Product Data Sheet

Product Name: Niclosamide monohydrate
Cat. No.: GC15901

Chemical Properties

Cas. No.	73360-56-2		
Chemical Name	5-chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide;hydrate		
SMILES	C1=CC(=C(C=C1[N+](=O)[O-])Cl)NC(=O)C2=C(C=CC(=C2)Cl)O.O		
Formula	$C_{13}H_{10}Cl_2N_2O_5$	M.Wt	345.13
Solubility	Soluble in DMSO	Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 ℃ and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20℃ for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		
Structure	Niclosamide monohydrate		

Protocol

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Kinase experiment:	All of the protein kinases are expressed either in Sf9 insect cells or in E.coli as recombinant GST-fusion proteins or His-tagged proteins. A radiometric protein kinase assay is used for measuring the kinase activity of the 22 protein kinases. Briefly, for each protein kinase, 50 µL reaction cocktail containing 60 mM HEPES-NaOH, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, 50 µg/mL PEG20000, 1 µM [γ-33P]-ATP, Niclosamide, adequate amount of enzyme and its substrate. The PKC-alpha assay additionally contain 1 mM $CaCl_2$, 4 mM EDTA, 5 µg/mL phosphatidylserine and 1 µg/mL 1, 2-Dioleyl-glycerol. The reaction cocktails are incubated at 37°C for 60 minutes and stop with 50 µL 2% (v/v) H_3PO_4. Incorporation of 33Pi is determined with a microplate scintillation counter. The activities and the IC50 values are calculated using Quattro Workflow V2.28.
Cell experiment:	Cells are plated in 96-well culture plates with cell density of 3-4 ×103 cells/well and treat with Niclosamide by adding 100 µL medium containing Niclosamide of various concentrations on the second day. After 72-hour's treatment, MTT is added to each well and incubated for additional 4-5 hours, and the absorbance is measured on a microplate reader at 570 nm. Cell growth inhibition is evaluated as the ratio of the absorbance of the sample to that of the control. The results are representative of at least 3 independent experiments.
Animal experiment:	Male nu/nu BALB/c mice are bred at the animal facility of Sun Yat-sen University. HL-60 cells are inoculated s.c. on the flanks of 4- to 6-wk-old mice. Tumors are measured every other day with use of calipers. Mice bearing HL-60 xenografts are randomized to receive treatment with normal saline (control) or p-niclosamide for 15 days (n=7 animals each). Tumor volumes are calculated by the following formula: a2×b×0.4, where a is the smallest diameter and b is the diameter perpendicular to a. After mice are euthanized, xenografts are dissected, weighed, or preserved.

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References:

[1]. Ren, X., et al., Identification of niclosamide as a new small-molecule inhibitor of the STAT3 signaling pathway. ACS Medicinal Chemistry Letters, 2010. 1(9): p. 454-459.
[2]. Wu CJ, et al. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother. 2004 Jul;48(7):2693-6.
[3]. Chen, M., et al., The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling. Biochemistry, 2009. 48(43): p.

10267-74.
[4]. Jin, Y., et al. Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF-kappaB pathway and generation of reactive oxygen species. Cancer Res, 2010. 70(6): p. 2516-27.

Background

Niclosamide monohydrate is an inhibitor of STAT3 with IC50 of 0.25 μM in HeLa cells and inhibits DNA replication in a cell-free assay.

Niclosamide monohydrate is an inhibitor of STAT3, inhibits STAT3-mediated luciferase reporter activity with an IC50 of 0.25 μM in HeLa cells. Niclosamide (1 μM) inhibits the EGF-induced nuclear translocation of STAT3 in Du145 cells. Niclosamide (< 2 μM) dose dependently inhibits the transcription of STAT3 downstream genes in Du145 cells. Niclosamide (< 10 μM) induces G0/G1 arrest and apoptosis of Du145 cancer cells in a dose dependent manner[1]. Niclosamide can block SARS-CoV replication at a micromolar concentration in Vero E6 cells infected with SARS-CoV[2]. Niclosamide (< 7.5 μM) promotes Frizzled1 endocytosis, downregulates Dishevelled-2 protein, and inhibits Wnt3A-stimulated beta-catenin stabilization and LEF/TCF reporter activity in U2OS cells[3]. Niclosamide inhibits the TNF-induced NF-κB reporter activity in a dose- and time-dependent manner in U2OS cells. Niclosamide (125 nM) inhibits NF-κB activation induced by p65, IKKα, IKKβ, IKKγ, and TAK1 in U2OS cells. Niclosamide (< 500 nM) completely block the time- and dose-dependent TNFα-induced alteration of the NF-κB-

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DNA complex in HL-60 cells. Niclosamide (< 10 nM) inhibits constitutive NF-κB activation in U266 cells. Niclosamide inhibits TNF-induced degradation of IκBα and relocation of p65 in a dose- and time-dependent manner in HL-60, Molm13, or AML primary cells. Niclosamide (500 nM) causes decrease in TNF-induced NF-κB-dependent gene products involved in cell survival in HL-60 cells. Niclosamide also inhibits the growth and induces robust apoptosis of AML cells associated with decreased Mcl-1 and XIAP levels and increased intracellular ROS levels[4].

Niclosamide (40 mg/kg/d, i.p.) suppresses growth of xenografted AML cells in nude mice bearing HL-60 xenograft tumors[4].

References:
[1]. Ren, X., et al., Identification of niclosamide as a new small-molecule inhibitor of the STAT3 signaling pathway. ACS Medicinal Chemistry Letters, 2010. 1(9): p. 454-459.
[2]. Wu CJ, et al. Inhibition of severe acute respiratory syndrome coronavirus replication by niclosamide. Antimicrob Agents Chemother. 2004 Jul;48(7):2693-6.
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