Title: NOVEL P-GLYCOPROTEIN INHIBITOR, METHOD FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

Abstract: A p-glycoprotein inhibitor of Formula 1 is capable of effectively preventing the development of multi-drug resistance directed to an anticancer agent in cancer cells, and greatly enhances the bioavailability of the drug, such as paclitaxel, which is not readily absorbed when orally administered.
NOVEL P-GLYCOPROTEIN INHIBITOR, METHOD FOR THE PREPARATION THEREOF AND PHARMACEUTICAL COMPOSITION COMPRISING THE SAME

Field of the Invention

The present invention relates to a novel p-glycoprotein inhibitor and a pharmaceutically acceptable salt thereof having an inhibitory activity against multi-drug resistance, a method for the preparation thereof and a pharmaceutical composition comprising the same as an active ingredient.

Background of the Invention

Multi-drug resistance (MDR) in cancer cells is a major impediment to successful chemotherapy in many types of cancer. MDR is a phenomenon where tumor cells develop cross-resistance against a group of structurally and functionally unrelated compounds after exposed to one cytotoxic agent. The drug resistance developing in tumor cells often results from an elevated expression of particular proteins, such as cell-mediated transporters, which results in an increased efflux of cytotoxic agents from the cancer cells, thereby lowering their intracellular concentration.

Specifically, MDR in tumor cells is often caused by an over-expression of p-glycoprotein (P-gp), a 170-kd ATP-dependent membrane transporter. This protein is an active efflux pump of chemotherapeutic drugs, natural products and hydrophobic peptides.

The expression of p-glycoprotein is usually highest in tumor cells derived from tissues that normally express p-glycoprotein, such as colon, kidney, pancreas and liver, resulting in a potential resistance to some cytotoxic agents even before chemotherapy is initiated. In other tumors, the expression of p-glycoprotein may be low at the time of diagnosis but increases after exposure to chemotherapeutic drugs, resulting in a development of MDR in those cells. The chemotherapeutic drugs that are most frequently associated with MDR are hydrophobic natural products such as taxan (e.g., paclitaxel and docetaxel),
vinca alkaloid (e.g., vinorelbine, vincristine and vinblastine), anthracyclins (e.g., doxorubicin, daunorubicin and epirubicin), epipodophyllotoxin (e.g., etoposide and teniposide), topotecan, daunomycin, and mitomycin C (D. W. Shen, et al., *Science* 232, 643-645, 1986).

It is also known that p-glycoprotein is present on the wall of the intestinal tract and inhibits the absorption of orally administered drugs. When an anticancer agent, such as paclitaxel or docetaxel, is orally administered, its absorption is significantly inhibited by the action of p-glycoprotein (Schinkel, et al., *Cell* 77, 491-502, 1994). Accordingly, when a p-glycoprotein inhibitor is administered in combination with an anticancer agent, it may be possible to facilitate the treatment of malignant tumor by way of allowing the agent to accumulate in multi-drug resistant cancer cells, due to the inhibitor’s activity blocking the p-glycoprotein’s efflux pump.

There have been numerous attempts to improve the efficacy of an anticancer agent using certain compounds that inhibit the action of p-glycoprotein in cancer cells. For example, verapamil (a calcium channel inhibitor) and cyclosporin A (an immunosuppressive agent) are known to be effective in reversing the MDR of cancer cells against anticancer agents. However, these compounds exhibit low binding affinities to p-glycoprotein and the administration thereof in high dosage may entail an unacceptable toxicity problem. Further, administration of conventional p-glycoprotein inhibitors often induces serious adverse effects such as blood pressure decline and immunity suppression (Tsuro T, et al., *Cancer Res.* 41, 1967-1972, 1981).

PCT Publication No: WO 94/07858 discloses a piperidin-2-carboxylic acid derivative as an effective MDR inhibitor. VX-710, one of the specific active compounds disclosed in the patent, shows a greater inhibitory activity at a μM concentration level than the conventional p-glycoprotein inhibitors, and prevents the development of MDR in cancer cells by directly binding to the p-glycoprotein’s efflux pump. However, it has been found that VX-710 inhibits the activity of cytochrome P450 enzyme. When administered together with paclitaxel or vinblastin in clinical trials, VX-710 inhibits the cytochrome P450-mediated metabolism of such drug, resulting in an undesirable increase of a cytotoxic agent in the serum and over-exposure of a patient to the cytotoxic
agent.

PCT Publication No: WO 92/12132 teaches an acridine derivative as a MDR inhibitor. GF-120918, one of the compounds described in this patent, strongly inhibits the ATPase activity of p-glycoprotein and breast cancer-resistance protein (BCRP). However, GF-120918 shows a low selectivity in inhibiting the p-glycoprotein’s efflux pump.

PCT Publication No: WO 98/17648 reports an anthranilic acid derivative that is active as an inhibitor of p-glycoprotein and thus may be used as a modulator of MDR in the treatment of cancers. XR-9576, one of the compounds mentioned in the patent, binds to p-glycoprotein with a high affinity and potently inhibits its activity, allowing various anticancer agents to accumulate in cancer cells. However, when clinically used to prevent the development of MDR, it causes undesired side effects.

Accordingly, there has continued to exist a need to develop an effective agent that can be used to resensitize multi-drug resistant cells to therapeutic or prophylactic drugs or to prevent the development of MDR in cancer cells without adverse side effects. The present inventors have endeavored to develop such agent and found that a chromone derivative having an anti-oxidative activity acts as a p-glycoprotein inhibitor for preventing MDR without causing adverse side effects. This chromone derivative may be used together with an anticancer agent against MDR cells.

**Summary of the Invention**

Accordingly, it is an object of the present invention to provide a novel p-glycoprotein inhibitor and a pharmaceutically acceptable salt thereof having MDR inhibitory activity, which enhances the oral absorption rate of a conventional anticancer agent.

