**S213726: A Chemical Probe for KEAP1**

Version 1.0 (date)

**Web link for more details: https://www.thesgc.org/chemical-probes/...**

**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**

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| **Chemical Probe Name** | S213726 |
| **Negative control compound** | S214489 |
| **Target(s) (synonyms)** | Kelch-like ECH-associated protein 1, KEAP1 (KLHL19) |
| **Recommended cell assay concentration** | 1 µM |
| **Suitability for *in* vivo 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**  |  |
| ***In vitro* 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**

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| **Chemical Probe** | **Negative Control** |
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| SMILEs:CCS(=O)(=O)c1nc(c(s1)N2CCN(C)C(=O)[C@H]2C)S(=O)(=O)c3ccccc3Cl | SMILEs:CN1CCN(CC1=O)c2sc(C=C)nc2S(=O)(=O)c3ccccc3Cl |
| InChiKey: XRAUKWUDYSUHJM-LLVKDONJSA-N | InChiKey: LQNJUPLCHMOONS-UHFFFAOYSA-N |
| Molecular Weight: 478.00 | 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. | 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 | Dissolution: Soluble in DMSO up to 50 mM |
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**Chemical Probe Profile**

***In vitro* Potency & Selectivity:** irreversible covalent binding; *k*obs very fast; *k*alkyl/*K*D = 1.46 x 10-2 min-1µM-1 (= 243 M-1s-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) EC50 = 61.8 nM; Nrf2 nuclear translocation assay (U2OS) EC50 = 136 nM; Ultra-selective by isoTOP-ABPP mass spectrometry (one off target PCIF1 Cys626).