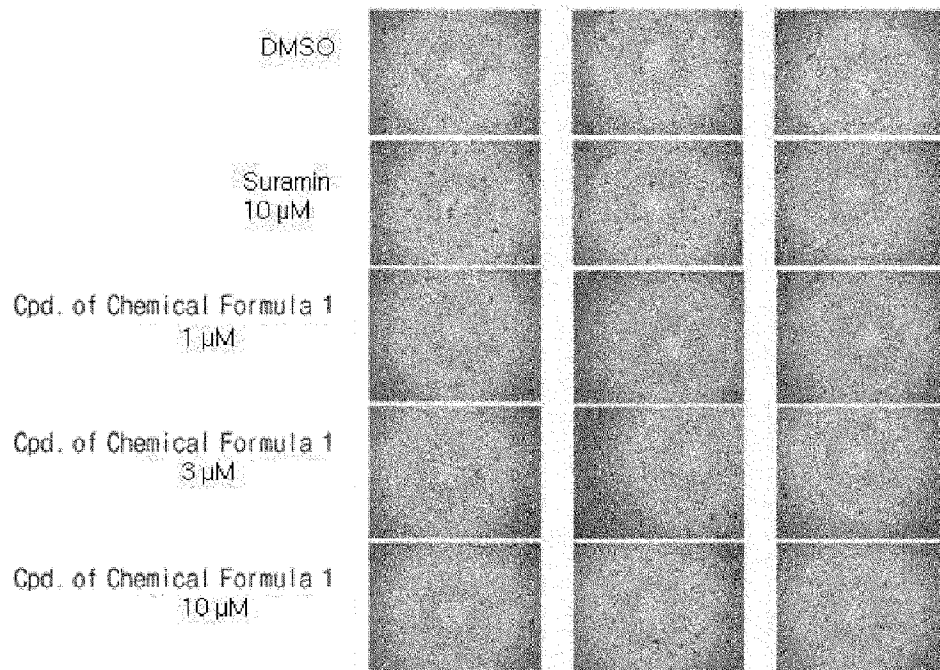
[Fig. 7]



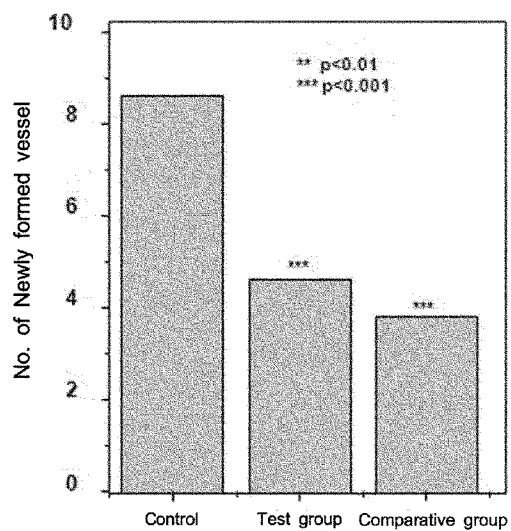
[Fig. 8]



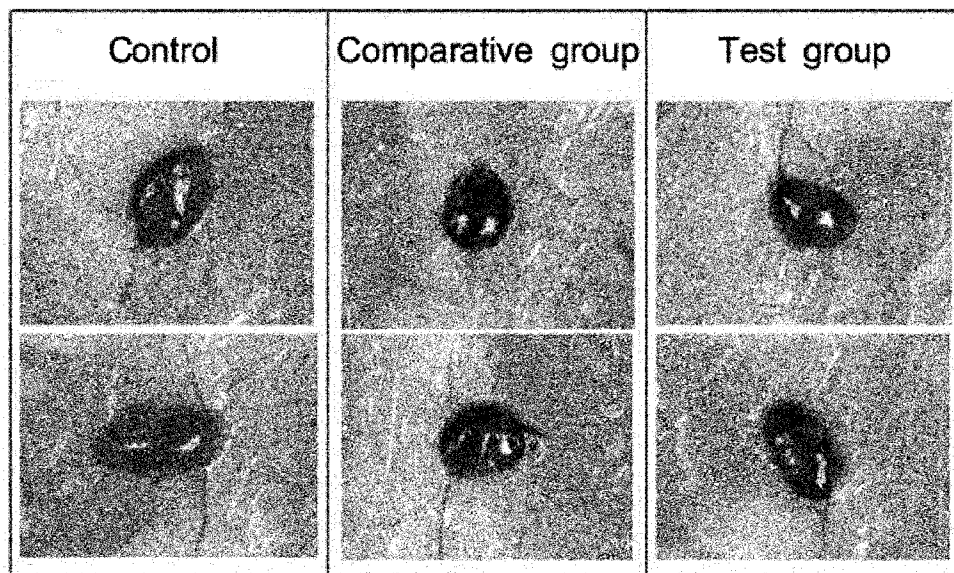
[Fig. 9]



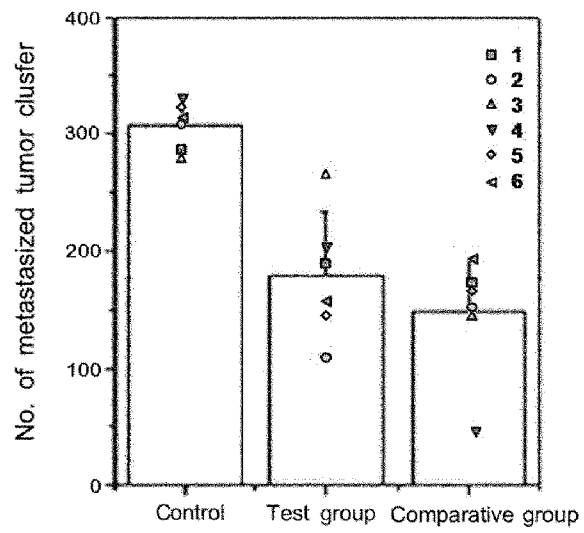
[Fig. 10]



[Fig. 11]



[Fig. 12]



[Fig. 13]

