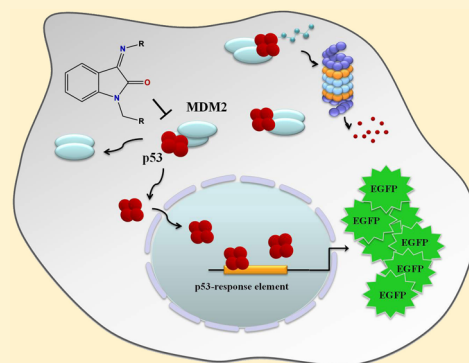


Discovery of Novel Isatin-Based p53 Inducers

P. Davidovich,^{*,†} V. Aksenova,^{†,#} V. Petrova,^{†,§} D. Tentler,^{†,‡} D. Orlova,[†] S. Smirnov,^{||} V. Gurzhiy,^{||}
A. L. Okorokov,[⊥] A. Garabadzhiu,[†] G. Melino,^{†,§} N. Barlev,^{†,‡} and V. Tribulovich[†][†]St. Petersburg Technological Institute, 26, Moskovskii Av., St. Petersburg 190013, Russia[‡]Institute of Cytology, 4, Tikhoretsky Av., St. Petersburg 194064, Russia[§]MRC Toxicology Unit, University of Leicester, Lancaster Road, Leicester LE1 9HN, United Kingdom^{||}St. Petersburg State University, Universitetsky Av. 26, St. Petersburg 198504, Russia[⊥]Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, United Kingdom

Supporting Information

ABSTRACT: A series of isatin Schiff base derivatives were identified during *in silico* screening of the small molecule library for novel activators of p53. The compounds selected based on molecular docking results were further validated by a high-content screening assay using U2OS human osteosarcoma cells with an integrated EGFP-expressing p53-dependent reporter. The hit compounds activated and stabilized p53, as shown by Western blotting, at higher rates than the well-known positive control Nutlin-3. Thus, the p53-activating compounds identified by this approach represent useful molecular probes for various cancer studies.



KEYWORDS: *in silico*, isatin Mannich and Schiff bases, p53 activators, *in vivo* assay, protein–protein interactions, MDM2

The p53 protein functions primarily as a sequence-specific transcription factor,¹ and it drives the expression of a large number of genes² in response to various stimuli.³ The current view is that p53, in addition to its crucial role in anticancer responses,⁴ also plays a role in inflammation.⁵ The attenuation of p53 activity (commonly caused by point mutations or overexpression of its negative regulatory proteins) leads to uncontrolled cell proliferation.

p53 has a wide network of protein–protein interactions⁶ (PPI), and some of the interacting proteins are key players in the regulation of tumor growth.⁷ However, among the wide variety of p53 PPIs, only several interacting proteins are directly responsible for p53 activity and stability. Therefore, blunting these critical p53-related PPIs has been shown to be a successful strategy for cancer treatment.⁸

There are several pharmacological methods to stimulate p53 antitumor activity: (a) small molecules (SM) that cause genotoxic stress (for example, doxorubicin);⁷ (b) reactivators of mutant p53, which revert the p53 molecule to the wild-type conformation;⁹ and (c) SM PPI inhibitors that block the ubiquitin-mediated proteasomal degradation of p53.⁸ The latter “class” of molecules seems to be the most promising as it generally exhibits lower toxicity levels.

As mentioned above, p53 is subject to ubiquitin-dependent degradation, and the principal p53-specific E3 ubiquitin ligase is mouse double minute (MDM2) protein. Perhaps not surprisingly, MDM2 is a major pharmacological target.

