# Product Data Sheet

Product Name: Ruxolitinib sulfate
Cat. No.: GC37575

## Chemical Properties

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|---|---|---|---|
| Cas. No. | 1092939-16-6 | | |
| SMILES | [H][C@@](C1CCCC1)(N2N=CC(C3=C4C=CNC4=NC=N3)=C2)CC#N.O=S(O)(O)=O | | |
| Formula | $C_{17}H_{20}N_6O_4S$ | M.Wt | 404.44 |
| 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 | Ruxolitinib sulfate | | |

## Protocol

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| **Kinase experiment:** | 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 (IC50) is calculated as Ruxolitinib concentration required for inhibition of 50% of the fluorescent signal[1]. |


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| **Cell experiment:** | Cells are seeded at 2 × 103/well of white bottom 96-well plates, treated with Ruxolitinib from DMSO stocks (0.2% final DMSO concentration), and incubated for 48 hours at 37°C with 5% CO2. 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 IC50 curves are fitted according to nonlinear regression analysis of the data using PRISM GraphPad[1]. |
| **Animal experiment:** | Mice[1]Mice are fed standard rodent chow and provided with water ad libitum. Ba/F3-JAK2V617F cells (105 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 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[1]. |


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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.

## Background

Janus-associated kinases (JAKs) are cytoplasmic tyrosine kinases that are required for activating the signaling of certain cytokines and growth factor receptors.[1,2] A JAK2 gene fusion mutation, $JAK2^{V617F}$, that causes unchecked activation of various growth factors

and cytokines, has been linked to myeloproliferative neoplasms (MPNs), including polycythemia vera, essential thrombocythemia, and primary myelofibrosis.[3] Ruxolitinib is a potent ATP mimetic that inhibits both JAK1 and JAK2 with $IC_{50}$ values of 2.7 and 4.5 nM, respectively and is relatively less selective for JAK3 ($IC_{50}$ = 322 nM).[3] It can block interleukin-6 (IL-6) signaling ($IC_{50}$ = 281 nM) and proliferation of $JAK2^{V617F+}$ Ba/F3 cells ($IC_{50}$ = 127 nM).[4] In primary cultures, ruxolitinib preferentially suppresses erythroid progenitor colony formation from $JAK2^{V617F+}$ polycythemia vera patients ($IC_{50}$ = 67 nM) *versus* healthy donors ($IC_{50}$ > 400 nM).[4] In a mouse model of $JAK2^{V617F+}$ MPN, 90 mg/kg ruxolitinib reduced splenomegaly, decreased circulating levels of IL-6 and TNF-α, eliminated neoplastic cells, and prolonged survival of the treated animals.[4]

1.Leonard, W.J., and O'Shea, J.J.JAKS AND STATS: Biological implicationsAnnu. Rev. Immunol.16293-322(1998) 2.Yamaoka, K., Saharinen, P., Pesu, M., et al.The Janus kinases (Jaks)Genome Biol.5(12)253(2004) 3.Verstovsek, S.Therapeutic potential of JAK2 inhibitorsHematology Am. Soc. Hematol. Educ. Program636-642(2009) 4.Quintás-Cardama, A., Vaddi, K., Liu, P., et al.Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: Therapeutic implications for the treatment of myeloproliferative neoplasmsBlood115(15)3109-3117(2010)


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