

ingredients included in the pharmaceutical composition or with a kit, a product, or a concomitant agent, thereby preventing, removing, or reducing harmful effects of cancer.

[0052] In the present disclosure, the term "pharmaceutically effective amount" refers to an amount sufficient to treat a disease with a reasonable benefit/risk ratio applicable to medical treatment, and the level of "therapeutically effective amount" may be determined by factors, which includes a patient's health condition, a cancer type, severity, drug activity, drug sensitivity, an administration method, an administration time, an administration route and an excretion rate, a period of treatment, combination or drugs simultaneously used, and other factors well known in the medical field. It is understood that a dose administered in the present disclosure may be lower for each compound in the composition relative to "therapeutically effective amount" defined for each compound used alone or in combination with treatments other than the combined therapy described above. In particular, the pharmaceutical composition, the kit, the product, and the concomitant agent of the present disclosure may reduce tumor development, reduce tumor burden, cause tumor regression in a mammalian host, and/or prevent occurrence of metastasis or cancer. In the cancer treatment, the pharmaceutical composition of the present disclosure may be administered in a therapeutically effective amount.

[0053] In an embodiment, a daily dose of the compound represented by Formula 1 of the present disclosure may be about 1 mg to about 400 mg, preferably, about 2 mg to about 240 mg, more preferably, about 5 mg to about 160 mg, and even more preferably, about 5 mg to about 120 mg. In addition, a daily dose of irinotecan which is the anticancer drug of the present disclosure may be about 10 mg/m² to about 350 mg/m², preferably, about 25 mg/m² to about 100 mg/m², and more preferably, about 25 mg/m² to about 75 mg/m². In some embodiments, the anticancer drug (e.g., irinotecan) is to be intravenously dosed at about 30 mg/m² to about 120 mg/m². In some embodiments, the anticancer drug (e.g., irinotecan) is to be dosed at about 50 mg/m², about 75 mg/m², or about 100 mg/m². In some embodiments, the anticancer drug (e.g., irinotecan) is to be intravenously dosed at about 125 mg/m², 180 mg/m², or about 350 mg/m². In some embodiments, the second pharmaceutical composition is to be intravenously dosed at about 30 mg/m² to about 120 mg/m². In some embodiments, the second pharmaceutical composition is to be dosed at about 50 mg/m², about 75 mg/m², or about 100 mg/m². However, due to the nature of cancer which is a

target disease of the pharmaceutical composition of the present disclosure, a dose of each ingredient may vary greatly depending on the conditions described above, and is not limited to the embodiments above.

[0054] As used herein, the term “about” or “approximately” refers to a $\pm 10\%$ variation from the recited quantitative value (and includes the recited quantitative value itself) unless otherwise indicated or inferred from the context. For example, unless otherwise stated or inferred from the context, a dose of about 100 mg/m^2 indicates a dose range of $100 \pm 10\% \text{ mg/m}^2$, i.e., from 90 mg/m^2 to 110 mg/m^2 , inclusive.

[0055] Regarding the pharmaceutical composition for co-administration of the present disclosure, a suitable administration cycle of (i) the compound represented by Formula 1 or the pharmaceutically acceptable salt thereof and (ii) the anticancer drug may be determined according to the dose. In the present disclosure, (i) the compound represented by Formula 1 or the pharmaceutically acceptable salt thereof may be administered once a day to once a week, and preferably, once a day, and (ii) the anticancer drug may be administered once a day to once a week, and preferably, once a week.

[0056] In an embodiment of the present disclosure, one cycle of administration of the pharmaceutical composition for co-administration of the present disclosure may be 14 days to 21 days, but is not limited thereto. In an embodiment of the present disclosure, when the (one) cycle of the administration is 14 days, (i) from the 1st day to the 7th day, from the 1st day to the 5th day, or from 2nd day to the 6th day, the compound represented by Formula 1 or the pharmaceutically acceptable salt may be orally administered once a day or administered by injection once a day, and (ii) on the 1st day or from the 2nd day to the 6th day, the anticancer drug may be administered by injection once a day.

