

Subject Review

Inhibition of the p53-MDM2 Interaction: Targeting a Protein-Protein Interface

Patrick Chène

Oncology Department, Novartis, Basel, Switzerland

Abstract

MDM2 inhibits p53 transcriptional activity, favors its nuclear export, and stimulates its degradation. Inhibition of the p53-MDM2 interaction with synthetic molecules should therefore lead to both the nuclear accumulation and the activation of p53 followed by the death of the tumor cells from apoptosis. Inhibitors of the p53-MDM2 interaction might be attractive new anticancer agents that could be used to activate wild-type p53 in tumors. This review describes our current knowledge on the properties of the existing p53-MDM2 antagonists. Because the discovery of modulators of protein-protein interactions is an emerging field in drug discovery, the strategy used for designing inhibitors of the p53-MDM2 interaction could serve as an example for other protein interfaces.

Introduction

The p53 protein has the structure of a transcription factor and is therefore made up of several domains. A transactivation domain and a proline-rich domain are found at its NH₂ terminus. A DNA-binding domain is in the middle of the protein. Finally, a tetramerization domain and a regulatory region are present at its COOH terminus. This structure allows the tetrameric p53 protein, on binding to DNA, to regulate the expression of several genes with different biological functions, such as cell cycle regulation, apoptosis, DNA repair, and differentiation (1). The central role of p53 in the cell suggests that the loss of p53 function may have dramatic consequences. This is demonstrated by the study of p53 gene knockout mice (2). Mice homozygous for inactivated p53 allele develop normally but are highly sensitive to tumors. By 6 months of age, 74% of the animals develop tumors, and within 10 months, all of them are dead. The importance of p53 in cancer is supported by the fact that the *p53* gene in human tumors is deleted or mutated in more than 50% of human cancers (3).

The loss of p53 is not the only way of inactivating p53 in tumors. This can also be the consequence of an overexpression

of the MDM2¹ protein. p53 and MDM2 form an autoregulatory feedback loop (4, 5). p53 stimulates the expression of the MDM2 protein, which in turn inhibits p53. MDM2 acts negatively on p53 in at least three different ways (Fig. 1A). Firstly, by binding to its transactivation domain, it inhibits its transcriptional activity (6). Secondly, MDM2, which acts as an ubiquitin ligase, promotes p53 degradation (7, 8). Thirdly, on binding to p53, MDM2 favors the export of p53 because it contains a nuclear export signal (9). The analysis of tumor samples shows that the *MDM2* gene is amplified in about 7% of these tissues (10) and various reports reveal that the MDM2 protein is overexpressed in different types of tumors (11, 12). It is very likely that the p53 pathway is not active in these tumors, because the overexpressed MDM2 protein constantly inhibits the p53 protein. Agents preventing this MDM2-mediated inactivation of p53 should permit the activation of p53 and as a consequence show anticancer activity.

Several strategies to activate the p53 pathway in tumors via inhibition of the MDM2 protein are under investigation (Fig. 1B). The first one is to decrease the cellular concentration of MDM2 with antisense oligodeoxynucleotides (13). A second possibility is to block the MDM2-mediated ubiquitination of p53 (14). The third strategy is to use mimics of p14ARF, a negative regulator of MDM2 (15). Finally, the fourth possibility is to prevent the interaction between p53 and MDM2 with molecules that bind at the interface between these two proteins. This latter drug discovery strategy will be the topic of this review.

Biochemical and Structural Properties of the p53-MDM2 Complex

The regions involved in the interaction between p53 and MDM2 were first identified in a yeast two-hybrid screen (16) and in immunoprecipitation experiments (17). The MDM2-binding domain on p53 was localized between residues 1 and 41 (16) or 1 and 52 (17) and the p53-binding domain on MDM2 between residues 1 and 118 (16) or 19 and 102 (17). Subsequently, site-directed experiments have demonstrated the importance of p53 residues Leu¹⁴, Phe¹⁹, Leu²², and Trp²³ (18), and a minimal MDM2-binding site on the p53 protein was mapped with p53-derived peptides between residues 18 and 23 (19).

The strength of the interaction between p53 peptides and MDM2 fragments has been determined by several methods.

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Requests for reprints: Patrick Chène, Oncology Department, Novartis, K125 443, CH-4002 Basel, Switzerland. Phone: 41-61-696-2050; Fax: 41-61-696-3835. E-mail: patrick_chene@yahoo.com

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¹The abbreviations used are *MDM2*, human gene and oncogene; *MDM2*, human protein and isoform; *mdm2*, mouse gene; *Mdm2*, mouse protein.

