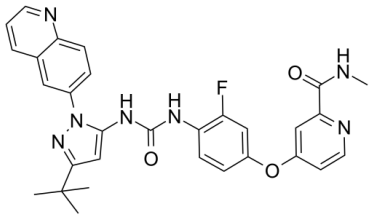
Data Sheet

Product Name:	Rebastinib
Cat. No.:	CS-1038
CAS No.:	1020172-07-9
Molecular Formula:	C30H28FN7O3
Molecular Weight:	553.59
Target:	Apoptosis; Bcr-Abl; FLT3; Src
Pathway:	Apoptosis; Protein Tyrosine Kinase/RTK
Solubility:	DMSO : 50 mg/mL (90.32 mM; ultrasonic and warming and heat to 80°C)



BIOLOGICAL ACTIVITY:

Rebastinib (DCC-2036) is a conformational control **Bcr-Abl** inhibitor for **Abl1^{WT}** and **Abl1^{T315I}** with **IC_{50}** of 0.8 nM and 4 nM, also inhibits SRC, KDR, FLT3, and Tie-2, and low activity to seen towards c-Kit. IC50 & Target: IC50: 0.75±0.11 nM (ABL1^{WT}), 2±0.3 nM (FLT3), 4±0.3 nM (KDR), 6±0.3 nM (TIE2), 34±6 nM (SRC)[1] **In Vitro:** Rebastinib (DCC-2036) inhibits ABL1^{native} and the gatekeeper mutant ABL1^{T315I} with IC_{50} of 0.8 nM and 4 nM, respectively. Rebastinib potently (IC_{50} 0.82 nM) inhibits u-ABL1^{native}, which is thought to exist predominantly in the inactive type II conformation. In addition, Rebastinib also strongly inhibits p-ABL1^{native} (IC_{50} 2 nM), which more readily adopts an active, Type I conformation. More significantly, Rebastinib potently inhibits both u-ABL1^{T315I} (IC_{50} 5 nM) and p-ABL1^{T315I} (IC_{50} 4 nM), both of which exist predominately in the Type I conformation due to stabilization of an activating hydrophobic spine by the T315I mutation. Rebastinib also potently inhibits ABL1H396P (IC_{50} 1.4 nM), which, like ABL1^{T315I}, is predisposed to exist predominately in a Type I activated conformation due to the restricted backbone torsional angles imposed by the mutant Pro396. In addition to ABL1, Rebastinib also inhibits the SRC family kinases LYN, SRC, FGR, and HCK, and PDGFRα, and PDGFR β with IC_{50} of 29±1, 34±6, 38±1, 40±1, 70±10 and 113±10 nM, respectively. Notably, Rebastinib spared c-KIT (IC_{50} 481 nM). Rebastinib effectively inhibits the proliferation of Ba/F3 cells expressing native BCR-ABL1^{native} (IC_{50} 5.4 nM). Rebastinib also inhibits proliferation of the Ph$^+$ cell line K562 (IC_{50} 5.5 nM). REBASTINIB (DCC-2036) also inhibits proliferation of several common TKI-resistant mutants of BCR-ABL1, including G250E, Q252H, Y235F, E255K, V299L, F317L, and M351T, at IC_{50}s ranging from 6-150 nM. Rebastinib effectively inhibits autophosphorylation of BCR-ABL1^{native} (IC_{50} 29 nM) and BCR-ABL1^{T315I} (IC_{50} 18 nM), as well as the phosphorylation of STAT5 in both cell lines (IC_{50} 28 nM and 13 nM, respectively)[1]. **In Vivo:** A single oral dose of Rebastinib (DCC-2036) at 100 mg/kg afforded circulating plasma levels that exceeded 12 μM for up to 24 hours (data not shown), and effectively inhibited BCR-ABL1 signaling for up to 8 hours in Ba/F3-BCR-ABL1^{T315I} leukemia cells isolated from BM and spleen of tumor-bearing mice, as assessed by intracellular flow cytometric staining for phospho-STAT5 and immunoblotting of tissue extracts for phospho-BCR-ABL1 and phospho-STAT5. Treatment of mice bearing Ba/F3-BCR-ABL1^{T315I} leukemia cells with Rebastinib at 100 mg/kg once daily by oral gavage significantly prolonged their survival, while STI571 at 100 mg/kg twice daily is ineffective. In this aggressive allograft model, Rebastinib (DCC-2036) is as effective for treatment of BCR-ABL^{T315I} leukemia as STI571 at 100 mg/kg twice daily is for treatment of BCR-ABL1^{native} leukemia, and reduced the leukemia cell burden in the spleens of treated mice[1].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: Rebastinib is dissolved in DMSO and stored, and then diluted with appropriate medium before use[1].[1]Ba/F3 cells (3×10^3 cells/well) or primary Ph+ leukemia cells (5×10^4 cells/well) are plated in triplicate in 96-well plates containing test compounds (e.g., Rebastinib (DCC-2036)). After 72h, viable cells are quantified by resazurin or MTT assay. Results represent an average of at least three independent experiments[1]. **Animal Administration:** Rebastinib is prepared in 0.5% CMC/1% Tween-80 (Mice)[1].[1]Mice[1] Ba/F3 cells (1×10^6) transformed to interleukin-3 independence by transduction with either BCR-ABL1^{native} or BCR-ABL1^{T315I} retrovirus are injected intravenously into syngeneic Balb/c recipients. Beginning day 3 post-injection, mice are treated with STI571 (100 mg/kg in

water twice daily via oral gavage) or with Rebastinib (DCC-2036) (100 mg/kg in 0.5% CMC/1% Tween-80, once daily via oral gavage) or with vehicle (0.5% CMC/1% Tween-80) alone. For induction of CML-like leukemia, bone marrow (BM) from male Balb/c donor mice is harvested 4d after intravenous administration of 150 mg/kg 5-FU, transduced with BCR-ABL1^{T315I} retrovirus, and 5×10^5 cells injected intravenously into sublethally irradiated (400 cGy) Balb/c recipients. Beginning at d5 post-transplant, cohorts are treated once daily by oral gavage with vehicle alone, or Rebastinib (DCC-2036) at 100 mg/kg. For induction of B-cell acute lymphoblastic leukemia, BM from donors not pretreated with 5-FU is transduced once with BCR-ABL1^{T315I} retrovirus and 1×10^6 cells injected into sublethally irradiated Balb/c recipients. Beginning at d8 post-transplant, cohorts are treated twice daily by oral gavage with vehicle alone, with Rebastinib (DCC-2036) at 60 mg/kg, with STI571 at 100 mg/kg (in water), or with BMS-354825 at 10 mg/kg (in 80 mM citric acid pH 3.1).

References:

[1]. Chan WW, et al. Conformational control inhibition of the BCR-ABL1 tyrosine kinase, including the gatekeeper T315I mutant, by the switch-control inhibitor DCC-2036. Cancer Cell. 2011, 19(4), 556-568.

CAIndexNames:

2-Pyridinecarboxamide, 4-[4-[[[[3-(1,1-dimethylethyl)-1-(6-quinolinyl)-1H-pyrazol-5-yl]amino]carbonyl]amino]-3-fluorophenoxy]-N-methyl-

SMILES:

CC(C)(C)C1=NN(C(NC(NC2=C(F)C=C(OC3=CC(C(NC)=O)=NC=C3)C=C2)=O)=C1)C4=CC=C5C(C=CC=N5)=C4

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