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Enhanced pharmacokinetic properties of 1,4-benzodiazepine-2,5-dione antagonists of the HDM2-p53 protein–protein interaction through structure-based drug design

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Abstract—Guided by structure-based drug design, modification of the 1,4-benzodiazepin-2,5-dione lead compound **1** resulted in the discovery of **19**, a potent and orally bioavailable antagonist of the HDM2-p53 protein–protein interaction (FP IC_{50} = 0.7 μM, $F \approx 100\%$).

The tumor suppressor protein, p53, is maintained at a low concentration in cells via redundant negative regulatory feedback loops, the main one involving the protein HDM2.[1] HDM2 binds the transactivation domain of p53, thereby targeting it for proteasomal degradation through its E3 ligase activity.[1] Antagonism of HDM2-p53 binding should increase levels of p53 and thus trigger apoptosis or cell-cycle arrest, providing an attractive approach to treating cancers possessing wildtype (wt) p53.[2–5]

Proof-of-concept studies have been performed using the HDM2 small-molecule inhibitors called 'Nutlins,' demonstrating that antagonizing HDM2 in wt-p53 tumor cell lines is a viable approach for cancer therapy.[6–8]

We previously reported 1,4-benzodiazepine-2,5-diones (BDPs) as potent antagonists of the HDM2-p53 interaction in vitro and in cell-based assays.[9–11] The lead compound **1** (Fig. 1) demonstrated rapid clearance and no bioavailability (data not shown).

The poor pharmacokinetic (PK) results were rationalized on the basis of several factors, including low solubility and poor cell membrane permeability. Although

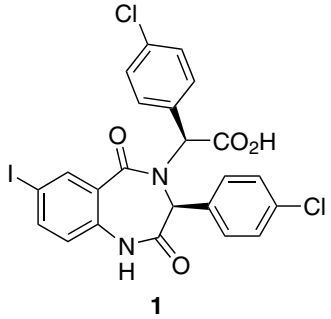

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Figure 1. The structure of the lead compound (**1**).

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the A log P[12] (4.85) does not violate the Lipinski 'Rule-of-Five,'[13] the calculated pK_a[14] of the acid moiety is 2.66 resulting in a low percentage of neutral species available (~0.002%) at physiological pH (7.4) to pass through the cell membrane.

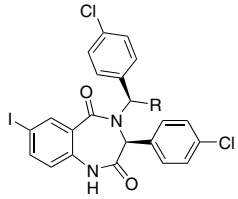
To improve cell permeability, modifications were made at the carboxylic acid site and tested for in vitro potency in the previously described fluorescence polarization (FP) assay (Table 1).[9,10] Compounds with acceptable potencies were tested for cell activity in the BrdU proliferation assay,[10,11] measuring for the preferential activity in MCF7 mammary carcinoma cells, which express wt-p53, over MDA MB231 mammary carcinoma cells expressing mutant p53.

Compound **2**, the homologue of **1**, proved to be essentially equipotent to **1** and had slightly better cell-based activity. This may be due to an increase in the acid pK_a (3.93),[14] thereby increasing the available neutral fraction at pH 7.4. Coupling of the acid with a primary amine resulted in either a loss of in vitro potency or little benefit to cell permeability as inferred from the BrdU IC_{50} values (**3**–**6**).

Since functionalization of the acid moiety was unproductive, it was hypothesized that removal of the acid would provide the best course of action. Compound **7** was synthesized using the 4-component Ugi condensation previously described,[9] but proved insoluble, precluding an accurate IC_{50} determination (Table 1). Thus, a solubilizing group was deemed necessary.

Inspection of the co-crystal structure of **1** with HDM2 (PDB ID: 1T4E)[10] demonstrated that substitution might be tolerated on the ring nitrogen (N1) since it is primarily solvent-exposed (Fig. 2). This hypothesis was tested by alkylating the ester of **1** using the Mitsunobu reaction, followed by saponification yielding the corresponding acid (Table 2).

Table 1. Modifications of the carboxylic acid moiety of **1**



Compound[a]	R	FP IC_{50} (μM)	BrdU IC_{50}[b] (μM)
1	$-CO_2H$	0.42	38 (128)
2	$-CH_2CO_2H$	0.54	24 (70)
3	$-C(O)NH(CH_2)_2OH$	2.12	na[c]
4	$-C(O)NH(CH_2)_2CO_2H$	0.85	na[c]
5	$-C(O)NH(CH_2)_4CO_2H$	0.87	31 (90)
6	$-C(O)NH(CH_2)_2NMe_2$	27	na[c]
7	H	na[c]	na[c]

[a] Racemic mixture of (*S*,*S*) and (*R*,*R*) isomers.
[b] MCF-7 (MDA MB231).
[c] Data not available.

