

METHOD FOR TREATING CHOLANGIOCARCINOMA WITH PTEN GENE MUTATION AND/OR DELETION

TECHNICAL FIELD

The present invention belongs to the technical field of medicines, and relates to a method for treating cancers and particularly to use of a proteasome inhibitor and an in-vivo active ingredient thereof in the preparation of a medicament for treating cholangiocarcinoma with PTEN gene mutation and/or deletion.

BACKGROUND

Cholangiocarcinoma is characterized by high malignancy and rapid progression. At the time of clinical visit, most patients are already at an advanced stage, with many experiencing recurrence and metastasis, resulting in a low operability. The therapeutic effect of conventional radiotherapy and chemotherapy on cholangiocarcinoma is very limited. For targeted therapy, immunotherapy, and other means showing clear therapeutic effect in other tumors, there are few successful examples in an attempt to treat cholangiocarcinoma. Academician Wang Hongyang reveals in his research paper "PTEN status determines the chemosensitivity to proteasome inhibition in cholangiocarcinoma" that nearly 40% of cholangiocarcinoma harbor deletion of PTEN, an important tumor suppressor gene, and this mutation significantly increases the proteasome activity, thus having a direct influence on the malignant behavior of tumor cells.

The Ubiquitin-Proteasome Pathway (UPP) is a main pathway for the systemic degradation of intracellular proteins, which participates in many physiologically important cellular processes, including signal transduction, immune response, unfolded protein response and cell cycle progress. This pathway is greatly associated with the onset of cardiovascular and cerebrovascular diseases, cancers, and neurodegenerative diseases. Proteasome inhibitors are used as therapeutic agents for diseases, such as cancers.

The first proteasome inhibitor bortezomib was approved by the FDA in 2003 for the treatment of recurrent myeloma. In 2004, the drug was approved to be marketed in the European Union for the treatment of multiple myeloma. In September 2005, the drug was introduced by Xi'an Janssen, and marketed initially in Guangzhou, China. On July 11, 2007, it was approved by the FDA for the treatment of relapsed or refractory mantle cell lymphoma

(MCL), becoming the only drug approved by the FDA for the treatment of MCL. The price of Velcade in the Chinese market is about 13,000 yuan per 3.5 mg, and the cost for one course of treatment is about 40,000 yuan. Such a high expense is a very heavy economic burden for many patients. Moreover, current clinical data shows that this drug also has more side effects, such as fatigue, nausea, diarrhea, and neuropathy.

Some other proteasome inhibitors have also been developed as drugs or are in the clinical trial and test stage, including carfilzomib, ONX0912, CEP-18770 and MLN9708. MLN9708 is a second-generation small-molecule proteasome inhibitor, which is used to treat various malignant gliomas in humans. After contact with an aqueous solution and plasma, MLN9708 is rapidly hydrolyzed into MLN2238. MLN2238 is a biologically active form of MLN9708. MLN2238 is an N-capped dipeptidyl leucine boronic acid, which inhibits the chymotrypsin-like proteolytic ($\beta 5$) site of 20S proteasome, with an IC₅₀ value of 3.4 nM and a K_i value of 0.93 nM. MLN2238 is an effective selective reversible inhibitor of the proteasome, and this reversibility is time dependent. However, the proteasome inhibitor has the problem of drug distribution in the treatment of solid tumors, for example, the clinical study of Bortezomib in patients with solid tumors failed to prove any therapeutic effect of the drug (Proteasome inhibitors in cancer therapy, *Nat Rev Clin Oncol.* 2017 Jul; 14(7): 417-433). Therefore, there is a key problem needed to be solved to develop a high-efficacy proteasome inhibitor with low price and low toxic and reduced side effects that is used for precise targeted therapy for cholangiocarcinoma based on PTEN mutation according to molecular typing, and to study its efficacy.

SUMMARY

In view of the defects in the prior art, an object of the present invention is to provide new use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof.

