

Product Data Sheet

Product Name: TRAM-34

Cat. No.: GC13040

Chemical Properties

Cas. No. 289905-88-0

Chemical Name 1-[(2-chlorophenyl)-diphenylmethyl]pyrazole

SMILES C1=CC=C(C=C1)C(C2=CC=CC=C2)(C3=CC=CC=C3Cl)N4C=CC=N4

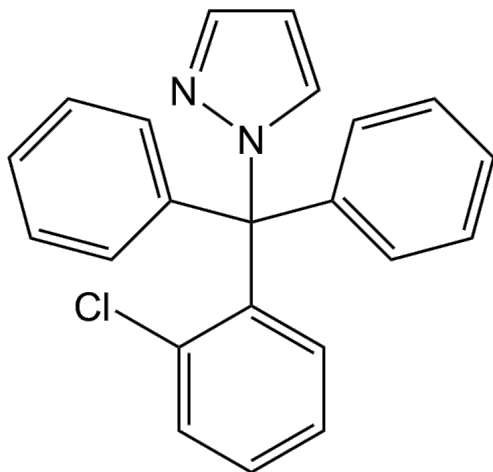
Formula $C_{22}H_{17}ClN_2$ M.Wt 344.84

Solubility $\geq 17.25\text{mg/mL}$ in DMSO Storage Store at -20°C

General tips For obtaining a higher solubility, please warm the tube at 37°C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution: ship with blue ice. All other available size: ship with RT, or blue ice upon request.

Structure



Protocol

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

**Kinase
experiment:**

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[2].

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

**Animal
experiment:**

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.

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

References:

- [1]. Wulff H, et al. Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+} -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 Ca^{2+} -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.

Background

TRAM-34 is a highly selective inhibitor of KCa3.1 channel with IC_{50} value of 20 nM [1]. The Ca^{2+} -binding protein calmodulin (CaM) confers Ca^{2+} sensitivity to KCa3.1 of KCa3.1. On the basis of crystal structure obtained for the C-terminal region of the rat KCa2.2 channel (rSK2) with CaM that the binding of Ca^{2+} to the CaM N-lobe results in CaM interlocking the C-terminal regions of two adjacent KCa3.1 subunits, leading to the formation of a dimeric structure. It is reported that many factors can increase KCa3.1, like balloon injury [2].

TRAM-34 is a KCa3.1 channel inhibitor and plays an important role in many diseases. When tested with human T cells, TRAM-34 treatment inhibited cells mobility and migration via blocking KCa3.1 channel [3]. In coronary smooth muscle cells isolated by laser capture microdissection, delivery of TRAM-34 via balloon catheter significantly blocked the KCa3.1 increase [4]. When tested with COS-7 cells, TRAM-34 inhibited KCa3.1 channel with K_d of 20 ± 3 nM and a Hill coefficient of 1.2 with 1 μM calcium in the pipette [1].

In male Wistar rat model of ischemic stroke, administration of TRAM-34 intraperitoneal (10 or 40 mg/kg, twice daily) for 7 days reduced infarction, neuronal death, microglia activation and neurological deficit via blocking KCa3.1 channel [5].

References:

- [1]. Wulff, H., et al., *Design of a potent and selective inhibitor of the intermediate-conductance Ca^{2+} -activated K^{+} channel, IKCa1: a potential immunosuppressant. Proc Natl Acad Sci U S A, 2000. 97(14): p. 8151-6.*
- [2]. Kaushal, V., et al., *The Ca^{2+} -activated K^{+} channel KCNN4/KCa3.1 contributes to microglia*

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

Product Data Sheet

- activation and nitric oxide-dependent neurodegeneration. J Neurosci, 2007. 27(1): p. 234-44.*
- [3].Chimote, A.A., et al., *Selective inhibition of KCa3.1 channels mediates adenosine regulation of the motility of human T cells. J Immunol, 2013. 191(12): p. 6273-80.*
- [4].Tharp, D.L., et al., *Local delivery of the KCa3.1 blocker, TRAM-34, prevents acute angioplasty-induced coronary smooth muscle phenotypic modulation and limits stenosis. Arterioscler Thromb Vasc Biol, 2008. 28(6): p. 1084-9.*
- [5].Chen, Y.J., 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. 31(12): p. 2363-74.*

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

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA