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Novel 1,4-benzodiazepine-2,5-diones as Hdm2 antagonists with improved cellular activity

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Abstract—The disruption of the p53-Hdm2 protein–protein interaction induces cell growth arrest and apoptosis. We have identified the 1,4-benzodiazepine-2,5-dione scaffold as a suitable template for inhibiting this interaction by binding to the Hdm2 protein. Several compounds have been made with improved potency, solubility, and cell-based activities.

p53 is a multifunctional protein that regulates cell proliferation by induction of cell growth arrest or apoptosis in response to DNA damage and/or stress stimuli.[1] Mutations in the p53 gene occur in about 50% of all human tumors,[2] and these mutations facilitate carcinogenesis. However, there are an estimated 50% of human tumors that have wild-type p53, although its function appears to be inactivated. The human double minute 2 protein (Hdm2) inhibits the transcriptional activity of p53 by binding to its transactivation domain and producing an autoregulatory feedback loop.[3] Blocking this protein–protein interaction raises the levels of p53 and brings the cell to growth arrest and apoptosis. The disruption of the binding between these two proteins is therefore an attractive therapeutic target for the treatment of wild-type p53 tumors.[4]

Although the strategy of inhibiting the p53-Hdm2 interaction has been demonstrated as feasible by a number of groups,[5] only a few have disclosed small molecules that specifically block this pathway.[6] We have recently reported the 1,4-benzodiazepine-2,5-dione **1** (Fig. 1) as a potent inhibitor of the p53-Hdm2 interaction.[7] We now report the improved potency observed from the introduction of an ortho amino functionality to the benzylic ring **2**. We believe this increased potency (**2** vs **1** FP-IC_{50} = 0.25 and 0.85 μM, respectively) is due to an extra donor hydrogen bond interaction between the amino group and the carbonyl of Val93 of Hdm2, as shown in Figure 2. However, **2** was found to be less potent than **1** on the p53 inducible gene 3 (PIG-3) production cell-based assay, which is typically used as a downstream protein marker to monitor levels of p53.[8] We hypothesize that this reduction of cell activity of **2** versus **1** is a consequence of the zwitterionic character of **2**, which limits the cross-membrane permeability and adversely modulates the availability of the compound to its intended target Hdm2. To obtain compounds that combine optimized potency, solubility, and cell-permeability characteristics, we synthesized novel 1,4-benzodiazepine-2,5-diones bearing (i) different solubilizing groups in position 1 and (ii) functionalized benzyl groups in position 4.

The synthesis of the target diazepines is reported in Schemes 1–7. The synthesis of the benzodiazepine scaffold could be achieved by the Ugi reaction, as shown in Scheme 4, or starting from isatoic anhydride (Scheme 7). The synthesis of various benzylamines necessary for the Ugi reaction is reported in Schemes 1–3. Accordingly, benzaldehydes **3a**–**d** were reduced to the corresponding alcohols **4a**–**d** using either sodium borohydride for those compounds with a methylene group in the benzylic position or methyl lithium for compounds with a methyl group in the benzylic position. Alcohols **4a**–**d** were reacted with phthalimide under Mitsunobu conditions to generate intermediates **5a**–**d**, which were deprotected with hydrazine to yield amines **6a**–**d**.

Keywords: Hdm2; p53; Benzodiazepinedione.
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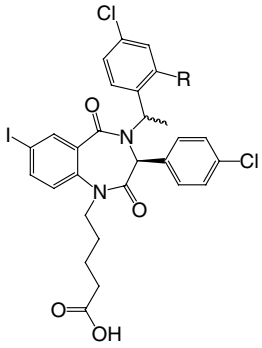


Compds	R	FP IC_{50} μM	PIG-3 production @ 1.1 μM	3.3 μM	10 μM	30 μM
1	H	0.85	60	79.7	208.7	484.3
2	NH_2	0.25		44	77	

Figure 1. IC_{50} and JAR cell normalized PIG-3 production (ELISA, ng/mg) of our previously published benzodiazepinediones.

