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Bioorganic & Medicinal Chemistry Letters 15 (2005) 1857–1861
Bioorganic & Medicinal Chemistry Letters

Structure-based design, synthesis, and biological evaluation of novel 1,4-diazepines as HDM2 antagonists

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Received 28 July 2004; revised 6 February 2005; accepted 7 February 2005

Abstract—Crystallographic analysis of ligands bound to HDM2 suggested that 7-substituted 1,4-diazepine-2,5-diones could mimic the α-helix of p53 peptide and may represent a promising scaffold to develop HDM2–p53 antagonists. To verify this hypothesis, we synthesized and biologically evaluated 5-[(3*S*)-3-(4-chlorophenyl)-4-[(*R*)-1-(4-chlorophenyl)ethyl]-2,5-dioxo-7-phenyl-1,4-diazepin-1-yl]valeric acid (**10**) and 5-[(3*S*)-7-(2-bromophenyl)-3-(4-chlorophenyl)-4-[(*R*)-1-(4-chlorophenyl)ethyl]-2,5-dioxo-1,4-diazepin-1-yl]valeric acid (**11**). Preliminary in vitro testing shows that **10** and **11** substantially antagonize the binding between HDM2 and p53 with an IC_{50} of 13 and 3.6 μM, respectively, validating the modeling predictions. Taken together with the high cell permeability of diazepine **11** determined in CACO-2 cells, these results suggest that 1,4-diazepine-2,5-diones may be useful in the treatment of certain cancers.

The *hdm2* oncogene product (HDM2) is an ubiquitin protein ligase[1,2] that suppresses the transcriptional activity of the tumor suppressor p53 and promotes its rapid degradation through the ubiquitin proteolysis pathway.[3–5] Since p53 is the most frequently inactivated protein in human cancers,[6] small molecule antagonists of the HDM2–p53 protein–protein interaction appear to offer an attractive strategy for cancer therapy, because it offers the possibility to up-regulate the p53 response.[7–9] After the recent publication by Roche scientists of the 'nutlins',[10] a series of imidazoline antagonists of HDM2–p53 interaction (compounds **1**–**3**, Chart 1) found to activate the p53 pathway in cancer cells, the p53/HDM2 target has received increasing attention in the scientific community.

Moreover, disruption of the protein–protein interaction with low-molecular weight compounds has been revisited since compounds **1**–**3** were found to activate the p53 pathway in cancer cells, leading to cell arrest, apoptosis and growth inhibition.[10] Crystallographic analysis established that the reported imidazolines **1**–**3** bind to HDM2 in the p53-helix binding site.[10]

Independently, from a library of α-(acylamino)amides screened with our proprietary high-throughput miniaturized thermal denaturation assay ThermoFluor®,[11] compound **4** (Chart 1) emerged as a new small molecule antagonist of the HDM2–p53 interaction (IC_{50} = 15 μM).[12] This hit **4** was shown to bind to HDM2 in the p53-binding pocket using a fluorescence polarization (FP) peptide displacement assay.[13] The rigidification of this highly flexible open structure by incorporation of an amide linkage between two of the three phenyl rings of compound **4** (Fig. 1) led to the discovery of the 1,4-benzodiazepine-2,5-dione **5**, which is approximately 10-fold more active than **4** (IC_{50} = 1.7 μM).[12,14] Further structure–activity relationship studies led to the identification of benzodiazepine **6** as one of the most potent HDM2 antagonists reported so far (IC_{50} = 220 nM).[14,15] Cinnamoyl amide **7** was also identified in our library to significantly antagonize the binding between HDM2 and p53 (IC_{50} = 30 μM).[12]

Keywords: HDM2–p53; Diazepine; Selenium; Cancer.
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0960-894X/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2005.02.018

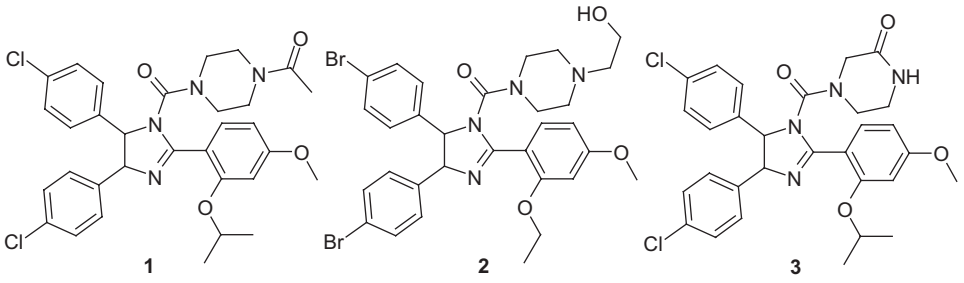


Chart 1. HDM2 antagonists.[10]

