

产品名称：2-[[**(2E)-3-(2-萘基)-1-氧代-2-丁烯基**]氨基]苯甲酸
产品别名：**BIBR 1532**

生物活性:				
Description	BIBR 1532 is a potent, selective and non-competitive telomerase inhibitor with IC ₅₀ of 100 nM in a cell-free assay.			
IC ₅₀ & Target	IC50: 100 nM (telomerase)			
In Vitro	BIBR 1532 non-competitively inhibits telomerase activity[1]. BIBR 1532 inhibits the proliferation of JVM13 leukemia cells with an IC50 of 52 μM, and similar effect also occurs in other leukemia cell lines such as Nalm-1, HL-60, and Jurkat. BIBR 1532 exerts antiproliferative effect on acute myeloid leukemia (AML) with IC50 of 56 μM with no effect on the proliferative capacity of normal hematopoietic progenitor cells[2]. BIBR 1532 (2.5 μM) reduces colony-forming ability, induces telomere length shortening and causes chemotherapeutic sensitization via inhibiting telomerase activity in MCF-7/WT and melphalan-resistant MCF-7/MInR cell lines[3]. BIBR 1532 is cytotoxic in a dose-dependent manner in T-cell prolymphocytic leukemia (T-PLL)[4]. BIBR 1532 in combination with carboplatin (a chemotherapeutic agent) eliminates ovarian cancer spheroid-forming cells in ES2, SKOV3, and TOV112D cell lines[5].			
Solvent&Solubility	<i>In Vitro:</i> DMSO : ≥ 100 mg/mL (301.79 mM) * "≥" means soluble, but saturation unknown.			
	Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg
		1 mM	3.0179 mL	15.0893 mL
		5 mM	0.6036 mL	3.0179 mL
		10 mM	0.3018 mL	1.5089 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℃，6 months；-20℃，1 month。 -80℃ 储存时，请在 6 个月内使用，-20℃ 储存时，请在 1 个月内使用。			
	<i>In Vivo:</i> 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 (7.54 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.54 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% corn oil Solubility: ≥ 2.5 mg/mL (7.54 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (7.54 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]. Pascolo E, et al. Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem. 2002 May 3;277(18):15566-72. [2]. El-Daly H, et al. Selective cytotoxicity and telomere damage in leukemia cells using the telomerase inhibitor BIBR1532. Blood. 2005 Feb 15;105(4):1742-9. [3]. Ward RJ, et al. Pharmacological telomerase inhibition can sensitize drug-resistant and drug-sensitive cells to chemotherapeutic treatment. Mol Pharmacol. 2005 Sep;68(3):779-86. [4]. R?th A, et al. Short telomeres and high telomerase activity in T-cell prolymphocytic leukemia. Leukemia. 2007 Dec;21(12):2456-62. [5]. Meng E, et al. Targeted inhibition of telomerase activity combined with chemotherapy demonstrates synergy in eliminating ovarian cancer spheroid-forming cells. Gynecol Oncol. 2012 Mar;124(3):598-605.
实验参考：	
Cell Assay	Cells are plated as triplicates in complete RPMI 1640 medium with various concentrations of BIBR1532. After 24 to 72 hours, water-soluble tetrazolium (WST-1) is added, which is transformed into formazan by mitochondrial reductase systems. The increase in the number of viable cells results in an increase of activity of mitochondrial dehydrogenases, leading to an increase of formazan dye formed, which is quantified by ELISA reader after 2, 3, and 4 hours of incubation. [1]
Kinase Assay	For the direct telomerase assay with the endogenous telomerase, 10 μL of telomerase-enriched extract is mixed with different concentrations of BIBR1532 in a final volume of 20 μL. After 15-minute preincubation on ice, 20 μL of the reaction mixture is added, and the reaction is initiated by transferring the tubes to 37°C. The final concentrations in the reaction mixture are 25 mM Tris-Cl (pH 8.3), 1 mM MgCl₂, 1 mM EGTA, 1 mM dATP, 1 mM dTTP, 6.3 μM cold dGTP, 15 μCi [α-³²P]dGTP (3000 Ci/mmol; NEN), 1.25 mM spermidine, 10 units of RNasin, 5 mM 2-mercaptoethanol, and 2.5 μM TS-primer (5'-AATCCGTCGAGCAGAGTT). For the recombinant enzyme, 1-7 μL of affinity-purified telomerase (containing less than 0.025 μM hTERT) are assayed in a final volume of 40 μL containing 50 mM Tris acetate (pH 8.5), 50 mM KCl, 1 mM MgCl₂, 1 mM spermidine, 5 mM 2-mercaptoethanol, 1 mM dATP, 1 mM dTTP, 2.5 μM dGTP, 15 μCi of [α-³²P]dGTP (3000 Ci/mmol) and 2.5 μM (TTAGGG)₃. The reaction is initiated by incubation at 37°C for 2 hours and stopped by addition of 50 μL of RNase mix (0.1 mg/mL RNaseA-100 u/mL RNaseT1 in 10 mM Tris-Cl (pH 8.3) and 20 mM EDTA) and incubation for 20 min at 37°C. Samples are deproteinated by adding 50 μL of 0.3 mg/mL proteinase K in 10 mM Tris-Cl (pH 8.3) and 0.5% w/v SDS, for a 30-minute incubation at 37°C. DNA is recovered by phenol extraction and ethanol precipitation, and the extension products are analyzed on an 8% (endogenous telomerase) or 12% (recombinant telomerase) polyacrylamide-urea gel. Dried gels are exposed to a Kodak phosphorimager screen, and the results are analyzed. [1]
References	[1]. Pascolo E, et al. Mechanism of human telomerase inhibition by BIBR1532, a synthetic, non-nucleosidic drug candidate. J Biol Chem. 2002 May 3;277(18):15566-72. [2]. El-Daly H, et al. Selective cytotoxicity and telomere damage in leukemia cells using the telomerase inhibitor BIBR1532. Blood. 2005 Feb 15;105(4):1742-9. [3]. Ward RJ, et al. Pharmacological telomerase inhibition can sensitize drug-resistant and drug-sensitive cells to chemotherapeutic treatment. Mol Pharmacol. 2005 Sep;68(3):779-86.

	[4]. R?th A, et al. Short telomeres and high telomerase activity in T-cell prolymphocytic leukemia. <u>Leukemia</u>. 2007 Dec;21(12):2456-62. [5]. Meng E, et al. Targeted inhibition of telomerase activity combined with chemotherapy demonstrates synergy in eliminating ovarian cancer spheroid-forming cells. <u>Gynecol Oncol</u>. 2012 Mar;124(3):598-605.
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源叶生物