Pivotal Role of an Aliphatic Side Chain in the Development of an HDM2 Inhibitor
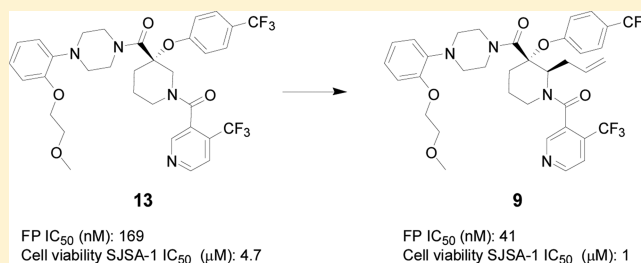
Yao Ma,*[†] Brian R. Lahue,[†] Craig R. Gibeau,[†] Gerald W. Shipps, Jr.,[†] Stéphane L. Bogen,[‡] Yaolin Wang,[‡] Zhuyan Guo,[‡] and Timothy J. Guzi[†]

[†]Discovery and Preclinical Sciences, Merck Research Laboratories, 33 Avenue Louis Pasteur, Boston, Massachusetts 02115, United States

[‡]Discovery and Preclinical Sciences, Merck Research Laboratories, 2015 Galloping Hill Road, Kenilworth, New Jersey 07033, United States
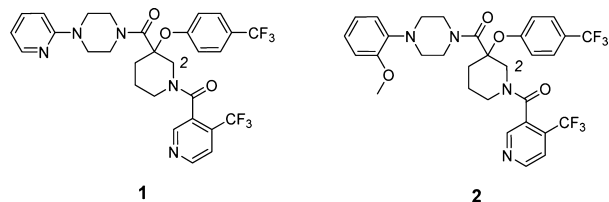
Ⓢ Supporting Information

ABSTRACT: Introduction of an aliphatic side chain to a key position of a novel piperidine-based HDM2 inhibitor scaffold resulted in significant potency gains, enabling further series progression.

13
FP IC$_{50}$ (nM): 169
Cell viability SJSA-1 IC$_{50}$ (μM): 4.7

9
FP IC$_{50}$ (nM): 41
Cell viability SJSA-1 IC$_{50}$ (μM): 1

KEYWORDS: HDM2, MDM2, p53, substituted piperidine, protein−protein interaction, small molecule inhibitor

The tumor suppressor protein p53 plays a central role in maintaining the integrity of the genome in a cell. It regulates the expression of a diverse array of genes responsible for DNA repair, cell cycle and growth arrest, and apoptosis.[1−5] The oncogene HDM2 is a ubiquitin protein ligase that negatively regulates tumor suppressor p53 and lessens its transcriptional activity as well as promoting p53 protein degradation.[6−9] Antagonists of the tumor suppressor p53-HDM2 interaction represent an attractive and novel mechanism in oncological therapeutics. Disrupting this HDM2-p53 protein−protein interaction with a small molecule is hypothesized to increase intracellular levels of p53 leading to decreased proliferation and increased apoptosis of tumor cells, which offers great therapeutic potential for the treatment of cancers.[10−13] Crystallographic studies of MDM2- (the murine homologue of human HDM2) and p53-derived peptides bound together have revealed a deep hydrophobic binding pocket involving residues Phe19, Trp23, and Leu26 of p53.[14] Since the discovery of Roche's Nutlin imidazoline series, which antagonizes this HDM2−p53 interaction,[15] several potent low

**Figure 1.** Novel piperidine-based HDM2 inhibitors.

molecular weight inhibitors have also been described.[16,17] Recently, we have reported the discovery of a novel series of substituted piperidines as small molecule antagonists of HDM2, which selectively inhibit proliferation of wild-type p53 cancer cell lines (Figure 1).[18,19] It has historically been challenging to overcome the large surface contacts of protein−protein interactions with a small molecule inhibitor. Herein we report a breakthrough made in our efforts to further optimize this substituted piperidine series through chemical modifications at the piperidine 2-position.