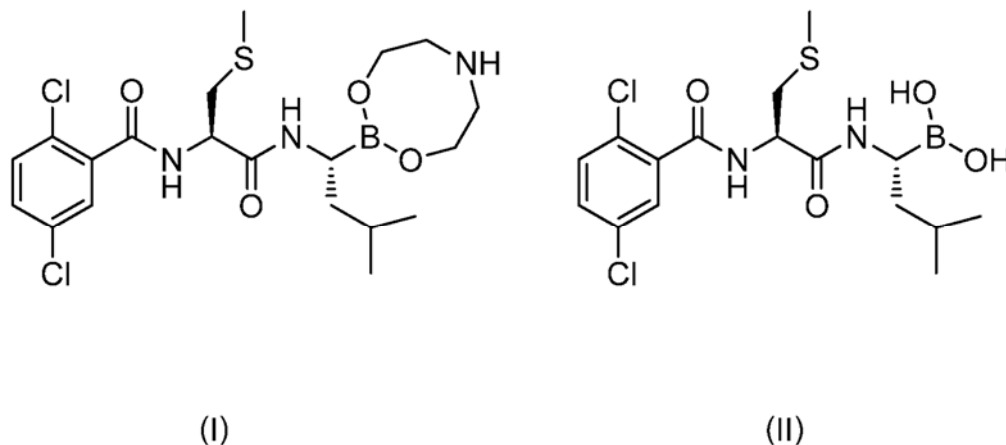
The objects of the present invention can be accomplished through the following technical solutions.

The present invention provides use of a therapeutically effective amount of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of medicaments for treating cholangiocarcinoma, where preferably the cholangiocarcinoma is cholangiocarcinoma with PTEN gene mutation and/or deletion.

The present invention further provides a method for treating cholangiocarcinoma, which includes administering a therapeutically effective amount of a proteasome inhibitor of Formula

(I) or (II) or a pharmaceutically acceptable salt thereof to a patient having cholangiocarcinoma, where preferably the cholangiocarcinoma is cholangiocarcinoma with PTEN gene mutation and/or deletion.

The proteasome inhibitor of Formula (I) or (II) according to the present invention has a structure shown below:



In some embodiments of the present application, the cholangiocarcinoma in the present invention includes intrahepatic cholangiocarcinoma, hilar cholangiocarcinoma, or distal cholangiocarcinoma. In some embodiments, the cholangiocarcinoma is intrahepatic cholangiocarcinoma.

The cholangiocarcinoma in the present invention is with PTEN gene mutation and/or deletion, where the PTEN gene mutation and/or deletion may be the decrease of PTEN gene transcription and protein abundance, or the complete deletion of PTEN gene. Whether the PTEN gene has mutation and/or deletion can be diagnosed by a common gene diagnosis method. Preferably, the use is in cholangiocarcinoma with complete deletion of PTEN gene.

In the present invention, the cholangiocarcinoma can be cholangiocarcinoma in different stages, for example, early, intermediate, advanced or metastatic cholangiocarcinoma, or recurrent cholangiocarcinoma.

The use in the present invention is specifically to administer a therapeutically effective amount of the proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof to a patient in need of treatment. Specifically, the dosage of the proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the treatment of cholangiocarcinoma can be appropriately selected according to the severity of the symptoms, the age, sex, weight, and sensitivity difference of the patients, the mode of administration, the administration time, the administration interval, and the preparation type, etc. For example, the dosage can be 1 μ g to 20 g per day, and it can be administered once a day or in multiple divided

times a day, for example, 2 times, 3 times, or 4 times.

In some specific embodiments of the present invention, the patient with cholangiocarcinoma in the present invention may be a patient that has disease progression after receiving previous chemotherapy, small molecule targeted therapy and/or radiotherapy. The previous chemotherapy can be those commonly used in the art, such as treatment with gemcitabine, 5-fluorouracil, paclitaxel, oxaliplatin, cisplatin and/or tegafur, gimeracil and oteracil, and the small molecule targeted therapy can also be those commonly used in the art, such as treatment with sunitinib.

The proteasome inhibitor of Formula (I) or (II) of the present invention or a pharmaceutically acceptable salt thereof can be administered through various routes, including, but not limited to, oral administration, intravenous administration, sublingual administration, intranasal administration, inhalation, and subcutaneous administration, etc. In some specific embodiments of the present invention, the proteasome inhibitor is administered by oral administration or injection. Preparations suitable for oral administration can be in any dosage form suitable for oral administration in the art, for example, including, but not limited to, tablets, capsules, powders, granules, dripping pills, pastes, and powders, etc., and tablets or capsules are preferred. The oral preparation can be prepared by a conventional method using a pharmaceutically acceptable carrier known in the art.

