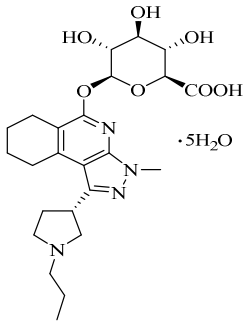
Use of PARP inhibitor TSL-1502 intermediate TSL-1502M

Technical Field

The invention belongs to the technical field of drug preparation, and specifically, relates to the use of an intermediate TSL-1502M for a PARP inhibitor TSL-1502.

Background Art

PARP inhibitor TSL-1502, a compound with a chemical name of (2S,3S,4S,5R,6S)-3,4,5-trihydroxy-6-(3-methyl-1-((S)-1-propylpyrrolidin-3-yl)-6,7,8,9-tetrahydro-3H-pyrazolo[3,4-c]isoquinolin-5-oxy)tetrahydro-2H-pyran-2-carboxylic acid (designated as TSL-1502), was first described as the compound in the paragraph 0177 in ZL201180002886.8 (Publication No. CN102510863A, Authorization No. CN102510863B, hereinafter the 2011 Patent), with the structural formula as Formula I.

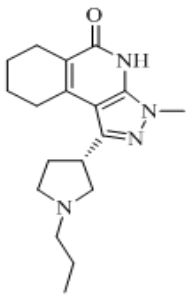


Formula I

The above patent discloses in claim 16 the specific tumor types such as head carcinoma, thyroid carcinoma, neck cancer, eye cancer, skin cancer, oral cancer, throat cancer, esophagus cancer, breast cancer, bone cancer, leukemia, myeloma, lung cancer, colon cancer, carcinoma of sigmoid, rectal cancer, gastric cancer, prostate cancer, breast cancer, ovarian cancer, kidney cancer, liver cancer, pancreatic cancer, brain cancer, intestinal cancer, heart cancer, adrenal carcinoma, subcutaneous tissue cancer, lymph node cancer, malinant melanoma, malignant glioma and the like. However, in fact, only the treatment effects in melanoma and human breast cancer cell line were proven in the Example 19, while other tumors were not mentioned.

TSL-1502M is an intermediate for preparation of TSL-1502, as described in the

patent application CN201910465780.X, with the title of invention as "AN INTERMEDIATE TSL-1502M OF A PARP INHIBITOR AND THE PREPARATION METHOD THEREOF", (application date of May 31, 2019, Publication No. CN112010850A), with the structural formula as Formula II,



Formula II

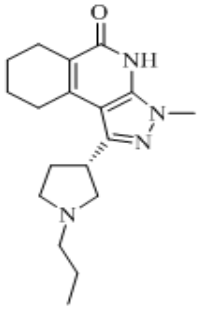
Formula II compound is designated as TSL-1502M, and it can be used as an intermediate to prepare TSL-1502, and can be used for the detection of TSL-1502, and is useful as a reference compound. However, the document did not disclose the medical use information for TSL-1502M.

Summary of the Invention

The invention is the unexpected discovery on the medical use, in particular the anti-tumor use during the study of TSL-1502M.

Specifically, the invention provides the use of TSL-1502M in the manufacture of a medicament for preventing and treating tumor.

The structure of said TSL-1502M is shown in Formula II:



Formula II

According to the invention, through the study, it is unexpectedly found that said compound has an anti-tumor effect, and the anti-tumor effect is mainly exerted by inhibiting proliferation of tumor cells and inducing cell apoptosis, wherein said tumor

cells include tumor cells with BRCA function defects, tumor cells with BRCA gene mutation, or tumor cells with normal BRCA function.

Thus, according to the anti-tumor effect of the invention, the tumor comprises the following tumors.

Said tumor is specifically breast cancer, pancreatic cancer, ovarian cancer, colon cancer.

Said tumor is preferably breast cancer, ovarian cancer, pancreatic cancer.

The invention further provides a pharmaceutical preparation composition comprising TSL-1502M, wherein the pharmaceutical preparation composition can be in any ingestible pharmaceutical form, such as tablet, sugar-coated tablet, film-coated tablet, enteric-coated tablet, capsule, hard capsule, soft capsule, oral liquid, buccal tablet, granule, electuary granule, pill, pulvis, ointment, pellet, suspension, powder, solution, injectable preparation, suppository, soft ointment, hard ointment, cream, spray, drop, patch.

