

Product Data Sheet

Product Name: Palomid 529

Cat. No.: GC12773

Chemical Properties

Cas. No. 914913-88-5

Chemical Name 8-(1-hydroxyethyl)-2-methoxy-3-[(4-methoxyphenyl)methoxy]benzo[c]chromen-6-one

SMILES CC(C1=CC2=C(C=C1)C3=CC(=C(C=C3OC2=O)OCC4=CC=C(C=C4)OC)OC)O

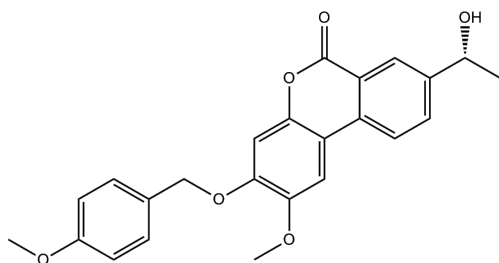
Formula $C_{24}H_{22}O_6$ M.Wt 406.43

Solubility $\geq 41\text{mg/mL}$ in DMSO with gentle warming 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.

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**Kinase
experiment:**

The proteins are produced with rabbit reticulocyte lysates that couples transcription and translation in a single reaction. The amount of template used in each reaction is determined empirically and expression is monitored in parallel reactions where [35S]methionine is incorporated into the receptor followed by gel electrophoresis and exposure to film. Binding reactions of the estrogen receptors (ER) and Palomid 529 (P529) are carried out in 100 mL final volumes in TEG buffer [10 mM Tris (pH 7.5), 1.5 mM EDTA, 10% glycerol]. In vitro transcribed-translated receptor (5 AL) is used in each binding reaction in the presence of 0.5 nM [3H]estradiol (E2). All compounds are routinely tested from 10^{-11} to 10^{-6} M and diluted in ethanol. The reactions are incubated at 4°C overnight and bound E2 is quantified by adding 200 mL dextran-coated charcoal. After a 15-min rotation at 4°C, the tubes are centrifuged for 10 min and 150 mL of the supernatant are added to 5 mL scintillation mixture for determination of cpm by liquid scintillation counting. The maximum binding is determined by competing bound E2 with only the ethanol vehicle. Controls for background are included in each experiment using 5 mL unprogrammed rabbit reticulocyte lysate. This value, typically 10% to 15% of the maximal counts, is subtracted from all values. The data are plotted and K_i s are calculated using the Prism software. Experiments are conducted at least thrice in duplicate[1].

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Cell experiment: The proliferation assay is carried out by seeding the HUVECs in 96-well plates at a density of 1,000 per well in complete medium. Following a 24-h plating period, the cells are starved for 24 h in 0.5% serum before being treated with Palomid 529 in the presence of 10 ng/mL basic fibroblast growth factor (bFGF) or VEGF in complete medium. After 48 h, cell number is determined using a colorimetric method as described by the supplier. The results are expressed as the percentage of the maximal bFGF or VEGF response in the absence of P529. Nonproliferating endothelial cells are assayed by growing HUVECs to quiescence in 96-well plates and treating with Palomid 529 (0, 100, 200, 300 and 400 nM) for 48 h. Initially, 5,000 cells per well are seeded and confluence is achieved the next day. The plates are incubated for another 24 h to ensure growth arrest before treatment with P529. Cell number is determined as outlined above[1].

Animal experiment: Mice[1] Four- to 6-wk-old female nude mice are pretreated with Palomid 529 (200 mg/kg/2d, i.p.) for 1 wk, and then 1×10^5 C6V10 rat glioma cells are injected s.c.. Treatment continued while tumors are allowed to grow for 21 d. U87 cells ($3 \times 10^6/100$ AL) are injected s.c. into nude mice. From day 3 after injection of tumor cells, mice are treated by micronized Palomid 529 (P529) at doses of 50 mg and 25 mg/kg/2 d i.p., respectively. Mice without drug treatment served as controls. U87 tumors are allowed to grow for 24 d. During drug treatment, tumor volumes are measured with a caliper and estimated as $\text{length} \times \text{width} \times \text{width} \times 0.53$. Animals are euthanized and the tumors are taken for immunohistologic and immunoblotting studies.

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References:

- [1]. Xue Q, et al. Palomid 529, a novel small-molecule drug, is a TORC1/TORC2 inhibitor that reduces tumor growth, tumor angiogenesis, and vascular permeability. *Cancer Res*, 2008, 68(22), 9551-9557.
- [2]. Weinberg MA, et al. RES-529: a PI3K/AKT/mTOR pathway inhibitor that dissociates the mTORC1 and mTORC2 complexes. *Anticancer Drugs*. 2016 Jul;27(6):475-87.
- [3]. Gravina GL, et al. The TORC1/TORC2 inhibitor, Palomid 529, reduces tumor growth and sensitizes to docetaxel and cisplatin in aggressive and hormone-refractory prostate cancer cells. *Endocr Relat Cancer*. 2011 Jul 1;18(4):385-400.

Background

Palomid 529 (P529) is a novel potent antitumour PI3K/Akt/mTOR inhibitor with a GI₅₀ of <35 μ M in the NCI-60 cell lines panel.[1] Palomid 529 inhibits both VEGF-driven and bFGF-driven endothelial cell proliferation with IC₅₀ of 20 nM and 30 nM, respectively.

PI3K activates AKT, then AKT activates CREB, inhibits p27, locates FOXO in the cytoplasm, activates PtdIns-3ps, and mTOR which can effect transcription of p70 or 4EBP1.[2,3]

In many cancers, this pathway is overactive, thus reducing apoptosis and allowing proliferation. This pathway is necessary, however, to promote growth and proliferation over differentiation of adult stem cells, neural stem cells specifically. Additionally, this pathway has been found to be a necessary component in neural long term potentiation.[4] Lowering the effect of the PI3K pathway and increasing the effect of GSK3 β and HB9 in NSCs is a potential way of generating these cells better for survival of the neurons. Amplifying the PI3K/AKT pathway increases this neural outgrowth which can determine appropriate treatment concentrations of bisperoxovanadium to stimulate axonal outgrowth but not cause cancer. Not only Palomid 529 inhibits radiation-induced overexpression of Id-1 and VEGF, but also down-regulates radiation-induced MMP-2 and MMP-9. [5]

Reference:

- [1]. Xue Q, et al. "Palomid 529, a novel small-molecule drug, is a TORC1/TORC2 inhibitor that reduces tumor growth, tumor angiogenesis, and vascular permeability." *Cancer Res*, 2008, 68(22), 9551-9557.
- [2]. Rafalski, V. A.; Brunet, A. "Energy metabolism in adult neural stem cell fate". *Progress in Neurobiology*. 2011, 93 (2): 182–203.
- [3]. Ojeda, L. et al. "Critical role of PI3K/Akt/GSK3 β in motoneuron specification from human neural

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stem cells in response to FGF2 and EGF". PLoS ONE 2011, 6 (8): e23414.

[4]. Sui, L; Wang, J; Li, B. M. "Role of the phosphoinositide 3-kinase-Akt-mammalian target of the rapamycin signaling pathway in long-term potentiation and trace fear conditioning memory in rat medial prefrontal cortex". *Learning & Memory* 2008, 15 (10): 762–76.

[5]. Diaz R, et al. "The novel Akt inhibitor Palomid 529 (P529) enhances the effect of radiotherapy in prostate cancer". *Br J Cancer* 2009, 100(6), 932-940.

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