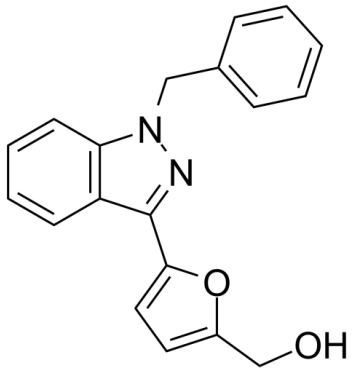
Data Sheet

Product Name:	Lificiguat
Cat. No.:	CS-3363
CAS No.:	170632-47-0
Molecular Formula:	C19H16N2O2
Molecular Weight:	304.34
Target:	Guanylate Cyclase
Pathway:	GPCR/G Protein
Solubility:	DMSO : ≥ 100 mg/mL (328.58 mM); H2O : < 0.1 mg/mL (insoluble)



BIOLOGICAL ACTIVITY:

Lificiguat binds to the β subunit of **soluble guanylyl cyclase(sGC)** with **K_d** of 0.6-1.1 μM in the presence of CO. IC50 & 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].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]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]. **Cell Assay:** Lificiguat (YC-1) is dissolved in DMSO and stored, and then diluted with appropriate media before use[2].[2]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]. **Animal Administration:** Lificiguat (YC-1) is dissolved in DMSO and diluted with saline[3][4].[3][4]Mice[3]

Fifty-eight female nu/nu mice (4 weeks-old) are used. MDA-MB-468 breast cancer cells (5×10^6 cells 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.

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.

CAIndexNames:

2-Furanmethanol, 5-[1-(phenylmethyl)-1H-indazol-3-yl]-

SMILES:

OCC1=CC=C(C2=NN(CC3=CC=CC=C3)C4=C2C=CC=C4)O1

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA