上海源叶生物科技有限公司
Shanghai yuanye Bio-Technology Co., Ltd
电话：021-61312973　　传真：021-55068248
网址：www.shyuanye.com
邮箱：shyysw@sina.com

产品名称：Preladenant
产品别名：瑞德南特；SCH-420814

生物活性：

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.
IC_{50} & Target	Ki: 1.1 nM (Adenosine A_{2A} receptor)[1]
In Vitro	Preladenant also completely antagonizes cAMP in cells expressing the recombinant human A_{2A} receptor. Preladenant is determined to has K_B values of 1.3 nM at the A_{2A} receptor; the value is in good agreement with the $K_i$value determined in the radioligand binding assay. A similar functional assay with A_{2B} receptor-expressing cells is used to demonstrate selectivity over A_{2B} receptors. In this assay, the K_B value for Preladenant is 1.2 μM, indicating that Preladenant is 923-fold selective for the A_{2A} receptor over the A_{2B} 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 Wilcoxin 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].
Solvent&Solubility	***In Vitro:*** **DMSO： 5 mg/mL** (**9.93 mM**; Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	1.9859 mL	9.9293 mL	19.8586 mL
5 mM	0.3972 mL	1.9859 mL	3.9717 mL
10 mM	---	---	---

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出

	现沉淀、析出现象，可以通过加热和/或超声的方式助溶 1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: 0.5 mg/mL (0.99 mM); Suspended solution; Need ultrasonic 此方案可获得 0.5 mg/mL (0.99 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 5.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: 0.5 mg/mL (0.99 mM); Suspended solution; Need ultrasonic 此方案可获得 0.5 mg/mL (0.99 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 5.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 0.5 mg/mL (0.99 mM); Clear solution 此方案可获得 ≥ 0.5 mg/mL (0.99 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 5.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Hodgson RA, et al. Characterization of the potent and highly selective A2A receptor antagonists preladenant and SCH 412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent [2]. Hodgson RA, et al. Preladenant, a selective A(2A) receptor antagonist, is active in primate models of movement disorders. Exp Neurol. 2010 Oct;225(2):384-90.

实验参考：

Cell Assay	HEK 293 cells stably expressing either human A_{2A} or A_{2B} 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 4×10^6 cells/mL in Hanks' balanced salt solution supplemented with 10 nM HPS, pH 7.4,, 5 mM $MgCl_2$, 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 (A_{2A}) or 5-N-cyclopropylcarboxamidoadenosine (A_{2B}) 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].
	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 only small

Animal Administration	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 of the animals. Animals are dosed with vehicle, Preladenant, or 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[2] 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.
Kinase Assay	Receptor binding is performed using membranes prepared from cells with recombinant expression of adenosine receptors as follows: human A_{2A} and HEK 293, rat A_{2A} and Chinese hamster ovary, human and rat A1 and Chinese hamster ovary, and human A_3 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 (A_1 and A_{2A}, Dulbecco's phosphate-buffered saline with 10 mM $MgCl_2$; A_3, 50 mM Tris-HCl, 120 mM NaCl, 10 mM $MgCl_2$). 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 ([^{3}H]SCH 58261, A_{2A}), 1 ([^{3}H]DPCPX, A_1), or 0.25 ([^{125}I]AB-MECA, A_3) nM. Nonspecific binding is defined by adding 100 nM CGS 15923 (A_{2A}), 100 nM NECA (A_1), or 100 nM DPCPX (A_3). Plates are incubated at room temperature with agitation for 1.5 h (A_{2A} and A_1) or 2 h (A_3). 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. IC_{50} values are determining by fitting the displacement curves using an iterative curve-fitting program. K_i values are calculated using the Cheng-Prusoff equation[1].
References	[1]. Hodgson RA, et al. Characterization of the potent and highly selective A2A receptor antagonists preladenant and SCH 412348 [7-[2-[4-2,4-difluorophenyl]-1-piperazinyl]ethyl]-2-(2-furanyl)-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c] pyrimidin-5-amine] in rodent [2]. Hodgson RA, et al. Preladenant, a selective A(2A) receptor antagonist, is active in primate models of movement disorders. Exp Neurol. 2010 Oct;225(2):384-90.