产品名称：**MRT67307**
产品别名：**MRT67307**

生物活性：

Description

MRT67307 is a dual inhibitor of the IKKε and TBK-1 with IC_{50}s of 160 and 19 nM, respectively. MRT67307 also inhibits ULK1 and ULK2 with IC_{50}s of 45 and 38 nM, respectively.

IC_{50} & Target

TBK1	IKKε	ULK2	ULK1	Autophagy
19 nM (IC_{50}, at 0.1 mM ATP)	160 nM (IC_{50}, at 0.1 mM ATP)	38 nM (IC_{50})	45 nM (IC_{50})	

In Vitro

MRT67307 actually enhances phosphorylation in $IKK\alpha^{-/-}$ MEFs, the IL-1-stimulated phosphorylation of p105 at Ser^{933} and RelA at both Ser^{468} and Ser^{536}. MRT67307 also enhances IL-1-stimulated activation of NF-κB-dependent gene transcription in wild-type MEFs. Treatment of macrophages with MRT67307 leads to an increase in the poly(I:C)- and LPS-stimulated phosphorylation of p105 and RelA and enhanced NF-κB transcriptional activity[1]. MRT67307 (10 μM) is sufficient to reduce phospho-ATG13 to control levels, and in line with the in vitro IC_{50} values, 10-fold less MRT68921 (1 μM) results in a similar reduction. MRT67307 and MRT68921 inhibit ULK and block autophagy in cells[2]. MRT67307 increases IL-10 production and suppresses proinflammatory cytokine production in macrophages. MRT67307 increases CREB-dependent gene transcription by promoting the dephosphorylation of CRTC3. MRT67307 does not inhibit the brain-specific kinases (BRSKs) and only inhibits the maternal embryonic leucine zipper kinase (MELK) and AMPK itself more weakly[3].

Solvent&Solubility

In Vitro:

DMSO： ≥ 100 mg/mL (215.24 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.1524 mL	10.7619 mL	21.5239 mL
	5 mM	0.4305 mL	2.1524 mL	4.3048 mL
	10 mM	0.2152 mL	1.0762 mL	2.1524 mL

*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。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

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：

——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

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (5.38 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (5.38 mM，饱和度未知) 的澄清溶液。

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。

	2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (5.38 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (5.38 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。 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。
References	[1]. Clark K, et al. Novel cross-talk within the IKK family controls innate immunity. Biochem J. 2011 Feb 15;434(1):93-104. [2]. Petherick KJ, et al. Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. J Biol Chem. 2015 May 1;290(18):11376-83. [3]. Clark K, et al. Phosphorylation of CRTC3 by the salt-inducible kinases controls the interconversion of classically activated and regulatory macrophages. Proc Natl Acad Sci U S A. 2012 Oct 16;109(42):16986-91.

实验参考：

Cell Assay	Cells are rinsed in ice-cold PBS and extracted in lysis buffer (50 mM Tris·HCl at pH 7.4, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium β-glycerol 1-phosphate, 1 mM DTT, 1 mM sodium orthovanadate, 0.27mol/Lsucrose, 1% (vol/vol) Triton X-100, 1 μg/mL aprotinin, 1 μg/ mL leupeptin, and 1 mM phenylmethylsulphonyl fluoride). Cell extracts are clarified by centrifugation at 14,000 × g for 10 min at 4°C, and protein concentrations are determined by using the Bradford assay. FLAG-CRTC3 is purified on anti-FLAG M2 agarose, whereas endogenous CRTC3 is immunoprecipitated from cell extracts by using anti-CRTC3 raised against the peptide CWKEEKHPGFR (S277D bleed 2) and coupled to Protein GSepharose. To detect proteins in cell lysates, 20 μg of protein extract is separated by SDS/PAGE. After transfer to PVDF membranes, proteins are detected by immunoblotting and visualized by treating the blots with ECL followed by autoradiography. The following antibodies are used for immunoblotting: pSer133 CREB, pSer171 CRTC2, total CRTC2, GAPDH, total STAT3, pTyr705 STAT3, FLAG (M2 clone), CRTC3, HA (3F10), and 14-3-3; and antibodies against pSer329 (S256D bleed 2) and pSer370 (S253D bleed 2) of CRTC3 are raised against the phosphopeptides GLQSSRpSNPSIQ and RLFSLpSNPSLST. [3]
Kinase Assay	Substrates and kinases are diluted in 50 mM Tris/HCl (pH 7.5), 0.1% 2-mercaptoethanol, 0.1 mM EGTA and 10 mM magnesium acetate. Reactions are initiated with [γ-^{32}P]ATP (2500 c.p.m./pmol) to a final concentration of 0.1 mM and terminated after 15 min at 30°C by the addition of SDS and EDTA (pH 7.0) to final concentrations of 1.0% (w/v) and 20 mM respectively. After heating for 5 min at 100°C and separation by SDS/PAGE, the phosphorylated proteins are detected by autoradiography. [1]
References	[1]. Clark K, et al. Novel cross-talk within the IKK family controls innate immunity. Biochem J. 2011 Feb 15;434(1):93-104. [2]. Petherick KJ, et al. Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. J Biol Chem. 2015 May 1;290(18):11376-83. [3]. Clark K, et al. Phosphorylation of CRTC3 by the salt-inducible kinases controls the interconversion of classically activated and regulatory macrophages. Proc Natl Acad Sci U S A. 2012 Oct 16;109(42):16986-91.