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Structure-Based Design of Potent Non-Peptide MDM2 Inhibitors

Ke Ding,† Yipin Lu,† Zaneta Nikolovska-Coleska,† Su Qiu,† Yousong Ding,† Wei Gao,† Jeanne Stuckey,‡ Krzysztof Krajewski,§ Peter P. Roller,§ York Tomita,|| Damon A. Parrish,⊥ Jeffrey R. Deschamps,⊥ and Shaomeng Wang*,†

Departments of Internal Medicine and Medicinal Chemistry and Comprehensive Cancer Center, and Life Sciences Institute, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109, Laboratory of Medicinal Chemistry, National Cancer Institute-Frederick, National Institutes of Health, Frederick, Maryland 21702, Lombardi Cancer Center, Georgetown University Medical Center, Washington, D.C. 20007, The Laboratory for the Structure of Matter, Naval Research Laboratory, 4555 Overlook Avenue, Washington, D.C. 20375

Received February 23, 2005; E-mail: shaomeng@umich.edu

The p53 tumor suppressor plays a central role in controlling cell cycle progression and apoptosis[1,2] and is an attractive cancer therapeutic target because its tumor suppressor activity can be stimulated to eradicate tumor cells.[1−3] A new approach to stimulation of the activity of p53 is through blocking its interaction with the MDM2 oncoprotein, an endogenous cellular inhibitor of p53, using non-peptide small-molecule MDM2 inhibitors.[3,4] The design of non-peptide small-molecule MDM2 inhibitors to block the p53−MDM2 interaction is being intensively pursued as a new strategy for anti-cancer drug design.[3,4]

Although potent, peptide-based small-molecule MDM2 inhibitors have been designed to block the p53−MDM2 interaction,[5] very few non-peptide, small-molecule MDM2 inhibitors have been reported to date.[3] Most of them have weak or modest binding affinities,[3] highlighting the challenge in designing potent, non-peptide, small-molecule MDM2 inhibitors. Recently, synthetic *cis*-imidazoline analogues (termed Nutlins) were reported as a class of potent, non-peptide, small-molecule MDM2 inhibitors.[4] In this communication, we wish to report a successful structure-based design of a new class of non-peptide MDM2 inhibitors based upon the spiro-oxindole core structure.

The structural basis of the p53−MDM2 interaction has been established by X-ray crystallography.[6] The crystal structure shows that the interaction between p53 and MDM2 is primarily mediated by three hydrophobic residues (Phe19, Trp23, and Leu26) of p53 and a small but deep hydrophobic cleft in MDM2. This hydrophobic cleft is an ideal site for designing small-molecule MDM2 inhibitors that can block the p53−MDM2 interaction and is the target site in our MDM2 inhibitor design.

Since the indole ring of Trp23 residue of p53 is buried deeply inside a hydrophobic cavity in MDM2 and its NH group forms a hydrogen bond with the backbone carbonyl in MDM2, Trp23 appears to be most critical for binding of p53 to MDM2. We have therefore searched for chemical moieties that can mimic the interaction of Trp23 with MDM2. In addition to the indole ring itself, we have found that oxindole can perfectly mimic the side chain of Trp23 for interaction with MDM2 (Figure 1).

Since many anticancer drugs are natural products or derivatives of natural products, we used substructure search techniques to identify natural products that contain an oxindole ring. Among the natural products we have identified, there were a number of natural alkaloids such as *spirotryprostatin A* and *Alstonisine* which contain a spiro-oxindole core structure (Figure 1). Our modeling studies showed that although these compounds fit poorly into the MDM2 cleft due to steric hindrance, the spiro(oxindole-3,3′-pyrrolidine) core structure may be used as the starting point for the design of a new class of MDM2 inhibitors (Figure 1). The oxindole can closely mimic the Trp23 side chain in p53 in both hydrogen-bonding formation and hydrophobic interactions with MDM2, and the spiropyrrolidine ring provides a rigid scaffold from which two hydrophobic groups can be projected to mimic the side chain of Phe19 and Leu26. We have designed candidate compounds using different R_1, R_2, and R_3 groups with different configurations and docked them into the MDM2 binding cleft using the GOLD program.[7] Our docking studies showed that compound **1a** in Figure 1 closely mimics p53 in its interaction with MDM2 (Supporting Information) and predicted that **1a** should have a good affinity to MDM2. The 6-chloro substituent on the oxindole ring in **1a** occupies a smaller hydrophobic cavity in MDM2 and was shown to improve the binding affinity of peptide-based MDM2 inhibitors.[5]

The designed compound **1a** was synthesized using an asymmetric 1,3-dipolar reaction[8] as the key step (Scheme 1). The absolute

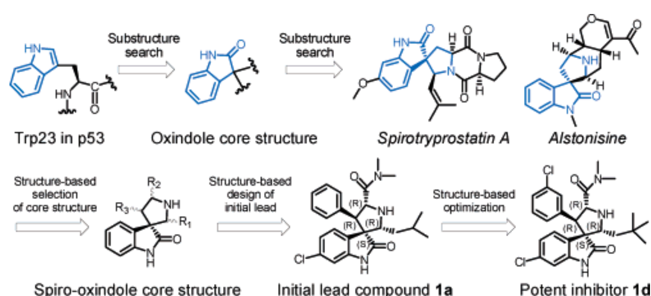

Figure 1. Structure-based strategy to design a new class of MDM2 inhibitors.

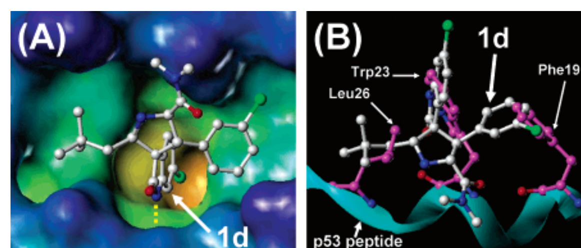

Figure 2. Predicted binding model using computational docking for initial lead compound **1a** and for an optimized compound **1d**. For **1a** and **1d**, carbon atoms are colored in light magenta, nitrogen atoms in blue, chloride atoms in green, and oxygen atoms in red. For MDM2 binding site, carbon atoms are colored in gray, nitrogen atoms in blue, oxygen atoms in red, and sulfur atoms in yellow. Hydrogen bonds are depicted with a dashed yellow line.

† Departments of Internal Medicine and Medicinal Chemistry and Comprehensive Cancer Center, University of Michigan.
‡ Life Sciences Institute, University of Michigan.
§ National Institutes of Health.
|| Georgetown University Medical Center.
⊥ Naval Research Laboratory.

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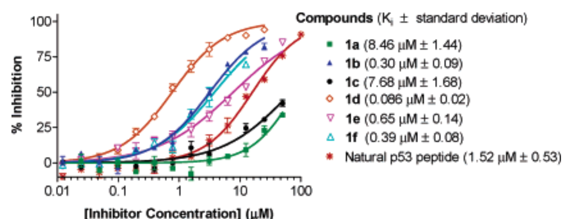
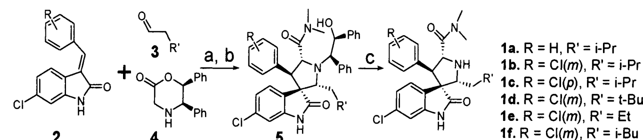


Figure 3. Competitive binding curves and K_i values of inhibitors to MDM2 as determined using a FP-based binding assay.

Scheme 1. Synthesis of Compound **1a** and Other Designed MDM2 Inhibitors[a]



[a] Reagents and conditions: (a) 4 Å molecular sieves, toluene, 70 °C; (b) dimethylamine/THF, room temperature; (c) $Pd(OAc)_4$, CH_2Cl_2−MeOH (1:1), 0 °C.

stereochemistry of **1a** and other designed analogues was determined by X-ray crystallographic analysis of one of the key intermediates **5** (Supporting Information).

To determine the ability of **1a** to disrupt the interaction between MDM2 and p53, we have established a fluorescence polarization-based (FP-based) binding assay using a recombinant human MDM2 protein and a p53-based peptide[5] labeled with a fluorescent tag (Supporting Information). This fluorescently labeled p53-based peptide has a K_d value of 1 nM to MDM2 (Supporting Information), consistent with its previously reported high-affinity for MDM2.[5] A natural p53 peptide (residues 16−27) was determined to have a K_i value of 1.52 μM (Figure 3) in this binding assay. Compound **1a** has a K_i value of 8.46 μM (Figure 3) in our FP-based assay and thus represents a novel lead compound.

