产品名称：3-(5-Hydroxymethyl-2-Furyl)-1-Benzylindazole
产品别名：利非西呱 ；Lificiguat；YC-1

生物活性：

Description	Lificiguat binds to the β subunit of soluble guanylyl cyclase(sGC) with Kd of 0.6-1.1 μM in the presence of CO.
IC_{50} & Target	Kd: 0.6-1.1 μM (sGC, in the presence of CO)[1]
In Vitro	Soluble guanylate cyclase (sGC) is a heterodimeric heme protein and the primary NO receptor. Lificiguat (YC-1) binds near or directly to the heme-containing domain of the beta subunit. In the absence of CO, Lificiguat (YC-1) binds with K_d=9-21 μM, depending on construct. In the presence of CO, these values decrease to 0.6-1.1 μM. Lificiguat (YC-1) greatly enhanced CO binding to heterodimeric sGC, as expected (K_d=1 μM). Lificiguat (YC-1) stimulates sGC two- to four-fold in the absence of NO but acts synergistically with CO or NO to achieve several hundred fold activation. Binding of Lificiguat(YC-1) can also overcome inhibitory phosphorylation of sGC[1]. Lificiguat (YC-1) is a soluble guanylyl cyclase (sGC) activator. HCC cell lines HepG2, BEL-7402 and HCCLM3 are incubated for 72 h with Sorafenib and/or Lificiguat (YC-1). Sorafenib or Lificiguat (YC-1) alone inhibits HCC cell proliferation in a dose-dependent manner. Moreover, combination of Sorafenib and Lificiguat (YC-1) significantly suppresses proliferation of HCC cells in a dose-dependent manner. In addition, at the ED_{50} doses for both Sorafenib and Lificiguat (YC-1), combination index values (CI)=0.93 in HepG2, 0.95 in BEL-7402 and 0.72 in HCCLM3 respectively, suggesting that Sorafenib and Lificiguat (YC-1) synergistically inhibit proliferation of HCC cells[2].
In Vivo	Lificiguat (YC-1) (30 or 60 mg/kg, i.p.) inhibits MDA-MB-468 tumor growth in a dose-dependent manner. The effect of the prodrug formulation of Lificiguat (YC-1), YC-1-S, in MDA-MB-468 tumor-bearing mice is also investigated. In vivo pharmacokinetic analysis reveal that YC-1-S is quickly converted into its active form. Mice are administered 20, 40 or 80 mg/kg YC-1-S p.o. YC-1-S also displays dose-dependent inhibition of MDA-MB468 tumor growth. Both Lificiguat (YC-1) and YC-1-S dose-dependently reduce tumor weight. Moreover, the mean body weight of mice is not affected by Lificiguat (YC-1) or YC-1-S compare with vehicle-treated groups[3]. Lificiguat (YC-1) is a potent NO-GC activator reported to improve rodent learning behavior when examined with the Morris water maze (MWM) and avoidance tests. Lificiguat (YC-1) enhances long-term potentiation (LTP) in hippocampal Schafer collateral-CA1 synapse via the NO-cGMP-PKG-dependent pathway and potentiated LTP induction in the amygdala, increases the activation of ERK, and potentiated the expression of brain-derived neurotrophic factor (BDNF) cAMP response element-binding protein (CREB) in response to fear memory test[4].

In Vitro:

DMSO : ≥ 100 mg/mL (**328.58 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	3.2858 mL	16.4290 mL	32.8580 mL
5 mM	0.6572 mL	3.2858 mL	6.5716 mL
10 mM	0.3286 mL	1.6429 mL	3.2858 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

Solvent&Solubility	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 (8.21 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.21 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 (8.21 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (8.21 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 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 (8.21 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (8.21 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]. Purohit R, et al. YC-1 binding to the β subunit of soluble guanylyl cyclase overcomes allosteric inhibition by the α subunit. Biochemistry. 2014 Jan 14;53(1):101-14. [2]. Kong J, et al. YC-1 enhances the anti-tumor activity of sorafenib through inhibition of signal transducer and activator of transcription 3 (STAT3) in hepatocellular carcinoma. Mol Cancer. 2014 Jan 13;13:7. [3]. Chang LC, et al. YC-1 inhibits proliferation of breast cancer cells by down-regulating EZH2 expression via activation of c-Cbl and ERK. Br J Pharmacol. 2014 Sep;171(17):4010-25. [4]. Komsuoglu Celikyurt I, et al. Effects of YC-1 on Learning and Memory Functions of Aged Rats. Med Sci Monit Basic Res. 2014 Aug 21;20:130-7.

实验参考：

Cell Assay	Cell proliferation assay is measured using a Cell Counting Kit-8 (CCK-8). Briefly, cells are cultured in 96-well plates at a concentration of 3×10^3/well, incubated for 24 h, and treated with Sorafenib and/or Lificiguat (YC-1). After 72 h treatment, CCK-8 reagent is added to each well. The absorbance is measured at 450 nm after 2.5 h incubation at 37°C using an automated ELISA plate reader. Any synergistic effects resulting from combination of the compounds are measured using Microsoft Excel software to determine the combination index values (CI>1: antagonistic effect, CI=1: additive effect, and CT<1: synergistic effect)[2].
	Mice[3] Fifty-eight female *nu/nu* mice (4 weeks-old) are used. MDA-MB-468 breast cancer cells (5×10^6 cells

Animal Administration	per mouse) are suspended in 0.1 mL of Matrigel solution (50% v/v Matrigel in PBS) and inoculated into the mammary fat pads of nude mice. When the tumor masses reach 100 mm^3, the tumor-bearing mice are randomly divided into groups for treatments with different Lificiguat (YC-1)/YC-1-S doses. The mice are i.p. injected with YC-1 (30 or 60 mg/kg) or administered YC-1-S p.o. Tumor size and mouse body weight are measured once every 3 days, and tumor volume (mm^3) is calculated using the equation: length×$(width)^2$×0.5. At the end of the experiments, mice are killed and tumor nodules are dissected and weighed. Tumor tissues are subjected to Western blotting. Rats[4] 4-month-old (200-250 g) and 24-month-old (550-600 g) male Wistar-albino rats are used. Lificiguat (YC-1) is prepared immediately prior to use and given intraperitoneally (i.p.) in a volume of 0.1 mL per 100 g body weight. All rats receives 1 mg/kg/day of Lificiguat (YC-1) for 2 weeks. DMSO is administered to 4-month-old and 24-month-old rats (n=10, for each group). Doses are selected to confirm the selected doses on locomotor activity; all results are measured.
Kinase Assay	CO dissociation constants are measured by titrating CO from a saturated solution into sGC protein and monitoring the appearance of the CO-bound Soret absorption band. The *Ms* sGC β_1(1-380) and *Bt* sGC β_1(1-197) samples are prepared in Ar-purged buffer supplemented with excess dithionite. CO binding experiments are performed in a 10 cm pathlength cuvette for *Ms* sGC-β_1(1-380) and *Ms* sGC-NT21 using a Cary 50 spectrophotometer with a modified sample holder. Binding data in the presence and absence of 50 μM Lificiguat (YC-1) is plotted using a single site saturation ligand binding model in SigmaPlot[1].
References	[1]. Purohit R, et al. YC-1 binding to the β subunit of soluble guanylyl cyclase overcomes allosteric inhibition by the α subunit. Biochemistry. 2014 Jan 14;53(1):101-14. [2]. Kong J, et al. YC-1 enhances the anti-tumor activity of sorafenib through inhibition of signal transducer and activator of transcription 3 (STAT3) in hepatocellular carcinoma. Mol Cancer. 2014 Jan 13;13:7. [3]. Chang LC, et al. YC-1 inhibits proliferation of breast cancer cells by down-regulating EZH2 expression via activation of c-Cbl and ERK. Br J Pharmacol. 2014 Sep;171(17):4010-25. [4]. Komsuoglu Celikyurt I, et al. Effects of YC-1 on Learning and Memory Functions of Aged Rats. Med Sci Monit Basic Res. 2014 Aug 21;20:130-7.