AV-412

Cat. No.: HY-10346
CAS No.: 451493-31-5
Molecular Formula: C₄₁H₄₄ClFN₆O₇S₂
Molecular Weight: 851.41
Target: EGFR
Pathway: JAK/STAT Signaling; Protein Tyrosine Kinase/RTK
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 : ≥ 28 mg/mL (32.89 mM)
* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

<table>
<thead>
<tr>
<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>1.1745 mL</td>
<td>5.8726 mL</td>
<td>11.7452 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.2349 mL</td>
<td>1.1745 mL</td>
<td>2.3490 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.1175 mL</td>
<td>0.5873 mL</td>
<td>1.1745 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 (2.94 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (2.94 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
AV-412 (MP412) is an EGFR inhibitor with IC₅₀s of 0.75, 0.5, 0.79, 2.3, 19 nM for EGFR, EGFR⁸⁵⁸⁸R, EGFR⁷⁹⁰M, EGFR⁸⁵⁸⁸R/⁷⁹⁰M and ErbB2, respectively.

IC₅₀ & Target

<table>
<thead>
<tr>
<th>IC₅₀ &amp; Target</th>
<th>EGFR 0.75 nM (IC₅₀)</th>
<th>EGFR⁸⁵⁸⁸R 0.5 nM (IC₅₀)</th>
<th>EGFR⁷⁹⁰M 0.79 nM (IC₅₀)</th>
<th>EGFR⁸⁵⁸⁸R/⁷⁹⁰M 2.3 nM (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ERB2 19 nM (IC₅₀)</td>
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</tbody>
</table>
In Vitro

AV-412 inhibits autophosphorylation of EGFR and ErbB2 with IC$_{50}$ of 43 and 282 nM, respectively. AV-412 also inhibits epidermal growth factor (EGF)-dependent cell proliferation with an IC$_{50}$ of 100 nM. AV-412 abrogates EGFR signaling in the gefitinib-resistant H1975 cell line, which harbors a double mutation of L858R and T790M in EGFR$^{[1]}$.

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

In Vivo

In animal studies using cancer xenograft models, AV-412 (30 mg/kg) demonstrates complete inhibition of tumor growth of the A431 and BT-474 cell lines, which overexpress EGFR and ErbB2, respectively. AV-412 suppresses autophosphorylation of EGFR and ErbB2 at the dose corresponding to its antitumor efficacy. When various dosing schedules are applied, AV-412 shows significant effects with daily and every-other-day schedules, but not with a once-weekly schedule, suggesting that frequent dosing is preferable for this compound. Furthermore, AV-412 shows a significant antitumor effect on the ErbB2-overexpressing breast cancer KPL-4 cell line, which is resistant to gefitinib$^{[1]}$.

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PROTOCOL

Kinase Assay $^{[1]}$

Recombinant intracellular kinase domains of EGFR, EGFR$_{L858R}$, EGFR$_{T790M}$, EGFR$_{L858R/T790M}$, and purified EGFR from A431 cell membranes are used. Kinase reactions are carried out in 8 mM MOPS (pH 7.0), 0.2 mM ethylenediaminetetraacetic acid (EDTA), 10 mM MnCl$_2$, 10 mM Mg acetate, 0.1 mg/mL poly(Glu, Tyr) 4:1, $[^{33}P]$-ATP, and 5–10 mU of enzyme, except that 250 µM of the GGMEDIYFEFMGGKKK peptide substrate is used for EGFR$_{T790M}$. Phosphorylation is initiated by the addition of ATP and is allowed to proceed for 40 min at room temperature. The reaction is stopped by the addition of 3% phosphoric acid, then aliquots of the reaction mixture are spotted onto a filtermat. After rinsing to remove peptides bound non-specifically, the filter is scintillation counted$^{[1]}$.

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Cell Assay $^{[1]}$

To test the effects of AV-412 on growth factor-dependent cell proliferation, A431 and A7r5 cells are cultured for 24 h at 37°C in the presence of 1 ng/mL epidermal growth factor and 50 ng/mL platelet-derived growth factor, respectively. The $^3$H-thymidine incorporation during this period is measured$^{[1]}$.

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Animal Administration $^{[1]}$

Mice: For studies examining the dosing schedule in relation to efficacy against TE-8 tumors, AV-412 is administered either once daily, every other day, or once per week for 2 weeks. Mice are killed 1 day after the final treatment, and the tumors are dissected and weighed. For evaluation of tumor phosphorylation, tumor-bearing mice are given a single administration of AV-412 and tumors are dissected 4 h later$^{[1]}$.

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REFERENCES