A-674563 hydrochloride

Cat. No.: HY-13254A
Molecular Formula: C₂₂H₂₃ClN₄O
Molecular Weight: 394.9
Target: Akt
Pathway: PI3K/Akt/mTOR
Storage: 4°C, stored under nitrogen
* In solvent: -80°C, 6 months; -20°C, 1 month (stored under nitrogen)

SOLVENT & SOLUBILITY

In Vitro
DMSO : 100 mg/mL (253.23 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass 1 mg</th>
<th>Mass 5 mg</th>
<th>Mass 10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>2.5323 mL</td>
<td>12.6614 mL</td>
<td>25.3229 mL</td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>0.5065 mL</td>
<td>2.5323 mL</td>
<td>5.0646 mL</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>0.2532 mL</td>
<td>1.2661 mL</td>
<td>2.5323 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% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.33 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.33 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
A-674563 hydrochloride is a potent and selective Akt1 inhibitor with Ki of 11 nM.

<table>
<thead>
<tr>
<th>IC₅₀ &amp; Target</th>
<th>Akt1</th>
<th>PKA</th>
<th>CDK2</th>
<th>GSK3β</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (Ki)</td>
<td>11 nM</td>
<td>16 nM</td>
<td>46 nM</td>
<td>110 nM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERK2</td>
<td>260 nM</td>
<td>PKCδ</td>
<td>RSK2</td>
<td>MAPK-AP2</td>
</tr>
<tr>
<td>(Ki)</td>
<td></td>
<td>360 nM</td>
<td>580 nM</td>
<td>11 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKCγ</td>
<td>1.2 μM</td>
<td>Chk1</td>
<td>CK2</td>
<td>SRC</td>
</tr>
<tr>
<td>(Ki)</td>
<td></td>
<td>2.6 μM</td>
<td>5.4 μM</td>
<td>13 μM</td>
</tr>
</tbody>
</table>
### In Vitro

A-674563 slows proliferation of tumor cells with an EC\(_{50}\) of 0.4 \(\mu\)M\(^1\). A563 (0-10 \(\mu\)M) significantly decreases GSK3 and MDM2 phosphorylation in STS cells. A563 shows inhibitory effect on all STS cell lines, with IC\(_{50}\) values at 48 hours ranging from 0.22±0.034 \(\mu\)M (SW684) to 0.35 ±0.06 \(\mu\)M (SKLMS1). A563 induces G2 cell cycle arrest and apoptosis in STS cells. A563 (1 \(\mu\)M/12 hr) upregulates the expression of GADD45A independent of p53\(^2\). A-674563 (10-1000 nM) is anti-proliferative and cytotoxic in cultured human melanoma cells, induces melanoma cell apoptotic death, inhibited by caspase inhibitors, and inhibits melanoma cells via Akt-dependent and -independent mechanisms\(^3\). A-674563 is cytotoxic and anti-proliferative when added to U937 and Aml progenitor cells, activates caspase-3/9 and apoptosis in U937 and Aml progenitor cells, and manipulates other signalings in Aml cells while blocking Akt\(^4\).

### In Vivo

A-674563 (40 mg/kg/d, p.o.) shows no significant monotherapy activity, but the efficacy of the combination therapy (A-674563+paclitaxel) is significantly improved in the PC-3 prostate cancer xenograft model. A-674563 (20, 100 mg/kg) increases plasma insulin in an oral glucose tolerance test\(^1\). A563 (20 mg/kg/bid; p.o.) exhibits slow tumor growth and a significant difference in tumor volume without significant weight loss of mice. A563-treated tumors express increased levels of GADD45\(\alpha\) and decreased levels of PCNA (a nuclear marker for proliferation). Additionally, TUNEL assay staining levels (marker for apoptosis) increase in the A563-treated specimens\(^2\). A-674563 (25, 100 mg/kg, lavage daily) potently inhibits A375 xenograft growth in mice\(^3\). A-674563 (15, 40 mg/kg) injection inhibits U937 xenograft in vivo growth, and improves mice survival\(^4\).

### Protocol

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

The cells on 96-well plates are gently washed with 200 \(\mu\)L of PBS. Alamar Blue reagent is diluted 1:10 in normal growth media. The diluted Alamar Blue reagent (100 \(\mu\)L) is added to each well on the 96-well plates and incubated until the reaction is complete. Analysis is done using an fmax Fluorescence Microplate Reader, set at the excitation wavelength of 544 nm and emission wavelength of 595 nm. Data are analyzed using SOFTmax PRO software. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

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

Immunocompromised male scid mice are at 6 to 8 weeks of age. The 1×10\(^6\) 3T3-Akt1 or 2×10\(^6\) MiaPaCa-2 and PC-3 cells in 50% Matrigel are inoculated s.c. into the flank. For early treatment studies, mice are randomly assigned to treatment groups and therapy is initiated the day after inoculation. Ten animals are assigned to each group, including controls. For established tumor studies, tumors are allowed to reach a designated size and mice are assigned to treatment groups of equal tumor size (n=10 mice per group). Tumor size is evaluated by twice weekly measurements with digital calipers. Tumor volume is estimated using the formula: V=L×W\(^2\)/2. A-443654 is given s.c. in a vehicle of 0.2% HPMC. A-674563 is given orally in a vehicle of 5% dextrose. Gemcitabine and paclitaxel are added to the assay. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

### Customer Validation


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### References


2. Zhu QS, et al. Soft tissue sarcoma cells are highly sensitive to AKT blockade: a role for p53-independent up-regulation of GADD45 alpha. Cancer Res,