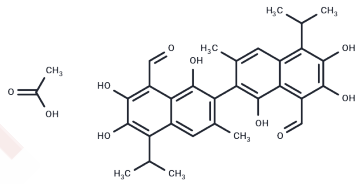
Data Sheet (Cat.No.T6396)

TargetMol

(R)-(-)-Gossypol acetic acid

Chemical Properties



CAS No. : 866541-93-7

Formula: C30H30O8·C2H4O2

Molecular Weight: 578.61

Storage: Powder: -20°C for 3 years
Actual storage temperature shall be subject to the COA.

Biological Description

Description	(R)-(-)-Gossypol acetic acid (AT101 acetate), the R-(-) enantiomer of Gossypol acetic acid, binds with Bcl-2, Bcl-xL, and Mcl-1 with Ki values of 0.32 μM, 0.48 μM, and 0.18 μM, respectively; it does not inhibit the BIR3 domain or BID. [Phase 2]
Targets(IC50)	Bcl-2 Family,Autophagy
In vitro	AT-101 inhibits a panel of different lymphoproliferative malignancies with IC50 ranged from 1.2 μM to 7.4 μM. AT-101 (10 μM) disrupts the Δψm in a concentration- and time-dependent manner in a diffuse large B-cell and in mantle cell lymphoma lines. AT-101 (1 μM or 2 μM) combined with carfilzomib (6 nM or 10 nM) induces apoptosis in HBL-2 and Granta cell lines. [2] AT-101 (20 μM for 24 hours) results in a median 72% apoptosis and down-regulation of Mcl-1 in CLL lymphocytes in both suspension culture as well as stromal coculture. Stromal cells express undetectable levels of antiapoptotic but high levels of activated ERK and AKT proteins and has low or no apoptosis with AT-101. [3] AT-101 induces apoptosis in a time- and dose-dependent fashion, with ED50 values of 1.9 mM and 2.4 mM in Jurkat T and U937 cells, respectively. AT-101 (10 μM) combined with radiation (32 Gy) induces more apoptosis than radiation alone and exceeds the sum of the effects caused by the single agent treatments. AT-101 activates SAPK/JNK in a dose- and time-dependent manner. [4] AT-101 (10 μM) induces apoptosis through activation of caspase-9, -3, and -7 in VCaP Cells. AT-101 (10 μM) decreases Bcl-2 and Mcl-1 expression in VCaP cells. [5] AT-101 (< 20 μM) is able to inhibit the growth of multiple myeloma cells despite the stimulatory growth effects provided by stromal cells in the bone marrow milieu. AT-101 (10 μM) induces apoptosis in multiple myeloma cells via the activation of caspases 3, caspases 9 and PARP. AT-101 (10 μM) promotes apoptosis in multiple myeloma cells by disrupting the Bax/Bcl-2 ratio and the mitochondrial membrane potential. [6]
In vivo	AT-101 is still detectable in plasma with average concentrations of 0.49 μM for the 35 mg/kg group and 0.39 μM for the 200 mg/kg group in SCID beige mice bearing RL-DLBCL xenograft. AT-101 peak plasma concentration is observed after 30 minutes of administration of the drug in both the dose levels, with the 200 mg/kg group showing a plasma average concentration almost 4 times greater than the 35 mg/kg group (7.88 μM and 27.78 μM respectively) in SCID beige mice. AT-101 (25 mg/kg to 100 mg/kg, orally) indefinitely results in earlier onset of weight loss equivalent to more than 10% of the pretreatment weight and death in SCID beige mice. AT-101 (35 mg/kg, orally per day for 10 days) plus intraperitoneal cyclophosphamide (Cy) and intraperitoneal rituximab (R)

