A Nonpeptidic Sulfonamide Inhibits the p53–mdm2 Interaction and Activates p53-Dependent Transcription in mdm2-Overexpressing Cells

Peter S. Galatin and Donald J. Abraham*

Department of Medicinal Chemistry and Institute for Structural Biology and Drug Discovery, Virginia Commonwealth University, Richmond, Virginia 23298

Abstract: The evaluation of a sulfonamide inhibitor of the p53–mdm2 interaction is presented. The compound was identified using 3D database searching techniques based on a computationally derived pharmacophore model of mdm2 binding. It inhibits the physical interaction of recombinant p53 and mdm2 in vitro and increases p53-dependent transcription in an mdm2-overexpressing cell line.

p53, the "guardian of the genome", is a tetrameric allosterically regulated protein that is activated by a number of genotoxic factors.[1–5] In response to these signals, p53 promotes the transcription of genes responsible for cell-cycle arrest, DNA repair, and apoptosis. The importance of p53 in forestalling cancer development is implicated by the fact that over 50% of clinically detected tumors demonstrate mutations in one or both p53 alleles. p53 can be inactivated by other mechanisms besides mutation, however. There are a number of cancers, particularly of the glia, bone, and soft tissues, which have wild-type p53 and overexpressed mdm2, a protein which can inhibit p53's ability to bind to DNA and activate transcription.[6–12] Analysis of the X-ray crystal structure of the p53–mdm2 complex reveals that the N-terminal portion of p53 forms an amphipathic α−helix, which inserts its hydrophobic face (Phe19, Trp23, and Leu26) into a deep groove in mdm2.[13] Much has been written about the importance of these hydrophobic residues, especially how mutation at even one of these positions significantly reduces the ability of p53 to bind to mdm2.[14,15]

Inhibition of the p53–mdm2 interaction is a potential avenue of targeted anticancer therapy. In recent years, a number of peptide inhibitors have been identified.[16–21] To overcome the pharmacokinetic shortcomings of peptides, the search is on for small molecules with similar potency.[22–24] Previously, we reported that hydropathic indices from the HINT molecular modeling program[25] (eduSoft, LLC) could serve as parameters in predictive QSAR regression equations for p53–mdm2 inhibition.[26] This work has led to a pharmacophoric model for mdm2 binding for use in the discovery of nonpeptidic agents potentially able to mimic the portions of p53 necessary to bind to mdm2 (Figure 1). 3D database searches of the National Cancer Institute database have yielded a sulfonamide compound[27] that demonstrates both low-micromolar inhibition of the p53–mdm2 interaction in vitro and cellular activation of p53-dependent transcription. This compound represents a novel lead compound for further p53–mdm2 inhibitor design.
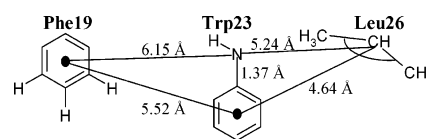

Figure 1. Schematic of the pharmacophore for mdm2 binding, as gauged by analysis with the HINT program. All atoms displayed are explicitly entered into the 3D database searching program. The thick dots in the center of the benzene rings are centroids, the spacial average of all six ring carbons. Dotted lines indicate distance measurements that are used as search constraints. The curved arc in Leu26 is an angle contraint.
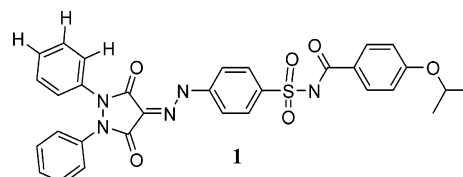

Figure 2. Sulfonamide compound NSC 279287 (CAS 59541-35-4).

Various permutations of the "mdm2-binding" pharmacophore were entered as searches. UNITY (Tripos, Inc.), when run in flex mode, attempts to fit query compounds to the target pharmacophore, rotating, and/or stretching bonds and torsional angles within the limits of chemical stability. Initial searches allowed a constraint tolerance of ±10%. When few database hits resulted, the search was rerun with a distance tolerance of ±20% (see Supporting Information).

