Product Data Sheet

Product Name: PF-03084014
Cat. No.: GC10998

Chemical Properties

Cas. No.	865773-15-5		
Chemical Name	(2S)-2-[[(2S)-6,8-difluoro-1,2,3,4-tetrahydronaphthalen-2-yl]amino]-N-[1-[1-(2,2-dimethylpropylamino)-2-methylpropan-2-yl]imidazol-4-yl]pentanamide		
SMILES	CCCC(C(=O)NC1=CN(C=N1)C(C)(C)CNCC(C)(C)C)NC2CCC3=CC(=CC(=C3C2)F)F		
Formula	$C_{27}H_{41}F_2N_5O$	M.Wt	489.64
Solubility	≥ 19.8mg/mL in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	PF-03084014		

Protocol

Kinase experiment [1]:

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com
Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

γ-secretase assay	A DNA fragment encoding amino acids 596 - 695 of the 695-aa isoform of APP (APP695) and the Flag sequence (DYKDDDDK) at the C terminus was generated by PCR amplification with suitably designed oligonucleotides and the APP695 cDNA. The Met that serves as the translation start site is residue 596 of APP695 (the P1 residue with respect to the β-secretase cleavage site). This DNA fragment was inserted into the prokaryotic expression vector pET2-21b. The recombinant protein, C100Flag, was overproduced in Escherichia coli [strain BL21(DE3)] and purified by Mono-Q column chromatography. C100Flag (1.7 μM) was incubated with cell membranes (0.5 mg/mL) in the presence of CHAPSO, CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate), or Triton X-100 (0, 0.125, 0.25, 0.5, or 1%) in buffer B (50 mM Pipes, pH 7.0/5 mM MgCl2/5 mM CaCl2/150 mM KCl) at 37°C. The reactions were stopped by adding RIPA (150 mM NaCl/1.0% NP-40/0.5% sodium deoxycholate/0.1% SDS/50 mM Tris HCl, pH 8.0) and boiling for 5 mins. The samples were centrifuged and the supernatant solutions were assayed for the Aβ peptides by ECL. The Aβ40- and Aβ42-related products from γ-secretase-mediated processing of C100Flag possess a Met at the N terminus and are thus defined as M-Aβ40 and M-Aβ42, respectively. Likewise, supernatant solution (0.125 mg/mL) from CHAPSO-extracted HeLa cell membranes (solubilized γ-secretase) was incubated with C100Flag (1.7 μM) in buffer B containing 0.25% CHAPSO and subsequently assayed for M-Aβ40 and M-Aβ42 by using ECL.

Cell experiment [2]:

Cell lines	Human T-ALL cell lines HPB-ALL

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Preparation method	This compound is soluble in DMSO. General tips for obtaining a higher concentration: Please warm the tube at 37℃ for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20℃ for several months.
Reaction Conditions	~ 1 μM; 7 days
Applications	In HPB-ALL cells, PF-03084014 inhibited cell growth through induction of cell cycle arrest and apoptosis.
Animal experiment [2]:	
Animal models	HPB-ALL xenograft mouse models
Dosage form	75 and 150 mg/kg; p.o.; b.i.d., for 14 days
Applications	In HPB-ALL models, PF-03084014 showed robust antitumor activity on 14-day twice daily dosing. PF-03084014 dose-dependently inhibited tumor growth, with a maximal tumor growth inhibition of ~ 92% at the dosage of 150 mg/kg.
Other notes	Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.

References:

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[1]. Li YM, Lai MT, Xu M, Huang Q, DiMuzio-Mower J, Sardana MK, Shi XP, Yin KC, Shafer JA, Gardell SJ. Presenilin 1 is linked with gamma-secretase activity in the detergent solubilized state. Proc Natl Acad Sci U S A. 2000 May 23;97(11):6138-43.

[2]. Wei P, Walls M, Qiu M, et al. Evaluation of selective γ-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. Molecular

cancer
therapeutics,
2010, 9(6):
1618-1628.

Background

PF-03084014 is a reversible and selective inhibitor of γ-secretase with IC50 value of 6.2 nM [1].

The Notch signaling pathway is triggered by the interaction of Notch ligand and Notch receptor located in the membrane of the adjacent cell. Then the NICD fragment is released through the cleavage caused by γ-secretase and subsequently regulates the transcription of the downstream genes. Since Notch signaling pathway appears to be important in the development of many cancers, the small-molecule inhibitors of γ-secretase are now developed as antitumor drugs in cancer treatment. PF-03084014 is one of these GSIs (γ-secretase inhibitors) that showed IC50 value of 6.2 nM in inhibiting the production of Aβ in HeLa cells. Meanwhile PF-03084014 has no significant inhibition effect on other receptors, proteases, ion channels and kinases, demonstrating its selectivity against γ-secretase [1, 2].

In CLL (chronic lymphocytic leukemia) cells from mutated CLL patients, the treatment of 10 μM PF-03084014 induced 29.63% apoptosis. PF-03084014 at concentration of 1 μM induced cell apoptosis with 7.84%. In Notch1-unmutated cells, PF-03084014 induced apoptosis with 22.04% and 4.44% at concentrations of 1 and 10 μM, respectively. PF-03084014 showed no significant apoptosis induction in normal T cells from Notch1-mutated CLL patients. In HPB-ALL cells harboring Notch1 mutations, PF-03084014 inhibited Notch receptor cleavage with IC50 value of 13.3 nM. It also down-regulated the expressions of Hes-1 and cMyc in the cells. It is found that PF-03084014 inhibits cell growth through induce cell cycle arrest at G0-G1 phase [1, 3].

In HPB-ALL tumor xenograft model, oral administration of PF-03084014 at dose of 50 mg/kg inhibited NICD production (70%-80%) after 24 hours. 150 mg/kg PF-03084014 caused maximal tumor growth inhibition of 92%. Besides that, the combination treatment of PF-03084014 and GEM significantly inhibited tumor growth and caused tumor regression in implanted PDA (pancreatic ductal adenocarcinoma) xenografts. It is also reported that the coadministration of PF-03084014 and dexamethasone can abrogate the gastrointestinal toxicity induced by PF-03084014 [1, 4].

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References:
1.Wei P, Walls M, Qiu M, et al. Evaluation of selective γ-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. Molecular cancer therapeutics, 2010, 9(6): 1618-1628.
2.Arcaroli J J, Quackenbush K S, Purkey A, et al. Tumours with elevated levels of the Notch and Wnt pathways exhibit efficacy to PF-03084014, a γ-secretase inhibitor, in a preclinical colorectal explant model. British journal of cancer, 2013, 109(3): 667-675.
3.López-Guerra M, Xargay-Torrent S, Rosich L, et al. The γ-secretase inhibitor PF-03084014 combined with fludarabine antagonizes migration, invasion and angiogenesis in NOTCH1-mutated CLL cells. Leukemia, 2014.
4.Yabuuchi S, Pai S G, Campbell N R, et al. Notch signaling pathway targeted therapy suppresses tumor progression and metastatic spread in pancreatic cancer. Cancer letters, 2013, 335(1): 41-51.

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