Importantly, unlike acetylation¹⁰ or methylation, MDM2-dependent ubiquitination of p53 on several lysine residues in its C-terminus significantly destabilizes the protein.¹⁰ In recent years, MDM2 has been recognized as the critical protein to target for p53 stabilization.¹¹

The p53 protein can be structurally divided into four domains: the N-terminal region that includes two trans-activation domains (TADs), the proline-rich region, the central core DNA-binding domain, and the C-terminal tetramerization and regulatory domain.¹²

The p53 TADs are the main interface for interaction with the regulatory protein MDM2. The α -helical part of TAD1 (key residues Phe19, Trp23, and Leu26) within the p53 protein interacts with MDM2 by binding to its N-terminal hydrophobic pocket. The p53-binding part of MDM2 is rigid, and its solved structure is widely used in molecular docking studies.¹³

Most of the MDM2 inhibitors mimic the structure of the TAD1 α -helix¹⁴ and hence form the same intermolecular contacts with the hydrophobic MDM2 pocket as the original p53. An alternative concept of stapled peptides was shown to be a successful strategy in attempts to inhibit MDM2.¹⁵ Nutlin-3 is the most studied SM inhibitor of the MDM2–p53 interaction; it has been examined through *in vitro* and *in vivo*¹⁶

Received: January 10, 2015

Accepted: July 6, 2015

Published: July 6, 2015

experiments. Several NMR¹⁷ and X-ray¹⁸ studies have specified the binding pose and details of its interactions with MDM2. Nutlin-3 was able to reactivate p53 in a nongenotoxic manner in clinical trials.¹⁹ To date, many Nutlin derivatives with higher affinities for MDM2 have been developed. Isoindolinones are derivatives of one promising “class” of inhibitors showing *in vivo* and *in vitro* activity.²⁰ Benzylideneindoline-2-ones have recently been presented as MDM2-p53 PPI inhibitors that exhibit *in cellulo* activity.²¹ More detailed information about PPI inhibitors is presented in the recently published review.²²

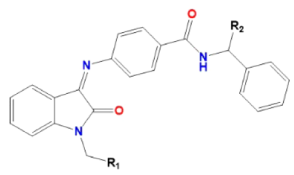
The isatin (1*H*-indole-2,3-dione) scaffold (benzylideneindoline-2-one analogue) is widely used in drug discovery studies.²³ N-Substituted isatin piperazine derivatives have been reported as selective inhibitors for aldehyde dehydrogenases in *in vitro* assays,²⁴ and the crystal structures of protein–ligand complexes have validated the mechanism of their action. Piperidine analogues show inhibitory activity against another class of metabolic proteins, carboxylesterases.²⁵ N-Alkylated isatins have recently been described as caspase-3 activity inhibitors.²⁶ Isatin-benzothiazole Schiff base derivatives have shown anticancer activity in breast tumor cell lines.²⁷

Here, we report the discovery of isatin Mannich and Schiff base derivatives (IMSBDs) that activate the p53 transcription factor. These compounds were identified by *in silico* screening (Table S2), and their activities were validated *in cellulo* using a human bone osteosarcoma epithelial U2OS cell line, which stably expressed a p53-driven enhanced green fluorescent protein (EGFP) reporter.

We have virtually screened the in-house library of 38,000 compounds available at the St. Petersburg Technological Institute. Analysis of the affinity data along with visual inspection allowed us to identify a list of potential MDM2-p53 PPI IMSBDs inhibitors. Because the identified IMSBDs can exist in two isomeric states,²⁸ they were treated as separate *E*- and *Z*-isomers. Some of the IMSBDs were poorly soluble, whereas others had low inhibitory activity and high cytotoxicity levels (only the N-substituted benzamide isatin Schiff base derivatives showed significant p53 activation levels (Table 1) accompanied by low toxicity).

The identified compounds represent a combination of Mannich²⁹ and Schiff bases that are known to have a tendency for hydrolysis. Therefore, we separately analyzed two different possible hydrolysis reactions (Figure S7) of IMSBD4 using

Table 1. EGFP Activation and Cytotoxicity after Treatment with IMSBDs



IMSBDs	R ₁ , R ₂	number of cells		
		IMSBDs/Nutlin	EGFP	
			p53 ⁺	p53 ⁻
1	Bn-piperazine, Me	8.70	0.04	0.27
2	Bn-piperidine, H	4.90	0.14	0.32
3	Bn-piperazine, H	4.20	0.10	0.19
4	Ph-piperazine, H	3.90	0.22	0.40
5	2-F-Ph-piperazine, H	3.60	0.11	0.24
6	methylbenzylamine, H	3.10	0.14	0.41

UV–vis spectrometry. As seen in Figure S6, incubation of the isatin Mannich base for 12 h at pH 7.5 and 37 °C led to its almost complete hydrolysis. In contrast, the isatin Schiff base exhibited a much lower rate of hydrolysis. Thus, we predicted that even though the selected compounds undergo hydrolysis, they still exert their biological functions as non-N-substituted Schiff bases.

