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# Discovery of 1-arylpyrrolidone derivatives as potent p53–MDM2 inhibitors based on molecule fusing strategy



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#### ABSTRACT

Introducing an aryl moiety to our previous pyrrolidone scaffold by molecule fusing strategy afforded two sets of isopropylether–pyrrolidone and  $\alpha$ -phenylethylamine–pyrrolidone derivatives. Two novel compounds **8b** and **8g** of the latter serial showed potent p53–MDM2 inhibitory activities with  $K_i$  values of 90 nM which were three-time higher than that of the parent compound. We also confirmed compound **8b** can activate p53 proteins in lung cancer A549 cells. The results offered us valuable information for further lead optimization.

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The human tumor suppressor p53, as one of the most potent transcriptional activator proteins, plays a protective role in normal tissues. The p53 is also short-lived in normal cells under physiological conditions and is mainly regulated by the murine double minute 2 protein (MDM2).<sup>1–3</sup> However, in approximately 50% of all human cancers p53 has been found to be inactivated. Overexpression of MDM2 was discovered as the main reason for p53 function impairment.<sup>4</sup> Recently, restoration of p53 activity by inhibiting the p53–MDM2 interaction has been demonstrated as a successful strategy for anticancer drug development.<sup>5,6</sup> To date, an increasing number of small molecule inhibitors have been generated and six excellent compounds RG7112, RO5503781, MK-8242, SAR405838, CGN097 and DS-3032b have entered clinical trials.<sup>7–14</sup>

In 2012, Zhuang et al. discovered a novel lead structure with pyrrolidone scaffolds by virtual screening method based on cocrystal structures of p53–MDM2 interaction.<sup>15,16</sup> Further hit optimization and SAR led to the discovery of two compounds **Z41** ( $K_i$  = 260.0 nM) and **Z60a** ( $K_i$  = 150.0 nM) which showed potent antitumor efficacy both in vitro and vivo (Fig. 1). These optimized compounds **Z41** and **Z60a** showed the similar interaction manner of MDM2 protein with benzoyl group and 4-bromophenyl group located in the Trp23 pocket and Leu26 pockets. However, imidazole group could not work well in concert with Phe19 pocket. According to binding mode of RG7112 which was the first generation small molecule inhibitor in clinical trial, two 4-chlorophenyl groups respectively entered Trp23 pocket and Leu26 pocket, while the 2-ethoxy-4-butylphenyl group projected into the Phe19 pocket (PDB code 4IPF).<sup>7</sup> In this paper, we present two novel classes of small-molecule inhibitors of p53–MDM2 protein–protein interaction based on pyrrolidone scaffold by a molecule fusing strategy (Fig. 2). Fourteen compounds with substituted phenyl groups on N1 position had synthesized and insight into the SAR of pyrrolidones was also revealed for further research.

The general synthetic route of target compounds is outlined in Scheme 1. Benzylamines **3a–3d** were prepared by the etherification of 2-fluoro-4-(trifluoro-methyl)benzonitrile **1** and four aliphatic alcohols, and subsequently reduction by LiAlH<sub>4</sub> in dried ether in good yield. With these benzylamines in hand, compounds **6a–6g** were readily obtained by an efficient three-component coupling reaction.<sup>15</sup> Followed Mitsunobu reaction and Leuckart-Wallach reaction afforded isopropylether–pyrrolidone derivatives **7a–7g** and  $\alpha$ -phenylethylamine–pyrrolidone derivatives **8a–8g**, respectively.

The binding constants  $K_i$  of all target compounds were measured by fluorescence polarization (FP) binding assay method. Nutlin-3 was used as the reference drug who was one of the most active small-molecule p53–MDM2 inhibitors. The results were



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Figure 1. The first small molecule inhibitor RG7112 of p53-MDM2 in clinical trial and active pyrrolidones developed by our group.



Figure 2. The molecule fusing strategy of 2-arylpyrrolidone derivatives.



