

A New Family of Small Molecules To Probe the Reactivation of Mutant p53

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The protein p53 is recognized as one of the most important guardians in the body that prevents tumor development.¹ Since its discovery, the roles of p53 have been the focus of research geared toward understanding the mechanisms of uncontrolled cell growth or cancer.² Specifically, when healthy cells are damaged, p53 levels increase, followed by inhibition of cell growth or programmed cell death (apoptosis).³ This regulation of damaged cells is initiated by a p53-DNA binding event. Mutated forms of p53 that lose the ability to bind DNA cannot arrest cell growth, and the proliferation of damaged cells results.^{2b} Mutant forms of p53 are present in approximately 50% of all human cancers.⁴ If mutant p53 can be induced to readopt the active form of wild-type p53, tumor suppressor function can be restored (reactivation).⁵ Molecules that reactivate mutant p53 could selectively target tumor cells due to the accumulation of mutated p53 in these cells.⁶

Recently, reactivation of mutant p53 by small organic molecules has been described.⁷ These studies relied on random screening of combinatorial libraries to identify small molecule reactivators. Among the molecules reported to reactivate mutant p53, a number contain heteroaromatic groups that interact primarily with DNA,⁸ which is undesirable for drug candidates due to nonspecific binding and possible mutagenic effects.⁶ In contrast, the molecule PRIMA-1 (Figure 1A) has been reported to interact directly with several p53 mutants, reactivating the protein to restore wild-type function.^{9,10} Currently, the molecular mechanism describing how a small organic molecule can refold a mutated protein remains unclear.

In our research on p53 reactivation, we have initiated a program to develop a synthetically accessible class of molecules that can be easily modified to examine structural activity relationships (SARs) and mechanism of biological activity or to optimize for anticancer activity. Our first goal was to develop a class of molecules with spatial arrangements of functional groups that loosely resemble PRIMA-1, but within an easily modifiable framework (Figure 1B). In particular, we sought to develop a new family of molecules that lacks the bicyclic core of PRIMA-1 and contains one hydroxymethyl side chain.

A variety of compounds were prepared bearing the general structure shown in Figure 1B. Initially, our molecules were prepared starting from 2-aminoacetophenone hydrochloride. From this starting material, compounds with one or two hydroxymethyl side chains were prepared (all molecules with one side chain were racemic) (Table 1, 1–7, 10–12).^{10,11}

This initial series of molecules was then studied for their ability to arrest cell growth in Saos-2 cells expressing a mutant p53.¹¹ While most compounds did not show the desired activity, molecule 7 (Figure 1C) was particularly effective at selectively killing cells

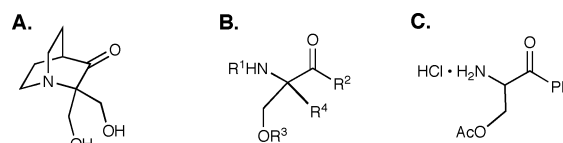


Figure 1. Organic molecules that reactivate mutant p53. (A) Structure of PRIMA-1. (B) General scaffold where R¹ to R⁴ can be varied to study effects on p53 reactivation. (C) One of the molecules that selectively kills Saos-2 cells expressing mutant p53 (referred to as molecule 7).

Table 1. Activity of Molecules on Cells Expressing Mutant p53



R¹, R², X, and Y can be altered synthetically to a variety of different groups.

product ^a	R ¹	X	Y	R ²	activity ^b	mutant ^b
1	Boc	CH ₂ OH	CH ₂ OH	Ph	NA	281
2	Boc	CH ₂ OAc	CH ₂ OAc	Ph	NA	281
3	H	CH ₂ OH	CH ₂ OH	Ph	NA	281
4	H	CH ₂ OAc	CH ₂ OAc	Ph	10 μM	281
5	H	H	H	Ph	NA	281
6	H	H	CH ₂ OH	Ph	NA	281
7	H	H	CH ₂ OAc	Ph	5–50 μM	175, 281
8	H	H	CH ₂ OAc	Ph	5–50 μM ^c	175, 281
9	H	H	CH ₂ OAc	Ph	5–50 μM ^d	175, 281
10	Ac	H	H	Ph	NA	175, 281
11	Ac	H	CH ₂ OH	Ph	TX ^e	175, 281
12	Ac	H	CH ₂ OAc	Ph	TX ^e	175, 281
13	H	H	CH ₂ OAc	Et	NA	175, 281
14	H	H	CH ₂ OAc	OMe	NA	281
15	H	H	CH ₂ OAc	morph.	NA	281
16	Me	H	CH ₂ OAc	Ph	TX ^{e,f}	175, 281
17	H	Me	CH ₂ OAc	Ph	NA	175, 281
43	H	H	CH ₂ OAc	<i>p</i> -F-Ph	TX ^{d,e}	175, 281
44	H	H	CH ₂ OAc	<i>p</i> -Me-Ph	100 μM ^d	175, 281
45	H	H	CH ₂ OAc	<i>p</i> -OMe-Ph	NA ^d	175, 281
46	H	H	CH ₂ OAc	<i>β</i> -naphthyl	NA ^d	175, 281

^a All compounds are (+, –) mixtures unless otherwise noted; entries 3–9 and 13–46 were isolated as HCl salts. ^b PRIMA-1 is active at 75 μM. See Supporting Information for cellular assay details; NA = no activity and no toxicity. ^c (S)-Enantiomer. ^d (R)-Enantiomer. ^e Compounds are toxic to all cells. ^f Compound was 86% ee favoring the (S)-enantiomer.

expressing R175H and R281G p53 mutants at concentrations lower than that of PRIMA-1 (Figure 2). Molecule 4 also showed selectivity for R281G mutants. The positive results obtained with racemic 7 underlined the need to produce both enantiomers of 7 (8 and 9). Therefore, an asymmetric synthesis was developed to produce enantiomerically pure material.¹¹ Interestingly, (S)- and (R)-7 showed activity comparable to that of (±)-7, indicating that chirality does not play a role in controlling the desired activity (Table 1, entries 7–9).

Our synthetic strategy can be readily modified to afford numerous other analogues of 7. Therefore, molecules 11–17 were prepared

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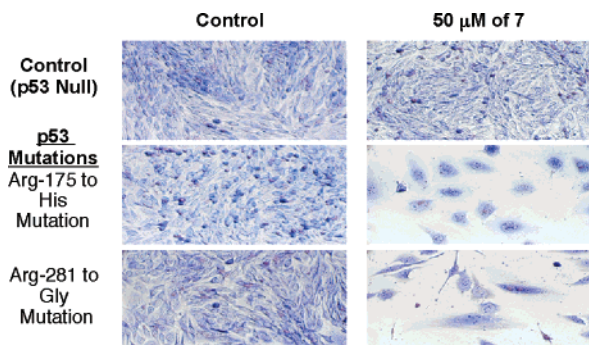


Figure 2. Cellular assay with Saos-2 cells expressing no p53 (control) or mutant p53 at positions 175 and 281 with 50 μ M of compound **7** (PRIMA-1 induces similar results at 75 μ M). Each panel represents the same magnification (20 $\times$).

to gain some insight into SAR for this series of molecules (Table 1).¹¹ While molecules **11**–**17** did not possess any ability to selectively arrest growth of cells expressing mutant p53, the results of these studies point to the importance of the phenyl group and a primary amine as essential features for the desired biological activity. If the phenyl ketone of **7** is changed to a methyl ester (**14**), morpholine amide (**15**), or even ethyl ketone (**13**), the desired activity is lost. Furthermore, attaching a methyl group to the nitrogen of **7** (molecule **16**) or a methyl group to the α -carbon of **7** (molecule **17**) eliminates the desired activity and in some cases results in nonspecific toxicity.

On the basis of the previous results, modifications to the phenyl ring were explored for effects on the desired biological activity. Therefore, molecules **43**–**46** were synthesized to test whether electron donating, withdrawing, or sterics on the aromatic ring affect the biological activity. From these data, an electron-withdrawing fluorine group raises the nonspecific cytotoxicity. On the other hand, an electron-releasing methoxy group and a naphthyl group result in loss of activity. A methyl group retains activity, although the optimal concentration is higher compared to that of molecule **7**.

Along with our new molecules, the activity of PRIMA-1 was compared in the same assays.¹¹ Although our active compounds, as well as PRIMA-1, selectively eliminated cells expressing mutant p53, we have been unable to detect any evidence of restoration of wild-type p53 properties, including induced expression of an artificial p53-responsive promoter–reporter, or endogenous target genes (e.g., p21CIP1, HDM2), or the reestablishment of the wild-type conformation in vitro.

In support of our findings, recent data by Wiman and co-workers demonstrated that a derivative of PRIMA-1 (PRIMA-1^{MET}), which is twice as effective as the parental compound, does not significantly alter mutant p53 protein levels, p53 post-translational modification (which is thought to be a prerequisite for its activity), or the induction of BAX, a p53 target gene.¹² However, PRIMA-1^{MET} was found to upregulate the expression of PUMA, a BH3-only pro-apoptotic factor. It is well-established that PUMA expression is regulated in a p53-dependent and -independent manner.¹³ These findings raise the issue of whether PRIMA-1 and our compounds directly restore wild-type activity to mutant p53. It remains a formal possibility that these molecules selectively target cells expressing

mutant p53 by other mechanisms such as directing p53 to the mitochondria¹⁴ or by blocking its gain-of-function activity, which protects against cell death.¹⁵ Future studies will be needed to determine whether mutant p53 is the actual target.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. Cellular assay procedure and data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>

