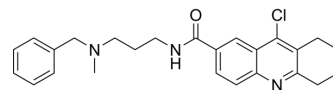


## HBX 19818

Cat. No.:	HY-17540		
CAS No.:	1426944-49-1		
Molecular Formula:	C <sub>25</sub> H <sub>28</sub> ClN <sub>3</sub> O		
Molecular Weight:	421.96		
Target:	Deubiquitinase		
Pathway:	Cell Cycle/DNA Damage		
Storage:	Powder	-20°C	3 years
		4°C	2 years
	In solvent	-80°C	2 years
		-20°C	1 year



### SOLVENT & SOLUBILITY

#### In Vitro

1M HCl : 100 mg/mL (236.99 mM; ultrasonic and adjust pH to 1 with HCl)  
 DMSO : 20 mg/mL (47.40 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	2.3699 mL	11.8495 mL	23.6989 mL
	5 mM	0.4740 mL	2.3699 mL	4.7398 mL
	10 mM	0.2370 mL	1.1849 mL	2.3699 mL

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

#### In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
 Solubility: ≥ 2 mg/mL (4.74 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
 Solubility: ≥ 2 mg/mL (4.74 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

HBX 19818 is a specific inhibitor of ubiquitin-specific protease 7 (USP7), with an IC<sub>50</sub> of 28.1 μM.

#### IC<sub>50</sub> & Target

IC<sub>50</sub>: 28.1 μM (USP7)<sup>[1]</sup>

#### In Vitro

HBX 19818 is an inhibitor of USP7, with an IC<sub>50</sub> of 28.1 μM. HBX 19818 shows no effects on USP8, USP5, USP10, CYLD, UCH-L1, UCH-L3 or on SENP1, a SUMO protease, with IC<sub>50</sub>s of > 200 μM. HBX 19818 selectively inhibits USP7 with IC<sub>50</sub> of 28.1 μM in human cancer cells. In addition, HBX 19818 (1.5, 4, 12, 36, or 100 μM) inhibits USP7 deubiquitination of polyubiquitinated p53. HBX 19818 (30 μM) also causes significantly higher levels of Mdm2 polyubiquitinated forms in USP7-overproducing HEK293 cells than those in DMSO-treated control cells. HBX 19818 inhibits HCT116 proliferation in a dose-dependent

manner, with an IC<sub>50</sub> of 0.2 μM<sup>[1]</sup>.

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

## PROTOCOL

### Kinase Assay <sup>[1]</sup>

The ability of HBX 19818 and HBX 28,258 to inhibit a panel of deubiquitinating enzymes, including UCH-L3 (13 pM), USP7 (100 pM), USP8 (1.36 nM), UCH-L1 (2.5 nM), USP5 (10 nM), USP20 (10 nM), and USP2 (500 pM), is tested using the UbAMC substrate (300 nM). The potential effects of HBX 19818 and HBX 28,258 are also tested on the enzymatic activities of SENP1 (80 pM), cathepsin-B (100 pM), and caspase-3 (100 pM) using the SUMO1-AMC (750 nM), ZRR-AMC (3 μM), and DEVD-AMC (250 nM) substrates, respectively. All enzymes are tested in USP7 reaction buffer (50 mM Tris-HCl [pH 7.6], 0.5 mM EDTA, 5 mM DTT, 0.01% Triton X-100, and 0.05 mg/mL serum albumin), except for two enzymes, USP8 (same buffer but pH 8.8) and caspase-3 (100 mM HEPES [pH 7.5], 10% sucrose, and 0.1% CHAPS). All enzymes are pre-incubated with DMSO or compounds (including HBX 19818) for 30 min at room temperature, and the enzymatic reaction is initiated by adding the substrate of interest. The reaction mixture is incubated at room temperature for 1 hr, and the reaction is stopped by adding acetic acid (100 mM). The reactions are monitored using the PHERAstar<sup>[1]</sup>.

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

### Cell Assay <sup>[1]</sup>

HCT116 cell proliferation is evaluated by incubating HCT116 cells for 30 min in culture medium containing 10 μM 5-bromo-2-deoxyuridine (BrdU), which is incorporated into the DNA of proliferating cells. Cells are then harvested by trypsin treatment, collected by centrifugation, and the pellet is resuspended and incubated in 70% ethanol for 30 min at 4°C. After centrifugation and supernatant removal, DNA is denatured by incubating it in 2 N HCl for 30 min at room temperature. The percentage of BrdU-containing cells is then determined by flow cytometry, making it possible to quantify proliferating cells. Cell cycle is evaluated after treatment with HBX 19818 for 24 hr, followed by fixing detached cells and trypsinized cells in 70% ethanol for 30 minutes at 4°C. Cells are then incubated in PBS supplemented with 1% BSA, 0.5% Tween 20, 50 μg/mL RNase A and 50 μg/mL propidium iodide for 30 minutes at 37°C. Samples are analyzed on a FACSort fluorocytometer. The percentage of cells in the different phases of the cell cycle is calculated using Multicycle software<sup>[1]</sup>.

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

## CUSTOMER VALIDATION

- Cell Metab. 2019 May 7;29(5):1166-1181.e6.
- Nat Struct Mol Biol. 2016 Apr;23(4):270-7.
- Nat Chem Biol. 2017 Dec;13(12):1207-1215.
- Cell Death Differ. 2022 Dec 16.
- Theranostics. 2020 Jul 23;10(20):9332-9347.

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## REFERENCES

[1]. Reverdy C, et al. Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. Chem Biol. 2012 Apr 20;19(4):467-77.

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

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