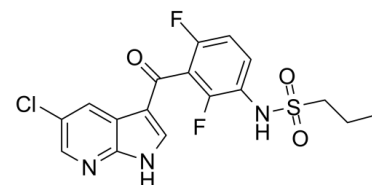


## Data Sheet

Product Name:	PLX-4720
Cat. No.:	CS-0094
CAS No.:	918505-84-7
Molecular Formula:	C <sub>17</sub> H <sub>14</sub> ClF <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S
Molecular Weight:	413.83
Target:	Raf
Pathway:	MAPK/ERK Pathway
Solubility:	DMSO : ≥ 50 mg/mL (120.82 mM)



### BIOLOGICAL ACTIVITY:

PLX-4720 is a potent and selective inhibitor of **B-Raf<sup>V600E</sup>** with **IC<sub>50</sub>** of 13 nM in a cell-free assay, equally potent to c-Raf-1(Y340D and Y341D mutations), and 10-fold selectivity for B-Raf<sup>V600E</sup> than wild-type B-Raf. **IC<sub>50</sub> & Target:** IC<sub>50</sub>: 13 nM (B-Raf<sup>V600E</sup>) **In Vitro:** PLX-4720 displays >10 times selectivity against wild type B-Raf, and >100 times selectivity over other kinases such as Frk, Src, Fak, FGFR, and Aurora A with **IC<sub>50</sub>** of 1.3-3.4 μM. PLX-4720 significantly inhibits the ERK phosphorylation in cell lines bearing B-Raf<sup>V600E</sup> with **IC<sub>50</sub>** of 14-46 nM, but not the cells with wild-type B-Raf. PLX-4720 significantly inhibits the growth of tumor cell lines bearing the B-Raf<sup>V600E</sup> oncogene, such as COLO205, A375, WM2664, and COLO829 with **GI<sub>50</sub>** of 0.31 μM, 0.50 μM, 1.5 μM, and 1.7 μM, respectively. In addition, PLX-4720 treatment at 1 μM induces cell cycle arrest and apoptosis exclusively in the B-Raf<sup>V600E</sup>-positive 1205Lu cells, but not in the B-Raf wild-type C8161 cells<sup>[1]</sup>. PLX-4720 treatment (10 μM) significantly induces > 14-fold expression of BIM in the PTEN<sup>+</sup> cells, compared with the PTEN<sup>-</sup> cell lines (4-fold), giving an explanation of the resistance of PTEN<sup>-</sup> cells to PLX-4720-induced apoptosis<sup>[2]</sup>. **In Vivo:** Oral administration of PLX-4720 at 20 mg/kg/day induces significant tumor growth delays and regressions in B-Raf<sup>V600E</sup>-dependent COLO205 tumor xenografts, without obvious adverse effects in mice even at dose of 1 g/kg. PLX-4720 at 100 mg/kg twice daily almost completely eliminates the 1205Lu xenografts bearing B-Raf<sup>V600E</sup>, while has no activity against C8161 xenografts bearing wild-type B-Raf. The anti-tumor effects of PLX-4720 correlate with the blockade of MAPK pathway in those cells harboring the V600E mutation<sup>[1]</sup>. PLX-4720 treatment at 30 mg/kg/day significant inhibits the tumor growth of 8505c xenografts by >90%, and dramatically decreases distant lung metastases<sup>[3]</sup>.

### PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** <sup>[1]</sup>For each enzyme (0.1 ng), 20-μL reactions are carried out in 20 mM Hepes (pH 7.0), 10 mM MgCl<sub>2</sub>, 1 mM DTT, 0.01% Tween-20, 100 nM biotin-MEK protein, various ATP concentrations, and increasing concentrations of PLX-4720 at room temperature. Reactions are stopped at 2, 5, 8, 10, 20, and 30 minutes with 5 μL of a solution containing 20 mM Hepes (pH 7.0), 200 mM NaCl, 80 mM EDTA, and 0.3% BSA. The stop solution also includes phospho-MEK Antibody, Streptavidin-coated Donor beads and Protein A Acceptor beads from the AlphaScreen Protein A Detection Kit. The antibody and beads are preincubated in stop solution in the dark at room temperature for 30 minutes. The final dilution of antibody is 1/2,000, and the final concentration of each bead is 10 μg/mL. The assay plates are incubated at room temperature for one hour then are read on a PerkinElmer AlphaQuest reader. **Cell Assay:** <sup>[1]</sup> Cells are treated with various concentrations PLX-4720 for 24, 48, and 72 hours. Cell proliferation is measured by using the CellTiter-Glo Luminescent Cell Viability Assay or MTT assay. For cell cycle analysis, supernatant and cells are collected, pelleted, and fixed with 70% ethanol. Before staining with propidium iodide (10 μg/mL), cells are incubated for 1 hour at 37°C in 0.5 mg/mL RNase I to rid samples of residual RNA contamination. Samples are then analyzed by using the EPICS XL apparatus. For the assessment of apoptosis, media and cells are harvested and pelleted before staining with annexin-FITC and propidium iodide. Samples are subsequently analyzed by using the EPICS XL apparatus. **Animal Administration:** <sup>[1]</sup>Metastatic melanoma cells (2×10<sup>6</sup>) are s.c. injected into the

flanks of SCID mice and allowed appr 2 weeks to reach 0.125 mm<sup>3</sup> in volume. Subsequently, the animals receive either 100 mg/kg PLX4720 (oral gavage) or vehicle control twice daily for 15 days. Tumor volume is recorded every 72 h. The average tumor size for each respective group is normalized to the tumor volume at the first day of treatment. After 15 days of treatment, animals are killed and tumors are excised, fixed in formalin, paraffin-embedded, and analyzed by immunohistochemistry.

### References:

- [1]. Tsai J, et al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A, 2008, 105(8), 3041-3046.
- [2]. Paraiso KH, et al. PTEN loss confers BRAF inhibitor resistance to melanoma cells through the suppression of BIM expression. Cancer Res, 2011, 71(7), 2750-2760.
- [3]. Nucera C, et al. B-Raf(V600E) and thrombospondin-1 promote thyroid cancer progression. Proc Natl Acad Sci U S A, 2010, 107(23), 10649-10654.
- [4]. Rizzolio S, et al. Neuropilin-1 upregulation elicits adaptive resistance to oncogene-targeted therapies. J Clin Invest. 2018 Aug 31;128(9):3976-3990.

### CAIndexNames:

1-Propanesulfonamide, N-[3-[(5-chloro-1H-pyrrolo[2,3-b]pyridin-3-yl)carbonyl]-2,4-difluorophenyl]-

### SMILES:

O=S(NC1=CC=C(C(C(C2=CNC3=NC=C(C=C23)Cl)=O)=C1F)F)(CCC)=O

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

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