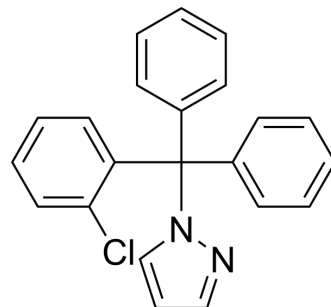


TRAM-34

Cat. No.:	HY-13519		
CAS No.:	289905-88-0		
Molecular Formula:	C ₂₂ H ₁₇ ClN ₂		
Molecular Weight:	345		
Target:	Potassium Channel		
Pathway:	Membrane Transporter/Ion Channel		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : 25 mg/mL (72.46 mM; Need ultrasonic)

Concentration	Solvent	Mass		
		1 mg	5 mg	10 mg
Preparing Stock Solutions	1 mM	2.8986 mL	14.4928 mL	28.9855 mL
	5 mM	0.5797 mL	2.8986 mL	5.7971 mL
	10 mM	0.2899 mL	1.4493 mL	2.8986 mL

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

In Vivo

- Add each solvent one by one: 20% SBE-β-CD in saline
Solubility: 5 mg/mL (14.49 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
Solubility: ≥ 2.08 mg/mL (6.03 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
Solubility: 2.08 mg/mL (6.03 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 90% corn oil
Solubility: ≥ 2.08 mg/mL (6.03 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

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

IC₅₀ & Target

K_d: 20 nM (IKCa1)^[1]

In Vitro

TRAM-34 selectively blocks the IKCa1 current (K_d=25 nM), TRAM-34 also blocks IKCa1 currents in human T84 colonic

epithelial cells with equivalent potency ($K_D=22$ 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 μ M calcium in the pipette^[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 μ M) TRAM-34 increased cell proliferation, whereas at higher concentrations (20-100 μ M) 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 17 β -oestradiol^[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

In Vivo

Mice (n=5) injected intravenously with a single dose of TRAM-34 (0.5 mg/kg; 29 μ M) 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 \approx 500-1,000 times the channel-blocking dose^[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 $22.6\pm 3.6\%$ in the controls (n=8) to $11.3\pm 2.8\%$ in rats treated with 10 mg/kg TRAM-34 (n=6, mean \pm s.e.m., $P=0.039$) and to $8.1\pm 1.9\%$ 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 ($P=0.11$)^[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay ^[2]

MCF-7 cell protein (250 μ g) 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 μ L. 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 μ L of hydroxylapatite (HAP, 60% in TEDG buffer) is added, the mixture is vortexed every 5 min over 15 min and centrifuged at 1000 \times 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 μ L of 100% ethanol is added and the mixture then incubated for 15 min and centrifuged at 1034 \times 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^[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

Animal Administration ^{[1][3]}

Mice^[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^[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 μ L/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.

CUSTOMER VALIDATION

- Nat Commun. 2022 Jun 21;13(1):3544.
- Haematologica. 2017 Oct;102(10):e415-e418.
- Life Sci. 2023 Dec 4, 122326.
- J Inflamm Res. 2021 Mar 5;14:719-735.
- Cell Calcium. 2022 Jun;104:102571.

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REFERENCES

- [1]. Wulff H, et al. Design of a potent and selective inhibitor of the intermediate-conductance Ca²⁺-activated K⁺ channel, IKCa1: a potential immunosuppressant. Proc Natl Acad Sci U S A. 2000 Jul 5;97(14):8151-6.
- [2]. Roy JW, et al. The intermediate conductance Ca²⁺-activated K⁺ channel inhibitor TRAM-34 stimulates proliferation of breast cancer cells via activation of oestrogen receptors. Br J Pharmacol. 2010 Feb 1;159(3):650-8.
- [3]. Chen YJ, et al. The KCa3.1 blocker TRAM-34 reduces infarction and neurological deficit in a rat model of ischemia/reperfusion stroke. J Cereb Blood Flow Metab. 2011 Dec;31(12):2363-74.

Caution: Product has not been fully validated for medical applications. For research use only.

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