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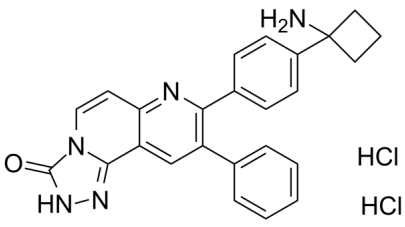
Certificate of Analysis

Catalog Number	BP10050
Product Name	MK-2206 dihydrochloride

Physical and Chemical Properties

Synonyms	MK-2206 2HCl
CAS No.	1032350-13-2
Chemical Formula	C25H23Cl2N5O
Molecular Weight	480.39
Solubility	DMSO: 12 mg/mL (25 mM) Ethanol: <1 mg/mL
Storage	Powder: -20°C for 2 years In solvent: -80°C for 1 year
Chemical Structure OR Tested Image	

Product Information

Description	MK-2206 is a highly specific inhibitor of Akt1/2/3 (IC50: 8/12/65 nM in cell-free assays) and no effect on the activities of 250 other protein kinases.
Targets&IC50	Akt3: 65 nM (cell free), Akt2: 12 nM (cell free), Akt1: 8 nM (cell free)
In vitro	Cells were seeded at a density of 2 to 3 × 103 per well in 96-well plates. Twenty-four hours after plating, varying concentrations of the drug, either as a single agent or in combination, were added to the wells. Cell proliferation was determined by using the CellTiter-Glo assay at 72 or 96 hours after dosing. The nature of the drug interaction was evaluated by using the combination index (CI) according to the method of Chou and Talalay. A commercial software package was obtained from Calcusyn. In combination with docetaxel, we tested three treatment sequences: (a) MK-2206 followed by docetaxel—cells were exposed to MK-2206 for 24 hours, and then after washout of MK-2206, cells were treated with docetaxel for an additional 72 hours; (b) docetaxel followed by MK-2206—cells were exposed to docetaxel for 24 hours, and then after washout of docetaxel, cells were treated with MK-2206 for an additional 72 hours; and (c) concurrent treatment—cells were exposed to both MK-2206 and docetaxel for 72 hours.
In vivo	When the mean tumor size reached 0.13 cm3 for the SK-OV-3 or 0.2 cm3 for the NCI-H292, HCC70, PC-3, and NCI-H460 models, the mice were randomized into control and treatment groups with approximately equivalent ranges of tumor volume between groups (n = 5 animals per group). The following vehicles were used to dose the compounds: 30% Captisol (Cydex) for MK-2206; 0.5% methylcellulose + 0.1% Tween 80 for erlotinib; distilled water for lapatinib; 0.73% ethanol in saline for docetaxel; and saline for carboplatin and gemcitabine. The control group received vehicle only. Tumor volume was measured with calipers twice a week. Animal body weight and physical signs were monitored during the experiments. Tumor volume was calculated, taking length to be the longest diameter across the tumor and width to be the perpendicular diameter, by using the following formula: (length × width)2 × 0.5. Relative tumor volume was assessed by dividing the tumor volume on different observation days with the starting tumor volume. Statistical significance was evaluated by using the two-way repeated ANOVA test followed by Dunnett's test or an unpaired t-test.

Analytical Data

HPLC	Shows Min >99% purity
H-NMR	Consistent with structure
Stability and Solubility Advice	Information on product stability, especially in solution, has rarely been reported and in most cases we can only provide a general guideline. We recommend that once the stock solution has been prepared, it be stored in equal quantities in sealed vials and used within 1 month. Avoid repeated freezing and thawing cycles. Storage conditions for some special products should be referred to their storage details.

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