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-SO₂F to lysine and histidine:
To test the reactivity of Ar-SO₂F 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-SO₂F amino acid 6 forming the product with high purity (Figure S1). Because of the reactivity of the -SO₂F 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 -SO₂-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. After assembly, peptides were treated with 4% N₂H₄ 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. 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/H₂O/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]²⁺ = calcd: 1055.3 (monoisotopic); obsd: 1055.4; ESI-MS (peptide 3) = (ES+) [M+2H]²⁺ = calcd: 1134.4 (monoisotopic); obsd: 1134.6; ESI-MS (peptide 4) = (ES+) [M+2H]²⁺ = calcd: 1134.4 (monoisotopic); obsd: 1134.6; ESI-MS (peptide 5) = (ES+) [M+2H]²⁺ = calcd: 1096.4 (monoisotopic); obsd: 1096.6.
ReBiL Assay:

ReBiL assay was essentially performed as described previously. 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. 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-SO₂F to lysine:

Conc (Sulfonyl fluoride) = 0.02 M
Conc (BocLysine) = 0.2 M

Reactivity of Ar-SO₂F to histidine:

Conc (Sulfonyl fluoride) = 0.02 M
Conc (Ac-His) = 0.2 M

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 α-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, IC₅₀ = 4.1 µM for peptide 2 and 2.7 µM for peptide 3; For p53-Mdm4 inhibition, IC₅₀ = 3.6 µM for peptide 2 and 3.5 µM for peptide 3. Data are shown as mean ± 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:**

