Data Sheet (Cat.No.T207869)

TargetMol

EN219-alkyne

Chemical Properties

CAS No. :

Formula: C22H19BrClN3O3

Molecular Weight:

Storage: Keep away from direct sunlight
Store at -20°C
Actual storage temperature shall be subject to the COA.

Biological Description

Description	EN219-alkyne is an alkyne-functionalized EN219 probe. EN219 is a synthetic covalent ligand with moderate specificity for the N-terminal cysteine (C8) of RNF114, exhibiting an IC50 of 470 nM. It inhibits RNF114-mediated autoubiquitination and p21 ubiquitination. EN219-alkyne acts as a click chemistry reagent, containing an alkyne group that can undergo a copper-catalyzed azide-alkyne cycloaddition reaction (CuAAc) with molecules containing azide groups.
Targets(IC50)	Others
In vitro	EN219-alkyne probe in situ labeling and pull-down study (using 231MFP cells as an example): Begin by treating 231MFP cells with either a DMSO carrier or 50 μM EN219-alkyne probe for 90 minutes. Collect the cells in PBS and lyse them using sonication. Prepare protein blot samples by aliquoting 1 mg of protein from the lysate in 500 μL into each sample, then sequentially add: 10 μL of 5 mM biotin-pyridine azide, and 50 μL of click reaction mixture (consisting of 5 mM TBTA in butanol, DMSO (4:1, v/v), 50 mM Cu(II) SO4, and 50 mM TCEP). Incubate the samples at room temperature with gentle stirring for 1 hour. After CuAAC, precipitate proteins by centrifugation at 6,500 g and wash twice with pre-cooled methanol (500 μL). Centrifuge samples in a pre-cooled 4°C centrifuge at 6,500 g for 4 minutes, remove excess methanol, and resuspend protein precipitates by probe sonication in 250 μL PBS containing 1.2% SDS. Denature proteome at 90°C for 5 minutes, then centrifuge at 6,500 g to precipitate insoluble components and dilute soluble proteome in 1.2 mL PBS (final SDS concentration in samples is 0.2%) to a total volume of 1450 μL, retaining 50 μL as input. Add 85 μL pre-washed 50% streptavidin-agarose bead slurry to each sample and incubate overnight at room temperature with gentle agitation. Rotate beads at 6,500 g for 2 minutes at room temperature and remove supernatant from each sample. Transfer beads to spin columns and wash 3 times with PBS. To elute, boil beads in 50 μL LDS sample buffer for 5 minutes, then collect the eluate by centrifugation for immunoblot analysis. En219 (1 μM for 90 min) interacts with RNF114 C8, TUBB1 C201, HSPD1 C442, and HIST1H3A C97, as confirmed by isotopic tandem orthogonal proteolysis (isoTOP-ABPP) analysis. The above information is based on published literature. Experimental procedures should be appropriately modified to meet specific research demands.

Tel:781-999-4286 E_mail:info@targetmol.com Address:34 Washington Street,Wellesley Hills,MA 02481