

## Product Data Sheet

Product Name: LY2784544

Cat. No.: GC13848

### Chemical Properties

Cas. No. 1229236-86-5

Chemical Name 3-[(4-chloro-2-fluorophenyl)methyl]-2-methyl-N-(5-methyl-1H-pyrazol-3-yl)-8-(morpholin-4-ylmethyl)imidazo[1,2-b]pyridazin-6-amine

SMILES CC1=CC(=NN1)NC2=NN3C(=C(N=C3C(=C2)CN4CCOCC4)C)CC5=C(C=C(C=C5)Cl)F

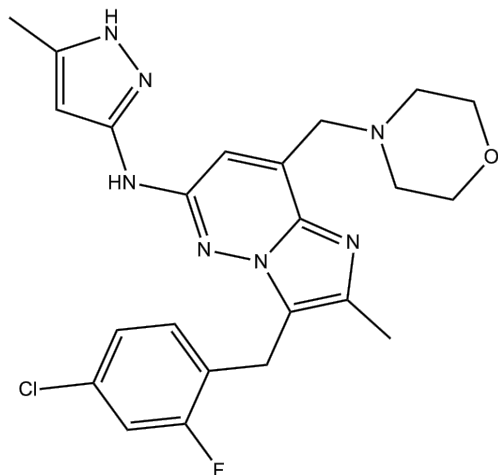
Formula  $C_{23}H_{25}ClFN_7O$  M.Wt 469.94

Solubility  $\geq 23.5$  mg/mL in DMSO,  $\geq 5.65$  mg/mL in EtOH with ultrasonic and warming  
Storage Store at  $-20^{\circ}\text{C}$

General tips For obtaining a higher solubility, please warm the tube at  $37^{\circ}\text{C}$  and shake it in the ultrasonic bath for a while. Stock solution can be stored below  $-20^{\circ}\text{C}$  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



### Protocol

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

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**Cell experiment:** Ba/F3 cells expressing JAK2V617F are placed in RPMI-1640-containing vehicle (DMSO) or Gandotinib (LY2784544) (concentration range, 0.001-20  $\mu$ M) ( $1 \times 10^4$  cells/96-well). Ba/F3 cells expressing wild-type JAK2 are treated similarly except IL-3 (2 ng/mL) is added. After a 72-hour incubation, cell proliferation is assessed by adding Cell Titer 96 Aqueous One Solution Reagent (20  $\mu$ L/well). The IC<sub>50</sub> for inhibition of cell proliferation is calculated using the GraphPad Prism 4 software[1].

**Animal experiment:** Mice[1]Dose- and time-dependent in vivo inhibition of JAK2V617F signaling is assessed by measuring inhibition of STAT5 phosphorylation in a mouse ascitic tumor model. Ba/F3-JAK2V617F-GFP cells ( $1 \times 10^7$ ) are implanted in the intraperitoneal cavity of severe combined immunodeficiency mice (SCID mice) and allowed to develop into ascitic tumors for 7 days. For dose-response studies (six animals/group), Gandotinib (LY2784544) is administered once by oral gavage (2.5, 5, 10, 20, 40, or 80 mg/kg), then 30 min later, ascitic tumor cells are collected, fixed, incubated for 2 h with Mouse-anti-pSTAT5 (pY694) Alexa Fluor 647 (1:10 dilution), and analyzed by flow cytometry. Time course studies are performed similarly, except the animals are treated with Gandotinib (LY2784544) at 20, 40 or 80 mg/kg and ascitic tumor cells collected at prespecified intervals of 0.25-6 h after dosing. Data are analyzed by the one-way analysis of variance, and Dunnett's test ( $\alpha=0.05$ ). Dose response data are analyzed with a four-parameter logistic curve-fitting program.

### References:

[1]. Ma L, et al. Discovery and characterization of LY2784544, a small-molecule tyrosine kinase inhibitor of JAK2V617F. Blood Cancer J. 2013, 3, e109.

### Background

LY2784544 is a potent, selective inhibitor of JAK2 with IC<sub>50</sub> value of 3 nM.[1]

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Janus kinase 2 (JAK2) belongs to the Janus kinase family and is a member of the type II cytokine receptor family. It is a non-receptor tyrosine kinase. The JAK2 mutation (V617F) within the autoinhibitory pseudokinase domain results in a gain-of-function activation of JAK2. The JAK2V617F mutant results in subsequent phosphorylation of signal transducer and activator of transcription 5 (STAT5). Then, STAT5 regulates the transcription of genes associated with cell growth, death and differentiation. The JAK2V617F mutant is oncogenic in cells, and sufficient for generating the myeloproliferative neoplasms (MPN) phenotypes in murine models. Mutant JAK2 kinase is an attractive therapeutic target for the therapy of MPNs. To selectively inhibit JAK2V617F but minimizing inhibition of wild-type JAK2 would be an optimal treatment approach.

LY2784544 is a potent and ATP-competitive inhibitor of JAK2 tyrosine kinase. LY2784544 has 8- and 16-fold selectivity for JAK2 than JAK1 and JAK3, respectively. LY2784544 more effective on inhibiting the phosphorylation of STAT5 in Ba/F3-TEL-JAK2 cells than in Ba/F3-TEL- JAK3 and – JAK1 cells with IC<sub>50</sub> of 0.191 (JAK2), 2.904 (JAK1) and 4.744 nM (JAK3). LY2784544 significantly inhibited STAT5 phosphorylation at a concentration range from 50 nM–20 nM in Ba/F3 cells expressing JAK2V617F. On the other hand, LY2784544 showed minimal inhibition of STAT5 phosphorylation at 50 and 200 nM concentrations in IL-3-stimulated Ba/F3 cells which express wild-type JAK2. LY2784544 significantly inhibited the cell proliferation of JAK2V617F-expressing cells with IC<sub>50</sub> = 55 nM. In JAK2V617F-expressing cells, LY2784544 induced apoptosis with EC<sub>50</sub> ± s.d. of 113 ± 0.023 nM measured by Caspase3/7-glo assays.[1]

LY2784544 with a TED<sub>50</sub> (threshold effective Dose 50) of 12.7 mg/kg, potently inhibits STAT5 phosphorylation in SCID mice bearing Ba/F3-JAK2V617F-GFP. In mouse hematologic disease model, LY2784544 also dose-dependently reduces Ba/F3-JAK2V617F-GFP tumor burden.[1]

### References:

1. Ma L, Clayton JR, Walgren RA, Zhao B, Evans RJ, Smith MC, Heinz-Taheny KM, Kreklau EL, Bloem L, Pitou C et al: Discovery and characterization of LY2784544, a small-molecule tyrosine kinase inhibitor of JAK2V617F. *Blood Cancer J* 2013, 3:e109.

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