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产品名称：Capadenoson
产品别名：卡帕诺生 ；BAY 68-4986

生物活性：

Description	Capadenoson is a selective agonist of adenosine-A1 receptor.
IC_{50} & Target	Adenosine A1 receptor[1]
In Vitro	To further elucidate the pharmacological properties of Capadenson, GTP shift assays are performed with the standard full A1-agonist CCPA and the A1-antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX). CCPA shows a $K_i$value of 4.2 nM in the binding assay on rat cortical brain membranes. In the presence of 1 mM GTP this K_i value shifts to a value of 64 nM. Therefore the GTP shift for CCPA is 15. DPCPX shows a GTP shift of 1 with virtually identical K_i values in the absence and presence of GTP. Capadenson shows a K_i value of 24 nM in the binding assay. In the presence of 1 mM GTP this K_i value shifts to a value of 116 nM resulting in a GTP shift of 5 for Capadenoson[1].
In Vivo	In the in vivo experiments, Wistar rats and SHR are pre-treated with Capadenoson at a concentration of 0.15 mg/kg for 5 days. On day 5, a stress test (physical restraint) is performed for 2 hours. The plasma concentration of Capadenoson measured 3 hours after drug intake remains constant in the 5 days prior to the restraint stress test and averaged 7.63 μg/L on day 4 and 5, respectively[1].

Solvent&Solubility

In Vitro:

DMSO : ≥ 50 mg/mL (**96.15 mM**)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.9230 mL	9.6148 mL	19.2297 mL
	5 mM	0.3846 mL	1.9230 mL	3.8459 mL
	10 mM	0.1923 mL	0.9615 mL	1.9230 mL

*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: 2.5 mg/mL (4.81 mM); Suspended solution; Need ultrasonic and warming

此方案可获得 2.5 mg/mL (4.81 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 25.0 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% corn oil Solubility: ≥ 2.5 mg/mL (4.81 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.81 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 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Bott-Flügel L, et al. Selective attenuation of norepinephrine release and stress-induced heart rate increase by partial adenosine A1 agonism. PLoS One. 2011 Mar 28;6(3):e18048. [2]. Bailey IR, et al. Optimization of Thermolytic Response to A1 Adenosine Receptor Agonists in Rats. J Pharmacol Exp Ther. 2017 Sep;362(3):424-430.

实验参考：

Animal Administration	Rats[1] A total of 14 Wistar rats and 18 SHR (body weight 200-50 g, all female) underwent experiments to evaluate the exocytotic, stimulation-induced NE release during electrical field stimulation. Rats are killed by an injection of pentobarbital i.p. (0.5 mL/100 mg body weight), and hearts are rapidly excised, and placed in ice cold Krebs-Henseleit solution (KHL). They are quickly mounted on a Langendorff apparatus for retrograde perfusion with KHL. Perfusion rate is kept constant at 10 mL/min, the temperature is adjusted to 37°C, and the pH to 7.4 through bubbling with 5% CO_2/95% O_2. Via an inflow line desipramine at a concentration of 10^{-7} M is added to the perfusion buffer. After an equilibration period of 20 minutes, electrical field stimulation is commenced via two metal paddles adjacent to both sides of the beating heart for 1 minute (5V, 6 Hz). We collected the efflux in plastic tubes the minute before, during, and 3 minutes after the stimulation. These are rapidly frozen in liquid nitrogen and stored at −20°C till analysis. The NE release is calculated as the cumulative release induced by the electrical stimulation. After the first stimulation (S1), the study drug Capadenoson at concentrations of 30 μg/L (6×10^{-8} M) or 300 μg/L(6×10^{-7} M), or 2-chloro-N6-cyclopentyladenosine (CCPA, 10−6 M), respectively, are added via separate perfusion lines for 30 minutes. After this time a second stimulation (S2) is executed to determine the effect of the drugs on NE release compared to the first stimulation. The effect of each pharmacological intervention is analysed by calculating the ratio of NE release induced by the second and first stimulation (S2/S1 ratio).
Kinase Assay	Membranes from the human cortex are prepared. [^{35}S]GTPγS binding is measured. Briefly, 5 μg of membrane protein is incubated in a total volume of 160 μL for 2 hr at 25°C in a shaking water bath. [^{35}S]GTPγS binding in control incubations and in the presence of capadenoson showed a linear time course up to this incubation time. Binding buffer contained 50 mM Tris/HCl, pH 7.4, 2 mM triethanolamine, 1 mM EDTA, 5 mM $MgCl_2$, 10 μM GDP, 1 mM dithiothreitol, 100 mM NaCl, 0.2 units/mL adenosine deaminase, 0.2 nM [^{35}S]GTPγS, and 0.5% bovine serum albumin. Non-specific binding is determined in the presence of 10 μM GTPγS. Incubations are terminated through filtration of the samples over multiscreen FB glass fiber filters followed by two washes with binding buffer. The filters are dried, coated with scintillator and counted for radioactivity. Binding curves of [^{35}S]GTPγS are analyzed by nonlinear regression using GraphPad Prism[1].

References	[1]. Bott-Flügel L, et al. Selective attenuation of norepinephrine release and stress-induced heart rate increase by partial adenosine A1 agonism. PLoS One. 2011 Mar 28;6(3):e18048. [2]. Bailey IR, et al. Optimization of Thermolytic Response to A1 Adenosine Receptor Agonists in Rats. J Pharmacol Exp Ther. 2017 Sep;362(3):424-430.