The pharmaceutical preparation of the invention is preferably in the form of a unit dose of the pharmaceutical preparation, and for example, when formulating in to a pharmaceutical preparation, the unit dose of the pharmaceutical preparation can comprise 0.1-1000mg of TSL-1502M according to the invention, with the remainder being pharmaceutically acceptable excipients. The pharmaceutically acceptable excipient can be 0.01-99.99% by weight of the total weight of the preparation.

The pharmaceutical preparation of the invention can determine the dosage regimen according to the condition of the patient when administered, such as 1-3 times daily, 1-20 tablets per time.

Preferably, the pharmaceutical preparation according to the invention can be oral preparation or injectable preparation.

Among others, the oral preparation is one selected from a group consisting of capsule, tablet, dripping pill, granule, concentrated pill, and oral liquid.

Among others, the injectable preparation is one selected from a group consisting of liquid, semi-solid, solid, and powder form, preferably injectable solution, and injectable powder.

Among the pharmaceutical preparation of the invention, the orally administered preparation can comprise excipients, such as binder, filler, diluent, tableting agent, lubricant, disintegrant, colorant, flavoring agent and wetting agent, and if necessary the tablet can be coated.

Suitable fillers include cellulose, mannitol, lactose and other similar fillers. Suitable disintegrants include starch, polyvinylpyrrolidinone and starch derivatives, for example, sodium starch glycolate. Suitable lubricants include, for example, magnesium stearate. Suitable pharmaceutically acceptable wetting agents include sodium lauryl sulfate.

The pharmaceutical preparation of the invention can be formulated into solid oral composition by conventional methods such as mixing, filling and tableting. By repeating the mixing, the active ingredient can be distributed into those compositions using a large amount of fillers.

The form of the oral liquid preparation can be for example aqueous or oily suspension, solution, emulsion, syrup or elixir, or can be a dry product that can be reformulated with water or other suitable carriers before use. Such liquid preparations can comprise conventional additives, such as suspending agent, for example, sorbitol, syrup, methylcellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel or edible hydrogenated oil, emulsifying agent, for example, lecithin, sorbitan monooleate or arabic gum; non-aqueous carrier (it can include edible oil), for example, almond oil, fractionated coconut oil, such as oily ester of the glycerol ester, propylene glycol or ethanol; preservative agent, for example, methylparaben or propylparaben or sorbic acid, and if necessary, can comprise conventional flavoring agent or colorant.

For an injectable preparation, a prepared liquid unit dosage form comprises the active ingredient of the invention and a sterile carrier. Depending on the carrier and the concentration, the compound can be suspended or dissolved. The preparation of the solution generally includes dissolving the active ingredient into a carrier, filtering and sterilizing before loading into a suitable vial or ampoule, and then sealing. The excipients, such as local anesthetic, preservative agent and buffer can also be

dissolved into such a carrier. In order to improve its stability, such composition can be frozen after loading in a vial, and be subjected to the water removal in vaccum.

When administered, the administered dosage of TSL-1502M can be 0.01mg-2000mg daily.

The anti-tumor effect as provided by the invention has the following charateristics.

1, The inventor found by experiments that, it is more sensitive in the cells with BRCA1/2 function defects (in particular breast cancer cells, pancreatic cancer cells, and ovarian cancer cells), and shows selectivity on the proliferation inhibiting effect of the cell, and the effect of TSL-1502M is superior to TSL-1502.

2, In the BRCA genotype mutated or normal tumor cells (in particular breast cancer cells), TSL-1502M shows enhancing effect on cytotoxic drugs.

3, TSL-1502M and olaparib can inhibit the DNA damage repair (preferably breast cancer), and the activity of TSL-1502M is stronger than olaparib.

4, TSL-1502 and TSL-1502M induce breast cancer cell cycle arrest in G_2/M phase (preferably breast cancer), and the activity of TSL-1502M is significantly stronger than TSL-1502.

5, TSL-1502, TSL-1502M and the positive reference drug olaparib all induce breast cancer cell apoptosis, wherein the activity of TSL-1502M is stronger than olaparib.

Brief Description of the Drawings

Figure 1: The effect of TSL-1502 and TSL-1502M on cultured cell proliferation *in vitro*.

Figure 2: The effect of the combination therapy of TSL-1502, TSL-1502M and cytotoxic drugs on the proliferation inhibition in MDA-MB-436, SW620 cells.

Figure 3: The effect of TSL-1502 and ABT-888 on inhibiting the MDA-MB-436 cell clonal formation.

