

Discovery of Novel 3,3-Disubstituted Piperidines as Orally Bioavailable, Potent, and Efficacious HDM2-p53 Inhibitors

Stéphane L. Bogen,^{*,†} Weidong Pan,[†] Craig R. Gibeau,[‡] Brian R. Lahue,[‡] Yao Ma,[‡] Latha G. Nair,[†] Elise Seigel,[†] Gerald W. Shipps, Jr.,[‡] Yuan Tian,[‡] Yaolin Wang,[§] Yinghui Lin,[§] Ming Liu,[§] Suxing Liu,[§] Asra Mirza,[§] Xiaoying Wang,[§] Philip Lipari,[§] Cynthia Seidel-Dugan,[§] Daniel J. Hicklin,[§] W. Robert Bishop,[§] Diane Rindgen,^{||} Amin Nomeir,^{||} Winifred Prosise,[⊥] Paul Reichert,[⊥] Giovanna Scapin,[⊥] Corey Strickland,[⊥] and Ronald J. Doll[†]

[†]Discovery Chemistry, Merck Research Laboratories, Kenilworth, New Jersey 07033, United States

[‡]Discovery Chemistry, Merck Research Laboratories, Boston, Massachusetts 02115, United States

[§]Discovery Biology, Merck Research Laboratories, Kenilworth, New Jersey 07033, United States

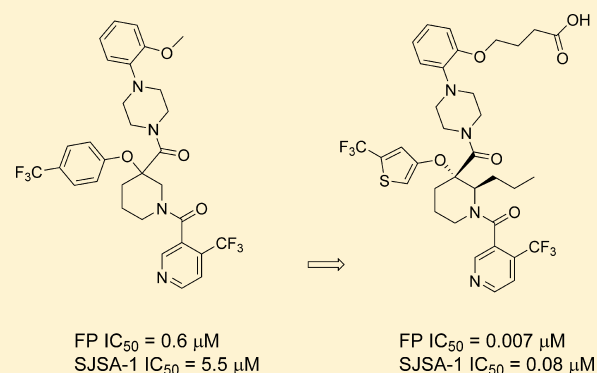
^{||}Pharmacokinetic, Pharmacodynamics and Drug Metabolism, Merck Research Laboratories, Kenilworth, New Jersey 07033, United States

[⊥]Structural Chemistry, Merck Research Laboratories, Kenilworth, New Jersey 07033, United States

S Supporting Information

ABSTRACT: A new subseries of substituted piperidines as p53-HDM2 inhibitors exemplified by **21** has been developed from the initial lead **1**. Research focused on optimization of a crucial HDM2 Trp23–ligand interaction led to the identification of 2-(trifluoromethyl)thiophene as the preferred moiety. Further investigation of the Leu26 pocket resulted in potent, novel substituted piperidine inhibitors of the HDM2-p53 interaction that demonstrated tumor regression in several human cancer xenograft models in mice. The structure of HDM2 in complex with inhibitors **3**, **10**, and **21** is described.

KEYWORDS: HDM2, p53, protein–protein interaction, cancer



Cancer is a disease of uncontrolled cell growth of various tissues and organs in the body. According to the American Cancer Society, about 1.7 million new cancer cases are expected to be diagnosed in 2015, with an estimated half million deaths in the US alone. Even with the use of conventional chemotherapy and newly approved targeted therapies, cancer still represents an unmet medical need.¹

The tumor suppressor p53 plays many critical roles in surveying and responding to various stress and damage signals. It regulates cell growth, migration, apoptosis, angiogenesis, metabolism, development, and stromal matrix cellular environment.^{2,3} The loss of p53 function predisposes cells to a cancerous state.⁴ HDM2 acts as a major negative regulator of p53 activity by repressing p53 transcriptional activity through its binding to p53 and targeting p53 for degradation in the proteasome through its ubiquitin E3 ligase activity. An inhibitor capable of blocking this HDM2-p53 interaction would lead to accumulation of p53 and activation of transcriptional and nontranscriptional activities of p53, leading to cell cycle arrest and apoptosis of tumor cells. In more than 50% of human cancers, p53 is inactivated due to overexpression of HDM2 protein,^{5–7} making the development

of small molecule inhibitors of HDM2-p53 a very attractive approach to cancer therapy.^{8–11}