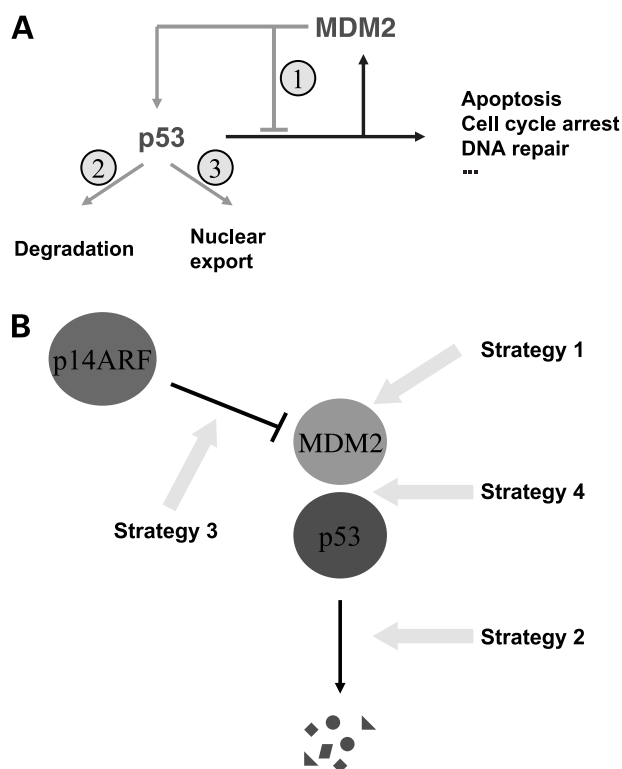


FIGURE 1. Regulation of p53 by MDM2. **A.** MDM2 regulates p53 in three different ways. On binding to p53 transactivation domain, it inhibits its transcriptional activity (1), promotes p53 degradation (2), and favors the export of p53 from the nucleus (3). Therefore, in the presence of MDM2, the p53 protein is inactivated and does not stimulate the expression of genes involved in apoptosis, cell cycle arrest, or DNA repair. In some tumors where MDM2 is overexpressed, p53 is constantly inhibited and tumor growth is favored. The inactivation of MDM2 in these tumors should activate the p53 pathway and as a possible consequence should activate apoptosis. **B.** Several strategies can be used to target MDM2 in tumors. Antisense oligonucleotides should decrease the cellular levels of MDM2 (Strategy 1). Compounds that inhibit the ubiquitin ligase activity of MDM2 could prevent p53 degradation (Strategy 2). p14ARF acts by blocking MDM2-dependent degradation and transcriptional silencing of p53. p14ARF mimics should therefore activate the p53 pathway (Strategy 3). Inhibitors of the p53-MDM2 interaction should release p53 from MDM2 and as a consequence should activate p53 tumor suppressor activity (Strategy 4).

Isothermal titration calorimetry at 35°C gives a apparent dissociation constant (K_d) of 600 and 420 nM for p53_{15–29} and p53_{1–57}, respectively, on binding to MDM2_{1–118} (20). Similar results were obtained with MDM2_{2–125} and p53_{15–29} at 15°C (21). Fluorescence anisotropy measurements at 25°C with Oregon Green p53_{1–35} and p53_{1–29} and with MDM2_{1–126} give a K_d of about 700 nM, but the shorter peptides, p53_{17–26} or p53_{18–26}, have a higher affinity ($K_d = 60$ nM; 22; see also Ref. 21). Measurements with the same technique but with fluorescein-labeled p53_{1–39} and MDM2_{17–125} give a K_d (4°C) value of 70 nM (23).

The regions corresponding to residues 13–119 of *Xenopus laevis* MDM2 and residues 17–125 of MDM2 have been crystallized in complex with p53_{15–29} and their structures were determined by X-ray crystallography at 2.3 and 2.6 Å resolution, respectively (20). The crystallized protein fragments (79% primary sequence similarity) have a similar structure. A large

cleft about 25 Å long and 10 Å wide is present at the surface of the MDM2 protein. The sides of the cleft are formed by two helices, and β-strands are present at its ends. Two antiparallel smaller helices form the bottom of the cleft (Fig. 2A). The same general structure of ¹⁵N-enriched MDM2_{1–118} is observed by ¹⁵N-heteronuclear single-quantum coherence nuclear magnetic resonance (NMR) spectroscopy (24).

Transfer nuclear Overhauser enhancement studies of p53-derived peptides show that these fragments do not have a stably folded structure in solution (25, 26) or form two type III β-turns (27). These measurements indicate that the peptide probably adopts the helical conformation observed in X-ray crystallography only when bound to MDM2. The structural organization of the p53 peptide on binding should therefore induce a negative entropic effect. The enthalpy and entropy values for p53_{1–35} binding to MDM2_{1–126} were estimated at –16.4 and –40.4 cal/mol, respectively (22), confirming an entropy loss on association. p53_{15–29} binds into the cleft present at the surface of MDM2 (Fig. 2B). The analysis of the angle of rotation around the N-C_α bond (φ angle) and the C_α-C' bond (ψ angle) indicates that residues 19–25 form an α-helix and that residues 17, 18, and 26–29 take a more extended conformation. The conformation of the bound peptide is stabilized by various H-bonds: Thr¹⁸ and Leu²², Phe¹⁹ and Trp²³, and Ser²⁰ and Lys²⁴ form intramolecular H-bonds via their backbone CO and NH; Leu²² makes a 3₁₀ helix type of H-bond with Leu²⁵; and Thr¹⁸ makes a H-bond via its backbone NH with the backbone CO of Asp²¹ and via its hydroxyl group with the carboxylic function of Asp²¹. These two H-bonds could contribute to the stability of the α-helix as has been demonstrated in other proteins (28). Computational alanine scanning applied to the p53-MDM2 interaction (29) shows that the mutation Thr¹⁸Ala does not affect the binding free energy. Because Thr¹⁸ is very important for p53 binding (21), the presence of alanine, a helix former (30), may compensate for the loss of the two H-bonds in Thr¹⁸Ala. A recent NMR study of MDM2 reveals that another region of this protein, between residues 16 and 24, is important for the interaction (31). These residues form a lid that closes over the p53-binding site. This lid competes with p53 for the p53-binding site. The binding of p53 to MDM2 should induce conformational changes because it leads to a displacement of the lid. Conformational changes within the MDM2 protein on p53 binding have been observed by NMR (21, 24).