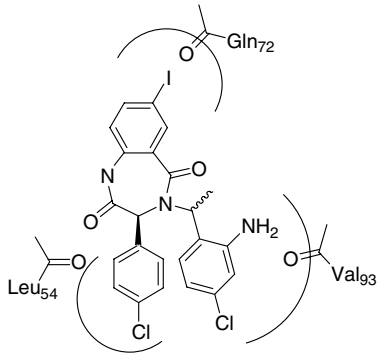
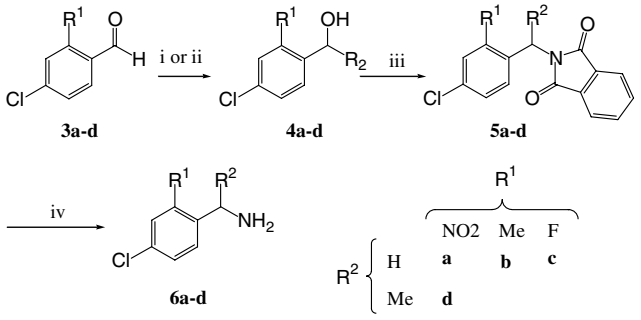
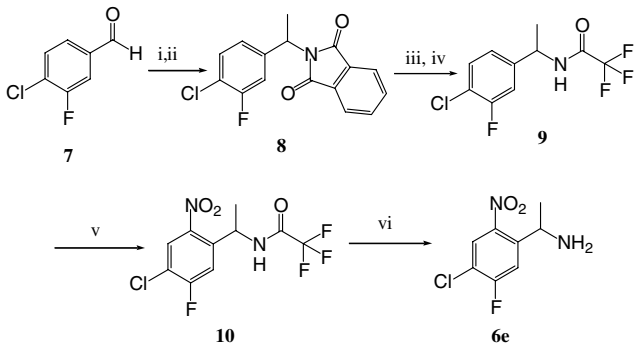


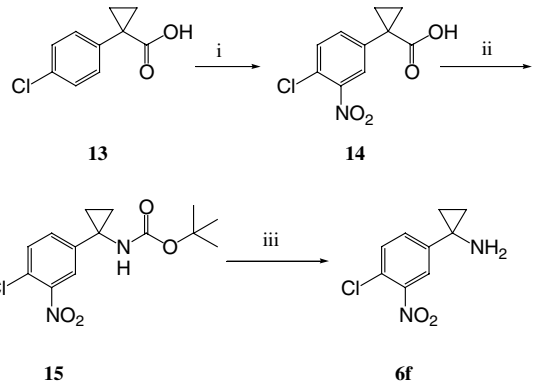
Figure 2. Schematic for the binding of the benzodiazepinedione **2** to Hdm2.



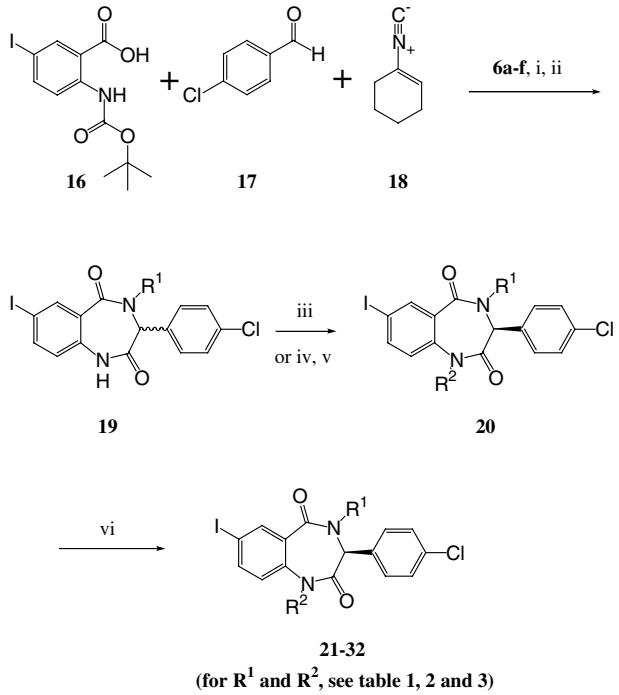
Scheme 1. Reagents and conditions: (i) $NaBH_4$, THF, rt, 1 h; (ii) MeLi, THF, −78 °C to rt, 0.5 h; (iii) phthalimide, Ph_3P, DIAD, THF, rt, 3 h; (iv) H_2NNH_2, THF, 80°C, 1 h.