Figure 4: The effect of TSL-1502M and AZD2281 on the Rad51 accumulation site formation.

Figure 5: The effect of TSL-1502M on the γ-H2AX phosphorylation in MDA-MB-436 cells.

Figure 6: The effect of TSL-1502, TSL-1502M and AZD2281, ABT888 on the cell

cycle in MDA-MB-436 cells.

Figure 7: The effect of TSL-1502, TSL-1502M and AZD2281 on inducing MDA-MB-436 cell apoptosis.

Detailed Description of the Invention

Example 1: The preparation of TSL-1502M

The Example 1 in the application CN201910465780.X, with the title of invention as "AN INTERMEDIATE TSL-1502M OF A PARP INHIBITOR AND THE PREPARATION METHOD THEREOF" is referred.

Reaction: In the reaction vessel, the main reagent (*S*)-3-methyl-1-(pyrrolidin-3-yl)-3,4,6,7,8,9-hexahydro-5*H*-pyrazolo[3,4-*c*]isoquinolin -5-one (2*S*,3*S*)-2,3-di(benzoyloxy)succinate (630.0g, 1mol) and ethanol (3800mL) were added, stirred, and cooled to 10ºC. Sodium cyanoborohydride (125.7g, 2mol) was added. Then propanal (116.2g, 2mol) was added dropwise, wherein during the dropwise addition, the temperature was less than 20ºC. After completing the dropwise addition, the reaction was continuously stirred at 10ºC~25ºC for 1h~2h.

Quenching: After the reaction was completed, hydrochloric acid having a concentration by mass of 36 to 38% (73g, 2mol) was slowly added dropwise to the reaction mixture. The pH was adjusted between 4 and 5. The addition speed was controlled. The large amount of generated gas was absorbed by 20% sodium hydroxide in order to prevent overflowing, and the temperature was controlled at 20ºC to 30ºC.

Post-treatment: S1, After quenching, the reaction solution was concentrated under reduced pressure to evaporate ethanol, at a temperature less than 70ºC.

S2, To the residue of S1, water and dichloromethane (the weight ratio of water and dichloromethane was 1: 4) were added, with an amount of 7 times of the weight of the compound of Formula I. At a temperature of 5ºC~15ºC, the pH of the mixture was adjusted to 9 to 10 with 40% sodium hydroxide aqueous solution. The solution was stirred, stood, and seperated, and the organic phase was kept.

S3, The aqueous phase obtained in S2 was continuously extracted once with dichloromethane. The obtained organic phase and the organic phase obtained in S2

were combined, and the aqueous phase was kept.

S4, The combined organic phase obtained in S3 was extracted with 40% sodium hydroxide aqueous solution. The obtained aqueous phase and the aqueous phase obtained in S3 were combined, and the organic phase was kept.

S5, The combined aquesou phase obtained in S4 was adjusted with 36~38% hydrochloric acid to pH 4 to 5. Then dichloromethane was added, with an amount of dichloromethane of 5 times of the weight of the compound of Formula I. The solution was stirred, stood, and seperated, and the organic phase was kept.

S6, The aqueous phase obtained in S5 was continuously extracted twice with dichloromethane. The obtained organic phase and the organic phase obtained in S4 and S5 were combined.

S7, The combined organic phase obtained in S6 was washed twice with 3% sodium bicarbonate aqueous solution, and then dried with anhydrous sodium sulfate. The sodium sulfate solid was removed by filtering. The filtrate was concentrated under reduced pressure, and then dried under vacuum to yield the intermediate of a PARP inhibitor TSL-1502M 226g.

Experimental Example 1

I, Experimental Material

1, Tested drug

TSL-1502: white powder, Lot. No. 120301, purity 99.56%, water content 16.65%, sealed, preserved at 2-8^{o}C, prepared by the Applicant;

TSL-1502M: white powder, Lot. No. 20150801, purity 98.6%, sealed, preserved at 2-8^{o}C, prepared by the Applicant;

ABT-888 (Veliparib): white powder, Lot. No. HM-069-8-20101112, purity 98.12%, sealed, preserved at 2-8^{o}C, purchased from Shanghai Haoyuan. ABT-888is a novel potent PARP-1 and PARP-2 inhibitor, mainly used in breast cancer.

