产品名称：N-[3-[[2-(4-氨基呋咱-3-基)-1-乙基-1H-咪唑并[4,5-C]吡啶-6-基]氧]苯基]-4-[[2-(4-吗啉基)乙基]氧]苯甲酰胺
产品别名： GSK 269962A

生物活性：

Description	GSK269962A is a potent ROCK inhibitor with IC_{50} s of 1.6 and 4 nM for recombinant human ROCK1 and ROCK2 respectively.					
IC_{50} & Target	ROCK1	ROCK2	RSK1	MSK1	AKT1	AKT2
	1.6 nM (IC_{50})	4 nM (IC_{50})	132 nM (IC_{50})	49 nM (IC_{50})	955 nM (IC_{50})	1350 nM (IC_{50})
	AKT3	CDK2	GSK3α			
	1510 nM (IC_{50})	3500 nM (IC_{50})	1260 nM (IC_{50})			
In Vitro	GSK269962A IC_{50} values of 1.6 nM toward recombinant human ROCK1. GSK269962A also exhibits more than 30-fold selectivity against a panel of serine/threonine kinases. GSK269962A induces vasorelaxation in preconstricted rat aorta with an IC_{50} of 35 nM. Both are highly potent toward human ROCK1 with IC_{50} of 1.6 nM for GSK269962A. On the other hand, GSK269962A has a significantly improved kinase selectivity profile with at least >30-fold selectivity against the panel of protein kinase tested [1].					
In Vivo	Oral administration of GSK269965A (0.3, 1, and 3 mg/kg) induces a dose-dependent reduction in blood pressure in spontaneously hypertensive rat (SHR). The reduction of blood pressure is acute and substantial. The maximal effect on blood pressure is observed approximately 2 h after oral gavages for both compounds. Under a similar setting, oral administration of Y-27632 (10 and 30 mg/kg) also induced a dose-dependent decrease of blood pressure. For all three Rho kinase inhibitors, the reduction of blood pressure is accompanied by an acute, dose-dependent increase in heart rate, presumably due to the activation of baroreflex mechanism [1].					

Solvent&Solubility

In Vitro:

DMSO : ≥ 30 mg/mL (52.58 mM)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	1.7525 mL	8.7627 mL	17.5254 mL
	5 mM	0.3505 mL	1.7525 mL	3.5051 mL
	10 mM	0.1753 mL	0.8763 mL	1.7525 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: ≥ 0.62 mg/mL (1.09 mM); Clear solution 此方案可获得 ≥ 0.62 mg/mL (1.09 mM，饱和度未知) 的澄清溶液。 以 1 mL 工作液为例，取 100 μL 6.2 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: 0.62 mg/mL (1.09 mM); Suspended solution; Need ultrasonic 此方案可获得 0.62 mg/mL (1.09 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 6.2 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: ≥ 0.62 mg/mL (1.09 mM); Clear solution 此方案可获得 ≥ 0.62 mg/mL (1.09 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 6.2 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Doe C, et al. Novel Rho kinase inhibitors with anti-inflammatory and vasodilatory activities. J Pharmacol Exp Ther. 2007 Jan;320(1):89-98.

实验参考：

Animal Administration	Rats [1]. Male Sprague-Dawley rats (350-400g) are anesthetized with 5% isoflurane in O_2 and killed by exsanguination. Aortic rings, approximately 2 to 3 mm in length, are suspended by two 0.1-mm diameter tungsten wire hooks in 10 mL tissue baths containing Krebs of the following composition: 112 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_2, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 11.0 mM dextrose, 0.01 mM indomethacin, and 0.01 mM L-NAME. Krebs is maintained at 37°C and aerated with 95% O_2, 5% CO_2, pH 7.4,. Changes in isometric force are measured under optimal resting tension (1 g) using FT03 force-displacement transducers coupled to model 7D polygraphs. After a 60-min equilibration period, the vessels are treated with standard concentrations of KCl (60 mM) and phenylephrine (1 μM). Cumulative concentration-response curves to phenylephrine are obtained for each tissue by dosing at 0.5-log unit intervals (1 nM to 10 μM). After several washes, each vessel is contracted to equilibrium with an EC_{80}concentration of phenylephrine, and tone is reversed by adding cumulative amounts of either GSK269962A or SB-772077-B at 0.5-log unit intervals (0.1 nM to 1 μM). Responses are expressed as percentage reversal of the tone established with phenylephrine.
Kinase Assay	The enzyme activity and kinetics of the purified ROCK1(3-543) are determined using scintillation proximity assay. In this assay, purified ROCK1 is incubated with peptide substrate (Biotin-Ahx-AKRRLSSLRA-CONH2), and ^{33}ATP and the subsequent incorporation of ^{33}P into the peptide is quantified by streptavidin bead capture. For IC_{50}determination, test compounds are dissolved at 10 mM in 100% DMSO, with subsequent serial dilution in 100% DMSO. Compounds are typically assayed over an 11-point dilution range with a concentration in the assay of 10 μM to 0.2 nM in 3-fold dilutions. For dose-response curves, data are normalized and expressed as percentage inhibition using the formula 100×[(U-C1)/(C2-C1)], where U is the unknown value, C1 is the average of the high signal (0%) control wells, and C2 is the average of the low signal (100%)

	control wells. Curve fitting is performed The results for each compound are recorded as pIC_{50} values [1].
References	[1]. Doe C, et al. Novel Rho kinase inhibitors with anti-inflammatory and vasodilatory activities. J Pharmacol Exp Ther. 2007 Jan;320(1):89-98.