产品名称：4-羟基-3-(2'-羟基-1,1'-联苯-4-基)-6-氧代-6,7-二氢噻吩并[2,3-B]吡啶-5-甲腈
产品别名：A-769662

生物活性：

Description	A-769662 is a potent, reversible AMPK activator with EC_{50} of 0.8 μM.
IC_{50} & Target	AMPK
	0.8 μM (EC_{50})
In Vitro	A-769662 is equally potent in activating the baculovirus expressed α1,β1,γ1 recombinant isoform of AMPK (EC50=0.7 μM). A-769662 and A-592107 activate AMPK purified from multiple tissues and species in a dose-responsive manner with modest variations in observed EC50s. EC50s determined for A-769662 using partially purified AMPK extracts from rat heart, rat muscle, or human embryonic kidney cells (HEKs) are 2.2 μM, 1.9 μM, or 1.1 μM, respectively[1]. A-769662 activates endogenous AMPK in LKB1-expressing (HEK293) and LKB1-deficient (CCL13) cells. A-769662 allosterically activates AMPK complexes containing γ1 harboring a substitution of arginine residue 298 to glycine (R298G). A-769662 inhibits dephosphorylation of Thr-172 in the mutant γ1-containing complexes to a similar degree as seen in the wild-type complexes[2]. A769662 (300 μM) has toxic effects on MEF cells. A769662 reversibly inhibits the proteasomal activity[3].
In Vivo	A-769662 (30 mg/kg, i.p.) significantly reduced the respiratory exchange ratio (RER) in the SD rat. There are 33% and 58% reductions of malonyl CoA levels in livers of animals treated with 30 mg/kg A-769662 (0.905 nmol/g) or 500 mg/kg metformin (0.574 nmol/g), respectively. A-769662 (30 mg/kg, b.i.d.) significantly decreases fed plasma glucose (30%-40% reduction), while the lower doses (3 and 10 mg/kg) of A-769662 had no effect on the in diabetic ob/ob mice[1].
Solvent&Solubility	***In Vitro:***
	DMSO： 100 mg/mL (277.48 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.7748 mL	13.8739 mL	27.7477 mL
	5 mM	0.5550 mL	2.7748 mL	5.5495 mL
	10 mM	0.2775 mL	1.3874 mL	2.7748 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 (6.94 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (6.94 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% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.94 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 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (6.94 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (6.94 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]. Cool B, et al. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab, 2006, 3(6), 403-416. [2]. Sanders MJ, et al. Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. J Biol Chem, 2007, 282(45), 32539-32548. [3]. Moreno D, et al, A769662, a novel activator of AMP-activated protein kinase, inhibits non-proteolytic components of the 26S proteasome by an AMPK-independent mechanism. FEBS Lett, 2008, 583(17), 2650-2654. [4]. Yerra VG, et al. Adenosine Monophosphate-Activated Protein Kinase Abates Hyperglycaemia-Induced Neuronal Injury in Experimental Models of Diabetic Neuropathy: Effects on Mitochondrial Biogenesis, Autophagy and Neuroinflammation. Mol Neurobiol. 2017 Apr;54(3):2301-2312.

实验参考：

Animal Administration	After acclimation *ob/ob* and lean mice are randomized to the various treatment groups by body weight and fed glucose levels (tail snip) at 8 AM. Baseline plasma insulin samples are also taken from a subset of the animals representing each treatment group (n=10 *ob/ob* and n=10 lean *ob/+* littermates). Two separate *ob/ob* and lean littermate studies are completed: 1) an initial 5 day study, and 2) a 14 day study to examine efficacy and more completely characterize the body weight change observed in the 5 day study. Treatment groups for the 5 day study are as follows: *ob/ob* vehicle (0.2% hydroxypropyl methylcellulose [HPMC], i.p., b.i.d.), A-592107 (10 or 100 mg/kg, i.p., b.i.d.), A-769662 (3 or 30 mg/kg, i.p., b.i.d.), AICAR (375 mg/kg, s.c., b.i.d.), or metformin (450 mg/kg, p.o., q.d., with vehicle in PM), and lean littermates treated with vehicle (i.p., b.i.d.). Treatment groups for the 14 day *ob/ob* and lean littermate study are as follows: *ob/ob* vehicle (0.2% HPMC, i.p., b.i.d.), A-769662 (3, 10, or 30 mg/kg, i.p., b.i.d.), or metformin, and lean littermates treated with vehicle or 30 mg/kg of A-769662 (i.p., b.i.d.). [1]
Kinase Assay	To assay glycogen phosphorylase b (GPb) activity, 1.5 μg/mL of rabbit GPb is added to a reaction mix containing 20 mM Na_2HPO_4 (pH 7.2), 2 mM $MgSO_4$, 1 mM β-NADP (β-nicotinamide adenine dinucleotide phosphate), 1.4 U/mL G-6-PDH (Glucose-6-Phosphate-Dehydrogenase) and 3 U/mL PGM (phosphoglucomutase). AMP or test compounds are added to the assay medium at the specified concentrations followed by the addition of glycogen (final concentration 1 mg/mL) to initiate the reaction. After incubating 10 min at 25°C, GPb activity is assessed by measuring absorbance at

	340 nm. [1]
References	[1]. Cool B, et al. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab, 2006, 3(6), 403-416. [2]. Sanders MJ, et al. Defining the mechanism of activation of AMP-activated protein kinase by the small molecule A-769662, a member of the thienopyridone family. J Biol Chem, 2007, 282(45), 32539-32548. [3]. Moreno D, et al, A769662, a novel activator of AMP-activated protein kinase, inhibits non-proteolytic components of the 26S proteasome by an AMPK-independent mechanism. FEBS Lett, 2008, 583(17), 2650-2654. [4]. Yerra VG, et al. Adenosine Monophosphate-Activated Protein Kinase Abates Hyperglycaemia-Induced Neuronal Injury in Experimental Models of Diabetic Neuropathy: Effects on Mitochondrial Biogenesis, Autophagy and Neuroinflammation. Mol Neurobiol. 2017 Apr;54(3):2301-2312.