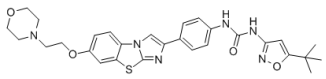
Data Sheet

Product Name:	Quizartinib
Cat. No.:	CS-0211
CAS No.:	950769-58-1
Molecular Formula:	$C_{29}H_{32}N_6O_4S$
Molecular Weight:	560.67
Target:	Autophagy; FLT3
Pathway:	Autophagy; Protein Tyrosine Kinase/RTK
Solubility:	DMSO : ≥ 33 mg/mL (58.86 mM)



BIOLOGICAL ACTIVITY:

Quizartinib is a uniquely potent and selective **Flt3** inhibitor with **K_d** (FLT3 binding affinity) of 1.6±0.7 nM in Biochemical assay. IC50 & Target: Kd: 1.6±0.7 nM (Flt3)[1]

In Vitro: Quizartinib (AC220) is a novel compound expressly optimized as a FLT3 inhibitor for the treatment of acute myeloid leukemia (AML). Quizartinib inhibits FLT3-WT and FLT3-ITD autophosphorylation with IC_{50} of 4.2±0.3 nM and 1.1±0.1 nM, respectively. Quizartinib inhibits MV4-11 and A375 cells with IC_{50} of 0.56±0.3 nM and >10 000 nM, respectively. Quizartinib inhibits FLT3 with low nanomolar potency in cellular assays and is highly selective when screened against the majority of the human protein kinome[1].

In Vivo: Quizartinib (AC220) inhibits FLT3 activity in vivo, significantly extends survival in a mouse model of FLT3-ITD AML at doses as low as 1 mg/kg when dosed orally once a day, eradicates tumors in a FLT3-dependent mouse xenograft model at 10 mg/kg, and potently inhibits FLT3 activity in primary patient cells. The oral bioavailability of Quizartinib, determined in rats by comparing oral and intravenous pharmacokinetics at 3 mg/kg, is approximately 40%. A single 10 mg/kg dose of Quizartinib is administered by oral gavage, and mice are killed at 2 time points after dosing, using groups of 4 animals each. Quantitation of total FLT3 and phospho-FLT3 in tumor samples revealed time-dependent inhibition of FLT3 autophosphorylation. FLT3 activity is inhibited by 90% at 2 hours, and 40% at 24 hours after administration. The extent of inhibition therefore correlated well with the expected free Quizartinib plasma levels, based on pharmacokinetic experiments[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]KinomeScan kinase binding assays are performed. For the FLT3 assay, a kinase construct that spanned the catalytic domain only (amino acids 592 to 969) is used. This construct does not include the juxtamembrane domain and is designed to measure the intrinsic binding affinity of the open FLT3 active site for inhibitors[1]. **Cell Assay:** Quizartinib (AC220) is dissolved in DMSO and stored, and then diluted with appropriate medium before use[1].[1]MV4-11 and RS4;11 cells are cultured in Iscove media with 10% fetal bovine serum (FBS) and RPMI complete with 10% FBS, respectively. For proliferation assays, cells are cultured overnight in low serum media (0.5% FBS), then seeded in a 96-well plate at 40 000 cells per well. Inhibitors (e.g., Quizartinib) are added to the cells and incubated at 37°C for 72 hours. Cell viability is measured using the Cell Titer-Blue Cell Viability Assay. To measure inhibition of FLT3 autophosphorylation, cells are cultured in low serum media (0.5% FBS) overnight and seeded at a density of 400 000 cells per well in a 96-well plate the following day. The cells are incubated with inhibitors (e.g., Quizartinib) for 2 hours at 37°C. To induce FLT3 autophosphorylation in RS4;11 cells, 100 ng/mL FLT3 ligand is added for 15 minutes after the 2-hour compound incubation. Cell lysates are prepared and incubated in 96-well plates precoated with a total FLT3 capture antibody. The coated plates are incubated with either a biotinylated antibody against FLT3 to detect total FLT3 or an antibody against phosphotyrosines to detect FLT3 autophosphorylation. In both cases, a SULFO-tagged streptavidin secondary antibody is used for electrochemiluminescence detection

on the Meso Scale Discovery platform[1]. **Animal Administration:** Quizartinib (hydrochloride salt) is formulated in 22% hydroxypropyl-β-cyclodextrin (Mice)[1].[1]Mice[1]

Female NU/NU or severe combined immunodeficient mice are used. Quizartinib (hydrochloride salt) is formulated in 22% hydroxypropyl-β-cyclodextrin, CEP-701 is formulated in 20% gelucire 44/14 in water (vol/vol), MLN-518 and sunitinib are formulated in 10 mM sodium citrate (pH 3.5), PKC-412 is formulated in 3:1 gelucire 44/14-propylene glycol (vol/vol), and sorafenib (toluene sulfonate salt) is formulated in 80% PEG-400. Compound concentrations are chosen to deliver the desired dose in a volume of 10 mL/kg. Compounds are administered by oral gavage and plasma samples collected 0.25, 0.5, 1, 2, 4, 6, and 24 hours after dosing. To collect plasma samples, eye bleeds (150 µL) are taken semilongitudinally using 3 groups of 3 animals each, taking 2 to 3 time points per animal to obtain a total of 3 independent plasma concentration time courses. Plasma samples and controls (25 µL) are extracted with 4 volumes of acetonitrile containing an internal standard and analyzed by liquid chromatography tandem mass spectrometry.

References:

[1]. Zarrinkar PP, et al. AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute myeloid leukemia (AML). Blood, 2009, 114(14), 2984-2992.

[2]. Puissant A, et al. SYK is a critical regulator of FLT3 in acute myeloid leukemia. Cancer Cell. 2014 Feb 10;25(2):226-42.

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