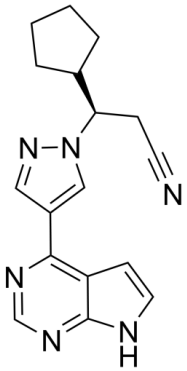
Data Sheet

Product Name:	Ruxolitinib
Cat. No.:	CS-0864
CAS No.:	941678-49-5
Molecular Formula:	$C_{17}H_{18}N_6$
Molecular Weight:	306.37
Target:	Apoptosis; Autophagy; JAK; Mitophagy
Pathway:	Apoptosis; Autophagy; Epigenetics; JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Stem Cell/Wnt
Solubility:	H_2O : < 0.1 mg/mL (ultrasonic;warming;heat to 60°C)



BIOLOGICAL ACTIVITY:

Ruxolitinib (INCB18424) is an orally active and selective **JAK1/2** inhibitor with **IC_{50}**s of 3.3 nM and 2.8 nM in cell-free assays, and has 130-fold selectivity for JAK1/2 over JAK3[1]. Ruxolitinib induces **autophagy** and kills tumor cells through toxic **mitophagy**[3]. IC50 & Target:IC50: 3.3 nM (JAK1), 2.8 nM (JAK2) ***In Vitro:***Ruxolitinib potently and selectively inhibits JAK2V617F-mediated signaling and proliferation, markedly increases apoptosis in a dose dependent manner, and at 64 nM results in a doubling of cells with depolarized mitochondria in Ba/F3 cells.

Ruxolitinib demonstrates remarkable potency against erythroid colony formation with IC_{50} of 67 nM, and inhibits proliferating of erythroid progenitors from normal donors and polycythemia vera patients with IC_{50} values of 407 nM and 223 nM, respectively[1]. ***In Vivo:***Ruxolitinib (180 mg/kg, orally, twice a day) results in survive rate of greater than 90% by day 22 and markedly reduces splenomegaly and circulating levels of inflammatory cytokines, and preferentially eliminated neoplastic cells, resulting in significantly prolonged survival without myelosuppressive or immunosuppressive effects in a JAK2V617F-driven mouse model[1].

In the Ruxolitinib group, the primary end point is reached in 41.9% of patients, as compared with 0.7% in the placebo group in the double-blind trial of myelofibrosis. Ruxolitinib results in maintaining of reduction in spleen volume and improvement of 50% or more in the total symptom score[2].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay:[1]Recombinant proteins are expressed using Sf21 cells and baculovirus vectors and purified with affinity chromatography. JAK kinase assays use a homogeneous time-resolved fluorescence assay with the peptide substrate (-EQEDEPEGDYFEWLE). Each enzyme reaction is carried out with Ruxolitinib or control, JAK enzyme, 500 nM peptide, adenosine triphosphate (ATP; 1mM), and 2% dimethyl sulfoxide (DMSO) for 1 hour. The 50% inhibitory concentration (IC_{50}) is calculated as INCB018424 concentration required for inhibition of 50% of the fluorescent signal. **Cell Assay:**Ruxolitinib is dissolved in 0.2% final DMSO.[1]Cells are seeded at 2×10^3/well of white bottom 96-well plates, treated with Ruxolitinib (INCB018424) from DMSO stocks (0.2% final DMSO concentration), and incubated for 48 hours at 37°C with 5% CO_2. Viability is measured by cellular ATP determination using the Cell-Titer Glo luciferase reagent or viable cell counting. Values are transformed to percent inhibition relative to vehicle control, and IC_{50} curves are fitted according to nonlinear regression analysis of the data using PRISM GraphPad. **Animal Administration:**Ruxolitinib is suspended in 5% dimethyl acetamide, 0.5% methocellulose.[1]Mice are fed standard rodent chow and provided with water ad libitum. Ba/F3-JAK2V617F cells (10^5 per mouse) are inoculated intravenously into 6- to 8-week-old female BALB/c mice. Survival is monitored daily, and moribund mice are humanely killed and considered deceased at time of death. Treatment with vehicle (5% dimethyl acetamide, 0.5% methocellulose) or Ruxolitinib (INCB018424) begin within 24 hours of cell inoculation, twice daily by oral gavage. Hematologic parameters are measured using a Bayer Advia120 analyzed, and statistical

significance is determined using Dunnett testing.

References:

[1]. Quintas-Cardama A, et al. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood, 2010, 115(15), 3109-3117.

[2]. Verstovsek S, et al. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. N Engl J Med, 2012, 366(9), 799-807.

[3]. Tavallai M, et al. Rationally Repurposing Ruxolitinib (Jakafi (®)) as a Solid Tumor Therapeutic.Front Oncol. 2016 Jun 13;6:142.

CAIndexNames:

1H-Pyrazole-1-propanenitrile, β-cyclopentyl-4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-, (βR)-

SMILES:

N#CC[C@H](C1CCCC1)N2N=CC(C3=C4C=CNC4=NC=N3)=C2

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