It is another object of the present invention to provide a method for preparing the p-glycoprotein inhibitor or a pharmaceutically acceptable salt thereof.

It is a further object of the present invention to provide a pharmaceutical composition comprising the p-glycoprotein inhibitor or a pharmaceutically
acceptable salt thereof as an active ingredient.

In accordance with one aspect of the present invention, there is provided a novel p-glycoprotein inhibitor of Formula 1 or a pharmaceutically acceptable salt thereof:

<Formula 1>

wherein,

R₁ to R₈ are each independently hydrogen, hydroxy, halogen, NO₂, C₁₋₅ alkyl or C₁₋₅ alkoxy.

In accordance with another aspect of the present invention, there is provided a method for preparing the p-glycoprotein inhibitor of Formula 1 or a pharmaceutically acceptable salt thereof, which comprises reacting a compound of Formula 2 with a compound of Formula 3 in a solvent in the presence of a condensing agent or a base:

<Formula 2>

<Formula 3>
wherein,
R₁ to R₈ are each independently hydrogen, hydroxy, halogen, NO₂, C₁₋₅ alkyl or C₁₋₅ alkoxy, and
R’ is OH, Cl or Br.

In accordance with a further aspect of the present invention, there is provided a pharmaceutical composition comprising the p-glycoprotein inhibitor of Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient together with a pharmaceutically acceptable carrier for inhibiting MDR.

**Detailed Description of the Invention**

The present invention provides a novel p-glycoprotein inhibitor of Formula 1 and a pharmaceutically acceptable salt thereof:

<Formula 1>

wherein,
R₁ to R₈ are each independently hydrogen, hydroxy, halogen, NO₂, C₁₋₅ alkyl or C₁₋₅ alkoxy.

Unlike the conventional p-glycoprotein inhibitors, e.g., cyclosporin A, cinchonine and verapamil, the compound of Formula 1 itself has no pharmacological activity and, consequently, causes no side effects while enhancing the bioavailability of a poorly absorbable drug by inhibiting p-glycoproteins located on the intestinal wall.

Representative examples of the preferred p-glycoprotein inhibitor of
Formula 1 are:

4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;

6-methyl-4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;

5-methoxy-4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;

5-hydroxy-4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;

6-fluoro-4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide; and

6-bromo-4-oxo-4'H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1'H-isouquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide.

The p-glycoprotein inhibitor of Formula 1 may be prepared by such a method as shown in Reaction Scheme 1:

< Reaction Scheme 1>
wherein,

R₁ to R₈ and R' have the same meanings as defined in Formulae 1 to 3.

In Reaction Scheme 1, the p-glycoprotein inhibitor of Formula 1 may be prepared by reacting an aminophenyl compound of Formula 2 with a carboxylic acid or acylhalide compound of Formula 3 in a solvent in the presence of a condensing agent or a base. It is preferable that 1 to 2 equivalents of the compound of Formula 3 are reacted with 1 equivalent of the compound of Formula 2. The reaction may be conducted at a temperature ranging from 0 to 50°C.

Representative examples of the condensing agent include 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide, N,N-dicyclohexylcarbodiimide, N,N-diisopropylcarbodiimide, 1-cyclohexyl-3-(2-(morpholinoethyl)carbodiimide methyl-p-toluenesulfonate and the like, preferably 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Such condensing agent may be used in an amount
ranging from 1 to 5 equivalents, preferably from 1 to 3 equivalents, per 1 equivalent of the compound of Formula 2.

In case a condensing agent is used in the reaction, 4-(dimethylamino)pyridine may be also used as a catalyst in an amount ranging from 0.05 to 0.3 equivalent based on 1 equivalent of the compound of Formula 2.

Representative examples of the base encompass triethylamine, dipropylethylamine, pyridine and the like, and such base may be used in an amount ranging from 1 to 2 equivalents relative to 1 equivalent of the compound of Formula 2. A suitable solvent is selected from dichloromethane, chloroform, \(N,N\)-dimethylformamide, tetrahydrofuran, 1,4-dioxane and the like, preferably dichloromethane and chloroform. The above reaction may be conducted at a temperature ranging from -20 to 100°C, preferably 0 to 50°C.

The compound of Formula 2 used in the preparation of the p-glycoprotein inhibitor of Formula 1 may be synthesized by the following steps:

1. reacting a compound of Formula 8 with an amine compound of Formula 9 in a solvent in the presence of a base to obtain a nitro compound of Formula 7;
2. subjecting the nitro compound of Formula 7 to hydrogenation in a solvent in the presence of a catalyst to obtain an amine compound of Formula 5;
3. reacting a compound of Formula 6 with thionyl chloride and reacting the resulting compound with the amine compound of Formula 5 in a solvent in the presence of a base to obtain a nitrophenyl compound of Formula 4; and
4. subjecting the nitrophenyl compound of Formula 4 to hydrogenation in a solvent in the presence of a catalyst.

\(<\text{Formula 4}>\)

\(<\text{Formula 5}>\)
<Formula 6>
\[ R_6 \]
\[ R_5 \]
\[ R_5 \]
\[ \text{OH} \]
\[ \text{NO}_2 \]

<Formula 7>
\[ R_7 \]
\[ \text{O}_2\text{N} \]
\[ \text{N} \]
\[ \text{R}_8 \]

<Formula 8>
\[ \text{Br} \]
\[ \text{O}_2\text{N} \]

<Formula 9>
\[ R_7 \]
\[ \text{HN} \]
\[ \text{R}_8 \]

wherein,
R_2 to R_8 have the same meanings as defined in Formula 1.

In step (1), it is preferable that 1 to 2 equivalents of the compound of Formula 9 are reacted with 1 equivalent of the compound of Formula 8. Representative examples of the base include pyridine, triethylamine, diisopropylethylamine, dimethylformamide and the like, and such base may be used in an amount ranging from 1 to 2 equivalents per 1 equivalent of the compound of Formula 8. A suitable solvent for this step is selected from water, methanol, ethanol, chloroform, dichloromethane, tetrahydrofuran, ethylether, hexane, toluene and the like, and step (1) may be conducted at a temperature ranging from 0 to 120°C.

