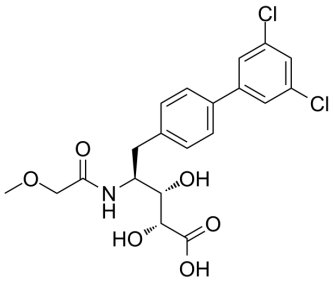
CC0651

Cat. No.:	HY-15301
CAS No.:	1319207-44-7
Molecular Formula:	$C_{20}H_{21}Cl_2NO_6$
Molecular Weight:	442.29
Target:	E1/E2/E3 Enzyme
Pathway:	Metabolic Enzyme/Protease
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 2 years -20°C 1 year



SOLVENT & SOLUBILITY

In Vitro

DMSO : ≥ 56 mg/mL (126.61 mM)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.2610 mL	11.3048 mL	22.6096 mL
5 mM	0.4522 mL	2.2610 mL	4.5219 mL
10 mM	0.2261 mL	1.1305 mL	2.2610 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

CC0651 is an allosteric inhibitor of the human Cdc34 ubiquitin-conjugating enzyme. CC0651 potently (IC_{50}=1.72 μM) inhibits the ubiquitination of $p27^{Kip1}$, as confirmed by dose-response analysis.

IC_{50} & Target

IC50: 1.72 μM ($p27^{Kip1}$ ubiquitination)[1]

In Vitro

CC0651 strongly impairs the rate of ubiquitin chain initiation on substrate by SCF^{Cdc4}, as measured by the monoubiquitination of Sic1 by K0 ubiquitin. CC0651 actually potentiates the formation of both ubiquitin dimers and monoubiquitinated hCdc34, concordant with the observed accumulation of the hCdc34 conjugate in cells treated with the ester derivative of CC0651. CC0651 completely inhibits the assembly of polyubiquitin chains and decreased formation of free triubiquitin and, to a lesser extent, hCdc34 monoubiquitin, but has no effect on production of diubiquitin[1]. CC0651 is an inhibitor of the E2 ubiquitin conjugating enzyme Cdc34A, acts by trapping a weak interaction between ubiquitin and the E2 donor ubiquitin binding site. A quantitative SCF ubiquitination assay with a β-Catenin substrate peptide yields a value of IC_{50} of 18±1 μM for CC0651 inhibition, similar to the effective concentrations observed in the NMR and TR-FRET assays[2].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]

For small-molecule screens, $p27^{Kip1}$ is ubiquitinated in a 15 µL reaction with 40 nM phospho-p27, 40 nM E1, 5 µM E2, 25 nM SCF^{Skp2}, 25 nM Cks1, and 27.8 µM biotinylated ubiquitin in 40 mM Tris-HCl [pH 7.5], 5 mM $MgCl_2$, 1 mM DTT, and 0.5 mM ATP at 23°C for 3 hr. The reaction is terminated with assay diluent, transferred to 384-well protein A plates coated with 62 ng SC528 antibody, incubated for 1 hr, and washed six times with 10 mM Tris-HCl [pH 7.6], 0.05% Tween20 prior to addition of 25 µL of 0.4 mg/mL europium streptavidin in HEPES [pH 7.6], 1% BSA, 0.2% Tween20 per well. Plates are incubated for 1 hr, washed, and read for Eu-time-resolved fluorescence after addition of 25 µL enhancement solution. For gel-based ubiquitination assays, CC0651 and its analogs are preincubated at the indicated concentrations with 0.5 µg E1, 1-4 µg hCdc34, and 50 ng SCF^{Cdc4}, 100 ng SCF^{Fbw7}, 150 ng $SCF^{\beta TrCP}$, or 50 ng SCF^{Skp2} for 10-45 min at 4°C in 20 µL reaction buffer (50 mM HEPES [pH 7.5], 10 mM $MgCl_2$, 2 mM ATP, and 50µM DTT). Reactions are initiated by addition of 1µg ubiquitin and respective SCF substrates (50 ng ^{His}Sic1 phosphorylated by Cln2-Cdc28, 50ng cyclin E phosphorylated by Cdk2, 25 ng biotinylated IκBα phosphopeptide [KKERLLDDHDpSGLDpSMKDEE], or 50 ng $p27^{Kip1}$ phosphorylated by cyclin E-Cdk2), incubated at 30°C for 1-3 hr, and products visualized by immunoblot[1].

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Cell Assay [1]

PC-3 and HCT116 cell lines are grown in DMEM supplemented with Penicillin/Streptomycin, 2 mM glutamine, and 10% fetal bovine serum (FBS). Lentiviral shRNA constructs for human CDC34, UBE2R2, and nontarget control are used. For proliferation assays, PC-3 cultures are seeded at 5000 cells/well in quadruplicate in 24-well plates, grown for 24 hr, and then infected with lentiviral supernatant. For small-molecule inhibition, cells are seeded in quadruplicate at 500 cells/well in 96-well plates, grown for 24 hr, and treated with compound. Proliferation is measured by MTT assay. PC-3 or HCT 116 cultures are synchronized in G0/G1 by serum starvation for 30 hr and then released by addition of 10% serum. For epistasis experiments, PC-3 cells are infected with CDC34 shRNA for 24 hr, followed by addition of inhibitor and assessment of proliferation after 4 days. Anti-Cdc34, anti-p27, anti-α-tubulin, anti-ubiquitin, and anti-cyclin E antibodies are used[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

REFERENCES

[1]. Ceccarelli DF, et al. An allosteric inhibitor of the human Cdc34 ubiquitin-conjugating enzyme. Cell. 2011 Jun 24;145(7):1075-87.

[2]. Huang H, et al. E2 enzyme inhibition by stabilization of a low-affinity interface with ubiquitin. Nat Chem Biol. 2014 Feb;10(2):156-63.

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

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