

产品名称: 2-[2-氟-4-[(2S)-2-吡咯烷基]苯基]-1H-苯并咪唑-7-甲酰胺
 产品别名: A-966492

生物活性:

Description	A-966492 is a novel and potent inhibitor of PARP1 and PARP2 with K_i of 1 nM and 1.5 nM, respectively.				
IC ₅₀ & Target	PARP-1	PARP-2			
	1 nM (K _i)	1.5 nM (K _i)			
In Vitro	A-966492 is one of the most potent PARP inhibitors. A-966492 displays excellent potency against the PARP-1 enzyme with a K_i of 1 nM and an EC ₅₀ of 1 nM in a whole cell assay. A-966492 significantly enhances the efficacy of TMZ in a dose-dependent manner. In addition, A-966492 is orally bioavailable across multiple species, crosses the blood–brain barrier, and appears to distribute into tumor tissue. A-966492 represents a promising, structurally diverse benzimidazole analogue and is being further characterized preclinically [1]				
In Vivo	A-966492 demonstrates good in vivo efficacy in a B16F10 subcutaneous murine melanoma model in combination with temozolomide and in an MX-1 breast cancer xenograft model both as a single agent and in combination with carboplatin. In addition, A-966492 has excellent pharmaceutical properties and has demonstrated in vivo efficacy in preclinical mouse tumor models in combination with TMZ and carboplatin, as well as single agent activity in a BRCA1-deficient MX-1 tumor model. A-966492 is further characterized in Sprague–Dawley rats, beagle dogs, and cynomolgus monkeys, with A-966492 demonstrating oral bioavailabilities of 34–72% and half-lives of 1.7–1.9 hours. In vivo, A-966492 demonstrates significant enhancement of the efficacy of TMZ in a murine B16F10 syngeneic melanoma model, with the A-966492 combination groups showing superior efficacy[1].				
Solvent&Solubility	In Vitro: DMSO : ≥ 100 mg/mL (308.31 mM) * "≥" means soluble, but saturation unknown.				
	Preparing Stock Solutions	<div><div>Solvent</div><div>Mass</div><div>Concentration</div></div>	1 mg	5 mg	10 mg
		1 mM	3.0831 mL	15.4154 mL	30.8309 mL
		5 mM	0.6166 mL	3.0831 mL	6.1662 mL
		10 mM	0.3083 mL	1.5415 mL	3.0831 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 (7.71 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.71 mM, 饱和度未知) 的澄清溶液。				

	<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> <p>2.请依序添加每种溶剂： 10% DMSO→ 90% (20% SBE-β-CD in saline) Solubility: \geq 2.5 mg/mL (7.71 mM); Clear solution 此方案可获得 \geq 2.5 mg/mL (7.71 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。</p> <p>3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: \geq 2.5 mg/mL (7.71 mM); Clear solution 此方案可获得 \geq 2.5 mg/mL (7.71 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。</p>
References	<p>[1]. Penning TD, et al. Optimization of phenyl-substituted benzimidazole carboxamide poly(ADP-ribose) polymerase inhibitors: identification of (S)-2-(2-fluoro-4-(pyrrolidin-2-yl)phenyl)-1H-benzimidazole-4-carboxamide (A-966492), a highly potent and efficacious</p>
实验参考：	
Cell Assay	<p>C41 cells are treated with A-966492 for 30 minutes in a 96-well plate. PARP are activated by damaging DNA with 1 mM H₂O₂ for 10 minutes. Cells are washed with ice-cold phosphate-buffered saline (PBS) once and fixed with prechilled methanol/acetone (7:3) at -20°C for 10 minutes. After they are air-dried, plates are rehydrated with PBS and blocked using 5% nonfat dry milk in PBS-Tween (0.05%) (blocking solution) for 30 minutes at room temperature. Cells are incubated with anti-PAR antibody 10H (1:50) in blocking solution at room temperature for 60 minutes followed by washing with PBS-Tween20 five times, and incubation with goat antimouse fluorescein 5(6)-isothiocyanate (FITC)-coupled antibody (1:50) and 1 μg/mL 40,6-diamidino-2-phenylindole (DAPI) in blocking solution at room temperature for 60 minutes. After washing with PBS-Tween20 5 times, analysis is performed using an fmax Fluorescence Microplate Reader set at the excitation and emission wavelength for FITC or the excitation and emission wavelength for DAPI. PARP activity (FITC signal) is normalized with cell numbers (DAPI). [1]</p>
Animal Administration	<p>A 0.2 cc amount of a 1:10 dilution of tumor brei in 45% Matrigel and 45% Spinner MEM is injected subcutaneously into the flank of female SCID mice on study day 0. Tumors are allowed to grow to the indicated size and then randomized to therapy groups (N=10 mice/group). PARP inhibitor therapy begin on day 14, with cisplatin treatment starting on day 16. At various intervals following tumor inoculation, the individual tumor dimensions are serially measured using calibrated microcalipers, and the tumor volumes are calculated. [1]</p>
Kinase Assay	<p>The enzyme assay is conducted in buffer containing 50 mM Tris, pH 8.0, 1 mM dithiothreitol(DTT), and 4 mM MgCl₂. PARP reactions contains 1.5 μM [³H]-NAD⁺ (1.6 μCi/mmol), 200 nM biotinylated histone H1, 200 nM sDNA, and 1 nM PARP-1 or 4 nM PARP-2 enzyme. Autoreactions utilizing SPA bead-based detection are carried out in 100 μL volumes in white 96-well plates. Reactions are initiated by adding 50 μL of 2X NAD⁺ substrate mixture to 50 μL of 2\times enzyme mixture containing PARP and DNA. These reactions are terminated by the addition of 150 μL of 1.5 mM benzamide</p>

	(appr 1×10^3 -fold over its IC_{50}). A 170 μ L amount of the stopped reaction mixtures is transferred to streptavidin-coated Flash Plates, incubated for 1 hour, and counted using a TopCount microplate scintillation counter. K_i data are determined from inhibition curves at various substrate concentrations. [1]
References	[1]. Penning TD, et al. <u>Optimization of phenyl-substituted benzimidazole carboxamide poly(ADP-ribose) polymerase inhibitors: identification of (S)-2-(2-fluoro-4-(pyrrolidin-2-yl)phenyl)-1H-benzimidazole-4-carboxamide (A-966492), a highly potent and efficacious</u>



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