TRAM-34

Cat. No.: HY-13519
CAS No.: 289905-88-0
Molecular Formula: C₂₂H₁₇ClN₂
Molecular Weight: 344.84
Target: Potassium Channel
Pathway: Membrane Transporter/Ion Channel
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: 25 mg/mL (72.50 mM; Need ultrasonic)
H₂O: < 0.1 mg/mL (insoluble)

Preparing Stock Solutions

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mM</td>
<td>2.8999 mL</td>
<td>14.4995 mL</td>
<td>28.9990 mL</td>
<td></td>
</tr>
<tr>
<td>5 mM</td>
<td>0.5800 mL</td>
<td>2.8999 mL</td>
<td>5.7998 mL</td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>0.2900 mL</td>
<td>1.4499 mL</td>
<td>2.8999 mL</td>
<td></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 (7.25 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: 2.5 mg/mL (7.25 mM); Suspended solution; Need ultrasonic
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (7.25 mM); Clear solution

BIOLOGICAL ACTIVITY

Description
TRAM-34 is a highly selective blocker of intermediate-conductance calcium-activated K⁺ channel (IKCa1) (Kᵩ=20 nM).

IC₅₀ & Target
Kᵩ: 20 nM (IKCa1)[¹]

In Vitro
TRAM-34 selectively blocks the IKCa1 current (Kᵩ=25 nM), TRAM-34 also blocks IKCa1 currents in human T84 colonic
epithelial cells with equivalent potency ($K_d=22 \text{ nM}$). TRAM-34 inhibits the cloned and the native IKCa1 channel in human T lymphocytes with a $K_d$ of 20-25 nM and is 200- to 1,500-fold selective over other ion channels. The dose-response curve reveals a $K_d$ of 20±3 nM and a Hill coefficient of 1.2 with 1 µM calcium in the pipette\cite{1}. TRAM-34, a specific inhibitor of $K_{Ca}$ 3.1 channels increased or decreased cell proliferation depending on the concentration. At intermediate concentrations (3-10 µM) TRAM-34 increased cell proliferation, whereas at higher concentrations (20-100 µM) TRAM-34 decreased cell proliferation. The enhancement of cell proliferation caused by TRAM-34 is blocked by the oestrogen receptor antagonists ICI182,780 and tamoxifen. TRAM-34 also increases progesterone receptor mRNA expression, decreased oestrogen receptor-α mRNA expression and reduced the binding of radiolabelled oestrogen to MCF-7 oestrogen receptor, in each case mimicking the effects of 17β-oestradiol\cite{2}.

**In Vivo**

Mice (n=5) injected intravenously with a single dose of TRAM-34 (0.5 mg/kg; 29 µM) appeared clinically normal during the 7-day study. The body-weight data of the TRAM-34-treated group (day 1:17.8 g; day 7: 27.0 g) are similar to control mice injected with the vehicle (day 1: 17.4 g; day 7: 23.4 g). Collectively, data from these limited toxicity studies suggest that TRAM-34 is not acutely toxic at ≈500-1,000 times the channel-blocking dose\cite{1}. Treatment with TRAM-34 results in a significant reduction in hematoxylin & eosin (H&E) defined lesion area with the mean infarct size being reduced from 22.6±3.6% in the controls (n=8) to 11.3±2.8% in rats treated with 10 mg/kg TRAM-34 (n=6, mean±s.e.m., P=0.039) and to 8.1±1.9% in rats treated with 40 mg/kg TRAM-34 (n=8; P=0.004). The treatment also tended to reduce brain shrinkage. However, the results are only statistically significant with 40 mg/kg TRAM-34 (P=0.013), but not for the 10 mg/kg group (P=0.11)\cite{3}.

**PROTOCOL**

**Kinase Assay** [2]

MCF-7 cell protein (250 µg) is incubated at room temperature for 2 h in TEDG buffer in the presence of 0.1 nM [2,4,6,7,16,17-3H(N)]-oestradiol ([3H]-E2) (110 Ci/mmol) in a total final volume of 500 µL. Non-specific binding is assessed in the presence of a 100-fold excess of non-radioactive E2. TRAM-34 and E2 standards are diluted in phenol red-free 5% DCC-FBS MEM containing supplements before being added to the cytosolic protein. A vehicle control comprised of 5% DCC-FBS MEM containing supplements with 0.7% DMSO. To separate ER-bound [3H]-E2 from unbound [3H]-E2, 250 µL of hydroxylapatite (HAP, 60% in TEDG buffer) is added, the mixture is vortexed every 5 min over 15 min and centrifuged at 1000×g for 10 min. The HAP-[3H]-E2-ER complex is washed with TEDG buffer, centrifuged and the wash step repeated. To elute [3H]-E2 from the HAP-[3H]-E2-ER complex, 500 µL of 100% ethanol is added and the mixture then incubated for 15 min and centrifuged at 1034×g for 10 min. The separated [3H]-E2 is removed and added to 2 mL of scintillation fluid. Radioactivity is quantified using a Beckman LS 5000TA scintillation counter. Competition of [3H]-E2 with TRAM-34 is assayed in quadruplicate on four independent protein extractions. An apparent dissociation constant of 0.135±0.034 nM (n=3) and a maximum binding capacity of 48.3±5.4 fmol/mg (n=3) are determined by Scatchard analysis\cite{2}. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Animal Administration** [1][3]

Mice\cite{1} Five CF-1BR mice (17-19 g) are injected intravenously with a single 1.0-ml dose of 0.5 mg/kg TRAM-34 (in mammalian Ringer solution with 1% ethanol and 2.5% BSA). Five control mice are injected with an equal volume of the vehicle. Mice are observed for adverse effects immediately after dosing, at 4 h after injection and daily for 7 days.

Rats\cite{3} Adult male Wistar rats weighing 160 to 180 g are used. Rats receive TRAM-34 at 10 mg/kg, 40 mg/kg or vehicle (Miglyol 812 neutral oil at 1 µL/g) twice daily intraperitoneally for 7 days starting 12 hours after reperfusion. Neurological deficits are scored according to a 4-score test and a tactile and proprioceptive limb-placing test as follows: (1) 4-score test (higher score for more severe neurological deficits): 0=no apparent deficit; 1=contralateral forelimb is consistently flexed during suspension by holding the tail; 2=decreasing grip ability on the contralateral forelimb while tail pulled; 3=spontaneous movement in all directions but circling to contralateral side when pulled by the tail; 4=spontaneous contralateral circling or depressed level of consciousness. (2) 14-score limb-placing test (lower score for more severe neurological deficits): proprioception, forward extension, lateral abduction, and
Adduction are tested with vision or tactile stimuli. For visual limb placing, rats are held and slowly moved forward or lateral toward the top of a table. Normal rats placed both forepaws on the tabletop. Tactile forward and lateral limb placing are tested by lightly contacting the table edge with the dorsal or lateral surface of a rat’s paw while avoiding whisker contact and covering the eyes to avoid vision.

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

REFERENCES

