**BIOLOGICAL ACTIVITY:**

Preladenant is a potent competitive antagonist of the human \textit{A2A} receptor (\textit{K}_i=1.1 \text{nM}) and has >1000–fold selectivity over all other adenosine receptors.

IC50 & Target: \textit{K}_i: 1.1 \text{nM} (Adenosine \textit{A2A} receptor)[1]

**In Vitro:** Preladenant also completely antagonizes cAMP in cells expressing the recombinant human \textit{A2A} receptor. Preladenant is determined to have \textit{K}_B values of 1.3 \text{nM} at the \textit{A2A} receptor; the value is in good agreement with the \textit{K}_i value determined in the radioligand binding assay. A similar functional assay with \textit{A2B} receptor–expressing cells is used to demonstrate selectivity over \textit{A2B} receptors. In this assay, the \textit{K}_B value for Preladenant is 1.2 \text{μM}, indicating that Preladenant is 923–fold selective for the \textit{A2A} receptor over the \textit{A2B} receptor[1].

**In Vivo:** 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 test[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 the minimum parkinsonian score. In addition, both the 12 mg/kg L–Dopa and L–Dopa+Preladenant groups are significantly improved on both minimum and mean parkinsonian scores relative to the 3 mg/kg L–Dopa group[2].

**PROTOCOL** (Extracted from published papers and Only for reference)

**Kinase Assay:**[1] Receptor binding is performed using membranes prepared from cells with recombinant expression of adenosine receptors as follows: human \textit{A2A} and HEK 293, rat \textit{A2A} and Chinese hamster ovary, human and rat \textit{A1} and Chinese hamster ovary, and human \textit{A3} and HEK 293. Radioligand competition binding assays are performed in 96–well plates in a total assay volume of 200 \text{μL} using a final test drug concentration range of between 0.1 and 3 \text{μM}. Membranes are diluted in assay buffer, pH 7.4 (A1 and A2A, Dulbecco’s phosphate–buffered saline with 10 mM MgCl\textsubscript{2}; A3, 50 mM Tris–HCl, 120 mM NaCl, 10 mM MgCl\textsubscript{2}). 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 ([\textsuperscript{3}H]SCH 58261, \textit{A2A}), 1 ([\textsuperscript{3}H]DPCPX, \textit{A1}), or 0.25 ([\textsuperscript{125}I]AB–MECA, \textit{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 \text{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\[^1\].

**Cell Assay:** Preladenant is dissolved in DMSO and stored, and then diluted with appropriate media before use\[^1\]. 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 4×10^5 cells/mL in Hanks' balanced salt solution supplemented with 10 nM 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 EC\(_{50}\) values are determined by fitting the curves using GraphPad Prism software\[^1\].

**Animal Administration:** Preladenant is prepared in 50% polyethylene glycol 400 (Rat and Mice)\[^1\]. Preladenant is dissolved in cyclodextrin, sonicated and administered p.o. for the study (Monkey)\[^2\]. Mice and Rat\[^1\]. 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 only small movements to keep its head above water. The duration of immobility is recorded during the last 4 min of the 6–min testing period by an observer blind to the treatment.

**References:**


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

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