AZD3759 is a potent, oral active, central nervous system-penetrant, EGFR inhibitor. At Km ATP concentrations, the IC\textsubscript{50}s are 0.3, 0.2, and 0.2 nM for EGFR\textsubscript{wt}, EGFR\textsuperscript{L858R}, and EGFR\textsuperscript{exon 19Del}, respectively.

**In Vitro:** At 2 mM of ATP concentrations, the IC\textsubscript{50}s are 102, 7.6, and 2.4 nM for EGFR\textsubscript{wt}, EGFR\textsuperscript{L858R}, and EGFR\textsuperscript{exon 19Del}, respectively. AZD3759 also inhibits pEGFR in H838\textsubscript{wt}, H3255\textsuperscript{L858R}, and PC-9\textsuperscript{exon 19Del} with IC\textsubscript{50} of 64.5, 7.2, and 7.4 nM, respectively. In cellular phosphorylation studies, AZD3759 also demonstrates 9-fold inhibition selectivity in EGFR-activating mutant cell lines over EGFR wild-type cell lines (H838 cell line).

**In Vivo:** Following oral dosing in rats at 2 mg/kg, absorption of AZD3759 is rapid with blood C\textsubscript{max} of 0.58 μM achieved at 1.0 h. Subsequently, blood concentrations of AZD3759 decline monoexponentially with a mean elimination half-life of 4.3 h, which is close to the same parameter obtained from intravenous dosing of 4.1 h. The bioavailability following an oral dose in rats is 91%. Blood pharmacokinetic parameters of AZD3759 in male dogs are determined following both a single dose intravenous infusion and oral administration. Following the IV dose in dogs, AZD3759 blood clearance is determined as 14 mL/min per kg, and the volume of distribution is 6.4 L/kg. Its elimination half-life is 6.2 h. Absorption of AZD3759 is rapid with blood C\textsubscript{max} (698 nM) occurring between 0.5 and 1.5 h. The oral bioavailability of AZD3759 is excellent at 90%. AZD3759 demonstrated significant dose-dependent antitumor efficacy (78% tumor growth inhibition at 7.5 mg/kg qd and tumor regression at 15 mg/kg qd, respectively, 4 weeks after treatment) with <20% body weight loss, whereas erlotinib had a limited effect in this model. At the end of the study, brain tissues are collected for histological assessment. Significantly decreased tumor area is observed by AZD3759 treatment at the doses of 7.5 and 15 mg/kg. In addition, modulation of pEGFR is detected by a single dose of AZD3759 at 15 mg/kg 1h after dosing, which confirmed target engagement by AZD3759.

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

**Kinase Assay:** AZD3759 is tested at a single 1 μM concentration across each of 124 kinases from Millipore kinase panel at an ATP concentration that is within 15 μM of their corresponding apparent Km values. The detailed protocols could be obtained from Millipore. In brief, recombinant kinases are incubated within appropriate buffer containing peptide substrate and radiolabelled γ-\textsuperscript{33}P-ATP together with presence or absence of required inhibitor concentration. The reaction is initiated by adding ATP/Mg\textsuperscript{2+} mix. After incubation for 40 minutes at room temperature, the reaction is stopped by adding 3% phosphoric acid solution. A portion of reaction mix is spotted onto P30 filtermat to trap peptide, and washed three times for 5 minutes with phosphoric acid to remove non-specific γ-\textsuperscript{33}P-ATP. The phosphorylated substrate is then measured by scintillation counting, which determined the level of kinase activity inhibition compared to control reactions.

**Cell Assay:** AZD3759 is prepared in DMSO and stored, and then diluted with appropriate medium before use. Cell proliferation assay is determined by MTS methods. Briefly, cells are seeded in 96-well plates (at a density to allow for logarithmic growth during the 72-hour assay) and incubated overnight at 37°C and 5% CO\textsubscript{2}. Cells are then exposed to
concentrations of compounds (e.g., AZD3759) ranging from 30 mM to 0.3 μM for 72 hours. For the MTS endpoint, cell proliferation is measured by the CellTiter AQueous Non-Radioactive Cell Proliferation Assay reagent. Absorbance is measured with a Tecan Ultra instrument. Predose measurements are made, and concentration needed to reduce the growth of treated cells to half that of untreated cells (GI50) values are determined using absorbance readings[1]. **Animal Administration:** AZD3759 is prepared in 1% methylcellulose (Rat)[1] Rat[1]. Male Han Wistar rats are orally dosed with the AZD3759 at 2 mg/kg in 1% methylcellulose. At 0.25, 0.5, 1, 2, 4 and 7 hour post-dose, cerebral spinal fluid (CSF) is collected from cisterna magna, and blood samples (>60 μL/time point/each site) are collected via cardiac puncture, into separate EDTA coagulated tubes, and then immediately diluted with 3-fold volume of water. Brain tissue is harvested and homogenized in 3x volume of 100 mM phosphate buffered saline (pH7.4). All samples are stored at -70°C prior to LC/MS/MS analysis.

**References:**


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

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