PP2

Cat. No.: HY-13805
CAS No.: 172889-27-9
Molecular Formula: C_{15}H_{16}ClN_{5}
Molecular Weight: 301.77
Target: Src
Pathway: Protein Tyrosine Kinase/RTK
Storage: Powder -20°C 3 years
         4°C 2 years
         In solvent -80°C 1 year
                     -20°C 6 months

SOLVENT & SOLUBILITY

In Vitro
DMSO: 50 mg/mL (165.69 mM; Need ultrasonic)
H$_2$O: < 0.1 mg/mL (insoluble)

Preparation of Stock Solutions

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Mass (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>3.3138 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.6628 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.3314 mL</td>
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<tr>
<td>1 mM</td>
<td>3.3138 mL</td>
<td>16.5689 mL</td>
<td>33.1378 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.6628 mL</td>
<td>3.1138 mL</td>
<td>6.6276 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.3314 mL</td>
<td>1.6569 mL</td>
<td>3.3138 mL</td>
</tr>
</tbody>
</table>

In Vivo
1. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 3 mg/mL (9.94 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
PP2 is a reversible and ATP-competitive Src family kinases inhibitor with IC$_{50}$s of 4 and 5 nM for Lck and Fyn, respectively.

IC$_{50}$ & Target
IC$_{50}$: 4 nM (Lck), 5 nM (Fyn)$^{[1]}$

In Vitro
At 10 μM, the effect of PP2 on cellular proliferation is not significant, indicating that, at this low concentration, the effect of PP2 on Gemcitabine cytotoxicity does not simply reflect a direct antiproliferative effect, but rather a potentiation of Gemcitabine-induced cytotoxicity. Above 20 μM, growth is increasingly suppressed, a finding consistent with reports in other human cancer cell lines. Although 10 μM PP2 is used in our study, at higher concentrations PP2 is reported to inhibit other intracellular kinases$^{[2]}$. PP2 is the most widely used commercially available Src family kinase inhibitor. PP2 inhibits Src family kinase activity with IC$_{50}$ of ~5 nM in vitro, concentrations to 10 μM are often necessary to achieve complete Src family kinase inhibition in cell culture$^{[3]}$.  


In Vivo

The tumor growth inhibition rate is 25% in the PP2 treatment group and 5% in the Gemcitabine treatment group (P>0.05). When administered in combination, PP2 and Gemcitabine produce a tumor growth inhibition rate of 98% (P<0.05). Hepatic metastasis occurred in 100% of control and Gemcitabine-treated groups; 88% of the PP2-treated group developed liver metastases. There are no detectable metastases in the group treated with PP2 and Gemcitabine in combination (P<0.05)\(^2\).

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

PROTOCOL

Cell Assay \(^2\)

Cell growth is determined by MTT assay and confirmed by cell counting. Results of the MTT assay have been shown to correlate well with \(^3\)Hthyidine incorporation in pancreatic cancer cell lines. Logarithmically growing cells are plated at 5×10\(^3\) cells/well in 96-well plates, allowed to adhere for 24 h, and cultured in the presence or absence of PP2 and Gemcitabine. Cell proliferation is determined after 96 h. Plates are read using a V\(_{\text{max}}\) microplate spectrophotometer at a wavelength of 570 nm corrected to 650 nm and normalized to controls. Each independent experiment is performed three times, with 10 determinations for each condition tested. The IC\(_{50}\) of Gemcitabine is calculated from these results. At identical time points, cells are trypsinized to form a single cell suspension. Intact cells, determined by trypan blue exclusion, are counted using a Neubauer hemocytometer, and the number of cells per mL is calculated and compared with the control group to confirm the MTT results\(^2\).

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Animal Administration \(^2\)

Mice\(^2\)

Male athymic nu/nu mice (age, 5 weeks; weight, 20-22 g; specific pathogen free) are anesthetized with i.p. ketamine (200 mg/kg) and xylazine (10 mg/kg) and inoculated with 10\(^6\) Gemcitabine-resistant PANC1\(^{\text{GemRes}}\) cells in 20 \(\mu\)L of PBS by surgical orthotopic implantation into the pancreas. After inoculation, mice are randomized to three treatment groups: (a) treatment group 1 (n=8) receive 2 mg/kg PP2 in 1% DMSO by i.p. injection three times a week; (b) treatment group 2 (n=8) receive Gemcitabine (100 mg/kg) in the same volume of 1% DMSO vehicle as received by group 1, three times a week; and (c) treatment group 3 (n=8) receive 2 mg/kg PP2 and 100 mg/kg Gemcitabine in the same volume of DMSO as groups 1 and 2, three times a week. The control group receive the same volume of 1% DMSO vehicle as the other groups, three times a week. Treatment is commenced 1 day after implantation, and necropsy is performed 4 weeks after implantation. Primary tumors are identified, weighed, and normalized to total body mass. Tumor growth inhibition rate is calculated using the following formula: tumor growth inhibition rate (\%)=(1−M\(_T\)/M\(_C\))×100, where M\(_T\) and M\(_C\) are the mean normalized tumor masses of treatment and control groups, respectively. Liver metastases are counted and confirmed histologically.

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REFERENCES