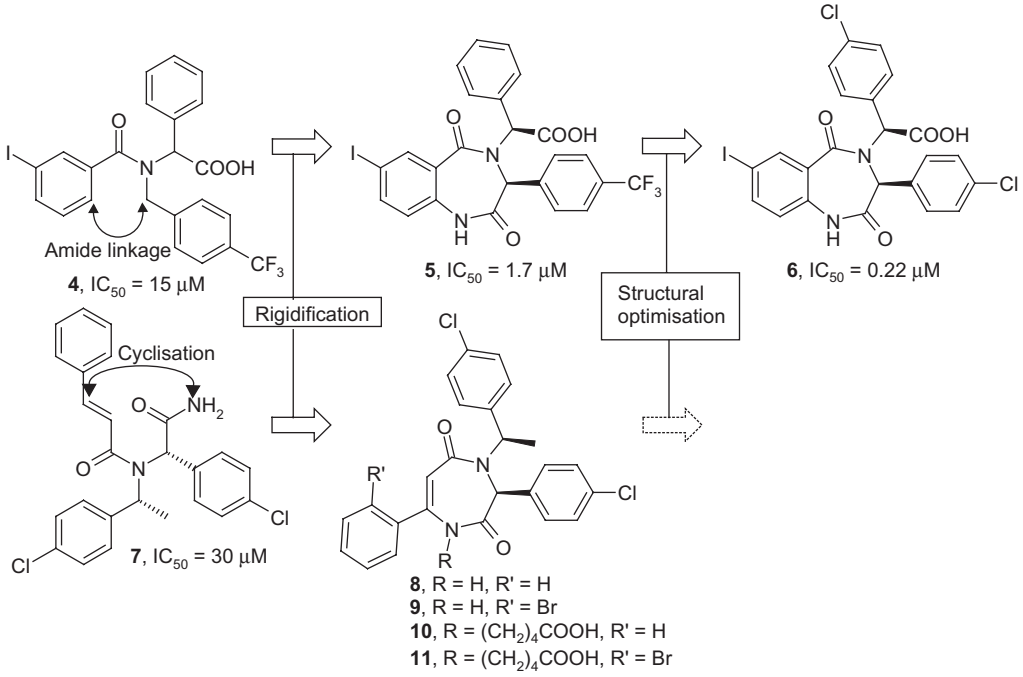


Figure 1. Design of HDM2 antagonists.

Following the same 'rigidification' strategy, which successfully led to the identification of the benzodiazepinediones **5** and **6**, we hypothesized that the 1,4-diazepinedione **8** (Chart 1) might act as a constrained version of **7** and thereby lock the structure in the desired bioactive conformation. Alternatively, **8** could be viewed as a *seco* analogue of compound **6**, where the phenyl ring of the benzodiazepine system has been detached from the diazepine ring. In this communication we report the rational design, synthesis and in vitro evaluation of an original series of potent diazepine antagonists of the HDM2–p53 binding. The CACO-2 cell permeability was also assessed for the target compounds **10** and **11**.

Recent crystallographic studies of HDM2 complexed with a p53-derived helical peptide have defined a deep hydrophobic binding pocket.[16] Side chains from the hydrophobic face of two turns of the p53 helix, Phe19, Trp23, and Leu26 (i, $i+4$, $i+7$), occupy this hydrophobic pocket on the HDM2 surface. The crystal structure of our lead compound **6** bound to the same site on HDM2 (PDB[17] ID: 1T4E) establishes that the (benzo)diazepinedione scaffold projects the three halophenyl rings of **6** into the regions occupied by the Phe19, Trp23, and Leu26 side chains in the peptide complex.[15] As such, the benzodiazepinedione scaffold serves as an α-helix mimetic, and other diazepine-based scaffolds are also of interest in this context. Although **4** can access the analogous conformation to that observed for **6** bound to HDM2, it is reasonable to presume that some or all of the difference observed in binding potency is due to the increased flexibility of **4** versus the constrained analog **6** (Chart 1). Alternative modes of cyclization or rigidification are of interest, since the 1,4-benzodiazepine-2,5-dione does present inherent limitations given the observed binding mode. One such limitation relates to access to the pocket occupied by Phe19 in the peptide complex. The orientation and rigidity of the iodophenyl ring precludes access to the bottom of the region occupied by the Phe19 side chain (Fig. 2). The observed SAR for phenyl substituents and alternative fused rings supports this contention.[12,14,15] Figure 2b shows that the orientation of Phe19 differs from that of the iodophenyl ring of **6**, and highlights the inaccessibility of the space immediately below the plane of the iodophenyl ring.

