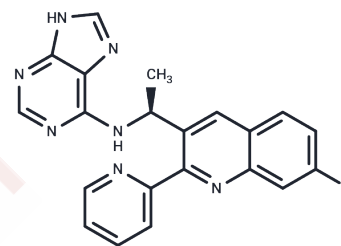


AMG319

Chemical Properties

CAS No. :	1608125-21-8
Formula:	C ₂₁ H ₁₆ N ₇
Molecular Weight:	385.4
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	AMG319 is a potent and selective PI3K δ inhibitor with an IC ₅₀ of 18 nM and exhibits >47-fold selectivity over other PI3Ks. [Phase 2]
Targets(IC ₅₀)	PI3K
In vitro	AMG319 inhibits anti-IgM/CD40L-induced B cell proliferation with IC ₅₀ of 8.6 nM and reduces pAkt level with IC ₅₀ of 1.5 nM. AMG319 also inhibits anti-IgD-induced CD-69 expression in HWB. [1]
In vivo	In female Lewis rats, AMG319 (3 mg/kg, p.o.) inhibits the KLH-induced inflammatory response by 88%. In the transgenic (IgMm) mice, AMG319 (, p.o.) inhibits in vivo pAKT with IC ₅₀ of 1.9 nM. [1]
Kinase Assay	PI3K Enzyme Assays: A PI3K Alphascreen assay is used to measure the activity of a panel of four phosphoinositide 3-kinases: PI3K α , PI3K β , PI3K γ , and PI3K δ . Enzyme reaction buffer is prepared using sterile water and 50 mM Tris-HCl, pH 7, 14 mM MgCl ₂ , 2 mM sodium cholate, and 100 mM NaCl. 2 mM DTT is added fresh on the day of the experiment. The Alphascreen buffer is made using sterile water and 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.10% Tween 20, and 30 mM EDTA. Then 1 mM DTT is added fresh on the day of the experiment. Compound source plates used for this assay are 384-well Greiner clear polypropylene plates containing test compounds at 5 mM and diluted 1:2 over 22 concentrations. Columns 23 and 24 contained only DMSO, as these wells comprised the positive and negative controls, respectively. Source plates are replicated by transferring 0.5 μ L per well into 384-well Optiplates. Each PI3K isoform is diluted in enzyme reaction buffer to 2 \times working stocks. PI3K α is diluted to 1.6 nM, PI3K β is diluted to 0.8 nM, PI3K γ is diluted to 15 nM, and PI3K δ is diluted to 1.6 nM. PI(4,5)P ₂ is diluted to 10 μ M, and ATP was diluted to 20 μ M. This 2 \times stock is used in the assays for PI3K α and PI3K β . For assay of PI3K γ and PI3K δ , PI(4,5)P ₂ is diluted to 10 μ M and ATP was diluted to 8 μ M to prepare a similar 2 \times working stock. Alphascreen reaction solutions are made using beads from the anti-GST Alphascreen kit. Two 4 \times working stocks of the Alphascreen reagents are made in Alphascreen reaction buffer. In one stock, biotinylated-IP ₄ is diluted to 40 nM and streptavidin-donor beads are diluted to 80 μ g/mL. In the second stock, PIP ₃ -binding protein is diluted to 40 nM and anti-GST-acceptor beads were diluted to 80 μ g/mL. As a negative control, a reference inhibitor at a concentration γ Ki (40 μ M) is included in column 24 as a negative (100% inhibition) control. Using a 384-well Multidrop, 10 μ L/well of 2 \times enzyme stock is added to columns

Kinase Assay	1-24 of the assay plates for each isoform. An amount of 10 μ L/well of the appropriate substrate 2 \times stock (containing 20 μ M ATP for the PI3K α and - β assays and containing 8 μ M ATP for the PI3K γ and - δ assays) is then added to columns 1-24 of all plates. Plates are then incubated at room temperature for 20 min. In the dark, 10 μ L/well of the donor bead solution is added to columns 1-24 of the plates to quench the enzyme reaction. The plates are incubated at room temperature for 30 min. Still in the dark, 10 μ L/well of the acceptor bead solution is added to columns 1-24 of the plates. The plates are then incubated in the dark for 1.5 h. The plates are read on an Envision multimode plate reader using a 680 nm excitation filter and a 520-620 nm emission filter.
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Solubility Information

Solubility	DMSO: 71 mg/mL (184.22 mM), Sonication is recommended. Ethanol: 71 mg/mL (184.22 mM), Sonication is recommended. H ₂ O: < 1 mg/mL (insoluble or slightly soluble), (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (5.19 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	2.5947 mL	12.9735 mL	25.9471 mL
5 mM	0.5189 mL	2.5947 mL	5.1894 mL
10 mM	0.2595 mL	1.2974 mL	2.5947 mL
50 mM	0.0519 mL	0.2595 mL	0.5189 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

Cushing TD, et al. J Med Chem. 2015, 58(1), 480-511.

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