

产品名称: **GW5074**

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生物活性:					
Description	GW 5074 is a potent and selective c-Raf inhibitor with IC₅₀ of 9 nM, and has no effect on the activities of JNK1/2/3, MEK1, MKK6/7, CDK1/2, c-Src, p38 MAP, VEGFR2 or c-Fms.				
IC ₅₀ & Target	c-Raf				
	9 nM (IC ₅₀)				
In Vitro	GW5074 is a potent and specific inhibitor of c-Raf with IC50 of 9 nM and has no effect of MKK6, MKK7, p38 MAP kinase and cdk5 in vitro. However, treatment of neuronal cultures with GW5074 permits accumulation of activating modifications on c-Raf and also B-Raf. The inhibition of LK-induced apoptosis by GW5074 in cerebellar granule neurons is not MEK-ERK-dependent. GW5074 delays down-regulation of Akt activity but inhibits apoptosis by an Akt-independent mechanism. GW5074 affects Ras, nuclear factor-kappa B and c-jun. GW5074 inhibits cell death caused by neurotoxins in granule cells and other neuronal types[1].				
In Vivo	GW5074 (5 mg/Kg) completely prevents extensive bilateral striatal lesions induced by 3-NP in mice[1]. GW5074 suppresses sidestream smoke-induced airway hyperresponsiveness in mice[2]. GW5074 completely abolishes chronic morphine-mediated AC superactivation I in CHO cells stably expressing the humanμ-opioid receptor[3].				
Solvent&Solubility	<i>In Vitro:</i> DMSO : ≥ 100 mg/mL (191.96 mM) * "≥" means soluble, but saturation unknown.				
	Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
		1 mM	1.9196 mL	9.5980 mL	19.1961 mL
		5 mM	0.3839 mL	1.9196 mL	3.8392 mL
		10 mM	0.1920 mL	0.9598 mL	1.9196 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℃, 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。				
	<i>In Vivo:</i> 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 (4.80 mM); Clear solution					
此方案可获得 ≥ 2.5 mg/mL (4.80 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。					
[1]. Chin PC, et al. The c-Raf inhibitor GW5074 provides neuroprotection in vitro and in an animal model of neurodegeneration through a MEK-ERK and Akt-independent mechanism. J Neurochem, 2004, 90(3).					

References	595-608. [2]. Lei Y, et al. The Raf-1 inhibitor GW5074 and dexamethasone suppress sidestream smoke-induced airway hyperresponsiveness in mice. <i>Respir Res</i>, 2008, 9(1), 71. [3]. Yue X, et al. Chronic morphine-mediated adenylyl cyclase superactivation is attenuated by the Raf-1 inhibitor, GW5074. <i>Eur J Pharmacol</i>. 2006 Jul 1;540(1-3):57-9.
实验参考:	
Cell Assay	Befiy, the tetrazolium salt MTT is added to the cultures at a final concentration of 1 mg/mL, and incubation of the culture is continued in the CO₂ incubator for a Hirthier 30 min at 37°C. The assay is stopped by adding lysis buffer [20% sodium dodecyl sulfate (SDS) in 50% N,N-dimethyl formamide, pH 4.7]. The absorbance is measured spectrophotometrically at 570 nm after an overnight incubation at room temperature. The absorbance of a well without cells is used as background and subtracted. Data are presented as mean±standard deviation. Statistical analysis is performed using ANOVA and Student-Neuman-Keuls' test. Besides MTT assays, viability is also quantified using the fluorescein-diacetate method and by DAPI staining (which reveals apoptotic nuclei as condensed or fragmented). The results using these assays are similar to those obtained with the MTT assay. [1]
Animal Administration	The Tru-Scan® activity monitoring system is used to assess locomotor activity on mice on the day following the 5 days of injection with saline, 3-NP or a combination of 3-NP and GW5074 (7 animals in each group). The animal is placed in a Perspex arena (25.9×25.9 cm) with infrared beams spaced at 0.6-inch intervals in the X-Y plane. The arena is also equipped with a second infrared beam system at the Z plane positioned 2.54 cm above the X-Y plane. In this system the movement of the animal is accurately assessed by interruptions in 17×17-grid system created by the infrared beams in both the X-Y and Z planes. The animal is allowed to remain in the arena for 15 min, with data collection performed during this period using the Tru Scan Line interface box and Tm Scan 99 software, operating through a Pentium PC. The following behavioral parameters are selected: (i) Total movements episodes: each movement in the floor plane is a series of coordinate changes with no rest for at least 1 sample interval; (ii) total movement distance: the sum of all vectored A-Y coordinate changes in the floor plane; (iii) mean velocity: the mean velocity of all X-Y coordinate change defined movements; and (iv) vertical plane entries: the total number of times any part of the animal entered the vertical plane (Z plane). [1]
Kinase Assay	Briefly, for each assay 5-10 mU of purified kinase is used. For GSK3β, cdk1, cdk2, cdk3, cdk5, the kinase is incubated with 1 μM GW5074 in a buffer containing 8 mM MOPS, pH 7.2, 0.2 mM EDTA, 10 mM magnesium acetate and [c-³³P-ATP] for 40 min at room temperature. Kinase activity is quantified by measuring ³³P incorporation by spotting an aliquot on P30 filters, washing in 50 mM phosphoric acid and scintillation counting. The buffer composition for c-Raf, JNK1, JNK2, JNK3, MEK1, MKK6, MKK7 is 50 mM Tris pH 7.5, 0.1 mM EGTA, 10 mM magnesium acetate and [c-³³P-ATP]. The peptide substrates used are as follows: For c-Raf, 0.66 mg/mL MBP; for cdks, 0.1 mg/mL histone H1; for JNKs, 3 μM ATF2; for MEK1, 1 μM MAPK2; for MKK6, 1 μM of SAPK2a and for MKK7, 2 μM JNK1α. [1]
References	[1]. Chin PC, et al. The c-Raf inhibitor GW5074 provides neuroprotection in vitro and in an animal model of neurodegeneration through a MEK-ERK and Akt-independent mechanism. <i>J Neurochem</i>. 2004, 90(3), 595-608. [2]. Lei Y, et al. The Raf-1 inhibitor GW5074 and dexamethasone suppress sidestream smoke-induced airway hyperresponsiveness in mice. <i>Respir Res</i>, 2008, 9(1), 71.

	[3]. Yue X, et al. Chronic morphine-mediated adenylyl cyclase superactivation is attenuated by the Raf-1 inhibitor, GW5074. Eur J Pharmacol. 2006 Jul 1;540(1-3):57-9.
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源叶生物