References

- (1) Lane D. P.; Fischer, P. M. *Nature* **2004**, 427, 789–790.
- (2) (a) Harris, C. C. *Carcinogenesis* **1996**, 17, 1187–1198. (b) Ribeiro, R. C.; Sandrini, F.; Figueiredo, B.; Zambetti, G. P.; Michalkiewicz, E.; Lafferty, A. R.; DeLacerda, L.; Rabin, M.; Cadwell, C.; Sampaio, G.; Cat, I.; Stratakis, C. A.; Sandrini, R. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, 98, 9330–9335. (c) Han, J. W.; Flemington, C.; Houghton, A. B.; Gu, Z.; Zambetti, G. P.; Lutz, R. J.; Zhu, L.; Chittenden, T. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, 98, 11318–11323. (d) Joerger, A. C.; Allen, M. D.; Fersht, A. R. *J. Biol. Chem.* **2004**, 279, 1291–1296. (e) 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. *Science* **2004**, 303, 844–848.
- (3) Reed, J. C. *Cancer Cell* **2003**, 3, 17–22.
- (4) Vogelstein, B.; Lane, D.; Levine, A. J. *Nature* **2000**, 408, 307–310.
- (5) DeDecker, B. S. *Chem. Biol.* **2000**, 7, 103–107.
- (6) (a) Sugikawa, E.; Hosoi T.; Yazaki, N.; Gamanuma, M.; Nakanishi, N.; Ohashi, M. *Anticancer Res.* **1999**, 19, 3099–3108. (b) Friedler, A.; Hansson, L. O.; Veprintsev, D. B.; Freund, S. M. V.; Rippin, T. M.; Nikolova, P. V.; Proctor, M. R.; Rudiger, S.; Fersht, A. R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, 99, 937–942. (c) North, S.; Pluquet, O.; Maurici, D.; Ghissassi, F. E.; Hainaut, P. *Mol. Carcinog.* **2002**, 33, 181–188. (d) Peng, Y.; Li, C.; Chen, L.; Sebt, S.; Chen, J. *Oncogene* **2003**, 22, 4478–4487.
- (7) Bykov, V. J. N.; Selivanova, G.; Wiman, K. G. *Eur. J. Cancer* **2003**, 39, 1828–1834.
- (8) (a) Foster, B. A.; Coffey, H. A.; Morin, M. J.; Rastinejad, F. *Science* **1999**, 286, 2507–2510. (b) Rippin, T. M.; Bykov, V. J. N.; Freund, S. M. V.; Selivanova, G.; Wiman, K. G.; Fersht, A. R. *Oncogene* **2002**, 21, 2119–2129.
- (9) Bykov, V. J. N.; Issaeva, N.; Shilov, A.; Hultcrantz, M.; Pugacheva, E.; Chumakov, P.; Bergman, J.; Wiman, K. G.; Selivanova, G. *Nat. Med.* **2002**, 8, 282–288.
- (10) Nielsen, A. T. *J. Org. Chem.* **1966**, 1053–1059.
- (11) See Supporting Information for details.
- (12) Bykov, V. J. N.; Zache, N.; Stridh, H.; Westman, J.; Bergman, J.; Selivanova, G.; Wiman, K. G. *Oncogene*, published online Feb 28, 2005, <http://dx.doi.org/10.1038/sj.onc.1208419>.
- (13) (a) Han, J.; Flemington, C.; Houghton, A. B.; Gu, Z.; Zambetti, G. P.; Lutz, R. J.; Zhu, L.; Chittenden, T. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, 98, 11318–11323. (b) Jeffers, J. R.; Parganas, E.; Lee, Y.; Yang, C.; Wang, J.; Brennan, J.; MacLean, K. H.; Han, J.; Chittenden, T.; Ihle, J. N.; McKinnon, P. J.; Cleveland, J. L.; Zambetti, G. P. *Cancer Cell* **2003**, 4, 321–328.
- (14) (a) Mihara, M.; Erster, S.; Zaika, A.; Petrenko, O.; Chittenden, T.; Pancoska, P.; Moll, U. M. *Mol. Cell.* **2003**, 11, 577–590. (b) Chipuk, J. E.; Kuwana, T.; Bouchier-Hayes, L.; Droin, N. M.; Newmeyer, D. D.; Schuler, M.; Green, D. R. *Science* **2004**, 303, 1010–1014. (c) Leu, J. I.; Dumont, P.; Hafey, M.; Murphy, M. E.; George, D. L. *Nat. Cell Biol.* **2004**, 6, 443–450.
- (15) (a) Matas, D.; Sigal, A.; Stambolsky, P.; Milyavsky, M.; Weisz, L.; Schwartz, D.; Goldfinger, N.; Rotter, V. *EMBO J.* **2001**, 20, 4163–4172. (b) Zalcenstein, A.; Stambolsky, P.; Weisz, L.; Muller, M.; Wallach, D.; Goncharov, T. M.; Krammer, P. H.; Rotter, V.; Oren, M. *Oncogene* **2003**, 22, 5667–5676.

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