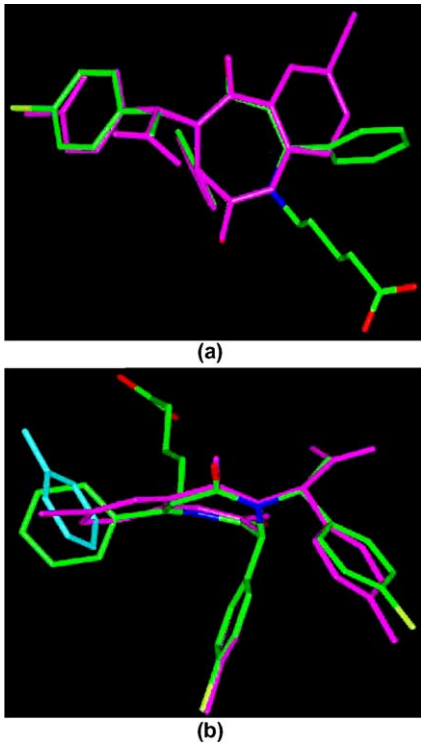
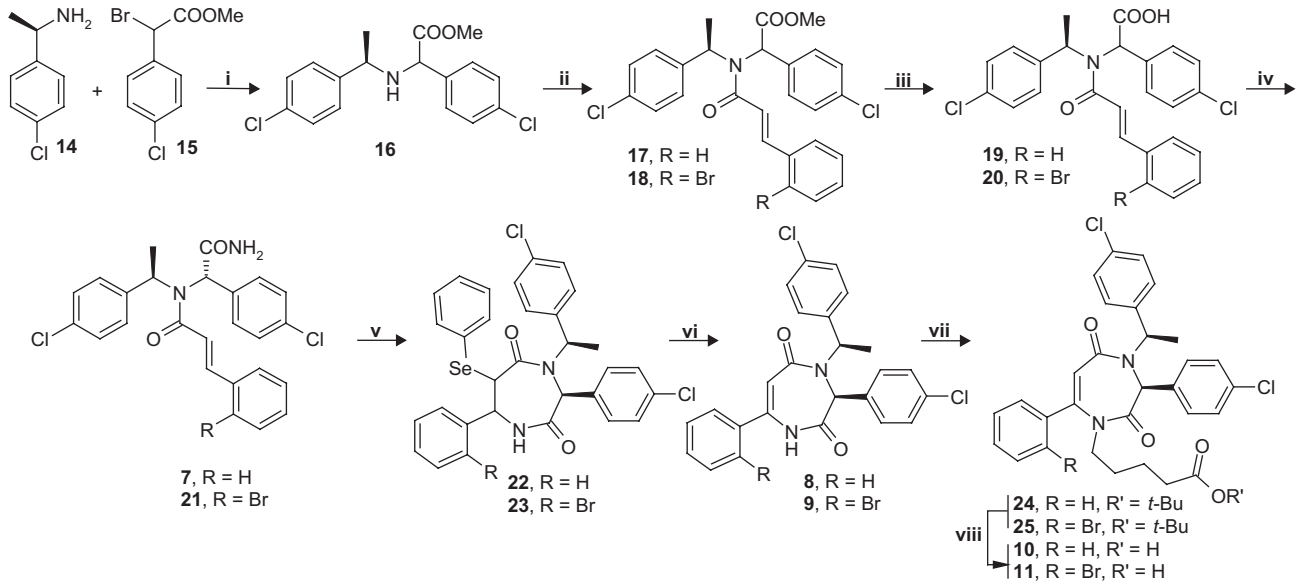


Figure 2. Superposition of a model of the low energy conformer of 1,4-diazepine-2,5-dione **10** (color-by-atom) onto the benzodiazepinedione **6** (purple) bound to HDM2. In the lower panel the Phe sidechain of a bound peptide from a different complex is also shown (light blue).