The present invention further provides a pharmaceutical composition for treating cholangiocarcinoma with PTEN gene mutation and/or deletion, which contains a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof, and at least a pharmaceutically acceptable carrier. The present invention further provides use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the chymotrypsin-like activity of the $\beta 5$ subunit of 20S proteasome.

The present invention further provides use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the caspase-like activity of the $\beta 1$ subunit.

The present invention further provides use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the caspase-like activity of the $\beta 2$ subunit.

Unless otherwise specified, for the purpose of the present application, the following terms used in the specification and claims have the following meanings.

The "patient" is a mammal, preferably a human.

"Pharmaceutically acceptable" means suitable for use in preparing a pharmaceutical

composition, generally safe and non-toxic, and not biologically or otherwise undesirable,, including being acceptable for human pharmaceutical use.

"Therapeutically effective amount" means an amount of a compound that is sufficient to control a disease when it is administered to a human for treating the disease.

"Treatment" means any administration of a therapeutically effective amount of a compound, and includes:

(1) inhibiting the disease (i.e., preventing the further development of the pathology and/or symptomatology) in a human experiencing or exhibiting the pathology or symptomatology of the disease, or

(2) ameliorating the disease (i.e., reversing the pathology and/or symptomatology) in a human experiencing or showing the pathology or symptomatology of the disease.

The method for treating cholangiocarcinoma provided in the present invention overcomes the problem of ineffectiveness of the existing proteasome inhibitor caused by drug distribution in the treatment of solid tumors, and shows good efficacy (the tumor inhibition rate is close to 60%) in patients with cholangiocarcinoma, especially patients with cholangiocarcinoma with PTEN gene mutation and/or deletion.

DETAILED DESCRIPTION

The present invention will be further described below in connection with specific examples, according to the common technical knowledge and conventional methods in the art. The examples below are merely some preferred embodiments of the present invention, which should not be regarded as limitations on the present invention. Some improvements can be made by those skilled in the art without departing from the scope of the present invention, which are also contemplated in the scope of the present invention.

Example 1: Study on anti-tumor activity and mechanism of Compound I in vitro

Test 1: Inhibition of compound on 20S proteasome and its subtypes.

The inhibitory effects of Compound I, active form Compound II and MLN9708 on different subunits of human 20S proteasome were studied by using purified specific fluorescent substrates for human 20S proteasome and various subunits. It is proved that Compound I of the present invention can selectively inhibit the chymotrypsin-like (CT-L) activity of the $\beta 5$ subunit of 20S proteasome, with an IC_{50} value of 2.22 ± 0.71 nM; the compound has a weaker inhibitory effect on the caspase-like (C-L) activity of the $\beta 1$ subunit, with an IC_{50} value of 32.69 ± 7.93 nM; and the compound has the weakest inhibitory effect on the trypsin-like (T-L) activity of

the $\beta 2$ subunit, with an IC_{50} value of 460.70 ± 170.06 nM. The inhibitory effects of active form Compound II on the 3 subunits ($\beta 5$, $\beta 1$, and $\beta 2$) of 20S proteasome are comparable to those of Compound I, with an IC_{50} value of 2.61 ± 0.75 , 24.27 ± 0.34 , and 410.03 ± 137.95 nM respectively. The IC_{50} values of the reference drug MLN9708 in inhibiting the 3 subunits ($\beta 5$, $\beta 1$, and $\beta 2$) are respectively 8.07 ± 2.47 , 48.25 ± 12.21 , and >2000 nM. The above results show that the activity of Compound II is comparable to that of Compound I, and both compounds can selectively inhibit the activity of the $\beta 5$ subunit of the proteasome, with an inhibitory effect that is stronger than that of the reference drug MLN9708 and an IC_{50} value that is about 3.09-3.64 times lower than that of MLN9708 (as shown in Table 1).

