Dasatinib (BMS-354825) Datasheet

### Technical Data

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular Weight (MW)</td>
<td>488.01</td>
</tr>
<tr>
<td>Formula</td>
<td>C_{22}H_{26}ClN_7O_2S</td>
</tr>
<tr>
<td>CAS No.</td>
<td>302962-49-8, 854001-07-3 (HCI)</td>
</tr>
<tr>
<td>Solubility (25°C)</td>
<td>DMF 98 mg/mL, Water &lt;1 mg/mL, Ethanol &lt;1 mg/mL</td>
</tr>
<tr>
<td>Storage</td>
<td>2 years -20°C Powder, 2 weeks 4°C in DMSO, 6 months -80°C in DMSO</td>
</tr>
</tbody>
</table>

### Biological Activity

#### Description
Dasatinib (BMS-354825, Sprycel) is a Src/Abl inhibitor for wild type Abl and Src with IC50 of 0.6 nM and 0.8 nM, respectively.

#### Targets
- Wild type Abl
- Src

**IC50**
- 0.6 nM
- 0.8 nM

### In vitro
Dasatinib is more effective than imatinib in inhibiting the proliferation of Ba/F3 cells expressing wild-type Bcr-Abl and Bcr-Abl mutants, with the exception of T315I. Dasatinib has a two-log (~325-fold) increased potency relative to imatinib. Dasatinib potently inhibits wild-type Abl kinase and all mutants except T315I over a narrow range. Dasatinib directly targets wild-type and mutant Abl kinase domains and inhibits autophosphorylation and substrate phosphorylation in a concentration-dependent manner. Dasatinib displays 325-fold greater potency compared with imatinib against cells expressing wild-type Bcr-Abl. The percent of colonies of TgE bone marrow cells are decreased from 100% in untreated wells to 4.12% in Dasatinib treated wells. In the presence of Dasatinib, the inhibition of the proliferation by WT and TgE bone marrow cells is statistically significant. Expression of LMP2A is able to promote B lymphocyte survival and proliferation, which can be inhibited by targeting Lyn and/or c-Abl kinases through Dasatinib. Dasatinib treatment inhibits Src signaling, decreases growth, and induces cell cycle arrest and apoptosis in a subset of thyroid cancer cells. Treatment with increasing doses of Dasatinib (0.019 μM to 1.25 μM) for 3 days inhibits the growth of the C643, TPC1, BCPAP, and SW1736 cell lines by about 50% at low nanomolar concentrations, while higher concentrations are required to inhibit the growth of the K1 cell line. Treatment with 10 nM or 50 nM Dasatinib results in a 9-22% increase of cells in the G1 population among BCPAP and SW1736 and K1 cells, and a corresponding 7-18% decrease in the percentage of cells in the S phase.

#### In vivo
Dasatinib reverses splenomegaly in LMP2A/MYC double transgenic mice. Dasatinib specifically prevents colony formation by LMP2A expressing bone marrow B cells and decreased spleen size in the TgE mice. Spleen mass is significantly decreased among Dasatinib treated Tg6/A-MYC mice when compared to the control group. Dasatinib inhibits lymphadenopathy in LMP2A/MYC double transgenic mice. Dasatinib reverses splenomegaly in Rag1KO mice engrafted with tumor cells from LMP2A/MYC double transgenic mice. Dasatinib therapy inhibits Lyn phosphorylation in B lymphocyte tumors expressing LMP2A.

### Clinical Trials
Dasatinib has entered in a phase II clinical trial in the treatment of hemangiopericytoma and gastrointestinal stromal tumor.

### Features

#### Protocol (Only for Reference)

**Kinase Assay:**

- **Kinase assays using wild-type and mutant glutathione S-transferase (GST)-Abl fusion proteins (c-Abl amino acids 220-498) are done. GST-Abl fusion proteins are released from glutathione-Sepharose beads before use.**
- The concentration of ATP is 5 μM. Immediately before use in kinase autophosphorylation and in vitro peptide substrate phosphorylation assays, GST-Abl kinase domain fusion proteins are treated with LAR tyrosine phosphatase. After 1-hour incubation at 30 °C, LAR phosphatase is inactivated by addition of sodium vanadate (1 mM). Immunoblot analysis comparing untreated GST-Abl kinase to dephosphorylated GST-Abl kinase is routinely done using phosphotyrosine-specific antibody 4G10 to confirm complete (>95%) dephosphorylation of tyrosine residues and c-Abl antibody CST 2862 to confirm equal loading of GST-Abl kinase. The Dasatinib concentration range is extended to 1,000 nM for mutant T315I. These same inhibitor concentrations are used for the in vitro peptide substrate phosphorylation assays. The three inhibitors are tested over these same concentration ranges against GST-Src kinase and GST-Lyn kinase.

