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产品名称: **Bay 60-7550**  
 产品别名: **BAY 607550**

生物活性:					
<b>Description</b>	Bay 60-7550 is a potent and selective PDE2 inhibitor with a $K_i$ of 3.8 nM.				
<b>IC<sub>50</sub> &amp; Target</b>	K <sub>i</sub> : 3.8±0.2 nM (PDE2)[1]				
<b>In Vitro</b>	Bay 60-7550 (1 μM) increases cGMP in the neuronal cultures compared with control [F(6,14)=12.97, p<0.05 for Bay 60-7550]. Bay 60-7550 in the presence of NMDA (30 μM) results in further increases in cGMP compared with NMDA alone. The NMDA receptor antagonist MK-801 (10 μM) blocks both Bay 60-7550+NMDA-induced elevation in cGMP in neuronal cultures[1]. Compared with untreated control cells, proliferation of PASCs from IPAH patients is significantly reduced by BAY 60-7550 (1 μM)[2].				
<b>In Vivo</b>	The PDE2 inhibitors Bay 60-7550 (1 mg/kg) reverses restraint stress-induced alterations in behavior, resulting in increased percentages of open-arm entries and open-arm time compared with the vehicle + restraint stress condition. In nonstressed mice, Bay 60-7550 produces a dose-dependent increase in percentages of open-arm entries and open-arm time compared with the vehicle-treated group; significant increases are observed at a dose of 3 mg/kg. In nonstressed mice, Bay 60-7550 increases, in a dose-dependent manner, the number of head-dips and time spent head-dipping, compared with vehicle-treated mice; significant increases are observed at doses of 1 and 3 mg/kg[1].				
<b>Solvent&amp;Solubility</b>	<b>In Vitro:</b> DMSO : ≥ 33.3 mg/mL (69.87 mM) * "≥" means soluble, but saturation unknown.				
		<b>Solvent Mass Concentration</b>	<b>1 mg</b>	<b>5 mg</b>	<b>10 mg</b>
	<b>Preparing</b>	1 mM	2.0983 mL	10.4916 mL	20.9833 mL
	<b>Stock Solutions</b>	5 mM	0.4197 mL	2.0983 mL	4.1967 mL
		10 mM	0.2098 mL	1.0492 mL	2.0983 mL
<p>*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。</p> <p>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。</p> <p><b>In Vivo:</b>        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</p> <p>1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline</p> <p>Solubility: ≥ 2.5 mg/mL (5.25 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.25 mM, 饱和度未知) 的澄清溶液。</p> <p>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。</p>					



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	<p>2.请依序添加每种溶剂: 10% DMSO→ 90% (20% SBE-β-CD in saline)</p> <p>Solubility: ≥ 2.5 mg/mL (5.25 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.25 mM, 饱和度未知) 的澄清溶液。</p> <p>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 20% 的 SBE-β-CD 生理盐水水溶液中, 混合均匀。</p> <p>3.请依序添加每种溶剂: 10% DMSO →90% corn oil</p> <p>Solubility: ≥ 2.5 mg/mL (5.25 mM); Clear solution</p> <p>此方案可获得 ≥ 2.5 mg/mL (5.25 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。</p> <p>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。</p>
<p><b>References</b></p>	<p>[1]. Masood A, et al. Anxiolytic effects of phosphodiesterase-2 inhibitors associated with increased cGMP signaling. J Pharmacol Exp Ther. 2009 Nov;331(2):690-9.</p> <p>[2]. Bubb KJ, et al. Inhibition of phosphodiesterase 2 augments cGMP and cAMP signaling to ameliorate pulmonary hypertension. Circulation. 2014 Aug 5;130(6):496-507.</p>
<p><b>实验参考:</b></p>	
<p><b>Cell Assay</b></p>	<p>Growth of human distal pulmonary artery smooth muscle cells isolated from patients with idiopathic pulmonary arterial hypertension (IPAH) or control cells from adults undergoing transplant or lung resection for suspected malignancy, are monitored following treatment with Bay 60-7550 (1 μM), ANP (1 μM), DETA-NONOate (10 μM), or Treprostinil (1 μM), alone or in combination[2].</p>
<p><b>Animal Administration</b></p>	<p>Mice[1]</p> <p>Male ICR mice weighing 28 to 35 g are used. Bay 60-7550 (0.5, 1, and 3 mg/kg), ND7001 (0.5, 1.0, and 3 mg/kg), Detanonoate (0.5 mg/kg), L-NAME (50 mg/kg), or Diazepam (1 mg/kg) is administered after restraint stress and 30 min before behavioral testing. Mice also are treated with Bay 60-7550 (3 mg/kg), ND7001 (3 mg/kg), Detanonoate, (0.5 mg/kg), L-NAME (50 mg/kg), or diazepam (1 mg/kg) in the absence of restraint stress; drugs are administered 30 min before the behavioral tests. Bay 60-7550 shows 50-fold selectivity for PDE2 compared with PDE1, 100-fold compared with PDE5, and greater than 200-fold compared with the other PDE families. ND7001 exhibits at least 100-fold selectivity for inhibition of PDE2 relative to other PDE families. For antagonism tests to assess the role of cGMP signaling in the behavioral effects of the PDE2 inhibitors, ODQ, an inhibitor of soluble guanylyl cyclase (20 mg/kg), is administered 20 min before Bay 60-7550 or ND7001.</p>
	<p>COS-7 cells are maintained in complete DMEM (containing 10% fetal calf serum, 100 units/mL penicillin G, 100 mg/mL streptomycin, and 400 μM L-alanyl-L-glutamine) at 37°C in 5% CO<sub>2</sub> atmosphere. A PDE2 expression plasmid is introduced into COS-7 cells using the FuGENE6 transfection reagent. Cells are lysed in solubilization buffer (275 mM NaCl, 1.5 mM MgCl<sub>2</sub>, 2 mM EGTA, 2% Triton X, 20% glycerol, and 40 mM Tris-HCl), and the cell lysates are used in the immunoprecipitation procedures. A protein A-agarose bead slurry (100 μL) is washed three times with ice-cold phosphate-buffered saline (100 mM NaCl, 2.7 mM KCl, 10.6 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.6</p>