The two large hydrophobic amino acids p53 Phe¹⁹ and Trp²³ are located face to face on the same side of the helix, and together with p53 Leu²⁶ (Fig. 2B), they point toward the MDM2 protein, where they are surrounded by several hydrophobic residues (Leu⁵⁴, Leu⁵⁷, Ile⁶¹, Met⁶², Tyr⁶⁷, Val⁷⁵, Val⁷⁵, Val⁹³, Phe⁸⁶, Ile⁹⁹, Phe⁹¹, and Ile¹⁰³) from the MDM2 protein. p53_{15–29} makes H-bonds between the backbone amide of Phe¹⁹ and the O_ε of MDM2 Gln⁷², between the N_ε of Trp²³ and the backbone carbonyl of MDM2 Leu⁵⁴, and between the backbone carbonyl of Asn²⁹ and the hydroxyl of MDM2 Tyr¹⁰⁰. The interaction between p53 and MDM2 is essentially hydrophobic, and 70% of the atoms at the interface are nonpolar. The calculated accessible surface area buried at the interface on MDM2_{25–109} and p53_{17–29} is about 660 and 809 Å², respectively. The gap volume (32) between both proteins is 892 Å³ and the gap volume index (ratio between the

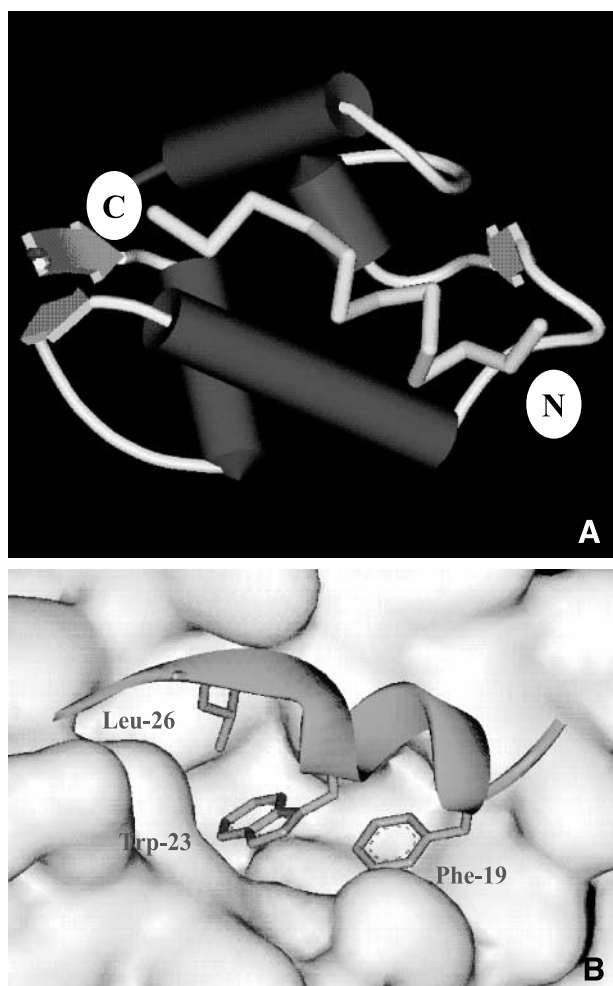


FIGURE 2. Structure of the p53-MDM2 complex. The structure of the p53-MDM2 complex was determined by X-ray crystallography at a resolution of 2.6 Å (20). **A.** Schematic representation of the secondary structure elements constituting MDM2_{25–109} (cylinders, α -helices; arrows, β -strands). The backbone of p53_{17–29} is represented. The NH₂ (N) and COOH (C) termini of the p53 peptide are indicated. **B.** The surface of MDM2_{25–109} and the backbone of p53_{17–29} are represented. The lateral chains of the three p53 residues (Phe-19, Trp-23, and Leu-26) are shown.

gap volume and the interface accessible surface area; 33) is 0.61 Å. This low value for the gap index shows that the two interacting interfaces are very complementary. Indeed, there is no bridging water molecule between both proteins (26, 34). The planarity (root mean square deviation of all interface atoms from the least-squares plane through the atoms; 33) of the MDM2 interface is 3.1, revealing that it is not flat but rather twisted.

This structural analysis reveals many factors important for the drug discovery process. Only one of the two partners (MDM2) has a structurally well-defined binding site. The inhibitors should therefore aim to mimic the other partner (p53). One of the two interfaces (p53) is formed by only one segment of contiguous amino acids, allowing the design of peptidic inhibitors (p53 mimics). Three p53 residues (Phe¹⁹, Trp²³, and

Leu²⁶) contribute to a large extent to the interaction and consequently to the binding energy of the p53 peptides (29). The inhibitors of the p53-MDM2 interaction will have to contain mimics of these amino acids. Two of the three H-bonds (via p53 Phe¹⁹ and p53 Trp²³) made by the p53 peptide are important for the interaction with MDM2. At least the most buried of these bonds (between p53 Trp²³ and MDM2 Leu⁵⁴) will have to be preserved to ensure sufficient affinity of the inhibitors. The interface between both proteins is rather small, suggesting that it might be possible to design inhibitors of relatively small size. This is important because molecules with molecular weights higher than 500 Da usually have a lower oral bioavailability. The interface is twisted. The less flat the interface between two partners, the greater the tendency of one of the two partners to be buried (here p53). The burial of an inhibitor is usually linked to its partial desolvation leading to a favorable entropic contribution in the binding energy. The p53-MDM2 interface is hydrophobic; therefore, inhibitors of the p53-MDM2 interaction will contain lipophilic substitutions. The presence of lipophilic groups usually improves the binding energy because of the favorable contribution of entropy. However, highly lipophilic inhibitors may show a decrease in bioavailability.

