

Product Data Sheet

Product Name: Rottlerin
Cat. No.: GC10820

Chemical Properties

Cas. No. 82-08-6

Chemical Name (Z)-1-(6-(3-acetyl-2,4,6-trihydroxy-5-methylbenzyl)-5,7-dihydroxy-2,2-dimethyl-2H-chromen-8-yl)-3-phenylprop-2-en-1-one

SMILES OC1=C2C(OC(C)(C)C=C2)=C(C(/C=C\C3=CC=CC=C3)=O)C(O)=C1CC(C(O)=C(C)C(O)=C4C(C)=O)=C4O

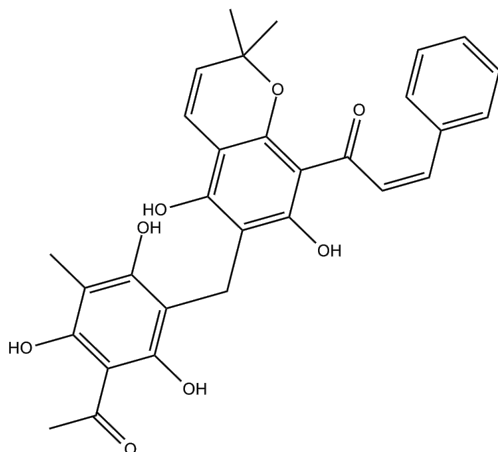
Formula $C_{30}H_{28}O_8$ M.Wt 516.55

Solubility >12.5mg/mL in DMSO (ultrasonic and warming and heat to 60°C) 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

Cell experiment [1]:

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Cell lines

HMVEC cells

Primary HMVEC cells were cultured in EBM-2 medium supplemented with EGM-2 Single Quots. All experiments were performed on near-confluent cultures from passages 3–9 made quiescent by incubation for 18–24h in serumfree EBM-2 medium. Cells were treated with 5 or 20 μ M Rottlerin for different times (2, 6, 24h) and then with TNFa (10ng/mL) for 4h. After treatment, cells were lysed in RIPA buffer (50mM Hepes–KOH, pH 7.4, 250mM NaCl, 1% NP-40, 5mM DTT, protease inhibitors). Total protein concentrations were determined using Bio-Rad protein assay reagent according to the manufacturer's instructions.

Preparation Method Equal amounts of proteins (30 μ g) were separated by SDS–PAGE using NOVEX 4–20% Tris-Glycine Pre-Cast Gel and transferred to a nitrocellulose membrane. The membranes were blocked for 1h with 5% non-fat dry milk in PBS, 0.1% Tween 20 and incubated with the primary antibodies for cyclin D-1, ECE-1, and β -actin. Then, the membranes were incubated with the appropriate secondary antibodies, and after washing, bound antibodies were visualized by enhanced chemiluminescence. Chemiluminescence was captured on X-ray film, and resulting band densities were scanned and quantified using NIH IMAGE software.

Reaction

Conditions

5 or 20 μ M; 2, 6, 24h

Applications

Rottlerin reduces cyclin D-1 to 50%, 40%, and 20% of control levels at 2, 6, and 24h, respectively.

Animal experiment [2]:

Animal models

Balb/c nude mice

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Preparation Method AsPC-1 cells (2×10^6 cells mixed with Matrigel, 50:50 ratio) were injected subcutaneously into the flanks of Balb/c nu/nu mice (4–6 weeks old, $n=7$). Rottlerin (0 or 20mg/kg) was administered to tumor-bearing mice through gavage (5 days per week, once daily) for 6 weeks. At the end of the experiment, all the mice from control and rottlerin-treated groups (seven mice in each group) were euthanized. Tumors from each mouse were isolated and divided into two groups, the first group for the Western blot analysis and the second group for immunohistochemistry.

Dosage form 20mg/kg/day for 6 weeks; p.o.

Applications Rottlerin significantly inhibited the growth of AsPC-1 pancreatic cancer xenografts in Balb/c nude mice, decreased the expression of cell proliferation markers (Ki67 and PCNA) in tumor tissues, increased the activation of apoptosis markers (caspase-3 and PARP), and suppressed the expression of proteins related to angiogenesis (VEGF, VEGFR, Cox-2, IL-8) and epithelial–mesenchymal transition (Slug, Snail, MMP-2, MMP-9), while upregulating the expression of E-cadherin.