Importantly, we found that the compounds bind with BSA, which is abundantly present in serum. The interaction of compounds with both BSA and MDM2 might also prevent these compounds from undergoing hydrolysis. For instance, Kimble-Hill et al.²⁴ reported successful crystallization of the nonhydrolyzed Mannich base (*N*-[(1-piperazinyl)methyl]isatin) with the ALDH3A1 protein using the same pH and buffer system in the crystallization assay as we used in our hydrolysis studies. Thus, the larger peak shifts in the UV–vis spectra in the culture media than in the buffer (Figure S6) can be explained by the effect of BSA binding.

We analyzed the ability of both ISBD isomers to dock to the p53-binding pocket of MDM2. The (*E*)-ISBDs docked to the p53-binding pocket with lower ΔG than the *Z*-form by approximately 1 kcal/mol. As seen in Figure 1, the (*E*)-ISBD2

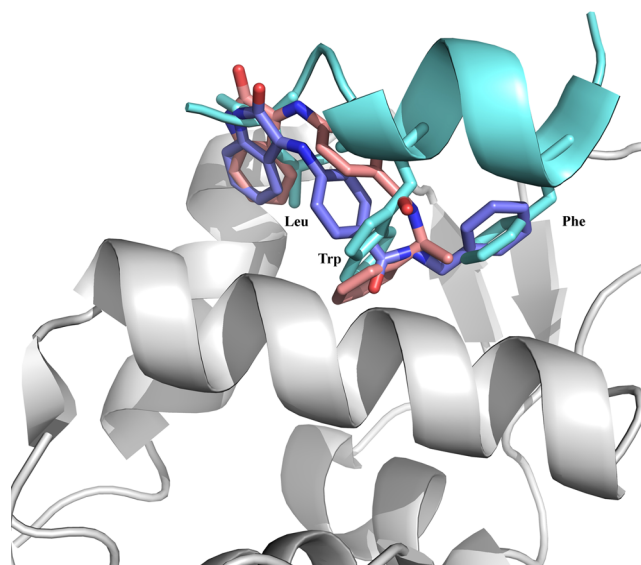


Figure 1. Superimposed representation of the docked poses of ISBD2 (blue) and ISBD1 (rose) with the p53 TAD1 α -helix (cyan).

isomer forms mainly hydrophobic interactions with the MDM2 p53-binding site (Leu pocket, π – π stacking interaction with His96 and Tyr100). The calculated affinity of (*S,E*)-ISBD1 to the p53-binding pocket of MDM2 is one-fold lower in K_i units than the corresponding value for ISBD2. We propose that the identified ISBDs can act as a p53 α -helix mimetic (Figure 1), whereby the phenyl ring of ISBD2 overlays the Phe19 residue, and the isatin core overlays the hydrophobic Leu26 of the p53 α -helix. Furthermore, the methylated aliphatic carbon in (*S,E*)-ISBD1 alters the conformation of the ligand, causing the phenyl ring to overlay the Trp23 residue.

One of the major limitations of p53-activating ISBDs is the presence of *E*-to-*Z* interconversion, which lowers the overall binding affinity to MDM2. As previously reported,²⁸ ISBDs crystallize preferentially in the (*E*)-conformation but convert to the (*Z*)-form when dissolved. The topology of the (*Z*)-form

differs substantially from that of the (*E*)-conformer (Figure S10).

We performed 2D NMR experiments on IMSBD4 to obtain the relationship of *E*-to-*Z* solution concentrations. The spectral data (Supporting Information) confirm the molar (*E*-to-*Z*) ratio to be 8:1 in polar DMSO ($\epsilon = 46.8$) and in less polar chloroform ($\epsilon = 4.7$). We suggest that the ratio between these isomers remains approximately the same when dissolved in culture medium for *in vivo* studies. We also confirmed the presence of the *E*-configuration in the solid state by solving the crystal structure of IMSBD4 (Table S1 and Figure S2).

To validate our *in silico* predictions, we decided to study the effect of SMs on p53 stabilization and activation. To this end, we developed a cell-based reporter system using the p53-positive U2OS cell line (Figure 2).

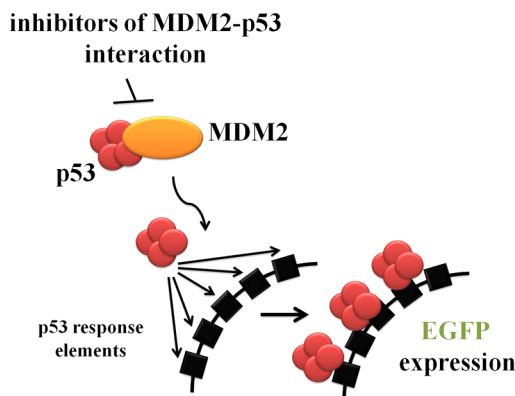


Figure 2. Schematic representation of the test-system containing five p53 response elements.

Unlike *in vitro* models, this approach is less prone to false-positive signals due to the lower background of off-target effects. The resulting cell line features an episomal plasmid that contains the *EGFP* coding sequence under the control of five repeated p53 response elements taken from the promoter region of the *p21* gene, which is the *bona fide* target of p53 transcriptional activity.

Upon treatment with activating SMs (e.g., MDM2-p53 PPI inhibitors), cellular p53 is stabilized by the acetylation modification.³⁰ Subsequently, p53 binds the response elements and triggers transcription of the *EGFP* gene, whose product emits fluorescence. Therefore, the efficacy of p53-activating SMs was measured indirectly as the number of *EGFP*-positive cells.

As shown in Figure 3, the presence of *EGFP*-positive cells (green) demonstrates that IMSBD4 activates p53-dependent transcription of the *EGFP* reporter.

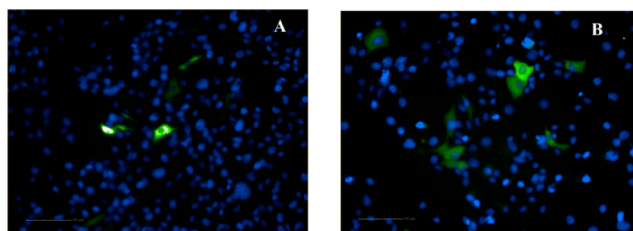


Figure 3. Representative image of the U2OS-pLV cell line response to treatment with Nutlin-3 (2.5 μM) (A) and IMSBD4 (2.5 μM) (B).

Quantitatively, the potency of compounds was estimated as the ratio between the percentage of *EGFP*-positive cells after treatment with IMSBDs (2.5 μM , 48 h) and the effect of a well-characterized nongenotoxic MDM2 inhibitor, Nutlin-3. *N*-Mannich bases of ISBDs substituted with *N*-(benzyl)benzamide demonstrated higher levels of p53 activation than the control Nutlin-3 inhibitor (Table 1). *EGFP* activation and cytotoxicity (Hoechst stain) values in p53⁺ and p53⁻ cells treated with various IMSBDs are shown as ratios between the effects of compounds and the effects mediated by Nutlin-3 treatment, which were arbitrarily set as 1.

As seen in Table 1, different IMSBDs activate p53 in U2OS-pLV cells (as measured by the number of cells “with *EGFP*”) at various levels but are consistently more effective than Nutlin-3. We also measured the cytotoxic effect of SMs by counting the number of cells that survived after the treatment. The cytotoxicity of IMSBDs was clearly and consistently higher in p53⁺ cells versus p53⁻ cells (Table 1, compare the last two rows), which indicates that these effects are p53 dependent. The fact that IMSBD cytotoxicity was higher than that of Nutlin-3 suggests that these compounds may have additional targets and/or activate the apoptotic p53 pathway, which is mechanistically different from the pathway activated by Nutlin-3 in these cells.

