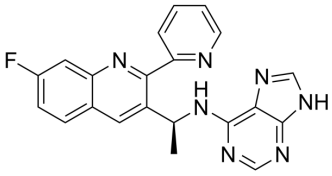
AMG319

Cat. No.:	HY-12948
CAS No.:	1608125-21-8
Molecular Formula:	$C_{21}H_{16}FN_7$
Molecular Weight:	385.4
Target:	PI3K
Pathway:	PI3K/Akt/mTOR

Storage:

Powder	-20°C	3 years
	4°C	2 years
In solvent	-80°C	2 years
	-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (129.74 mM; Need ultrasonic)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.49 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.49 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.49 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

AMG319 is a potent and selective PI3Kδ kinase inhibitor with IC_{50} of 18 nM.

IC_{50} & Target

PI3Kδ	PI3Kγ	PI3Kβ	PI3Kα
18 nM (IC_{50})	850 nM (IC_{50})	2.7 μM (IC_{50})	33 μM (IC_{50})

In Vitro

AMG319 inhibits PI3Kδ, PI3Kγ, PI3Kβ and PI3Kα with IC_{50}s of 18 nM, 850 nM, 2.7 μM and 33 μM, respectively. AMG319, a compound with an IC_{50} of 16 nM in a human whole blood assay (HWB), excellent selectivity over a large panel of protein

kinases, and a high level of in vivo efficacy as measured by two rodent disease models of inflammation. AMG319 has minimal CYP3A4/2D6 inhibition and does not inhibit CYPs (1A2, 2C8, 2C9, 2C19, 2E1, all >20 μM). There is no time dependent inhibition (TDI) against CYPs 3A4, 2D6, 1A2, and 2C9 nor CYP induction (3A4, 2D6, 1A2, 2B6) as measured in hepatocytes. AMG319 is clean in a hERG binding assay (>25 μM), and an Ames micronucleus test proved negative. AMG319 has minimal effects in a BSEP assay up to >200 μM[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

The study is performed to determine the correlation between biochemical coverage (i.e., pAKT) with functional activity in vivo. AMG319 achieves this coverage at the 3 mg/kg level, which also coveres the human whole blood assay (HWB) (CD-69) IC_{90} at trough for a full 24 h period. The lower doses 0.1, 0.3, and 1 mg/kg cover trough concentrations between the HWB IC_{50} and IC_{90} and evince partial efficacy. Similarly, the plasma concentration of AMG319 covers the IC_{90} at the 1 mg/kg dose of the mouse anti-IgM pAKT in vitro assay[1].

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PROTOCOL

Kinase Assay [1]

A PI3K Alphascreen assay is used to measure the activity of a panel of four phosphoinositide 3-kinases: PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ. 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 μL per well into 384-well Optiplates. Each PI3K isoform is diluted in enzyme reaction buffer to 2× working stocks. PI3Kα is diluted to 1.6 nM, PI3Kβ is diluted to 0.8 nM, PI3Kγ is diluted to 15 nM, and PI3Kδ is diluted to 1.6 nM. PI(4,5)P2 is diluted to 10 μM, and ATP is diluted to 20 μM. This 2× stock is used in the assays for PI3Kα and PI3Kβ. For assay of PI3Kγ and PI3Kδ, PI(4,5)P2 is diluted to 10 μM and ATP is diluted to 8 μM to prepare a similar 2× working stock. Alphascreen reaction solutions are made using beads from the anti-GST Alphascreen kit. Two 4× working stocks of the Alphascreen reagents are made in Alphascreen reaction buffer. In one stock, biotinylated-IP_4 is diluted to 40 nM and streptavadin-donor beads are diluted to 80 μg/mL. In the second stock, PIP_3-binding protein is diluted to 40 nM and anti-GST-acceptor beads are diluted to 80 μ g/mL. The plates are incubated at room temperature for 30 min. Still in the dark, 10 μL/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[1].

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Cell Assay [1]

B cells are purified from human peripheral blood mononuclear cells (PBMCs) by negative selection. Approximately 3×10^4 purified B cells per well are seeded into a 96-well plate. Compounds (e.g., AMG319) are dissolved in DMSO at a concentration of 10 mM, and a 10-point, 3-fold serial dilution of the compound is carried out in DMSO. Then 0.5 μL of compound is added to each well in duplicates so that the final DMSO concentration is 0.25% and the highest compound concentration is 10 μM. After preincubating for 30 min, B cells are treated with 2 μg/mL of anti-human IgM antibody plus 300 ng/mL human CD40L or 5 ng/mL human IL-4 plus 200 ng/mL of CD40L as a counterscreen to evaluate the off-target effects. The plates are incubated at 37°C and 5% CO_2 for 72 h, then pulsed with 0.5 μCi per well ^3H thymidine for 18 h, and B cells are collected to count the incorporation of ^3H thymidine[1].

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Animal Administration [1]

Mice[1]

IgM membrane only homozygous transgenic mice (6- to 12-week-old female) are orally dosed with AMG319 or vehicle control (n=5 per group). At 15 min after treatment, mice are tail iv injected with 50 μg of Endotoxin-free FITC-labeled μ chain specific anti-IgM or PBS only control. Blood and spleen tissue are collected after 30 min of stimulation for drug concentration and B cell pAKT analysis via flow cytometry. Briefly, blood and dispersed splenocytes are fixed with BD Phosflow lyse/fix buffer, pelleted, and permeabilized with cold 90% MeOH. Cells are then stained with pAKT and Alexa-647 secondary and B220-Pacific Blue for FACS analysis. Stimulated B220+/anti-IgM FITC+ B cells are analyzed for pAKT levels

with B220+/FITC-B cells from anti-IgM untreated mice serving as a control. Mice are maintained and experiments are performed.

Rats[1]

Female Lewis rats (N=8/dose group) are dosed po with AMG319 or vehicle (2% HPMC, 1% Pluronic F68, 10% Captisol, pH 2.0) once a day for 10 days at various doses. Two hours after the first dosing, 200 μL of PBS containing 60 μg of KLH is administered to each rat intravenously. Ten days after the KLH priming, rats are euthanized and blood is taken by cardiac puncture for the measurement of KLH specific IgG and IgM by ELISA.

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REFERENCES

[1]. Cushing TD, et al. Discovery and in vivo evaluation of (S)-N-(1-(7-fluoro-2-(pyridin-2-yl)quinolin-3-yl)ethyl)-9H-purin-6-amine (AMG319) and related PI3Kδ inhibitors for inflammation and autoimmune disease. J Med Chem. 2015 Jan 8;58(1):480-511.

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