Table 1. Summary of IC_{50} of compounds in inhibiting 20S proteasome (n=3)

Compound	IC_{50} (nM, Mean $\pm$ SD)		
	$\beta 1$	$\beta 2$	$\beta 5$
Compound I	32.69 ± 7.93	460.70 ± 170.06	2.22 ± 0.71
Compound II	24.27 ± 0.34	410.03 ± 137.95	2.61 ± 0.75
MLN9708	48.25 ± 12.21	>2000	8.07 ± 2.47

Test 2: Inhibitory effect of compounds on the CT-L activity of proteasome in cholangiocarcinoma cells

Cholangiocarcinoma cells RBE, HuCCT1, and HCCC-9810 were incubated with Compound I and MLN9708 for 1 h, respectively, and the inhibitory effects of the medicaments on the chymotrypsin-like (CT-L) activity of the $\beta 5$ subunit of 20S proteasome in the cells were detected. The PTEN gene was completely expressed in RBE cells, the PTEN gene transcription and protein abundance decreased in HuCCT1 cells (hemizygous deletion), and the PTEN gene was completely deleted in HCCC-9810 cells (exons 6-9 were deleted).

The results are shown in Table 2. Compound I and MLN9708 both have an inhibitory effect on the CT-L activity of 20S proteasome in cholangiocarcinoma cells. The inhibitory activity of Compound I on the three types of cells is significantly higher than that of MLN9708. The inhibitory effect on the CT-L activity of 20S proteasome in HCCC-9810 cells with complete deletion of PTEN is the highest, with an IC_{50} value of 23.09, which is significantly lower than that of MLN9708.

The above results show that Compound I can inhibit the CT-L activity of the proteasome in cholangiocarcinoma cells, with an effect significantly higher than that of MLN9708.

Table 2. Summary of IC₅₀ of compounds in inhibiting the CT-L activity of the proteasome in cholangiocarcinoma cells

Cell line	IC ₅₀ (nM)	
	Compound I	MLN9708
RBE	28.93	49.40
HCCC-9810	23.09	55.62
HuCCT1	112.4	474.7

Test 3: Inhibitory effect of compounds on the proliferation of cholangiocarcinoma cells

The effects of Compound I and MLN9708 on the proliferation of cholangiocarcinoma cells were detected by using the CellTiter-Glo detection kit.

As shown in Table 3, Compound I has a significantly higher inhibitory activity for HCCC-9810, HuCCT1, and RBE cells compared with MLN9708, and particularly has a highly selective proliferation inhibiting activity for cholangiocarcinoma cells with complete PTEN deletion, with a selectivity of about 1.7 times compared with cells with complete PTEN expression.

Table 3. Summary of IC₅₀ of Compound I and MLN9708 in inhibiting cholangiocarcinoma cell proliferation (n=3)

Cell line	PTEN gene expression	IC ₅₀ (nM)	
		Compound I	MLN9708
RBE	Complete expression	10.08	128.7
HCCC-9810	Complete deletion (exons 6-9 are deleted)	5.868	62.09
HuCCT1	Partial deletion (hemizygous deletion)	12.55	26.78

Example 2. Study on drug distribution of compounds

30 SD rats (male:female 1:1) weighing 195-217 g were orally administered with 0.6 mg/kg Compound I. Samples were taken from each group of 6 animals at 5 sampling time points including 0 h before administration, 5 min, 1 h, 4 h and 24 h after administration.

After euthanasia with CO₂, at least 1.0 mL of blood was collected by cardiac puncture, and EDTA-K2 was used for anticoagulation. After the blood was collected and mixed, it was placed on ice. 100 µL of the whole blood sample was aspirated and added into an EP tube with 200 µL of 0.5% formic acid in acetonitrile added in advance, in an amount of the whole blood:0.5% formic acid in acetonitrile (v:v) =1:2, and vortex mixed evenly. The whole blood sample was stored in an ultra-low temperature refrigerator. After collection, the blood sample (or the

remaining blood sample) was centrifuged within 30 min to separate the plasma (centrifugation condition: 11000 rpm, 5 min, 2-8°C). A certain amount of the plasma sample was aspirated, added into an EP tube with 0.5% formic acid in acetonitrile added in advance, in an amount of plasma:0.5% formic acid in acetonitrile (v:v)=1:2 (for example, 100 µL plasma + 200 µL 0.5% formic acid in acetonitrile), and vortex mixed evenly. The plasma sample was stored in an ultra-low temperature refrigerator.

