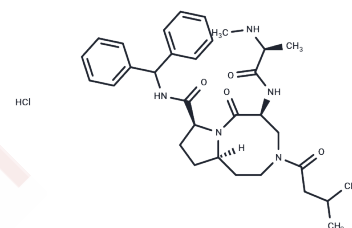


Xevinapant hydrochloride

Chemical Properties

CAS No. :	1071992-57-8
Formula:	C ₃₂ H ₄₄ ClN ₅ O ₄
Molecular Weight:	598.19
Storage:	Powder: -20°C for 3 years In solvent: -80°C for 1 year Actual storage temperature shall be subject to the COA.



Biological Description

Description	Xevinapant hydrochloride (AT-406 HCl) , an orally active antagonist of multiple inhibitor of apoptosis proteins(IAP), inhibits progression of human ovarian Y binding to XIAP-BIR3, cIAP1-BIR3 and cIAP2-BIR3 with Ki of 66.4 nM, 1.9 nM, and 5.1 nM, 50- to 100-fold higher affinities than the Smac AVPI peptide.
Targets(IC50)	Apoptosis,IAP
In vitro	AT-406 is a Smac mimetic and appears to mimic closely the AVPI peptide in both hydrogen bonding and hydrophobic interactions with XIAP, with additional hydrophobic contacts with W323 of XIAP. AT-406 is more sensitive to these IAPs than Smac AVPI peptide with 50-100 fold binding affinities. AT-406 (at 1 μM) completely restores the activity of caspase-9, which is suppressed by 500 nM XIAP BIR3 in a cell-free system. In MDA-MB-231 cell, AT-406 induces rapid cellular cIAP1 degradation and also pulls down the cellular XIAP protein. AT-406 effectively inhibits lots of human cancer cell lines and shows IC50 of 144 and 142 nM in MDA-MB-231 cell and SK-OV-3 ovarian cell, with low toxicity against normal-like human breast epithelial MCF-12F cells and primary human normal prostate epithelial cells. AT-406 induces apoptosis in MDA-MB-231 cell by inducing activation of caspase-3 and cleavage of PARP. [1]
Kinase Assay	Fluorescence Polarization Based Assays for XIAP, cIAP1, and cIAP2 BIR3 Proteins: FL-AT-406 (the fluorescently tagged AT-406) is employed to develop a set of new FP assays for determination of the binding affinities of Smac mimetics to XIAP, cIAP-1, and cIAP-2 BIR3 proteins. The Kd value of FL-AT-406 to each IAP protein is determined by titration experiments using a fixed concentration of FL-AT-406 and different concentrations of the protein up to full saturation. Fluorescence polarization values are measured using an Infinite M-1000 plate reader in Microfluor 2 96-well, black, round-bottom plates. To each well, FL-AT-406 (2, 1, and 1 nM for experiments with XIAP BIR3, cIAP-1 BIR3, and cIAP-2 BIR3, respectively) and different concentrations of the protein are added to a final volume of 125 μL in the assay buffer (100 mM potassium phosphate, pH 7.5, 100 μg/mL bovine γ-globulin, 0.02% sodium azide, with 4% DMSO). Plates are mixed and incubated at room temperature for 2-3 hours with gentle shaking. The polarization values in millipolarization units (mP) are measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Equilibrium dissociation constants (Kd) are then calculated by fitting the sigmoidal dose-dependent FP increases as a function of protein concentrations using Graphpad Prism 5.0 software. In competitive binding experiments for XIAP3 BIR3, AT-406 is incubated with 20 nM XIAP BIR3 protein and 2 nM

Kinase Assay	FL-AT-406 in the assay buffer (100 mM potassium phosphate, pH 7.5; 100 µg/mL bovine γ-globulin; 0.02% sodium azide). In competitive binding experiments for cIAP1 BIR3 protein, 3 nM protein and 1 nM FL-AT-406 are used. In competitive binding experiments for cIAP2 BIR3, 5 nM protein and 1 nM FL-AT-406 are used. For each competitive binding experiment, polarization values are measured after 2-3 hours of incubation using an Infinite M-1000 plate reader. The IC50 value, the inhibitor concentration at which 50% of the bound tracer is displaced, is determined from the plot using nonlinear least-squares analysis. Curve fitting is performed using the PRISM software. A Ki value for AT-406 is calculated.
Cell Research	Cells are seeded in 96-well flat bottom cell culture plates at a density of (3-4) × 10 ³ cells/well with AT-406 and incubated for 4 days. The rate of cell growth inhibition after treatment with different concentrations of AT-406 is determined by assaying with (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium monosodium salt (WST-8). WST-8 is added to each well to a final concentration of 10%, and then the plates are incubated at 37 °C for 2-3 hours. The absorbance of the samples is measured at 450 nm using a TECAN ULTRA reader. Concentration of AT-406 that inhibited cell growth by 50% (IC50) is calculated by comparing absorbance in the untreated cells and the cells treated with AT-406. (Only for Reference)

Solubility Information

Solubility	DMSO: 100 mg/mL (167.17 mM), Sonication is recommended. Ethanol: 100 mg/mL (167.17 mM), Sonication is recommended. H2O: <1 mg/mL, (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 8 mg/mL (13.37 mM), Sonication is recommended. <i>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.</i>

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.6717 mL	8.3585 mL	16.7171 mL
5 mM	0.3343 mL	1.6717 mL	3.3434 mL
10 mM	0.1672 mL	0.8359 mL	1.6717 mL
50 mM	0.0334 mL	0.1672 mL	0.3343 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

Cai Q, et al. J Med Chem, 2011, 54(8), 2714-2726.

Inhibitor · Natural Compounds · Compound Libraries · Recombinant Proteins

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