Structure-based Design of High Affinity Peptides Inhibiting the Interaction of p53 with MDM2 and MDMX

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MDM2 and MDMX function as key regulators of p53 by binding to its N terminus, inhibiting its transcriptional activity, and promoting degradation. MDM2 and MDMX overexpression or hyperactivation directly contributes to the loss of p53 function during the development of nearly 50% of human cancers. Recent studies showed that disrupting p53-MDM2 and p53-MDMX interactions can lead to robust activation of p53 but also revealed a need to develop novel dual specific or MDMX-specific inhibitors. Using phage display we identified a 12-residue peptide (pDI) with inhibitory activity against MDM2 and MDMX. The co-crystal structures of the pDI and a single mutant derivative (pDI6W) liganded with the N-terminal domains of human MDMX and MDM2 served as the basis for the design of 11 distinct pDI-derivative peptides that were tested for inhibitory potential. The best derivative (termed pDIQ) contained four amino acid substitutions and exhibited a 5-fold increase in potency over the parent peptide against both MDM2 (IC50 = 8 nM) and MDMX (IC50 = 110 nM). Further structural studies revealed key molecular features enabling the high affinity binding of the pDIQ to these proteins. These include large conformational changes of the pDIQ to reach into a hydrophobic site unique to MDMX. The findings suggest new strategies toward the rational design of small molecule inhibitors efficiently targeting MDMX.

The p53 tumor suppressor is a potent inducer of cell cycle arrest, apoptosis, cellular senescence, and innate immunity. It is activated in response to oncogenic transformation, extrinsic stress, and viral infection to protect higher organisms from cancer (1–3). p53 also facilitates maternal reproduction through induction of the growth factor leukemia inhibitory factor (LIF) that promotes embryo implantation (4). p53 activity is kept at minimal levels in unstressed cells by interactions with MDM2 and MDMX. MDM2 is an ubiquitin E3 ligase for p53 and an important regulator of p53 stability by forming a negative feedback loop (5, 6). The MDM2 homolog MDMX also binds to p53 and inhibits p53-dependent transcription (7). Loss of MDM2 or MDMX leads to embryonic lethality (8–10). Therefore, the expression of MDM2 and the expression of MDMX are both necessary for regulation of p53 during normal development.

Genetic or functional inactivation of p53 is an obligatory step during cancer development. In human tumors that retain wild type p53, amplification of MDM2 or MDMX serves as an alternative mechanism of p53 inactivation in a subset of tumors (11, 12). Furthermore, MDM2 activity is controlled by the tumor suppressor ARF (alternative reading frame) encoded by the INK4a locus (2). Deletion/epigenetic silencing of ARF occur in most tumors expressing wild type p53, resulting in hyperactive MDM2 and lack of p53 response to oncogenic stress in malignant tumors (13, 14). ARF has also been shown to promote MDMX degradation by MDM2 (15). Loss of ARF expression may result in MDMX stabilization that further inactivates p53. Therefore, MDM2 and MDMX are directly involved in p53 functional inactivation in ~50% of tumors, making them attractive drug targets.

Both MDM2 and MDMX regulate p53 by binding to a short amphipathic α-helix in its N-terminal transactivation domain. Earlier studies of MDM2-p53 binding and determination of MDM2-p53 crystal structure formed the foundation for recent development of small molecule disruptors of MDM2-p53 binding (16–20). These compounds, such as Nutlin 3a and MI-219, provided proof-of-concept for the anti-tumor potential of MDM2 inhibitors (21, 22). Importantly, Nutlin 3a and MI-219 do not inhibit MDMX, and the efficacy of Nutlin 3a is compromised in cells overexpressing MDMX (23–25). Furthermore, even in tumor cells without MDMX amplification, knockdown of MDMX by small interfering RNA still showed anti-tumor potential and cooperative effects with Nutlin in activating p53 (23, 24, 26). Therefore, MDMX expression contributes to p53 inactivation, suggesting that targeting both MDM2 and MDMX is needed to achieve optimal activation of p53.