Recently, multiple teams have reported the discovery of small molecules interfering with the HDM2-p53 protein–protein interaction (PPI).^{12–19} We previously disclosed the discovery of gem-disubstituted piperidines^{20–22} **1** with HDM2 binding activity. Detailed SAR and computational studies highlighted the pivotal role of an aliphatic side chain on the piperidine ring conformation leading to potent small molecule inhibitor **2** of the HDM2-p53 PPI (Figure 1).²³ Moreover, we demonstrated that this side chain at position 2 of the piperidine could tolerate polar functional groups providing the opportunity to improve physical properties. Similarly, transformation of the methoxy-ethylene side chain to hydroxyl-ethylene led to compound **3**, which showed good permeability and an excellent pharmacokinetic profile in mouse.^{24,25} More importantly, the structure of HDM2

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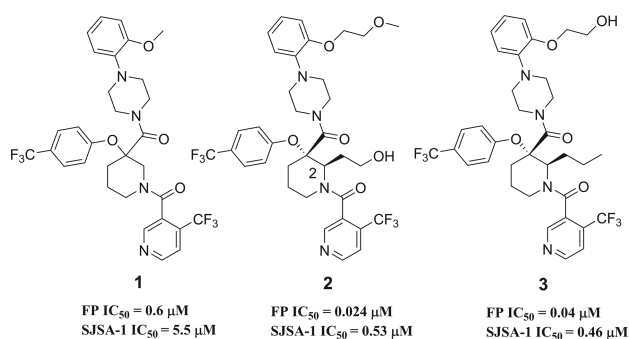


Figure 1. Optimization of lead 1 to 3 (see Table 1 for definitions).

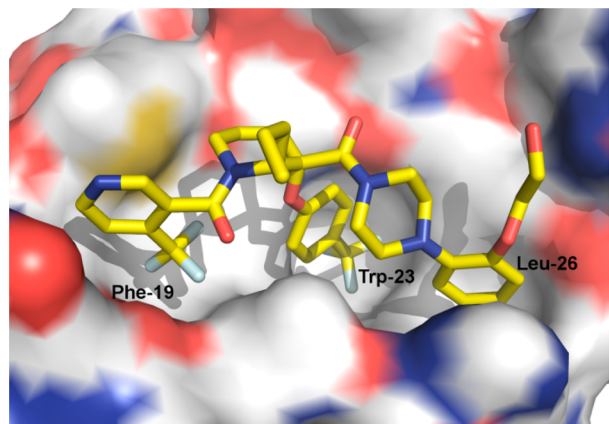
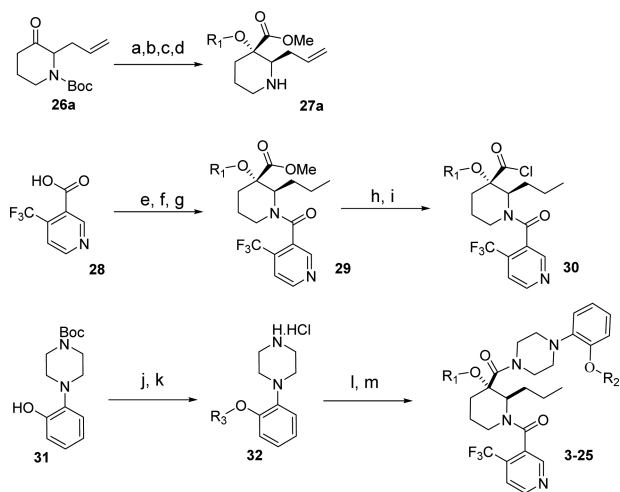


Figure 2. Structure of HDM2 in complex with 3. The inhibitor is shown as stick and the protein as surface, colored according to atom type. The Phe-19, Trp-23, and Leu-26 subsites are indicated.

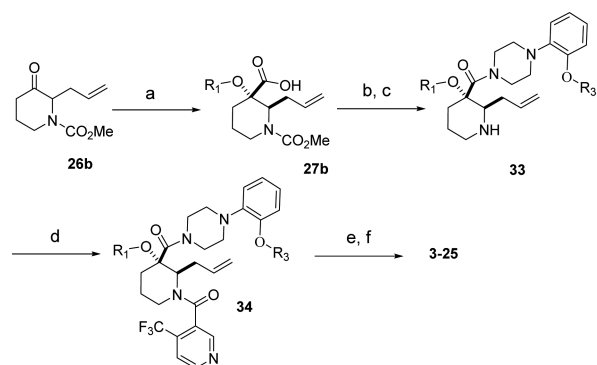
Scheme 1. Synthesis of 3–25 (General Method Method 1)^a



^aReagents and conditions: (a) R₁-OH, NaOH, CHCl₃, 0–40 °C, 25–50%; (b) ACN/MeOH (1:1), 2.0 M TMSCHN, 20 °C, 0.5 h, 40–50%; (c) 50% TFA/DCM, 0 °C to rt, 3 h, (100% yield); (d) Chiralcel AD column, 90:10 heptanes/iPrOH; (e) benzene, thionyl chloride, reflux/cat DMF, 100%; (f) 27, DCM/DIPEA/0 °C, 100%; (g) H₂, Pd/C, EtOH; (h) KOH, MeOH reflux, 100%; (i) (COCl)₂, DMF, DCM 0 °C; (j) Cs₂CO₃, DMF, R₃-Br (50–90% yield); (k) 4.0 N HCl dioxane 100%; (l) 30, THF/H₂O, 0 °C to rt, 0.5 h (90–100% yield); (m) final deprotection step.

in complex with 3 was solved using X-ray diffraction data (Figure 2).

Scheme 2. Synthesis of 3–25 (General Method 2)^a



^aReagents and conditions: (a) R₁-OH, NaOH, CHCl₃, 0–40 °C, 25–50%; (b) 32, DMF/DCM (1:1), HATU, DIPEA, 45 °C, 12 h, 40–60%; (c) DCM, TMS-I, 0 °C, 5 h, (100% crude yield); (d) DMF/DCM (1:1), HATU, DIPEA, 45 °C, 12 h, 40–80%; (e) H₂, Pd/C, EtOH; (f) final deprotection step and Chiralcel OD column purification.

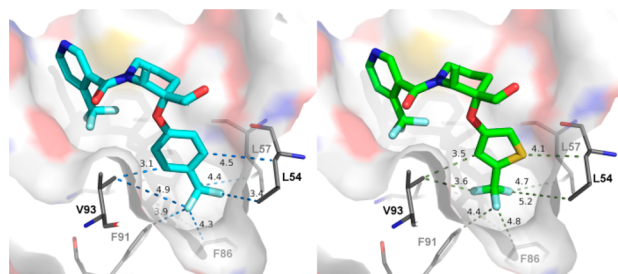
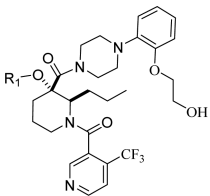
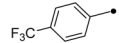
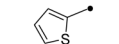
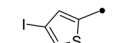
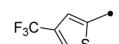
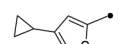
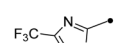
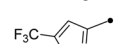
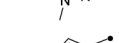
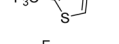
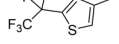
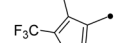
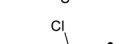


Figure 3. Comparison of 3 and 10 binding on the Trp-23 subsite.