After the blood sample was collected, brain, heart, liver, spleen, lung and kidney were collected from the animals. Each tissue was washed with physiological saline to avoid cross-contamination, patted dry with filter paper, and then placed in a labeled tube/self-sealed bag (one tube/self-sealed bag for each tissue). The sample was temporarily placed on ice before being stored in an ultra-low temperature refrigerator.

The sample was thoroughly homogenized by a high-speed homogenizer to prepare a homogenate of the tissue sample. The concentrations of the compound in the plasma, whole blood and tissue homogenate were determined by LC-MS/MS.

Results: After 0.6 mg/kg of Compound I is orally administered to rats, the concentrations of Compound II in the tissues are shown in Table 4 to Table 7. The comparison of exposure of Compound II in different tissues and plasma is shown in Table 8.

After 0.6 mg/kg of Compound I is orally administered, the order of exposure of Compound II in male and female rats is spleen > liver > kidney > lung > heart > whole blood > plasma > brain. According to the results, after 0.6 mg/kg of Compound I is orally administered to rats, Compound II is highly distributed in the target organ liver, with the exposure in liver tissue being 36.9-51.0 times that in plasma. This indicates a significant clinical application value.

Table 4. Concentration (ng/g or ng/mL) of Compound II in various tissues 5 min after 0.6 mg/kg of Compound I is orally administered to rats

Tissue	Rat No.						Male	Female
	201M	202M	203M	204F	205F	206F	Mean	Mean
Whole blood	BQL	24.0	3.48	55.5	18.9	21.75	13.7	32.1
Brain	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Heart	BQL	6.91	BQL	20.9	8.27	8.81	NA	12.7
Kidney	9.15	8.01	BQL	29.4	7.81	23.4	8.58	20.2
Liver	7.22	34.3	BQL	151	48.6	128	20.8	109
Lung	BQL	15.1	BQL	64.0	15.3	28.7	NA	36.0
Plasma	BQL	27.09	3.75	102.9	27.9	27.0	15.4	52.6

Spleen	BQL	BQL	BQL	25.2	7.10	7.73	NA	13.3
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(BQL: below the quantification limit: 5.00 ng/g or 0.300 ng/mL; NA: not applicable; /: insufficient homogenate or no sample, the same below)

Table 5. Concentration (ng/g or ng/mL) of Compound II in various tissues 1 h after 0.6 mg/kg of Compound I is orally administered to rats

Tissue	Rat No.						Male	Female
	301M	302M	303M	304F	305F	306F	Mean	Mean
Whole blood	12.24	35.1	42.6	24.81	12.45	21.99	30.0	19.8
Brain	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Heart	11.2	23.1	33.9	19.0	14.0	33.4	22.7	22.1
Kidney	33.9	90.8	116	75.7	56.8	89.8	80.2	74.1
Liver	80.4	194	259	240	205	308	178	251
Lung	19.5	48.8	46.7	40.3	33.3	70.4	38.3	48.0
Plasma	7.92	20.97	20.97	8.49	7.80	12.54	16.6	9.61
Spleen	57.3	155	206	143	92.2	223	139	153

Table 6. Concentration (ng/g or ng/mL) of Compound II in various tissues 4 h after 0.6 mg/kg of Compound I is orally administered to rats

Tissue	Rat No.						Male	Female
	401M	402M	403M	404F	405F	406F	Mean	Mean
Whole blood	29.1	36.9	34.8	55.2	26.16	34.2	33.6	38.5
Brain	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Heart	28.3	50.6	32.1	80.5	48.4	69.5	37.0	66.1
Kidney	104	160	111	217	133	172	125	174
Liver	173	345	166	439	298	334	228	357
Lung	52.4	132	63.5	213	163	175	82.6	184
Plasma	3.51	4.98	3.60	8.58	3.96	5.28	4.03	5.94
Spleen	173	301	211	530	279	400	228	403

Table 7. Concentration (ng/g or ng/mL) of Compound II in various tissues 24 h after 0.6 mg/kg of Compound I is orally administered to rats

Tissue	Rat No.						Male	Female
	501M	502M	503M	504F	505F	506F	Mean	Mean
Whole blood	69.3	26.31	55.8	35.7	59.7	83.1	50.5	59.5
Brain	BQL	BQL	BQL	BQL	BQL	BQL	NA	NA
Heart	82.1	38.4	54.4	43.5	65.8	115	58.3	74.8
Kidney	213	105	215	121	263	231	178	205
Liver	325	180	319	280	428	407	275	372
Lung	102	61.0	118	80.7	116	180	93.7	126
Plasma	9.27	3.93	7.32	3.54	5.73	5.94	6.84	5.07
Spleen	414	254	376	269	374	475	348	373