The small molecules developed against MDM2 are generally inactive for MDMX (22, 27). Recent structural studies using humanized zebra fish MDMX and human MDMX in complex with the p53 N-terminal peptide revealed extensive similarity between the p53-binding domains of MDM2 and MDMX in...
overall folding and the shapes of their p53-binding pockets. However, a few sequence differences result in a smaller hydrophobic cleft in MDMX that prevents efficient binding by Nutlin (28, 29). Future development of inhibitors against MDMX can be facilitated by identification of a high affinity artificial ligand that target MDMX and/or MDM2. We recently identified a

**FIGURE 1. Crystal structures of MDMX and MDM2 liganded with the pDI peptide and derivatives thereof.** The structures of the pDI, pDI6W, and pDIQ peptides (shown in orange) were determined in complex with MDMX and MDM2 (shown in gray). Indicated in cyan are the amino acid substitution sites. The major structural difference between MDMX and MDM2 is the α2'-helix, part of which constitutes the peptide-binding site (highlighted in blue in the pDIQ liganded structures). The MDMX-pDIQ complex crystallized with two monomers in the asymmetric unit, and only chain A is displayed.
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peptide (pDI) using phage display that selects for sequence with maximal binding to MDM2 and MDMX (30). pDI is 300-fold more potent than p53 peptide in disrupting MDM2-p53 and MDMX-p53 binding. Using a similar phage display strategy, Pazgier et al. (31) recently also identified a different, more potent peptide inhibitor (pMI) in their screen. The co-crystal structures of pMI and pDI in complex with the MDM2 and MDMX N-terminal domains have recently been reported (31, 32), revealing the structural basis for the inhibitory action of these two different peptides.

Here, we determined the crystal structures of MDM2 and MDMX in complex with the pDI and derivatives thereof. Based on the structural information obtained from the pDI and a single mutant peptide (pDI6W), we designed a quadruple mutant peptide (pDIQ) that displays high affinity for MDMX and is the most potent inhibitor against MDM2 reported to date. The findings provide important clues about the molecular basis for the potency and selectivity of MDM2 and MDMX inhibitors and should inspire new strategies toward the design of drug-like small molecule inhibitors specifically targeting MDMX.

**EXPERIMENTAL PROCEDURES**

**Materials**—The chemicals and reagents were purchased from Sigma unless otherwise noted. The peptides (>95% purity) were supplied by Genscript (Piscataway, NJ).

**Expression and Purification of Human MDM2 and MDMX**—The MDM2 N-terminal domain spanning residues 17–125 was cloned into pGEX2T; a shorter construct (residues 24–109) was cloned into a pDEST-His-MBP vector provided by Dr. David S. Waugh (33). The pDEST-His-MBP vector was also used to express the N-terminal domain of human MDMX (residues 23–111). The MDM2 and MDMX N-terminal constructs were expressed in *Escherichia coli* BL21-Gold (DE3). The proteins were purified by affinity chromatography using glutathione-Sepharose (GE Healthcare) for MDM2 (17–125) or nickel-nitrilotriacetic acid Superflow (Qiagen) for MDM2 (24–109) and MDMX (23–111). Eluted proteins were cleaved with thrombin or tobacco etch virus protease (33) and then mixed with the pDI, pDI6W, or pDIQ peptide. The respective MDM2/X-peptide complexes were further purified using SP Sepharose (GE Healthcare; elution buffer: 50 mM HEPES, 0.015–0.5 mM NaCl, 5% glycerol, 5 mM dithiothreitol, pH 6.8) followed by size exclusion chromatography on Superdex 75 (GE Healthcare; elution buffer: 50 mM Tris, 150 mM NaCl, 5 mM dithiothreitol, 1 mM EDTA, pH 7.5).

**Crystallization**—The MDM2-X peptide complexes were crystallized at 19 °C by the hanging drop vapor diffusion method at a protein concentration of ~10 mg/mL, supplemented with an additional half molar equivalent of the respective peptide. The MDMX (23–111)-pDI and pDI6W complexes

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**TABLE 1**

| Name   | Sequence | MDM2 IC<sub>so</sub> | MDMX IC<sub>so</sub> |
|--------|----------|----------------------|-----------------------|
| p53    | ETPSDLW | 2000                 | 6000                  |
| pMI    | TSPARYW | 20                   | 40                    |
| pDI    | LTFEHALTS | 44                   | 550                   |
| 6W     | LTTFEHALTS | 36                   | 250                   |
| 6S     | LQTREH WALS | Inactive | Inactive |
| 1E6N   | ETPFSDIEHEALS | Inactive | Inactive |
| 6N     | ETPFSDI | 400                  | 4000                  |
| 6W9S   | LTFEHALTS | 125                  | 500                   |
| 6W8S9S | LTFEHALTS | 130                  | 800                   |
| 4T6W   | LTFEHW | 20                   | 200                   |
| 1E6W   | ETPFSDIBEALS | 24                   | 180                   |
| 6W11L  | ETPFSDIEHEALS | 20                   | 140                   |
| pDIQ   | ETPFSDI | 8                    | 110                   |

