Kinase Assay:

**Protocol**

Kinase inhibition is investigated using one of three assay formats: 1. [[32]P]Phosphoryl transfer, luciferase-coupled chemiluminescence, or AlphaScreen tyrosine kinase technology. IC50s are calculated by nonlinear regression analysis using XFLI-32P-Phosphoryl Transfer Kinase Assay Reactions are performed in 384-well white, clear bottom, high-binding microtiter plates (Greiner, Monroe, NC). Plates are coated with 2 µg/well of protein substrate in a 50 µL volume of coating buffer contained 40 µg/mL substrate (poly(Glu, Tyr) 4:1, 22.5 mM Na2CO3, 27.5 mM NaHCO3, 50 mM NaCl and 3 mM Na3. Coated plates are washed once with 50 µL of assay buffer following overnight incubation at room temperature (RT). Test compounds and enzymes are combined with 32P-PAT (3.3 µCi/nmol) in a total volume of 20 µL. The reaction mixture is incubated at RT for 2 hours and terminated by aspiration. The microtiter plates are subsequently washed 6 times with 0.05% Tween-PBS buffer (PBST). Scintillation fluid (50 µL/well) is added and incorporated 32P is measured by liquid scintillation spectrometry using a MicroBeta scintillation counter (Perkin Elmer, Wellesley, MA).Luciferase-Coupled Chemiluminescence Assay Reactions are conducted in 384-well white, medium binding microtiter plates (Greiner). In a first step enzyme and compound are combined and incubated for 60 minutes; reactions are initiated by addition of ATP and peptide substrate (poly(Glu, Tyr) 4:1) in a final volume of 20 µL, and incubated at RT for 2-4 hours. Following the kinase reaction, a 20 µL aliquot of Kinase Glo (Promega, Madison, WI) is added and luminescence signal is measured using a Victor plate reader (Perkin Elmer).

**Features**

Kinase analysis can be performed using a range of different formats. Selleckchem offers a variety of products to facilitate your research. Please contact us for more information or to place an order.

**Biological Activity**

**Description**

XL880 (GSK1363089, EXEL-2880) is an ATP-competitive inhibitor of MET and KDR with IC50s of 0.4 nM and 0.9 nM, respectively.

**Targets**

MET

KDR

**IC50**

0.4 nM

0.9 nM

**In vitro**

XL880 inhibits HGF receptor family tyrosine kinases with IC50 values of 0.4 nM for Met and 3 nM for Ron. XL880 also inhibits KDR, Flk-1, and Flk-4 with IC50 values of 0.9 nM, 6.8 nM and 2.8 nM, respectively. XL880 inhibits colony growth of B16F10, A549 and H1299 cells with IC50 of 40 nM, 29 nM and 165 nM, respectively. 1 A recent study indicates XL880 affects cell growth differently in gastric cancer cell lines MKN-45 and KATO-III. XL880 inhibits phosphorylation of MET and downstream signaling molecules in MKN-45 cells, while targets FGFR2 in KATO-III cells. 2

**In vivo**

A single 100 mg/kg oral gavage dose of XL880 results in substantial inhibition of phosphorylation of B16F10 tumor Met and ligand (e.g., HGF/Vegf)-induced receptor phosphorylation of Met in liver and Flk-1/KDR in lung, which both persisted through 24 hours. Treatment with XL880 (30-100 mg/kg, once daily, oral gavage) results in reduction in tumor burden. The lung surface tumor burden is reduced by 50% and 58% following treatment with 30 and 100 mg/kg XL880, respectively. XL880 treatment of mice bearing B16F10 solid tumors also results in dose-dependent tumor growth inhibition of 64% and 87% at 30 and 100 mg/kg, respectively. For both studies, administration of XL880 is well tolerated with no significant body weight loss. 1 XL880 is developed to target abnormal signaling of HGF through Met and simultaneously target several receptors tyrosine kinase involved in tumor angiogenesis. XL880 caused tumor hemorrhage and necrosis in human xenografts within 2 to 4 hours, and maximal tumori necrosis is observed at 96 hours (after five daily doses), resulting in complete regression. 3

**Clinical Trials**

A Phase II study of XL880 about Recurrent or Metastatic Squamous Cell Cancer of the Head and Neck has been completed.

**Features**

**Protocol (Only for Reference)**

**Kinase Assay**

**Chemical Structure**

**Return Policy**

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www.selleckchem.com/datasheet/XL880(GSK1363089,EXEL-2880)-DataSheet.html
Cell Assay:[1]

<table>
<thead>
<tr>
<th>Cell Lines</th>
<th>B16F10, A549, and HT29 cells</th>
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<tbody>
<tr>
<td>Concentrations</td>
<td>40 nM</td>
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<tr>
<td>Incubation Time</td>
<td>12 to 14 days</td>
</tr>
</tbody>
</table>

Methods

B16F10, A549, and HT29 cells (1.2 × 10^3 per well) are mixed with soft agar and seeded in a 96-well plate containing 10% FBS and EXEL-2880 over a base agar layer. For normoxic conditions, the plates are incubated (37°C) for 12 to 14 days in 21% oxygen, 5% CO₂, and 74% nitrogen, whereas incubation (37°C) under hypoxic conditions is done in a hypoxia chamber in 1% oxygen, 5% CO₂, and 94% nitrogen. The number of colonies is evaluated under each condition following addition of 50% Alamar Blue and fluorescence detection.

Animal Study:[1]

<table>
<thead>
<tr>
<th>Animal Models</th>
<th>B16F10 tumor cells (2 × 10^5) are implanted via i.v. tail vein injection into athymic nude mice (Ncr or BALB/c) 5 to 8 weeks old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>0.9% normal saline</td>
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<tr>
<td>Doses</td>
<td>100 mg/kg</td>
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<td>Administration</td>
<td>Administered via oral gavage</td>
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</table>

References


Customer Reviews

Dr. Zhang of Tianjin Medical University
Foretinib (GSK1363089, XL880) purchased from Selleck.
After starved in serum-free medium for 24h, MDA-MB-231 cells incubated with the indicated concentrations of XL-880 for 3h, followed by 20-minute stimulation of 100ng/ml EGF

Additional Notes

- Please keep the product under -20°C for long-term storage.

- Not for human, veterinary diagnostic or therapeutic use.

- Specific storage and handling information for each product is indicated on the product datasheet. Most Selleck products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality, but save your shipping charges by using the most economical storage conditions for an overnight shipment. Upon receipt of the product, follow the storage recommendations on the product datasheet.