Inhibitors of the p53-MDM2 Interaction

Thus far, no potent synthetic low molecular weight compound inhibitor of the p53-MDM2 interaction is available. Only chalcone derivatives (Fig. 3A; 24) and some polycyclic compounds (Fig. 3B; 35) have been described. The potency of these molecules is low and they cannot be considered as drug candidates. A fungal (*Fusarium*) metabolite, chlorofusin (Fig. 3C), has been described as an inhibitor of the p53-MDM2 interaction (36). This compound has an IC₅₀ of about 5 μ M in an ELISA and in surface plasmon resonance. Recent evidences show that chlorofusin binds at the NH₂ terminus of MDM2 (37), but the exact binding mode of this compound is not determined. This natural compound could bind at the interface between p53 and MDM2 or could affect the interaction via an allosteric effect.

Most of the data about the inhibition of the p53-MDM2 interaction have been obtained with peptides. Initial work has shown that p53-derived peptides inhibit the p53-MDM2 interaction (16, 19), suggesting that peptides could be used as tool compounds to study this interaction. The potency of the p53-derived peptides was low and their optimization was needed. The display of peptide libraries on phages was first used (34) and a 12-mer phage-derived peptide (peptide 2; Table 1) 29 times more potent *in vitro* than the corresponding wild-type peptide was obtained (peptide 1; Table 1; 38). Truncations of peptides 2–8 residues led to peptide 3 with an IC₅₀ value of about 9 μ M (Table 1; 38), revealing that a minimal length of eight amino acids is required to keep an activity in the micromolar range. The three important amino acids corresponding to Phe¹⁹, Trp²³, and Leu²⁶ are present in peptide 3 as expected from the structural analysis. The phage display experiments also show that a tyrosine is strongly favored at position 22 in the phage-derived peptide (34). The hydroxyl group of Tyr²² can form a H-bond with the carboxylate of p53 Glu¹⁷, enhancing the helicity of the peptide (its

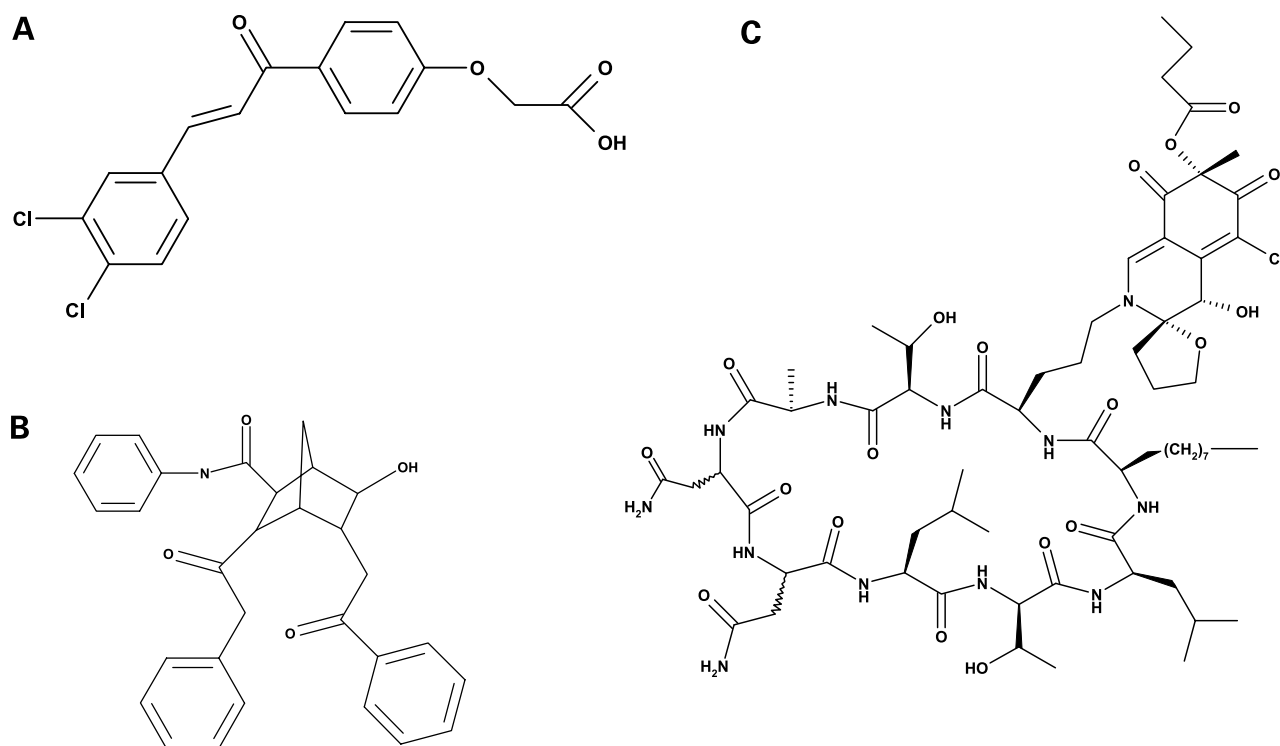


FIGURE 3. Natural and synthetic inhibitors of the p53-MDM2 interaction. **A.** Chalcone derivative (compound B-1; 24). **B.** Polycyclic compound (compound syc-7; 35). **C.** chlorofusin (36).