[0057] In an embodiment of the present disclosure, when the (one) cycle of the administration is 21 days, (i) from the 1st day to the 7th day, from the 1st day to the 5th day or from 2nd day to the 6th day, the compound represented by Formula 1 or the pharmaceutically acceptable salt may be orally administered once a day or administered by injection once a day, and (ii) on the 1st day, or from the 2nd day to the 6th day, the anticancer drug may be administered by injection once a day.

[0058] In some embodiments, the first pharmaceutical composition is to be dosed as a cycle, wherein the cycle comprises administering the first pharmaceutical composition for about 14 days or about 21 days. For example, in some embodiments, the first pharmaceutical composition is administered to a subject in need thereof for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days.

[0059] In some embodiments, the second pharmaceutical composition is to be dosed as a cycle, wherein the cycle comprises administering the second pharmaceutical composition for about 14 days or about 21 days. In some embodiments, the second pharmaceutical composition is to be dosed as a cycle, wherein the cycle is repeated at least once. For example, in some embodiments, the second pharmaceutical composition is administered to a subject in need thereof for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 days.

[0060] In some embodiments, in one cycle, the first pharmaceutical composition is to be administered from the 1st day to the 7th day, from the 1st day to the 5th day, or from 2nd day to the 6th day; and the second pharmaceutical composition is to be administered on the 1st day, or from 2nd to 6th day, wherein the first pharmaceutical composition is to be administered once daily, the second pharmaceutical composition is to be administered once daily, and the one cycle has a duration of 14 days or 21 days. In some embodiments, in one cycle, the first pharmaceutical composition containing 40 mg, 20 mg, or 10 mg of the compound of Formula 1, or a pharmaceutically acceptable salt thereof, is to be administered once daily, and the second pharmaceutical composition is to be administered once daily at a intravenously dose of 100 mg/m².

[0061] The pharmaceutical composition for co-administration of the present disclosure may be administered as an individual therapeutic agent, may be administered again in combination with other therapeutic agents, may be administered sequentially or simultaneously with a conventional therapeutic agent, and may be administered once or multiple times. In consideration of all of the factors described above, it is important to administer an amount capable of obtaining the maximum effect with a minimum amount without side effects.

[0062] In the present disclosure, the term "kit", "product", or "concomitant agent" specifically defines "kit of components" in the sense that the compound represented by Formula 1 as defined above may be administered independently or used in combination with

formulations, wherein the first pharmaceutical composition and second pharmaceutical composition are administered simultaneously or sequentially.

[0073] In some embodiments, the method or use comprises administering the first pharmaceutical composition and the second pharmaceutical composition each in the form of a separate dosage form. In some embodiments, the method or use comprises administering the first pharmaceutical composition in an oral dosage form, and the second pharmaceutical composition in an injection dosage form. In some embodiments, the method or use comprises administering the first pharmaceutical composition in a tablet, and the second pharmaceutical composition intravenously.

[0074] In some embodiments, the method or use comprises administering the first pharmaceutical composition and the second pharmaceutical composition each once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition is to be dosed at about 1 mg to about 180 mg. In some embodiments, the method or use comprises administering the first pharmaceutical composition is to be dosed at about 5 mg to about 170 mg. In some embodiments, the method or use comprises administering the first pharmaceutical composition is to be dosed at about 10 mg to about 160 mg. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dose of about 10 mg, about 20 mg, about 40 mg, about 80 mg, about 120 mg, or about 160 mg per administration. In some embodiments, the first pharmaceutical composition comprises about 1 mg to about 50 mg of the compound represented by Formula 1. In some embodiments, the first pharmaceutical composition comprises about 5 mg to about 40 mg of the compound represented by Formula 1. In some embodiments, the first pharmaceutical composition comprises about 5 mg, about 10 mg, about 20 mg, or about 40 mg of the compound represented by Formula 1. In some embodiments, the first pharmaceutical composition is administered to a subject in need thereof with at least 8 ounces of water.