The diazepinedione scaffold embodied in **8**–**11** (Fig. 1) was designed as an alternative to the benzodiazepinedione parent. The former essentially represents the parent with the iodophenyl ring removed and modified at the newly unfused position (Fig. 1). Conformational analysis indicated that the preference for the bound conformation observed for **6** would be maintained in the modified scaffold. This new scaffold would therefore provide an alternate mode of access to the Phe19-binding region, while maintaining the orientation of the other two side chain mimics of the parent **6** (Fig. 1). We conjectured that the limited flexibility of the phenyl–diazepinedione linkage might allow for improved (or alternative) access to the deepest part of the Phe19 binding region that is not achievable with the benzodiazepinedione framework. In the binding models that were explored, the acid solubilizing moieties of **10** and **11** are expected to be largely solvent exposed and to not have a major impact on binding. This hypothesis was previously verified in the benzodiazepine series: compounds bearing a valeryl solubilizing group in position 1 instead of position 4 were synthesized and found to be equipotent with **6**.[12] With the aim of verifying this model-based hypothesis, **10** and **11** were synthesized and tested for their ability to antagonize the HDM2–p53 protein–protein interaction. To the best of our knowledge, there is only one example of the 'nonfused' 1,4-diazepine-2,5-dione reported in the literature.[18] This cyclic proline derivative **13** was generated by the cyclofunctionalization of an olefinic (*S*)-*N*-(α,β-unsaturated)acylprolinamide **12** using an interesting organoselenium-induced cyclofunctionalization (Scheme 1). The structural identity and stereochemistry of compound **13** have been unambiguously determined by X-ray diffraction analysis.[18] Thus, the organoselenium-induced lactamization reported by Chung et al.[18] was found to be a suitable reaction for the synthesis of the desired HDM2 antagonists **10** and **11**. These two diazepines (**10** and **11**) were synthesized according to the procedure outlined in Scheme 2.

Scheme 1. Synthesis of diazepine **13** by the phenylselenium-induced lactamization of **12**.[18]

Alkylation of the (*R*)-benzylamine **14** with ethyl α-bromo-4-chlorophenylacetate **15** afforded the ester **16**, which was successively acylated with cinnamoyl chlorides and saponified to give the desired acids **19** and **20**. Reaction of **19** and **20** with ammonium chloride, HOBT and *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate afforded, after separation from the undesired diastereomers by silica gel chromatography, the two key amide substrates **7** and **21** required for the organoselenium-induced lactamization reaction. The identity of the diastereomers **7** and **21** was investigated using a series of NMR experiments in conjunction with molecular modeling, which have established that the active diastereomer as having the (*R*,*S*) configuration. Treatment of electron-deficient olefins **7** and **21** with phenylselenium bromide, silver triflate and dimethylformamide in dry acetonitrile gave the desired seleno seven-membered bislactams **22** and **23**. The oxidative intramolecular deselenylation of compounds **22** and **23** with hydrogen peroxide in tetrahydrofuran afforded the target diazepines **8** and **9**. Introduction of the acid solubilizing group was achieved by the alkylation of **8** and **9** with 5-bromovaleric acid *tert*-butyl ester and subsequent deprotection of the acid moiety to afford the desired final diazepine products **10** and **11**. The new diazepines **10** and **11** (Fig. 1) prepared in the present study were first tested for their ability to antagonize the binding between HDM2 and p53 in the fluorescence peptide (FP) displacement assay as described earlier.[13,14] The polarization of a fluorescein-labeled p53 peptide analog was measured by exciting at 485 nm and monitoring emission at 530 nm. The change in polarization upon displacement of the peptide from HDM2 (residues 17–125) by an antagonist was expressed as a percent with respect to the fluoresceinated peptide control.[13] Results are summarized in Table 1. Although compounds **8** and **9** are not soluble enough to be tested in this standard assay, the diazepine **10** was found to be twofold more potent than the corresponding open structure **7**, validating the rigidification approach suggested by the modeling experiments. This effect was even stronger with the more constrained bromo derivative **11**, which was found to be approximately



Scheme 2. Reagents and conditions: (i) K_2CO_3, TBAI, CH_3CN; (ii) PhCH=CHCOCl, pyridine, $CHCl_3$; (iii) NaOH, MeOH, H_2O, THF; (iv) NH_4Cl, HOBT, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DIPEA, DMF; (v) PhSeBr, AgOTf, DMF, CH_3CN; (vi) H_2O_2, THF; (vii) $Br(CH_2)_4COOt$-Bu, K_2CO_3, DMF; (viii) TFA, CH_2Cl_2.

