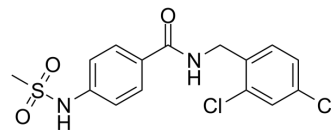


ML335

Cat. No.:	HY-104005		
CAS No.:	825658-06-8		
Molecular Formula:	C ₁₅ H ₁₄ Cl ₂ N ₂ O ₃ S		
Molecular Weight:	373.25		
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 : 155 mg/mL (415.27 mM; Need ultrasonic and warming)				
		Solvent Concentration	Mass 1 mg	5 mg	10 mg
	Preparing Stock Solutions	1 mM	2.6792 mL	13.3958 mL	26.7917 mL
		5 mM	0.5358 mL	2.6792 mL	5.3583 mL
10 mM		0.2679 mL	1.3396 mL	2.6792 mL	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (6.70 mM); Clear solution				

BIOLOGICAL ACTIVITY

Description	ML335 is a selective activator of both TREK-1 and TREK-2.
IC ₅₀ & Target	TREK-1, TREK-2
In Vitro	<p>Xenopus oocyte two-electrode voltage-clamp measurements show that ML335 and ML402 activate K_{2p}2.1 and K_{2p}10.1 but not K_{2p}4.1 (14.3±2.7 μM, K_{2p}2.1-ML335; 13.7±7.0 μM, K_{2p}2.1-ML402; 5.2±0.5 μM, K_{2p}10.1-ML335; and 5.9±1.6 μM, K_{2p}10.1-ML402). Swapping the Lys271 equivalent between K_{2p}2.1 and K_{2p}4.1 results in a clear phenotype reversal for ML335 and M402 activation. ML335 and ML402 activate K_{2p}2.1 in HEK293 cells similar to their effects in Xenopus oocytes (5.2±0.8 μM and 5.9±1.6 μM for ML335 and ML402, respectively (n≥3))^[1].</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

PROTOCOL

Cell Assay ^[1]

Mouse K₂P2.1, human K₂P4.1, and mutants are expressed from a previously described pIRES2-EGFP vector in HEK293T cells (ATTC). 70% confluent cells are transfected (in 35-mm diameter wells) with LipofectAMINE 2000 for 6 h, and plated onto coverslips coated with Matrigel. Effects of ML335, ML402 and arachidonic acid on K₂P2.1 current at 0 mV are measured by whole-cell patch-clamp experiments 24 h after transfection. Acquisition and analysis are performed using pCLAMP9 and an Axopatch 200B amplifier. Pipette resistance ranges from 1 to 1.5 MΩ. Pipette solution contains the following: 145 mM KCl, 3 mM MgCl₂, 5 mM EGTA and 20 mM HEPES (pH 7.2 with KOH). Bath solution contains the following: 145 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 3 mM MgCl₂ and 20 mM HEPES (pH 7.4 with NaOH). K₂P2.1 currents are elicited by a 1 s ramp from -100 to +50 mV from a -80 mV holding potential. After stabilization of the basal current, ML335 and ML402 are perfused at 200 mL per hour until potentiation is stably reached^[1].

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

REFERENCES

[1]. Lolicato M, et al. K₂P2.1 (TREK-1)-activator complexes reveal a cryptic selectivity filter binding site. *Nature*. 2017 Jul 20;547(7663):364-368.

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

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