Compounds identified by 3D searching of the NCI chemical database were requested from the Chemistry and Synthesis branch of the National Cancer Institute. Previous reports have indicated that approximately 65% of listed compounds are actually available. For the compounds we requested, the percentage was lower, around 40–50%.

Many of the compounds identified by the search still contain largely peptidic cores. They feature unnatural linkages and *O*-benzyl caps that would probably retard enzymatic hydrolysis to a significant degree. However, only a half of compound hits completely lack peptide bonds. Future database searches will focus on commercial chemical databases featuring fewer peptidic structures.

The p53–mdm2 binding assay was initially calibrated using the peptide [16]QETFSDLWKLLP[27], which inhibits the p53–mdm2 interaction strongly, essentially 100% at 100 μM. Fitting the dose–response curve to a three-parameter sigmoidal pharmacodynamic equation yields an IC_{50} of 12.9 ± 2.33 μM (Figure 3A). This is in line with values reported by other groups, ranging from 9.6 to 14 μM. Most of the National Cancer Institute compounds do not demonstrate dose–response pharmacodynamics. The exception is sulfonamide **1** (Figure 2), with an IC_{50} of 31.8 ± 6.02 μM (Figure 3B). NCI compounds were selected to screen as they met the criteria established by molecular modeling analysis of past active compounds. The large number of inactive compounds is not surprising, given that the models are based on a rather incomplete understanding of the p53–mdm2 interface molecular architecture.
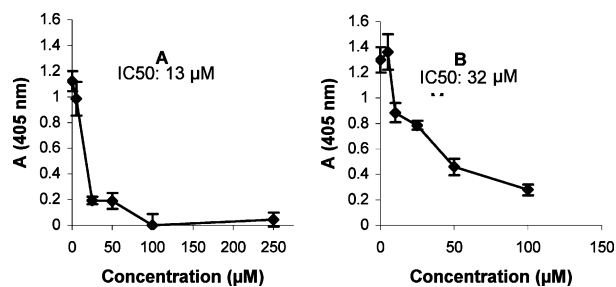
Figure 3. Dose−response curves for p53−mdm2 binding vs increasing concentration of tested compounds (A) positive control peptide [16]QETFSDLWKLLP,[27] and (B) **1**, and IC_{50} values. Plotted values and error bars represent the mean value ± standard error of four replicates.

Figure 4. A. p53 luciferase reporter system measurements in mdm2-overexpressing SJSA-1 cell line incubated with the following compounds: (A) Negative control (1% DMSO), (B) control peptide (100 μM), (C) control peptide (5 mM), (D) **1** (1 μM), and (E) **1** (100 μM). Bar heights and err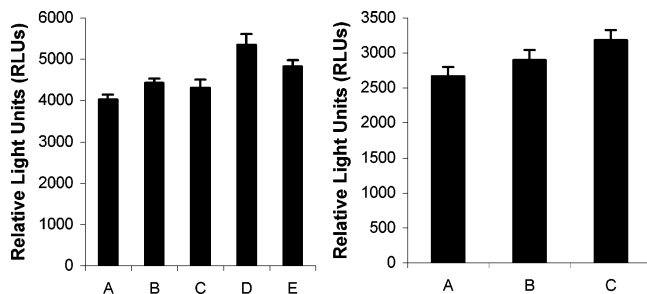or bars represent the mean value ± standard error of eight replicates. B. p53 luciferase reporter system measurements in mdm2-overexpressing SJSA-1 cell line incubated with the following compounds: (A) Negative control (1% DMSO), (B) control peptide (50 μM), and (C) **1** (50 μM). Bar heights and error bars represent the mean value ± standard error of four replicates.

Cell Culture Experiments. After demonstrating the efficacy of **1** in inhibiting the physical interaction between p53 and mdm2 in vitro, the next step was to see if this compound would induce an increase in p53's transcriptional activator activity in tumor cells overexpressing the mdm2 oncogene. SJSA-1 osteosarcoma cells stably transfected with the firefly luciferase gene under a p53-dependent promoter sequence were utilized. Compound **1** was again compared with the peptide inhibitor control. Exposure to 100 μM of the p53-derived peptide for 24 h results in a minimal increase in luciferase activity (<10%, Figure 4A). The minor enhancement of the peptide may be due to its size; it is a 12-mer peptide with a molecular mass over 1500 Da and may not enter the cells readily. In contrast, **1** leads to double the increase in p53-dependent transcriptional activity seen with the control peptide at the same concentration (Figures 4A and 4B). A 20% increase in transcriptional activity may seem small but is impressive considering prior work by other groups. A bacterial thioredoxin fusion protein, incorporating the sequence of IP3, a synthetic peptide with low-nanomolar IC_{50} of the p53−mdm2 interaction in vitro, was encoded in a plasmid microinjected into various cancer cell lines.[28] While the plasmid could induce p53 accumulation, transactivation, and cell-cycle arrest in cancer cell lines with a low level of mdm2 expression, it was quite ineffectual against SJSA-1. Though less potent in vitro, **1** appears to demonstrate superior pharmacokinetics in

a whole-cell environment. There is no biological activity gained by increasing concentration from 1 μM to 50 μM, and the signal actually decreases at 100 μM. This trend may be a result of oversaturation of import mechanisms and/or inhibitory effects.

In summary, sulfonamide compound **1** represents a lead compound in the development of potent inhibitors of the oncogenic p53−mdm2 interaction. Through this preliminary step in the drug design cycle, compound size has been reduced from a 12-mer peptide (MW over 1700) to a smaller molecule (MW about 600). This led to only a 3-fold drop in potency, quite acceptable given the number of interacting functional groups that have been pared from the full-length peptide. The compound is still fairly large, containing functional groups (such as one of the two phenyl groups on the pyrazolidindione ring) that could be removed or replaced to increase potency.

Supporting Information Available: Table of database hits screened in bioassays and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Kastan, M. B.; Onyekwere, O.; Sidransky, D.; Vogelstein, B.; Craig, R. W. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.* **1991**, *51*, 6304−11.
(2) Maltzman, W.; Czyzyk, L. UV irradiation stimulates levels of p53 cellular tumor antigen in nontransformed mouse cells. *Mol Cell Biol.* **1984**, *4*, 1689−94.
(3) Graeber, T. G.; Peterson, J. F.; Tsai, M.; Monica, K.; Fornace, A. J., Jr.; Giaccia, A. J. Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol.* **1994**, *14*, 6264−77.
(4) Linke S. P.; Clarkin, K. C.; Di Leonardo, A.; Tsou, A.; Wahl, G. M. A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. *Genes Dev.* **1996**, *10*, 934−47.
(5) Nigro J. M.; Aldape K. D.; Hess, S. M.; Tlsty, T. D. Cellular adhesion regulates p53 protein levels in primary human keratinocytes. *Cancer Res.* **1997**, *57*, 3635−9.
(6) Momand, J.; Jung, D.; Wilczynski, S.; Niland, J. The MDM2 gene amplification database. *Nucleic Acids Res.* **1998**, *26*, 3453−9.
(7) Reifenberger, G.; Liu, L.; Ichimura, K.; Schmidt, E. E.; Collins, V. P. Amplification and overexpression of the MDM2 gene in a subset of human malignant gliomas without p53 mutations. *Cancer Res.* **1993**, *53*, 2736−9.
(8) Biernat, W.; Kleihues, P.; Yonekawa, Y.; Ohgaki, H. Amplification and overexpression of MDM in primary (de novo) glioblastomas. *J. Neuropathol. Exp. Neurol.* **1997**, *56*, 180−5.
(9) Oliner, J. D.; Kinzler, K. W.; Meltzer, P.S.; George, D.L.; Vogelstein, B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **1992**, *358*, 80−83.
(10) Hung, J.; Anderson, R. p53: functions, mutations and sarcomas. *Acta Orthop. Scand.* **1997**, *273 Suppl.*, 68−73.
(11) Lonardo, F.; Ueda, T.; Huvos, A. G.; Healey, J.; Ladanyi, M. p53 and MDM2 alterations in osteosarcomas: correlation with clinicopathologic features and proliferative rate. *Cancer* **1997**, *79*, 1541−7.
(12) Takami, K.; Inui, H.; Nagayama, K.; Watatani, M.; Yasutomi, M.; Kurahasi, H.; Mori, T.; Takai, S. I.; Nishisho, I. Low grade amplification of MDM2 gene in a subset of human breast cancers without p53 alterations. *Breast Cancer* **1994**, *1*, 95−102.
(13) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N.P. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **1996**, *274*, 948−53.
(14) Lin, J.; Chen, J.; Elenbaas, B.; Levine, A. J. Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes Dev.* **1994**, *8*, 1235−46.
(15) Uesugi, M.; Verdine, G. L. The alpha-helical FXXPhiPhi motif in p53: TAF interaction and discrimination by MDM2. *Proc. Natl. Acad. Sci.* **1999**, *96*, 14801−6.