The difference in activity between IMSBD2–IMSBD6 is not significant (compared to IMSBD1), which supports the hypothesis that the common Schiff base is the active part of IMSBDs and that the variable Mannich base has no significant effect on the activity of SMs. Slight differences in the activity can be explained by the differences in physical properties (e.g., logP and PSA) of IMSBDs, whereas the binding poses of ISBD and IMSBD to MDM2 are generally similar (Figure S9).

To directly assess the effect of IMSBDs on the stability of the p53 protein, we performed Western blotting (WB), which allows measurement of the intracellular levels of the p53 protein. The representative WB data using IMSBD4 are shown in Figure 4. Treatment of U2OS-pLV cells with IMSBD4 for 12 h resulted in p53 stabilization comparable to the effect of Nutlin-3 but was less pronounced than Doxorubicin (DOXO).

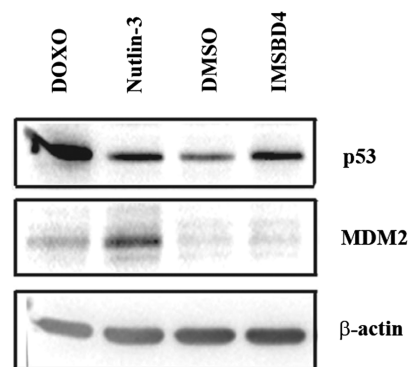


Figure 4. WB analysis of the p53 protein levels after treatment with different compounds.

We also examined the levels of MDM2 expression as it is the transcriptional target of p53, and the MDM2 gene product ubiquitinates p53.³¹ As shown in Figure 4, although IMSBD4 does significantly increase the level of p53, it has little or no influence on the protein level of MDM2. In contrast, Nutlin-3 and Doxorubicin increased the level of MDM2 in U2OS-pLV cells. This discrepancy also indicates that IMSBDs may stabilize

p53 by a different mechanism(s) from the one exerted by Nutlin-3. For instance, a close ISBD analogue, 4-hydroxybenzylideneindolin-2-one, has been reported as a micromolar inhibitor of Polo-like kinase 4, which is indirectly involved in the regulation of p53.³² However, the exact molecular mechanism of p53 stabilization requires additional investigation.

Using *in silico* and *in vivo* studies, we have identified several isatin-based derivatives that display strong p53 stimulatory effects. Based on *in silico* modeling, we assume that these novel pharmacological activators of p53 may disrupt the PPI between p53 and MDM2. However, further studies are required to establish the precise molecular mechanism of this phenomenon.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures, analytical and computational data, and biological assay details. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmchemlett.5b00011.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: davidovich.pavel@technolog.edu.ru.

Present Address

#National Institutes of Health, Bethesda, Maryland 20892, United States.

Author Contributions

The manuscript was written through contributions of all authors.

Funding

The work was supported by the Ministry of Education and Science of Russia (contracts 11.G34.31.0069, 14.B25.31.0013, and 14.132.21.1334) and by the BBSRC (BB/E019862/1).

Notes

The authors declare no competing financial interest.