The compound binds along the region of the HDM2 surface that has been shown to interact with p53, thus effectively disrupting this PPI. The affinity of compound 3 is mostly due to the hydrophobic interactions between the compound and three surface pockets that have been shown to bind p53, residues Phe19, Trp23, and Leu26.^{26–28} On the basis of the crystal structure, the trifluoromethylphenyl group binds in the Trp23 pocket. Although the installation of novel moieties that would bind in the deep Trp 23 pocket would have to take place early in the synthesis, it was envisioned that research optimization focused on this interaction could lead to enhanced binding potency. Thus, bioisosteric replacements of the phenyl group were studied.²⁹ Using the general chemistry outlined in Schemes 1 and 2, a series of analogues of lead 3 were prepared to examine the effect of substitution on binding interactions in the Trp 23 pocket. As described earlier,^{20,23,24} the team capitalized on the Bargellini reaction of protected 3-piperidinone 26a and 26b with various alcohol moieties to assemble in one-pot the gem-disubstituted piperidine core of type 27a and 27b.²⁰ Efficient acid chloride coupling procedures were developed to install 4-(trifluoromethyl)-nicotinic acid 28 as well as 1-(2-alkoxyphenyl)piperazine hydrochloride of type 32 into final targets. Another synthetic approach relied on standard HATU coupling procedures, installing first 1-(2-alkoxyphenyl)-piperazine moieties 32 then 4-(trifluoromethyl)-nicotinic acid 28 to deliver compounds of type 34.

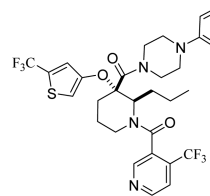
Of the initial analogues, 2-alkoxy-4-(trifluoromethyl)-thiophene bioisosteric replacement of the trifluoromethyl phenyl group in 3 was especially promising. Compound 27 with R₁ = 4-iodo-2-alkoxythiophene was envisioned as a key intermediate to

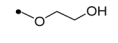
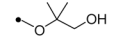
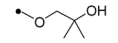
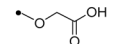
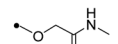

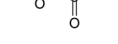
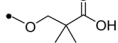
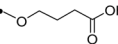
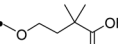
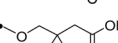
Table 1. Trp23 Substitution Structure–Activity Relationships^a


#	R1	FP (μM) ^a	IC ₅₀ (μM)	SJSA-1 IC ₅₀ (μM)
3		0.04	0.46	
4		2.9 ^b	-	
5		0.09 ^{b,c}	-	
6		0.17 ^b	-	
7		3.5 ^{c,d}	-	
8		1.1 ^b	-	
9		6.3 ^b	-	
10		0.015	0.18	
11		0.025	1.1	
12		0.058	-	
13		0.13	-	
14		0.47	-	

^aBiochemical p53-HDM2 binding fluorescent polarization (FP) assay and antiproliferative osteosarcoma SJSA-1 assay results for compounds 3–14. Fluorescence polarization (FP) peptide displacement assay value was determined as described in Zhang et al.¹⁵ For comparison Nutlin-3a fluorescence polarization (FP) peptide displacement IC₅₀ assay value obtained in house was 0.07 μM . In our antiproliferative assay, Nutlin-3a had an IC₅₀ value of 1.9 μM using osteosarcoma SJSA-1 cells. ^bRacemic mixture. ^cAllyl group at position 2 of piperidine. ^dPhenoxybutanoic acid side chain.

access targets 6 as well as 4, 5, and 7 (Table 1), via a sequence relying on regioselective transmetalation of 2,4-dibromothiophene followed by selective halide displacements. As seen in the poor activity with compound 7, substitution with both iodine and trifluoromethyl at position 4 (compounds 5 and 6 respectively) turned out to be optimal when compared to 4. Modification to a thiazole analogue in 8 resulted in 6-fold loss in potency where pyrazole analogue 9 was the least active of the series. Then the 4-alkoxy-2-(trifluoromethyl)thiophene isomer was prepared. Compound 10 demonstrated significant improvement in both biochemical (FP) and cellular (SJSA-1) inhibition assay not only versus 2-thiophene isomer 6 but original lead 3 as well. The structure of HDM2 in complex with 10 was solved and revealed (Figure 3) that the trifluoromethyl thiophene provides a better complementarity to the pocket than the trifluoromethyl phenyl

