BIOLOGICAL ACTIVITY:
RN–1734 is selective antagonist of the TRPV4 channel, completely antagonizes 4αPDD–mediated activation of TRPV4 with comparable, low micromolar IC50 values for all three species (hTRPV4: IC50 = 2.3 μM, mTRPV4: IC50 = 5.9 μM, rTRPV4: IC50 = 3.2 μM). IC50 value: 2.3 μM (hTRPV4), 5.9 μM (mTRPV4), IC50 = 3.2 μM (rTRPV4)
Target: TRPV4
in vitro: RN–1734 completely inhibits both ligand– and hypotonicity–activated TRPV4. In addition, RN–1734 is selective for TRPV4 in a TRP selectivity panel including TRPV1, TRPV3 and TRPM8, and could thus be a valuable pharmacological probe for TRPV4 studies. [1]

PROTOCOL (Extracted from published papers and Only for reference)
Cell assay [1] Cells expressing TRPV4 were cultured in DMEM medium containing 5% PenStrep, 5% glutamax, 200 μg/mL hygromycin, 5 μg/mL blasticidin and 10% heat inactivated FBS. Twenty–four hours prior to the assay, cells were transferred to PDL–coated, plastic 96–well black–walled plates and grown using culture media supplemented with 1 μg/mL doxycycline and 1 μM ruthenium red. The assay medium itself comprises DMEM + 0.25 mg/mL BSA. Plates are cooled for 1 min by being placed on a metal block kept at −20°C, the medium aspirated and ice–cold wash buffer I (PBS without Ca2+/Mg2+) is added to the cells. The wash buffer is aspirated and 45Ca2+ in medium is added to each well. The agonist EC50 curves of 4αPDD and RN–1747 are determined using concentrations ranging from 13 nM to 30 μM. For IC50 determination, the agonists used are 4αPDD (2 μM), RN–1747 (1 μM) and 30% hypotonicity (addition of 30% water to the cell media). Compounds are tested at concentrations ranging from 13 nM to 30 μM. The cells are then incubated on a shaker for 10 min before the medium is aspirated and the cells are washed in wash buffer II (PBS with Ca2+/Mg2+). Scintillation fluid is added and cells incubated in it for at least 15 min before counting using a MicroBeta liquid scintillation counter.

References:

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