Chemistry. The synthesis of this series of 2-substituted piperidine-based p53-HDM2 inhibitors was carried out in a fashion analogous to that described previously for 2-unsubstituted compounds **1** and **2** (Figure 1).[18,19] Beginning with N-benzyl-3-piperidone **3**, which was readily converted to the corresponding methyl carbamate **4** in a single step under conditions described by Brubaker,[20] the resulting ketone was subjected to an enamine alkylation with allyl bromide to provide **5** (Scheme 1). The requisite quaternary center was then introduced through a Bargellini reaction[18,21,22] with 4-trifluoromethylphenol to give racemic carboxylic acid **6**, which was converted to corresponding methyl ester for ease in handling. Chiral HPLC conditions were developed to resolve **7** to a single enantiomer (refer to Supporting Information for details). The carbamate protecting group on **7** was removed through the action of trimethylsilyliodide, and the resulting free

Scheme 1. Synthesis of the 2-Substituted Piperidine Core and Key Analogues[a]
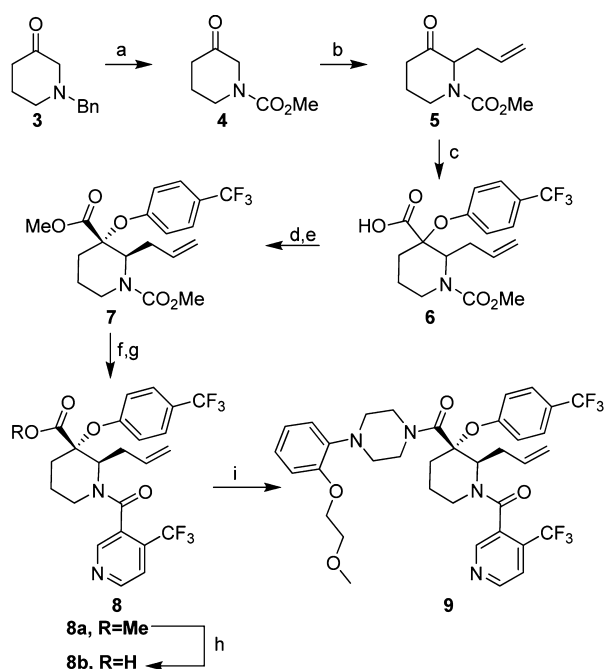
[a]Reagents and conditions: (a) methyl chloroformate, Et$_3$N, CHCl$_3$ 0 °C to r.t., 57%; (b) 1. pyrrolidine, benzene reflux; 2. allyl bromide, MeCN, 70 °C, 67%; (c) 4-trifluoromethylphenol, NaOH, CHCl$_3$, 0–40 °C; (d) TMS-CHN$_2$, MeCN, MeOH, 0 °C to r.t., ~40% overall yield from **5**; (e) chiral HPLC (see Supporting Information for the condition), 27%; (f) iodotrimethylsilane, CH$_2$Cl$_2$, r.t., >95%; (g) 4-trifluoronicotinic acid, HATU, Et$_2$N^{i}Pr, CH$_2$Cl$_2$, rt to 45 °C, >85%; (h) KOH, EtOH, H$_2$O, 65 °C, 88%; (i) HATU, 1-[2-(2-methoxy–ethoxy)-phenyl]-piperazine, Et$_2$N^{i}Pr, CH$_2$Cl$_2$ r.t. to 40 °C, 53%.

Scheme 2. Modification of the Olefin[a]
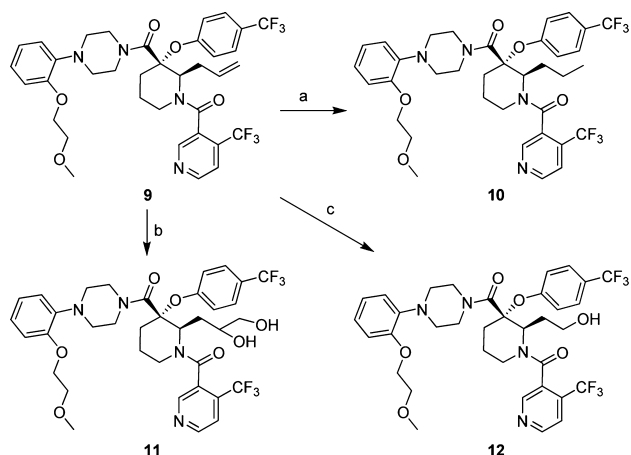
[a]Reagents and conditions: (a) H$_2$, Pd/C, EtOAc, 87%; (b) OsO$_4$, NMO, THF, H$_2$O, r.t., ~18% for combined two isomers; (c) 1. OsO$_4$, NaIO$_4$, 2,6-lutidine, dioxane, H$_2$O, r.t.; 2. NaBH$_4$, MeOH, r.t., 25%.

