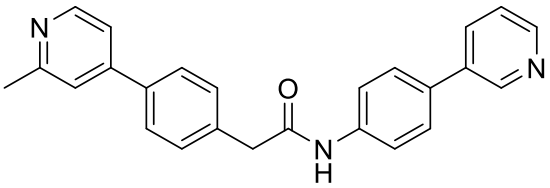
Product data sheet

MedKoo Cat#: 406605 Name: Wnt-C59 CAS#: 1243243-89-1 Chemical Formula: $C_{25}H_{21}N_3O$ Exact Mass: 379.16846 Molecular Weight: 379.45	
Product supplied as:	Powder
Purity (by HPLC):	≥ 98%
Shipping conditions	Ambient temperature
Storage conditions:	Powder: -20°C 3 years; 4°C 2 years.
	In solvent: -80°C 3 months; -20°C 2 weeks.

1. Product description:
Wnt-C59 is a potent porcupine (PORCN) inhibitor. Wnt-C59 inhibits PORCN activity in vitro at nanomolar concentrations, as assessed by inhibition of Wnt palmitoylation, Wnt interaction with the carrier protein Wntless/WLS, Wnt secretion, and Wnt activation of β-catenin reporter activity. In mice, Wnt-C59 displayed good bioavailability, as once daily oral administration was sufficient to maintain blood concentrations well above the IC(50). C59 blocked progression of mammary tumors in MMTV-WNT1 transgenic mice while downregulating Wnt/β-catenin target genes. Porcupine (PORCN) is a membrane bound O-acyltransferase that is required for Wnt palmitoylation, secretion, and biologic activity.

2. CoA, QC data, SDS, and handling instruction
SDS and handling instruction, CoA with copies of QC data (NMR, HPLC and MS analytical spectra) can be downloaded from the product web page under "QC And Documents" section. Note: copies of analytical spectra may not be available if the product is being supplied by MedKoo partners. Whether the product was made by MedKoo or provided by its partners, the quality is 100% guaranteed.

3. Solubility data

Solvent	Max Conc. mg/mL	Max Conc. mM
DMSO	50	131.77

4. Stock solution preparation table:

Concentration / Solvent Volume / Mass	1 mg	5 mg	10 mg
1 mM	2.64 mL	13.18 mL	26.35 mL
5 mM	0.53 mL	2.64 mL	5.27 mL
10 mM	0.26 mL	1.32 mL	2.64 mL
50 mM	0.05 mL	0.26 mL	0.53 mL

5. Molarity Calculator, Reconstitution Calculator, Dilution Calculator
Please refer the product web page under section of "Calculator"

6. Recommended literature which reported protocols for in vitro and in vivo study
In vitro study

1. Cheng Y, Phoon YP, Jin X, Chong SY, Ip JC, Wong BW, Lung ML. Wnt-C59 arrests stemness and suppresses growth of nasopharyngeal carcinoma in mice by inhibiting the Wnt pathway in the tumor microenvironment. Oncotarget. 2015 Jun 10;6(16):14428-39. doi: 10.18632/oncotarget.3982. PMID: 25980501; PMCID: PMC4546477.

2. Proffitt KD, Madan B, Ke Z, Pendharkar V, Ding L, Lee MA, Hannoush RN, Virshup DM. Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. Cancer Res. 2013 Jan 15;73(2):502-7. doi: 10.1158/0008-5472.CAN-12-2258. Epub 2012 Nov 27. PMID: 23188502.

In vivo study

1. Cheng Y, Phoon YP, Jin X, Chong SY, Ip JC, Wong BW, Lung ML. Wnt-C59 arrests stemness and suppresses growth of nasopharyngeal carcinoma in mice by inhibiting the Wnt pathway in the tumor microenvironment. Oncotarget. 2015 Jun 10;6(16):14428-39. doi: 10.18632/oncotarget.3982. PMID: 25980501; PMCID: PMC4546477.

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7. Bioactivity

Biological target:

Wnt-C59 (C59) is a PORCN inhibitor for Wnt3A-mediated activation of a multimerized TCF-binding site driving luciferase with IC50 of 74 pM in HEK293 cells.

