***General***

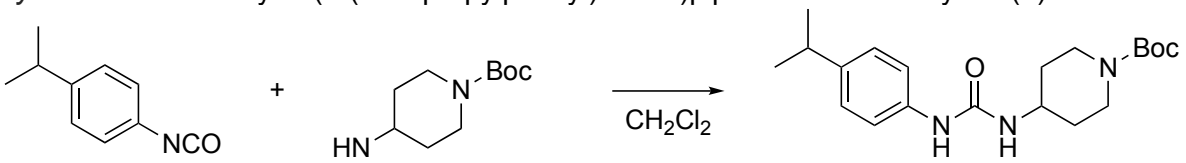
All reagents and solvents were purchased from commercial suppliers and used directly without further purification. All syntheses were carried out in a dry argon atmosphere unless otherwise specified. Reactions were monitored by thin-layer chromatography (TLC, on Merck $F_{254}$ silica gel 60 aluminum sheets, spots were either visible under light or UV-light (254 mm) or stained with an oxidizing solution ($KMnO_4$ stain). The same TLC system was used to test purity, and all final products showed a single spot on TLC. Column chromatography was performed on silica gel to purify the synthesized compounds further if needed.

$^1$H-NMR spectra were recorded on an Agilent 500 MHz DDR2 NMR spectrometer with deuterated dimethyl sulfoxide (DMSO-$d_6$) containing TMS as an internal standard. $^{13}$C-NMR spectra were recorded on an Agilent 500 MHz DDR2 NMR spectrometer at 125 MHz.

The purity of the inhibitors reported in this manuscript was additionally determined either by 1) HPLC-UV using a Shimadzu Prominence HPLC system equipped with Phenomenex Luna2 C18 reverse phase column (C18, 4.6 mm x 150 mm, 5 µm) coupled with a Shimadzu SPD-20A UV-vis detector (Detection at 230 nm) with linear gradient from 5% acetonitrile in water to 100 % acetonitrile in 30 min; or by 2) H-NMR. The lowest obtained purity was reported. The inhibitor was dissolved in EtOH at 100 µM and 10 µL was injected on HPLC. Purity was based on the percent of total peak area at 230 nm using HPLC-UV. This purity estimate was compared with that from the H-NMR and the data agreed closely. The high resolution mass spectrum (HRMS) was determined by Waters Xevo GS-XX Quadrupole/Time-of-Flight systems.

The synthesis, $IC_{50}$ and *Ki* of 1-(3-fluoro-4-(trifluoromethoxy)phenyl)-3-(1-isobutyrylpiperidin-4-yl)urea, [1] 1-(3-fluoro-4-(trifluoromethyl)phenyl)-3-(1-isobutyrylpiperidin-4-yl)urea, 1-(1-isobutyrylpiperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)urea and 1-(1-isobutyrylpiperidin-4-yl)-3-(4-(trifluoromethyl)phenyl)urea have been reported elsewhere. [1, 2]

*Synthesis of* ***tert***-butyl 4-(3-(4-isopropylphenyl)ureido)piperidine-1-carboxylate (**1**)




**(1)**

4-Isopropylphenyl isocyanate (72 mg, 0.45 mmol) and *tert*-Butyl 4-aminopiperidine-1-carboxylate (99 mg, 0.49 mmol) was dissolved in $CH_2Cl_2$ (10 mL) and stirred at rt for 168h. The reaction was purified by flash chromatography (Solvent A is hexane, Solvent B is Ethyl Acetate, see Table S1) to yield (123 mg, 0.34 mmol, 76% yield).

**Table S1: Details of flash chromatography purification for compound (1)**

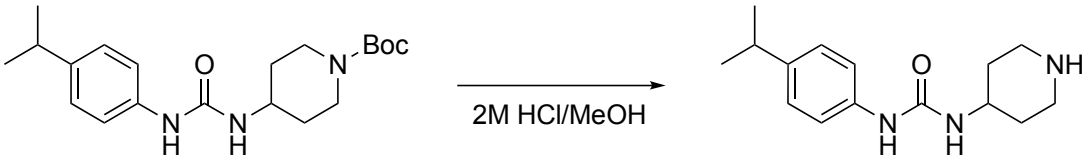
| Min | %B |
|---|---|
| 0 | 0 |
| 1.8 | 1 |
| 6.3 | 22.5 |
| 24.4 | 22.5 |
| 7.5 | 70 |
| 5 | 70 |
| 1 | 0 |
| 5 | 0 |

**NMR chemical shifts of the purified compound (1):**

$^{1}$H NMR (500 MHz, D6 DMSO) $\delta$ 8.21 (s, 1H), 7.26 (d, *J*=10 Hz, 2H), 7.07 (d, J=10 Hz, 2H), 6.10 (d, J=5 Hz, 1H), 3.75-3.85 (m, 2H), 3.64-3.58 (m, 1H), 2.89 (br, 2H), 2.79 (sept, J=7.5 Hz, 1H), 1.79-1.77 (m 2H), 1.40 (s, 9H), 1.26-1.20 (m, 2H), 1.15 (d, J=5 Hz, 6H).

$^{13}$C NMR (125 MHz, D6 DMSO) $\delta$ 154.61, 153.95, 141.10, 138.16, 126.40, 117.87, 78.70, 46.10, 32.78, 32.05, 28.14, 24.12.