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**TABLE 2**

|          | MDMX-pDI | MDMX-pDI6W | MDMX-pDIQ | MDMX-pDI6W | MDMX-pDIQ |
|----------|----------|------------|-----------|------------|-----------|
| Data collection |          |            |           |            |           |
| Space group | C2       | C2         | P21212    | I222       | P21212    |
| Unit cell dimensions (Å) | 70.2 b = 27.2 c = 51.8 | 70.4 b = 27.6 c = 52.1 | 44.9 b = 53.7 c = 87.3 | 37.2 b = 68.8 c = 92.4 | 43.8 b = 50.6 c = 39.2 |
| a = γ = 90° β = 124.4 | a = γ = 90° β = 124.6 | a = γ = 90° β = 90° | a = β = γ = 90° | a = β = γ = 90° |
| Resolution range | 25–1.8 (1.86–1.8) | 30–1.73 (1.79–1.73) | 35–1.8 (1.86–1.8) | 46–2.1 (2.18–2.1) | 29–1.78 (1.84–1.78) |
| Unique reflections | 7677 (748) | 8289 (664) | 20193 (1953) | 7261 (74) | 8640 (833) |
| Completeness (%) | 92.7 (94.70) | 93.9 (77.00) | 99.7 (99.00) | 99.8 (100.00) | 98.1 (99.30) |
| R<sub>merge</sub> (%) | 4.4 (8.90) | 4.9 (9.10) | 6.2 (47.60) | 11.9 (31.80) | 3.7 (11.60) |
| Protein atoms | 699 | 699 | 2 × 699 | 793 | 690 |
| Average B-factor (Å<sup>2</sup>) | 15.0 | 15.7 | 27.3 | 25.0 | 32.2 |
| Ligand atoms | 107 | 109 | 2 × 112 | 109 | 112 |
| Average B-factor (Å<sup>2</sup>) | 14.1 | 15.5 | 29.2 | 23.6 | 31.5 |
| Solvent molecules | 116 | 123 | 139 | 116 | 55 |
| Average B-factor (Å<sup>2</sup>) | 27.9 | 29.7 | 37.4 | 34.3 | 42.8 |
| RMSD bonds (Å) | 0.011 | 0.016 | 0.016 | 0.008 | 0.015 |
| RMSD angles (°) | 1.4 | 1.5 | 1.5 | 1.1 | 1.6 |
| R<sub>cryst</sub> (%) | 16.2 | 16.0 | 20.7 | 19.6 | 21.1 |
| R<sub>free</sub> (%) | 20.7 | 21.7 | 22.5 | 22.9 | 24.9 |

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| Coordinate error | From Luzzati plot (Å) | 0.21 | 0.21 | 0.24 | 0.28 | 0.28 |
| Cross-validated estimated | From SigmaA (Å) | 0.14 | 0.13 | 0.22 | 0.23 | 0.19 |
were crystallized from 1.4 M sodium/potassium phosphate, pH 8.2; 15% ethylene glycol was included for cryo-protection. The MDMX (23–111)-pDIQ complex was crystallized from 2.1 M ammonium sulfate, 10 mM Tris-HCl, pH 7.4, 5% propanol; 25% glycerol was included for cryo-protection. The MDM2 (17–125)-pDI6W complex crystallized from 2.2 M L-Malic acid, 100 mM Tris-HCl, pH 8.0; no cryo-protectant was added. The MDM2 (24–109)-pDIQ crystallized from 30% PEGmme2000, 100 mM Tris-HCl, pH 8.5, 200 mM MgCl₂; 10% ethylene glycol was included for cryo-protection.

Data Collection, Structure Solution, and Refinement—X-ray diffraction data were recorded at −180 °C using the oscillation method on single flash-frozen crystals (detector, Rigaku HTC image plate; x-rays, CuKα, focused by mirror optics; generator, Rigaku Micro-Max 007–HF). The data were processed with HKL2000 (HKL Research, Inc., Charlottesville, VA). The structures were determined by molecular replacement using the coordinates of human MDM2-p53 (Protein Data Bank entry 1T4F) and human MDMX-p53 (Protein Data Bank entry 3DAB) as search models. The program package CNS (34) was employed for phasing and refinement; model building was performed with O (35). Refinement cycles were performed using data to the highest resolution with no sigma cut-off applied. Several rounds of minimization, simulated annealing (starting temperature, 2500 K), and restrained individual B-factor refinement were carried out. The data collection and refinement statistics are summarized in Table 2. Figs. 1–5 and 7 were drawn with Pymol (DeLano Scientific, Palo Alto, CA).

Enzyme-linked Immunosorbent Assay and Fluorescence Polarization Assays—GST-DM2–1–150 and GST-DMX–1–200, and full-length His₆-p53 were expressed in E. coli and affinity-purified under non-denaturing conditions. ELISA plates were incubated with 2.5 μg/ml His₆-p53 in phosphate-buffered saline (PBS) for 16 h. After washing with PBS + 0.1% Tween 20 (PBST), the plates were blocked with PBS + 5% nonfat dry milk + 0.1% Tween 20 (PBSMT) for 0.5 h. GST-HDM2 and MDMX (5 μg/ml) were mixed with peptides in PBSMT + 10% glycerol + 10 mM dithiothreitol and added to the wells. The plates were washed with PBST after incubating for 1 h at room temperature and incubated with MDM2 antibody 4B2 and MDMX antibody 8C6 in PBSMT for 1 h, followed by washing and incubation with horseradish peroxidase rabbit anti-mouse Ig antibody for 1 h. The plates were developed by incubation with TMB peroxidase substrate (KPL) and measured by absorbance at 450 nm. Fluorescence polarization assay was performed using N-terminally conjugated fluorescein isothiocyanate-p53 (LSQETFSDLWKLLPEN) and fluorescein isothiocyanate-pDI peptides. Proteins (2–4000 nM) and peptides (2 nM) were mixed in fluorescence polarization buffer (25 mM HEPES, pH 7.4, 0.1% Tween 20, 150 mM NaCl, 10 mM dithiothreitol). The mixtures were incubated at 25 °C for 1 h and analyzed for fluorescence polarization. The Kₘ values were determined from fluorescence polarization data using the method described by Zhang et al. (36).

CD Analysis—Circular dichroism measurements were made using an Aviv Model 215 spectrometer. The peptides were prepared at a concentration of 0.1 mM in 10 mM Tris-HCl, pH 7.5, 5% methanol, and 10% trifluoroethanol. The measurements were taken at 222 nm.

The abbreviations used are: GST, glutathione S-transferase; ELISA, enzyme-linked immunosorbent assay; PBS, phosphate-buffered saline.
were performed at 23 °C. Blank scan (buffer) was subtracted from the spectra, and values of ellipticity were expressed in units of deg cm²/mole. The amphipathic α-helical peptide melittin was used as a standard.

RESULTS AND DISCUSSION

Characterization of a High Affinity MDM2- and MDMX-binding Peptide—We have recently identified a 12-residue peptide inhibitor of MDM2 and MDMX by screening a phage display library for peptides that bind to GST-MDM2–1-150 and GST-MDMX–1–200 (30). Both screens selected the same peptide sequence (1LTFEHYWAQLTS) as the highest affinity ligand for MDM2 and MDMX. This peptide was named pDI for peptide dual inhibitor. The pDI is distinct from the equivalent p53 sequence (p53p, 17ETFSDLWKLLPE28) but retains the key p53 residues Phe19, Trp23, and Leu26, which bind to three distinct hydrophobic pockets of MDM2 (16). The binding affinity of pDI to MDM2 and MDMX was analyzed using fluorescence polarization yielding dissociation constants of 1 nM for the MDM2-pDI and 3 nM for the MDMX-pDI interaction. By comparison, the $K_d$ values for p53p binding to MDM2 and MDMX were 160 and 260 nM, respectively, which is in good agreement with the previously determined $K_d$ values obtained by isothermal titration calorimetry (37). Inhibition of full-length p53 binding to MDM2 or MDMX by the pDI (and all other peptides studied here) was assessed by ELISA, yielding IC₅₀ values of 44 and 550 nM, respectively (Table 1).