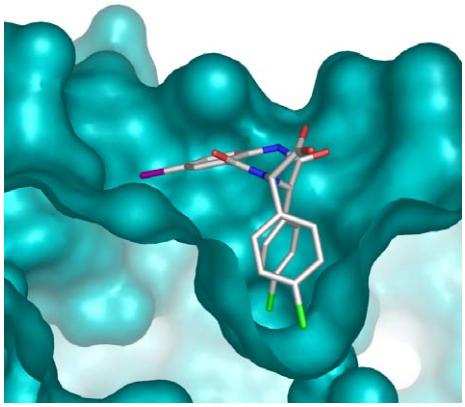
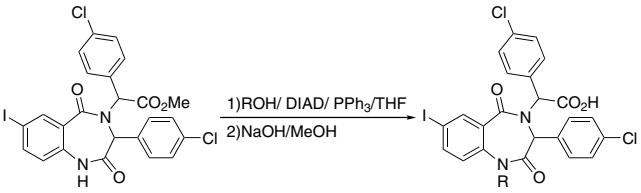


Figure 2. Cut-away view of the crystal structure of **1** bound to HDM2 (protein solvent-accessible surface, cyan; C, gray; O, red; N, blue; Cl, green; iodo, and purple; PDB ID: 1T4E).[10]

Table 2. Alkylation of **1** at N1 with solubilizing groups



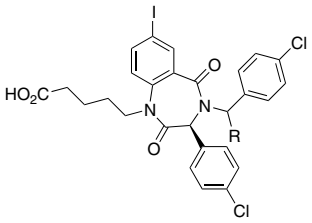
Compound[a]	R	FP IC_{50} (μM)
1	–H	0.42
8	$-CH_3$	2.18
9	$-CH_2C(O)NHMe$	3.33
10		4.35
11	$-CH_2CO_2H$	10.2
12	$-(CH_2)_2CO_2H$	1.09
13	$-(CH_2)_4CO_2H$	0.51
14	$-(CH_2)_2NMe_2$	36

[a] Racemic mixture of (*S*,*S*) and (*R*,*R*) isomers.

Tolerance to substitution of N1 is very sensitive to chain length. Short-chain solubilizing groups resulted in at least a 5-fold reduction in binding affinity (**8**–**11**), with the acid **11** showing the greatest potency loss. When the chain linking the carboxylic acid moiety to N1 was extended by one methylene, binding was improved 10-fold (**12** vs **11**). Expanding the chain length further (**13**) produced a compound essentially equipotent to the unsubstituted benzodiazepine (**1**). In contrast, utilizing a basic solubilizing group resulted in an essentially inactive molecule (**14**). On the basis of these results, the pentanoic acid group was selected as the substituent of choice for further investigation.

BDP analogues incorporating the pentanoic acid side chain as a solubilizing group were synthesized (Table 3). The ester precursor to **13** (**15**) showed a decrease in binding affinity, as did the diethylamido derivative (**16**). These results suggest that the carboxylate may be interacting with the HDM2 protein in a way not accessible to the ester or amide derivatives. Indeed, one of the two monomers in the unit cell of

Table 3. Optimization of BDPs with pentanoic acid side chain



Compound	R	FP IC_{50} (μM)	BrdU IC_{50}[e] (μM)
13[a]	$-CO_2H$	0.51 ± 0.03	38 (128)
15[a]	$-CO_2Me$	2.82 ± 0.06	25 (67)
16[a]	$-C(O)NEt_2$	2.13 ± 0.27	9 (37)
17[b]	H	8.22 ± 1.5	13 (60)
18[c]	(*S*)-CH_3	13.3 ± 1.9	na[f]
19[c]	(*R*)-CH_3	0.70 ± 0.02	7 (67)
20[d]	*c*–C_3H_5	2.15 ± 0.13	na[f]

[a] Racemic mixture of (*S*,*S*) and (*R*,*R*) isomers.
[b] Racemic at C3.
[c] Chirality at C3 is (*S*).
[d] Racemic mixture of (*S*,*R*) and (*R*,*S*) isomers.
[e] MCF-7 (MDA MB231).
[f] Data not available.