AZD2281 (Olaparib): white powder, Lot. No. 20131105, purity 99.15%, sealed, preserved at 2-8^{o}C, purchased from Shanghai Mode. AZD2281 is a PARP inhibitor, with approved indication including ovarian cancer, breast cancer, pancreatic cancer, prostate cancer;

Temozolomide(abbreviated as TMZ): white powder, Lot. No. 20150418, sealed, preserved at 2-8ºC. Temozolomide is an imidazole-tetrazine derivative, mainly used in glioblastoma multiforme.

SN-38 (active metabolite of irinotecan, 7-ethyl-10-hydroxycamptothecin. cas 号为 86639-52-3): ligh yellow powder, purity 99.1%, Lot. No. E060206, sealed, preserved at 2-8ºC, purchased from Shanghai Junjie Biotechnology. Irinotecan is a semi-synthesized derivative of camptothecin, mainly used in colorectal cancer.

2, Formulation method of the drug

TSL-1502, TSL-1502M, AZD2281 and ABT-888 were formulated into 10 mM stock solution with DMSO, packaged and stored at -70ºC. TMZ was formulated into 200mM stock solution with DMSO, , packaged and stored at -70ºC.

3, Cell line selection criteria and cell line source

By referring to the pharmacological study on the FDA approved PARP inhibitors Olaparib (LYNPARZA), Rucaparib (RUBRACA) and Niraparib (ZEJULA), the BRCA1/2 mutated-type and wild-type cells were selected to evaluate the in vitro anti-tuomr activity of TSL-1502 and its metabolites. Cell source and culture condition are shown in Table 1.

Table 1: Cell source and Type

Cell Line	Source	Tissue Source and Type	BRCA Genotype	Culture Condition
V-C8	Delivered abroad	Chinese Hamster Ovarian cell derived line	BRCA2 defective	10% FBS containing F-10
V-C8 #13-5	Delivered abroad	Chinese Hamster Ovarian cell derived line	Normal	10% FBS containing F-10
MDA-MB-436	cell library	human breast cancer cell	BRCA1 mutated	10%FBS and 10 μg/ml insulin containing L-15
Capan-1	ATCC	humanpancreatic cancer cell	BRCA2 mutated	20%FBS containing IMDM
HCC1937	ATCC	human breast cancer cell	BRCA1 mutated	10% FBS containing RPMI1640
MDA-MB-468	Cell library	human breast cancer cell	Normal	10% FBS containing L-15

UWB1.289	ATCC	human ovarian cancer cell	BRCA1-null	3% FBS containing RPMI1640/IMDM
UWB1.289 BRCA1	ATCC	human ovarian cancer cell	BRCA1-expressed	3% FBS containing RPMI1640/IMDM
SW620	ATCC	human colon cancer cell	Normal	10% FBS containing L-15

4, Reagents and Instruments

RPMI1640, L-15, F-10, DMEM and IMDM were purchased from Gibco BRL. FBS was purchased from Gibco BRL. SRB was purchased from Sigma. Anti-Rad51 primary antibody was purchased from Santa Cruz. Anti-P-γ-H2AX and PARP primary antibody were purchased from Cell Signaling Technology. Anti-β-Tubulin primary antibody was purchased from Sigma. horserdish peroxidase-labeled goat anti-rabbit and goat anti-mouse secondary antibody were purchased from Calbiochem. Alexa Fluor® 488 goat anti-rabbit fluoresence secondary antibody was purchased from Molecular Probes. Immobilon Western HRP Substrate luminal reagent was purchased from Millipore. Other conventional chemical reagents were domestic analytical pure (AR) reagents.

Multifunctional microplate reader Synergy H4 was purchased from BioTek. carbon dioxide incubator (Model 3111) was purchased from Thermo. Inverted microscope XDS-1B was purchased from Chongqing Optoelectronic Instrument Co., Ltd. Flow cytometer (FACS Calibur flow cytometer) was purchased from Beckman Dickson. Laser confocal microscope was purchased from Olympus. Western blot imager was purchased from GlinxScience Instruments.

II, Experimental Method

1, Sulfonyl Rhodamine B protein staining method(SRB method)

A certain number of cells in logarithmic phase were inoculated in 96-well plates. After 24 hours of adherent growth, drugs with different concentrations (1, 3, 10, 30, 100, 300, 1000, 3000, 10000 nM) were added. After 10 days of drug action, the cells were fixed with trichloroacetic acid, and then stained with SRB solution. Finally, Tris solution was added to dissolve SRB, and the OD value was measured by a microplate reader at the wavelength of 510nm. The cell growth inhibition rate was calculated according to the following formula.

