Product Datasheet

Technical Data

Formula	$C_{52}H_{79}N_5O_{12}$					
Molecular Weight	966.21	**CAS No.**	221877-54-9			
Solubility (25°C)*	In vitro	DMSO	100 mg/mL (103.49 mM)			
		Ethanol	100 mg/mL (103.49 mM)			
		Water	Insoluble			
	In vivo (Add solvents to the product individually and in order)	Oral Administration	Homogeneous suspension	CMC-NA	≥5mg/ml	Taking the 1 mL working solution as an example, add 5 mg of this product to 1 ml of CMC-Na solution, mix evenly to obtain a homogeneous suspension with a final concentration of 5 mg/ml.

* <1 mg/ml means slightly soluble or insoluble.
* Please note that Capsid Bio tests the solubility of all compounds in-house, and the actual solubility may differ slightly from published values. This is normal and is due to slight batch-to-batch variations.
* Room temperature shipping (Stability testing shows this product can be shipped at 2-8°C for storage purposes.)

Preparing Stock Solutions

Concentration \ Volume / Mass	1 mg	5 mg	10 mg
1 mM	1.0350 mL	5.1749 mL	10.3497 mL
5 mM	0.2070 mL	1.0350 mL	2.0699 mL
10 mM	0.1035 mL	0.5175 mL	1.0350 mL
50 mM	0.0207 mL	0.1035 mL	0.2070 mL

Biological Activity

Description	Zotarolimus (ABT-578, A 179578), an analogue of rapamycin, inhibits **FKBP-12** binding with an **IC50** of 2.8 nM.
Targets	FKBP-12 [1] 2.8 nM
In vitro	Zotarolimus (ABT-578) is a semi-synthetic analogue of rapamycin, made by substituting a tetrazole ring for the native hydroxyl group at position 42 in rapamycin. This compound is highly effective in inhibiting both smooth muscle cell and endothelial cell proliferation, with IC50 values of 2.9 nM and 2.6 nM, respectively. [1] It is mechanistically similar to sirolimus in having high-affinity binding to the immunophilin FKBP12 and comparable potency for inhibiting in vitro proliferation of both human and rat T cells. Zotarolimus inhibits Con A-induced human T cells and rat T cells proliferation with IC50 of 7.0 nM and 1337 nM respectively. [2]
In vivo	Zotarolimus (ABT-578) effectively reduces neointima formation in a 28-day, well-characterized swine model of coronary artery restenosis. It appears effective in preventing neointimal thickening, reducing late loss from 1.03 to 0.62 mm with a 47% reduction in TVF compared with bare metal stents (15.4% with the Driver stent to 8.1% with the Endeavor stent). [1] This compound is efficacious in suppressing adjuvant DTH, EAE, and cardiac allograft rejection with ED50 values of 1.72, 1.17, and 3.71 mg/kg/day, respectively. [2]
Features	Zotarolimus has a shorter in vivo half-life and is also demonstrated in rats to have less potent systemic immunosuppression than rapamycin.

Protocol (from reference)

Kinase Assay:[1]

- **Binding Affinity to FKBP12**

  96-well microtiter plates are first coated with FKBP-12 CMP-KDO synthetase fusion protein at 10 μg/mL, 100 μL/well for 2-3 h, followed by addition of 50 μL/well of buffer A (2% BSA and 0.2% Tween-20 in D-PBS) for 30-60 min. Microtiter plates are then washed three times with buffer B (0.2% Tween in D-PBS, pH adjusted to 7.4). Fifty microlitres of buffer A (for maximum), 20 μM FK506 in buffer A (for background), or various concentrations of zotarolimus (ABT-578) (10 pM-1 μM) in buffer A are added to each well followed by addition of 50 μL of A-79397 (an FK506 analogue)-alkaline phosphatase conjugate in buffer A. Microtiter plates are incubated at room temperature for 2-2.5 h followed by three washes with buffer B. About 100 μL of pNPP (p-nitrophenyl-phosphate) in 0.1 M aminomethylpropanol are added to each well and plates are incubated at room temperature for 90-120 min. Absorbance at 405 nM is read using an ELISA plate

Cell Assay:[1]

- **Cell lines**

  Human coronary artery cells
- **Concentrations**

  ~1 μM
- **Incubation Time**

  5 days
- **Method**

  Cell proliferation is assayed by measuring tritiated thymidine incorporation in vitro. Human coronary artery cells (hCa) are seeded into tissue culture flasks for expansion and applied to 96-well plates at desired density in complete media (5000 hCaSMC; 10 000 hCaEC). After 2 days, complete media is replaced with incomplete media to synchronize cells and induce G0 state. Two days later, incomplete media are removed and replaced with complete media (serum/growth factors) to induce G0 to G1 transition. Complete media also contain Zotarolimus (ABT-578) at desired concentrations to determine its effects on cell proliferation. On day 7, ^{3}H-thymidine is added to cells to monitor DNA synthesis, and cells are harvested after overnight incorporation of radioactivity. After an incubation period of 72 h, 25 μL (1 μCi/well) of ^{3}H-thymidine are added to each well. The cells are incubated at 37°C for 16-18 h to allow for incorporation of ^{3}H-thymidine into newly synthesized DNA and the cells harvested onto 96-well plates containing bonded glass

fibre filters . The filter plates are air-dried overnight, MicroScint-20 (25 μL) added to each filter well and counted. Drug activity is determined by the inhibition of ^{3}H-thymidine incorporation into newly synthesized DNA relative to cells grown in complete media.

Animal Study:[2]

- **Animal Models**
  Male Sprague-Dawley rats
- **Dosages**
  2.5 mg/kg
- **Administration**
  intravenous or oral

References

https://pubmed.ncbi.nlm.nih.gov/16449248/

https://pubmed.ncbi.nlm.nih.gov/17438408/

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