preorganization) and therefore its potency (see Ref. 29 for a discussion). In addition, the side chain of Tyr²² can generate more van der Waals interactions with the aliphatic part of MDM2 Val⁹³ and K94.

The p53 peptides take a helical structure when bound to MDM2 but adopt several conformations in solution (25, 26), leading to a negative contribution of entropy in the binding energy. To minimize this effect and thus to enhance the affinity of the peptides, nonnatural amino acids were introduced to preorganize the peptides in solution (39, 40). The presence of four α -aminoisobutyric acids (Aib), which promote a helical conformation (41), at positions 20, 22, 25, and 27 leads to peptide 4 with an IC₅₀ of 5.2 μ M (Table 1). Circular dichroism and ¹H NMR measurements confirm an increased preference of peptide 4 for helical structures (40). Similarly, the presence of one Aib at position 21 and one 1-aminocyclopropanecarboxylic acid (Ac₃c, a nonnatural amino acid that stabilizes 3₁₀

helices; 42) at position 25 leads to a 4-fold increase in potency (peptide 5; Table 1; 39). NMR studies confirm a higher preorganization of peptide 5. Because peptides 4 and 5 are still flexible in solution, their cyclization could further enhance their preorganization and therefore their potency.

Taking advantage of the structural information, two complementary strategies have been adopted to improve the affinity of peptide 5. The first one was to replace Tyr²² with a phosphonomethylphenylalanine (Pmp; peptide 6; Table 1; 39). The structure of the p53-MDM2 complex shows a spatial proximity between the hydroxyl group of p53 Tyr²² and the amine group of MDM2 Lys⁹⁴ (Fig. 4). The replacement of p53 Tyr²² by Pmp could induce the formation of a salt bridge with the amino group of MDM2 Lys⁹⁴, therefore enhancing the binding affinity. This single amino acid replacement led to ~7-fold increase in potency. The second strategy takes advantage of the fact that the natural peptide does not occupy

Table 1. Peptidic Inhibitors of the p53-MDM2 Interaction

Peptides	Sequence	IC ₅₀ (μ M)
1	Ac-Gln-Glu-Thr-Phe ¹⁹ -Ser-Asp-Leu-Trp ²³ -Lys-Leu-Leu ²⁶ -Pro-NH ₂	8.7 (39)
2	Ac-Met-Pro-Arg-Phe ¹⁹ -Met-Asp-Tyr-Trp ²³ -Glu-Gly-Leu ²⁶ -Asn-NH ₂	0.3 (39)
3	Ac-Phe ¹⁹ -Met-Asp-Tyr-Trp ²³ -Glu-Gly-Leu ²⁶ -Asn-NH ₂	8.9 (39)
4	Ac-Glu-Thr-Phe ¹⁹ -Aib-Asp-Aib-Trp ²³ -Lys-Aib-Leu ²⁶ -Aib-Glu-NH ₂	5.2 (40)
5	Ac-Phe ¹⁹ -Met-Aib-Tyr-Trp ²³ -Glu-Ac3c-Leu ²⁶ -Asn-NH ₂	2.2 (39)
6	Ac-Phe ¹⁹ -Met-Aib-Pmp-Trp ²³ -Glu-Ac3c-Leu ²⁶ -Asn-NH ₂	0.3 (39)
7	Ac-Phe ¹⁹ -Met-Aib-Pmp-6CITrp ²³ -Glu-Ac3c-Leu ²⁶ -Asn-NH ₂	0.005 (39)

Note: The IC₅₀ values of the different peptides have been determined *in vitro* with the MDM2-p53 peptide inhibition ELISA (34).