Table 8. Exposure (AUC_{0-t}, ng·h/g or ng·h/mL) of Compound II in various tissues and plasma after 0.6 mg/kg of Compound I is orally administered to rats

Tissue	Male rats		Female rats		AUC ratio
	AUC	AUC ratio	AUC	AUC ratio	
	(ng·h/g or ng·h/mL)	(tissue/plasma)	(ng·h/g or ng·h/mL)	(tissue/plasma)	(female/male)
Whole blood	957	6.17	1093	6.66	1.14
Brain	*	NA	*	NA	NA
Heart	1056	6.81	1558	9.50	1.48
Kidney	3376	21.8	4206	25.6	1.25
Liver	5727	36.9	8368	51.0	1.46
Lung	1970	12.7	3480	21.2	1.77
Plasma	155	1.00	164	1.00	1.06
Spleen	6385	41.2	8667	52.8	1.36

(*: The pharmacokinetic parameters are not calculated by the software; NA: Not applicable)

Example 3: Study on in-vivo anti-tumor activity of Compound I - subcutaneous patient-derived tumor xenograft (PDX) model CC6679 established with human cholangiocarcinoma

This study was carried out by Crown Bioscience (Beijing) Co., Ltd. Tumor tissue was collected from tumor-bearing mice of HuPrime® (https://www.crownbio.cn/wp-content/uploads/2023/01/Crownbio_HuPrime_Factsheet_CN.pdf) cholangiocarcinoma

xenograft model CC6679 (PTEN frameshift mutation, p.N323KfsTer2), cut into a tumor mass with a diameter of 2-3 mm, and inoculated subcutaneously at the right front shoulder of Balb/c nude mice. When the average tumor volume of the tumor-bearing mice reached about 104.46 mm³, they were randomly grouped and administered. The dosage of Compound I was 1.0 mg/kg, 1.2 mg/kg, and 1.0/1.5 mg/kg respectively (1.0 mg/kg in the first week and 1.5 mg/kg in the later two weeks). After the start of the administration, the weight and tumor size of the mice were measured twice a week. The tumor volume was calculated by a formula: tumor volume (mm³) = 1/2 x (a x b²) (where a represents the long diameter and b represents the short diameter).

The experimental results are shown in Table 9. Compound I (1.0, 1.2, 1.0/1.5 mg/kg, i.g., BIW, D1-19) inhibits the growth of subcutaneous transplanted tumor in nude mice in a dose-dependent manner, with a tumor inhibition rate of 38.32%, 39.63% and 52.32% respectively. It can be seen that Compound I has a good therapeutic effect on cholangiocarcinoma with PTEN gene mutation and/or deletion. Especially, when Compound I is given at a dosage of 1.0 mg/kg in the first week and 1.5 mg/kg in the later two weeks, the tumor inhibition rate is close to 60%.

Table 9. Summary of in-vivo anti-tumor efficacy of Compound I

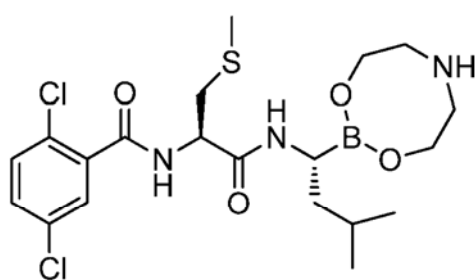
Administration	Dose (mg/kg)	Day 19			
		Tumor volume (mean ± SEM)	ΔT/ΔC (%)	TGI (%)	p Value
Vehicle control	--	677±109.41	-	-	-
Compound I	1.0	457.58±72.68	61.68	38.32%	0.301
Compound I	1.2	450.11±92.95	60.37	39.63%	0.272
Compound I	1.0/1.5	377.44±88.94	47.68	52.32%	0.104

Note: TGI: $1 - (T_i - T_0) / (C_i - C_0) \times 100\%$. Because the value of Bartlett's test is > 0.05 and the value of Kruskal Wallis test is > 0.05, Dunnett's test is used for pairwise comparison among all groups; and p value is calculated from the absolute tumor volume.

