For Research Use Only

AZ304

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Catalog Number: CM05663

产品信息

Catalog Number:
CM05663

CAS号:
942507-42-8

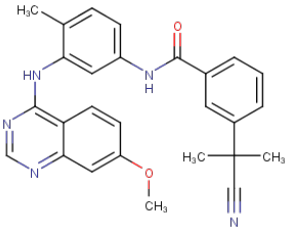
分子式:
$C_{27}H_{25}N_5O_2$

主要靶点:
c-Fms|p38 MAPK|Raf|Autophagy

主要通路:
MAPK 信号通路|蛋白酪氨酸激酶|MAPK 信号通路|自噬

分子量:
451.52

溶解度:
Ethanol:4 mg/mL;H2O:Insoluble;DMSO:50 mg/mL (110.74 mM)



靶点活性

B-Raf (V600E):38 nM (cell free)|CSF1R:35 nM (cell free)|p38:6 nM (cell free)|B-Raf:79 nM (cell free)|C-Raf:68 nM (cell free)

体外活性

AZ304 showed potent inhibitory activities to the kinase domains of wild type BRAF, V600E mutant BRAF and wild type CRAF in vitro, with IC50 values of 79 nM, 38 nM, and 68 nM, respectively. Consistent with BRAF kinase inhibition in vitro, AZ304 potently reduced ERK phosphorylation (p-ERK), with a mean EC50 of 65 nM in the V600E mutant BRAF containing melanoma cell line A375; an EC50 of 60 nM was obtained for the wild type BRAF melanoma cell line SK-MEL-31. AZ304 markedly inhibited cell proliferation in mutant BRAF cancer cell lines and effectively reduced cell growth in selected cell lines harboring wild type BRAF/RAS or mutant RAS. The GI50 values ranged from 0.08–7.72 μM in mutant BRAF cell lines, 0.43–11.7 μM in wild type BRAF/RAS cell lines, and 0.9–16.66 μM in mutant RAS cell lines.

体内活性

Compared with vehicle-treated controls, treatment with AZ304 or Cetuximab alone resulted in reduced tumor growth in both xenograft models. Furthermore, the AZ304 and Cetuximab combination caused dramatic tumour growth inhibition and even shrinking in the Caco-2 xenograft model.

动物实验

Female 4–6 weeks old athymic BALB/c nude mice were purchased from Shanghai SLAC Laboratory Animal Centre. RKO/Caco-2 cells (1 × 10^7) in 200 μl PBS were injected subcutaneously into the right scapular region of mice. After the average tumour size reached 150 – 200 mm^3, animals were randomly divided into 4 groups, each containing three mice and were treated with vehicle only (CON) which orally received 0.5% HPMC and injected with 0.9% saline, AZ304 only (AZ304 dissolved in 0.5% HPMC, 10 mg/kg by oral gavage twice daily), Cetuximab only (40 mg/kg by intraperitoneal injection twice per week), or their combination (A+C) for 10 days. Tumors were measured with a caliper every 2 days, so did body weights. Tumor volume was calculated using the formula V = 1/2 (width^2 × length). Mice were terminated by CO2 inhalation when the tumor diameters reached 1.5 cm, according to the protocol filed with the Guidance of Institutional Animal Care and Use Committee of China Medical University.

细胞实验

Briefly, the cells were treated with DMSO or multiple concentrations of AZ304 for 3 days. The cell growth was determined using the CellTiter 96 Aqueous One Cell Proliferation Assay. Percentage of net growth at day 3 (100%) relative to day 0 (0%) was calculated and the concentration of compound required to inhibit growth by 50% (GI50) determined. The assays were done in triplicate across different plates. The proliferation assays in selected colorectal cancer cell lines were measured using an MTT assay. First of all, cultured cells were seeded into 96-well plates (2000–5000 cells per well). After incubation for 24 h, the cells were pretreated with DMSO or AZ304 for 1 h. Then the indicated doses of Cetuximab were added. Cells were incubated for a further 48 or 72 h. For EGF stimulating assay, cells were incubated in reduced serum medium overnight and then treated with AZ304 or AZ304 + EGF (20 ng/ml) for 72 h. Twenty microliters of MTT solution (5 mg/ml) was added to each well followed by 4 h incubation at 37 °C. The cell culture medium was removed and the cells were lysed in 200 μl DMSO and the results were measured using a microplate reader.

储存

Powder: -20°C for 3 years | In solvent: -80°C for 1 year | Shipping with blue ice.