(16) Oliner, J. D.; Pietenpol, J. A.; Thiagalingam, S.; Gyuris, J.; Kinzler, K. W.; Vogelstein, B. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* **1993**, *362*, 857–60.

(17) Picksley, S. M.; Vojtesek, B.; Sparks, A.; Lane, D. P. Immunochemical analysis of the interaction of p53 with MDM2: fine mapping of the MDM2 binding site on p53 using synthetic peptides. *Oncogene* **1994**, *9*, 2523–9.

(18) Böttger, V.; Böttger, A.; Howard, S. F.; Picksley, S. M.; Chène, P.; Garcia-Echeverria, C.; Hochkeppel, H.-K.; Lane, D. P. Identification of novel mdm2 binding peptides by phage display. *Oncogene* **1996**, *13*, 2141–7.

(19) Kay, B. K.; Kurakin, A. V.; Hyde-DeRuyscher, R. From peptides to drugs via phage display. *Drug Discovery Today* **1998**, *3*, 370–8.

(20) Bottger, A.; Bottger, V.; Garcia-Echeverria, C.; Chene, P.; Hochkeppel, H. K.; Sampson, W.; Ang, K.; Howard, S. F.; Picksley, S. M.; Lane, D. P. Molecular characterization of the hdm2-p53 interaction. *J. Mol. Biol.* **1997**, *269*, 744–56.

(21) García-Echeverría, C.; Chène, P.; Blommers, M. J. J.; Furet, P. Discovery of potent antagonists of the interaction between human double minute 2 and tumor suppressor p53. *J. Med. Chem.* **2000**, *43*, 3205–8.

(22) Hansen, S.; Kaluza, B.; Menta, E.; Schumacher, R. European Patent EP0947494A1, **2000**.

(23) Stoll, R.; Renner, C.; Hansen, H.; Palme, S.; Klein, C.; Belling, A.; Zeslawski, W.; Kamionka, M.; Rehm, T.; Mühlhahn, P.; Schumacher, R.; Hesse, F.; Kaluza, B.; Voelter, W.; Engh, R. A.; Holak, T. A. Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53. *Biochemistry* **2001**, *40*, 336–44.

(24) Luke, R. W. A.; Jewsbury, P. J.; Cotton, R. International Patent WO0015657, 2000.

(25) Kellogg, G. E.; Joshi, G. S.; Abraham, D. J. New tools for modeling and understanding hydrophobicity and hydrophobic interactions. *Med. Chem. Res.* **1992**, *1*, 444–53.

(26) Galatin, P. S.; Abraham, D. J. QSAR: Hydropathic analysis of inhibitors of the p53-mdm2 interaction. *Proteins* **2001**, *45*, 169–75.

(27) Wrzeciono, U.; Zapak-Wojsznis, E. Sulfonamide. 8. Mitteilung: Sulfonamidderivate des 1,2-Diphenyl-3,5-dioxopyrazolidins. *Pharmazie* **1975**, *582*, 92–4.

(28) Böttger, A.; Böttger, V.; Sparks, A.; Liu, W.-L.; Howard, S. F.; Lane, D. P. Design of a synthetic mdm2-binding mini protein that activates the p53 response in vivo. *Curr. Biol.* **1997**, *7*, 860–9.