In vivo	show significantitumor volume control compared to any other treatment group. [2] AT-101 (15 mg/kg, p.o., 5 days/week) as a single agent in intact mice significantly reduces the development of VCaP tumor growth compared to untreated tumors at weeks 2 to 6. AT-101 in combination with surgical castration delays the onset of androgen-independent VCaP tumor growth compared to castration-only or AT-101-only groups in mice. [5]
Kinase Assay	Fluorescence-Polarization-Based Binding Assay: For competitive binding experiments, Bcl-2 protein (40 nM) and FAM-Bid peptide (2.5 nM) are preincubated in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 μg/mL bovine gamma globulin; 0.02% sodium azide, 5 μL of a solution in DMSO of AT101 is added to the Bcl-2/FAM-Bid solution in Dynex 96-well, black, round-bottom plates to produce a final volume of 125 μL. For each experiment, a control containing Bcl-2 and Flu-Bid peptide (equivalent to 0% inhibition), and another control containing only FAM-Bid, are included on eachassay plate. After 4 hours incubation, the polarization values in milipolarization units (mP) weremeasured at an excitation wavelength at 485 nm and an emission wavelength at 530 nm using the Ultra plate reader. IC50,the inhibitor concentration at which 50% of bound peptide is displaced, is determined from the plot using nonlinear leastsquares analysis and curve fitting performed using GraphPad Prizm 4 software. The unlabeled Bid BH3 peptide is used as the positive control. PF for Bcl-xL protein, Bak BH3 peptide labeled with 6-carboxyfluorescein succinimidyl ester (FAM-Bak) instead of the FAM-Bim to maximize the signal. It is determined that FAM-Bak has a Kd of 6 nM to Bcl-xL protein. The competitive binding assay for Bcl-xL is same as that for Bcl-2 with the following exceptions. 30 nM of Bcl-xL protein and 2.5 nM of FAM-Bak peptide in the following assay buffer: 50 mM Tris-Bis, pH 7.4 and 0.01% bovine gamma globulin. PF for Mcl-1 protein, FAM-Bid peptide and human Mcl-1 protein are used. It is determined that FAM-Bid peptide binds to human Mcl-1 protein with a Kd of 1.71 nM. The competitive binding assays for Mcl-1 are performed in the same manner as that for Bcl-2 with the following exceptions. 5 nM Mcl-1 and 1 nM Flu-Bid peptide in the following assay buffer: 25 mM Tris, pH 8.0; 150 mM NaCl and 0.05% Pluronic acid
Cell Research	Cells are counted and resuspended at an approximate concentration of 3×105 cells/well in a 24-well plate. AT-101 is diluted in DMSO that is maintained at a final concentration of less than 0.5%. Concentrations of AT-101 from 1 nM to 10 μM are used in most experiments. Following incubation at 37 ℃ in a 5% CO2 humidified incubator, 100 μL from each well is transferred to a 96-well opaque-walled plate; cell-Titer-Glo Reagent is added in a 1:1 ratio. Contents are mixed for 2 minutes on an orbital shaker to induce cell lysis. The plates are allowed to incubate at room temperature for 10 minutes before recording luminescence with a Synergy HT Multi-Detection Microplate Reader. In the schedule dependency experiments, serial dilutions of each drug are prepared in ratios relative to their IC50. Cells are preincubated with AT-101 for up to 72 hours, while 4-HC is added for a 24-hour period, being added at time 0, 24 hours, and 48 hours from the start of incubation. Each experiment is performed in triplicate and repeated at least twice.(Only for Reference)

Solubility Information

Solubility	DMSO: 83.33 mg/mL (144.02 mM),Sonication is recommended. H2O: < 1 mg/mL (insoluble or slightly soluble), Ethanol: 82 mg/mL (141.72 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)

In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 3.3 mg/mL (5.7 mM),Sonication is recommended. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.7283 mL	8.6414 mL	17.2828 mL
5 mM	0.3457 mL	1.7283 mL	3.4566 mL
10 mM	0.1728 mL	0.8641 mL	1.7283 mL
50 mM	0.0346 mL	0.1728 mL	0.3457 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Wang G, et al. J Med Chem. 2006, 49(21), 6139-6142.
Paoluzzi L, et al. Blood, 2008, 111(11), 5350-5358.
Balakrishnan K, et al. Blood, 2009, 113(1), 149-153.
Zerp SF, et al. Radiat Oncol, 2009, 23(4), 47.
Loberg RD, et al. Neoplasia, 2007, 9(12), 1030-1037.