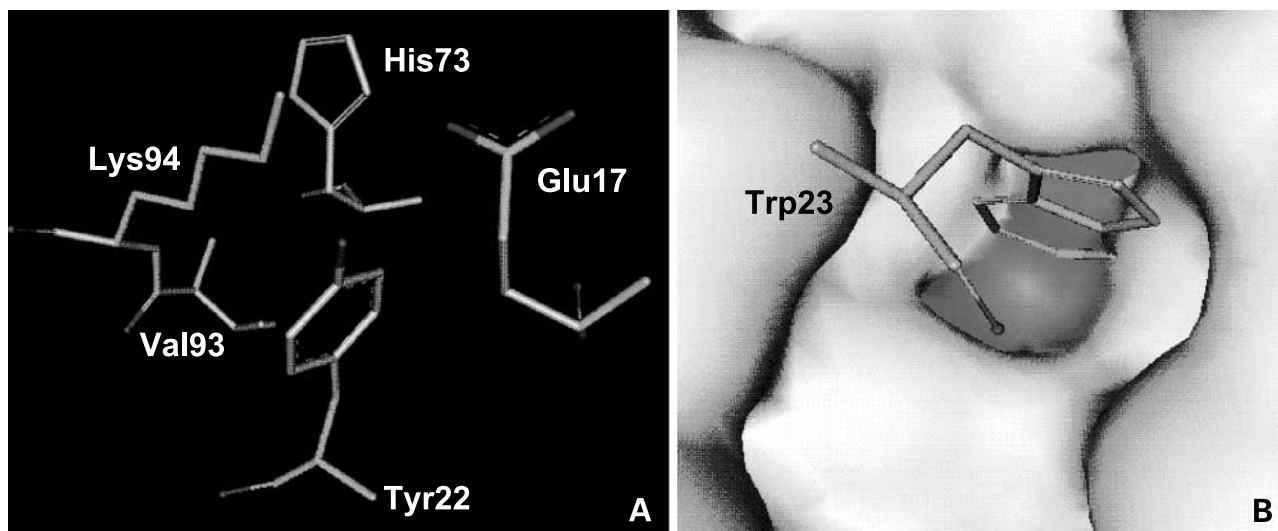


FIGURE 4. Strategies to improve the affinity of the p53 peptides. **A.** A tyrosine residue was introduced at position 22 in the original structure of the p53-MDM2 complex (20), and the location of this new lateral chain was manually adjusted to avoid sterical clashes. The residues in the close environment (6 Å) of Tyr22 are represented. His73, Val93, and Lys94 are from MDM2 and Glu17 is from p53. **B.** Small cavity in the p53-binding site. The surface of the MDM2 protein is in gray and the small cavity is in dark gray. p53 Trp23 is represented.

a small hydrophobic cavity (formed by residues Leu⁵⁷, Phe⁸⁶, Ile⁹⁹, and Ile¹⁰³) present in the cleft (Fig. 4). This cavity, which is also detected with the PROFEC program (29), is in front of C₁₂ (referred to below as position 6) of p53 Trp²³. Because an increase in the number of hydrophobic contacts usually enhances affinity, Trp²³ was substituted at position 6 with various hydrophobic groups (39). The best result was achieved with a chlorine [6-chloro-tryptophan (6ClTrp); peptide 7; Table 1]. The computational methyl-to-hydrogen scanning mutagenesis of the phenyl ring of p53 Trp²³ also predicts a gain in binding energy when a methyl group is present at position 6 (29). Altogether, the introduction of a Pmp at position 22 and a 6ClTrp at position 23 leads to an ~440-fold increase in the potency.

The IC₅₀ values of different peptidic inhibitors have recently been analyzed using hydropathic interactions software (43). This study shows that the three terminal atoms of the phenyl ring of Phe¹⁹, the phenyl ring and the indole nitrogen of Trp²³, and the isopropyl group of Leu²⁶ are the key pharmacophores.

Biological Effect of the Peptidic Inhibitors

In Vitro Validation

Because the potency of the nonpeptidic compounds available today is low, only the peptidic inhibitors were used to study the effect of inhibition of the p53-MDM2 interaction in tumor cells. Different strategies have been used to introduce the p53 peptides in cells. Peptide 2 has been inserted into the *Escherichia coli* thioredoxin protein (referred to below as TIP peptide; 44) or fused to the glutathione *S*-transferase protein (referred to below as GST; 45). Peptide 7 has been used without further modification (46, 47).

Immunoprecipitation experiments show that the GST peptide binds to the MDM2 protein in tumor cells preventing its interaction with p53 (45). This leads to a redistribution of the

cellular p53 protein, which is found predominantly in the nucleus in the presence of the GST peptide (45). The peptides also induce the accumulation of p53 (44–47). This effect is expected because the inhibition of the p53-MDM2 interaction prevents the MDM2-mediated degradation of p53. The accumulation of p53 leads to its transcriptional activation (44–47). The peptide-mediated activation of p53 affects the proliferation of tumor cell lines. The TIP peptide induces a decrease in the number of S-phase bromodeoxyuridine-positive T22 cells, suggesting a cell cycle arrest (44). The GST peptide decreases colony formation in a colony-forming assay, induces an increase in the number of cells with sub-G₁ and G₀-G₁ contents of DNA as measured by fluorescence-activated cell sorting, and finally stimulates cell death from apoptosis as determined in a terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay (45). Peptide 7 induces a cell cycle arrest in the colon carcinoma HCT116 cell line and apoptosis in SJSA-1 and JAR (choriocarcinoma) cells (47). The peptide 7-mediated apoptosis in the SJSA-1 occurs via the activation of caspase-3 (46). Apoptosis seems to be preferentially in tumor cells that overexpress the MDM2 protein (47). The effect of peptide 7 was also analyzed on nontumor cells (47). The effect of peptide 7 in two nontumor cell lines, NHDF 710 (normal human dermal fibroblasts) and HMEC 2595 (human mammary epithelial cells), was compared in a proliferation assay with its effect in SJSA-1 cells. Peptide 7, which activates p53 in the three cell lines, has a greater effect on the proliferation of SJSA-1 cells than on that of the two nontumor cell lines.

