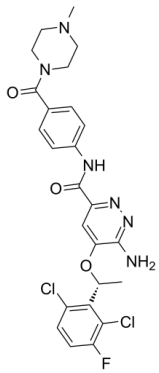
Data Sheet

Product Name:	X-376
Cat. No.:	CS-6077
CAS No.:	1365267-27-1
Molecular Formula:	$C_{25}H_{25}Cl_2FN_6O_3$
Molecular Weight:	547.41
Target:	ALK; c-Met/HGFR
Pathway:	Protein Tyrosine Kinase/RTK
Solubility:	DMSO : 100 mg/mL (182.68 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

X-376 is a potent and highly specific **ALK** tyrosine kinase inhibitor (TKI) (**IC_{50}**=0.61 nM). X-376 is a less potent inhibitor of **MET** (**IC_{50}** =0.69 nM). X-376 displays potent anti-tumor activity[1]. IC50 & Target: IC50: 0.61 nM (ALK), 0.69 nM (MET)[1] **In Vitro:** The ability of X-376 to inhibit the growth of different cancer cell lines harboring ALK fusions or point mutations is tested. X-376 is potent in H3122 lung cancer cells harboring EML4-ALK E13;A20 (IC_{50}: 77 nM). X-376 is also potent in H2228 lung cancer cells harboring EML4-ALK E6a/b; A20 (IC_{50}: 57 nM). Furthermore, X-376 is potent in SUDHL-1 lymphoma cells harboring NPM-ALK (IC_{50}: 32 nM). X-376 also inhibits SY5Y neuroblastoma cells harboring ALK F1174L, MKN-45 gastric carcinoma cells harboring MET dependent, HepG2 cells and PC-9 lung cancer cell lines harboring EGFR exon 19 del with IC_{50}s of 142 nM, 150 nM, 15.137 μM and 3.062 μM, respectively[1]. **In Vivo:** The effects of X-376 in vivo against H3122 xenografts are examined. A pharmacokinetic study reveals that X-376 shows substantial bioavailability and moderate half-lives in vivo. Nude mice harboring H3122 xenografts are treated with X-376 at 50 mg/kg bid. X-376 significantly delays the growth of tumors compared to vehicle alone. In the xenograft experiments, X-376 appears well-tolerated in vivo. Mouse weight is unaffected by X-376 treatment. Drug-treated mice appear healthy and do not display any signs of compound related toxicity. To further assess potential side effects of X-376, additional systemic toxicity and toxico-kinetic studies are performed in Sprague Dawley (SD) rats. Following 10 days of repeated oral administration of X-376 at 25, 50, 100 mg/kg in SD rats, all animals survive to study termination. The no significant toxicity (NST) levels are determined to be 50 mg/kg for X-376. At NST levels, X-376 achieves an AUC of 41 μM×hr and a C_{max} of 5.04 μM[1].

PROTOCOL (Extracted from published papers and Only for reference)

Cell Assay: X-376 is dissolved in DMSO and then diluted before use[1].[1]For viability experiments, cells are seeded in 96-well plates at 25%-33% confluency and exposed to drugs. **The human lung adenocarcinoma cell lines H3122 and H2228** are treated with **X-376 (10, 30, 100, 300 and 1000 nM)**. **SUDHL-1 lymphoma cells** are treated with **X-376 (5, 10, 30, 100 and 300 nM)**. **SY5Y neuroblastoma cells** are treated with **X-376 (30, 100, 300 and 1000 nM)**. At **72 hours** post X-376 addition, Cell Titer Blue Reagent is added and fluorescence is measured on a Spectramax spectrophotometer. All experimental points are set up in hextuplicate replicates and are performed at least two independent times. IC_{50}s are calculated using GraphPad Prism version 5 for Windows. The curves are fit using a nonlinear regression model with a log (inhibitor) vs. response formula[1].
Animal Administration: X-376 is formulated in 0.5% HPMC, 0.4% Tween80, 99.1% DI water[1].[1]Mice[1]
Nude mice (nu/nu) are injected with H3122 cells. Once tumors reach an average volume of 450 mm^3, a total of 27 athymic mice harboring H3122 tumors are randomized and dosed via oral gavage with **50 mg/kg X-376** or the control vehicle. Two, five, and fifteen hours after the single treatment (3 tumors/timepoint/group), mice are sacrificed and serum is collected for assessment of drug concentration using an LC-MS based bioanalytical method.

References:

[1]. Lovly CM, et al. Insights into ALK-driven cancers revealed through development of novel ALK tyrosine kinaseinhibitors. Cancer Res. 2011 Jul 15;71(14):4920-31.

CAIndexNames:

3-Pyridazinecarboxamide, 6-amino-5-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-N-[4-[(4-methyl-1-piperazinyl)carbonyl]phenyl]-

SMILES:

O=C(C1=NN=C(N)C(O[C@@H](C2=C(Cl)C=CC(F)=C2Cl)C)=C1)NC3=CC=C(C(N4CCN(C)CC4)=O)C=C3

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