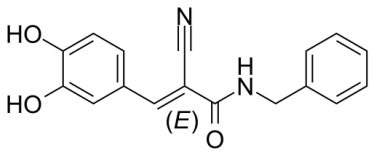
Data Sheet

Product Name:	AG-490
Cat. No.:	CS-0108
CAS No.:	133550-30-8
Molecular Formula:	C17H14N2O3
Molecular Weight:	294.30
Target:	Autophagy; EGFR; STAT
Pathway:	Autophagy; JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Stem Cell/Wnt
Solubility:	DMSO : ≥ 50 mg/mL (169.89 mM)



BIOLOGICAL ACTIVITY:

AG-490 is an tyrosine kinase inhibitor, inhibits **EGFR** and **Stat-3**. IC50 & Target: EGFR and Stat-3[1] **In Vitro:** AG490 inhibits the activation of Stat-3 by selectively blocking JAK2. AG490 is used to selectively inhibit JAK/Stat-3 activation. At a dose of 10 μM, Stat-3 phosphorylation is decreased by >95% and cell viability is maintained. AG490 at a dose of 10 μM results in >95% decrease in pStat-3 in EGF-stimulated A431 cells with no effect on Stat-3 mass[1]. AG-490 is a potent inhibitor of the JAK3/STAT, JAK3/AP-1, and JAK3/MAPK pathways and their cellular consequences. AG-490 abolishes IL-2-inducible [^{3}H]thymidine incorporation in a dose-dependent manner, displaying an IC_{50} of 25 μM. AG-490 potently inhibits IL-2-mediated proliferation in T cells, results distinct from previous studies that showed this agent induced apoptosis in ALL cells while exerting apparently no effects on the growth of mitogen-stimulated normal T cells[2]. **In Vivo:** AG490 significantly inhibits the development of type 1 diabetes (T1D) (p = 0.02, p = 0.005; at two different time points). Monotherapy of newly diagnosed diabetic NOD mice with AG490 (1 mg/mouse) markedly results in disease remission in treated animals (n=23) in comparision to the absolute inability (0%; 0/10, p=0.003, Log-rank test) of DMSO and sustained eugluycemia is maintained for several months following drug withdrawal[3]. AG490 (1-10 μg) significantly attenuates λ-carrageenan-induced thermal hyperalgesia in a dose-dependent manner. AG490 also reduces mechanical hyperalgesia[4].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: AG490 is dissolved in DMSO and stored, and then diluted with appropriate media before use[1].[1]A colorimetric cell proliferation assay is performed using the CellTiter 96 kit. Briefly, A431 cells are plated in 96-well plates (2000 cells/well) and cultured in DMEM/HAM's F-12 supplemented with 10% FCS for 24 h. Cells are incubated in serum-free media for 24 h. EGF (10 ng/mL) is added to all wells. Tyrphostin AG1478 (0.25 mM) and AG490 (10 mM) are added alone or in combination and the culture is incubated for the appropriate time. Medium is aspirated and CellTiter 96 Aqueous One Solution Reagent (20 μL) is added to each well. The plates are incubated at 37°C for up to 1 h and absorbance recorded at 490 nm using a 96-well plate reader. Data are derived from at least three independent experiments (in triplicate) for the both single agents and combination studies. IC_{50} values for Tyrphostin AG1478 (EGFR inhibitor) and AG490 (JAK/STAT inhibitor) are determined. The growth inhibitory effects of the combination are quantified using the Calucsyn software program[1]. **Animal Administration:** AG490 is dissolved in sterile DMSO and brought to final volume by sterile PBS followed by several pipetting to bring the compound into solution (Mice)[3].

AG490 is dissolved in 3.5% DMSO prior to the start of an experiment on each study (Rats)[4].[3][4]Mice[3]

Female NOD/LtJ, NOD.Scid, and BALB/c mice are used. One vial of compound containing 5 mg of AG490 is injected into5 mice (1 mg/mouse) via the i.p route. The control groups are receive the same volume of the vehicle under the same regimens and conditions.

Rats[4]

A total of 28 Male Sprague-Dawley rats (250-300 g) are used. The experiments are performed in rats 48 h after λ-carrageenan injection. A total of 4 groups (n=6) of rats are randomly included in the dose-response study. Group 1 is the vehicle control, which receive 100 μL i.pl. injection of 3.5% DMSO in saline. Groups 2-4 are injected with 3 different doses of AG490 (1, 5 or 10 μg). To study

the effects of naloxone on AG490-induced antinociception, an additional group of rats (group 5; n=4) is observed. Group 5 is co-administered with AG490 (10 μg) and Naloxone (10 μg). The drugs are administered i.pl. in a volume of 100 μl. As reported earlier, the in vivo pharmacological effects of AG490 are observed 4 h after treatment. Thus, the behavioral tests are performed before (baseline assessment) and 4 h after treatment. First, the rats are subjected to the thermal hyperalgesia test; 10 min later, the paw pressure test is performed on the same set of rats. All the experiments are performed between 8:00 a.m. and 2:00 p.m. to reduce the confounding influence of diurnal variations, and all the procedures are performed in a blinded fashion.

References:

[1]. Dowlati A, et al. Combined inhibition of epidermal growth factor receptor and JAK/STAT pathways results in greater growth inhibition in vitro than single agent therapy. Mol Cancer Ther. 2004 Apr;3(4):459-63

[2]. Wang LH, et al. JAK3, STAT, and MAPK signaling pathways as novel molecular targets for the tyrphostin AG-490 regulation ofIL-2-mediated T cell response. J Immunol. 1999 Apr 1;162(7):3897-904.

[3]. Davoodi-Semiromi A, et al. The tyrphostin agent AG490 prevents and reverses type 1 diabetes in NOD mice. PLoS One. 2012;7(5):e36079.

[4]. Cheppudira BP, et al. Anti-hyperalgesic effects of AG490, a Janus kinase inhibitor, in a rat model of inflammatory pain. Biomed Rep. 2015 Sep;3(5):703-706.

CAIndexNames:

2-Propenamide, 2-cyano-3-(3,4-dihydroxyphenyl)-N-(phenylmethyl)-, (2E)-

SMILES:

O=C(NCC1=CC=CC=C1)/C(C#N)=C/C2=CC=C(O)C(O)=C2.[(E)]

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