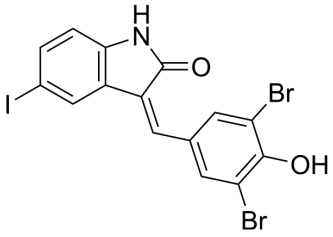
GW 5074

Cat. No.:	HY-10542
CAS No.:	220904-83-6
Molecular Formula:	$C_{15}H_8Br_2INO_2$
Molecular Weight:	520.94
Target:	Raf; Apoptosis
Pathway:	MAPK/ERK Pathway; Apoptosis
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



SOLVENT & SOLUBILITY

In Vitro

DMSO : 100 mg/mL (191.96 mM; ultrasonic and warming and heat to 60°C)

Preparing Stock Solutions	Mass / Solvent / 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

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (4.80 mM); Suspended solution

BIOLOGICAL ACTIVITY

Description

GW 5074 is a potent and selective c-Raf inhibitor with IC_{50} 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[1][2].

IC_{50} & Target

c-Raf
9 nM (IC_{50})

In Vitro

GW5074 is a potent and specific inhibitor of c-Raf with IC_{50} of 9 nM and has no effect of MKK6, MKK7, p38 MAP kinase and cdks 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].

	MCE has not independently confirmed the accuracy of these methods. They are for reference only.
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]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	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-^{33}P-ATP] for 40 min at room temperature. Kinase activity is quantified by measuring ^{33}P 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-^{33}P-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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [1]	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 CO_2 incubator for a Hirther 30 min at 37°C. The assay is stopped by adding lysis buffer [20% sodium dodecyi sulfate (SDS) in 50% N,N-dimethyl formamide, pH 4.7], The absorbance is measured spectrophotometrically at 570 nm after an ovemight 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 perfomied using ANOVA and Student-Neuman-Keuls' test. Besides MTT assays, viability is also quantitied using the tluorescein-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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [1]	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) witb 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 flor plane is a series of coordinate changes with no rest for at least I 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). MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- Oncogene. 2023 Apr 5.
- Carbohydr Polym. 2023 Apr 1;305:120533.
- Cell Death Discov. 2023 Apr 28;9(1):139.
- Biochem J. 2019 Mar 12;476(5):875-887.
- Sci Rep. 2020 Jul 7;10(1):11158.

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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. J Neurochem, 2004, 90(3), 595-608.

[2]. Lei Y, et al. The Raf-1 inhibitor GW5074 and NSC 34521 suppress sidestream smoke-induced airway hyperresponsiveness in mice. Respir Res, 2008, 9(1), 71.

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