Scheme 1. The synthetic route of 2-arylpyrrolidone derivatives **7a-7g** and **8a-8g**. Reagents and conditions: (i) NaH, ROH, 0 °C-25 °C, 55–64%; (ii) LiAlH<sub>4</sub>, Et<sub>2</sub>O, rt, 60–66%; (iii) 1,4-dioxane, rt, 35–55%; (iv) PPh<sub>3</sub>, DIAD, THF, *i*-PrOH, rt, 41–59%; (v) CH<sub>3</sub>COOH, *R*-α-phenylethylamine, microwave, 120 °C, 12–15%.

Table 1           MDM2 binding affinity of isopropylether-pyrrolidones 7a-7g										
Compds	Nutlin-3	7a	7b	7c	7d	7e	7f	7g	Z41	
$K_{i}$ ( $\mu$ M)	0.09	1.37	>100	0.72	0.71	>100	1.72	0.32	0.26	

Table 2	
MDM2 binding affinity of $\alpha$ -phenylethylamine-pyrrolidones <b>8a–8g</b>	

Compds	Nutlin-3	8a	8b	8c	8d	8e	8f	8g	Z60a
$K_{\rm i}(\mu{\rm M})$	0.09	8.18	0.09	3.02	>100	>100	37.7	0.09	0.26

summarized in Tables 1 and 2. As expected, most isopropyletherpyrrolidone derivatives indicated potent inhibitory activity with  $K_i$  values ranging from 0.26  $\mu$ M to 1.72  $\mu$ M. However, two compounds **7b** and **7e** failed to show p53–MDM2 binding inhibitor activity. Similarly, all the  $\alpha$ -phenylethylamine–pyrrolidone derivatives showed potent p53–MDM2 inhibitory activity except for **8d** and **8e**. To our delight, compounds **8b** and **8g** displayed excellent p53–MDM2 inhibitory activity with  $K_i$  values of 90 nM which were 3-time than that of the compound **Z60a**. As shown in Figure 3, three aromatic substituents of compound **8g** mimic



Figure 3. Binding modes of compound 8g in three key subpockets of MDM2. The figure was generated using PyMol (http://pymol.sourceforge.net/).



**Figure 4.** Cellular activity of compound **8b** for the p53 activation and MDM2 inhibition detected by Western blotting assay (A549 cells, 4 h treatment).

Phe19, Trp23 and Leu26 residues of p53 which is similar to the binding mode of RG7112.<sup>7,15</sup> Furthermore, both of the compounds showed equivalent inhibitory activity compared to the most famous active Nutlin-3. Structure–activity relationship analysis demonstrated that the fluorine substitution of pyrrolidone reduced inhibitory activity. For example, compounds **7e** and **8e** with fluorine on position 3 of N1 phenyl group indicated absent activity at 100  $\mu$ M.

The inhibition of the p53–MDM2 interaction is expected to activate p53, resulting in an increased level in the cells with wild-type p53. To confirm whether target compounds can activate p53 and increase the levels of MDM2 proteins in wild-type cells, compound **8b**, as one of the most two active pyrrolidones, was selected for Western blot assay. Consistent with the predictions, compound **8b** caused a dose dependent increase in the levels of p53 and slight

affect of MDM2 proteins in A549 cells (Fig. 4), indicating a good activation of p53.

In summary, a series of 2-arylpyrrolidone derivatives which was designed by a molecule fusing strategy were prepared in four steps with good yields. Among all targeted fourteen compounds, two phenylethylamine–pyrrolidone derivatives **8b** and **8g** were obtained with 3-time enhanced activities compared to parent compound **Z60a**. It was noted that fluorine on position 3 of N1 phenyl revealed disappear activity. In particular, compound **8b** was confirmed as a p53 proteins activator in A549 cells. Therefore, this study offered us valuable information for further lead optimization.

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014. 04.063.

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