Supporting Information

Use of a Retroinverso p53 Peptide as an Inhibitor of MDM2

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Peptide Preparation.

Peptide **2**, **4**-**5** were synthesized by the solid-phase method on the automated peptide synthesizer 341A (Applied Biosystems) using 9-fluoromethylcarboxy (Fmoc) chemistry. N-Fmoc protected amino acid building blocks were purchased from Novabiochem. Peptides **2**-**5** and biotinylated wt-p53 peptide were synthesized on Fmoc-Rink resin (Novabiochem). Global deprotection of side chain protecting groups, simultaneous cleavage off the resin and the precipitation of the crude products were carried out following a published protocol.[1] The crude mixture was purified by preparative scale reverse-phase HPLC to the purity >95% as determined by analytical scale HPLC. The desired peptides were characterized by MALDI-TOF mass spectrometry. Peptide **1** was purchased from Princeton University Synthesizing/Sequencing Laboratory. Peptide **3** and alanine substituted versions of peptide **2** and **5** were purchased from Tufts University Core Facility. Peptide **2**: HRMS cacld for $C_{84}H_{127}N_{20}O_{27}$ (MH^+) 1847.9174, found 1847.9332; peptide **3**: HRMS calcd for $C_{84}H_{127}N_{20}O_{27}$ 1848.0540, found 1849.77 (MH^+); peptide **4**: HRMS cacld for $C_{84}H_{127}N_{20}O_{27}$ (MH^+) 1847.9174, found 1847.4903; peptide **5**: HRMS cacld for $C_{84}H_{127}N_{20}O_{27}$ (MH^+) 1847.9174, found 1847.8910; biotin wt-p53 peptide: HRMS cacld for $C_{90}H_{136}N_{22}O_{27}SNa$ (MNa^+) 2011.9558, found 2011.9577.

Circular Dichroism (CD) Studies.

CD spectra were recorded on an AVIV 62DS spectrometer (Aviv Instruments) equipped with thermoelectric control of the cell jacket temperature, using 0.1cm pathlength cuvettes at 5ºC. Peptides were dissolved at a concentration of 100μM in 10mM PBS pH 7.2, containing 2,2,2-trifluoroethanol (TFE) in increasing amounts (0-60% v/v). For each concentration of peptide sample, three scans of data spanning from 200nm to 260nm in a step size of 0.5nm with 1 second averaging time at each wavelength, were collected. Experimental ellipticity data were converted into molar ellipticities and plotted against wavelength after noise suppression according to the manufacturer's protocol. The α helical content was calculated from CD data using the complex analysis provided in CDNN program, which has a basis set from 18 proteins and makes predictions with the neural network algorithm developed by Böhm.[2] Table S1 shows the α helical content for peptide **1**-**5** in 0 % and 60 % TFE.

Table S1.

peptide	% α-helicity	
	0% TFE	60% TFE
1	6	17
2	6	13
3	6	12
4	6	59
5	6	59

Protein Purification.

The plasmid encoding GST-hMDM2 (1-118) was a generous gift from Professor Arnold J. Levine. BL21 cells were transformed with the plasmid encoding GST-MDM2 and cultures were grown in 2 x YT medium containing ampicillin (50 μg/mL) at 37ºC. After addition of IPTG to a final concentration of 1 mM, the temperature was adjusted to 27ºC, and the culture was further incubated for 3 h. The cells were harvested by centrifugation and lysed using B-per® Bacterial Protein Extraction Reagent (Pierce) following the manufacturer's protocol. Following elution with glutathione, the protein was concentrated into a storage buffer (50 mM Tris-HCl, pH 7.4, 50 mM NaCl and 10 mM β-mercaptoethanol). The protein was analyzed by 12 % SDS-PAGE. The protein concentration was determined by Bradford assay.

Inhibition ELISA.

Inhibition ELISA to evaluate the inhibitory potency of the p53 peptide isomers were adapted from a method described by Stoll *et al.*[3] A streptavidin coated 96 well microtiter plate (Pierce) was blocked with blocking buffer (SuperBlock® Blocking Buffer in PBS, Pierce) followed by rinsing with wash buffer (PBST; PBS/0.05% Tween 20) twice. The plate was incubated for 1 hour with 50 μL per well of 125 nM solution of biotinylated p53-15mer peptide in wash buffer for 1 h, which was then washed with wash buffer three times. GST-MDM2 (1-118) at 20 nM was preincubated with inhibitors in incubation buffer (PBST, 10% blocking buffer, 5mM DTT, 5 or 10 % DMSO) for 30 min in a 96 well polypropylene microtiter plate (Fisher). The mixed solution of MDM2 and inhibitors were transferred to the plate with the p53 peptide immobilized and incubated for 1h. The wells were washed with wash buffer for 5 times. The anti-MDM2, N-20 (Santa Cruz Biotechnology), in PBST containing 10% blocking buffer was added at 800 fold dilution. After 1 h, the wells were washed thoroughly with wash buffer for 5 times and the horseradish peroxidase conjugated anti-rabbit IgG (Santa Cruz Biotechnology) diluted 10000 fold in PBST containing 10% blocking buffer was added to be incubated for another 1h. The wells were washed again with wash buffer 5 times and the peroxidase substrate TMB solution mixed with 30% hydrogen peroxide solution was added. After 10 minutes, the reaction was quenched by 50% acetic acid solution in 1N sulfuric acid solution. OD_{450nm} was determined with a HTS 700 Plus Bio Assay Reader (Perkin Elmer). All the steps in this assay were performed at room temperature.

Experiments were performed in triplicate to determine IC_{50} values unless noted otherwise. Calculation of % p53-MDM2 binding was processed as follows:

[% p53-MDM2 binding] = ([$OD_{450\ nm}$ inhibitor]-[$OD_{450\ nm}$ blank])/
([$OD_{450\ nm}$ no inhibitor]-[$OD_{450\ nm}$ blanks]) x 100

Blank condition contains no MDM2 protein. IC_{50} values for the peptide isomers are obtained by non-linear regression curve fitting to a Hill's equation using the Prism program (Graphpad). The experiments for Ala substituted series of peptide **2** and **5** did not reach 100 % inhibition below 1 mM inhibitor concentration, therefore the IC_{50} values were estimated with data with $OD_{450\ nm}$ values representing 50 % inhibition.

AM1 Calculations.

AM1 calculations were performed with GAUSSIAN 98; the default thresholds for wave function and gradient convergence were employed. A variety of initial conformations of the p53 peptide fragment, the retro p53 peptide fragment, and the retroinverso p53 peptide fragment were generated; some were modeled on the existing X-ray conformation of the p53 peptide fragment,[4] and others contained arbitrary side chain conformations. All were subjected to full geometry optimization with AM1. A summary of the results is shown in Table S2. Note: 1 au = 627.503 kcal/mol.

Table S2.

Calculation	Energy (au)	Delta (kcal/mol)
p53 peptide (**1**) fragment, optimization started from the X-ray conformation	-0.9300092	+10.4
p53 peptide (**1**) fragment, lowest energy geometry from among several optimizations of initial structures with arbitrary side chain conformations	-0.9466500	0.0
Retro p53 peptide (**4**) fragment, lowest energy geometry from several optimizations of initial structures with arbitrary side chain conformations	-0.9415760	+3.2
Retroinverso p53 peptide (**5**) fragment, lowest energy geometry among several optimizations of initial structures modeled on both the normal p53 X-ray conformation and several containing arbitrary side chain conformations	-0.9159709	+19.3

Peptide Sequences.

Peptide **1** H_2N-S-Q-E-T-F-S-D-L-W-K-L-L-P-E-N-CO_2H
Peptide **2** AcNH-S-Q-E-T-F-S-D-L-W-K-L-L-P-E-N-$CONH_2$
Peptide **3** AcNH-S-Q-E-T-F-S-D-L-W-K-L-L-P-E-N-$CONH_2$ (all-D-peptide)
Peptide **4** AcNH-N-E-P-L-L-K-W-L-D-S-F-T-E-Q-S-$CONH_2$
Peptide **5** AcNH-N-E-P-L-L-K-W-L-D-S-F-T-E-Q-S-$CONH_2$ (all-D-peptide)

Peptide **2** (F19A) AcNH-S-Q-E-T-A-S-D-L-W-K-L-L-P-E-N-$CONH_2$
Peptide **2** (W23A) AcNH-S-Q-E-T-F-S-D-L-A-K-L-L-P-E-N-$CONH_2$
Peptide **2** (L26A) AcNH-S-Q-E-T-F-S-D-L-W-K-L-A-P-E-N-$CONH_2$
Peptide **5** (F19A) AcNH-N-E-P-L-L-K-W-L-D-S-A-T-E-Q-S-$CONH_2$(all-D-peptide)
Peptide **5** (W23A) AcNH-N-E-P-L-L-K-A-L-D-S-F-T-E-Q-S-$CONH_2$(all-D-peptide)
Peptide **5** (L26A) AcNH-N-E-P-A-L-K-W-L-D-S-F-T-E-Q-S-$CONH_2$(all-D-peptide)
Biotinylated wt p53 peptide Biotin-S-G-S-G-Q-E-T-F-S-D-L-W-K-L-L-P-$CONH_2$

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References

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