In step (2), a representative example of the catalyst is a metallic catalyst such as palladium, platinum, zinc, iron and the like, and such metallic catalyst may be used in an amount ranging from 0.1 to 0.3 equivalent based on 1 equivalent of the compound of Formula 7. A suitable solvent for this step is chosen from methanol, ethanol, chloroform, dichloromethane, tetrahydrofuran, ethylether, hexane, toluene and the like, and step (2) may be conducted at a
temperature ranging from 0 to 50°C.

In step (3), it is preferable to employ the compound of Formula 5 in an amount ranging from 1 to 1.5 equivalents relative to 1 equivalent of the compound of Formula 6. Representative examples of the base are pyridine, triethylamine, diisopropylethylamine and the like, and such base may be used in an amount ranging from 1 to 3 equivalents per 1 equivalent of the compound of Formula 6. A suitable solvent for this step is water, methanol, ethanol, chloroform, dichloromethane, tetrahydrofuran, ethylether, hexane, toluene or the like, and step (3) may be conducted at a temperature ranging from 0 to 50°C.

In step (4), a preferred example of the catalyst is a metallic catalyst such as palladium, platinum, zinc, iron and the like, and such metallic catalyst may be used in an amount ranging from 0.1 to 0.3 equivalent relative to 1 equivalent of the compound of Formula 4. A suitable solvent for this step is selected from methanol, ethanol, chloroform, dichloromethane, tetrahydrofuran, ethylether, hexane, toluene and the like, and step (4) may be conducted at a temperature ranging from 0 to 50°C.

Furthermore, the present invention encompasses, within its scope, a pharmaceutically acceptable salt of the p-glycoprotein inhibitor of Formula 1 derived from an inorganic or organic acid. A preferred inorganic or organic acid is selected from hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, acetic acid, glycolic acid, lactic acid, pyruvic acid, malonic acid, succinic acid, glutamic acid, fumaric acid, malic acid, mandelic acid, tartaric acid, citric acid, ascorbic acid, palmitic acid, maleic acid, hydroxymaleic acid, benzoic acid, hydroxybenzoic acid, phenylacetic acid, cinnamic acid, salicylic acid, methanesulfonic acid, benzensulfonic acid and toluenesulfonic acid.

The present invention also provides a method for treating a mammal, including a human, which suffers from a cancer, comprising administering to the mammal an effective amount of the compound of Formula 1 or a pharmaceutically acceptable salt thereof:

(a) to improve or increase the efficacy of an anticancer agent;
(b) to increase or restore the sensitivity of a tumor to the anticancer agent; or
(c) to reduce or reverse MDR of a tumor to the anticancer agent regardless of whether the MDR is acquired, induced or innate.

The p-glycoprotein inhibitor of the present invention may be administered in combination with an anticancer agent which is not readily absorbed in the digestive tract due to the inhibitory action of p-glycoprotein. Thus, in a further aspect, the present invention provides a composition comprising the p-glycoprotein inhibitor of Formula 1 or a pharmaceutically acceptable salt thereof together with an anticancer agent, which is effective in:

(a) improving or increasing the efficacy of the anticancer agent;

(b) increasing or restoring the sensitivity of a tumor to the anticancer agent; or

(c) reducing or reversing MDR of a tumor to the anticancer agent regardless of whether the MDR is acquired, induced or innate.

Preferred examples of the anticancer agent comprise taxan (e.g., paclitaxel and docetaxel), vinca alkaloid (e.g., vincristine, vinblastine and vinorelbine), anthracycline (e.g., daunomycin, daunorubicin, doxorubicin and aclarubicin), camptothecin (e.g., topotecan and irinotecan), podophyllotoxin (e.g., etoposide and VP16), mitoxantrone, actinomycin, colchicine, gramicidine D, and amsacrine.

In a further aspect, the present invention provides a pharmaceutical composition comprising the p-glycoprotein inhibitor of Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient together with a pharmaceutically acceptable carrier, for the treatment of a mammal which suffers from a cancer:

(a) to improve or increase the efficacy of an anticancer agent;

(b) to increase or restore the sensitivity of a tumor to the anticancer agent; or

(c) to reduce or reverse MDR MDR of a tumor to the anticancer agent regardless of whether the MDR is acquired, induced or innate.

The p-glycoprotein inhibitor of Formula 1 according to the present invention may be formulated for oral, buccal, parenteral or rectal administration. Among these, formulations for oral or parenteral administration are preferred.

For oral administration, the pharmaceutical composition of the present
invention may take the form of tablets or capsules prepared in the conventional manner together with at least one pharmaceutically acceptable excipient, such as a binding agent (e.g., pregelatinised maize starch, polyvinylpyrrolidone and hydroxypropyl methylcellulose); a filler (e.g., lactose, microcrystalline cellulose and calcium hydrogen phosphate); a lubricant (e.g., magnesium stearate, talc and silica); and a disintegrant (e.g., sodium lauryl sulphate and sodium starch glycolate). These tablets may be coated by the methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by the conventional means together with at least one pharmaceutically acceptable additive such as a suspending agent (e.g., sorbitol syrup, a cellulose derivative and a hydrogenated edible fat); an emulsifying agent (e.g., lecithin and acacia); a non-aqueous vehicle (e.g., almond oil, oily ester, ethyl alcohol and fractionated vegetable oil); and a preservative (e.g., methyl or propyl-p-hydroxybenzoate and sorbic acid). These preparations may also contain at least one buffer salt or at least one flavouring, colouring or sweetening agent as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active ingredient.

For buccal administration, the pharmaceutical composition may take the form of tablets or lozenges formulated in the conventional manner.

The pharmaceutical composition of the present invention may be formulated for parenteral administration such as bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form e.g., in ampoules or in multi-dose containers, with added preservative. The pharmaceutical composition may take the form of suspensions, solutions or emulsions in oily, aqueous or alcoholic vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in a powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

The pharmaceutical composition of the present invention may also be formulated for rectal administration, for example, suppositories or retention
enemas containing conventional suppository bases such as cocoa butter or other glycerides.

A proposed daily dose of the p-glycoprotein inhibitor of the present invention for administration to a human (of approximately 70 kg body weight) ranges about from 0.1 to 100 mg/kg, more preferably about from 1 to 20 mg/kg. It may be necessary to make routine variations to the dosage, and the route of administration depending on the age and condition of the patient. For example, a daily dose of about 1 mg/kg may be appropriate for administration by infusion in a human. The daily dose may be given as a single unit or multiple subunits.

The following Examples are intended to further illustrate the present invention without limiting its scope.

**Example 1: Synthesis of 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide**

1) **Synthesis of 4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isquinoline-2-yl)-ethyl]-phenyl amine**

2.30 g of 2-(4-nitrophenyl)ethyl bromide and 2.29 g of 6,7-dimethoxy-1,2,3,4-tetrahydro isoquinoline hydrochloride salt were dissolved in 150 ml of N,N'-dimethylformamide, followed by adding 4.15 g of potassium carbonate and 1.80 g of sodium iodide thereto, and the mixture was allowed to react at 100°C for 12 hrs. After the reaction was completed, 150 ml of distilled water was added to the reaction mixture, followed by extraction three times with 200 ml portion of ethyl acetate. The combined organic layer was washed with saturated NaCl and dried over magnesium sulfate. The solution was filtered under a reduced pressure and the solvent was removed by evaporation, to obtain a residue 2.8 g. The residue was dissolved in 30 ml of ethyl acetate and recrystallized therefrom, to obtain 2.40 g of a nitro derivative. The nitro derivative was dissolved in a mixture of 150 ml of tetrahydrofuran and 150 ml of methanol and 0.24 g of Pd/C was added thereto. The mixture was reduced...
under an atmospheric hydrogen pressure for 18 hrs. The reduction mixture was filtered through a cellite pad under a reduced pressure, and the pad was washed with methanol. The filtrate and the methanol wash solution were combined, and the solvent was removed there from under a reduced pressure, to obtain 2.03 g of the title compound (yield: 92%).

$^1$H-NMR(CDC$_3$) $\delta$ : 6.97(d, 2H), 6.57(d, 2H), 6.53(s, 1H), 6.47(s, 1H), 3.77(s,6H), 3.57(s, 2H), 3.50(s, 2H), 2.71(m, 8H)

2) Synthesis of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy benzamide

1.14 g of 4,5-dimethoxy-2-nitrobenzoic acid was added to 20 ml of toluene and 0.73 ml of thionyl chloride was added thereto. The mixture was allowed to react at 100°C for 2 hrs. After the reaction was completed, the solvent was removed under a reduced pressure, to obtain a residue (1.13 g). The residue was dissolved in 20 ml of dichloromethane and cooled to 0°C, to which 1.56 g of 4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenyl amine synthesized in step (1) and a mixture of 1.05 ml of triethylamine and 20 ml of dichloromethane were added. The resulting mixture was heated to room temperature and allowed to react at that temperature for 8 hrs. After the reaction was completed, the reaction mixture was washed with saturated ammonium chloride and saturated NaCl. The organic layer was dried over magnesium sulfate, followed by filtration under a reduced pressure, and the solvent was removed by evaporating under a reduced pressure. The resulting residue was purified by column chromatography, to obtain 2.50 g of a nitro derivative. 2.50 g of the nitro derivative was dissolved in a mixture of 30 ml of ethanol and 30 ml of dichloromethane, and 0.25 g of Pd/C was added thereto. The reaction mixture was reduced under an atmospheric hydrogen pressure for 12 hrs. The reduction mixture was filtered through a cellite pad under a reduced pressure, and the pad was washed with ethanol. The filtrate and the methanol wash solution were combined, and the solvent was removed there from under a reduced pressure, to obtain 2.24 g of the title compound (yield: 95%).
\[ ^1\text{H-NMR(CDC\textsubscript{3}) } \delta : 8.96(s, 1H), 7.58(d, 2H), 7.21(s, 1H), 7.03(d, 2H), 6.54(s, 1H), 6.46(s, 1H), 4.10(s, 2H), 3.81(s, 6H), 3.77(s, 6H), 3.77(s, 2H), 3.06(s, 6H). \]

3) Synthesis of 4-oxo-4\textit{H}-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1\textit{H}-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

2.5 g of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1\textit{H}-isoquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide synthesized in step (2) and 0.97 g of chromone-2-carboxylic acid were dissolved in 5 ml of dichloromethane, and 0.1 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide chloride salt and 0.005 g of 4-(dimethylamino)pyridine were added thereto, followed by allowing the mixture to react at room temperature for 12 hrs. After the reaction was completed, the reaction mixture was washed with 50 ml of distilled water, and the organic layer was separated. The organic layer was dried over magnesium sulfate, filtered and concentrated under a reduced pressure. The resulting residue was purified by column chromatography, to obtain 2.2 g of the title compound (yield: 66%).

\[ ^1\text{H-NMR(CDC\textsubscript{3}) } \delta : 12.80(s, 1H), 8.53(s, 1H), 8.23(d, 1H), 8.85(s, 1H), 7.73(m, 2H), 7.59(d, 2H), 7.47(t, 1H), 7.32(d, 2H), 7.23(s, 1H), 7.12(s, 1H), 6.62(s, 1H), 6.55(s, 1H), 3.98(d, 6H), 3.86(s, 6H), 3.68(s, 2H), 2.95(m, 2H), 2.83(m, 6H) \]

Example 2: Synthesis of 6-methoxy-4-oxo-4\textit{H}-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1\textit{H}-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