Inhibition Rate = (OD Value$_{\text{Control Well}}$ − OD Value$_{\text{Drug Well}}$) / OD Value$_{\text{Control Well}}$×100%

The IC_{50} of the half inhibitory concentration was calculated based on the inhibition rate at each concentration.

2, Clonal Formation Experiment

Cells were inoculated in 6-well plates at a density of 200 cells per well. After adherent growth for 24 hours, drugs with different concentrations (0.3, 1, 3, 10, 30, and 100 nM) were added, and the culture medium and drugs were changed every 5 days. After 20 days, they were fixed with methanol: glacial acetic acid (3: 1), stained with crystal violet, counted, and photographed.

3. Immunofluorescence Method

The cells pasted on the small cover glass were treated with drugs, fixed with 4% paraformaldehyde, and penetrated and ruptured in phosphate buffer solution containing 0.3% Triton X-100 (pH7.4). After blocking, the anti-Rad51 primary antibody was added for incubation at 4 ºC. Alexa Fluor ® 488 goat anti-rabbit fluoresence secondary antibody was used for incubation, and finally DAPI staining was performed. After the slices were sealed, they were photographed under laser confocal microscope.

4, Western Blot Method

4.1 Detection of γ-H2AX phosphorylation

MDA-MB-436 was inoculated into a 6-well plate (2×10^5/ml) and treated with different concentrations of TSL-1502M or AZD2281(100, 1000, and 10000 nM) for 1.5 h, followed by 1mM TMZ for 0.5 h. Then, 1×SDS gel loading buffer (50 mM Tris-HCl (pH 6.8), 100 mM DTT, 2% SDS, 10% glycerol, and 0.1% bromophenol blue) were added to lyse the cells. The cell lysate was heated and denatured in a boiling water bath, and was subjected to SDS-PAGE electrophoresis. After the electrophoresis was completed, the protein was transferred to PVDF membrane by a wet transfer system. The PVDF membrane was placed in a blocking solution (5% defatted milk powder diluted in TBS/T) for blocking at room temperature, and was subjected to I and II anti-reaction. After the membrane was washed, the color was developed using the Immunologic Western HRP Substrate Luminal Reagent and photographed using the Western blot Imager.

4.2 Detection of cell apoptosis

MDA-MB-436 was inoculated into a 6-well plate (1×10^5/ml) and treated with different concentrations of TSL-1502, TSL-1502M (0.01, 0.1, 1 μM) or AZD2281(0.1, 1, 10 μM) for 120 h. Then, 1×SDS gel loading buffer (50 mM Tris-HCl (pH 6.8), 100

mM DTT, 2% SDS, 10% glycerol, and 0.1% bromophenol blue) were added to lyse the cells. The cell lysate was heated and denatured in a boiling water bath, and was subjected to SDS-PAGE electrophoresis. After the electrophoresis was completed, the protein was transferred to PVDF membrane by a wet transfer system. The PVDF membrane was placed in a blocking solution (5% defatted milk powder diluted in TBS/T) for blocking at room temperature, and was subjected to I and II anti-reaction. After the membrane was washed, the color was developed using the Immunologic Western HRP Substrate Luminal Reagent and photographed using the Western blot Imager.

5. Flow cytometry

MDA-MB-436 cells were inoculated in 6-well plates (2×10^5/ml) and collected 48h after drug treatment. After fixation with ethanol overnight, RNase and propidium iodide were added, mixed, and stained in the dark at 37 C for 30 minutes. Finally, the DNA content in cells was detected by flow cytometer (FACSCalibur flow cytometer). The number of cells in each group was 1×10^4 cells. The experimental results were analyzed with ModFit Lt Mac V3.0 software.

III, Experimental Results

1, TSL-1502, TSL-1502M inhibit the proliferation of various in vitro cultured tumor cells

TSL-1502, AZD2281, and ABT-888 were tested for cell proliferation inhibition after 240 hours of exposure (replacement of fresh drug every 120 hours). The results are shown in Table 2 and Figure 1.

