**NS-018 maleate**

**Cat. No.:** HY-19631  
**CAS No.:** 1354799-87-3  
**Molecular Formula:** C_{25}H_{24}FN_{7}O_{4}  
**Molecular Weight:** 505.5  
**Target:** JAK  
**Pathway:** Epigenetics; JAK/STAT Signaling; Stem Cell/Wnt  
**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: ≥ 30 mg/mL (59.35 mM)

* *≥* means soluble, but saturation unknown.*

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 mM</td>
<td>1.9782 mL</td>
<td>9.8912 mL</td>
<td>19.7824 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.3956 mL</td>
<td>1.9782 mL</td>
<td>3.9565 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.1978 mL</td>
<td>0.9891 mL</td>
<td>1.9782 mL</td>
</tr>
</tbody>
</table>

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

**BIOLOGICAL ACTIVITY**

**Description**  
NS-018 maleate is a highly active and orally bioavailable JAK2 inhibitor, with an IC_{50} of 0.72 nM, 46-, 54-, and 31-fold selectivity for JAK2 over JAK1 (IC_{50}, 33 nM), JAK3 (IC_{50}, 39 nM), and Tyk2 (IC_{50}, 22 nM).

**IC_{50} & Target**  
IC_{50}: 0.72 nM (JAK2), 33 nM (JAK1), 39 nM (JAK3), 22 nM (Tyk2)[1]

**In Vitro**  
NS-018 maleate is a highly active JAK2 inhibitor, with an IC_{50} of 0.72 nM, 46-, 54-, and 31-fold selectivity for JAK2 over JAK1 (IC_{50}, 33 nM), JAK3 (IC_{50}, 39 nM), and Tyk2 (IC_{50}, 22 nM). NS-018 also inhibits Src-family kinases, especially SRC and FYN, and weakly inhibits ABL and FLT3 with 45- and 90-fold selectivity for JAK2, respectively. NS-018 shows potent inhibitory activity against cell lines JAK2V617F or MPLW515L mutations or the TEL-JAK2 fusion gene (expressing a constitutively activated JAK2) with IC_{50} of 11-120 nM, but has only minimal cytotoxicity against most other hematopoietic cell lines that have no constitutively activated JAK2[1]. NS-018 (0.5 μM) preferentially suppresses colony-forming unit-granulocyte/macrophage (CFU-GM) formation from myelodysplastic syndrome (MDS)-derived bone marrow mononuclear cells (BMMNCs). NS-018 (1 μM) suppresses the phosphorylation of STAT3 (the
downstream kinase of JAK2) in CFU-GM-forming cells from MDS patients[2].

| In Vivo | NS-018 (12.5, 25, 50, 100 mg/kg, p.o.) potently prolongs the survival of mice and reduces splenomegaly in a mouse Ba/F3-JAK2V617F disease model. NS-018 (25, 50 mg/kg, p.o.) significantly reduces leukocytosis, hepatosplenomegaly and extramedullary hematopoiesis, improves nutritional status, and prolongs survival in JAK2V617F transgenic mice[1]. |

**PROTOCOL**

**Kinase Assay**[1]

The kinase domains of human JAK1, JAK2, JAK3 and TYK2 are incubated in a reaction medium containing serial dilutions of NS-018, biotinylated peptide substrate, ATP and MgCl2 in a streptavidin-coated plate for 1 h at 30°C. Phosphorylated substrates are spectrophotometrically detected with horseradish peroxidase-linked antibody (PY-20) and TMB (3,3’,5,5’-tetramethylbenzidine) solution. The concentrations require to give 50% inhibition (IC50) are estimated by fitting the absorbance data to a logistic curve with SAS version 8.2. The inhibitory effect of NS-018 is tested against a panel of 53 kinases[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Cell Assay**[2]

Bone marrow mononuclear cells (BMMNCs) from healthy volunteers and myelodysplastic syndrome (MDS) patients are incubated in MethoCult GF H4434 methylcellulose medium containing various hematopoietic cytokines at $1.0 \times 10^5$ cells/mL with or without NS-018 at 37°C in a humidified atmosphere of 5% CO2. Commercially available purified normal human CD34-positive (CD34+) BM cells are used as a control. Burst-forming unit-erythroid (BFU-E) and colonyforming unit-granulocyte/macrophage (CFU-GM) colonies are counted under an inverted microscope on day 14 of culture[2].

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**Animal Administration**[1]

Female BALB/c nude mice are placed in blanket cages in an environment maintained at 21-25°C and 45-65% relative humidity, with artificial illumination for 12 h and a ventilation frequency of at least 15 times/h. They are allowed free access to food pellets and tap water. Ba/F3-JAK2V617F cells ($10^6$ per mouse) are inoculated intravenously into 7-week-old mice. Administration of vehicle (0.5% methylcellulose) or NS-018 twice daily by oral gavage begins the day after cell inoculation. Survival is monitored daily, and moribund mice are humanely killed and their time of death is recorded for purposes of survival analysis. In a parallel study, all mice are humanely killed after 8 days of administration, and their spleens are removed and weighed[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**REFERENCES**

