

# S213726: A Chemical Probe for KEAP1

Version 1.0 (5<sup>th</sup> October 2023)

## Overview

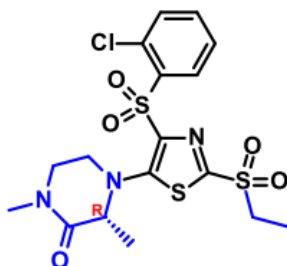
KEAP1 is a substrate adaptor protein of the CUL3 E3 ubiquitin ligase complex and is a key regulator of the cellular response to oxidative and electrophilic stress. Its best known substrate is the transcription factor Nrf2 which regulates the expression of antioxidant proteins. Inhibitors of KEAP1, which block its E3 ligase activity and stabilise Nrf2 from degradation, protect cells from oxidative damage and have potential application across multiple therapeutic areas. Servier in collaboration with CMD Oxford have developed S213726 as a potent and selective covalent ligand for the BTB domain of KEAP1. S214489 is a closely-related negative control compound.

## Summary

Chemical Probe Name	S213726
Negative control compound	S214489
Target(s) (synonyms)	Kelch-like ECH-associated protein 1, KEAP1 (KLHL19)
Recommended cell assay concentration	1 $\mu$ M
Suitability for <i>in vivo</i> use and recommended dose	S213726 has been tested in db/db mice at 100 mg/kg PO and anti-oxidant responses observed up to 24 hr.
Publications	
Orthogonal chemical probes	
<i>In vitro</i> assay(s) used to characterise	LC-MS, isoTOP-ABPP mass spectrometry
Cellular assay(s) for target-engagement	ARE reporter assay, Nrf2 nuclear translocation

## Chemical Probe & Negative Control Structures and Use

Chemical Probe



SMILES:

CCS(=O)(=O)c1nc(c(s1)N2CCN(C)C(=O)[C@H]2C)S(=O)(=O)c3ccccc3Cl

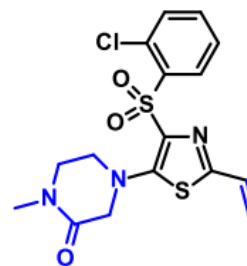
InChiKey: XRAUKWUDYSUHJM-LLVKDONJSA-N

Molecular Weight: 478.00

Storage: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C. DMSO stocks should be aliquoted in single-use volumes (and not refrozen). DMSO stocks older than 3-6 months should be tested for activity before use.

Dissolution: Soluble in DMSO up to 50 mM

Negative Control



SMILES:

CN1CCN(CC1=O)c2sc(C=C)nc2S(=O)(=O)c3ccccc3Cl

InChiKey: LQNJUPLCHMOONS-UHFFFAOYSA-N

Molecular Weight: 397.90

Storage: As a dry powder or as DMSO stock solutions (10 mM) at -20 °C. DMSO stocks should be aliquoted in single-use volumes (and not refrozen). DMSO stocks older than 3-6 months should be tested for activity before use.

Dissolution: Soluble in DMSO up to 50 mM

## Chemical Probe Profile

***In vitro* Potency & Selectivity:** irreversible covalent binding;  $k_{obs}$  very fast;  $k_{alkyl}/K_D = 1.46 \times 10^{-2} \text{ min}^{-1} \mu\text{M}^{-1}$  (= 243  $\text{M}^{-1}\text{s}^{-1}$ )  
Selective against other BTB, Kelch and kinase domains tested by LC-MS or DSF.

**Potency in Cells and Cellular Target Engagement:** ARE reporter assay (HEPG2)  $EC_{50} = 61.8 \text{ nM}$ ; Nrf2 nuclear translocation assay (U2OS)  $EC_{50} = 136 \text{ nM}$ ; Ultra-selective by isoTOP-ABPP mass spectrometry (one off target PCIF1 Cys626).