In BRCA-deficient or mutated cells (V-C8, MDA-MB-436, Capan-1 and UWB1.289), the proliferation inhibition effect of TSL-1502M was significant, and the IC_{50} were, respectively, 0.2±0.0nM, 0.9±0.2nM, 100.6±73.8 nM, 1.9±0.4nM). The inhibition effect was significantly stronger than its prodrug TSL-1502 (IC_{50} were, respectively, 132.1±4.7nM, 79.6±12.5nM, ≈10000 nM, 935.7±526.6nM), and the reference drugs AZD2281 (IC_{50} were, respectively, 18.6±1.3 nM, 4.0±0.7nM, 586.1±89.4 nM, ≈1nM (The inhibition rate was slightly weaker than TSL-1502M)) and ABT-888 (IC_{50} were, respectively, 314.2±24.4 nM, 114.7±60.8nM, NA, 2102.5±1470.1nM) ;

However, in BRCA1 mutated HCC1937 cells, the proliferation inhibitions of TSL-1502M, TSL-1502, AZD2281 and ABT-888 were all weak.

In BRCA-normal cells (V-C8#13-5 and UWB1.289 BRCA1), the proliferation

inhibition effects of TSL-1502M, TSL-1502, AZD2281 and ABT-888 were all weak. However, in MDA-MB-468 cells with normal BRCA function, TSL-1502M and AZD2281 showed proliferation inhibition effect to some extent (IC_{50} were, respectively, 73.6±19.5 nM and 541.1±93.8 nM) .

In summary, TSL-1502M is more sensitive to the cells with BRCA1/2 function defects, and shows selectivity on the proliferation inhibition effect in the cells. The function features on selectivity are similar to AZD2281 and ABT-888, and the inhibition effect is stronger than TSL-1502, AZD2281 and ABT-888.

Table 2: The effect on the proliferation of in vitro cultrated cell (n=2)

Cell Line	IC50 (nM, Mean ± SD)			
	TSL-1502	TSL-1502M	AZD2281	ABT-888
V-C8	132.1±4.7	0.2±0.0	18.6±1.3	314.2±24.4
V-C8#13-5	>10000	>10000	≈10000	>10000
Capan-1	≈10000	100.6±73.8	586.1±89.4	NA
HCC1937	>10000	>10000	≈10000	>10000
MDA-MB-468	>10000	73.6±19.5	541.1±93.8	NA
UWB1.289	935.7±526.6	1.9±0.4	≈1	2102.5±1470.1
UWB1.289 BRCA1	>10000	2110.0±865.5	2917.5±79.9	>10000
MAD-MB-436	79.6±12.5	0.9±0.2	4.0±0.7	114.7±60.8

2, TSL-1502, TSL-1502M enhances cytotoxic drug anti-tumor activity

The anti-tumor effect of the combination of TSL-1502 and TSL-1502M and cytotoxic drug were detected. The results are shown in Tables 3-1, 3-2 and Figure 2.

In BRCA1 mutated MDA-MB-436 cells, 30, 100, 300 nM TSL-1502, 3, 10, 30 nM TSL-1502M, 10, 30, 100 nM AZD2281 or 30, 100, 300 nM ABT-888 were combined with 10, 30 μM TMZ for 120 hours.

TSL-1502, TSL-1502M and the reference compound AZD2281, ABT-888 can all enhance the proliferation inhibition effect of TMZ on MDA-MB-436 cell.

In BRCA-normal SW620 cells, 3, 10μM TSL-1502M or 3, 10μM AZD2281 and 0.1, 1nM irinotecan active metabolite SN38 were combined for 144 hours. TSL-1502M and the reference compound AZD2281 can all enhance the proliferation inhibition effect of SN38 on SW620 cell.

The above results indicate that, in BRCA genotype mutated or normal tumor cells, TSL-1502M shows enhancing effect on cytotoxic drugs.

2.1 The proliferation inhibition effect of TSL-1502 and TSL-1502M on MDA-MB-436 cells: see Table 3-1

Table 3-1: The proliferation inhibition effect on MDA-MB-436 cells. Inhibition Rate (%, Mean±SD)

		AZD2281			
		0 nM	10 nM	30 nM	100 nM
TMZ	0 μM	0	25.0±3.6	36.4±4.1	47.0±5.2
	10μM	2.7±2.5	42.0±0.4	56.8±1.6	61.5±2.2
	30μM	9.1±4.8	59.8±2.9	69.6±3.5	71.7±1.4
		ABT888			
		0 nM	30 nM	100 nM	300 nM
TMZ	0 μM	0	4.9±0.8	18.9±6.6	23±3.2
	10μM	0.9±2.2	18.1±1.4	30.1±0.7	40±0.5
	30μM	6.2±3.5	38.2±1.5	50.5±0.3	60±2.6
		TSL-1502			
		0 nM	30 nM	100 nM	300 nM
TMZ	0 μM	0	1.3±2.1	15.6±3.3	30.2±1
	10μM	3.8±0.8	7±4.2	24.6±2.7	48.1±2.8
	30μM	11.2±2.4	22.7±2.1	42.3±3	63.4±3
		TSL-1502M			
		0 nM	3 nM	10 nM	30 nM
TMZ	0 μM	0	28.6±0.1	61.9±1.3	67.5±1.5
	10μM	-0.9±0.8	45.2±1.3	69.3±0.6	74.1±1.7
	30μM	8.5±3.5	63.8±3.3	75.6±1.1	80.2±3.5

