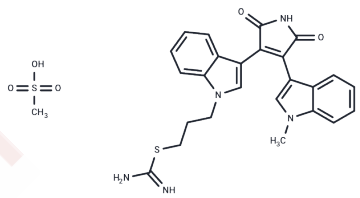
Data Sheet (Cat.No.T6643)

TargetMol

Ro 31-8220 Mesylate

Chemical Properties



CAS No. : 138489-18-6

Formula: C25H23N5O2S·CH4O3S

Molecular Weight: 553.65

Storage: Powder: -20°C for 3 years | In solvent: -80°C for 1 year
Actual storage temperature shall be subject to the COA.

Biological Description

Description	Ro 31-8220 Mesylate (Bisindolylmaleimide IX mesylate) is a pan-PKC inhibitor for PKC-α/βI/βII/γ/ε (IC50: 5/24/14/27/24 nM), and also shows potent inhibition against MSK1, MAPKAP-K1b, S6K1, and GSK3β.
Targets(IC50)	JNK,PKC
In vitro	Within MLP/mice, Ro 31-8220 (6 mg/kg/d, s.c.) significantly enhances myocardial contractility.
In vivo	RO31-8220 effectively inhibits the growth of A549 cells (IC50: 0.78 μM) and MCF-7 cells (IC50: 0.897 μM). In platelets with low adrenergic response, RO31-8220 amplifies the phosphorylation of Akt, thereby enhancing adrenalin-induced platelet aggregation. It significantly reduces the secretion of apolipoprotein E from primary human macrophages by inhibiting the vesicular transport of the apoE gene to the plasma membrane, without significantly affecting the mRNA or protein levels of ApoE. RO31-8220 inhibits the activity of rat brain protein kinase C (IC50: 23 nM) without selectivity towards PKC-α/β/γ/ε isoforms. Additionally, RO31-8220 exerts an inhibitory effect on voltage-dependent sodium channels.
Kinase Assay	Assay of PKC Activity : Assay mixtures contain 0.2 mg/mL peptide-gamma, 10 μM MgCl2, 0.6 mM CaCl2, 10 μM [γ-32P]ATP, 1.25 mg/mL phosphatidylserine and 1.25 ng/mL phorbol 12-myristate 13-acetate in 20 mM Hepes (pH 7.5), 2 mM EDTA, 1 mM dithiothreitol and 0.02% (w/v) Triton X-100. Peptide-γ is a synthetic peptide, GPRPLFCRKGSLRQKW, resembling the PKC-γ pseudosubstrate site, except that a serine residue replaces the pseudosubstrate alanine, converting the peptide from an inhibitor into a substrat. The assays are started by the addition of 2.5 m-units of enzyme, incubated at 30 °C for 10 min and terminated by spotting on to P81 paper, followed by extensive washing in 75 mM orthophosphoric acid. The papers are then washed in ethanol, dried, and incorporated radioactivity is determined by liquidscintillation spectroscopy.
Cell Research	Human A549 lung and MCF-7 breast carcinoma cells are obtained from the European Collection of Animal Cell Cultures. Cells (passage number 10-30) are cultured in an atmosphere of 5% carbon dioxide, the former in Ham's F-12 medium with penicillin/streptomycin, the latter in minimum essential medium (Eagle's modification) with additional pyruvate (1 mM) and non-essential amino acids. Both media are supplemented with 10% FCS and glutamine (2 mM). Cells are subcultured routinely twice

Cell Research	weekly to maintain logarithmic growth. For cell proliferation studies cells are seeded and incubated with 3 ml of medium including agents, which is replenished at intervals of 48 h (A549) or 72 h (MCF-7). Following incubation for 4 days (A549) or 6 days (MCF-7) with drugs, cell number is assessed using a Coulter Counter Model ZM. In order to achieve PKC depletion, cells are incubated for 24 h with bryostatin 1 (1 μM). Under these conditions growth inhibition caused by bryostatin 1 is negligible. Bryostatin is removed by extensive washing of the cells followed by a 2 h recovery period. In previous work using the A549 cell line this washing procedure has been shown to eliminate bryostatin-mediated effects. The cells are then incubated for a further 24 h with staurosporine, RO 31-8220, UCN-01 or H-7. In some experiments cells are incubated with inhibitor for 48 rather than 24 h, in this case bryostation was not removed and left in the incubate. After removal of agents inhibition of DNA synthesis is evaluated by measurement of [3H]Tdr incorporation into cell. Radioactivity is counted using a Packard 1500 Tricarb scintillation counter. (Only for Reference)

Solubility Information

Solubility	Ethanol: 2.8 mg/mL (5.06 mM),Sonication is recommended. DMSO: 250 mg/mL (451.55 mM),Sonication is recommended. (< 1 mg/ml refers to the product slightly soluble or insoluble)
In vivo Formulation	10% DMSO+40% PEG300+5% Tween 80+45% Saline: 2 mg/mL (3.61 mM),Sonication is recommended. 10% DMSO+90% Saline: 10 mg/mL (18.06 mM),Solution. *Please add the solvents sequentially, clarifying the solution as much as possible before adding the next one. Dissolve by heating and/or sonication if necessary. Working solution is recommended to be prepared and used immediately. The formulation provided above is for reference purposes only. In vivo formulations may vary and should be modified based on specific experimental conditions.*

Preparing Stock Solutions

	1mg	5mg	10mg
1 mM	1.8062 mL	9.031 mL	18.062 mL
5 mM	0.3612 mL	1.8062 mL	3.6124 mL
10 mM	0.1806 mL	0.9031 mL	1.8062 mL
50 mM	0.0361 mL	0.1806 mL	0.3612 mL

Please select the appropriate solvent to prepare the stock solution, according to the solubility of the product in different solvents. Please use it as soon as possible.

Note: The dilution table applies only to solid products. For liquid products, please calculate the stock solution based on the stated concentration and/or density.

Reference

Wilkinson SE, et al. Biochem J. 1993, 294 (Pt 2), 335-337.
Chen X, Xi Z, Liang H, et al. Melatonin prevents mice cortical astrocytes from hemin-induced toxicity through activating Nrf2/HO-1 signaling in vitro. Frontiers in Neuroscience. 2019, 13: 760
Davies SP, et al. Biochem J. 2000, 351(Pt 1), 95-105.
Courage C, et al. Br J Cancer. 1995, 71(4), 697-704.
Kim SY, et al. BMB Rep. 2011, 44(2), 140-145.
Karunakaran D, et al. J Biol Chem. 2013, 288(7), 5186-5197.
Chen X, Xi Z, Liang H, et al. Melatonin prevents mice cortical astrocytes from hemin-induced toxicity through activating Nrf2/HO-1 signaling in vitro[J]. Frontiers in Neuroscience. 2019, 13: 760.