CYC065

Cat. No.: HY-101212
CAS No.: 1070790-89-4
Molecular Formula: C₂₁H₃₁N₇O
Molecular Weight: 397.52
Target: CDK
Pathway: Cell Cycle/DNA Damage
Storage: Powder -20°C 3 years
         4°C  2 years
         In solvent -80°C 6 months
                   -20°C 1 month

SOLVENT & SOLUBILITY

In Vitro

DMSO: ≥ 100 mg/mL (251.56 mM)
* "≥" means soluble, but saturation unknown.

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent concentration</th>
<th>Mass, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>2.5156</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>12.578</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>25.156</td>
</tr>
</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.29 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
CYC065 is a second-generation, orally available ATP-competitive inhibitor of CDK2/CDK9 kinases\(^1\) with IC\(_{50}\)s of 5 and 26 nM, respectively\(^2\).

IC\(_{50}\) & Target
CDK2   CDK9

In Vitro
CYC065 blocks cells in the G1 phase of the cell cycle and inhibits cell growth specifically in cyclin E1 (CCNE1)-overexpressing
uterine serous carcinomas (USCs). USC cell lines expressing high CCNE1 mRNA and protein levels to be significantly more sensitive to treatment with CYC065 in vitro when compared with low CCNE1-expressing cell lines (IC$_{50}$ mean±s.d.=124.1±57.8 nM in CCNE1-overexpressing USC cell lines vs 415±117.5 nM in CCNE1 low expressors, respectively; P=0.0003). Importantly, low concentrations of CYC065 (i.e., 100 nM) causes an arrest in the G1 phase of the cell cycle only in the CCNE1-overexpressing USC cell lines (i.e., USC-ARK-2, USC-ARK-7) [1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

To evaluate the therapeutic potential of CYC065 as a single agent, USC-ARK-2-derived xenografts are treated daily with CYC065 (22.5 mg/kg) for a 3-week period. Tumor size and mouse weight are recorded two times a week. The daily administration of CYC065 results in a significant reduction of tumor growth compared with the vehicle-treated mice (P=0.012 starting at day 9 of the treatment). No significant weight loss is reported during the entire treatment period [1].

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PROTOCOL

Cell Assay [1]

The effect of CYC065 on the viability and IC$_{50}$ of USC-ARK-1, USC-ARK-2, USC-ARK-7, USC-ARK-4 and USC-ARK-6 USC primary cell lines is determined in flow-cytometry assay. Briefly, tumour cells are plated in six-well plates and treated with a titration of CYC065 concentrations (i.e., ranging from 100 to 500 nM). After 72 h, cells are harvested, washed and stained with propidium iodide (Pi; 5 μg/mL) for flow cytometric counts. The percentage of viable cells is then normalised considering the vehicle-treated cells as 100% viable. Half-maximal inhibitory concentration values are determined using GraphPad Prism5 version 6. For drug combination studies, USC-ARK-1 and USC-ARK-2 cell lines are incubated with the combination of Taselisib and CYC065 at multiple paired concentrations including the IC$_{50}$, the IC$_{50}$/2 and the IC$_{50}$*$2$ of each cell line to the corresponding drug (i.e., 10 nM of Taselisib and 198 nM of CYC065 for USC-ARK-1 and 50 nM of Taselisib and 62.5 nM of CYC065 for USC-ARK-2). Synergism is assessed by the combination index (CI). CI values <1 define a synergistic activity of the combination treatment. The CI values are calculated using the CompuSyn software [1].

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Animal Administration [1]

Mice [1]

The in vivo efficacy of CYC065 used as a single agent is evaluated on xenograft mouse models derived from the CCNE1-amplified USC-ARK-2 USC cell line. Xenografts derived from the CCNE1-amplified, PIK3CA-mutated USC-ARK-1 cell line are used for evaluating the in vivo combination of CYC065 and Taselisib. Briefly, 5-7-week-old SCID mice are injected into the subcutaneous region with USC cells. A minimum of five animals per group are used. Treatments are administrated by oral gavage starting 1 week after tumor implantation when the size of the tumor is 0.125-0.150 cm$^3$. Uterine serous carcinoma-ARK-2-derived xenografts are divided into two groups: one group of animal receive the vehicle, whereas the experimental group receive CYC065 (22.5 mg/kg daily for 3 weeks). Uterine serous carcinoma-ARK-1-derived xenografts are instead divided into four groups: one group receive the vehicle (0.5% methylcellulose-0.2% Tween-80), one group receive CYC065 (22.5 mg/kg daily for 3 weeks), one group receive Taselisib (10 mg/kg daily, 5 days per week per 3 weeks) and the last group receive the combination of CYC065 and Taselisib. The size of the tumor at the initiation of treatment is 0.125-0.150 cm$^3$. Mouse weight and tumor size is recorded two times a week for the entire experimental period. Tumor volume is calculated.

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REFERENCES

