

**Product Data Sheet**

**Chemical Properties**

**Product Name:** CHIR-99021 (CT99021)

**Cas No.:** 252917-06-9

**M.Wt:** 465.34

**Formula:** C22H18Cl2N8

**Synonyms:** CHIR9021, CHIR-99021, CHIR 99021, CT99021, GSK-3 Inhibitor XVI

**Chemical Name:** 6-((2-((4-(2,4-dichlorophenyl)-5-(5-methyl-1H-imidazol-2-yl)pyrimidin-2-yl)amino)ethyl)amino)nicotinonitrile

**Canonical SMILES:** N#CC1=CC=C(NCCNC2=NC(C3=NC=C(C(N3)C(C4=CC=C(Cl)C=C4Cl) =N2)N=C1

**Solubility:** >23.3mg/mL in DMSO

**Storage:** Store at 4°C

**General tips:** For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

**Shopping Condition:** Evaluation sample solution: ship with blue ice
All other available size: ship with RT, or blue ice upon request

**Biological Activity**

**Targets:** GSK-3

**Pathways:** PI3K/Akt/mTOR Signaling >> GSK-3

**Description:**

CHIR-99021 is a highly specific glycogen synthase kinase-3 (GSK-3) inhibitor which can inhibit both isoforms with IC50 of 10 nM (GSK-3α) and 6.7 nM (GSK-3β).

CHIR-99021 was proved to promote self-renewal and maintain pluripotency of both B6 and
BALB/c ES cells via stabilizing the downstream effectors like c-Myc and β -catenin[1]. In J1 mESC cells, CHIR-99021 played an important role in maintaining the colony morphology as well as the self-renewal when combined with leukemia inhibitory factor (LIF). CHIR-99021 has shown to regulate multiple signaling pathways which involve Wnt/β-catenin, TGF-β, Nodal and MAPK, and the expression of epigenetic regulatory genes like Dnmt3[2]. CHIR-99021 has been demonstrated to promote DN3 thymocytes proliferation and differentiation in the absence of pre-TCR signaling, Notch1 signaling or CXCL12[3]. However, study has also found that higher concentration (10 ?M but not 1 ?M or 3 ?M) of CHIR99021 might selectively inhibit differentiation by activating IL-7 signaling pathway[3].

Reference:

Protocol

Cell experiment:

Cell lines
Human embryonic stem cells (ESCs)

Preparation method
The solubility of this compound in DMSO is >10 mM. General tips for obtaining a higher concentration: Please warm the tube at 37 °C for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Reacting conditions
8 μM, 24 hours

Applications
On day 0, differentiation was initiated with 8 μM CHIR-99021 for 24 h conferring canonical Wnt/β-catenin activation, followed by Wnt inhibition on day 3 by addition of 4 μM IWR-1 for 48 h. For all groups tested, first signs of eGFP-fluorescence and first beating EBs were observed on day 6 with a constant increase until d10, reaching almost 100% of beating EBs for the groups seeded with 666-2000 cells per aggregate. Flowcytometry analysis of dissociated EBs on day 10 showed the highest yield of 5.9 Nkx2-5-eGFP+ cells for the group seeded with 666 cells/aggregate; higher cell numbers per aggregate resulted in lower yields. Immunofluorescence stainings of EB cryosections and dissociated/reseeded cells confirmed a high content of Nkx2-5+ and cTnT+ cardiomyocytes, thereby demonstrating efficient cardiomyogenic differentiation of human
ESC-derived EBs after aggregation on agarose microwells and induction with small molecule-based media.

**Animal experiment [3]:**

<table>
<thead>
<tr>
<th>Animal models</th>
<th>Akita type 1 diabetic mice and wild-type mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dosage form</td>
<td>Intraperitoneal injection, 50 mg/kg, daily</td>
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<tr>
<td>Applications</td>
<td>At 15 min after the propranolol injection, the 2-min average HF fraction increased from 46.8 ± 2.9% before CHIR-99021 treatment to 67.8 ± 5.1% after CHIR-99021 treatment. Treatment of Akita mice with CHIR-99021 increased SREBP-1 from 0.53 ± 0.07- to 1.17 ± 0.11-fold. CHIR-99021 treatment increased GIRK4 levels from 0.28 ± 0.06- to 1.08 ± 0.14-fold of those in WT mice, which was significantly higher than in placebo.</td>
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<tr>
<td>Other notes</td>
<td>Please test the solubility of all compounds indoor, and the actual solubility may slightly differ with the theoretical value. This is caused by an experimental system error and it is normal.</td>
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</table>

**Reference:**


**Product Validation**

![Intracellular antibody FACS staining](image)

Intracellular antibody FACS staining for Nestin and TUJ1 indicated a stark contrast in the number of neurons generated by LSB (2% TUJ1+) compared to LSB3i (75% TUJ1+). When one or two of the three inhibitors used in 3i were added, the same level of TUJ1 cells was not achieved; however, CHIR (CHIR99021) with either SU5402 or DAPT achieved >53% neurons, indicating a requirement for CHIR in the formation of TUJ1+ neurons. Results are presented as mean ± s.e.m.
Caution

FOR RESEARCH PURPOSES ONLY.

NOT FOR HUMAN, VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Specific storage and handling information for each product is indicated on the product datasheet. Most ApexBio products are stable under the recommended conditions. Products are sometimes shipped at a temperature that differs from the recommended storage temperature. Short-term storage of many products are stable in the short-term at temperatures that differ from that required for long-term storage. We ensure that the product is shipped under conditions that will maintain the quality of the reagents. Upon receipt of the product, follow the storage recommendations on the product data sheet.

ApexBio Technology
www.apexbt.com

7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com