[0075] In some embodiments, the method or use comprises administering the second pharmaceutical composition is to be intravenously dosed at about 30 mg/m² to about 120 mg/m². In some embodiments, the method or use comprises administering the second pharmaceutical composition is to be intravenously dosed at about 50 mg/m², about 75 mg/m², or about 100 mg/m². In some embodiments, the method or use comprises administering the

first pharmaceutical composition and the second pharmaceutical composition in one cycle, wherein the one cycle has a duration of about 14 or 21 days. In some embodiments, the cycle is repeated at least once. In some embodiments, the second pharmaceutical composition comprises an anticancer drug (e.g., irinotecan) at a concentration of about 20 mg/mL. In some embodiments, the second pharmaceutical composition comprises 5% dextrose or 0.9% sodium chloride. In some embodiments, the anticancer drug (e.g., irinotecan) is administered for about 90 minutes. In some embodiments, the anticancer drug (e.g., irinotecan) is administered for more than about 90 minutes.

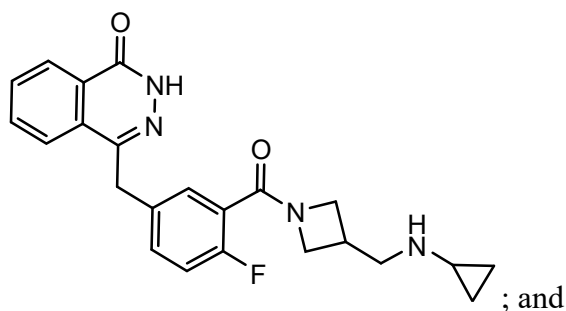
[0076] In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 40 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 50 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 40 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 75 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 10 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 20 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 40 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 80 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m². In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 120 mg, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m².

[0077] In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 40 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 50 mg/m² once daily. In some embodiments, the method or use comprises administering the first

pharmaceutical composition at a dosage of about 40 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 75 mg/m² once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 10 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m² once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 20 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m² once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 40 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m² once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 80 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m² once daily. In some embodiments, the method or use comprises administering the first pharmaceutical composition at a dosage of about 120 mg once daily, and intravenously administering the second pharmaceutical composition at a dosage of about 100 mg/m² once daily.

[0078] In some embodiments, the present disclosure provides a method of treating cancer, wherein the method comprises administering to a subject in need thereof (i) a first pharmaceutical composition comprising a compound represented by Formula 1 or a pharmaceutically acceptable salt thereof;

<Formula 1>



treatment concentration. Here, DMSO was used in a final content not exceeding 1.1 % to treat the cells.

[0091] As cell lines, five human-derived gastric cancer cell lines (i.e., AGS, SNU-668, SNU-216, SNU-484, and SNU-638) were used. The source of the cell lines is the Korean Cell Line Bank (KCLB). As a medium, a complete growth medium consisting of 90 % RPMI1640 and 10 % heat-inactivated fetal bovine serum was used.

1.2. Thawing and subculturing of frozen cells

[0092] Frozen cells were thawed in a water bath at 37 °C, suspended in a culture medium, and then centrifuged at 1,200 rpm for 2 minutes. The precipitated cells were suspended in a culture medium, spread on a culture dish, and cultured at 37 °C in 5 % CO₂. Reflecting the growth rate of each cell, the cells were cultured periodically at intervals of 3 days to 5 days.

1.3. Cell plating and drug treatment

[0093] To remove the cells from the culture dish, the cells were treated with trypsin and allowed for a reaction in an incubator at 37 °C in 5 % CO₂. The cells collected after the completion of the reaction were suspended in a culture medium, and centrifuged at 1,200 rpm for 3 minutes. The cell suspension was put into a hemocytometer to calculate the number of cells per unit volume. According to the conditions set for each cell line, the cell suspension was plated into a 96-well plate and then stabilized for 24 hours at 37 °C in 5 % CO₂.

