Tosedostat

Cat. No.: HY-14807

CAS No.: 238750-77-1

Molecular Formula: $C_{21}H_{30}N_2O_6$

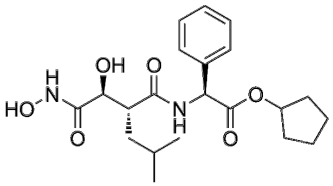
Molecular Weight: 406.47

Target: Aminopeptidase

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 : 100 mg/mL (246.02 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	2.4602 mL	12.3010 mL	24.6021 mL
	5 mM	0.4920 mL	2.4602 mL	4.9204 mL
	10 mM	0.2460 mL	1.2301 mL	2.4602 mL

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

In Vivo

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   Solubility: ≥ 2.5 mg/mL (6.15 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)
   Solubility: ≥ 2.5 mg/mL (6.15 mM); Clear solution
3. Add each solvent one by one: 10% DMSO >> 90% corn oil
   Solubility: ≥ 2.5 mg/mL (6.15 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Tosedostat (CHR-2797) is an orally active aminopeptidase inhibitor. CHR-2797 exerts antiproliferative effects against a range of tumor cell lines[1].

IC_{50} & Target

Aminopeptidase[1]

In Vitro

Treatment of HL-60 cells with Tosedostat (CHR-2797) leads to an increase in the secretion of Stanniocalcin 2 (STC2) protein into the growth medium. Increases in SLC7A11 expression are detectable at 60 nM Tosedostat and as early as 2 h

	posttreatment. The IC_{50}s for inhibition of proliferation by Tosedostat in U-937 and HuT 78 cell lines are 10 nM and >10 μM, respectively. Tosedostat treatment increases expression of amino acid deprivation response (AADR) genes in U-937 cells but not in HuT 78 cells[1]. By 24 h with 0.01 μM Tosedostat the mean MCA production is reduced to 77.8% of the untreated control cells; similarly the MCA production is reduced to 51.3% with 1 μM, 38.5% with 5 μM, and 35.3% with 10 μM Tosedostat[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
In Vivo	Tosedostat (CHR-2797) is active as an anticancer agent in vivo in rodent cancer models, and a dose-response relationship has been shown in two models. The effect of Tosedostat is less apparent when the tumor burden is higher before treatment [1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

PROTOCOL

Kinase Assay [1]	Cells are seeded at a density of 4×10^4/mL, cultured for 24 h, then treated with 0.06 to 6 μM Tosedostat (CHR-2797) for 24 h. After treatment, 5×10^4 cells are washed with PBS and seeded in 100 μL Cys/Met-free RPMI 1640 containing Tosedostat, supplemented with 10% dialyzed FBS. 1.5 μCi [^{35}S]Cys/Met (>1,000 Ci/mmol) is added, and incubation continued for 1 h at 37°C. Cells are captured onto 96-well GF/B filter plates and washed twice with PBS before precipitation with 10% ice-cold trichloroacetic acid (TCA) for 1 h at 4°C. Precipitated proteins are washed four times with ice-cold 10% TCA and air-dried for 1 h. UltimaGold scintillation cocktail is added and allowed to mix for 1 h before scintillation counting using a scintillation counter[1]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [2]	Leukemic cells are washed and suspended in phosphate buffered saline (PBS). 100 μL of cell suspension (1×10^5 cells/mL) is mixed with 100 μL of Tosedostat (CHR-2797) (0.01 to 10 μM) and 200 μM L-alanine 4-methyl-coumaryl-7-amide (ala-MCA) in a 96 well plate in duplicate. The aminopeptidase activity is measured by detecting the fluorescent 7-amino-4-methylcoumarin (MCA) liberated from the cleavage of ala-MCA by cellular aminopeptidases (excitation 355 nm, emission 460 nm)[2]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [3]	A breeding colony of NOD/SCID IL2R gamma^{null} mice are used in this study. The mice are inoculated subcutaneously in the right flank with 2×10^6 H929 myeloma cells in 50 μL RPMI-1640 and 50 μL Matrigel™ Basement Membrane Matrix Growth Factor Reduced. The mice are assigned into the following four treatment groups (10 animals per group): no treatment, Tosedostat 75 mg/kg, CHR-3996 30 mg/kg, and Tosedostat 75 mg/kg with concomitant CHR-3996 30 mg/kg. Tosedostat is administered daily by intra-peritoneal injection beginning four days after the tumour cells are inoculated. Caliper measurements of the longest perpendicular tumour diameters (length) and width are performed every other day to estimate the tumour volume[3]. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

REFERENCES

[1]. Krige D, et al. CHR-2797: an antiproliferative aminopeptidase inhibitor that leads to amino acid deprivation in human leukemic cells. Cancer Res. 2008 Aug 15;68(16):6669-79.

[2]. Jenkins C, et al. Aminopeptidase inhibition by the novel agent CHR-2797 (tosedostat) for the therapy of acute myeloid leukemia. Leuk Res. 2011 May;35(5):677-81.

[3]. Smith EM, et al. The combination of HDAC and aminopeptidase inhibitors is highly synergistic in myeloma and leads to disruption of the NFκB signalling pathway. Oncotarget. 2015 Jul 10;6(19):17314-27.

[4]. Emma M Smith, et al. The combination of HDAC and aminopeptidase inhibitors is highly synergistic in myeloma and leads to disruption of the NFκB signalling pathway. Oncotarget. 2015 Jul 10;6(19):17314-27.

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