In vitro activity

It was found that Wnt-C59 (C59) indeed functions as a bona fide PORCN inhibitor using a number of cell-based assays. C59 inhibits WNT3A-mediated activation of a multimerized TCF-binding site driving luciferase (Super8xTopFlash; STF) with an IC50 of 74 pmol/L (Fig. 1A). As expected for a PORCN inhibitor, Wnt secretion into culture medium is completely abrogated by C59 treatment (Fig. 1A, inset). Consistent with C59 targeting PORCN, overexpression of PORCN rescues the inhibition of WNT3A-mediated STF activity, similar to that of an unrelated PORCN inhibitor IWP-1 (refs. 21, 22; Fig. 1B). Wnt acylation is required for binding to the carrier protein WLS. WNT3A and WNT8A coimmunoprecipitate with WLS, but this interaction is blocked when cells have been pretreated with C59 (Fig. 1C). Using alkyne palmitic acid and click chemistry, it was found that C59 prevents incorporation of palmitate into WNT3A, consistent with inhibition of PORCN activity (Fig. 1D). C59 inhibits the activity of all splice variants of murine PORCN (Fig. 2A). In preliminary studies, we found that very high concentrations of C59 were required to produce developmental phenotypes in Xenopus embryogenesis. Consistent with this, while Xenopus laevis PORCN was active when expressed in PORCN-null human cells, its activity was resistant to inhibition by C59 (Fig. 2A). Because the Xenopus protein is 77% identical to human PORCN, this provides genetic evidence that PORCN is the molecular target of C59, suggests a mechanism for C59 drug resistance to emerge, and indicates that less related MBOAT proteins would also be unaffected by C59. Showing that inhibition of PORCN is likely to prevent all Wnt-mediated signaling, it was found that 9 of 9 β-catenin activating Wnts and 4 of 4 additional noncanonical Wnts lost activity when cells were treated with C59 (Fig. 2B and C). In summary, C59 is a nanomolar inhibitor of mammalian PORCN acyltransferase activity and blocks activation of all evaluated human Wnts. Thus, it's possible that C59 administration will prevent all human and murine Wnt-dependent signaling.

Reference: Cancer Res. 2013 Jan 15;73(2):502-7. http://cancerres.aacrjournals.org/cgi/pmidlookup?view=long&pmid=23188502

In vivo activity

Both SUNE1 and HNE1 cell lines were chosen due to their distinct growth dynamics for animal studies. The hypothesis is if Wnt signaling was dominant in the control of cellular stemness and proliferation, it would be possible to demonstrate the inhibitory effects by Wnt-C59 in these cell lines. Wnt-C59 treatment obviously delayed the growth of SUNE1 cells immediately after administration of tested chemicals in animals, but expression of active β-catenin protein was detected in tumor stroma. The findings suggested that Wnt-C59 was not able to fully control the CSC microenvironment to favor SUNE1-induced tumor growth in animals. It was also possible that some unknown negative feedback or redundant regulatory mechanisms finally dominated the signaling networks that caused restoration of Wnt activities and tumor cell proliferation in SUNE1 cells. To detect long-term effects of Wnt-C59 in animals, which have not been reported yet, the tumor growth of HNE1 cells was investigated. These cells require a longer latency period to form growing tumors in mice, reflecting greater intra-tumoral heterogeneity in this cell line. In the control group, surviving tumor cells, presumably CSCs, expanded in the injection sites and formed progressively growing tumors very quickly after the latency period. In contrast, injected tumor cells died and did not form visible and progressively growing tumors after the treatment of Wnt-C59 in animals. It was not possible to directly show the presence of CSCs in injected HNE1 cells, but the sphere inhibition assay demonstrates that Wnt-C59 could safely eliminate cells with stemness properties in an irreversible manner.

Reference: Oncotarget. 2015 Jun 10;6(16):14428-39. https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/25980501/

Note: The information listed here was extracted from literature. MedKoo has not independently retested and confirmed the accuracy of these methods. Customer should use it just for a reference only.