Structural Characterization of the MDM2 and MDMX pDI Peptide Complexes—In an attempt to understand the differences in binding affinities for MDM2 and MDMX, we first determined the crystal structure of MDMX liganded with the pDI peptide (Fig. 1 and Table 2). Subsequently, both MDM2 and MDMX were co-crystallized with a derivative peptide, pDI6W, which contained a single Y6W mutation and displayed increased inhibitory properties over the parent peptide (Table 1). The structures of the pDI6W peptide served as a template for the design of the pDIQ peptide. The overall structures of the N-terminal domains of MDM2 and MDMX liganded with the pDI and derivative peptides are very similar, as expected from the previously determined MDM2/X structures (16, 38). The major structural difference between MDM2 and MDMX is the orientation of the C-terminal helix (α₂', residues 96–106 for MDM2 and 95–105 for MDMX) relative to the binding site of p53 and the pDI peptide (Fig. 1; see also Fig. 5). The pDI peptides all bind to MDM2 and MDMX in a helical conformation via residues Phe⁻³-Trp⁻⁷-Leu⁻¹⁰ to the same groove that harbors the Phe₁⁹-Trp₂₃-Leu₂₆ triplet of the p53 helix (16). The interaction pattern between the triplet residues and their binding sites in MDM2/X is dominated by hydrophobic interactions. In addition, the indole nitrogen of Trp(P)⁷ establishes a hydrogen bond with the carbonyl oxygen of Leu₅₄MDM2 or Met₅₃MDMX. This hydrogen bond makes it possible for the indole ring to intercalate into the MDM2/X residues His₇₇ and Lys₉₃ (MDM2 residues His₇₅ and Lys₉₁) and the peptide residue His(P)⁷. The hydroxyl group of Tyr(P)⁶ and the indole nitrogen of Trp(P)⁶ are hydro-
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Amino acid substitutions were introduced into the pDI6W peptide and analyzed by ELISA to determine their potency in disrupting MDM2-p53 and MDMX-p53 interactions. Replacing Trp(P)⁶ with Ser or Asn (6S, 6N, and 1E6N of Table 1) reduced or even abolished inhibitory potency, corroborating the above mentioned notion that ring systems in this position confer stability through an increased number of van der Waal’s interactions (Δ < 4 Å) than Tyr(P)⁶, which possibly contributes to the improved binding potential of the pDI6W over the pDI parent peptide.

Molecular Basis for the High Affinity of the pDIQ Peptide—

Upon binding to MDMX, with up to 2 Å displacement shifts of the C terminus from residues 8–12 (Fig. 4). This is accompanied by a loss of a helical structure of residues 10 and 11 (Figs. 1 and 4), which in turn allows Leu(P)¹¹ to interact with Tyr(P)⁹⁹ of the MDMX α²'-helix and the side chains of Met⁵₃ and Val⁴⁹ of the α₂-helix (Fig. 5). It appears that the increase in hydrophobicity of the Leu¹¹ side chain in the pDIQ versus the Thr¹¹ side chain in pDI6W causes this structural change upon binding to MDMX. By contrast, the pDIQ in complex with MDM2 retains complete helical conformation, directing Leu(P)¹¹ away from the α²'-helix and toward the α₂-helix. Here, Leu(P)¹¹ interacts with the side chains of Leu⁶⁴ and Phe⁶⁵ through van der Waal’s forces, whereas the ε-amino group of Lys⁶⁵ is hydrogen-bonded to its carbonyl oxygen (Fig. 5).

The conformational changes in the pDIQ peptide appear to be induced mainly by residue Tyr⁹⁹ of the α²'-helix (Fig. 5; see also Fig. 7). The orientation of this helix with respect to the peptide-binding site differs considerably between MDMX and MDM2. As a result, this tyrosine residue appears to exert a major role in the binding of the pDIQ to MDM2 and MDMX. In MDM2, Tyr¹⁰⁰ prevents Leu(P)¹¹ from reaching into the hydrophobic groove formed between the α²'-helix and the α₂-helix. As a consequence, the C terminus of