Analysis of the predicted binding model of **1a** to MDM2 (Supporting Information) suggests that **1a** can be further optimized for its interaction with MDM2. For example, its phenyl ring binds to the hydrophobic binding pocket occupied by the side chain of Phe19, but there is additional room available. Similarly, its isobutyl group fills in the hydrophobic binding pocket occupied by Leu26, but a slightly larger hydrophobic group could be accommodated. We have therefore designed new analogues of **1a** to optimize further the interactions at these two hydrophobic binding sites.

Modeling studies predicted that introduction of a chlorine atom at the *meta*-position to the phenyl ring in **1a** can occupy the additional room available at this binding site and effectively improve the hydrophobic interaction, while a chlorine atom at either the *para*- or the *ortho*-position of the phenyl ring in **1a** is less optimal (Supporting Information). Compound **1b** with an *m*-Cl substituent was synthesized (Scheme 1) and found to have a K_i value of 300 nM, 28 times more potent than **1a**. To further confirm our prediction, **1c** with a *p*-Cl substituent was synthesized and found to be 26 times less potent than **1b** with a K_i value of 7.68 μM.

As indicated above, the isobutyl group in **1a** is less than optimal, and we further optimized the hydrophobic interaction at this site using **1b** as the template. Modeling studies predicted that a 2,2-dimethylpropyl group should enhance the hydrophobic interaction (Figure 2 and Supporting Information). The resulting compound **1d** containing such a 2,2-dimethylpropyl group has a K_i value of 86 nM in our FP-based assay and is thus 98 times more potent than the initial lead compound **1a**.

To further confirm the importance of the hydrophobic interaction at this site, we have synthesized **1e** and **1f** with, respectively, a hydrophobic group smaller or larger than that in **1d**. Modeling studies predicted that both **1e** and **1f** should be less potent than **1d**, and in fact, FP-based binding experiments showed that **1e** and **1f** with K_i values of 0.65 and 0.39 μM, respectively, are substantially less potent than **1d**.

One major advantage of non-peptide MDM2 inhibitors over peptide-based inhibitors is their superior cell permeability.[3,4] Based upon their mode of action,[1,3,4] it is predicted that a potent, non-peptide small-molecule MDM2 inhibitor will be effective in inhibition of cell growth in cancer cells with wild-type p53 and will have a weaker activity in cancer cells with either mutated or deleted p53.[4] We evaluated our designed MDM2 inhibitors in p53 wild-type LNCaP human prostate cancer cells[9] for their ability to inhibit cell growth and found that, as predicted, the potent MDM2 inhibitor **1d** is a highly effective inhibitor of cell growth, with an IC_{50} value of 0.83 μM. Compounds **1a**, **1b**, **1c**, **1e**, and **1f** inhibit cell growth with IC_{50} values of 9.7, 2.1, 6.7, 2.7, and 1.9 μM, respectively. It is significant that their activities in inhibition of cell growth in LNCaP cells correlate almost perfectly with their binding affinities to MDM2.

The cellular selectivity of these compounds was evaluated in human prostate cancer PC-3 cells with a deleted p53.[9] Consistent with our predictions, these MDM2 inhibitors are much less potent in PC-3 cells than in LnCaP cells (Supporting Information). For example, **1d** has an IC_{50} value of 22.5 μM in PC-3 cells, 27 times less than that in LNCaP cells.

One potential concern in the development of MDM2 inhibitors as new anti-cancer drugs is that such inhibitors may be equally toxic to normal cells with wild-type p53. We thus evaluated **1d** in normal human prostate epithelial cells[9] with wild-type p53 and determined that **1d** has an IC_{50} value of 10.5 μM in inhibition of cell growth, 13 times less toxic than to LNCaP cancer cells. This shows that our designed MDM2 inhibitors have good selectivity between cancer and normal cells with wild-type p53.

In summary, we have described a successful structure-based design of a novel class of potent, non-peptide small-molecule MDM2 inhibitors to target the p53-MDM2 interaction. Our studies provide a convincing example that a structure-based strategy can be employed to design highly potent, non-peptide, small-molecule inhibitors to target protein−protein interaction, which remains a very challenging area in chemical biology and drug design. Further optimization of this class of promising MDM2 inhibitors may ultimately lead to the development of an entirely new type of anticancer drugs.

Supporting Information Available: An Experimental Section including information on the synthesis and chemical data for **1a**−**1f**, molecular modeling methods and results for **1a**−**1f**, experimental procedure for the fluorescence polarization-based binding assay, and details on the cellular growth inhibition assay and results. This material is available free of charge via the Internet at http://pubs.acs.org.

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