

Product Data Sheet

Product Name: C527
Cat. No.: GC13892

Chemical Properties

Cas. No. 192718-06-2

Chemical Name 2-(4-fluorophenyl)naphtho[2,3-d]oxazole-4,9-dione

SMILES O=C1C2=C(N=C(C3=CC=C(F)C=C3)O2)C(C4=CC=CC=C41)=O

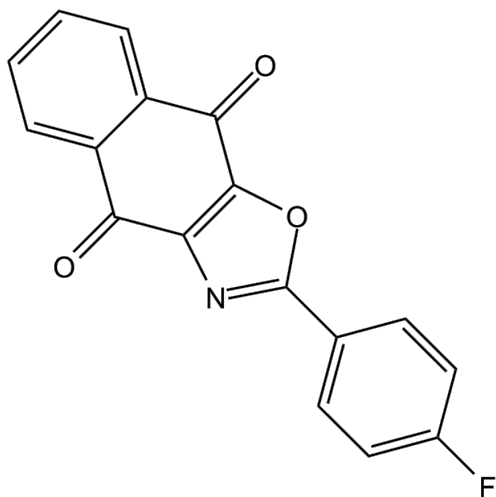
Formula $C_{17}H_8FNO_3$ M.Wt 293.25

Solubility <2.93mg/mL in DMSO Storage 4°C, protect from light

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

Kinase experiment [1]:

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

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In Vitro Deubiquitination Assays

Purified USP5 enzyme was purchased from Boston Biochem. UCH-L1 and UCH-L3 were as reported previously. USP12/46 was prepared in our laboratory as described. The in vitro enzymatic assays were performed as described previously using ubiquitin-AMC (Ub-7-amido-4methylcoumarin; Boston Biochem) as a substrate in a reaction buffer containing 20 mM HEPES-KOH (pH 7.8), 20 mM NaCl, 0.1 mg/ml ovalbumin, 0.5 mM EDTA and 10 mM dithiothreitol. The fluorescence was measured by FluoStar Galaxy Fluorometer (BMG Labtech). For the Ub-vinylsulfone (VS) assay, the proteins were incubated with Ub-VS (Boston Biochem) at 0.5 μ M final concentration for 45 min at 30 °C, followed by the immunoblotting analysis.

Cell experiment [1]:

Cell lines

human U20S osteosarcoma cells, Hela cells

Preparation method

The solubility of this compound in DMSO is <2.93mg/mL. General tips for obtaining a higher concentration: Please warm the tube at 37°C for 10 minutes and/or shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Reacting condition 0.5, 1 and 2 μ M; 24 h

Applications

In human U20S osteosarcoma cells, C527 promoted ID1 degradation in a dose-dependent way. In Hela cells, C527 increased the levels of Ub-FANCD2 and Ub-FANCI. Pre-treatment with C527 also enhanced the cytotoxicity of DNA damaging agents including Mitomycin C and Camptothecin. C527 inhibited Camptothecin induced the Rad51 foci formation and reduced homologous recombination (HR) activity.

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References:

1. Mistry H, Hsieh G, Buhrlage SJ et al. Small-molecule inhibitors of USP1 target ID1 degradation in leukemic cells. *Mol Cancer Ther.* 2013 Dec;12(12):2651-62. doi: 10.1158/1535-7163.MCT-13-0103-T. Epub 2013 Oct 15.

Background

C527, also named heterocyclic tricyclic 1,4-dihydro-1,4-dioxo-1H-naphthalene, is an inhibitor of USP1 (deubiquitinating enzyme 1) / USF1 (USP1-associated factor 1) complex with IC₅₀ value of $0.88 \pm 0.03 \mu\text{M}$ in vivo. C527 is a pan-deubiquitinating enzyme inhibitor in vitro, with a high nanomolar IC₅₀ for the USP1/UAF1 complex.

C527 inhibited the DUB activity of the USP12/USP46 complex and other DUB enzymes in vitro. However, the IC₅₀ of C527 for these DUB enzymes was higher in comparison to USP1/UAF1 complex. C527 had considerably less inhibitory effect on UCH-L1 and UCH-L3, a different subclass of deubiquitinating enzymes, referred to as the ubiquitin C- terminal hydrolases, even though they are also cysteine proteases.

In several leukemic cell lines, C527 promote ID1 degradation, and cause cytotoxicity. In mouse osteosarcoma cells, C527 promotes the degradation of ID1 and the concurrent upregulation of p21. In human U2OS osteosarcoma cells, C527 promoted the dose-dependent degradation of ID1. In HeLa cells, C527 treatments caused an increase in the levels of Ub-FANCD2 and Ub-FANCI, and inhibited Camptothecin induced the Rad51 foci. Pre-treatment of HeLa cells with USP1 inhibitor caused an enhancement in the cytotoxicity of Mitomycin C and Camptothecin.

Reference:

1. Mistry H, Hsieh G, Buhrlage SJ et al. Small-molecule inhibitors of USP1 target ID1 degradation in leukemic cells. *Mol Cancer Ther.* 2013 Dec;12(12):2651-62. doi: 10.1158/1535-7163.MCT-13-0103-T. Epub 2013 Oct 15.

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