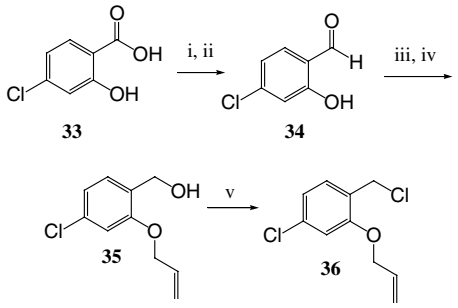
Scheme 2. Reagents and conditions: (i) MeLi, THF, −78 °C to rt, 1 h; (ii) phthalimide, Ph_3P, DIAD, THF, −78 °C to rt, 0.5 h; (iii) H_2NNH_2, THF, 80 °C, 1 h; (iv) pyridine, trifluoroacetic anhydride, 0 °C to rt, 16 h; (v) concd H_2SO_4, KNO_3, 0 °C to rt, 16 h; (vi) LiOH, THF–H_2O, rt, 3 days.



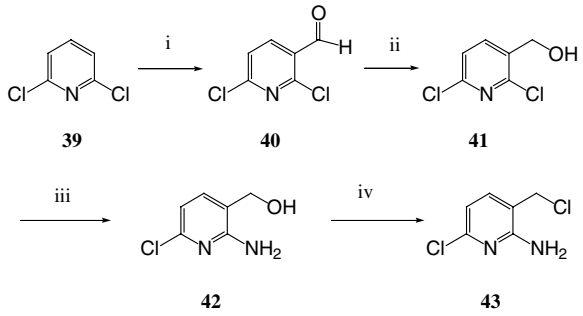
Scheme 3. Reagents and conditions: (i) concd H_2SO_4, KNO_3, 0 °C to rt, 16 h; (ii) DPPA, THF, *t*-BuOH, reflux, 3 h; (iii) TFA, CH_2Cl_2, rt, 1 h.



Scheme 4. Reagents and conditions: (i) MeOH, rt, 2 days; (ii) AcCl, rt to 60 °C, 1 h; (iii) chromatographic separation of diastereoisomers, Br-R^2, Bu_4NI, K_2CO_3, THF, 80 °C, 2 h; (iv) 1-bromo-4-chlorobutane, K_2CO_3, THF, 80 °C, 2 h; (v) 1-methylpiperazine, K_2CO_3, THF, 80 °C, 2 h; (vi) NH_4Cl, Zn^0, AcOH, 80 °C, 3 h.



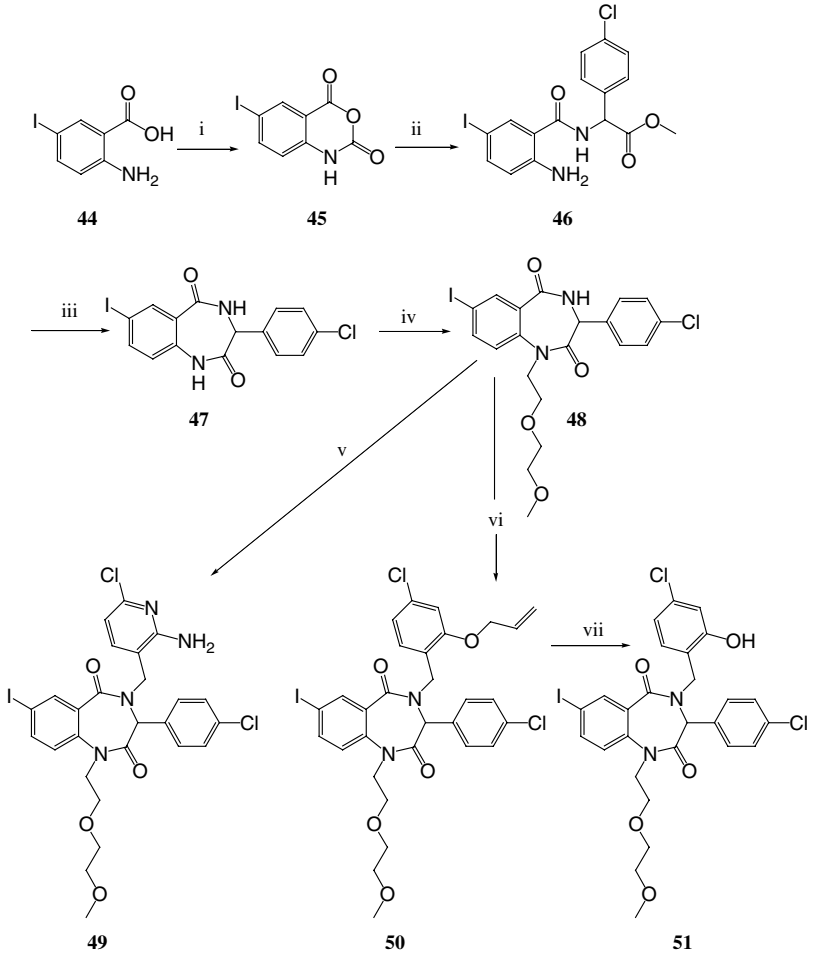
Scheme 5. Reagents and conditions: (i) borane-methyl sulfide complex, THF, 0 °C to reflux, 16 h; (ii) DDQ, CH_2Cl_2, rt, 4 h; (iii) 3-bromopropene, $CsCO_3$, THF, reflux, 3 h; (iv) $NaBH_4$, THF, rt, 2 h; (v) $SOCl_2$, CH_2Cl_2, rt, 1 h.