the HDM2/**1** co-crystal structure shows a hydrogen bond between the antagonist carboxylate and the side chain hydroxyl of Ser-17, the N-terminal residue in the construct we typically used (Fig. 3).[10] Furthermore, binding affinity is not greatly affected by extension of the N-terminus (HDM2[2-188] vs HDM2[17-125]; 0.39 μM vs 0.59 μM, respectively), suggesting that this binding contact may not be an artifact of truncation. Thus, the BDP carboxylate-Ser17 contact may be an important contributor to the binding observed for these antagonists.

Conformational factors may also contribute to the observed effects of acid modification (Table 3). Removal of the acid function (**17**) yielded a 16-fold loss in binding affinity. Conformational analysis with **13** established that the presence of the carboxylate favors the observed binding conformation (Fig. 2) through steric repulsion with the ring carbonyl oxygen, essentially locking out rotation around the single N–C bond (Fig. 4a). Removal

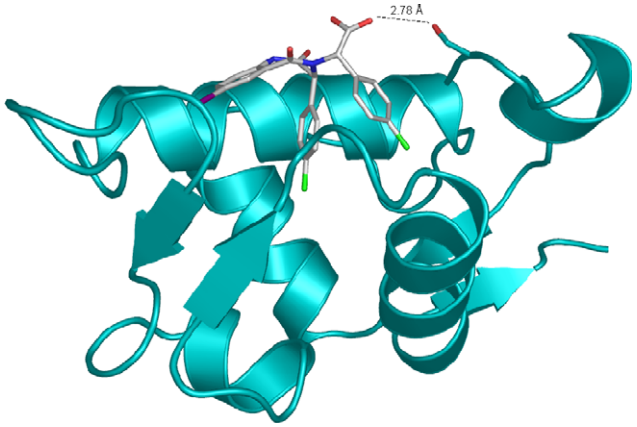


Figure 3. Crystal structure of **1** bound to HDM2 demonstrating the HDM2 Ser17-BDP carboxylate H-bond (PDB ID: 1T4E).[10]

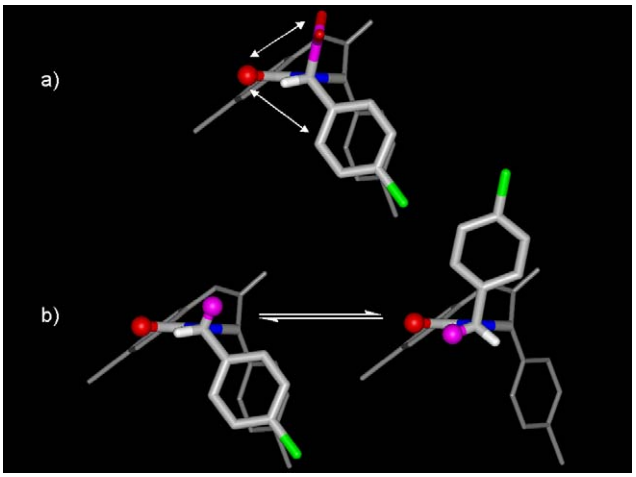


Figure 4. (a) Steric repulsion between the carboxylate and amide carbonyl favoring the bound conformation of **1** (and **13**). (b) The two main conformers of **7** (and **17**) looking down the C–N bond. The benzylic hydrogens were differentially colored to highlight steric interactions with the amide carbonyl.

of the acid lowers the barrier to rotation, permitting multiple conformations (Fig. 4b).

We tested our hypothesis regarding the importance of conformational rigidity using an enantiomerically pure α-methylbenzylamine in the BDP synthesis. Compounds **18** and **19** were generated from the *S*- and *R*-enantiomers, respectively. Consistent with the importance of conformational bias through restricted rotation, the (*R*)-enantiomer (**19**), for which the observed binding conformation is favored, has similar binding affinity to that of the parent acid **13** (Table 3).

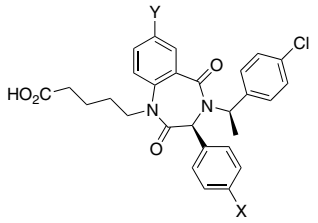
In contrast, the (*S*)-enantiomer (**18**), favoring a different conformation that, if bound, would leave one of the three major hydrophobic interaction sites vacant, is much less active (Figs. 2 and 4). The larger cyclopropyl analogue (**20**) has a similar potency to the ester or amide (Table 3).

