

Product Data Sheet

Product Name: A66
Cat. No.: GC17550

Chemical Properties

Cas. No. 1166227-08-2

Chemical Name (2S)-1-N-[5-(2-tert-butyl-1,3-thiazol-4-yl)-4-methyl-1,3-thiazol-2-yl]pyrrolidine-1,2-dicarboxamide

SMILES CC1=C(SC(=N1)NC(=O)N2CCCC2C(=O)N)C3=CSC(=N3)C(C)(C)C

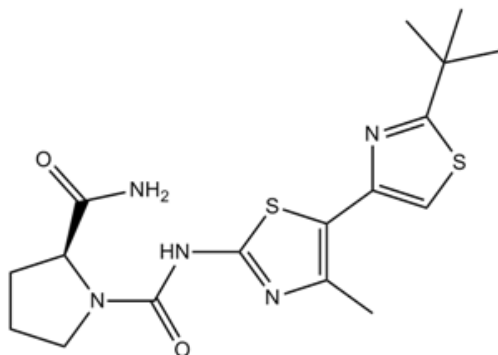
Formula $C_{17}H_{23}N_5O_2S_2$ M.Wt 393.53

Solubility $\geq 19.7\text{mg/mL}$ in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C 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



Protocol

Cell experiment [1]:

Cell lines Human rhabdomyosarcoma Rh30 cell line

Caution: Product has not been fully validated for medical applications. For research use only.

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Preparation Method Human rhabdomyosarcoma Rh30 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. Myristoylated tag was ligated in tandem with each p110 gene sequences and then inserted into pBabe-Puro plasmid. The resulting constructs were co-transfected with pGP and pE-Ampho plasmids into 293FT cells to obtain retrovirus, which were then used to infect Rh30 cells. After selection with 1µg/mL puromycin for 1 week, cells stably expressing myristoylated PI3K isoforms were pooled for validation and named as Rh30-Myr-p110. Rh30-Myr-p110 cells grown to 50% confluence in 12-well plates continued to be incubated in serum-free medium for 12h and were then exposed to A66 at 100nM for 1h.

Reaction Conditions 100nM; 1h

Applications A66 significantly inhibited the phosphorylation of Akt in human rhabdomyosarcoma Rh30-Myr-p110 cells.

Animal experiment [2]:

Animal models Male CD1 mice

Preparation Method For glucose tolerance tests and pyruvate tolerance tests the mice were starved overnight and for the insulin tolerance test food was withdrawn 2h prior to the start of the experiments. A66 was dosed intraperitoneally 1h after the end of the dark cycle and 1h prior to the intraperitoneal dosing with glucose or pyruvate (2g/kg) or insulin (0.75unit/kg).

Dosage form 10mg/kg; 24h; i.p.

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Applications A66 induced significant impairments in the insulin tolerance test and glucose tolerance test, and an increase in glucose production during a pyruvate tolerance test in mice.

References:

- [1] WANG X, LI J P, YANG Y, et al. A pharmacological model reveals biased dependency on PI3K isoforms for tumor cell growth [J]. *Acta Pharmacol Sin*, 2013, 34(9): 1201-7.
- [2] SMITH G C, ONG W K, REWCASTLE G W, et al. Effects of acutely inhibiting PI3K isoforms and mTOR on regulation of glucose metabolism in vivo [J]. *Biochem J*, 2012, 442(1): 161-9.

Background

A66 is a highly specific and selective p110 α inhibitor with an IC₅₀ value of 32nM^[1, 2]. A66 can also inhibit p110 α mutation such as p110 α E545K and p110 α H1047R, which have the IC₅₀ values of 30nM and 43nM^[1]. A66 can inhibit tumor necrosis factor induced necroptosis^[3].

A66 (100nM; 24h) markedly opposed the increased expression of *Ido1* and *Tgfb1* transcripts by TGF- β in mouse plasmacytoid dendritic cells^[4]. A66 (100nM; 1h) reduced lipid kinase activity in MT (polyoma middle T antigen) tumor cells^[5]. A66 (100nM; 1h) significantly inhibited the phosphorylation of Akt in human rhabdomyosarcoma Rh30 cells tranfected p110 retrovirus^[6].

A66 (10mg/kg; i.p. ; 24h) induced an increase in glucose production during a pyruvate (2g/kg; i.p.) tolerance test in mice^[7]. A66 (10mg/kg; i.p. ; 21 day) significant delayed in growth of SK-OV-3 cells derived xenograft tumours in mice xenograft model (SK-OV-3 cells were subcutaneously inoculated on the right flank of the mice)^[1].

References:

- [1] JAMIESON S, FLANAGAN J U, KOLEKAR S, et al. A drug targeting only p110alpha can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types [J]. *Biochem J*, 2011, 438(1): 53-62.
- [2] SUN M, HILLMANN P, HOFMANN B T, et al. Cancer-derived mutations in the regulatory subunit p85alpha of phosphoinositide 3-kinase function through the catalytic subunit p110alpha [J]. *Proc Natl*

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Acad Sci U S A, 2010, 107(35): 15547-52.

[3] HU S, CHANG X, ZHU H, et al. PI3K mediates tumor necrosis factor induced-necroptosis through initiating RIP1-RIP3-MLKL signaling pathway activation [J]. Cytokine, 2020, 129(155046).

[4] IACONO A, POMPA A, DE MARCHIS F, et al. Class IA PI3Ks regulate subcellular and functional dynamics of IDO1 [J]. EMBO Rep, 2020, 21(12): e49756.

[5] UTERMARK T, RAO T, CHENG H, et al. The p110alpha and p110beta isoforms of PI3K play divergent roles in mammary gland development and tumorigenesis [J]. Genes Dev, 2012, 26(14): 1573-86.

[6] WANG X, LI J P, YANG Y, et al. A pharmacological model reveals biased dependency on PI3K isoforms for tumor cell growth [J]. Acta Pharmacol Sin, 2013, 34(9): 1201-7.

[7] SMITH G C, ONG W K, REWCASTLE G W, et al. Effects of acutely inhibiting PI3K isoforms and mTOR on regulation of glucose metabolism in vivo [J]. Biochem J, 2012, 442(1): 161-9.

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