Table 1. Binding competition between HDM2 and p53 determined with the fluorescence peptide (F1 9mer) assay[13]

Compound	HDM2-FP_1 binding IC_{50} (μM)
5[12]	1.7
6[12]	0.2
7[12]	30
10	13
11	3.6

10-fold more potent than **7** (IC_{50} of 3.6 and 30 μM, respectively). Furthermore, compound **11** was tested in a CACO-2 cell monolayer assay,[19] a widely accepted in vitro model to predict intestinal drug permeability in humans.[20] The apparent permeability coefficient (P_{app}) from the apical to the basolateral side was determined to be 27.1×10^{-6} cm/s. Based on this excellent P_{app} coefficient, compound **11** is expected to have good gastrointestinal permeability.[19,20]

In summary, we have described the structure-based design of an original series of 1,4-diazepine-2,5-diones that act as antagonists of the HDM2–p53 protein–protein interaction. These compounds were synthesized enantiomerically pure, using the previously reported organoselenium-induced cyclofunctionalization followed by a reductive elimination of the seleno moiety and the subsequent introduction of the acid solubilizing group in position 1. Conformational constraint yielded improved potency, with the IC_{50} decreasing from 30 μM for unconstrained analogue (*S*)-α-(4-chlorophenyl)-α-[(*R*)-*N*-[1-(4-chlorophenyl)ethyl]-*N*-(cinnamoyl)amino]acetamide (**7**) to 3.6 μM for the constrained version 5-[(3*S*)-7-(2-bromophenyl)-3-(4-chlorophenyl)-4-[(*R*)-1-(4-chlorophenyl)ethyl]-2,5-dioxo-1,4-diazepin-1-yl]valeric acid (**11**), providing one of the most potent series of small molecule HDM2 antagonists reported to date. Moreover, diazepine **11** exhibits good CACO-2 cell permeability (P_{app} apical to basolateral = 27.1×10^{-6} cm/s). These promising preliminary results have encouraged us to undertake systematic structural optimization of the three phenyl groups in positions 3, 4, and 7 of the diazepine heterocycle, the results of which will be reported in due course together with cell-based activity.

More generally, these results strongly support the hypothesis that, like the benzodiazepine-2,5-dione scaffold, the 1,4-diazepine-2,5-dione *seco* bioisoster is mimicking the α-helix of the natural p53 protein with respect to the HDM2 binding interaction. These and related scaffolds could serve in the future as templates for the design of novel, nonpeptidic small molecule α-helix proteomimetics in the quest for antagonists of protein–protein interactions.

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11. The ThermoFluor® screening method is a high-throughput, direct binding assay that measures the affinity of compounds toward a target protein. Hits are identified by a shift in T_m greater than three times the standard deviation (~0.2 °C) at a standard concentration. By measuring the compound-dependent difference in protein melting temperature (T_m) at a series of compound concentrations, the dissociation constant (K_d) of that compound can be calculated. More details can be found in: Pantoliano, M. W.; Petrella, E. C.; Kwasnoski, J. D.; Lobanov, V. S.; Myslik, J.; Graf, E.; Carver, T.; Asel, E.; Springer, B. A.; Lane, P.; Salemme, F. R. *J. Biomol. Screen.* **2001**, *6*, 429.
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13. Typical FP assay conditions were as follows: 20 nM HDM2 (17–125), 5 nM fluorescein-labeled p53-derived 9-mer peptide (N-terminal fluorescein-RFMDYWEGL, K_d = 20 nM), 50 mM Hepes pH 7.5, 150 mM NaCl, 3 mM β-octyl glucopyranoside, and 2.5% DMSO. Compound, HDM2 and peptide were incubated for 15–20 min at room temperature prior to reading the fluorescence polarization (excitation = 485 nM, emission = 530 nM).
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