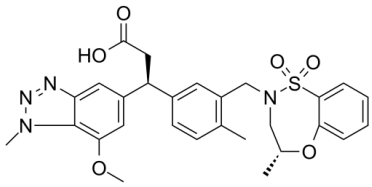
Data Sheet

Product Name:	KI696
Cat. No.:	CS-0020903
CAS No.:	1799974-70-1
Molecular Formula:	C28H30N4O6S
Molecular Weight:	550.63
Target:	Keap1-Nrf2
Pathway:	NF-κB
Solubility:	DMSO : 125 mg/mL (227.01 mM; Need ultrasonic)



BIOLOGICAL ACTIVITY:

KI696 is a high affinity probe that disrupts the **Keap1/NRF2** interaction. KI696 is a potent and selective inhibitor of the **KEAP1/NRF2** interaction. IC50 & Target: Target: Keap1-NRF2[1] **In Vitro:** KI696 (compound 7) exhibits very high affinity for the KEAP1 Kelch domain (ITC K_d=1.3 nM with the exception of the organic anion transporting polypeptide 1B1 (OATP1B1) (IC_{50}=2.5 μM), the bile salt export pump BSEP (IC_{50}=4.0 μM), and the phosphodiesterase PDE3A (IC_{50}=10 μM), no significant cross-reactivity is observed. No cytotoxicity is observed towards BEAS-2B cells with KI696 at concentrations up to 10 μM. KI696 increases NRF2 Nuclear Translocation in Normal Human Bronchial Epithelial cells. KI696 increases mRNA expression of the NRF2-dependent genes NQO1 and GCLM in NHBE cells transfected with the non-targeting siRNA, while NRF2 gene silencing significantly decreases compound activity. KI696 increases NQO1 Activity in an NRF2-Dependent Manner. Treatment with tBHP clearly has a detrimental effect on cell health and appearance while pre-treatment of cells with 1 μM KI696 before the exposure to tBHP maintained cell morphology consistent with the DMSO control. KI696 Induces the Expression of NRF2-Regulated Genes in COPD patient-derived bronchial epithelial cells[1]. **In Vivo:** KI696 induces the expression of each of the Nqo1, Ho-1, Txnrd1, Srxn1, Gsta3, Gclc genes in a dose-dependent manner, with maximum increases over vehicle controls of 37-(Nqo1), 17-(Ho-1), 9-(Txnrd1), 28-(Srxn1), 15-(Gsta3) and 13-fold (Gclc) occurring at the 50 μmol/kg dose. EC_{50} values are 44.0, 25.7, 42.6, 33.8, 28.4, and 44.1 μmol/kg, respectively, giving an average EC_{50} value of 36.4±3.4 μmol/kg. KI696 attenuates ozone-Induced pulmonary inflammation. KI696 restores ozone-induced depletion of lung GSH levels. KI696 is administered to rats at 10, 35 and 50 μmol/kg by IV infusion, resulting in steady state compound concentrations in the blood of 407±44 nM, 946±50 nM and 1437±186 nM, respectively, over the 6 hour infusion period. Exposure to ozone 24 hours post-dose produces a significant depletion in lung levels of the anti-oxidant molecule, GSH, which is restored by KI696 in a dose-dependent manner[1].

PROTOCOL (Extracted from published papers and Only for reference)

Kinase Assay: [1]Inhibition of the Kelch domain-NRF2 interaction is determined using a fluorescence polarisation-based competition assay in a black 384-well microplate. Each well contained 2 nM 5′-TAMRA-NRF2 peptide (AFFAQLQLDEETGEFL) and 7 nM human KEAP1 (residues 321-609) in 50 μL of assay buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 2 mM 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 1 mM DTT, 0.005% BSA, 1% DMSO). After 1 hour at room temperature, fluorescence polarisation (excitation 485 nm/emission 520 nm) is measured using a BMG Pherastar FS plate reader. IC_{50} values are determined by fitting the data to a four parameter logistic fit[1]. **Cell Assay:** [1]**BEAS-2B cells** are plated in 384 well black clear-bottomed plates and are incubated overnight (37°C, 5% CO_2). On day 2, the plates are centrifuged and 50 nL of compound (**KI696**) or controls are added to the cells for 48 hours. On day 4, the medium is aspirated from the plate and crude cell lysates are made by using 1X lysis buffer with Complete, Mini, EDTA-free Protease Inhibitor. After lysis, the plates are incubated for 20 minutes at room temperature and the MTT cocktail is prepared for measurement of NQO1 activity. The samples are analyzed on an Envision plate reader, reading absorbance at 570 nm for five individual readings with 10 minute intervals. Product formation is measured kinetically and the pEC50 of NQO1 specific activity induction is calculated by plotting the change in absorbance versus log [compound] followed

by 4-parameter fitting[1]. **Animal Administration:** [1]Rats[1]

To examine the effect of KI696 under conditions of oxidative stress, rats are administered **KI696 at 35 μmol/kg** (the approximate average EC_{50} value for gene expression) by IV infusion over 6 hours, and after 24 hours are exposed to ozone (1 ppm for 3 hours). Fifteen minutes following the termination of ozone exposure, the numbers of total cells, neutrophils and mononuclear cells in the bronchoalveolar lavage (BAL) fluid are measured[1].

References:

[1]. Davies TG, et al. Monoacidic Inhibitors of the Kelch-like ECH-Associated Protein 1: Nuclear Factor Erythroid 2-Related Factor 2 (KEAP1:NRF2) Protein-Protein Interaction with High Cell Potency Identified by Fragment-Based Discovery. J Med Chem. 2016 Apr 28;59(8):3991-4006.

CAIndexNames:

1H-Benzotriazole-5-propanoic acid, β-[3-[[(4R)-3,4-dihydro-4-methyl-1,1-dioxido-2H-5,1,2-benzoxathiazepin-2-yl]methyl]-4-methylphenyl]-7-methoxy-1-methyl-, (βS)-

SMILES:

COC1=C(N2C)C(N=N2)=CC([C@H](C3=CC(CN(C[C@H]4C)S(=O)(C5=CC=CC=C5O4)=O)=C(C)C=C3)CC(O)=O)=C1

Tel: 732-484-9848 Fax: 888-484-5008 E-mail: sales@ChemScene.com

Address: 1 Deer Park Dr, Suite Q, Monmouth Junction, NJ 08852, USA