■ REFERENCES

- (1) Brady, C. A.; Jiang, D.; Mello, S. S.; Johnson, T. M.; Jarvis, L. A.; Kozak, M. M.; Broz, D. K.; Basak, S.; Park, E. J.; McLaughlin, M. E.; Karnezis, A. N.; Attardi, L. D. Distinct p53 Transcriptional Programs Dictate Acute DNA-Damage Responses and Tumor Suppression. *Cell* **2011**, *145*, 571–583.
- (2) Soria, C.; Estermann, F. E.; Espantman, K. C.; O'Shea, C. C. Heterochromatin silencing of p53 target genes by a small viral protein. *Nature* **2010**, *466*, 1076–1081.
- (3) Manfredi, J. J. p53 and Apoptosis: It's Not Just in the Nucleus Anymore. *Mol. Cell* **2003**, *11*, 552–554.
- (4) Cooks, T.; Harris, C. C.; Oren, M. Caught in the cross fire: p53 in inflammation. *Carcinogenesis* **2014**, *35*, 1680–1690.
- (5) Rokudai, S.; Laptenko, O.; Arnal, S. M.; Taya, Y.; Kitabayashi, I.; Prives, C. MOZ increases p53 acetylation and premature senescence through its complex formation with PML. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 3895–3900.
- (6) Hoe, K. K.; Verma, C. S.; Lane, D. P. Drugging the p53 pathway: understanding the route to clinical efficacy. *Nat. Rev. Drug Discovery* **2014**, *13*, 217–236.
- (7) Vassilev, L. T. p53 Activation by Small Molecules: Application in Oncology. *J. Med. Chem.* **2005**, *48*, 4491–4499.
- (8) 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.
- (9) Wassman, C. D.; Baronio, R.; Demir, Ö.; Wallentine, B. D.; Chen, C.-K.; Hall, L. V.; Salehi, F.; Lin, D.-W.; Chung, B. P.; Hatfield, W. G.; Chamberlin, R. A.; Luecke, H.; Lathrop, R. H.; Kaiser, P.; Amaro, R. E. Computational identification of a transiently open L1/S3 pocket for reactivation of mutant p53. *Nat. Commun.* **2013**, *4*, 1407–1416.
- (10) Marouco, D.; Garabadiu, A. V.; Melino, G.; Barlev, N. A. Lysine-specific modifications of p53: a matter of life and death? *Oncotarget* **2013**, *4*, 1556–1571.
- (11) Hardcastle, I. R. Targeting the MDM2–p53 Protein–Protein Interaction: Design, Discovery, and Development of Novel Anticancer Agents. In *Cancer Drug Design and Discovery*, 2nd ed.; Neidle, S., Eds.; Academic Press: San Diego, CA, 2014; pp 391–426.
- (12) Chillemi, G.; Davidovich, P.; D'Abramo, M.; Mametnabiev, T.; Garabadzhiu, A. V.; Desideri, A.; Melino, G. Molecular dynamics of the full-length p53 monomer. *Cell Cycle* **2013**, *12*, 3098–3108.
- (13) Warner, W. A.; Sanchez, R.; Dawoodian, A.; Li, E.; Momand, J. Identification of FDA-approved Drugs that Computationally Bind to MDM2. *Chem. Biol. Drug Des.* **2012**, *80*, 631–637.
- (14) Persico, M.; Ramunno, A.; Maglio, V.; Franceschelli, S.; Esposito, C.; Carotenuto, A.; Braccaccio, D.; De Pasquale, V.; Pavone, L. M.; Varra, M.; Orteca, N.; Novellino, E.; Fattorusso, C. New Anticancer Agents Mimicking Protein Recognition Motifs. *J. Med. Chem.* **2013**, *56*, 6666–6680.
- (15) Chang, Y. S.; Graves, B.; Guerlavais, V.; Tovar, C.; Packman, K.; To, K.-H.; Olson, K. A.; Kesavan, K.; Gangurde, P.; Mukherjee, A.; Baker, T.; Darlak, K.; Elkin, C.; Filipovic, Z.; Qureshi, F. Z.; Cai, H.; Berry, P.; Feyfant, E.; Shi, X. E.; Horstick, J.; Annis, D. A.; Manning, A. M.; Fotouhi, N.; Nash, H.; Vassilev, L. T.; Sawyer, T. K. Stapled α -helical peptide drug development: A potent dual inhibitor of MDM2 and MDMX for p53-dependent cancer therapy. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, E3445–E3454.
- (16) Endo, S.; Yamato, K.; Hirai, S.; Moriwaki, T.; Fukuda, K.; Suzuki, H.; Abei, M.; Nakagawa, I.; Hyodo, I. Potent *in vitro* and *in vivo* antitumor effects of MDM2 inhibitor Nutlin-3 in gastric cancer cells. *Cancer Sci.* **2011**, *102*, 605–613.
- (17) Showalter, S. A.; Bruschiweiler-Li, L.; Johnson, E.; Zhang, F.; Bruschweiler, R. Quantitative Lid Dynamics of MDM2 Reveals Differential Ligand Binding Modes of the p53-Binding Cleft. *J. Am. Chem. Soc.* **2008**, *130*, 6472–6478.
- (18) Anil, B.; Riedinger, C.; Endicott, J. A.; Noble, M. E. M. The structure of an MDM2–Nutlin-3a complex solved by the use of a validated MDM2 surface-entropy reduction mutant. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2013**, *69*, 1358–1366.
- (19) Secchiero, P.; Bosco, R.; Celeghini, C.; Zauli, G. Recent Advances in the Therapeutic Perspectives of Nutlin-3. *Curr. Pharm. Des.* **2011**, *17*, 569–577.
- (20) Bertamino, A.; Soprano, M.; Musella, S.; Rusciano, M. R.; Sala, M.; Vernieri, E.; Di Sarno, V.; Limatola, A.; Carotenuto, A.; Cosconati, S.; Grieco, P.; Novellino, E.; Illario, M.; Campiglia, P.; Gomez-Monterrey, I. Synthesis, *in Vitro*, and *in Cell* Studies of a New Series of [Indoline-3,2'-thiazolidine]-Based p53 Modulators. *J. Med. Chem.* **2013**, *56*, 5407–5421.
- (21) Zheng, G.-H.; Shen, J.-J.; Zhan, Y.-C.; Yi, H.; Xue, S.-T.; Wang, Z.; Ji, X.-Y.; Li, Z.-R. Design, synthesis and *in vitro* and *in vivo* antitumor activity of 3-benzylideneindolin-2-one derivatives, a novel class of small-molecule inhibitors of the MDM2–p53 interaction. *Eur. J. Med. Chem.* **2014**, *81*, 277–288.
- (22) Zhao, Y.; Aguilar, A.; 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.
- (23) Vine, K. L.; Matesic, L.; Locke, J. M.; Skropeta, D. Recent Highlights in the Development of Isatin-Based Anticancer Agents. *Adv. Anticancer Agents Med. Chem.* **2013**, *2*, 254–312.
- (24) Kimble-Hill, A. C.; Parajuli, B.; Chen, C.-H.; Mochly-Rosen, D.; Hurley, T. D. Development of Selective Inhibitors for Aldehyde Dehydrogenases Based on Substituted Indole-2,3-diones. *J. Med. Chem.* **2014**, *57*, 714–722.