Scheme 6. Reagents and conditions: (i) *n*-BuLi, diisopropylamine, DMF, THF, −78 °C to rt, 1 h; (ii) $NaBH_4$, THF, rt, 1 h; (iii) NH_3, Cu^0, EtOH, 120 °C, 6 h; (iv) HCl (gas), $SOCl_2$, CH_2Cl_2, rt, 1 h.

The synthesis of the amine **6e** is reported in Scheme 2. Aldehyde **7** was reacted with methyl lithium followed by Mitsunobu reaction with phthalimide to give intermediate **8**, which was successively deprotected with hydrazine, followed by reprotection with trifluoroacetic anhydride to give amide **9**. Subsequent nitration of **9** with potassium nitrate in concentrated sulfuric acid provided the corresponding nitro derivative **10**, which was deprotected with lithium hydroxide to give the target benzylamine **6e**.

The synthesis of cyclopropylamine **6f** is depicted in Scheme 3. The commercially available acid **13** was nitrated with concentrated nitric acid to give **14**, followed by a Curtius rearrangement to produce carbamate **15**, which was deprotected in the presence of TFA to give amine **6f**.



Scheme 7. Reagents and conditions: (i) triphosgene, DIEA, THF-CH_2Cl_2, rt, 16 h; (ii) amino-(4-chlorophenyl)acetic acid methyl ester, NEt_3, DMF, 100 °C, 10 h; (iii) *t*-BuOK, THF, rt, 1 h; (iv) 1-bromo-2-(2-methoxyethoxy)ethane, K_2CO_3, THF, 80 °C, 2 h; (v) **43**, $CsCO_3$, THF, 80 °C, 3 h; (vi) **36**, $CsCO_3$, THF, 80 °C; (vii) $Cl_2PdP(Ph_3)_2$, $NaBH_4$, THF, rt, 1 h.

The benzylamines **6a–f** were engaged in a four-component Ugi reaction with 4-chlorobenzaldehyde **17**, Boc-protected anthranilic acid **16**, and 1-isocyanocyclohexene **18** (reaction inducer), which after treatment with acetyl chloride and in situ cyclization provided the target diazepinediones **19** as diastereomeric mixtures. Chromatographic separation of diastereomers and subsequent alkylation with the corresponding solubilizing groups afforded nitroderivatives **20**, which were reduced with zinc, to produce target compounds **21–32**, as racemic mixtures (Scheme 4).

