Temozolomide

Cat. No.: HY-17364
CAS No.: 85622-93-1
Molecular Formula: C₆H₆N₆O₂
Molecular Weight: 194.15
Target: DNA Alkylator/Crosslinker; Autophagy; Apoptosis
Pathway: Cell Cycle/DNA Damage; Autophagy; Apoptosis
Storage: -20°C, protect from light, stored under nitrogen
* In solvent: -80°C, 6 months; -20°C, 1 month (protect from light, stored under nitrogen)

**SOLVENT & SOLUBILITY**

<table>
<thead>
<tr>
<th>In Vitro</th>
<th>DMSO : 20.83 mg/mL (107.29 mM; Need ultrasonic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O : 2.86 mg/mL (14.73 mM; Need ultrasonic)</td>
<td></td>
</tr>
</tbody>
</table>

### Preparing Stock Solutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>5.1507 mL</td>
<td>25.7533 mL</td>
<td>51.5066 mL</td>
</tr>
<tr>
<td>5 mM</td>
<td>1.0301 mL</td>
<td>5.1507 mL</td>
<td>10.3013 mL</td>
</tr>
<tr>
<td>10 mM</td>
<td>0.5151 mL</td>
<td>2.5753 mL</td>
<td>5.1507 mL</td>
</tr>
</tbody>
</table>

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

### In Vivo

1. Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline
   Solubility: ≥ 2.5 mg/mL (12.88 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.08 mg/mL (10.71 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.08 mg/mL (10.71 mM); Clear solution
4. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (10.71 mM); Clear solution
5. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.08 mg/mL (10.71 mM); Clear solution
6. Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)
   Solubility: ≥ 0.26 mg/mL (1.34 mM); Clear solution

**BIOLOGICAL ACTIVITY**

**Description**

Temozolomide (NSC 362856) is an oral active DNA alkylating agent that crosses the blood-brain barrier. Temozolomide is
also a proautophagic and proapoptotic agent. Temozolomide is effective against tumor cells that are characterized by low levels of O6-alkylguanine DNA alkyltransferase (OGAT) and a functional mismatch repair system. Temozolomide has antitumor and antiangiogenic effects\(^1\)\(^2\).

<table>
<thead>
<tr>
<th>IC(_{50}) &amp; Target</th>
<th>DNA alkylator(^1)</th>
</tr>
</thead>
</table>

**In Vitro**

Temozolomide (TZM) is a methylating agent that crosses the blood-brain barrier and is indicated for malignant gliomas and metastatic melanomas. Temozolomide is effective against tumor cells that are characterized by low levels of O\(^6\)-alkylguanine DNA alkyltransferase (OGAT) and a functional mismatch repair system (MR)\(^1\). Determination of the IC\(_{50}\) for Temozolomide (TZM) in different cell lines gave values ranging from 14.1 to 234.6 \(\mu\)M that fell into two clearly differentiated groups: cell lines with low IC\(_{50}\) values (<50 \(\mu\)M), which include A172 (14.1±1.1 \(\mu\)M) and LN229 cells (14.5±1.1 \(\mu\)M), and those with high IC\(_{50}\) values (>100 \(\mu\)M), which include SF268 (147.2±2.1 \(\mu\)M) and SK-N-SH cells (234.6±2.3 \(\mu\)M)\(^3\).

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

**In Vivo**

Temozolomide (TZM), as a single agent, does not significantly increase median survival time (MST) with respect to control. Noteworthy, intracranial injection of NU1025, immediately before the administration of 100 or 200 mg/kg Temozolomide, significantly increases lifespans with respect to controls or to groups treated with Temozolomide only. When Temozolomide is fractionated, the increase in lifespan (ILS) obtained with this schedule is higher than that observed when NU1025 is combined with a single injection of Temozolomide (statistical comparison of survival curves: NU1025 intracranially+Temozolomide 100 mg/kg×2 vs NU1025+Temozolomide 200 mg/kg; \(P=0.023\)\(^1\)).

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**PROTOCOL**

**Cell Assay**\(^1\)

The murine lymphoma cell line L5178Y of DBA/2 (H-2\(^d\)/H-2\(^d\)) origin is cultured in RPMI-1640 containing 10% fetal calf serum and antibiotics. Inhibition of PARP is obtained by treating cells (10\(^5\) cells/mL) with 8-hydroxy-2-methylquinazolin-4\([3\rangleH\]-1 (NU1025), at a concentration (25 \(\mu\)M) that abrogates PARP activity. Cells are then exposed to Temozolomide (7.5-125 \(\mu\)M) and are cultured for 3 days. Cell growth is evaluated by counting viable cells in quadruplicate, and apoptosis is assessed by flow cytometry analysis of DNA content. Long-term survival is analyzed by colony-formation assay\(^1\).

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**Animal Administration**\(^1\)

Male B6D2F1 (C57BL/6×DBA/2) mice are used. L5178Y cells (10\(^4\) in 0.03 mL RPMI-1640) are then injected intracranially, through the center-middle area of the frontal bone to a 2-mm depth, using a 0.1-mL glass microsyringe and a 27-gauge disposable needle. To evaluate tumor cell growth, brains are fixed in 10% phosphate-buffered formaldehyde, and histologic sections (5 \(\mu\)m) are cut along the axial plane, stained with hematoxylin-eosin, and analyzed by light microscopy. Temozolomide is dissolved in DMSO (40 mg/mL), diluted in saline (5 mg/mL), and administered intraperitoneally on day 2 after tumor injection at 100 mg/kg or 200 mg/kg, doses commonly used for in vivo preclinical studies. Because cytotoxicity induced by Temozolomide and PARP inhibitors can be improved by fractionated modality of treatment, in selected groups a total dose of 200 mg/kg Temozolomide is divided in 2 doses of 100 mg/kg given on days 2 and 3.

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**CUSTOMER VALIDATION**

- Brain. 2021 Mar 3;144(2):615-635.
REFERENCES