[0094] The 10 mM stock solution containing irinotecan was serially diluted in DMSO at a ratio of 1/10 to prepare a 100X stock solution for each treatment concentration, and a 1000X stock solution containing 1 mM hydrochloride compound of Formula 1 was prepared, so as to prepare a stock solution with a final treatment concentration of 1 µM. To minimize the effect of the solvent on the cells, each well contained 1.1 % DMSO. The stock solution of each concentration was added to a culture medium to have a 10X concentration, and then the cells were treated at a 1X concentration. Each cell line was treated with irinotecan alone or in combination of hydrochloride compound of Formula 1 and irinotecan, each for 5 days.

The fold change (ratio) = IC_{50} in the presence of DMSO / IC_{50} in the presence of 1 μ M hydrochloride of Compound 1

[0098] Referring to Table 1, the IC_{50} values of the five cell lines in Example 1 were found to be 1.3 times to 3.1 times lower than those in Comparative Example 1. Accordingly, it was confirmed that, in the experiment using the gastric cancer cell lines, the pharmaceutical composition of the present disclosure exhibited excellent inhibitory effect on the proliferation of tumor cells, compared to the administration of the anticancer drug alone.

Experimental Example 2

2.1. Preparation and treatment of DMSO stock solution

[0099] Hydrochloride of the compound represented by Formula 1 (M.W. 442.92, hereinafter, referred to as 'hydrochloride compound of Formula 1') was obtained from Ildong Pharmaceutical. Oxaliplatin (M.W. 397.29) was obtained from Sigma Aldrich.

[0100] Each of these drugs was dissolved in 100 % DMSO to prepare a 10 mM stock solution, which was then divided into single doses and stored at -20 °C.

[0101] The 10 mM stock solution containing hydrochloride compound of Formula 1 and oxaliplatin was serially diluted in DMSO at a ratio of 1/10 to prepare a stock solution for each treatment concentration. Here, DMSO was used in a final content not exceeding 1.1 % to treat the cells.

[0102] As cell lines, five human-derived gastric cancer cell lines (i.e., AGS, SNU-668, SNU-216, SNU-484, and SNU-638) were used. The source of the cell lines is the KCLB. As a medium, a complete growth medium consisting of 90 % RPMI1640 and 10 % heat-inactivated fetal bovine serum was used.

2.2. Thawing and subculturing of frozen cells

[0103] Frozen cells were thawed in a water bath at 37 °C, suspended in a culture medium, and then centrifuged at 1,200 rpm for 2 minutes. The precipitated cells were suspended in a culture medium, spread on a culture dish, and cultured at 37 °C in 5 % CO₂. Reflecting the growth rate of each cell, the cells were cultured periodically at intervals of 3 days to 5 days.

2.3. Cell plating and drug treatment

[0104] To remove the cells from the culture dish, the cells were treated with trypsin and allowed for a reaction in an incubator at 37 °C in 5 % CO₂. The cells collected after the completion of the reaction were suspended in a culture medium, and centrifuged at 1,200 rpm for 3 minutes. The cell suspension was put into a hemocytometer to calculate the number of cells per unit volume. According to the conditions set for each cell line, the cell suspension was plated into a 96-well plate and then stabilized for 24 hours at 37 °C in 5 % CO₂.

[0105] The 10 mM stock solution containing oxaliplatin was serially diluted in DMSO at a ratio of 1/10 to prepare a 100X stock solution for each treatment concentration, and a 1000X stock solution containing 1 mM hydrochloride compound of Formula 1 was prepared, so as to prepare a stock solution with a final treatment concentration of 1 µM. To minimize the effect of the solvent on the cells, each well contained 1.1 % DMSO. The stock solution of each concentration was added to a culture medium to have a 10X concentration, and then the cells were treated at a 1X concentration. Each cell line was treated with oxaliplatin alone or in combination of hydrochloride compound of Formula 1 and oxaliplatin, each for 5 days.

