

Supporting Information

**Proximity-enabled bioreactivity to generate covalent peptide inhibitors of p53-Mdm4**

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## Reagents:

His tagged Mdm4 was purchased from Sino Biological Inc. (cat. Number 15395-H07E-10). Reagents and amino acids for peptide synthesis were purchased from ChemImpex, Novabiochem (Merck) or Sigma. Alkenyl amino acids were purchased from AAPPTec.

## Reactivity of Ar-SO<sub>2</sub>F to lysine and histidine:

To test the reactivity of Ar-SO<sub>2</sub>F towards lysine and histidine, we initially used Uaa **6**, the synthesis of which will be described in a separate publication. Both lysine and histidine reacted with the Ar-SO<sub>2</sub>F amino acid **6** forming the product with high purity (Figure S1). Because of the reactivity of the -SO<sub>2</sub>F group in **6** to nucleophilic attack by piperidine, direct incorporation of **6** into peptides using Fmoc-based solid phase peptide synthesis failed. To overcome this limitation, we designed Uaa **1a,b**, the -SO<sub>2</sub>-F group of which is introduced after complete assembly of the peptide chain on solid support.

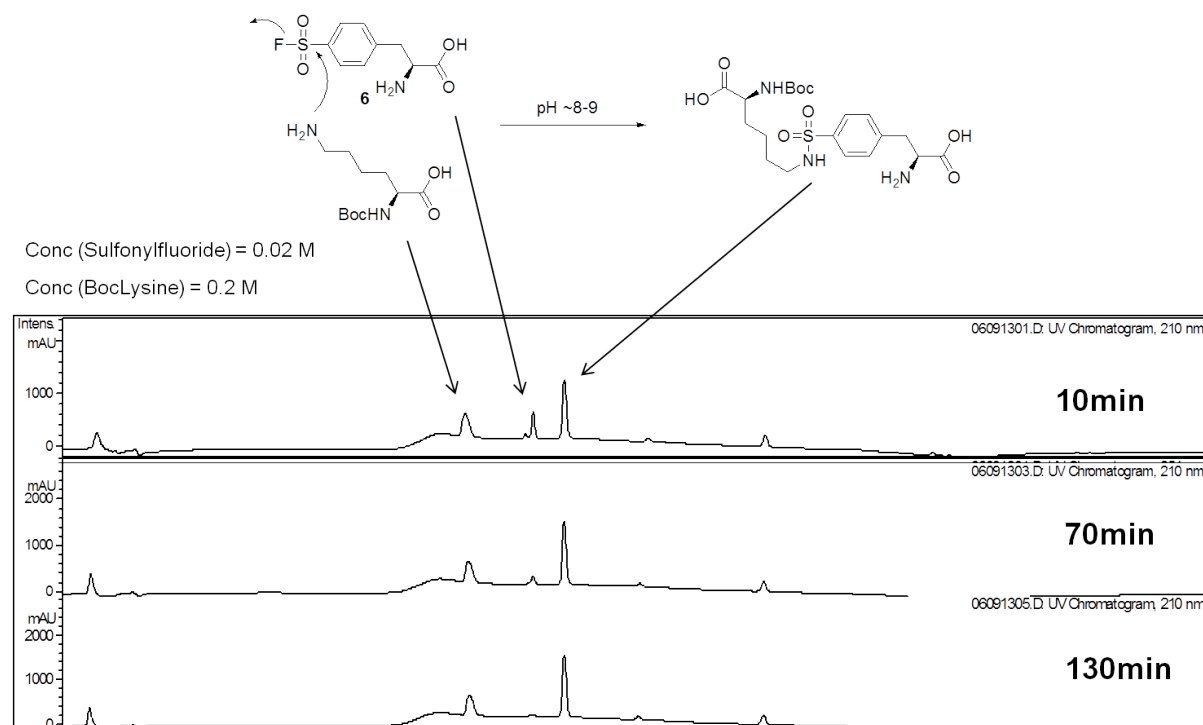
## Peptide synthesis:

The stapled peptides were synthesized on a 0.3 mmol scale by standard protocols using Fmoc chemistry. As solid support a Rink amide resin was used (Novabiochem Rink Amide MBHA resin, 100-200 mesh, loading = 0.6 mmole/g). Fmoc amino acids were coupled in five-fold excess with HBTU/DIEA (1:2) in DMF for 30 min. The Fmoc-protected olefin building blocks were coupled in 2.5-fold excess using HBTU/DIEA (1:1) in DMF for 45 min. Fmoc deprotection was realized by treating the peptide-bound resin with 20% (v/v) piperidine/DMF for 15 min. After assembly of the linear peptide chain the N-terminus of **2**, **3**, **4** and **5** was acetylated using a solution of acetic anhydride and DIEA in DMF. Ring-closing metathesis (RCM) was performed as described previously.<sup>1</sup> After assembly, peptides were treated with 4% N<sub>2</sub>H<sub>4</sub> solution in order to remove ivDde and subsequently 3-(fluorosulfonyl)benzoic acid was coupled to the amino side chain of diaminopropionic acid (Dap) using five-fold excess with PyBOP/DIEA (1:2) in DMF for 30 min. 3-(fluorosulfonyl)benzoic acid was prepared previously according to the procedure from ref.<sup>1</sup> by adding 3-(chlorosulfonyl)benzoic acid to a solution of KF/HF for 1h. Final deprotection and cleavage of the peptides from the resin was realized using TFA/H<sub>2</sub>O/TIS (85/5/5, v/v). Crude peptides were precipitated by the addition of cold diethyl ether and purified on a reversed-phase C18 column (Waters; XBridge Prep C18). Composition and purity of the stapled peptides was confirmed by LC-MS mass spectrometry using a C18 column (Phenomenex, Gemini). ESI-MS (peptide **2**) = (ES+) [M+2H]<sup>2+</sup> = calcd: 1055.3 (monoisotopic); obsd: 1055.4; ESI-MS (peptide **3**) = (ES+) [M+2H]<sup>2+</sup> = calcd: 1134.4 (monoisotopic); obsd: 1134.6; ESI-MS (peptide **4**) = (ES+) [M+2H]<sup>2+</sup> = calcd: 1134.4 (monoisotopic); obsd: 1134.6; ESI-MS (peptide **5**) = (ES+) [M+2H]<sup>2+</sup> = calcd: 1096.4 (monoisotopic); obsd: 1096.6.