0.5 g of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1\textit{H}-isoquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide obtained in step (2) of Example 1 was dissolved in 5 ml of dichloromethane, and 0.21 g of 6-methylchromone-2-carbonyl chloride and 0.1 ml of triethylamine were added there to, followed by allowing the mixture to react at room temperature for 12 hrs. After the
reaction was completed, the reaction mixture was washed with 50 ml of distilled water, and the organic layer was separated. The organic layer was dried over magnesium sulfate, filtered and concentrated under a reduced pressure. The resulting residue was purified by column chromatography, to obtain 0.4 g of the title compound (yield: 58%).

\[ \text{H-NMR(CDC}_3\text{) } \delta : 12.75(\text{s, } 1\text{H}), 9.16(\text{s, } 1\text{H}), 8.01(\text{s, } 1\text{H}), 7.80(\text{s, } 1\text{H}), 7.59(\text{m, } 4\text{H}), 7.31(\text{d, } 2\text{H}), 7.20(\text{s, } 1\text{H}), 7.11(\text{s, } 1\text{H}), 6.62(\text{s, } 1\text{H}), 6.55(\text{s, } 1\text{H}), 3.98(\text{d, } 6\text{H}), 3.85(\text{s, } 6\text{H}), 3.68(\text{s, } 2\text{H}), 2.96(\text{m, } 2\text{H}), 2.82(\text{m, } 6\text{H}) \]

**Example 3: Synthesis of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl][phenylcarbamoyl-4,5-dimethoxy-phenyl]-amide**

1) Synthesis of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid

a) Synthesis of 1-(2-hydroxy-6-methoxy-phenyl)-ethanone

10.0 g of 2',6'-dihydroxy-acetophenone was dissolved in 70 ml of acetone, and 9.1 g of potassium carbonate and 4.0 ml of iodomethane were added thereto, followed by allowing the mixture to react at 55°C for 8 hrs. After the reaction was completed, the solvent was removed under a reduced pressure. The resulting residue was mixed with 500 ml of water and extracted twice with 250 ml portion of methylenechloride. The combined organic layer was washed with saturated NaCl, dried over magnesium sulfate, filtered and concentrated under a reduced pressure, to obtain a residue which gave 10.4 g of the title compound (yield: 95%).

\[ \text{H-NMR(CDC}_3\text{) } \delta : 13.25(\text{s, } 1\text{H}), 7.34(\text{t, } 1\text{H}), 6.57(\text{d, } 1\text{H}), 6.38(\text{d, } 1\text{H}), 3.87(\text{s, } 3\text{H}), 2.68(\text{s, } 3\text{H}) \]

b) Synthesis of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid

8.9 g of metallic sodium was treated with 120 ml of ethanol to prepare a sodium ethoxide solution. 10.3 g of 1-(2-hydroxy-6-methoxy-phenyl)-ethanone obtained in step (a) was dissolved in 35 ml of diethyl oxalate, and added to the sodium ethoxide solution. The resulting mixture was allowed to
react at 100°C for 16 hrs. After the reaction was completed, the reaction mixture was cooled to room temperature and the solvent was removed under a reduced pressure, to obtain a residue. After dissolving the residue in water, the resulting mixture was acidified with 2 N HCl, and extracted with ethyl acetate. The organic layer was washed with saturated NaCl, dried over magnesium sulfate, filtered and concentrated under a reduced pressure, to obtain a residue which gave 13.2 g of the title compound (yield: 97%).

$^1$H-NMR(DMSO) $\delta$: 7.72(t, 1H), 7.16(d, 1H), 7.01(d, 1H), 6.69(s, 1H), 3.85(s, 3H)

2) Synthesis of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

0.2 g of 2-amino-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide obtained in step (2) of Example 1 was reacted with 0.14 g of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid obtained in step (b) of Example 3 according to the same method as described in step (3) of Example 1, to obtain 0.15 g of the title compound (yield: 52%).

$^1$H-NMR(CDC$_3$) $\delta$: 12.76(s, 1H), 8.47(s, 1H), 7.80(s, 1H), 7.60(m, 3H), 7.30(d, 2H), 7.28(s, 1H), 7.09(d, 2H), 6.85(d, 1H), 6.61(s, 1H), 6.54(s, 1H), 3.97(d, 6H), 3.94(s, 3H), 3.84(s, 6H), 3.69(s, 2H), 2.96(m, 2H), 2.79(m, 6H)

Example 4: Synthesis of 5-hydroxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

1) Synthesis of 5-hydroxy-4-oxo-4H-chromene-2-carboxylic acid

1.0 g of 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid was dissolved in 10 ml of methylenechloride under atmospheric nitrogen pressure, cooled to -78°C, and 4.6 ml of boron tribromide was added in portions thereto. After stirring for 30 min, the mixture was heated slowly to room temperature and stirred for 6 hrs. The reaction mixture was diluted with water and extracted
with methylenechloride. The organic layer was dried over magnesium sulfate, filtered and concentrated under a reduced pressure, to obtain a residue which gave 0.6 g of the title compound (yield: 64%).

\[^1\text{H-NMR(DMSO)}\, \delta : 12.19(\text{s, 1H}), 7.70(\text{t, 1H}), 7.12(\text{d, 1H}), 6.90(\text{s, 1H}), 6.84(\text{d, 1H})\]

2) Synthesis of 5-hydroxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

0.23 g of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide obtained in step (2) of Example 1 was reacted with 0.14 g of 5-hydroxy-4-oxo-4H-chromene-2-carboxylic acid obtained in step (1) of Example 4 according to the same method as described in step (3) of Example 1, to obtain 0.18 g of the title compound (yield: 56%).

