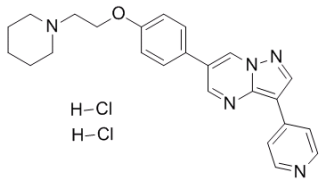
Data Sheet

Product Name:	Dorsomorphin (dihydrochloride)
Cat. No.:	HY-13418
CAS No.:	1219168-18-9
Molecular Formula:	C24H27Cl2N5O
Molecular Weight:	472.41
Target:	AMPK; Autophagy
Pathway:	Autophagy; Epigenetics; PI3K/Akt/mTOR
Solubility:	H_2O: ≥ 48 mg/mL; DMSO: 5.2 mg/mL (Need ultrasonic)



BIOLOGICAL ACTIVITY:

Dorsomorphin dihydrochloride is a potent and selective **AMPK** inhibitor, that is competitive with ATP, with **Ki** of 109±16 nM in the absence of AMP.

IC50 & Target: Ki: 109±16 nM (AMPK)[1]

In Vitro: HT1080 cells are treated with 10 μM Dorsomorphin for 2 h under 2DG stress. Immunoblot analysis reveals that phosphorylation levels of the catalytic α subunit of AMPK are increased by exposure of HT1080 cells to 2DG, whereas both basal and 2DG–induced phosphorylation levels are clearly reduced when Dorsomorphin is added. Measurements of cellular kinase activity using an ELISA–based assay system confirmed that Dorsomorphin does reduce the endogenous AMPK activity regardless of cell culture conditions[2].

In Vivo: Administration of Dorsomorphin over 24 h leads to a 60% increase in total serum iron concentrations. Dorsomorphin treatment is therefore effective in reducing basal levels of hepcidin expression and increasing serum iron concentrations in adult mice[3].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [2]HT1080 cells are seeded in 24–well plates (2×10^4 cells per well) and treated with Dorsomorphin in the presence or absence of glucose or 10 mM 2DG for 2 h. HT1080 cells that overexpressed the wild–type and dominant negative AMPKα1 are prepared by transfecting plasmid DNA (pAMPKα1–wt, pAMPKα1–D168A and pcFlag as a control) in 6–well plates, seeding in 24–well plate and treating with UPR inhibitors. Cells are lysed with cell lysis buffer (20 mM Tris–HCl, pH 7.5, 250 mM NaCl, 10% glycerol, 0.5% NP–40, 1 mM EDTA, 1 mM EGTA, 0.2 mM PMSF, 1 μg/mL pepstatin, 0.5 μg/mL leupeptin, 5 mM NaF, 2 mM Na_3Vo_4, 2 mM β–glycerophosphate, 1 mM DTT). Relative AMPK kinase activity (mean±SD of duplicate determinations) to control sample (vehicle or pcFlag under normal growth conditions) is determined using the CycLex AMPK kinase assay kit[2].

Cell Assay: Dorsomorphin is dissolved in DMSO (10 mM) and stored, and then diluted with appropriate media (DMSO 0.5%) before use[2].[2]HeLa and 786–O cells are treated with various concentrations of Dorsomorphin (0, 0.3, 1, 3, 10 μM), Versipelostatin and Phenformin in the presence or absence of 10 mM 2DG or 1 μg/mL of Tunicamycin as a stressor for 30 h in 96–well plates. For the combination study, 786–O cells are treated with various concentrations of UPR inhibitors in the presence or absence of 10 mM 2DG for 24 h. The medium is then replaced with fresh growth medium, and cells are cultured for a further 15 h. Subsequently, MTT is added to the culture medium, and the absorbance of each well is determined. For the viability assay under glucose–withdrawal conditions, HT1080 cells are treated with various concentrations of Dorsomorphin and phenformin in 12–well plates in the presence or absence of glucose for 18 h, seeded in 96–well plates with growth medium, and then cultured for a further 48 h before MTT is added. Relative cell survival (mean±SD of quadruplicate determinations) is calculated by setting each control absorbance from untreated cells as 100%. The effects of drug combinations at concentrations producing 80% cell growth inhibition (IC_{80}) are analyzed using the isobologram

method[2].

Animal Administration: Dorsomorphin is prepared as a stock solution in DMSO[3].[3]Mice[3]
12–week–old C57BL/6 mice raised on a standard diet are injected via the tail vein with 0.2 g/kg of Dextran or 0.2 g/kg of iron–dextran USP. Dextran is injected with vehicle only, whereas iron–dextran is injected with either vehicle or Dorsomorphin (10 mg/kg). 1 h after injection, mice are killed and liver segments are collected in 500 μL of SDS–lysis buffer and mechanically homogenized. 20 μL of liver extracts are resolved by SDS–PAGE and immunoblotted. Total RNA is harvested using Trizol from mechanically homogenized mouse livers (6 h after injection with a single intraperitoneal dose of Dorsomorphin (10 mg/kg) or DMSO).

References:

[1]. Zhou G, et al. Role of AMP–activated protein kinase in mechanism of metformin action. J Clin Invest. 2001 Oct;108(8):1167–74.

[2]. Saito S, et al. Compound C prevents the unfolded protein response during glucose deprivation through a mechanism independent of AMPK and BMP signaling. PLoS One. 2012;7(9):e45845.

[3]. Yu PB, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol. 2008 Jan;4(1):33–41.

[4]. Zhang DY, et al. Role of autophagy and its molecular mechanisms in mice intestinal tract after severe burn. J Trauma Acute Care Surg. 2017 Oct; 83(4):716–724.

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