PD0325901

Cat. No.: HY-10254
CAS No.: 391210-10-9
Molecular Formula: \( \text{C}_{16}\text{H}_{14}\text{F}_{3}\text{IN}_{2}\text{O}_{4} \)
Molecular Weight: 482.19
Target: MEK; Autophagy
Pathway: MAPK/ERK Pathway; Autophagy
Storage: Powder: -20°C 3 years, 4°C 2 years, In solvent: -80°C 6 months, -20°C 1 month

SOLVENT & SOLUBILITY

In Vitro

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>≥ 56 mg/mL (116.14 mM)</td>
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<tr>
<td>* &quot;≥&quot; means soluble, but saturation unknown.</td>
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Preparing Stock Solutions:
- 1 mM: 2.0739 mL
- 5 mM: 0.4148 mL
- 10 mM: 0.2074 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.18 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.18 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.18 mM); Clear solution

BIOLOGICAL ACTIVITY

Description: PD0325901 is a selective and cell permeable MEK inhibitor with an IC₅₀ of 0.33 nM.

IC₅₀ & Target:
- MEK1: 0.33 nM (IC₅₀)
- Autophagy
In Vitro

PF0325901 shows higher permeability, and should be able to achieve higher systemic exposures than CI-1040[1]. PD0325901 is exquisitely specific and highly potent against purified MEK, revealing a $K_{i\text{app}}$ of 1 nM against activated MEK1 and MEK2. PD0325901 is roughly 500-fold more potent than CI-1040 with respect to its cellular effects on phosphorylation of ERK1 and ERK2, displaying subnanomolar activity. PD0325901 prevents the growth of melanoma cell lines. PD0325901 inhibits the growth of TPC-1 cells and K2 cells with $GC_{50}$ of 11 nM and 6.3 nM, respectively. PD0325901 significantly prevents the growth of PTC cells harboring a BRAF mutation at very low concentration (10 nM) and only moderately increases the growth of the PTC cells carrying the RET/PTC1 rearrangement at the same concentration. PD0325901 effectively inhibits the phosphorylation of ERK1/2 in multiple PTC cell lines[2].

In Vivo

PD0325901 (25 mg/kg, p.o.) inhibits phosphorylation of ERK by more than 50% at 24 hours post-dosing. The dose required to produce a 70% incidence of complete tumor responses (C26 model) is 25 mg/kg/day versus 900 mg/kg/day for PD0325901 and CI-1040, respectively. Anticancer activity of PD 0325901 has been demonstrated for a broad spectrum of human tumor xenografts. PD0325901 (20-25 mg/kg/day, p.o.) treatment in mice, shows no tumor growth inoculated with PTC cells bearing a BRAF mutation. For PTC with the RET/PTC1 rearrangement, the average tumor volume of the orthotopic tumor is decreased by 58% as compared with controls. PTC cells carrying a BRAF mutation are more sensitive to PD0325901 than are PTC cells carrying the RET/PTC1 rearrangement[2].

PROTOCOL

Kinase Assay [1]

Incorporation of $^{32}$P into myelin basic protein (MBP) is assayed in the presence of a glutathione S-transferase fusion protein containing p44MAP kinase (GST-MAPK) and a glutathione S-transferase protein containing p45MEK (GST-MEK). The assay solution contained 20 mM HEPES, pH 7.4, 10 mM MgCl$_2$, 1 mM MnCl$_2$, 1 mM EGTA, 50 mM [$\gamma$-$^{32}$P]ATP, 10 mg GST-MEK, 0.5 mg GST-MAPK and 40 mg MBP in a final volume of 100 mL. Reactions are stopped after 20 minutes by addition of trichloroacetic acid and filtered through a GF/C filter mat. $^{32}$P retained on the filter mat is determined using a 1205 Betaplate. PD0325901 is assessed at various dose ranges in order to determine dose response curves. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Cell Assay [2]

PTC cells ($1 \times 10^4$) are seeded in 24-well plates with 1 mL of medium for 4 days in a 37°C incubator. MEK inhibitor PD0325901 at varying concentrations is added to the cells in triplicate on day 0. MTT dissolved in 0.8% NaCl solution at 5 mg/mL is added to each well (0.2 mL) on day 2 to test $GC_{50}$ or every day for cell growth curves. The cells are incubated at 37°C for 3 hours with MTT. The liquid is then aspirated from the wells and discarded. Stained cells are dissolved in 0.5 mL of DMSO and their absorption at 570 nm is measured using a Synergy HT multidetection microplate reader. For $GC_{50}$, cell growth is calculated as $100 \times (T−T_0)/(C−T_0)$, where $T$ is the optical density of the wells treated with inhibitors after a 48-hour period, $T_0$ is the optical density at time zero, and $C$ is the control optical density with DMSO only. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration [2]

Mice (10-14 per group) are anesthetized s.c. with a cocktail. K2 and TPC-1 cells stably infected with a retrovirus expressing luciferase ($5 \times 10^5$ cells in 5 µL RPMI1640 medium) are inoculated into the thyroid gland, and the mice are monitored weekly for tumor growth by Xenogen using Living Image 3.0 software. One week after inoculation, PD0325901 is dissolved in 80 mM citric buffer (pH 7) by sonication and given to mice daily by oral gavage (20-25 mg/kg) for 3 weeks (5 consecutive days/week). Mice are sacrificed only due to tumor burden or loss of 20% of body weight. Tumor sizes are measured with calipers and tumor volume ($V$) is calculated by the formula ($V$=length×width×depth). Control mice are given 80 mM citric buffer (pH 7) alone. All in vivo experiments are done at least twice. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
CUSTOMER VALIDATION

- **Biomaterials.** 2018 Oct;180:12-23.
- **Neuro Oncol.** 2019 Mar 18;21(4):486-497.

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REFERENCES


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Tel: 609-228-6898  Fax: 609-228-5909  E-mail: tech@MedChemExpress.com
Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA