Brigatinib

Cat. No.: HY-12857
CAS No.: 1197953-54-0
Molecular Formula: C_{29}H_{39}ClN_{7}O_{2}P
Molecular Weight: 584.09
Target: Anaplastic lymphoma kinase (ALK)
Pathway: Protein Tyrosine Kinase/RTK
Storage: Powder -20°C 3 years
4°C 2 years
In solvent -80°C 2 years
-20°C 1 year

SOLVENT & SOLUBILITY

In Vitro
Ethanol: 10 mg/mL (17.12 mM; Need ultrasonic and warming)
DMSO: 2 mg/mL (3.42 mM; Need ultrasonic)

Preparing Stock Solutions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>1.7121 mL</td>
<td>8.5603 mL</td>
<td>17.1206 mL</td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>0.3424 mL</td>
<td>1.7121 mL</td>
<td>3.4241 mL</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>0.1712 mL</td>
<td>0.8560 mL</td>
<td>1.7121 mL</td>
<td></td>
</tr>
</tbody>
</table>

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

In Vivo
1. Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 1 mg/mL (1.71 mM); Clear solution
2. Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 1 mg/mL (1.71 mM); Clear solution
3. Add each solvent one by one: 10% EtOH >> 90% corn oil
   Solubility: ≥ 1 mg/mL (1.71 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 0.5 mg/mL (0.86 mM); Clear solution
5. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 0.5 mg/mL (0.86 mM); Clear solution
6. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 0.5 mg/mL (0.86 mM); Clear solution

BIOLOGICAL ACTIVITY
**Description**

Brigatinib (AP-26113) is a highly potent, selective and orally active ALK inhibitor, with an IC₅₀ of 0.6 nM. Brigatinib can be used for research of NSCLC\(^1\).

**IC₅₀ & Target**

IC₅₀: 0.6 nM (ALK)\(^1\)

**In Vitro**

Brigatinib potently inhibits the in vitro kinase activity of ALK (IC₅₀, 0.6 nM) and all five mutant variants tested, including G1202R (IC₅₀, 0.6-6.6 nM).

Brigatinib demonstrates a high degree of selectivity, only inhibiting 11 additional native or mutant kinases with IC₅₀ <10 nM. These include ROS1, FLT3, and mutant variants of FLT3 (D835Y) and EGFR (L858R; IC₅₀, 1.5-2.1 nM).

Brigatinib exhibits more modest activity against EGFR with a T790M resistance mutation (L858R/T790M), native EGFR, IGF1R, and INSR (IC₅₀, 29-160 nM) and does not inhibit MET (IC₅₀ >1000 nM).

In cellular assays, brigatinib inhibits ALK and ROS1 with IC₅₀ of 14 and 18 nM, respectively.

Brigatinib inhibits FLT3 and IGF-1R with about 11-fold lower potency (IC₅₀, 148-158 nM) and inhibits mutant variants of FLT3 and EGFR with 15- to 35-fold lower potency (IC₅₀, 211-489 nM).

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Brigatinib inhibits cell growth with GI₅₀ values ranging from 503 to 2,387 nM in three ALK-negative ALC and NSCLC cell lines\(^1\).

Brigatinib inhibits ALK activity and abrogates proliferation of ALK addicted neuroblastoma cell lines, with IC₅₀ of 75.27 ± 8.89 nM.

Brigatinib inhibits both the ALK-I1171N and the ALK-G1269A mutant receptors at 10 and 4 nM levels, respectively\(^3\).

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

**In Vivo**

Brigatinib (10, 25, or 50 mg/kg once daily, p.o.) leads to a dose-dependent inhibition of tumor growth in ALK\(^+\) Karpas-299 (ALCL) and H2228 (NSCLC) xenograft mouse models. Brigatinib markedly enhances survival of mice bearing ALK\(^+\) brain tumors compared with PF-02341066\(^1\).

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

**PROTOCOL**

**Kinase Assay\(^1\)**

In vitro HotSpot\(^{SM}\) kinase profiling of 289 kinases is performed. The assay is conducted in the presence of 10 μM \(^{33}\)P-ATP, using brigatinib concentrations ranging from 0.05 nM to 1 μM.

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

**Cell Assay\(^3\)**

Cells are seeded at 15,000 per well with serial dilutions of the indicated inhibitors. After 72 hours cell viability is assessed by resazurin. IC₅₀ values are calculated with GraphPad Prism 6.0 by fitting data to a log (inhibitor concentration) vs. normalized response (variable slope) equation. Each experiment is performed in duplicate and repeated at least three times.

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

**Animal Administration\(^2\)**

Mice: (1) Eight- to 10-week-old female SCID/beige mice are injected intravenously with 5×10⁶ H3122 cells per mouse and are randomly selected into treatment groups (n=10) when the average tumor size reaches appr 300 mm\(^3\) (day zero). Treatments are administered orally for up to 21 consecutive days at a 10 mL/kg dose volume. Subcutaneous tumors are measured two or three times weekly. Tumor volume (in mm\(^3\)) is calculated using the formula (L×W\(^2\))/2. When a tumor reaches 10% of the body weight of the host, the animal is euthanized via CO\(_2\) asphyxiation. (2) Eight- to 10-week old female SCID/beige mice are injected subcutaneously with 2.5×10⁶ Karpas-299 cells per mouse and are randomly selected into treatment groups (n=10) when the average tumor size reached appr 180 mm\(^3\) (day zero). Treatments are administered orally for 14 consecutive days at a 10 mL/kg dose volume. Tumor volume is measured and calculated as described for the H3122 model.

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