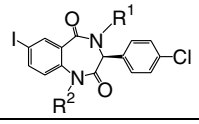
Iodoisatoic anhydride **45** (Scheme 7) was used as starting material in an alternative route to the synthesis of substituted benziodiazepinediones. Accordingly, **45** was reacted with (amino)(4-chloro phenyl)acetic acid methyl ester to provide the 3-substituted benzodiazepine core **47** followed by successive substitution at the N4-position and then at the N1-position (Scheme 7). Schemes 5 and 6 show the synthesis of the benzylic chloride intermediates. In Scheme 5, commercially available 4-chloro salicylic acid **33** was reduced to the corresponding alcohol with borane-methyl sulfide complex and then reoxidized to the aldehyde **34** using DDQ. Protection of the phenol with allyl bromide and reduction back to the alcohol yielded **35**. Finally, conversion of the alcohol to the chloride using thionyl chloride produced **36**.

In Scheme 6, 2,5-dichloropyridine **39** was converted to the corresponding aldehyde **40** in the presence of LDA and DMF. Reduction of the aldehyde with sodium borohydride gave alcohol **41**, which was treated with ammonia to selectively displace the chloride in position 2 leading to intermediate **42**. Transformation of the benzylic alcohol to the chloride **43** was achieved by treatment with thionyl chloride.

Alkylation of diazepine **48** with the two chloro derivatives **43** and **36** afforded 4-substituted derivatives **49** and **50**, respectively (Scheme 7). Subsequent unmasking of the phenol was achieved by treatment of **50** with sodium borohydride and dichloro bis (triphenylphosphine)palladium (II) to give phenol **51**.

Tables 1–3 show the IC_{50} values obtained in an FP assay[8] and JAR cell normalized PIG-3 production.[9] The introduction of a methyl group on the benzylic position of **52** to give **2** resulted in a 3-fold improvement in FP-IC_{50} value (Table 1). Although this is a significant increase in potency, there is no difference between the cellular PIG-3 production of these two compounds at 1.1 or 3.3 μM. We hypothesized that this is due to the zwitterionic character of these compounds, which limits their cell permeability. Accordingly, the replacement of the valeric acid side chain of **2** with a non-charged solubilizing group, such as the methoxyethoxyethyl side chain of **21**, led to a decrease in potency in the FP assay, but showed better results in the PIG-3 production exper-

Table 1. FP-IC_{50} and JAR cell normalized PIG-3 production (ELISA, ng/mg) of analogues bearing ethylene glycol in position 1 as the solubilizing group



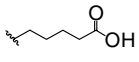
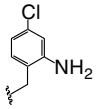
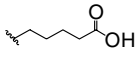
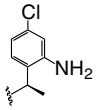
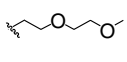
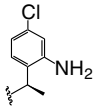
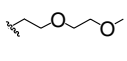
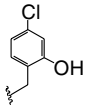
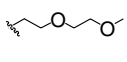
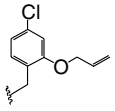
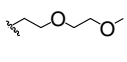
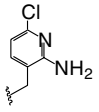
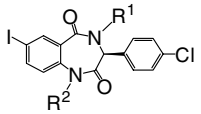
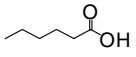
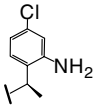
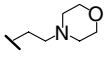
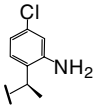
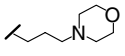
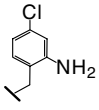
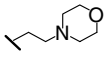
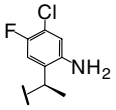
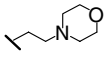
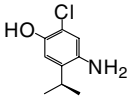
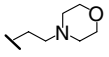
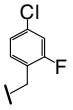
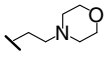
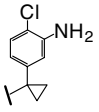
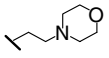
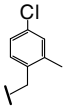
Compound	R^2	R^1	FP-IC_{50} (μM)	PIG-3 (μM) at		
				1.1	3.3	10
52			0.81	47	74	196
2			0.25	44	77	
21			0.70	78	218	375
49			2.44	62.3	74	99
50			>12.5			
51			11.6			

Table 2. FP-IC_{50} and JAR cell normalized PIG-3 production (ELISA, ng/mg) of analogues bearing a morpholino side chain in position 1 as a solubilizing group



Compound	R^2	R^1	FP-IC_{50} (μM)	PIG-3 (μM) at		
				1.1	3.3	10
2			0.25	44	77	
22			0.79	79	234	395
23			3.6	55	184	375
27			2.6	37	110	313
28			2.7	34	54	111
26			8.6			
24			25			
25			19			

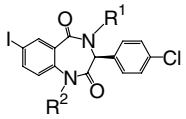
iment, indicating that this compound is able to penetrate the cell, unlike **2** and **52**. The replacement of the ortho-anilino substituent of **21**, a hydrogen bond donor, with the hydroxy group of **49** caused no significant loss of activity (assuming 3-fold loss of activity due to the lack of a benzylic methyl group). A significant decrease in activity is observed, however, with the protected hydroxyl group of **50**, as well as more soluble 2-amino-5-chloro-pyridine **51**.