gen-bonded with water molecules. Both the Tyr(P)⁶ and Trp(P)⁶ ring systems interact with the aliphatic portion of Lys⁷³ and the C-β atom of His³² through hydrophobic forces. However, the indole ring of Trp(P)⁶ establishes more van der Waals interactions (Δ < 4 Å) than Tyr(P)⁶, which possibly contributes to the improved binding potential of the pDI6W over the pDI parent peptide.
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The pDIQ peptide retains helical conformation, positioning Leu(P)11 over the α2-helix. By contrast, Tyr99 of MDMX accommodates the binding of Leu(P)11 to the hydrophobic groove. It appears that the conformational flexibility in the C-terminal region of the pDIQ peptide ensures the high affinity binding to both MDM2 and MDMX. Remarkably, the pDIQ is 5-fold more active against MDMX than the parent pDI peptide, despite the partial loss of the energetically favored helical structure. This indicates the high binding potential of the groove and MDM2/X. The other amino acid substitutions were designed to increase the solubility of the peptide, at the same time strengthening the helical conformation through intrapeptide hydrogen bonds or through bridging water molecules. It has been suggested that the p53 N-terminal region is unstructured in solution but forms an α-helix upon binding to the hydrophobic pockets of MDM2 or MDMX. (39) To test whether the inhibitory potency of our derivative peptides is reflected by a higher degree of helical conformation in solution, circular dichroism studies were performed (Fig. 6). The most potent peptides, pDIQ and 1E6W (Table 1), exhibited the strong double-helical characteristics expected for α-helices, with pDIQ being most pronounced. The α-helical feature was less prominent in the 6W peptide and absent in the inactive 6S peptide. These results indicate that the improved inhibitory action of peptides is not only due to an increase in noncovalent interactions between the peptide and the protein (as seen for the Y6W and T11L substitutions) but also due to the enhanced stability of the α-helical conformation in solution.

Implications for the Design of Small Molecule Inhibitors against MDMX—Currently, small molecule inhibitors of MDM2 with anti-tumor activity such as Nutlin and MI-219 are generally ineffective against MDMX (24, 25, 30, 40). Superposition of the complexes of MDM2-pDIQ and MDM2-Nutlin2 reveals the almost perfect fit of the three hydrophobic residues of Nutlin with the side chains of the peptidic FWL triplet (Fig. 7). However, the structural differ-
ences of the α2′ helices of both proteins directly impact the binding site of Leu(P)10. For MDM2, Tyr100 accommodates the binding of Nutlin, whereas Tyr99 in MDMX causes a potential steric clash with the Nutlin molecule. Although the efficient binding of the pDIQ peptide to MDMX is made possible through structural flexibility of the C-terminal region of the pDIQ peptide, small molecules such as Nutlin cannot undergo these conformational changes. Therefore, the design of p53 antagonists that selectively target MDMX over MDM2 must take into account these distinct differences of the peptide-binding site. One strategy could be the design of small molecules centering on the Leu(P)10 site and extending to the Trp(P)7 and Leu(P)11 sites, rather than centering on the Trp(P)7 site and extending to the Phe(P)3 and Leu(P)10 sites as in the case for Nutlin. To keep their size small, scaffolds designed in this manner will have to lack the functionality to bind to the Phe(P)3 site (Phe(P)3 is ~10 Å away from Leu(P)10). This will likely result in a loss of bind-

FIGURE 6. High affinity pDI peptides adopt helical conformation in solution. The peptides were analyzed by circular dichroism spectroscopy at 0.1 mM concentration at 23 °C. Melittin is a representative α-helical peptide from bee venom that served as a positive control. The IC50 values for each peptide against MDM2 are indicated.
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In conclusion, the results from this work demonstrate that peptides identified by phage display as MDM2 antagonists do not necessarily exhibit maximum binding affinity. Rather, such peptides may serve as ideal starting points for the structure-based design of inhibitors with substantially improved potency. In this study, the structure-activity analysis of a limited number of amino acid substitutions resulted not only in a 5-fold increase in inhibitory activity over the parent peptide but also revealed large conformational changes in the peptide required to bind efficiently to MDMX. These structural changes are induced by a hydrophobic site in MDMX, which probably has not yet received due attention in the design of small molecule inhibitors specifically targeting MDMX. The findings reported here should facilitate the rational design of novel MDMX-specific or MDM2/MDMX dual specific inhibitors as potential cancer therapies.

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