Synthesis of 1-(4-isopropylphenyl)-3-(piperidin-4-yl)urea (**2**)



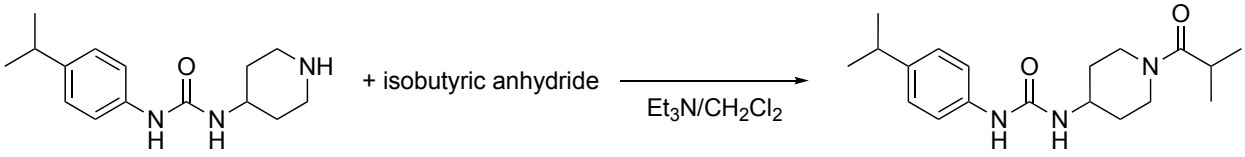

**(2)**

KSL-59 boc (103 mg, 0.28 mmol) was dissolved in 2M HCl in MeOH and heated to 55 ˚C for 144h. The solvent was then removed by rotavap and the pH was brought to pH 12 by slowly adding KOH pellets. The solution was stirred at rt until the product precipitated out. The product was vacuum filtered and left to dry overnight (48 mg, 0.18 mmol, 64% yield).

**NMR chemical shifts of the purified compound (2):**

$^1$H NMR (500 MHz, D6 DMSO) $\delta$ 8.18 (s, 1H), 7.25 (d, J=5 Hz, 2H), 7.07 (d, J=10 Hz, 2H), 6.03 (d, J=10 Hz, 1H), 3.47 (s, 1H), 3.31 (s, 2H by water peak), 2.87 (d, J=10 Hz, 2H), 2.78 (quint, J=6.3 Hz, 1H), 2.64?? , 2.47 (s, 1H by DMSO peak), 1.73 (d, J=10 Hz, 2H), 1.23-1.19 (m, 1H), 1.15 (d, J=5 Hz, 6H)

Synthesis of 1-(1-isobutyrylpiperidin-4-yl)-3-(4-isopropylphenyl)urea (**3**)




**(2)** **(3)**

2 was dissolved in 10 mL of DCM and stirred for 168h at rt. The product was then purified by flash chromatography (Solvent A is hexane, Solvent B is Ethyl Acetate, see Table S2) yielding the final product 3 (41.6 mg, 0.115 mmol, 99%). Note that product 3 is referred to in the main text as Ligand 10.

**Table S1: Details of flash chromatography purification for compound (3)**

| Min | %B |
|---|---|
| 0 | 0 |
| 5 | 0 |
| 3 | 2 |
| 3 | 6 |
| 2 | 10 |
| 10 | 35 |
| 16 | 35 |
| 7 | 72 |
| 13 | 72 |
| 0.3 | 0 |
| 5 | 0 |

**Characterization of product (3):**

High Resolution Mass Spectrometry (calculated for [H+ ]: C19H30N3O2): 332.2338; found (ESI(+), [M-H+ ]): 332.2342

Melting point (˚ C): 152-155

$^1$H NMR (500 MHz, D6 DMSO) $\delta$ 8.22 (s, 1H), 7.27 (d, J=10 Hz, 2H), 7.07 (d, J=5 Hz, 2H), 6.12 (d, J=10 Hz, 1H), 4.02 (dd, J=175 Hz, J=12.5 Hz, 2H), 3.72-3.65 (m, 1H), 3.16 (t, J=12.5 Hz, 1), 2.87 (sept, J=6.7 Hz, 1H), 2.82-2.76 (m, 2H), 1.84 (dd, J=35 Hz, J=15 Hz, 2H), 1.15 (d, J=5 Hz, 7H?), 0.99 (t, J=7.5 Hz, 6H).

C NMR 174.11, 154.59, 141.06, 138.14, 126.37, 121.84, 117.84, 46.27, 43.53, 33.02, 32.75, 31.97, 29.00, 24.09.

1. Lee KS, Liu JY, Wagner KM, Pakhomova S, Dong H, Morisseau C, Fu SH, Yang J, Wang P, Ulu A, Mate CA, Nguyen LV, Hwang SH, Edin ML, Mara AA, Wulff H, Newcomer ME, Zeldin DC, Hammock BD. Optimized inhibitors of soluble epoxide hydrolase improve in vitro target residence time and in vivo efficacy. J Med Chem. 2014;57(16):7016-30. Epub 2014/08/01. doi: 10.1021/jm500694p. PubMed PMID: 25079952; PMCID: PMC4148150.
2. Lee KSS, Ng JC, Yang J, Hwang SH, Morisseau C, Wagner K, Hammock BD. Preparation and evaluation of soluble epoxide hydrolase inhibitors with improved physical properties and potencies for treating diabetic neuropathic pain. Bioorg Med Chem. 2020;28(22):115735. Epub 20200831. doi: 10.1016/j.bmc.2020.115735. PubMed PMID: 33007552; PMCID: PMC7914304.
3. Lee KS, Morisseau C, Yang J, Wang P, Hwang SH, Hammock BD. Forster resonance energy transfer competitive displacement assay for human soluble epoxide hydrolase. Anal Biochem. 2013;434(2):259-68. Epub 2012/12/12. doi: 10.1016/j.ab.2012.11.015. PubMed PMID: 23219719; PMCID: PMC3632402.