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产品名称：RS-127445 Hydrochloride
产品别名：RS 127445；MT 500

生物活性：

Description	RS 127445 is a novel high affinity, selective 5-HT2B receptor antagonist with pKi of 9.5.			
IC_{50} & Target	sPLA2	5-$HT_{1B/D}$ Receptor	5-HT_3 Receptor	5-HT_5 Receptor
	5.5 (pKi)	<6 (pKi)	<6 (pKi)	<6 (pKi)
	5-HT_6 Receptor	5-HT_{2A} Receptor	5-HT_{2C} Receptor	5-HT_{2B} Receptor
	<6 (pKi)	6.3 (pKi)	6.4 (pKi)	9.5 (pKi)
In Vitro	RS-127445 is found to has nanomolar affinity for the 5-HT_{2B} receptor (pK_i=9.5±0.1) and 1,000 fold selectivity for this receptor as compared to numerous other receptor and ion channel binding sites. RS-127445 potently displaces [^{3}H]-5-HT from human recombinant 5-HT_{2B} receptors expressed in CHO-K1 cells. The affinity (pK_i value) of RS-127445 for the 5-HT_{2B} receptor is 9.5±0.1 (n=9). RS-127445 is selective for the 5-HT_{2B} receptor, having approximately 1000 fold lower affinity for the human recombinant 5-HT_{2A}, 5-HT_{2C}, 5-HT_5, 5-HT_6 and 5-HT_7receptors, a 5-HT_{1A} receptor in rat brain membranes, a 5-$HT_{1B/D}$ receptor in bovine caudate, and a monoamine uptake site in rabbit platelets. RS-127445 potently blocks the 5-HT (10 nM) evoked increases in intracellular calcium concentrations in the HEK-293 cells expressing the 5-HT_{2B} receptor (pIC_{50} of 10.4±0.1). In cells expressing human recombinant 5-HT_{2B} receptors, RS-127445 potently antagonizes 5-HT-evoked formation of inositol phosphates (pK_B=9.5±0.1) and 5-HT-evoked increases in intracellular calcium (pIC_{10}=10.4±0.1). RS-127445 also blocks 5-HT-evoked contraction of rat isolated stomach fundus (pA_{2B}=9.5±1.1) and (±)α-methyl-5-HT-mediated relaxation of the rat jugular vein (pA_2=9.9±0.3)[1].			
In Vivo	In rats, the fraction of RS-127445 that is bioavailable via the oral or intraperitoneal routes is 14 and 60% respectively. Intraperitoneal administration of RS-127445 (5 mg/kg) produced plasma concentrations predicted to fully saturate accessible 5-HT2B receptors for at least 4 h.RS-127445 (5 mg/kg) is administered to rats by oral, intraperitoneal and intravenous routes. Peak plasma concentrations are rapidly achieved with the highest concentrations being found at the first time-point measured following intravenous and intraperitonael administration (0.08 h) and by 0.25 h following dosing by the oral route of administration. RS-127445 is cleared from plasma with an estimated terminal elimination half-life of approximately 1.7 h. The bioavailability of RS-127445, when administered by the oral and intraperitoneal routes is approximately 14 and 62% of that obtained by intravenous administration. Approximately 60% of an intraperitoneal dose and 14% of the oral dose of RS-127445 (5 mg/kg) is bioavailable[1]. RS-127445 (1-30 mg/kg), dose-dependently reduces faecal output, reaching significance at 10 and 30 mg/kg (n=6-11). In blood and brain, >98% of RS-127445 is protein-bound[2].			
	In Vitro: **DMSO : ≥ 31 mg/mL (97.55 mM)** * "≥" means soluble, but saturation unknown.			

	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
Preparing	1 mM	3.1467 mL	15.7337 mL	31.4673 mL

Solvent&Solubility	**Stock Solutions**	5 mM	0.6293 mL	3.1467 mL	6.2935 mL
		10 mM	0.3147 mL	1.5734 mL	3.1467 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 (7.87 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.87 mM，饱和度未知) 的澄清溶液。

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% (20% SBE-β-CD in saline)

Solubility: ≥ 2.5 mg/mL (7.87 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.87 mM，饱和度未知) 的澄清溶液。

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。

3.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 2.5 mg/mL (7.87 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.87 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]. Bonhaus DW, et al. RS-127445: a selective, high affinity, orally bioavailable 5-HT2B receptor antagonist. Br J Pharmacol. 1999 Jul;127(5):1075-82.

