**BIological Activity:**

Brigatinib is a highly potent and selective ALK inhibitor, with \( \text{IC}_{50} \) of 0.6 nM.

**In Vitro:** Brigatinib potently inhibits the in vitro kinase activity of ALK (\( \text{IC}_{50} \), 0.6 nM) and all five mutant variants tested, including G1202R (\( \text{IC}_{50} \), 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; \( \text{IC}_{50} \), 1.5-2.1 nM). Brigatinib exhibits more modest activity against EGFR with a T790M resistance mutation (L858R/T790M), native EGFR, IGF1R, and INS (\( \text{IC}_{50} \), 29-160 nM) and does not inhibit MET (\( \text{IC}_{50} \) >1000 nM). In cellular assays, brigatinib inhibits ALK and ROS1 with IC50s of 14 and 18 nM, respectively. Brigatinib inhibits FLT3 and IGF-1R with about 11-fold lower potency (\( \text{IC}_{50} \), 148-158 nM) and inhibits mutant variants of FLT3 and EGFR with 15- to 35-fold lower potency (\( \text{IC}_{50} \), 211-489 nM). Brigatinib inhibits cell growth with \( \text{GI}_{50} \) values ranging from 503 to 2,387 nM in three ALK-negative ALCL and NSCLC cell lines[1]. Brigatinib inhibits 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].

**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 crizotinib[1]. Brigatinib (10, 25, 50 mg/kg, p.o.) results in dose-dependent antitumor activity, with tumor regressions in a mouse model of NSCLC[2].

**Protocol (Extracted from published papers and Only for reference)**

**Kinase Assay:**[1]In vitro HotSpot\textsuperscript{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. **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. \( \text{IC}_{50} \) 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. **Animal Administration:**[2](1) Eight- to 10-week-old female SCID/beige mice are injected intravenously with 5×10\textsuperscript{6} H3122 cells per mouse and are randomly selected into treatment groups (n=10) when the average tumor size reaches appr 300 mm\textsuperscript{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\textsuperscript{3}) is calculated using the formula \((L\times W^2)/2\). When a tumor reaches 10% of the body weight of the host, the animal is euthanized via CO\textsubscript{2} asphyxiation. (2) Eight- to 10-week old female SCID/beige mice are injected subcutaneously with 2.5×10\textsuperscript{6} Karpas-299 cells per mouse and are randomly selected into treatment groups (n=10) when the average tumor size reaches appr 180 mm\textsuperscript{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.
References:


Caution: Product has not been fully validated for medical applications. For research use only.

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