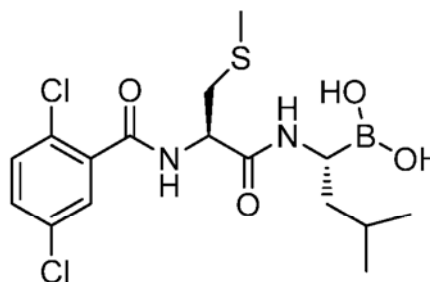
CLAIMS

What is claimed is:

1. Use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of medicaments for treating cancers, wherein the cancers are cholangiocarcinoma with PTEN gene mutation and/or deletion:



(I)



(II)

2. The use according to claim 1, wherein the cholangiocarcinoma is hilar cholangiocarcinoma, intrahepatic cholangiocarcinoma, or distal cholangiocarcinoma.

3. The use according to claim 1, wherein the cholangiocarcinoma is intrahepatic cholangiocarcinoma.

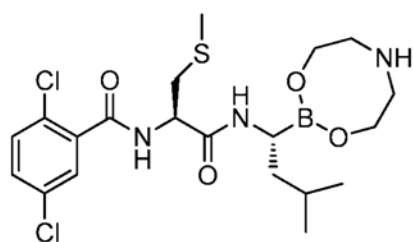
4. The use according to claim 1, wherein the PTEN gene mutation and/or deletion is the decrease of PTEN gene transcription and protein abundance, or the complete deletion of PTEN gene.

5. The use according to claim 4, wherein the PTEN gene mutation and/or deletion is the complete deletion of PTEN gene.

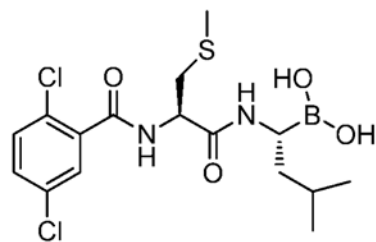
6. The use according to claim 1, wherein the cholangiocarcinoma is early, intermediate, advanced or metastatic cholangiocarcinoma, or recurrent cholangiocarcinoma.

7. The use according to claim 1, wherein the proteasome inhibitor of Formula (I) or (II) or the pharmaceutically acceptable salt thereof is administered through oral administration, intravenous administration, sublingual administration, intranasal administration, inhalation, or subcutaneous administration, preferably oral administration or injection, and further preferably oral administration.

8. Use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the chymotrypsin-like activity of the $\beta 5$ subunit of 20S proteasome:

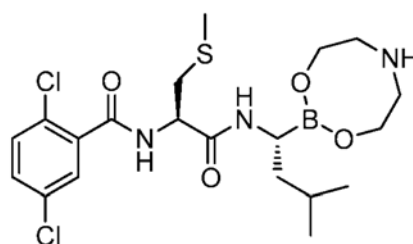


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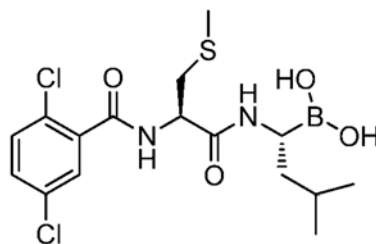


(II)

9. Use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the caspase-like activity of the $\beta 1$ subunit:

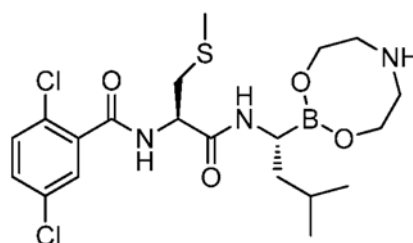


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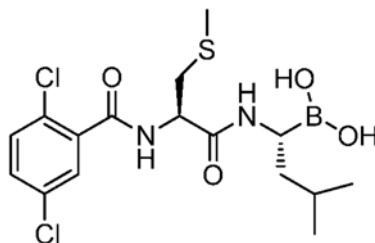


(II)

10. Use of a proteasome inhibitor of Formula (I) or (II) or a pharmaceutically acceptable salt thereof in the preparation of an inhibitor of the caspase-like activity of the $\beta 2$ subunit:



(I)



(II)