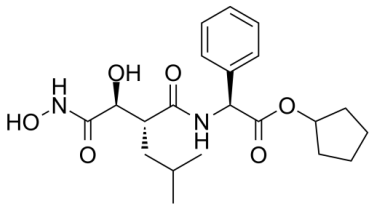
Data Sheet

Product Name:	Tosedostat
Cat. No.:	CS-0003570
CAS No.:	238750-77-1
Molecular Formula:	C21H30N2O6
Molecular Weight:	406.47
Target:	Aminopeptidase
Pathway:	Metabolic Enzyme/Protease
Solubility:	DMSO : 25 mg/mL (61.51 mM; Need ultrasonic and warming)



BIOLOGICAL ACTIVITY:

Tosedostat is an **aminopeptidase** inhibitor. IC50 & 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]. **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].

PROTOCOL (Extracted from published papers and Only for reference)

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]. **Cell Assay:** Tosedostat (CHR-2797) is stored at -20°C as a 10 mM stock in dimethyl sulfoxide (DMSO), and diluted with RPMI culture medium prior to use.[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]. **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].

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.

CAIndexNames:

Benzeneacetic acid, -[[(2R)-2-[(1S)-1-hydroxy-2-(hydroxyamino)-2-oxoethyl]-4-methyl-1-oxopentyl]amino]-, cyclopentyl ester, (S)-

SMILES:

O=C(OC1CCCC1)[C@@H](NC([C@@H]([C@H](O)C(NO)=O)CC(C)C)=O)C2=CC=CC=C2

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