In Vivo Validation

Harbour *et al.* (48) have linked the Tat transduction sequence to p53_{16–27} (referred to below as Tat peptide) and used the resulting transducible peptide both *in vitro* and *in vivo*. *In vitro*, the Tat peptide induces apoptosis as shown in a

TUNEL assay. This effect is tumor cell specific because the Tat peptide was not toxic to nontumor cells. Moreover, a mutated form of the Tat peptide, which contains the mutation Trp²³Ala, did not induce cell death in tumor cells. The Tat peptide was next used in New Zealand White rabbits bearing intraocular retinoblastoma. Two injections of the peptide (200 μ M) were realized over 3 days into the interior chamber. The tumors started to dissolve 24 h after the injection of the Tat peptide, and at the end of the treatment, a reduction of about 76% of the total mass of the tumor was observed. The tumor cells were positive in TUNEL staining, suggesting an induction of apoptosis. In contrast, no effect was observed in tumors treated with the mutated Tat peptide. The Tat peptide induced damages only to the tumor and not to the surrounding ocular tissues (lens, cornea, retina, etc.). These *in vivo* experiments are very encouraging, because they suggest that inhibitors of the p53-MDM2 have an anticancer activity *in vivo*.

p53 and MDM2 Family Members

In the last years, p53-related genes (p63 and p73) and a *mdm2*-related gene (*mdmx*) have been identified (49–51). These proteins share many properties with p53 or MDM2, but

they also have distinct cellular functions (52, 53). p53 has an overall primary sequence similarity of 69% and 62% with p73 and p63, respectively, and MDM2 of 37% with mdmx. This homology suggests that p53 and MDM2 can associate with other members of the p53 and MDM2 families. Indeed, it has been established that mdmx binds to p53 (49). The good sequence conservation (58% identity) between MDM2 and mdmx within their p53-binding region enables a molecular model of the mdmx protein to be established based on the structure of MDM2 (Fig. 5). The two structures are very similar, and it is not surprising that inhibitors of the p53-MDM2 interaction also disrupt the p53-mdmx interaction (54). Small structural differences exist between MDM2 and mdmx, suggesting that it might be possible to design compounds, which more specifically inhibit one of the two interactions (54). p73 and p63 associate with MDM2 (55, 56) as expected from their strong homology with p53. Measurements carried out in a homogeneous time-resolved fluorescence assay show that the p73_{1–30} and p53_{7–42} peptides inhibit the p53-MDM2_{1–188} interaction in similar fashion: IC₅₀ = 690 and 1000 nM respectively (room temperature; 57). Similar results were obtained by isothermal calorimetry (21). However, experiments with fluorescein-labeled peptides give a *K_d* (4°C) value of 430 nM for the interaction between p73_{1–37} and MDM2_{17–125},

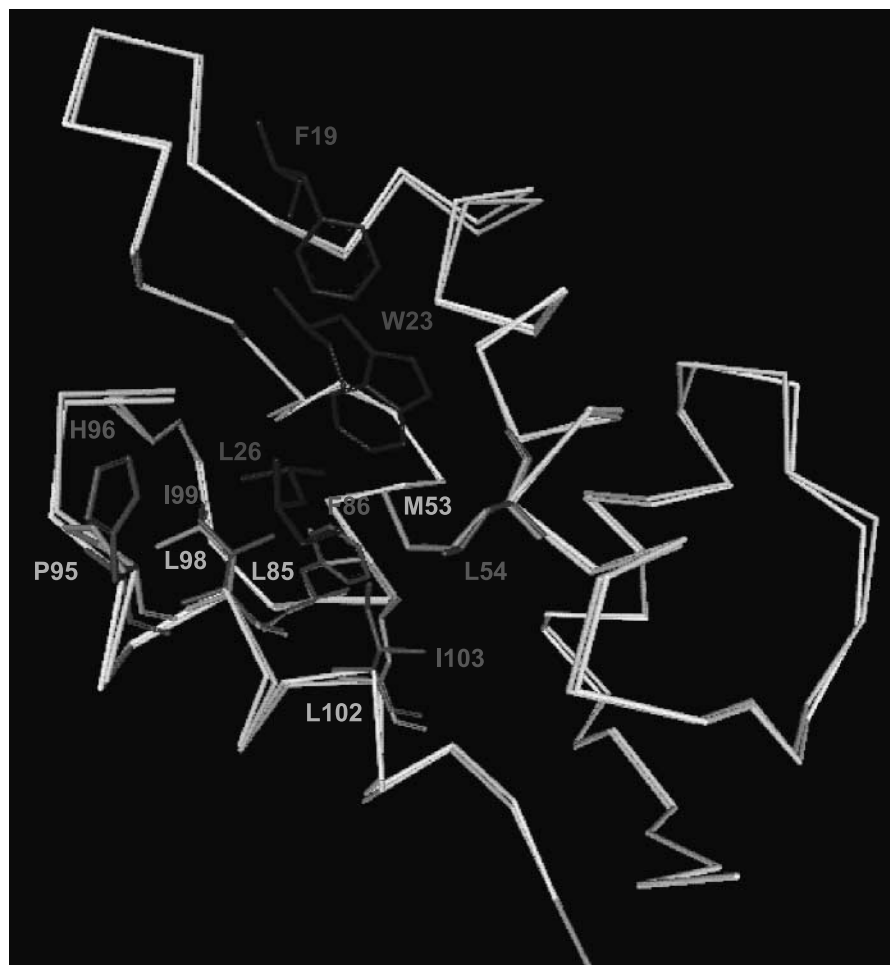


FIGURE 5. Molecular model of mdmx. A molecular model of human mdmx_{7–107} was established from the structure of MDM2_{25–109} (PDB code: 1YCR; 20). The conformation of the lateral chain of the amino acids from the p53-binding site has been manually adjusted to allow the presence of p53_{17–29}. The polypeptidic chains of MDM2_{25–109} and mdmx_{7–107} are represented in *white*. The nonconserved amino acids between mdmx and MDM2 are represented. The three residues (Phe¹⁹, Trp²³, and Leu²⁶) from p53_{17–29} are shown.