2.4. MTT assay

[0106] After the treatment of each drug, 100 µL of 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolimbromide solution (Sigma Aldrich) was added to each well, the plate was cultured at 37 °C for 2 hours, and the culture medium was removed. After formazan crystals were dissolved in 100 µL of DMSO, a Synergy H4 microplate reader (BioTek) was used to measure absorbance of each cell at 570 nm.

[0107] The absorbance was calculated as activity expressed in percent at each concentration of each drug, as compared with a negative control group (100 %), which is the group including a solvent (DMSO) only. IC₅₀ was calculated by nonlinear regression analysis in 3 or 4 parameter fits (GraphPad Prism 7.04).

[0108] According to the MTT assay, the sensitivity was confirmed through the viability of each cell line in oxaliplatin with or without 1 µM of hydrochloride compound of Formula 1, and results thereof are shown in FIG. 2. As shown in FIG. 2, the administration of oxaliplatin alone exhibited an effect of apoptosis, whereas the co-administration of

oxaliplatin with compound of Formula 1 resulted in a higher apoptosis rate. Accordingly, it was confirmed that the inhibitory effect on cancer growth obtained by such co-administration increased in a dose-dependent manner. The calculated IC₅₀ values are shown in Table 2.

[Table 2]

Drug administered	IC ₅₀ value (μM)				
	SNU-484	SNU-216	SNU-668	AGS	SNU-638
Example 2 (administration of hydrochloride compound of Formula 1 and oxaliplatin)	1.52	(n.a.)	14.3	3.05	11.1
Comparative Example 2 (administration of oxaliplatin alone)	10.4	(n.a.)	11.2	2.38	30.3
The fold change (ratio)	6.8	(n.a.)	0.8	0.8	2.7

5

The fold change (ratio) = IC₅₀ in the presence of DMSO / IC₅₀ in the presence of 1 μM hydrochloride of Compound 1

[0109] Referring to Table 2, the IC₅₀ values of the specific cell lines in Example 2 were found to be 2.7 times to 6.8 times lower than those in Comparative Example 2. Accordingly, it was confirmed that, in the experiment using the gastric cancer cell lines, the pharmaceutical composition of the present disclosure exhibited excellent inhibitory effect on the proliferation of tumor cells, compared to the administration of the anticancer drug alone.

10

Experimental Example 3

3.1. Cell culture

[0110] MKN45 tumor cells were cultured in RPMI1640 supplemented with 10 % fetal bovine serum at 37 °C in 5 % CO₂. The cells were harvested before tumor transplantation was performed, and then counted using a cell counter.

3.2. Tumor transplantation

[0111] To perform tumor transplantation, 0.1 ml of PBS tumor suspension (3×10^6 of MKN45 tumor cells) were subcutaneously inoculated into the upper right flank of each mouse.

3.3. Randomization

[0112] Randomization was performed when the average tumor size of the mouse reached about 150 mm³. A total of 40 mice were used for the test, and 10 mice per group were assigned to 4 groups as shown in Table 3 (Example 3 and Comparative Examples 3 to 5). Randomization was performed based on a "matched distribution" method (Study Director™ software, version 3.1.399.19). Here, the grouping date was marked as 0.

[Table 3]

Division	Number of mice	Drug administered	Level administered (mg/kg)	Solution administered (mg/mL)	Volume administered (mL/kg)	ROA	Frequency and duration of administration
Example 3	10	Hydrochloride compound of Formula 1	2	0.2	10	p.o.	QD×22 days
		Irinotecan	5	1	5	i.v.	QW×4 times
Comparative Example 3	10	Vehicle	-	-	10	p.o.	QD×22 days
Comparative Example 4	10	Hydrochloride compound of Formula 1	2	0.2	10	p.o.	QD×22 days
Comparative Example 5	10	Irinotecan	5	1	5	i.v.	QW×4 times