Figure 4b can be used to demonstrate the two BDP conformations. For **19**, the pink hydrogen represents the methyl group so the conformer on the left is favored due to minimization of the steric repulsion between the carbonyl oxygen and methyl substituent. Conversely, for **18**, the white hydrogen represents the methyl group so minimizing steric interactions would favor the right-hand conformer.

The cell-based activity of **19** was enhanced 5-fold over **1** (and **13**), suggesting that the compound was more cell permeable. This was consistent with the observed CACO-2 assay results (Table 4). Additional analogues were prepared based on the SAR developed for the original lead series,[9] and these proved to be similar in potency to **19** in both in vitro and cell-based assays (Table 4).

With compounds possessing desirable early ADME properties in hand, the PK profile of **19** was determined in mice. Compound **19** was administered intravenously (iv) and orally (po) at 5 and 40 mg/kg, respectively.

Table 4. FP, proliferation, and CACO-2 assay results of BDP analogues



Compound[a]	X	Y	FP IC_{50} (μM)	BrdU IC_{50}[b] (μM)	CACO-2 P_{app}($\times 10^{-6}$) A → B(B → A)
19	Cl	I	0.70 ± 0.02	7 (67)	7.34 (13.0)
21	CF_3	I	0.98 ± 0.02	11 (30)	10.3 (9.6)
22	OCF_3	I	1.04 ± 0.03	19 (67)	na[c]
23	Cl	C≡CH	0.71 ± 0.01	10.3 (69)	20.9 (16.1)
24	Cl	C≡CCH_3	1.11 ± 0.08	10.7 (30)	19.9 (9.1)

[a] Pure enantiomer with chirality as shown.
[b] MCF-7 (MDA MB231).
[c] Data not available.

The time course of the plasma concentration of **19** is shown in Figure 5.

The iv dosing shows a relatively smooth decrease in plasma concentration over time, with a half-life ($t_{1/2}$) of 3.1 h. The clearance of **19** was 13.2 mL/min/kg and the volume of distribution was 3.0 L/kg (both values are normalized for animal weight).

Oral administration of **19** resulted in a C_{max} of 6.3 μM after 6 h (T_{max}). The $t_{1/2}$ was 2.5 h. The truncated area-under-the-curve values were used to determine the approximate bioavailability of 100% in mice.

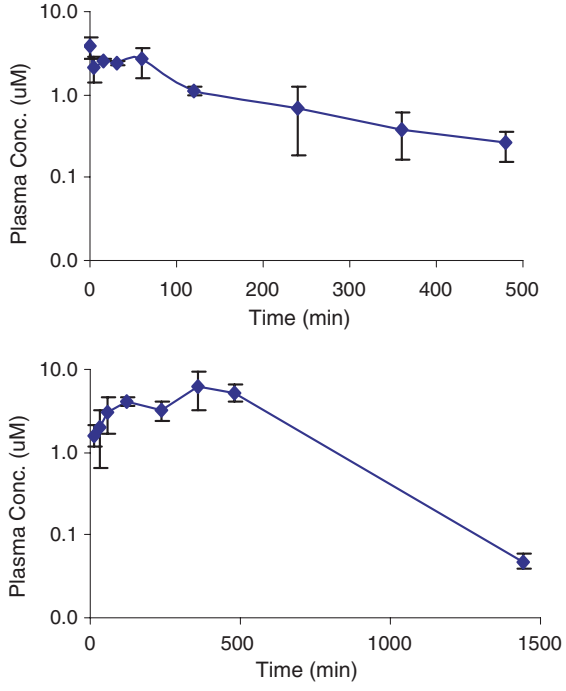


Figure 5. Average plasma concentration versus time profiles of **19** after iv (above) and po (below) administration to mice at doses of 5 and 40 mg/kg, respectively (±standard error for $n = 3$).

In summary, structural modification of the poorly bioavailable BDP inhibitor of the HDM2-p53 protein–protein interaction (**1**) was performed under the guidance of structure-based design. Several iterations ultimately led to the discovery of an orally bioavailable and potent drug-like antagonist (**19**). With **19** or one of the structurally related analogues in hand (**21**–**24**), proof-of-concept in vivo studies were performed and have been described in detail elsewhere.[11]

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