which is rather different from the K_d value of 70 nM obtained with p53₁₋₃₉ (23). p73 and p63 also interact with mdmX (49, 55). Altogether, these data suggest that inhibitors of the p53-MDM2 interaction should also affect complexes formed by the other p53 and MDM2 family members. Recent data obtained with the Tat peptide indicate that it might well be the case (48). This peptide induces the accumulation of p73 in a cell line that expresses a mutated form of p53.

Conclusion

p53 is altered in many human cancers, and new reports are published every month showing that it plays a central role in the tumor cell. This makes p53 one of the most attractive targets for cancer therapy. However, after many years of intense research, very few drugs targeting p53 have been identified (58). Among the different strategies evaluated by pharmaceutical companies, the design of modulators of the p53-MDM2 interaction is very often explored. Three reasons can explain this interest. First, the regulation of p53 by MDM2 is well characterized at the biological level. MDM2 negatively controls p53, and the cell naturally inhibits the p53-MDM2 interaction to activate p53 under stress conditions. Therefore, agents that prevent this interaction should stimulate p53 activity in tumor cells. Second, the structure of the p53-MDM2 complex has been known for many years (20). This information is an invaluable help to determine the drugability of the p53-MDM2 interface and to guide the drug discovery process. Third, a clear pharmacophore model has been established with the help of site-directed mutagenesis experiments and with the results obtained with the peptidic inhibitors. More recently, it has been shown that the peptidic inhibitors could activate the p53 pathway both *in vitro* and *in vivo* and that apoptosis could be induced in tumor cells overexpressing MDM2. Therefore, we know today that the inhibitors of the p53-MDM2 interaction have a potential as new anticancer agents, but no drug-like molecules have been identified. The best nonpeptidic compounds described in the literature have a weak activity in biochemical assays and the peptides, even if they are more potent, are by no mean druggable molecules.

Why has it not been possible to identify better inhibitors thus far? It is difficult to know precisely the strategies used by the pharmaceutical companies to identify inhibitors of the p53-MDM2 interaction, but random screens have certainly been carried out. In this approach, the compound libraries are screened in *in vitro* assays to identify active compounds. The disclosed results show, thus far, that this approach has not really been successful. One of the reasons for this failure might be the good complementarity of the p53-MDM2 interface. To show a reasonable activity, a compound should fill the Phe¹⁹, Trp²³, and Leu²⁶ subpockets present at the surface of MDM2, keeping some of the hydrogen bonds present in the p53-MDM2 complex. The probability of finding such a compound in the screened libraries might be very low. Moreover, minor modifications of the pharmacophores reduce drastically the potency of the inhibitors (39). Therefore, even if a compound can fill the three subpockets, the presence of a substitution at the wrong position could decrease its potency to a large extent. It might therefore be very difficult to identify potent inhibitors of the p53-MDM2 interaction in random screens and different approaches should be tried.

Two strategies can be envisaged. The first one is the *de novo* synthesis of inhibitors. This approach uses the structural and the biochemical information available. It consists in filling in a stepwise fashion the different subpockets present at the surface of the MDM2 protein. For example, filling the subpocket corresponding to p53 Trp²³ could be an interesting starting point. The results obtained with the peptides show that p53 Trp²³ contributes to a large extent to the binding energy. Mimics of p53 Trp²³ that keep the very important hydrogen bond made with MDM2 Leu⁵⁴ could therefore be used first and substitutions filling the two other subpockets could subsequently be added. This stepwise design also allows optimization of the linkers used to attach the mimics of p53 Phe¹⁹, Trp²³, and Leu²⁶. These linkers should bring the different mimics into the position where they fit the best in the MDM2 subpockets, and in addition, they should be sufficiently rigid to avoid large negative entropic changes on binding. The second strategy to identify inhibitors of the p53-MDM2 interaction is again to screen the compound libraries. However, these libraries, in contrast to the ones used in classical random screens, should be made up of very small molecules. This should help to identify compounds that bind into the different subpockets present at the surface of the MDM2 protein. These molecules should show independently a weak affinity for MDM2, but when brought together with the proper linkers, they should lead to more potent compounds. NMR could be used to identify these weak binding compounds (59). This second strategy has the advantage over the first one in that it may permit to identify in a short time mimics of p53 Phe¹⁹, Trp²³, and Leu²⁶. This approach will only be successful if the screened libraries contain the correct compounds, and as for the *de novo* approach if linkers bringing together the binding compounds in the correct orientation in the final molecule are identified.

In summary, today, we still can say that targeting the p53-MDM2 interface is an attractive drug discovery approach to activate p53 in tumors. However, if no promising inhibitors are identified within the next few years, it can be foreseen that the drug discovery activities in this area will be questioned.

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