BI-847325

Cat. No.: HY-18955
CAS No.: 1207293-36-4
Molecular Formula: C₂₉H₂₈N₄O₂
Molecular Weight: 464.56
Target: MEK; Aurora Kinase; Apoptosis
Pathway: MAPK/ERK Pathway; Cell Cycle/DNA Damage; Epigenetics; 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 : ≥ 36 mg/mL (77.49 mM)
* “≥” means soluble, but saturation unknown.

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Mass Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
</tr>
<tr>
<td>1 mM</td>
<td></td>
<td>2.1526 mL</td>
<td>10.7629 mL</td>
<td>21.5257 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td></td>
<td>0.4305 mL</td>
<td>2.1526 mL</td>
<td>4.3051 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td></td>
<td>0.2153 mL</td>
<td>1.0763 mL</td>
<td>2.1526 mL</td>
</tr>
</tbody>
</table>

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

BIOLICAL ACTIVITY

Description
BI-847325 is an ATP competitive dual inhibitor of MEK and aurora kinases (AK) with IC₅₀ values of 4 and 15 nM for human MEK2 and AK-C, respectively.

IC₅₀ & Target
| MEK2 | 4 nM (IC₅₀) |
| Aurora C | 15 nM (IC₅₀) |

In Vitro
BI 847325 inhibits the activity of X. laevis AK-B with an IC₅₀ of 3 nM; the IC₅₀ values for human AK-A and AK-C are 25 and 15 nM, respectively. BI 847325 also inhibits human MEK1 and MEK2 with respective IC₅₀ values of 25 and 4 nM. BI 847325 at 1,000 nM inhibits 6 enzymes by more than 50% (LCK, MAP3K8, FGFR1, AMPK, CAMK1D and TBK1) and the IC₅₀ values are below 100 nM only for LCK (5 nM) and MAP3K8 (93 nM). Proliferation is inhibited in A375 and Calu-6 cell lines with GI₅₀ values of 7.5 nM and 60 nM, respectively[1].

In Vivo
Daily oral administration of BI 847325 at 10 mg/kg shows efficacy in both BRAF- and KRAS-mutant xenograft models.
BI 847325 administered once weekly at 70 mg/kg inhibits both MEK and AK in KRAS-mutant tumors\(^1\).

### PROTOCOL

#### Kinase Assay\(^1\)

Assays are run in the presence of 100 \(\mu\)M ATP using 10 \(\mu\)M of substrate. 30 \(\mu\)L PROTEIN-MIX in 25% DMSO and incubated for 15 min at room temperature. 10 \(\mu\)L PEPTIDE-MIX is added, the mixture is incubated for 60 min at RT and stopped by adding 180 \(\mu\)L 6.4% TCA (final concentration: 5%). Incorporated phosphate is measured in a scintillation counter and IC\(_{50}\) values are calculated using a sigmoidal curve analysis program with variable hill slope\(^1\).

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

#### Cell Assay\(^1\)

Cells are plated in 96-well format and BI 847325 is added 24 hours after cell seeding. At the same time, a “time zero” untreated cell plate is fixed. Compound is serially diluted and assayed over 8 concentrations in triplicates. After 72 h incubation, cells are fixed and stained with fluorescent nuclear dye. Concentration-response curves are analyzed using a four-parameter log-logistic function without upper or lower limitation. GI\(_{50}\) are calculated\(^1\).

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#### Animal Administration\(^1\)

Mice: Tumor grafted female BomTac:NMRI-Foxn1\(^{nu}\) mice are used in the study. BI 847325 is dissolved in 0.5% Natrosol 250 HX with 3% Tween 80 and sonicated until a homogenous suspension is obtained, then 1 M HCl is added and the suspension is vortexed and sonicated again. MEK inhibitors GSK 1120212 and AZD 6244 are suspended in 1% or 0.5% Natrosol, respectively. An administration volume of 10 mL/kg body weight is used and compounds are administered orally with a gavage needle at the indicated dose and schedule. Tumor volumes are measured and mice are inspected daily for clinical signs and body weight is determined daily\(^1\).

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### REFERENCES