(25) Hyatt, J. L.; Moak, T.; Hatfield, M. J.; Tsurkan, L.; Edwards, C. C.; Wierdl, M.; Danks, M. K.; Wadkins, R. M.; Potter, P. M. Selective Inhibition of Carboxylesterases by Isatins, Indole-2,3-diones. *J. Med. Chem.* **2007**, *50*, 1876–1885.

(26) Chu, W.; Rothfuss, J.; Zhou, D.; Mach, R. H. Synthesis and evaluation of isatin analogs as caspase-3 inhibitors: Introduction of a hydrophilic group increases potency in a whole cell assay. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2192–2197.

(27) Solomon, V. R.; Hu, C.; Lee, H. Hybrid pharmacophore design and synthesis of isatin–benzothiazole analogs for their anti-breast cancer activity. *Bioorg. Med. Chem.* **2009**, *17*, 7585–7592.

(28) Davidovich, P.; Novikova, D.; Tribulovich, V.; Smirnov, S.; Gurzhiy, V.; Melino, G.; Garabadzhiu, A. First X-ray structural characterization of isatin Schiff base derivative. NMR and theoretical conformational studies. *J. Mol. Struct.* **2014**, *1075*, 450–455.

(29) Roman, G. Mannich bases in medicinal chemistry and drug design. *Eur. J. Med. Chem.* **2015**, *89*, 743–816.

(30) Barlev, N. A.; Liu, L.; Chehab, N. H.; Mansfield, K.; Harris, K. G.; Halazonetis, T. D.; Berger, S. L. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. *Mol. Cell* **2001**, *8*, 1243–1254.

(31) Brooks, C. L.; Gu, W. p53 Ubiquitination: MDM2 and Beyond. *Mol. Cell* **2006**, *21*, 307–315.

(32) Yu, B.; Yu, Z.; Qi, P.-P.; Yu, D.-Q.; Liu, H.-M. Discovery of orally active anticancer candidate CFI-400945 derived from biologically promising spirooxindoles: Success and challenges. *Eur. J. Med. Chem.* **2015**, *95*, 35–40.