- **Cell Lines**
  - Ba/F3 cell lines
- **Concentrations**
  - ~32 nM
- **Incubation Time**
  - 72 hours

**Proliferation is measured using a methanethiosulfonate-based viability assay. IC50 and IC90 values are reported for the in vitro peptide substrate phosphorylation assays. The three inhibitors are tested over these same concentration range is extended to 1,000 nM for mutant T315I. These same inhibitor concentrations are used for the in vitro peptide substrate phosphorylation assays, GST-Abl kinase domain fusion proteins are treated with LAR tyrosine phosphatase. After 1-hour incubation at 30 °C, LAR phosphatase is inactivated by addition of sodium vanadate (1 mM). Immunoblot analysis comparing untreated GST-Abl kinase to dephosphorylated GST-Abl kinase is routinely done using phosphotyrosine-specific antibody 4G10 to confirm complete (>95%) dephosphorylation of tyrosine residues and c-Abl antibody CST 2862 to confirm equal loading of GST-Abl kinase. The Dasatinib concentration range is extended to 1,000 nM for mutant T315I. These same inhibitor concentrations are used for the in vitro peptide substrate phosphorylation assays. The three inhibitors are tested over these same concentration ranges against GST-Src kinase and GST-Lyn kinase.

**Cell Assay:**

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  - Ba/F3 cell lines
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**Methods**

**Proliferation** is measured using a methanethiosulfonate-based viability assay. IC50 and IC90 values are reported as the mean of three independent experiments done in quadruplicate. The inhibitor concentration ranges are 0 nM to 32 nM (Dasatinib). The Dasatinib concentration range is extended to 200 nM for mutant T315I.

**Animal Study**

- **Animal Models**: EμLMP2A (TgE and Tg6 strains), MYC (λ-MYC), and LMP2A/λ-MYC double transgenic mice (Tg6/λ-MYC)
- **Formulation**: DMSO
- **Doses**: 30 mg/kg
- **Administration**: Administered via i.p.

**References**


**Customer Reviews**

Data from [Invest New Drugs, 2010. October, Ahead of Print]

**Dasatinib (BMS-354825)** purchased from Selleckchem, cells were either left untreated (Unt.) or exposed to Dasatinib (Das.) for 24 h. Equal amounts of cell lysates were analyzed for ERK1/2, p38 and CREB phosphorylation by Western blot using antibodies specific for the native form of the kinases and for residues that are phosphorylated (P-) in each kinase upon activation. One of three experiments with similar results is shown. Protein bands were quantified by densitometry and level of PERK1/2, P-p38 and P-CREB expressed as arbitrary units were calculated for each cell line after normalization to total ERK1/2, p38 and CREB respectively.

Data from [Biochem Pharmacol, 2011. May, Ahead of Print]

**Dasatinib (BMS-354825)** purchased from Selleckchem. Capacity of the TKI to overcome the AML-typical differentiation blockage. The myeloid cell lines MOLM-13 and HL-60 were incubated for 6 days with 0.01% DMSO (serving as a negative solvent control), 1 mM of ATRA (serving as a positive control), as well as with the indicated doses of the four TKI. (A) Representative May–Gruenwald–Giemsa staining of MOLM-13 cells, (B) quantitation of the percentage of MOLM-13 cells exhibiting at least two morphological signs of differentiation (that is a decrease in cytoplasmic basophilia, a reduction of the nucleo-cytoplasmic ratio, appearance of nuclear lobulation and/or cytoplasmic granules). Percentages were evaluated by examining at least 100 cells/condition; (C) representative FACS overlays of MOLM-13 cells depicting TKI-induced CD11b expression (black line) as compared to the isotype (shaded grey); (D) quantitation of TKI-induced CD11b-expression in MOLM-13 cells; (E) representative slides depicting morphological staining of MOLM-13 cells assessed in the NBT-reduction assay; (F) respective quantitative assessment demonstrating the NBT-reducing capacity under the different drugs; (G) representative May–Gruenwald–Giemsa staining of HL-60 cells.

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