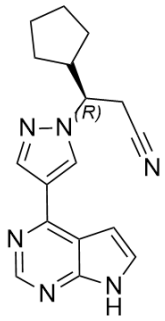
Ruxolitinib

Cat. No.:	HY-50856
CAS No.:	941678-49-5
Molecular Formula:	$C_{17}H_{18}N_6$
Molecular Weight:	306.37
Target:	JAK; Autophagy; Mitophagy
Pathway:	Epigenetics; JAK/STAT Signaling; Stem Cell/WntAutophagy
Storage:	Powder -20°C 3 years 4°C 2 years In solvent -80°C 6 months -20°C 1 month



Solvent & Solubility

In Vitro

DMSO : ≥ 36 mg/mL (**117.50 mM**)

H_2O : < 0.1 mg/mL (insoluble)

* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	3.2640 mL	16.3201 mL	32.6403 mL
5 mM	0.6528 mL	3.2640 mL	6.5281 mL
10 mM	0.3264 mL	1.6320 mL	3.2640 mL

Please refer to the solubility information to select the appropriate solvent.

In Vivo

1. Add each solvent one by one: **10% DMSO >> 90% (20% SBE-β-CD in saline)**
   Solubility: ≥ 2.67 mg/mL (8.71 mM); Clear solution
2. Add each solvent one by one: **10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline**
   Solubility: ≥ 2.67 mg/mL (8.71 mM); Clear solution
3. Add each solvent one by one: **10% DMSO >> 90% corn oil**
   Solubility: ≥ 2.67 mg/mL (8.71 mM); Clear solution

BIOLOGICAL ACTIVITY

Description

Ruxolitinib is a potent 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.

IC_{50} & Target	JAK2	JAK1	Tyk2	JAK3

	2.8 nM (IC_{50})	3.3 nM (IC_{50})	19 nM (IC_{50})	428 nM (IC_{50})

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]. Ruxolitinib (15 mg twice daily) treatment leads a total of 28% of the patients to have at least a 35% reduction in spleen volume at week 48 in patients with myelofibrosis, as compared with 0% in the group receiving the best available therapy. The mean palpable spleen length has decreased by 56% with Ruxolitinib but has increased by 4% with the best available therapy at week 48. Patients in the ruxolitinib group has an improvement in overall quality-of-life measures and a reduction in symptoms associated with myelofibrosis[3].

PROTOCOL

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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Cell Assay [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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.
Animal Administration [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. MCE has not independently confirmed the accuracy of these methods. They are for reference only.

CUSTOMER VALIDATION

- **Nat Med**. 2018 Aug;24(8):1143-1150.

- **Cancer Discov**. 2018 May;8(5):616-631.
- **Sci Transl Med**. 2018 Jul 18;10(450). pii: eaaq1093.
- **Blood**. 2014 Dec 18;124(26):3924-31.
- **Blood**. 2013 Nov 21;122(22):3628-31.

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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]. Harrison C, et al. JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. N Engl J Med. 2012 Mar 1;366(9):787-98.

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