amine was subjected to standard amidation conditions with 4-trifluoromethylnicotinic acid to give intermediate **8a**. Ester hydrolysis of **8a** provided carboxylic acid **8b**, which underwent amidation with the corresponding piperazine building block to give final compound **9**, the absolute stereochemistry of which was confirmed by analysis of its X-ray cocrystal structure with HDM2.
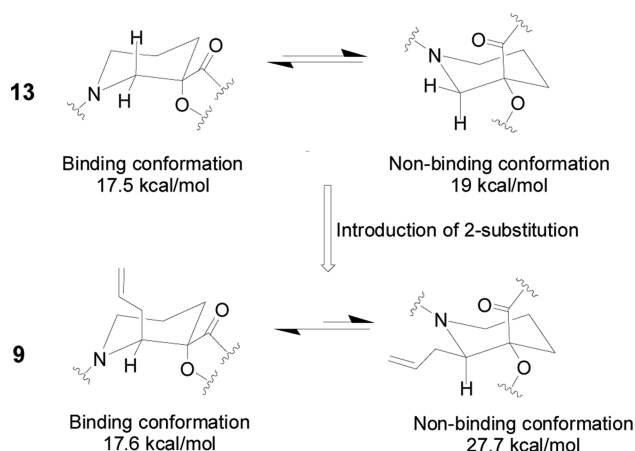
Table 1. Biochemical and Cellular Activities of Compounds 9–14[a]

Compound		FP IC$_{50}$ (nM)	Cell viability SJSA-1 IC$_{50}$ (µM)
13		169	4.7
9		41	1
10		24	1.2
11a (diastereomer 1)		24	0.55
11b (diastereomer 2)		16	0.42
12		24	0.53

[a]All compounds were tested as HCl salts. Fluorescent polarization was measured by reading the plate using the Analyst AD (Molecular Device). IC$_{50}$ was determined as described in Zhang et al.[23]

The allyl substituent in the 2-position of the piperidine ring in compound **9** served as a synthetic handle for further elaboration at this position (Scheme 2). Reduction of the olefin in **9** by hydrogenation led to compound **10**. Polarity was introduced at this position in the form of two hydroxyl groups through dihydroxylation of the double bond in **9** to give diols **11**, which in turn were converted to alcohol **12** through in situ sodium periodate-promoted cleavage followed by reduction of the resulting aldehyde.

Results and Discussion. While it was anticipated that improvements to the in vitro potency and concomitant cell-based activity of C2-unsubstituted compounds such as **13**[18] could be made through modifications of the largely hydro-phobic binding interactions with the HDM2 protein,

Figure 2. Impact of side chain substitution on the piperidine ring conformations.

Table 2. Cytochrome P450 Inhibition of Select HDM2 Inhibitors

| | cytochrome P450 inhibition | | | | | |
| | coincubation (IC$_{50}$, μM) | | | preincubation (IC$_{50}$, μM) | | |
compound	3A4	2D6	2C9	3A4	2D6	2C9
13	4.9	>30	>30	0.3	>30	>30
9	2.1	>30	>30	<0.3	>30	>30
11a	>30	>30	>30	7.5	>30	>30
12	13.3	>30	>30	0.9	>30	>30

optimization of the overall DMPK profile was expected to require the introduction of polar substituents to the inhibitor in such a way as to not interfere with binding to HDM2. The addition of an allyl group as a starting point for synthetic diversification as described in the previous paragraph was targeted to do just this; however, introduction of this simple aliphatic side chain itself provided an unexpected yet significant impact on binding efficiency. Compared to unsubstituted analogue **13**, the addition of allyl (**9**) and *n*-propyl (**10**) groups at C2 of the piperidine core provided a 4- and 7-fold improvement, respectively, in the biochemical fluorescence polarization (FP) peptide displacement assay[23] (Table 1). As a result of this intrinsic potency gain, activity of these compounds in the cellular viability assay with osteosarcoma SJSA-1 cells translated to similar levels of improvement with both **9** and **10** displaying IC$_{50}$ values around 1 μM. As anticipated, the incorporation of more polar functional groups such as diols **11a/b** and the primary alcohol **12** did not negatively impact the binding affinity with biochemical IC$_{50}$ values from 16 to 24 nM.

In the absence of binding activities with the protein, the piperidine core as a six-membered cyclic ring should have several conformations coexisting in solution; however, only one of them was found to fit into HDM2's p53-binding pocket. The energy required for the 2-unsubstituted piperidine **13** to adopt the chair conformation necessary for binding to HDM2 (17.5

kcal/mol) was equivalent to that of the ring-flipped conformation, within the resolution (5 kcal/mol) of the Sybyl software used to perform the calculations (Figure 2). However, the binding conformation of the 2-allyl analogue **9** was calculated to be more than 10 kcal/mol more favorable than the nonbinding conformer (17.6 vs 27.7 kcal/mol), likely due to the unfavorable steric interactions of the two amides with the adjacent axial allyl substituent in the latter. As a result, by introducing the side chain at the piperidine-2 position, exchange between ring conformations has been significantly slowed down with the preferred binding conformation existing predominantly in the absence of HDM2. Locking the orientation of the substituents on the piperidine core in this way improved the binding affinity of 2-substituted compounds such as **9** considerably.

As potent HDM2 inhibitors, this series of compounds exhibited a ~3× improvement in the cell Titer-Glo assay with SJSA-1 osteosarcoma cells compared to well-studied Nutlin-3a (Figure 3).[15,16] As with Nutlin-3a, these compounds were only active against wild-type (wt)-p53 cell lines like SJSA-1 with no activity observed in the mutant-p53 cell lines, such as the endometrial cancer cell line SKUT-1, indicating on-target mechanism-based antiproliferative activity. Thus, a ~30× toxicity window was achieved between the wt-p53 and mutant-p53 cancer cell lines.

As anticipated, DMPK analysis of compound **9** indicated a similar cytochrome P450 (CYP) profile to that of the 2-unsubstituted compound **13** as both lipophilic molecules did not inhibit CYP 2D6 and 2C9 isoforms but exhibited both direct (coincubation IC$_{50}$ = 2.1–4.9 μM) and metabolism/mechanism-based CYP 3A4 inhibition (coincubation IC$_{50}$/preincubation IC$_{50}$ $\approx$ 7–16-fold) (Table 2). The introduction
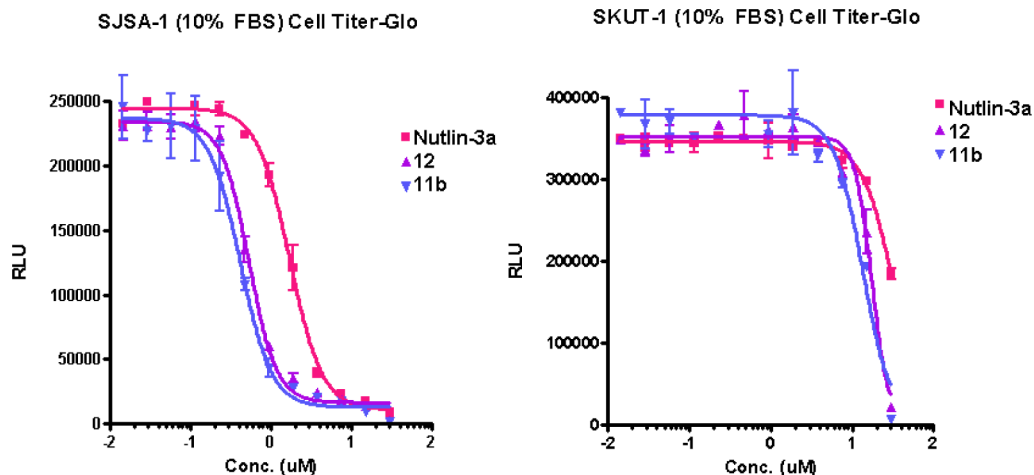
Figure 3. Novel HDM2 inhibitors selectively inhibit wt-p53 cancer cell lines.

of one (**12**) or two (**11a**) polar hydroxyl groups decreased the direct inhibition of CYP 3A4 with IC_{50} values of 13.3 and >30 μM, respectively. However, both compounds displayed CYP 3A4 metabolism/mechanism-based inhibition (coincubation IC_{50}/preincubation $IC_{50} \approx 4-15$-fold), which led to a more thorough investigation that will be the subject of a future publication.

In summary, while continuing to optimize the substituted piperidine as a novel series of HDM2 inhibitors, significant potency enhancement was achieved by introducing an aliphatic side chain at the piperidine 2-position. As a result, corresponding cell-based activities were improved while maintaining a favorable toxicity window between wt-p53 and mutant-p53 cell lines for this new series inhibitor. Introduction of this side chain afforded a great opportunity for further optimization of activity and DMPK profiles toward the discovery of an improved small molecule HDM2 antagonist.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and LC−MS characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(Y.M.) E-mail: yaoma1@yahoo.com.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Levine, A. J. p53, the cellular gatekeeper for growth and division. *Cell* **1997**, *88*, 323−331.

(2) Vousden, K. H.; Lu, X. Live or let die: the cell's response to p53. *Nat. Rev. Cancer* **2002**, *2*, 594−604.

(3) Oren, M. Decision making by p53: life, death and cancer. *Cell Death Differ.* **2003**, *10*, 431−442.

(4) Fridman, J. S.; Lowe, S. W. Control of apoptosis by p53. *Oncogene* **2003**, *22*, 9030−9040.

(5) Vazquez, A.; Bond, E. E.; Levine, A. J.; Bond, G. L. The genetics of the p53 pathway, apoptosis and cancer therapy. *Nat. Rev. Drug Discovery* **2008**, *7*, 979−987.

(6) Chen, J.; Wu, X.; Lin, J.; Levine, A. J. mdm-2 inhibits the G1 arrest and apoptosis functions of the p53 tumor suppressor protein. *Mol. Cell. Biol.* **1996**, *16*, 2445−2452.

(7) Momand, J.; Zambetti, G. P.; Olson, D. C.; George, D.; Levine, A. J. The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation. *Cell* **1992**, *69*, 1237−1245.

(8) Momand, J.; Wu, H. H.; Dasgupta, G. MDM2: master regulator of the p53 tumor suppressor protein. *Gene* **2000**, *242*, 15−29.

(9) Oliner, J. D.; Pietenpol, J. A.; Thiagalingam, S.; Gyuris, J.; Kinzler, K. W.; Vogelstein, B. Oncoprotein MDM2 conceals the activation domain of tumor suppressor p53. *Nature* **1993**, *362*, 857−860.

(10) Momand, J.; Jung, D.; Wilczynski, S.; Niland, J. The MDM2 gene amplification database. *Nucleic Acids Res.* **1998**, *26*, 3453−3459.

(11) Polsky, D.; Bastian, B. C.; Hazan, C.; Melzer, K.; Pack, J.; Houghton, A.; Busam, K.; Cordon-Cardo, C.; Osam, I. HDM2 protein overexpression, but not gene amplification, is related to tumorigenesis of cutaneous melanoma. *Cancer Res.* **2001**, *61*, 7642−7646.

(12) Chene, P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. *Nat. Rev. Cancer* **2003**, *3*, 102−109.

(13) Bottger, A.; Bottger, 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*, 860−869.

(14) 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*, 948−953.

(15) Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; Filipovic, Z.; Kong, N.; Kammlott, U.; Lukacs, C.; Klein, C.; Fotouhi, N.; Liu, E. A. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **2004**, *303*, 844−848.

(16) Hu, C.-Q.; Hu, Y.-Z. Small molecule inhibitors of the p53-MDM2. *Curr. Med. Chem.* **2008**, *15*, 1720−1730.

(17) Patel, S.; Player, M. R. Small-molecule inhibitors of the p53-HDM2 interaction for the treatment of cancer. *Expert Opin. Invest. Drugs* **2008**, *17*, 1865−1882.

(18) Ma, Y.; Lahue, B.; Shipps, G.; Brookes, J.; Wang, Y. Substituted piperidines as HDM2 inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1026−1030.

(19) Ma, Y.; Lahue, B.; Shipps, G.; Wang, Y.; Bogen, S.; Voss, M.; Nair, L.; Tian, Y.; Doll, R.; Guo, Z.; Strickland, C.; Zhang, R.; McCoy, M.; Pan, W.; Siegel, E.; Gibeau, C. Preparation of substituted piperidines that increase p53 activity and the uses thereof. U.S. Pat. Appl. Publ. US2008004287, 2008.

(20) Brubaker, A.; Colley, M. Synthesis and pharmacological evaluation of some 6-substituted 7-methyl-1,4-dioxa-7-azaspiro[4.5]-decanes as potential dopamine agonists. *J. Med. Chem.* **1986**, *29*, 1528.

(21) Cvetovich, R. J.; Chung, J. Y. L.; Kress, M. H.; Amato, J. S.; Matty, L.; Weingarten, M. D.; Tsay, F.-R.; Li, Z.; Zhou, G. An efficient synthesis of a dual PPAR α/γ agonist and the formation of a sterically congested α-aryloxyisobutyric acid via a Bargellini reaction. *J. Org. Chem.* **2005**, *70*, 8560−8563.

(22) Rychnovsky, S. D.; Beauchamp, T.; Vaidyanathan, R.; Kwan, T. Synthesis of chiral nitroxides and an unusual racemization reaction. *J. Org. Chem.* **1998**, *63*, 6363−6374.

(23) Zhang, R.; Mayhood, T.; Lipari, P.; Wang, Y.; Durkin, J.; Syto, R.; Gesell, J.; McNemar, C.; Windsor, W. Fluorescence polarization assay and inhibitor design for MDM2/p53 interaction. *Anal. Biochem.* **2004**, *331*, 138−146.