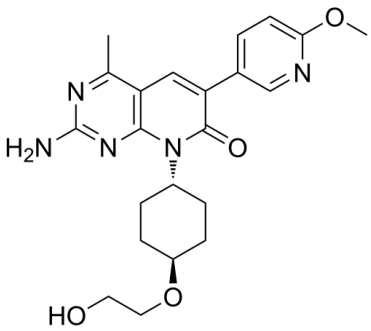
Data Sheet

Product Name:	PF-04691502
Cat. No.:	CS-0919
CAS No.:	1013101-36-4
Molecular Formula:	$C_{22}H_{27}N_5O_4$
Molecular Weight:	425.48
Target:	Autophagy; mTOR; PI3K
Pathway:	Autophagy; PI3K/Akt/mTOR
Solubility:	DMSO : 50 mg/mL (ultrasonic)



BIOLOGICAL ACTIVITY:

PF-04691502 is a potent and selective inhibitor of **PI3K** and **mTOR**. PF-04691502 binds to human PI3Kα, β, δ, γ and mTOR with **K_i**s of 1.8, 2.1, 1.6, 1.9 and 16 nM, respectively. IC50 & Target: Ki: 1.2 nM (mouse PI3Kα), 1.8 nM (human PI3Kα), 2.1 nM (human PI3K β), 1.6 nM (human PI3Kδ) ,1.9 nM (human PI3Kγ), 16 nM (human mTOR)[1] ***In Vitro:*** PF-04691502 inhibits recombinant mouse PI3Kα in an ATP-competitive inhibitor. PF-04691502 potently inhibits AKT phosphorylation on S473 and T308 in all the 3 cancer cell lines with IC_{50} values of 3.8 to 20 nM and 7.5 to 47 nM, respectively. Using a 96-well plate-based P-S6RP(S235/236) ELISA assay, PF-04691502 potently inhibits mTORC1 activity with an IC_{50} of 32 nM. PF-04691502 inhibits cell proliferation of BT20, SKOV3, and U87MG with IC_{50} values of 313, 188, and 179 nM, respectively. In PIK3CA-mutant and PTEN-deleted cancer cell lines, PF-04691502 reduces phosphorylation of AKT T308 and AKT S473 (IC_{50} of 7.5-47 nM and 3.8-20 nM, respectively) and inhibits cell proliferation (IC_{50} of 179-313 nM). PF-04691502 inhibits mTORC1 activity in cells as measured by PI3K-independent nutrient stimulated assay, with an IC_{50} of 32 nM and inhibits the activation of PI3K and mTOR downstream effectors including AKT, FKHRL1, PRAS40, p70S6K, 4EBP1, and S6RP[1]. ***In Vivo:*** Nude mice bearing U87MG tumors are administered orally once a day with PF-04691502 at 0.5, 1, 5, and 10 mg/kg (maximum tolerated dose, MTD). Treatment with 10 mg/kg results in a significant reduction of P-AKT(S473) levels at 1 hour postdosing, and persistent inhibition is observed for 8 hours. P-AKT(S473) recovers to above baseline 24 hours after 10 mg/kg treatment. For P-S6RP(S235/236), a similar inhibition time course is observed, but after 24 hours of treatment, P-S6RP levels remain lower than vehicle tumors. Modulation of the AKT downstream effector, P-PRAS40(T246), and mTOR downstream effector, P-4EBP1(T37/46), is observed. The PF-04691502-treated tumors are also evaluated by immunohistochemistry for levels of P-AKT(S473), total AKT, P-S6RP, and total S6RP. Phosphorylation of AKT and S6RP are significantly reduced at 4 hours after a single dose of PF-04691502 at 10 mg/kg. Dose-dependent tumor growth inhibition (TGI) is obtained in the U87MG xenograft model and approximately 73% TGI is observed at the MTD dose of 10 mg/kg[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]The biochemical protein kinase assays for class I PI3K and mTOR are assessed. The fluorescence polarization assay for ATP competitive inhibition is done as follows: mPI3Kα dilution solution (90 nM) is prepared in fresh assay buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 5 mM DTT, 0.05% CHAPS) and kept on ice. The enzyme reaction contained 0.5 nM mouse PI3Kα (p110α/p85α complex purified from insect cells), 30 μM PIP2, PF-04691502 (0, 1, 4, and 8 nM), 5 mM $MgCl_2$, and 2-fold serial dilutions of ATP (0-800 μM). Final DMSO is 2.5%. The reaction is initiated by the addition of ATP and terminated after 30 minutes with 10 mM EDTA. In a detection plate, 15 uL of detector/probe mixture containing 480 nM GST-Grp1PH domain and 12 nM TAMRA tagged fluorescent PIP3 in assay buffer is mixed with 15 uL of kinase reaction mixture. The plate is shaken for 3 minutes, and incubated for 35 to 40 minutes before reading on an LJL Analyst HT[1]. **Cell Assay:** PF-04691502 is prepared in DMSO and stored,

and then diluted with appropriate medium (DMSO 0.1%) before use[1].[1]BT20, U87MG, and SKOV3 cells are plated at 3,000 cell/well in 96-well culture plates in growth medium with 10% FBS. Cells are incubated overnight and treated with DMSO (0.1% final) or serial diluted compound for 3 days. Resazurin is added to 0.1 mg/mL. Plates are incubated at 37°C in 5% CO_2 for 3 hours. Fluorescence signals are read as emission at 590 nm after excitation at 530 nm. IC_{50} values are calculated by plotting fluorescence intensity to drug concentration in nonlinear curves. U87MG and SKOV3 cells are plated in 96-well plates overnight and caspase-3/caspase-7 activity is assessed with the Caspase-Glo 3/7 Assay Kit[1]. **Animal Administration:** PF-04691502 is formulated in 0.5% methylcellulose in water suspension and given orally once a day[1].[1]Mice[1]
Female nu/nu mice (6-8 weeks old) are used. Tumor cells for implantation are harvested and resuspended in serum-free medium mixed with matrigel (1:1). SKOV3, U87MG, or NSCLC cells ($2.5\text{-}4\times10^6$) are implanted subcutaneously into the hind flank region. Treatment started when average tumor size is 100 to 200 mm^3. PF-04691502 is formulated in 0.5% methylcellulose in water suspension and given orally once a day. Animal body weights and tumor volumes are measured every 2 to 3 days. Tumor volume is determined with Vernier calipers and calculated. Percentage of tumor growth inhibition (TGI) is calculated. Data are presented as mean±SE. Comparisons between treatment groups and vehicle group are done using 1-way ANOVA by Dunnett's tests. Student's t test is used to determine the P value for the comparison of 2 groups.

References:

[1]. Yuan J, et al. PF-04691502, a potent and selective oral inhibitor of PI3K and mTOR kinases with antitumor activity. Mol Cancer Ther. 2011 Nov;10(11):2189-99.

CAIndexNames:

Pyrido[2,3-d]pyrimidin-7(8H)-one, 2-amino-8-[trans-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-

SMILES:

OCCO[C@@H]1CC[C@@H](N2C(N=C(N)N=C3C)=C3C=C(C4=CC=C(OC)N=C4)C2=O)CC1

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