2.2: The proliferation inhibition effect of TSL-1502 and TSL-1502M on SW620 cells. See Table 3-2

Table 3-2: The proliferation inhibition effect on SW620 cells. Inhibition Rate (%, Mean±SD)

		AZD2281		
		0μM	3μM	10μM
SN38	0 nM	0	15.4±23.0	70.2±18.2
	0.1nM	1.0±11.2	32.2±27.9	94.5±5.0
	1nM	73.5±8.0	92.0±9.3	97.1±1.4
		TSL-1502M		
		0μM	3μM	10μM
SN38	0 nM	0	18.8±13.4	37.2±17.9
	0.1nM	0.0±4	37.7±16.6	56.3±12.7
	1nM	62.3±18.1	98.0±0.7	98.3±1.3

3, TSL-1502 inhibits the clonal formation of tumor cells with BRCA function defects

TSL-1502 prodrug needs long time to generate the anti-tumor effect. Therefore, when detecting the inhibition effect of TSL-1502 on on clonal formationof MDA-MB-436 cells (BRCA1 genotype mutated), the effect duration was prolonged to 20 days. The results are shown in Figure 3. TSL-1502 and the reference compound can all inhibit the clonal formation of MDA-MB-436 cells in a dose-dependent manner. The IC_{50} of TSL-1502 on inhibiting the clonal formation was 9.5±1.4nM, and the IC_{50}s of AZD2281 and ABT-888 on inhibiting the clonal formation were, respectively, 1.7±0.1 and 9.5±1.4nM. In summary, TSL-1502 and the reference drugs AZD2281 and ABT-888 can all inhibit the clonal formation of tumor cells with BRCA function defects, while the inhibition effect of TSL-1502 is stronger than ABT-888 and is weaker than AZD2281.

4, TSL-1502M selectively induces the formation of Rad51 accumulation sites.

PARP inhibition can induce DNA damage, which in turn increases the spontaneous formation of Rad51 accumulation site in functionally normal cells undergoing homologous recombination. In BRCA1/2-deficient cells, however, homologous recombination repair is dysfunctional, and the formation of Rad51 accumulation site is not induced. We detected the effect of TSL-1502M and AZD2281 on the formation of Rad51 accumulation sites using immunofluorescence, as shown in Figure 4.

After 24 hours treatment of 10 μM TSL-1502M or 30 μM AZD2281, in cells with BRCA1/2 normal function (V-C8 #13-5), Rad51 accumulation site was significantly induced, while in cells with BRCA1/2 function defects (V-C8), Rad51 accumulation site could not be induced.

It is indicated that, TSL-1502M can selectively inducse the formation of Rad51 accumulation sites in the cells with BRCA1/2 normal function, which features are similar to AZD2281.

5, The combination of TSL-1502M and TMZ significantly enhances the phosphorylation of MDA-MB-436 cell γ-H2AX

Phosphorylated histone H2AX(γ-H2AX) is a marker of DNA damage. We detected the effect of TSL-1502M combined with TMZ on DNA damage repair in BRCA1 mutant MDA-MB-436 cells. The effects of TMZ combined with TSL-1502M and the

reference control compound AZD2281 on γ-H2AX are presented in Figure 5 and indicates the follows.

After the treatment of the combination of TSL-1502M or reference compound AZD2281 and TMZ, the phosphorylation of γ-H2AX was significantly enhanced.

The above results indicates that, TSL-1502M and AZD2281 can inhibit DNA damage repair, and the activity of TSL-1502M is stronger than AZD2281.

6, TSL-1502inducing cell arrest in G2/M phase

By treating with TSL-1502, TSL-1502M and the reference compound AZD2281 on MDA-MB-436 cells for 48 hours, the effect on cell cycle was detected by flow cytometry. The results are shown in Figure 6.

