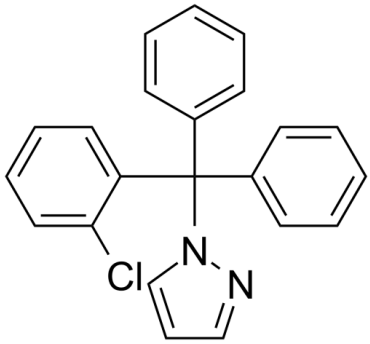
Data Sheet

Product Name:	TRAM-34
Cat. No.:	CS-1921
CAS No.:	289905-88-0
Molecular Formula:	C22H17ClN2
Molecular Weight:	344.84
Target:	Potassium Channel
Pathway:	Membrane Transporter/Ion Channel
Solubility:	H2O : < 0.1 mg/mL (insoluble); DMSO : 25 mg/mL (72.50 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

TRAM-34 is a highly selective blocker of intermediate-conductance calcium-activated K^+ channel (**IKCa1**) ($\mathbf{K_d}$=20 nM). IC50 & Target: Kd: 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 μM 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 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[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[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)[3].

PROTOCOL (Extracted from published papers and Only for reference)

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-^{3}H(N)]-oestradiol ([^{3}H]-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 [^{3}H]-E2 from unbound [^{3}H]-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-[^{3}H]-E2-ER complex is washed with TEDG buffer, centrifuged and the wash step repeated. To elute [^{3}H]-E2 from the HAP-[^{3}H]-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 [^{3}H]-E2 is removed and added to 2 mL of scintillation fluid. Radioactivity is quantified using a Beckman LS 5000TA scintillation counter. Competition of [^{3}H]-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]. **Animal Administration:** TRAM-34 is prepared in 1% ethanol and 2.5% BSA (Mice)[1].
TRAM-34 is prepared in Miglyol 812 neutral oil (Rat)[3].[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.
Rat[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.

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.

CAIndexNames:

1H-Pyrazole, 1-[(2-chlorophenyl)diphenylmethyl]-

SMILES:

ClC1=CC=CC=C1C(N2N=CC=C2)(C3=CC=CC=C3)C4=CC=CC=C4

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