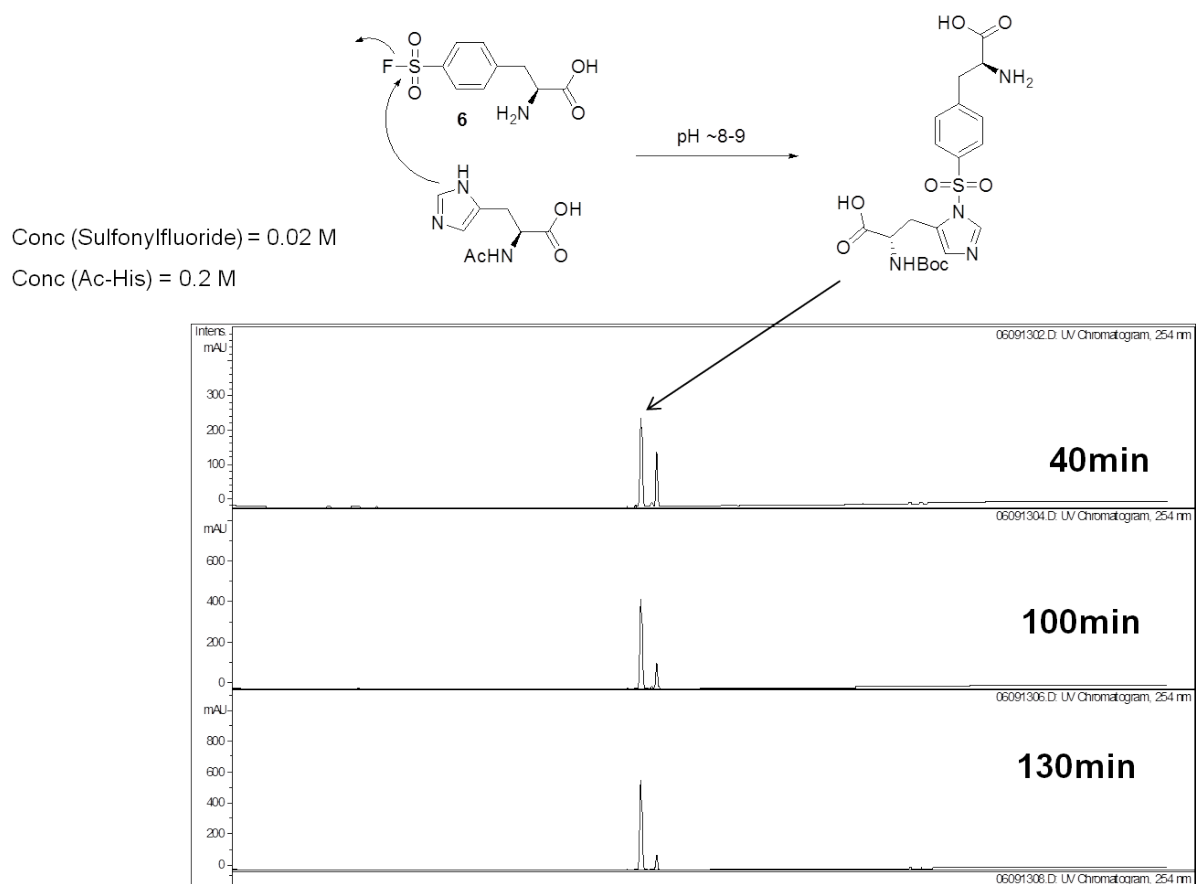
**ReBiL Assay:**

ReBiL assay was essentially performed as described previously.<sup>2</sup> Cellular lysates obtained from p53-Mdm2, p53-Mdm4, or Brca1-Bard1 reporter cells (Saos-2) were incubated with peptides in the presence of serum (FBS) in a 384-well plate at room temperature for 60 minutes. Steady-Glo was added, and luminescence was read in a Tecan M200 luminometer at 26 °C. Mass spectrometric analyses of Mdm4 samples were performed as previously described.<sup>3</sup> Cell viability was measured on the p53-null Saos-2 cells by CellTiter Glo assay per Promega's instructions. Peptides were incubated with the Saos-2 cells at 37 °C for 8 h.

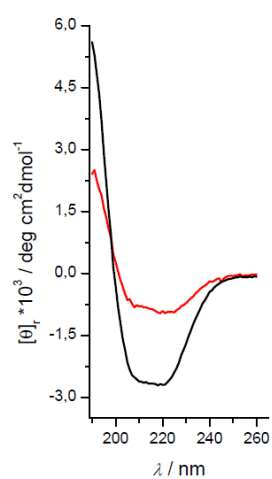
### Reactivity of Ar-SO<sub>2</sub>F to lysine:



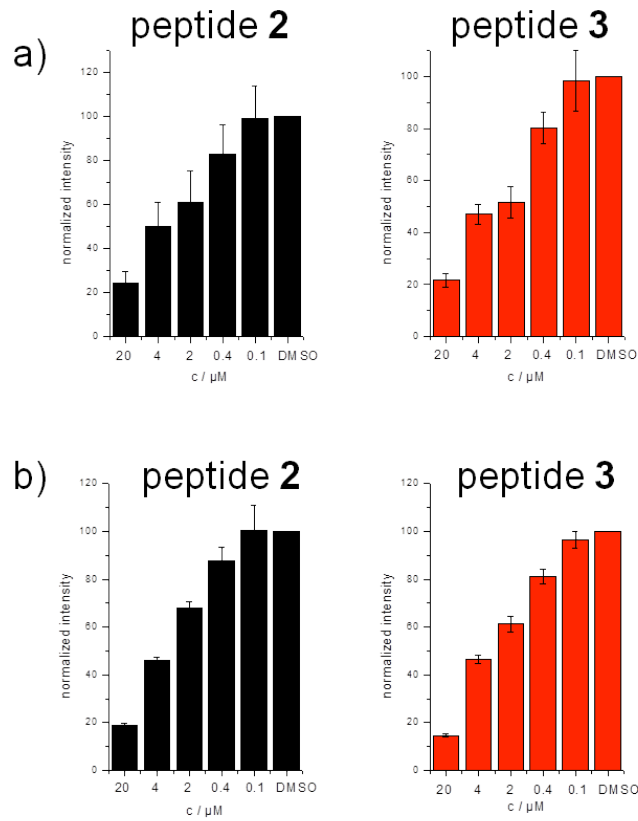
### Reactivity of Ar-SO<sub>2</sub>F to histidine:



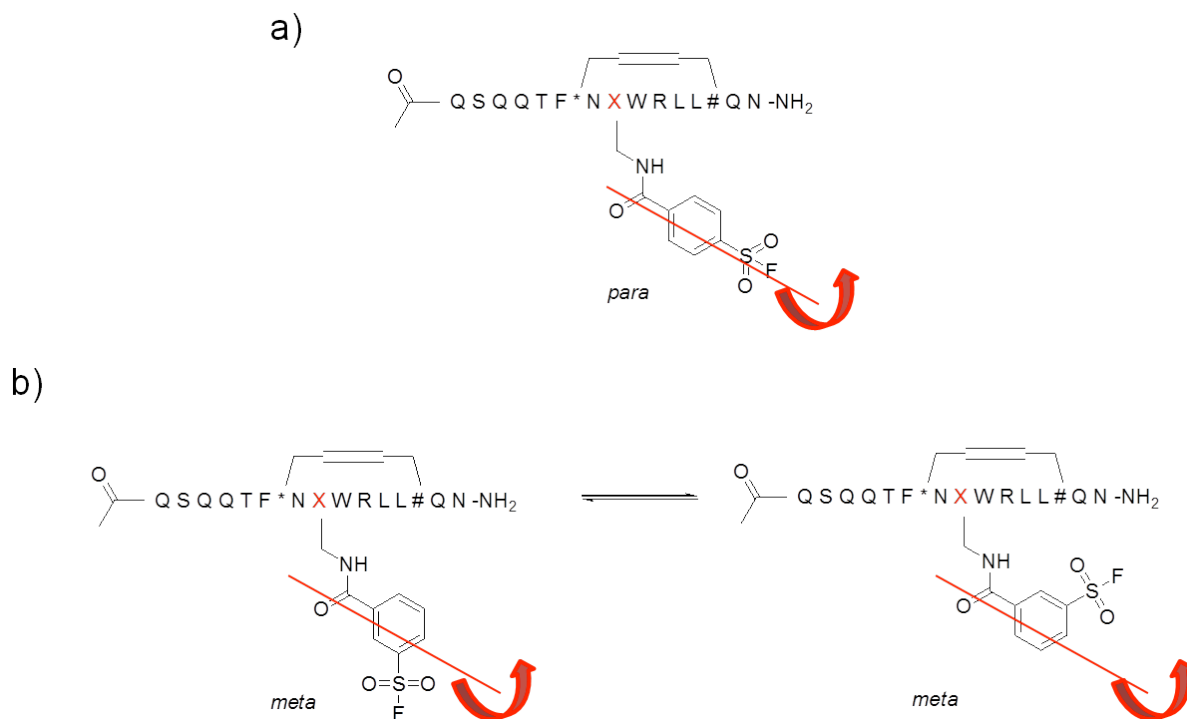
**Figure S1.** Aryl sulfonyl fluoride Uaa **6** reacts with the side chain of either lysine or histidine as determined by HPLC-MS.



**Figure S2.** CD spectra of **4** (black line) and **5** (red line) showing typical shapes for  $\alpha$ -helical conformation. CD spectra were recorded on a Bio-Logic MOS450 spectrometer in Na-phosphate (10 mM) buffer containing NaF (100 mM) and at pH 7.5 using a 0.1 cm quartz cuvette (Helma) at 25 °C.



**Figure S3.** ReBiL cell lysate assay showed that peptides **2** and **3** both inhibited a) p53-Mdm2 interaction and b) p53-Mdm4 interaction with similar activity. The assay was performed using cell lysates of Saos-2 cells. For p53-Mdm2 inhibition,  $IC_{50} = 4.1 \mu\text{M}$  for peptide **2** and  $2.7 \mu\text{M}$  for peptide **3**; For p53-Mdm4 inhibition,  $IC_{50} = 3.6 \mu\text{M}$  for peptide **2** and  $3.5 \mu\text{M}$  for peptide **3**. Data are shown as mean  $\pm$  s.e.m. ( $n = 5$ ) and normalized to the luminescent reading of DMSO treated cells.



**Figure S4.** Substitution of the  $-\text{SO}_2\text{F}$  group on the *meta* position (b) can afford more flexibility and accessibility than the *para* position (a) in reaching and reacting with lysine or histidine side chain in proximity.

**Supplemental Reference:**

- [1] Kim, Y.-W.; Grossmann, T.N.; Verdine, G.L. *Nat. Prot.* **2011**, 6, 761-771
- [2] Li, Y.-C.; Rodewald, L.W.; Hoppmann, C.; Wong, E. T.; Lebreton, S.; Safar, P.; Patek, M.; Wang, L.; Wertman, K.F.; Wahl, G.M. *Cell Reports* **2014**, 9, 1946–1958
- [3] Xiang, Z.; Ren, H.; Hu, Y.S.; Coin, I.; Wei, J.; Cang, H.; Wang, L. *Nat. Methods* **2013**, 10, 885-888.