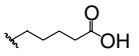
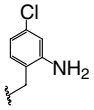
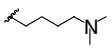
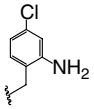
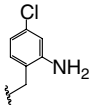
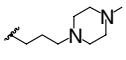
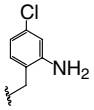
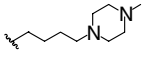
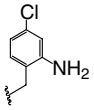
Compounds with morpholino side chains as a solubilizing group are shown in Table 2. The introduction of the morpholino group attached to the template with a two-carbon (**22**) or three-carbon (**23**) linker yielded an increase in PIG-3 production. Further modifications to the benzylic moiety of these morpholine derivatives were attempted to improve cell potency. Replacing the methyl group on the benzylic position with a cyclopropyl and moving the amino hydrogen bond donor to the meta position, as in **24** result in a significant loss of activity. When the *ortho*-amine hydrogen bond donor group is replaced by a methyl group (**25**), or a fluoro group (**26**), a loss of activity is also observed. Similarly, introduction of an electron-withdrawing fluoro substituent para to the amino group (**27**) resulted in a loss of more than 3-fold potency. Similar loss of potency was observed when the functional group in the para position of the amine is an electron-donating hydroxyl, as in **28**.[10]

Table 3 shows compounds with solubilizing chains bearing *N*-methylpiperazine or dimethylamino groups. Although, introduction of a butyl and propyl dimethylamines **29** and **31**, and the butylpiperazine **32** led to compounds with reduced cell activity, 4-methylpiperazinopropyl derivative **30** was found to be equipotent to the methoxyethoxyethyl **21** and morpholinoethyl **22** derivatives.

In summary, we have reported novel 1,4-benzodiazepine-2,5-diones with potent disrupting capacity of the binding between p53 and Hdm2. The SAR analysis

Table 3. FP-IC_{50} and JAR cell normalized PIG-3 production (ELISA, ng/mg) of analogues bearing *N*-methylpiperazine or dimethylamine functional groups in position 1 as solubilizing groups



Compound	R^2	R^1	FP-IC_{50} (μM)	PIG-3 (μM) at		
				1.1	3.3	10
52			0.81	47	74	196
29			2.83	32	79	−136
31			12.2	36	68	−92
30			5.41	57	198	385
32			5.66	43	125	32

of the benzylic position shows that the amino group in the ortho position can be replaced by a hydroxyl group, but not by methyl or fluoro, validating our hypothesis that hydrogen bond donor groups in this position can establish an extra interaction with the carbonyl of Val93 of Hdm2. Moreover, the replacement of the valeric acid solubilizing group of **2** with methoxyethoxyethyl (**21**), morpholinoethyl (**22**), and 4-methylpiperazinopropyl (**30**) resulted in the discovery of new lead compounds with improved cell-based activity as measured by the level of PIG-3 production.

References and notes

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8. The evaluation of the inhibitory activity of our compounds is based on the displacement of a peptide substrate attached in the N-terminus with fluorescein (FP assay).
9. In order to confirm that these compounds were stimulating p53 activity, we measured the induction of PIG-3 (p53 inducible gene 3) Polyak, K.; Xia, Y.; Zweier, J. L.; Kinzler, K. W.; Vogelstein, B. *Nature* **1997**, *389*, 300, JAR choriocarcinoma cells, which overexpress both Hdm2 and p53, were treated with various antagonists and cellular PIG-3 protein was measured by ELISA (Exalpha, Watertown, MA). Results greater than 100 ng PIG-3 normalized to total protein were considered positive for specific activation of p53.
10. Compound **28** was isolated as a byproduct of the reduction of the nitro functional group in the synthesis of compound **27**.