Table 2. Leu 26 Substitution Structure–Activity Relationships^a


#	R2	FP (μM)	IC ₅₀ (μM)	SJSA-1 IC ₅₀ (μM)	HCT116 IC ₅₀ (μM)
10		0.015	0.18	0.39	
15		0.025	1.1	-	
16		0.023	0.6	-	
17		0.002	1.1	-	
18		0.004	0.11	0.47	
19		0.011	0.08	-	
20		0.004	0.26	-	
21		0.007	0.09	0.18	
22		0.010	0.28	-	
23		0.033	-	-	
24		0.007	0.17	-	

^aBiochemical p53-HDM2 binding fluorescent polarization (FP) assay, antiproliferative osteosarcoma SJSA-1, and colorectal HCT116 assay results for compounds 10, 15–24.

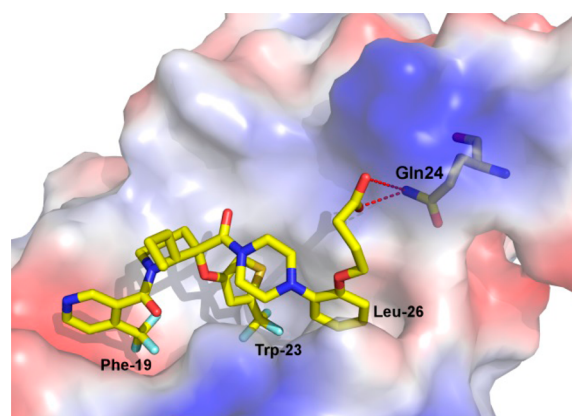


Figure 4. Structure of HDM2 in complex with 21. The inhibitor is shown as stick and the protein as surface, colored according to atom type. The Phe-19, Trp-23, and Leu-26 subsites are indicated as well as Gln 24 residue.

moiety. The trifluoromethyl thiophene is equidistant from all the side chains that line the binding pocket, while the trifluoromethyl phenyl is asymmetrically bound and in close contact with the side

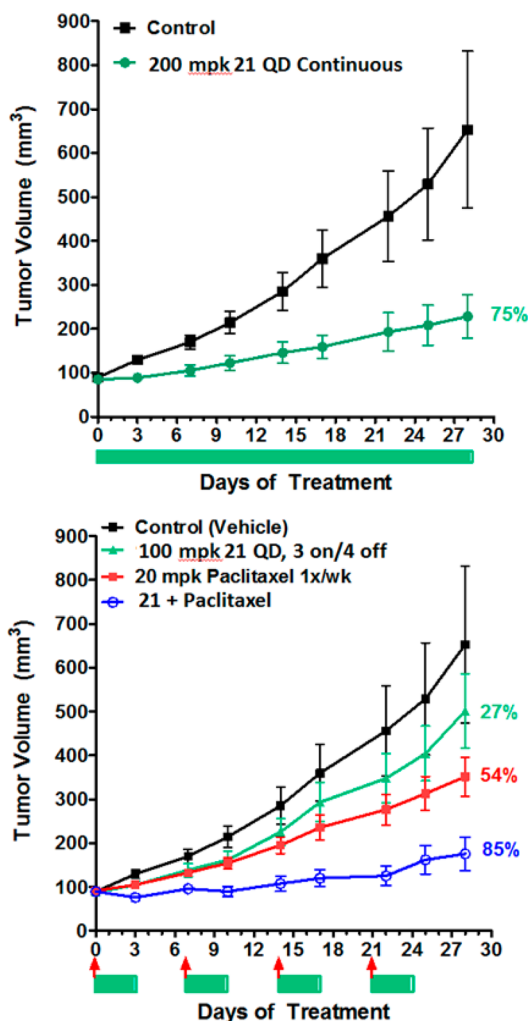


Figure 7. Efficacy of **21** as single agent and in combination with Cytotoxic in A549 non-small cell lung cancer (NSCLC) xenograft model. Oral *in vivo* efficacy profile of **21** on nude mice implanted with A549 NSCLC. *N* = 10 per group. Bars and arrows below the graph indicates treatment days of **21** (bar) and Paclitaxel (arrow).

CYP's (3A4, 2D6, 2C8, 2C9, 1A2; IC_{50} s > 50 μ M). While **21** was not a substrate for cytochrome P450 3A4, it was found to have a potential for time-dependent inhibition that could require close monitoring when part of a combination regimen with other cancer therapies. Plasma protein binding (% free fraction) was similar across human, monkey, dog, and mouse (4%, 5%, 2%, and 3%, respectively) with excellent permeability (Caco-2 = 180 nm/s). Human half-life projection of **21** based on observed preclinical PK by applying allometric scaling is estimated to be 6 h, consistent with an oral BID therapeutic. Compound **21** showed no cardiovascular liabilities with 20% hERG (VC) inhibition at 6 μ M, translating in 80-fold window for a human dose as high as 1000 mg BID.

Since compound **21** demonstrated the best cellular anti-proliferative potency and selectivity, activity for additional cancer cell lines was evaluated and confirmed cell growth inhibition in a broad panel of human tumor cell lines expressing wild-type p53 (Figure 5). Thus, several cell lines were implanted in nude mice for study in cancer xenograft models. Efficacy of **21** both as a single agent (in SJSA-1 osteosarcoma) as well as in combination with cytotoxics in A549 nonsmall cell lung (NSCLC) and in A2780 ovarian cancer xenograft model were evaluated. Single

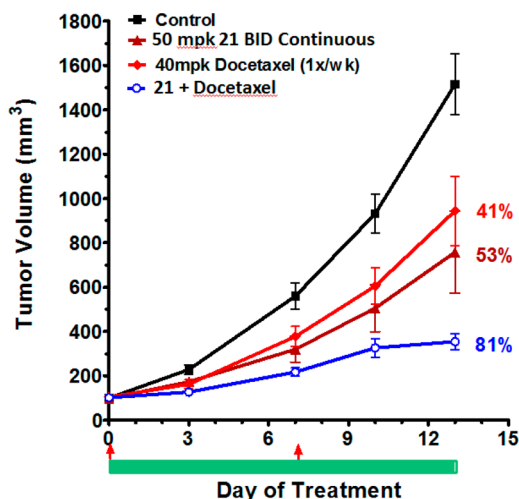


Figure 8. Efficacy of **21** as single agent and in combination with Cytotoxic in A2780 ovarian cancer xenograft model. Oral *in vivo* efficacy profile of **21** on nude mice implanted in A2780 ovarian cancer xenograft model. *N* = 10 per group. Bar and arrows below the graph indicates treatment days of **21** (bar) or Docetaxel (arrow).

agent efficacy of **21** in an SJSA-1 osteosarcoma model following oral administration of **21** at either intermittent schedule (oral daily dosing with cycle of 3 days on, 4 days off) or daily continuous at 100 and 200 mpk resulted in tumor regression in the SJSA-1 osteosarcoma xenograft model (Figure 6) without any observable toxicity (no body weight loss was observed in any treatment group compare to vehicle control group). Single agent efficacy of **21** in an A549 (NSCLC) model following oral administration of **21** daily at 200 mg/kg resulted in 75% tumor growth inhibition (Figure 7). Intermittent dosing of **21** (daily for 3 days followed by 4 days off schedule) in combination with cytotoxic paclitaxel (20 mg/kg, intraperitoneal, weekly) in the A549 NSCLC model resulted in increased antitumor response compared to either single agent used alone. To further exemplify activity of **21**, wild-type p53 mice bearing A2780 tumor xenograft were treated with 50 mg/kg **21** (oral, twice daily) or 40 mg/kg docetaxel (ip, weekly, 2X) or the combination of the two agents (Figure 8). Continuous daily dosing of **21** at 50 mpk BID resulted in 41% tumor growth inhibition while combination with cytotoxic Docetaxel resulted in increased antitumor response (81% tumor growth inhibition) in the A2780 ovarian cancer xenograft model compared to either single agent used alone.

In summary, the structure of HDM2 in complex with a novel piperidine-based inhibitor was described. Investigation of bioisosteric replacements of moieties that binds into the Trp23 pocket led to a new subseries of HDM2-p53 inhibitors with improved potency as exemplified by **10**. Exploration of various functional groups binding in the Leu 26 pocket led to the discovery of **21** as a potent, selective, and orally active HDM2-p53 inhibitor that demonstrated tumor growth inhibition in a variety of cancer cell lines expressing wild-type p53 *in vivo*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.5b00472.

General synthesis and characterization data for key intermediates and final compounds **3**, **4**, **6**, **8**, **10**, **14**, **15**, **17–19**, and **21–25** (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: stephane.bogen@merck.com.

Notes

The authors declare no competing financial interest.

REFERENCES

- (1) American Cancer Society, Inc. Surveillance Research, 2013.
- (2) Mandinova, A.; Lee, S. W. The p53 pathway as a target in cancer therapeutics: obstacles and promise. *Sci. Transl. Med.* **2011**, *3*, 64rv1.
- (3) Raycroft, L.; Wu, H. Y.; Lozano, G. Transcriptional activation by wild-type but not transforming mutants of the p53 antioncogene. *Science* **1990**, *249*, 1049–1051.
- (4) Hainaut, P.; Hollstein, M. p53 and human cancer: the first ten thousand mutations. *Adv. Cancer Res.* **2000**, *77*, 81–137.
- (5) Vousden, K. H.; Lane, D. P. p53 in health and disease. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 275–283.
- (6) Bond, G. L.; Hu, W.; Levine, A. J. MDM2 is a central node in the p53 pathway: 12 years and counting. *Curr. Cancer Drug Targets* **2005**, *5*, 3–8.
- (7) Wade, M.; Wang, Y. V.; Wahl, G. M. The p53 orchestra: Mdm2 and Mdmx set the tone. *Trends Cell Biol.* **2010**, *20*, 299–309.
- (8) Poyurovsky, M. V.; Prives, C. Unleashing the power of p53: lessons from mice and men. *Genes Dev.* **2006**, *20*, 125–131.
- (9) Brown, C. J.; Lain, S.; Verma, C. S.; Fersht, A. R.; Lane, D. P. Awakening guardian angels: drugging the p53 pathway. *Nat. Rev. Cancer* **2009**, *9*, 862–873.
- (10) Wade, M.; Li, Y. C.; Wahl, G. M. MDM2, MDMX and p53 in oncogenesis and cancer therapy. *Nat. Rev. Cancer* **2013**, *13*, 83–96.
- (11) Zhao, Y.; Aguilar, A.; Denzil Bernard, D.; Wang, S. Small-Molecule Inhibitors of the MDM2–p53 Protein–Protein Interaction (MDM2 Inhibitors) in Clinical Trials for Cancer Treatment. *J. Med. Chem.* **2015**, *58*, 1038–1052.
- (12) 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.
- (13) Hu, C.-Q.; Hu, Y.-Z. Small molecule inhibitors of the p53-MDM2. *Curr. Med. Chem.* **2008**, *15*, 1720–1730.
- (14) 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.
- (15) Zhao, Y.; Yu, S.; Sun, W.; Liu, L.; Lu, J.; McEachern, D.; Shargary, S.; Bernard, D.; Li, X.; Zhao, T. Discovery of Potent and Orally Active p53-MDM2 Inhibitors RO5353 and RO2468 for Potential Clinical Development. *J. Med. Chem.* **2013**, *56*, 5553–5561.
- (16) Gonzalez-Lopez de Turiso, F.; Sun, D.; Rew, Y.; Bartberger, M. D.; Beck, H. P.; Canon, J.; Chen, A.; Chow, D.; Correll, T. L.; Huang, X.; et al. Rational design and binding mode duality of MDM2-p53 inhibitors. *J. Med. Chem.* **2013**, *56*, 4053–4070.
- (17) Zak, K.; Pecak, A.; Rys, B.; Wladyka, B.; Domling, A.; Weber, L.; Holak, T. A.; Dubin, G. Diaryl- and triaryl-pyrrole derivatives: inhibitors of the MDM2–p53 and MDMX–p53 protein–protein interactions. *Expert Opin. Ther. Pat.* **2013**, *23*, 425–448.
- (18) Zhang, Z.; Chu, X.-J.; Liu, J.-J.; Ding, Q.; Zhang, J.; Bartkovitz, D.; Jiang, N.; Karnachi, P.; So, S.-S.; Tovar, C.; Filipovic, Z. M.; Higgins, B.; Glenn, K.; Packman, K.; Vassilev, L.; Graves, B. Discovery of potent and orally active p53-MDM2 inhibitors RO5353 and RO2468 for potential clinical development. *ACS Med. Chem. Lett.* **2014**, *5*, 124–127.
- (19) Gonzalez, A. Z.; Eksterowicz, J.; Bartberger, M. D.; Beck, H. P.; Canon, J.; Chen, A.; Chow, D.; Duquette, J.; Fox, B. M.; Fu, J.; Huang, X.; Houze, J. B.; Jin, L.; Li, Y.; Li, Z.; Ling, Y.; Lo, M.-C.; Long, A. M.; McGee, L. R.; McIntosh, J.; McMinn, D. L.; Oliner, J. D.; Osgood, T.; Rew, Y.; Saiki, A. Y.; Shaffer, P.; Wortman, S.; Yakowec, P.; Yan, X.; Ye, Q.; Yu, D.; Zhao, X.; Zhou, J.; Olson, S. H.; Medina, J. C.; Sun, D. Selective and potent morpholinone inhibitors of the MDM2–p53 protein–protein interaction. *J. Med. Chem.* **2014**, *57*, 2472–2488.
- (20) Ma, Y.; Lahue, B.; Shipps, G.; Brookes, J.; Wang, Y. Substituted piperidines as HDM2 inhibitors. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1026–1030.
- (21) Wang, Y.; Zhang, R.; Ma, Y.; Lahue, B.; Shipps, G. Method of Using Substituted Piperidines that Increase p53 Activity. U.S. Pat. Appl. Publ. US2008004286, 2008.
- (22) 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. US200800428, 2008.
- (23) Ma, Y.; Lahue, B. R.; Gibeau, C. R.; Shipps, G. W., Jr.; Bogen, S. L.; Wang, Y.; Guo, Z.; Guzi, T. J. Pivotal role of an aliphatic side chain in the development of an HDM2 inhibitor. *ACS Med. Chem. Lett.* **2014**, *5*, 572–575.
- (24) Pan, W.; Lahue, B. R.; Ma, Y.; Nair, L. G.; Shipps, G. W., Jr.; Wang, Y.; Doll, R.; Bogen, S. L. Core modification of substituted piperidines as novel inhibitors of HDM2–p53 protein–protein interaction. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 1983–1986.
- (25) Caco2 permeability = 99 nm/sec (AP to BL) ; Mouse PK (PO/IV, 20/2 mpk) AUC = 8.7 $\mu\text{M}\cdot\text{h}$; F = 92%.
- (26) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; Levine, A. J.; Pavletich, N. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **1996**, *274*, 948–953.
- (27) Chen, J.; Marechal, V.; Levine, A. Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell. Biol.* **1993**, *13*, 4107–4114.
- (28) 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*, 744–756.
- (29) Meanwell, N. J. *Med. Chem.* **2011**, *54*, 2529–2591. Lima, L. M.; Barreiro, E. J. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr. Med. Chem.* **2005**, *12*, 23–49.
- (30) 100 μM for **21**, 10 μM for **10**, 12 μM for **18** (solubility assessed at pH 7.4 in PB).
- (31) Cl values following IV dosing of compound **21**: Mouse (2 mpk, 20% HPBCD, 133 mL/min/kg), monkey (1 mpk, 40% HPBCD, 23 mL/min/kg), dog (1 mpk, 40% HPBCD, 4.1 mL/min/kg).