\[^1\text{H-NMR(CDC}_3\text{)}\, \delta : 12.85(\text{s, 1H}), 12.22(\text{s, 1H}), 8.50(\text{s, 1H}), 7.86(\text{s, 1H}), 7.64(\text{d, 1H}), 7.58(\text{d, 2H}), 7.31(\text{d, 2H}), 7.15(\text{t, 1H}), 7.14(\text{s, 1H}), 6.87(\text{d, 1H}), 6.61(\text{s, 1H}), 6.55(\text{s, 1H}), 3.97(\text{d, 6H}), 3.85(\text{s, 6H}), 3.68(\text{s, 2H}), 2.95(\text{m, 2H}), 2.84(\text{m, 6H})\]

Example 5: Synthesis of 6-fluoro-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

0.2 g of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide obtained in step (2) of Example 1 was reacted with 0.11 g of 6-fluorochromene-2-carbonyl chloride according to the same method as described in Example 2, to obtain 0.18 g of the title compound (yield: 64%).

\[^1\text{H-NMR(CDC}_3\text{)}\, \delta : 12.84(\text{s, 1H}), 8.54(\text{s, 1H}), 7.86(\text{dd, 1H}), 7.76(\text{s, 1H}), 7.73(\text{dd, 1H}), 7.57(\text{d, 2H}), 7.48(\text{dt, 1H}), 7.32(\text{d, 2H}), 7.22(\text{s, 1H}), 7.11(\text{s, 1H}), 6.62(\text{s, 1H}), 6.55(\text{s, 1H}), 3.99(\text{d, 6H}), 3.85(\text{s, 6H}), 3.67(\text{s, 2H}), 2.93(\text{m, 2H}), 2.82(\text{m, 6H})\]
Example 6: Synthesis of 6-bromo-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide

0.2 g of 2-amino-N-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenyl-4,5-dimethoxy-benzamide obtained in step (2) of Example 1 was reacted with 0.18 g of 6-bromochromene-2-carboxylic acid according to the same method as described in step (3) of Example 1, to obtain 0.17 g of the title compound (yield: 55%).

$^1$H-NMR(CDCl$_3$) δ : 12.85(s, 1H), 8.54(s, 1H), 8.36(s, 1H), 7.86(dd, 1H), 7.77(s, 1H), 7.58(m, 3H), 7.32(d, 2H), 7.24(s, 1H), 7.12(s, 1H), 6.62(s, 1H), 6.56(s, 1H), 3.99(d, 6H), 3.86(s, 6H), 3.70(s, 2H), 2.96(m, 2H), 2.87(m, 6H)

Example 7: Synthesis of 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide methanesulfonate

1.0 g of 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide obtained in Example 1 was dissolved in 70 ml of methanol and stirred for about 30 min. 0.10 ml of methanesulfonic acid diluted with 5 ml of methanol was added dropwise thereto at 0°C. After 10 min, the reaction mixture was stirred at room temperature for 6 hrs. 0.98 g of the title compound was obtained in a yield of 86%.

$^1$H-NMR(CDCl$_3$) δ : 8.59(s, 1H), 8.34(d, 1H), 8.01(t, 1H), 7.94-7.91(m, 3H), 7.76-7.70(m, 2H), 7.58(d, 2H), 7.12-7.17(t, 1H), 7.03(s, 1H), 6.98(s, 1H), 4.14(s, 3H), 4.11(s, 3H), 4.02(s, 6H), 3.79-3.67(m, 4H), 3.34-3.21(m, 6H), 2.87(s, 3H)

Preparation Example 1: Formulation for oral administration (1)

To prepare an oral formulation comprising 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isooquinoline-2-yl)-
ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide obtained in Example 1 as an active ingredient, the following ingredients were mixed and pressed into a single tablet:

<table>
<thead>
<tr>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective compound</td>
</tr>
<tr>
<td>Corn starch</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Magnesium stearic acid</td>
</tr>
</tbody>
</table>

Further, other oral formulations comprising each of the p-glycoprotein inhibitors obtained in Examples 2 to 7 as an active ingredient were prepared by the same method as described above. In this case, 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide methanesulfonate of Example 7 was used in an amount of 114 mg.

**Preparation Example 2: Formulation for oral administration (2)**

To prepare an oral formulation comprising 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide obtained in Example 1 as an active ingredient, a soft gelatin capsule was prepared by using the following ingredients:

<table>
<thead>
<tr>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective compound</td>
</tr>
<tr>
<td>Corn starch</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Crystalline cellulose</td>
</tr>
<tr>
<td>Magnesium stearic acid</td>
</tr>
</tbody>
</table>

Further, other oral formulations comprising each of the p-glycoprotein inhibitors obtained in Examples 2 to 7 as an active ingredient were prepared by the same method as described above. In this case, 4-oxo-4H-chromene-2-
carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide methanesulfonate of Example 7 was used in an amount of 114 mg.

**Preparation Example 3: Injection formulation (1)**

An injection formulation comprising 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide obtained in Example 1 as an active ingredient was prepared by using the following ingredients:

<table>
<thead>
<tr>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt of effective compound</td>
</tr>
<tr>
<td>5% glucose solution</td>
</tr>
</tbody>
</table>

Further, other injection formulations comprising each of the pglycoprotein inhibitors obtained in Examples 2 to 7 as an active ingredient were prepared by the same method as described above. In this case, 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide methanesulfonate of Example 7 was used in an amount of 23 mg.

**Preparation Example 4: Injection formulation (2)**

An injection formulation comprising 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide obtained in Example 1 as an active ingredient was prepared by using the following ingredients:

<table>
<thead>
<tr>
<th>Quantity (mg/tablet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective compound</td>
</tr>
<tr>
<td>Polyethylene glycol 400</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
</tbody>
</table>
Further, injection formulations comprising each of the p-glycoprotein inhibitors obtained in Examples 2 to 7 as an active ingredient were prepared by the same method as described above. In this case, 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide methanesulfonate obtained in Example 7 was used in an amount of 23 mg.