References:

- [1] Valacchi G, Pecorelli A, Sticozzi C, et al. Rottlerin exhibits antiangiogenic effects in vitro. *Chem Biol Drug Des.* 2011;77(6):460-470.
- [2] Huang M, Tang SN, Upadhyay G, et al. Rottlerin suppresses growth of human pancreatic tumors in nude mice, and pancreatic cancer cells isolated from Kras(G12D) mice. *Cancer Lett.* 2014;353(1):32-40.

Background

Rottlerin, a natural product purified from *Mallotus Philippinensis*, is a specific PKC inhibitor, with IC₅₀ values for PKC δ of 3-6 μ M, PKC α , β , γ of 30-42 μ M, PKC ϵ , η , ζ of 80-100 μ M [1]. Rottlerin also acts as

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a direct mitochondrial uncoupler, and stimulates autophagy by targeting a signaling cascade upstream of mTORC1^[2]. Rottlerin is often used in the research of viral infections, neurodegenerative diseases and metabolic disorders^{[3][4]}.

In vitro, treatment of human microvascular endothelial cells (HMVECs) with Rottlerin (5 or 20μM; 2, 6, 24h) significantly inhibits the proliferation of cells by reducing thymidine incorporation by 75%–80%, dose-dependently inhibits tube formation on Matrigel with complete inhibition at 20μM after 18h, and reduces cyclin D-1 mRNA to 50%, 40%, and 20% of control levels at 2, 6, and 24h, respectively, with a similar trend observed for protein levels^[5]. Additionally, Rottlerin exhibits significant antiproliferative effects on various cell lines, with IC₅₀ values of 12μM for C6 cells (24h), 5μM for C6 cells (48h), 7μM for T98G cells (48h), and 9μM for U138MG cells (48h); reduces the number of S-phase cells in C6, T98G, and U138MG cells at 5μM (24h); and induces DNA laddering in HL-60 cells at 5μM (24h)^[6].

In vivo, Rottlerin (20mg/kg/day; p.o.; 6 weeks) significantly inhibited the growth of AsPC-1 pancreatic cancer xenografts in Balb/c nude mice, decreased the expression of cell proliferation markers (Ki67 and PCNA) in tumor tissues, increased the activation of apoptosis markers (caspase-3 and PARP), and suppressed the expression of proteins related to angiogenesis (VEGF, VEGFR, Cox-2, IL-8) and epithelial–mesenchymal transition (Slug, Snail, MMP-2, MMP-9), while upregulating the expression of E-cadherin^[7].

References:

- [1] Gschwendt M, Müller HJ, Kielbassa K, et al. Rottlerin, a novel protein kinase inhibitor. *Biochem Biophys Res Commun*. 1994;199(1):93-98.
- [2] Balgi AD, Fonseca BD, Donohue E, et al. Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling. *PLoS One*. 2009;4(9):e7124.
- [3] Lama Z, Gaudin Y, Blondel D, Lagaudrière-Gesbert C. Kinase inhibitors tyrphostin 9 and rottlerin block early steps of rabies virus cycle. *Antiviral Res*. 2019;168:51-60.
- [4] Zhang D, Anantharam V, Kanthasamy A, Kanthasamy AG. Neuroprotective effect of protein kinase C delta inhibitor rottlerin in cell culture and animal models of Parkinson's disease. *J Pharmacol Exp Ther*. 2007;322(3):913-922.
- [5] Valacchi G, Pecorelli A, Sticozzi C, et al. Rottlerin exhibits antiangiogenic effects in vitro. *Chem Biol Drug Des*. 2011;77(6):460-470.
- [6] Parmer TG, Ward MD, Hait WN. Effects of rottlerin, an inhibitor of calmodulin-dependent protein

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kinase III, on cellular proliferation, viability, and cell cycle distribution in malignant glioma cells. *Cell Growth Differ.* 1997;8(3):327-334.

[7] Huang M, Tang SN, Upadhyay G, et al. Rottlerin suppresses growth of human pancreatic tumors in nude mice, and pancreatic cancer cells isolated from Kras(G12D) mice. *Cancer Lett.* 2014;353(1):32-40.

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