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<b>Kinase Assay</b>	<p>mM NaH<sub>2</sub>PO<sub>4</sub>) and mixed with the 5 µg of PDE2 antibody and 100 µL (2 µg/µL) of the lysate sample and rotated overnight at 4°C. The bead/sample mixture is then centrifuged at 1000g to separate the beads from the supernatant. The beads are resuspended in 100 µL of ice-cold lysis buffer (20 mM Tris, pH 7.4, 140 mM NaCl, 0.75 mM MgCl<sub>2</sub>, 1 mM EGTA, 1% Triton X-100, and 20% glycerol, containing protease and phosphatase inhibitors) to elute the PDE2 for use in the enzyme activity assays. The PDE2 activity assay is done. The recombinant PDE2 enzyme derived from COS-7 cell expression and diluted in KHEM buffer (50 mM KCl, 50 mM HEPES, 10 mM EGTA, and 1.9 mM MgCl<sub>2</sub>, pH 7.2) is mixed with different concentrations of PDE2 inhibitors (Bay 60-7550, ND7001, and EHNA) and [<sup>3</sup>H]cGMP/cGMP (5 µM) as the substrate. The mixture is then incubated for 30 min at 37°C (100 µL of reaction volume). To convert the [<sup>3</sup>H]GMP to [<sup>3</sup>H]guanosine, samples are incubated with snake venom from <i>Crotalus atrox</i> for 30 min at 37°C. The samples are then vortexed with a freshly prepared slurry of Dowex/water/ethanol [1:1:1, v/v] and then centrifuged for 10 min. [<sup>3</sup>H]Guanosine in the supernatant is then quantified by liquid scintillation counting. Bay 60-7550 is dissolved in dimethyl sulfoxide, EHNA is dissolved in distilled water, and ND7001 is dissolved in ethanol as 10 mM stocks and then diluted for use in assays with 20 mM Tris, pH 7.4; final concentrations of the respective solvents did not affect the assay. IC<sub>50</sub> values at a single substrate concentration are determined by nonlinear regression analysis of the log concentration-response curves for each PDE2 inhibitor; K<sub>i</sub> values are calculated[1].</p>
<b>References</b>	<p>[1]. Masood A, et al. Anxiolytic effects of phosphodiesterase-2 inhibitors associated with increased cGMP signaling. <i>J Pharmacol Exp Ther</i>. 2009 Nov;331(2):690-9.</p> <p>[2]. Bubb KJ, et al. Inhibition of phosphodiesterase 2 augments cGMP and cAMP signaling to ameliorate pulmonary hypertension. <i>Circulation</i>. 2014 Aug 5;130(6):496-507.</p>

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