Test Example 1: Tests for inhibiting multi-drug resistance and increasing anticancer agent's activity

Multi-drug resistance inhibitory activities of the compounds synthesized in Examples 1 to 7 in cancer cell lines MCF-7 (human breast cancer cells) and MCF-7/Dx were examined as follows. MCF-7/Dx cells isolated from MCF-7 by a series of doxorubicin treatment were resistant cancer cell lines expressing p-glycoprotein and showing drug resistance. MCF-7/Dx cells were cultured in RPMI1640 medium containing glutamine, sodium bicarbonate, gentamicin and amphotericin supplemented with 5% fetal bovine serum, under the condition of 37°C, 5% CO₂/95% O₂ and 100% humidity, and subcultured at intervals of 3 to 4 days. Cells were detached from the culture plate by treating with 0.25% trypsin containing 3 mM 1,2-cyclohexanedianiminetetra acetic acid.

MCF-7 and MCF-7/Dx cells were inoculated into a 96-well microplate at a concentration of 2×10³ cells/well and cultured in the above culture medium for 24 hrs. When the cells were observed to have adhered to the bottom of the microplate, the culture medium was removed, 100 μl of a paclitaxel solution having a concentration of 10⁻¹¹ to 10⁻⁶ M was added to each well alone or in combination with one of the compounds of Examples 1 to 6 at a concentration of 50 nM, and then, the microplate was incubated at 25°C for 72 hrs. After the incubation was completed, the culture medium was removed from each well, and the cells were treated with 10% trichloroacetic acid for 1 hr to fix them. After removing the trichloroacetic acid, the microplate was washed with water and dried at room temperature. A staining solution prepared by dissolving 0.4% SRB (sulforhodamine B) in 1% acetic acid was added to the wells, and the
microplate was kept at room temperature for 30 min to stain the cells. The well plate was washed with 1% acetic acid to remove unbound SRB. The stained cells were treated with 10 μl of trisma base solution (pH 10.3 to 10.5) to elute SRB from the cells, and the absorbance of each well at 520 nm was measured using a microplate reader. The \( ED_{50} \) value representing paclitaxel inhibition of the cell growth by the extent of 50% was calculated from the measured absorbance and shown in Table 1. The increase in the anticancer activity of paclitaxel against the resistant cancer cell line MCF7/Dx was determined by comparing the \( ED_{50} \) value obtained with the compound of Example (\( T_{ED50} \)) with that without (\( C_{ED50} \)). The results are represented as resistance inhibitory effect (\( C_{ED50}/T_{ED50} \)).

<table>
<thead>
<tr>
<th></th>
<th>( ED_{50} ) of paclitaxel (nM)</th>
<th>MCF7</th>
<th>MCF7/Dx</th>
<th>Resistance inhibitory effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td>11.5</td>
<td>294.6</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 1</td>
<td>13.7</td>
<td>3.9</td>
<td>75.5</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 2</td>
<td>8.6</td>
<td>4.1</td>
<td>71.9</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 3</td>
<td>8.5</td>
<td>4.0</td>
<td>73.7</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 4</td>
<td>6.5</td>
<td>5.4</td>
<td>54.6</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 5</td>
<td>11.9</td>
<td>5.1</td>
<td>57.8</td>
<td></td>
</tr>
<tr>
<td>Compound of Example 6</td>
<td>9.1</td>
<td>8.1</td>
<td>36.4</td>
<td></td>
</tr>
</tbody>
</table>

As shown in Table 1, it was found that each of the compounds of Examples 1 to 6 effectively inhibited the multi-drug resistance of MCF-7/Dx to paclitaxel at a concentration of 50 nM.

**Test Example 2: Test for in vivo absorption of paclitaxel**
A comparative study to determine the bioavailability of orally administered paclitaxel and that observed when paclitaxel is administered in combination with each of the compounds of Examples was conducted as follows. A control group was administered only paclitaxel. The experimental group was administered with 12 mg of the compound of Example 1 (composition: 12 mg of the compound of Example 1 in 4 ml of 5% dextrose + 1.2 μg of methanesulfonic acid) and 20 mg/kg of paclitaxel (composition: 6 mg of paclitaxel in 0.5 ml of Cremophor EL + 0.5 ml of ethanol), and the control group was administered with a vehicle (4 ml of 5%-dextrose + 1.2 μg of methanesulfonic acid) and 20 mg of paclitaxel (composition: 6 mg of paclitaxel in 0.5 ml of Cremophor EL + 0.5 ml of ethanol).

14 to 15 week-old Sprague-Dawley rats (Daehan Biolink Co., Ltd.) were divided into two groups, each consisting of 5 to 8 rats. The rats were acclimated for more than 7 days allowing free access to food and water. And then, the rats were put on a fast over a period of 24 hrs, while they were allowed to free access to water. The rats were orally administered with the experimental or control preparation in an amount corresponding to 20 mg/kg of paclitaxel. Blood samples were taken directly from the hearts of the rats before administration, and 1, 2, 4, 6, 8 and 24 hours after the administration, respectively. Each blood sample was centrifuged at 4°C, 12,000 rpm for 5 min to obtain a serum sample. To 200 μl each of the serum sample, 400 μl of acetonitrile as an internal standard was added and the mixture was shaken to obtain an extract. The extract was centrifuged at 4°C, 12,000 rpm for 5 min to obtain a supernatant. 50 μl of the supernatant was subjected to HPLC under the following conditions:

Semi-HPLC system: Shiseido SI-1 model,
Analysis column: Capcell Pak C_{18} UG120 (5 μm, 1.5× 250 mm),
Pre-column: Capcell Pak C_{18} MF Ph-1 (4.6× 10 mm),
Concentration column: Capcell Pak C_{18} UG120 (5 μm, 1.5× 35 mm),
Mobile phase for pre-column: 20% acetonitrile,
Mobile phase for analysis column: 55% acetonitrile,
Injection volume: 5 μl,
Flow rate: 5 μl/min,
Detection: 227 nm.

