G-5555

Cat. No.: HY-19635
CAS No.: 1648863-90-4
Molecular Formula: C₂₅H₂₅ClN₆O₃
Molecular Weight: 492.96
Target: PAK
Pathway: Cell Cycle/DNA Damage; Cytoskeleton
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: ≥ 27 mg/mL (54.77 mM)
≥" means soluble, but saturation unknown.

Preparation of Stock Solutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>2.0286 mL</td>
<td>10.1428 mL</td>
<td>20.2856 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.4057 mL</td>
<td>2.0286 mL</td>
<td>4.0571 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.2029 mL</td>
<td>1.0143 mL</td>
<td>2.0286 mL</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% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (5.07 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (5.07 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (5.07 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
G-5555 is a potent p21-activated kinase 1 (PAK1) inhibitor with \( K_i \)s of 3.7 nM and 11 nM for PAK1 and PAK2, respectively.

IC₅₀ & Target
<table>
<thead>
<tr>
<th>IC₅₀ Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK1</td>
</tr>
<tr>
<td>PAK2</td>
</tr>
</tbody>
</table>
**In Vitro**

G-5555 is a potent PAK1 inhibitor with a $K_i$ of 3.7 nM. G-5555 shows excellent kinase selectivity and inhibits only eight out of the 235 kinases tested other than PAK1 with inhibition >70%: PAK2, PAK3, KHS1, Lck, MST3, MST4, SIK2, and YSK1. The IC$_{50}$s of G-5555 against SIK2, PAK2, KHS1, MST4, YSK1, MST3 and Lck are 9, 11, 10, 20, 34, 43, 52 nM, respectively. In general, G-5555 demonstrates high selectivity for the group I PAKs. There is negligible activity for G-5555 against the hERG channel with IC$_{50}$ more than 10 $\mu$M in a patch clamp assay. G-5555 potently inhibits PAK2, with a $K_i$ of 11 nM. In an array of 23 breast cancer cell lines, G-5555 has significantly greater growth inhibitory activity in cell lines that are PAK-amplified compared to non-amplified lines.

**In Vivo**

G-5555 exhibits low blood clearance and an acceptable half-life. Good oral exposure (AUC = 30 $\mu$M•h) and high oral bioavailability (F = 80%) are achieved. In an H292 non-small cell lung cancer (NSCLC) xenograft study in mice, G-5555 inhibits phosphorylation of the PAK1/2 downstream substrate mitogen-activated protein kinase 1 (MEK1) S298 and, when administered at an oral dose of 25 mg/kg b.i.d., imparts 60% tumor growth inhibition in this model and a PAK1 amplified breast cancer xenograft model, MDAMB-175.

**PROTOCOL**

**Kinase Assay** [1]

The 10 µL assay mixtures contain 50 mM HEPES (pH 7.5), 0.01% Brij-35, 10 mM MgCl$_2$, 1 mM EGTA, 2 µM FRET peptide substrate, and PAK enzyme (20 pM PAK1; 50 pM PAK2; 90 pM PAK4). Incubations are carried out at 22°C in black polypropylene 384-well plates. Prior to the assay, enzyme, FRET peptide substrate and serially diluted test compounds (G-5555, etc.) are preincubated together in assay buffer (7.5 µL) for 10 minutes, and the assay is initiated by the addition of 2.5 µL assay buffer containing 4× ATP (160 µM PAK1; 480 µM PAK2; 16 µM PAK4). Following the 60-minute incubation, the assay mixtures are quenched by the addition of development reagent, and 1 hour later the emissions of Coumarin (445 nm) and Fluorescein (520 nm) are determined after excitation at 400 nm.

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

**Animal Administration** [1]

Mice

Three mice in each of the two groups are administered 25 mg/kg oral suspension dose twice, with the second dose given 6 hours after the first dose. The dose volumes are 5 mL/kg for the IV group and 10 mL/kg for the PO groups. Following administration of G-5555, 15 µL of blood is collected at each time point are stored at -70 to -80°C until analysis.

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

**CUSTOMER VALIDATION**


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**REFERENCES**


[2]. Rudolph J, et al. Chemically Diverse Group I p21-Activated Kinase (PAK) Inhibitors Impart Acute Cardiovascular Toxicity with a Narrow Therapeutic