[2]. Bassil AK, et al. Inhibition of colonic motility and defecation by RS-127445 suggests an involvement of the 5-HT2B receptor in rodent large bowel physiology. Br J Pharmacol. 2009 Sep;158(1):252-8

实验参考：

HEK-293 cells expressing the human 5-HT_{2B} receptor are incubated with [^{3}H]-myoinositol (1.67 μCi/mL) in 162 cm^2 flasks overnight at 37°C in an inositol free Ham's F12 medium containing 10% dialyzed foetal bovine serum. The cells are harvested, washed five times with phosphate buffered saline and resuspended in inositol free Ham's F12 media at density of approximately 3×10^6 cells/mL. RS-127445 (10 μM) is initially dissolved in 10% (v/v) DMSO with 90% inositol free Ham's F12 medium. Subsequent dilutions are made with inositol free Ham's F12 medium. 5-HT is dissolved in inositol free Ham's F12 medium containing 100 mM LiCl and 1 mM ascorbate.

Cell Assay	RS-127445, vehicle or other antagonists are pre-incubated with 240 μL of cell suspension at 37°C for 20 min. The reactions are initiated by addition of 5-HT. Sixty minutes later, the reactions are terminated by adding 50 μL of ice-cold 20% perchloric acid, chilled in an ice-water bath for 10 min and then neutralized with 160 μL of 1 N KOH. Each sample is diluted with 2 ml of 50 mM Tris-HCl, pH 7.4 at room temperature. The aqueous portion (2.2 mL) is transferred onto Dowex AG1X8 columns (1 ml, 1 : 1, w/v) which had been washed with 5 ml of distilled water. The columns are then washed with 18 ml of distilled water and the inositol phosphates are eluted with 3 ml of 1 N HCl. The eluted radioactivity is determined by liquid scintillation spectroscopy using a Packard 1900CA analyzer[1].
Animal Administration	Rats[1] Male Sprague-Dawley rats (200 g) are used. To compare the plasma kinetics of RS-127445 following different routes of administration, 90 rats are distributed into three treatment groups of 30 rats each. A single dose of RS-127445 (5 mg/kg) dissolved (2.5 mg/mL) in ethanol:propylene glycol : water (10 : 50 : 40, v:v:v), is administered to each rat. At 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 h after dosing, the rats are anaesthetized and blood samples are collected by cardiac puncture. Mice[2] Adult male C57BL/6J mice (25-30 g) are used. The effects of RS-127445 (1 nM-10 μM, single concentration per tissue, 15 min contact time) or vehicle (5 or 50 μL DMSO) are expressed as the percentage change in amplitude compared with the mean amplitude of four pre-drug, post-EFS contractile responses. The results are analysed using a two-sample equal variance t-test.
Kinase Assay	CHO-K1 cells expressing human 5-HT_{2A}, 5-HT_{2B} or 5-HT_{2C} receptors are harvested using 2 mM EDTA in phosphate buffered saline. Cell membranes are prepared by four cycles of homogenization (Brinkman P10 disrupter) and centrifugation (48,000×g for 15 min). As previously described, each assay is established so as to achieve steady state conditions and to optimize specific binding. For the 5-HT_{2A} receptor, membranes from 1×10^6cells are incubated with 0.2 nM [^{3}H]-ketanserin at 32°C for 60 min. Nonspecific binding is determined using 10 μM methysergide. For the 5-HT_{2B} receptor, membranes from 1.5×10^6 cells are incubated with 0.2 nM [^{3}H]-5-HT at 4°C for 120 min. Nonspecific binding is determined using 10 μM 5-HT. For the 5-HT_{2C} receptor, membranes from 3×10^5cells are incubated with 0.5 nM [^{3}H]-mesulergine at 32°C for 60 min. Nonspecific binding is determined using 10 μM methysergide. Assays are terminated by vacuum filtration through glass fibre filters (GF/B) which had been pretreated with 0.1% polyethyleneimine. Total and bound radioactivity is determined by liquid scintillation counting. Greater than 90% specific binding is achieved in each of these assays.The selectivity of RS-127445 for 5-HT_{2B}receptors is further examined by testing the compound for affinity at over 100 additional ion channel or receptor binding sites. These studies are conducted by Cerep using standard protocols[1].
References	[1]. Bonhaus DW, et al. RS-127445: a selective, high affinity, orally bioavailable 5-HT2B receptor antagonist. Br J Pharmacol. 1999 Jul;127(5):1075-82. [2]. Bassil AK, et al. Inhibition of colonic motility and defecation by RS-127445 suggests an involvement of the 5-HT2B receptor in rodent large bowel physiology. Br J Pharmacol. 2009 Sep;158(1):252-8