The observed time-dependent changes in the in-blood paclitaxel concentration are shown in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>AUC (ng·hr/ml)</th>
<th>( T_{\text{max}} ) (hr)</th>
<th>( C_{\text{max}} ) (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>440± 205</td>
<td>2.0</td>
<td>78± 31</td>
</tr>
<tr>
<td>Compound of Example 1</td>
<td>3,040± 1,131</td>
<td>1.0</td>
<td>1,187± 420</td>
</tr>
<tr>
<td>Compound of Example 3</td>
<td>3,645± 1,230</td>
<td>1.0</td>
<td>1,110± 518</td>
</tr>
<tr>
<td>Compound of Example 6</td>
<td>3,480± 1,210</td>
<td>1.0</td>
<td>1,020± 530</td>
</tr>
</tbody>
</table>

*1 Area under the curve of blood concentration till 24 hrs  
*2 Maximum blood concentration  
*3 Time at the maximum blood concentration

As can be seen in Table 2, when the inventive compound is administered together with an anticancer agent, such as paclitaxel which is normally not readily absorbable in the digestive tract, the bioavailability of the drug becomes markedly enhanced, far higher than the level observed when the drug is administered alone.

While the invention has been described with respect to the above specific embodiments, it should be recognized that various modifications and changes may be made to the invention by those skilled in the art which also fall within the scope of the invention as defined by the appended claims.
What is claimed is:

1. A compound of Formula 1 or a pharmaceutically acceptable salt thereof:

![Formula 1](image)

wherein,

R₁ to R₈ are each independently hydrogen, hydroxy, halogen, NO₂, C₁₋₅ alkyl or C₁₋₅ alkoxy.

2. The compound of claim 1, which is selected from the group consisting of:

- 4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;
- 6-methyl-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;
- 5-methoxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;
- 5-hydroxy-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl)-amide;
- 6-fluoro-4-oxo-4H-chromene-2-carboxylic acid (2-4-[2-(6,7-dimethoxy-3,4-dihydro-1H-isoquinoline-2-yl)-ethyl]-phenylcarbamoyl-4,5-dimethoxy-
phenyl)-amide;

6-bromo-4-oxo-4\textit{H}-chromene-2-carboxylic acid (2-4\textbf{-[2-\textbf{(6,7-dimethoxy-3,4-dihydro-1\textit{H}-isoquinoline-2-yl)}-ethyl]-phenylcarbamoyl-4,5-dimethoxy-phenyl})-amide; and

a pharmaceutically acceptable salt thereof.

3. A method for preparing the compound of Formula 1, which comprises reacting a compound of Formula 2 with a compound of Formula 3 in a solvent in the presence of a condensing agent or a base:

\[\text{<Formula 2>}\]

\[\text{<Formula 3>}\]

wherein,

\(R_1\) to \(R_8\) are each independently hydrogen, hydroxy, halogen, NO\(_2\), C\(_{1-5}\) alkyl or C\(_{1-5}\) alkoxy, and

\(R'\) is OH, Cl or Br.

4. The method of claim 3, wherein the compound of Formula 2 is prepared by the steps of:

(1) reacting a compound of Formula 8 with a compound of Formula 9 in a solvent in the presence of a base to obtain a compound of Formula 7;

(2) subjecting the compound of Formula 7 to hydrogenation in a solvent in the presence of a catalyst to obtain a compound of Formula 5;

(3) reacting a compound of Formula 6 with thionyl chloride and reacting the resulting compound with the compound of Formula 5 in a solvent in the presence of a base to obtain a compound of Formula 4; and
(4) subjecting the compound of Formula 4 to hydrogenation in a solvent in the presence of a catalyst:

![Formula 4]

![Formula 5]

![Formula 6]

![Formula 7]

![Formula 8]

![Formula 9]

wherein,

R_2 to R_8 are each independently hydrogen, hydroxy, halogen, NO_2, C_1-5 alkyl or C_1-5 alkoxy.

5. A pharmaceutical composition for inhibiting multi-drug resistance
comprising the compound of Formula 1 or a pharmaceutically acceptable salt thereof as an active ingredient together with a pharmaceutically acceptable carrier:

<Formula 1>

wherein,

R₁ to R₈ are each independently hydrogen, hydroxy, halogen, NO₂, C₁₋₅ alkyl or C₁₋₅ alkoxy.

6. The composition of claim 5, which further comprises an anticancer agent.

7. The composition of claim 6, wherein the anticancer agent is selected from the group consisting of paclitaxel, docetaxel, vincristine, vinblastine, vinorelbine, daunomycin, doxorubicin, topotecan, irinotecan, actinomycin and etoposide.
# INTERNATIONAL SEARCH REPORT

**INTERNATIONAL APPLICATION**

**International application No.**
PCT/KR2004/002557

**A. CLASSIFICATION OF SUBJECT MATTER**

**IPC7 C07D 405/12**

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)

IPC7 C07D 405/12

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

Korean patents and applications for inventions since 1975

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

STN[Registry]

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>US 6011069 A (NISSHIN FLOUR MILLING CO., LTD.) 4 Jan. 2000 See the whole document.</td>
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</tr>
<tr>
<td>D, A</td>
<td>WO 98/17648 A1 (XENOVA LTD.) 30 Apr. 1998 See the whole document.</td>
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</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:
  *A* document defining the general state of the art which is not considered to be of particular relevance
  *E* earlier application or patent but published on or after the international filing date
  *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
  *O* document referring to an oral disclosure, use, exhibition or other means
  *P* document published prior to the international filing date but later than the priority date claimed

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

24 NOVEMBER 2004 (24.11.2004)

Date of mailing of the international search report

25 NOVEMBER 2004 (25.11.2004)

Name and mailing address of the ISA/KR

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Facsimile No. 82-42-472-7140

Authorized officer

LEE, Mi Jeong

Telephone No. 82-42-481-5601

Form PCT/ISA/210 (second sheet) (January 2004)
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