Preladenant

Cat. No.: HY-10889
CAS No.: 377727-87-2
Molecular Formula: C₂₅H₂₉N₉O₃
Molecular Weight: 503.56
Target: Adenosine Receptor
Pathway: GPCR/G Protein
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 : 5 mg/mL (9.93 mM; Need ultrasonic)
H₂O : < 0.1 mg/mL (insoluble)

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>1.9859 mL</td>
<td>9.9293 mL</td>
<td>19.8586 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>0.3972 mL</td>
<td>1.9859 mL</td>
<td>3.9717 mL</td>
</tr>
<tr>
<td>10 mM</td>
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</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: 0.5 mg/mL (0.99 mM); Suspended solution; Need ultrasonic
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 0.5 mg/mL (0.99 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 0.5 mg/mL (0.99 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
Preladenant is a potent and competitive antagonist of the human adenosine A₂A receptor with a \( K_i \) of 1.1 nM and has over 1000-fold selectivity over other adenosine receptors.

IC₅₀ & Target
\[ K_i: 1.1 \text{ nM (Adenosine A}_2\text{A receptor)} \]

In Vitro
Preladenant also completely antagonizes cAMP in cells expressing the recombinant human A₂A receptor. Preladenant is
Preladenant (1 mg/kg) inhibits L-Dopa-induced behavioral sensitization after repeated daily administration, which suggests a reduced risk of the development of dyskinesias. Preladenant exhibits antidepressant-like profiles in models of behavioral despair, namely the mouse tail suspension test and the mouse and rat forced swim tests[1]. Preladenant produces a dose-dependent reduction in parkinsonian scores at doses of 1 mg/kg (min score: 9.0) and 3 mg/kg (min score: 6.5). A subthreshold dose of Preladenant reduces minimum and mean parkinsonian scores in animals treated with 3 mg kg of L-Dopa to 5.25 and 6.88 respectively. A Wilcoxon test is used to compare individual treatments against vehicle. Preladenant (3 mg/kg), L-Dopa (3, 6, and 12 mg/kg), and the combination of Preladenant and L-Dopa (1 or 3 mg/kg+3 mg/kg) are all significantly improved on both minimum and mean parkinsonian scores relative to the 3 mg/kg L-Dopa group[2].

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

### In Vivo

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### PROTOCOL

**Kinase Assay**[1]

Receptor binding is performed using membranes prepared from cells with recombinant expression of adenosine receptors as follows: human A2A and HEK 293, rat A2A and Chinese hamster ovary, human and rat A1 and Chinese hamster ovary, and human A3 and HEK 293. Radioligand competition binding assays are performed in 96-well plates in a total assay volume of 200 μL using a final test drug concentration range of between 0.1 and 3 μM. Membranes are diluted in assay buffer, pH 7.4 (A1 and A2A, Dulbecco’s phosphate-buffered saline with 10 mM MgCl2; A3, 50 mM Tris-HCl, 120 mM NaCl, 10 mM MgCl2). To remove endogenous adenosine from the membrane preparations, 4 U/mL adenosine deaminase is added to the membranes, which are then incubated at room temperature for 15 min. Radioligand is added to a final concentration of 0.5 ([3H]SCH 58261, A2A), 1 ([3H]DPCPX, A1), or 0.25 ([125I]IB-MECA, A3) nM. Nonspecific binding is defined by adding 100 nM CGS 15923 (A2A), 100 nM NECA (A1), or 100 nM DPCPX (A3). Plates are incubated at room temperature with agitation for 1.5 h (A2A and A1) or 2 h (A3). Membranes are filtered onto Packard GF-B filter plates and washed in ice-cold assay buffer using a Brandel cell harvester to separate bound and free radioligand. The plates are dried before addition of 45 μL of Microscint 20 to each well. IC50 values are determined by fitting the displacement curves using an iterative curve-fitting program. Kᵢ values are calculated using the Cheng-Prusoff equation[1].

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**Cell Assay**[1]

HEK 293 cells stably expressing either human A2A or A2B receptors are grown to confluence, harvested using enzyme-free cell dissociation buffer and pelleted by centrifugation (1000g; 5 min). The cells are washed and diluted to a final density of 4×10⁶ cells/mL in Hanks’ balanced salt solution supplemented with 10 mM HPS, pH 7.4, 5 mM MgCl₂, and 0.2% bovine serum albumin. Preladenant is diluted in the above buffer with inclusion of the following components to achieve the respective final assay concentrations: 0.25% DMSO, 2 U/mL adenosine deaminase, and 100 μM Ro 201724. Cell suspensions (20 μL) are preincubated for 15 min at room temperature in 96-well plates containing 25 μL of vehicle or Preladenant. CGS-21680 (A2A) or 5-N-cyclopropylcarboxamidoadenosine (A2B) at 10-fold the desired concentration is then added, and the reactions are incubated for 15 min at 37°C. The reactions are terminated by the addition of 50 μL of assay/lysis buffer. The concentration response curves for CGS-21680 in the presence and absence of Preladenant are plotted, and the EC50 values are determined by fitting the curves using GraphPad Prism software[1].

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**Animal Administration**[1][2]

Mice and Rats[1]

Male CD rats and male CD1 mice are used. Preladenant is administered orally in 50% polyethylene glycol 400 at a dose volume of 3 to 5 mL/kg. In the forced swim test (FST), mice are placed individually into glass cylinders filled to a depth of 10 cm with water (25°C) and left for 6 min. A mouse is judged to be immobile when it floats in an upright position and made despair, namely the mouse tail suspension test and the mouse and rat forced swim tests[1]. Preladenant produces a dose-dependent reduction in parkinsonian scores at doses of 1 mg/kg (min score: 9.0) and 3 mg/kg (min score: 6.5). A subthreshold dose of Preladenant reduces minimum and mean parkinsonian scores in animals treated with 3 mg kg of L-Dopa to 5.25 and 6.88 respectively. A Wilcoxon test is used to compare individual treatments against vehicle. Preladenant (3 mg/kg), L-Dopa (3, 6, and 12 mg/kg), and the combination of Preladenant and L-Dopa (1 or 3 mg/kg+3 mg/kg) are all significantly improved on both minimum and mean parkinsonian scores relative to the 3 mg/kg L-Dopa group[2].

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SCH 412348 1 h before behavioral testing. Each rat is placed individually in a cylinder of water (25°C) and left to swim for 15 min before being removed and dried in a heated enclosure and returned its home cage. Twenty-four hours later (test day), the animal is re-exposed to the conditions, and the total immobility time during a 5-min period is recorded. In addition, the duration of time that the rats spent climbing the sides of the cylinder is recorded. On test day, each animal is dosed with Preladenant, SCH 412348, or vehicle 1 h before behavioral testing.

Monkeys

Six female cynomolgus (Macaca fascicularis) monkeys (weighing 3.5-4.2 kg) are used. The animals are rendered parkinsonian by subcutaneous (sc) administrations of MPTP (2-3 mg/kg) once per week until a stable parkinsonian syndrome (unchanged disability score of 8 or greater for at least a month) developed as measured by a parkinsonian disability scale. At least 2 months after the final administration of MPTP, the monkeys are treated chronically with Prolopa (L-Dopa/benserazide, 100/25 mg) until clear and reproducible dyskinesias developed. The present experiment with L-Dopa and Preladenant (1 mg/kg and 3 mg/kg, p.o.) is performed in these monkeys.

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**REFERENCES**


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**CAUTION:** Product has not been fully validated for medical applications. For research use only.

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