TSL-1502, TSL-1502M and the reference compound AZD2281 can all induce the increase of G_2/M phase cells, that is, induce the cell cycle G_2/M phase arrest in a dose-dependent manner. .

The effect of TSL-1502M inducing G_2/M phase cycle arrest is similar to AZD228. At a dosage of 10μM, they all significantly induce G_2/M phase arrest. The effect of TSL-1502 inducing G_2/M phase cycle arrest is weaker than TSL-1502M and the reference compound AZD2281. At a dosage of 10μM, the effect of inducing G_2/M phase arrest is weak.

The above results indicate that, TSL-1502 and TSL-1502M induce MDA-MB-436 cell cycle arrest in G_2/M phase, and the activity of TSL-1502M is significantly stronger than TSL-1502, and is similar to the reference compound AZD2281.

7, TSL-1502, TSL-1502M induces cell apoptosis

By treating MDA-MB-436 cells with TSL-1502, TSL-1502M and the reference drugs AZD2281 for 120 hours, Western Blot was used to detect the cleavage changes of PARP, an apoptotic marker. The results are shown in Figure 7.

TSL-1502 had a weak induced PARP cleavage at 1μM, more pronounced at 10μM;

TSL-1502M could induce PARP cleavage obviously at 0.01μM;

The reference drug AZD2281 showed a weak induced PARP cleavage at 0.1μM, more pronounced at 1μM and 10 μ M.

The results indicate that TSL-1502 and TSL-1502M, like reference drug AZD2281, could induce apoptosis of MDA-MB-436 cells, and the activity of TSL-1502 was weaker than that of AZD2281 and that of TSL-1502M was stronger than that of AZD2281.

Experiment Conclusion:

The invention studies the in vitro antitumor activity and action mechanism of TSL-1502 and a metabolite TSL-1502M, mainly including:

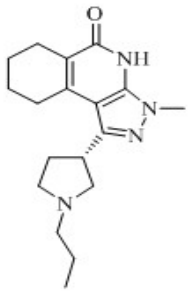
1) TSL-1502M has significant inhibitory effect on the proliferation of BRCA -deficient or mutant cells, such as V-C8, MDA-MB-436 and UWB1.289 cells with IC_{50} values of 0.2±0.0nM, 0.9±0.2nM and 1.9±0.4nM, respectively. This inhibition was significantly stronger than that of its prodrugs, TSL-1502 (IC_{50} of 132.1±4.7nM, 79.6±12.5nM, and 935.7±526.6nM, respectively), and the reference drugs, AZD2281 (IC_{50} of 18.6±1.3 nM, 4.0±0.7nM, and ≈1nM, slightly weaker than TSL-1502M), and ABT-888 (IC_{50} of 314.2±24.4 nM, 114.7±60.8nM, and 2102.5±1470.1nM, respectively)

In BRCA-normal cells, such as V-C8#13-5 and UWB1.289 BRCA1 cells, the proliferation inhibitions of TSL-1502M, TSL-1502, AZD2281 and ABT-888 were all weak.

2) TSL-1502 can inhibit the clone formation of MDA-MB-436 cells. TSL-1502M significantly enhanced the proliferation inhibition effect of temozolomide on MDA-MB-436 cells (BRCA1 mutated) and irinotecan active metabolite SN38 on SW620 cells (BRCA1/2 normal), and selectively induced the formation of a Rad51 accumulation sites in BRCA1/2 functionally normal cells. The combination with temozolomide induced a significant increase in the phosphorylation of γ-H2AX in MDA-MB-436 cells. It can induce G2/M phase arrest of MDA-MB-436 cells and induce apoptosis. The action features of TSL-1502M is similar to that of the reference compound AZD2281, and the activity of TSL-1502M is stronger than that of AZD2281.

What is claimed:

1. The use of the compound of Formula II and SN38 in the manufacture of a medicament for preventing and treating colon tumor.



Formula II.

2. The use according to claim 1, wherein the drug is in any ingestible pharmaceutical form.

3. The use according to claim 2, wherein the drug is selected from a group consisting of tablet, capsule, oral liquid, buccal tablet, granule, pill, pulvis, ointment, pellet, powder, injectable preparation, suppository, ointment, spray, drop, patch, dripping pill.

4. The use according to claim 3, wherein the injectable preparation is selected from a group consisting ofliquid, semi-solid, solid, powder form .

5. The use according to claim 4, wherein the injectable preparationis one of injectable solution and injectable powder.