

产品名称: (2S)-N1-(2-叔丁基-4'-甲基[4,5'-联噻唑]-2'-基)-1,2-吡咯烷二甲酰胺
产品别名: A66

生物活性:					
Description	A66 is a highly specific and selective p110α inhibitor with an IC ₅₀ of 32 nM.				
IC ₅₀ & Target	p110α	p110α E545K	p110α H1047R	p110γ	PI3K-C2β
	32 nM (IC ₅₀)	30 nM (IC ₅₀)	43 nM (IC ₅₀)	3480 nM (IC ₅₀)	462 nM (IC ₅₀)
	PI4Kβ				
	236 nM (IC ₅₀)				
In Vitro	A66 is a potent inhibitor of the wild-type and oncogenic forms of p110α but not other class-I PI3K isoforms[1]. The p110α-specific inhibitor A66 (0.7 μM) induces a 75-80% reduction in focus formation by the highly transforming iSH2 mutants KS459delN, DKRMNS560del, and K379E. The p110α-specific inhibitor A66 reduced phosphorylation of Akt on T308 by all p85 mutants[2].				
In Vivo	The optimal dosing strategy for xenograft studies is determined by investigating the drug pharmacokinetics after a dose of 10 mg/kg of body weight by intraperitoneal injection in CD-1 mice. Despite a short half-life of only 0.42 h, the large C _{max} (8247 nM) of A66 S that is reached 30 min after dosing ensured that the AUC _{0-inf} (area under the curve from zero time to infinity) (6809 nM•h) is similar to that of BEZ-235 (7333 nM•h), which has a longer half-life of 2.73 h. Furthermore, the A66 on SK-OV-3 tumour tissue is tested using a single dose of 100 mg/kg of body weight to determine whether a long-lasting effect of the drug could be achieved on target tissues. These studies show that A66 causes a profound reduction in the phosphorylation of Akt/PKB and p70 S6 kinase, but not of ERK (extracellular-signal-regulated kinase), at both 1 and 6 h after dosing. Levels of A66 in plasma are determined to be 21.1±1.2 μM and 9.1±1.1 μM at 1 and 6 h after drug injection, whereas levels of A66 in the tumor are 22.7±2.1 μM and 16.0±1.3 μM at the same time points [1].				
Solvent&Solubility	In Vitro: DMSO : 50 mg/mL (127.06 mM; Need ultrasonic)				
		<div> <div>Solvent</div> <div>Mass</div> <div>Concentration</div> </div>	1 mg	5 mg	10 mg
	Preparing	1 mM	2.5411 mL	12.7055 mL	25.4110 mL
	Stock Solutions	5 mM	0.5082 mL	2.5411 mL	5.0822 mL
		10 mM	0.2541 mL	1.2706 mL	2.5411 mL
<p>*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。</p> <p>储备液的保存方式和期限：-80℃，6 months；-20℃，1 month。-80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。</p> <p>In Vivo:</p> <p>请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：</p> <p>——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶</p> <p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline</p> <p>Solubility: ≥ 2.5 mg/mL (6.35 mM); Clear solution</p>					

	<p>此方案可获得 ≥ 2.5 mg/mL (6.35 mM, 饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中, 混合均匀向上述体系中加入 50 μL Tween-80, 混合均匀; 然后继续加入 450 μL 生理盐水定容至 1 mL。</p> <p>2.请依序添加每种溶剂: 10% DMSO\rightarrow 90% (20% SBE-β-CD in saline)</p> <p>Solubility: ≥ 2.5 mg/mL (6.35 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.35 mM, 饱和度未知) 的澄清溶液。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中, 混合均匀。</p> <p>3.请依序添加每种溶剂: 10% DMSO \rightarrow 90% corn oil</p> <p>Solubility: ≥ 2.5 mg/mL (6.35 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (6.35 mM, 饱和度未知) 的澄清溶液, 此方案不适用于实验周期在半个月以上的实验。</p> <p>以 1 mL 工作液为例, 取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中, 混合均匀。</p>
References	<p>[1]. Jamieson S, et al. <u>A drug targeting only p110α can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types.</u> <i>Biochem J</i>, 2011, 438(1), 53-62.</p> <p>[2]. Sun M, et al. <u>Cancer-derived mutations in the regulatory subunit p85α of phosphoinositide 3-kinase function through the catalytic subunit p110α.</u> <i>Proc Natl Acad Sci U S A</i>, 2010, 107(35), 15547-15552.</p>
实验参考:	
Animal Administration	<p>Mice[1]</p> <p>Age-matched specific pathogen-free Rag1^{-/-} or NIH-III mice are subcutaneously inoculated on the right flank with 5\times10⁶ U87MG, SK-OV-3 or HCT-116 cells in PBS. Tumour diameter as measured by electronic calipers is used to calculate tumour volume (mm³) based on the formula (L\timesw²)$\times$$\pi$/6 (where L=longest tumour diameter and w=perpendicular diameter). A66 is administered in 20% 2-hydroxypropyl-β-cyclodextrin in water, whereas BEZ-235 is administered in 10% ethanol. Control mice are administered the A66 dosing vehicle alone. The drugs are dosed by intraperitoneal injection as the free base equivalent at a dosing volume of 10 mL/kg of body weight. For tumour pharmacodynamic studies, mice are administered a single dose of A66 or the control vehicle when tumors reached approximately 8-9 mm in diameter. Animals are killed 1 or 6 h after dosing and the tumors are removed, biopulverized and assayed for protein concentration. For antitumor efficacy studies, dosing began when tumors are well established, averaging approximately 7 mm in diameter. Doses are administered once daily (QD) or twice daily (BID) with injections separated by a minimum of approximately 8 h. Different dosing schedules are used for the three xenograft models depending on the rate of tumor growth and the body weight tolerance of control mice. Animals are dosed daily for 21 days or twice daily for 16 days (SK-OV-3), daily for 14 days (U87MG) and daily for 7 days (HCT-116). Animals are monitored daily for any signs of emerging toxicity and body weight is recorded. Mice are killed if they developed moderate signs of toxicity or if body weight loss exceeded 20% of starting weight. TGI (tumour growth inhibition) is calculated on the final day of dosing by determining the relative tumour size of drug-treated mice as a percentage of the average relative tumour size of control mice. The statistical significance of TGI values is determined by one-way ANOVA with Bonferroni multiple comparison analysis using GraphPad Prism 5.02.</p>

Kinase Assay	<p>IC₅₀ values are evaluated using the PI3K (human) HTRF Assay. p85α/p110δ is obtained from Invitrogen. All other isoforms are produced in-house by co-expressing full-length human p85α with the indicated human full-length catalytic subunit containing a histidine tag at the N-terminus to allow purification. The PI3Ks are titrated and used at a concentration between their EC₆₅-EC₈₀ values. PI3K activity in immunoprecipitates is assayed using an antibody to the N-SH2 (N-Src homology 2) domain of p85α. Assays for other lipid kinases and protein kinases are performed by the National Centre for Protein Kinase Profiling and Invitrogen Drug Discovery Services [1].</p>
References	<p>[1]. Jamieson S, et al. <u>A drug targeting only p110α can block phosphoinositide 3-kinase signalling and tumour growth in certain cell types.</u> Biochem J, 2011, 438(1), 53-62.</p> <p>[2]. Sun M, et al. <u>Cancer-derived mutations in the regulatory subunit p85α of phosphoinositide 3-kinase function through the catalytic subunit p110α.</u> Proc Natl Acad Sci U S A, 2010, 107(35), 15547-15552.</p>



源叶生物