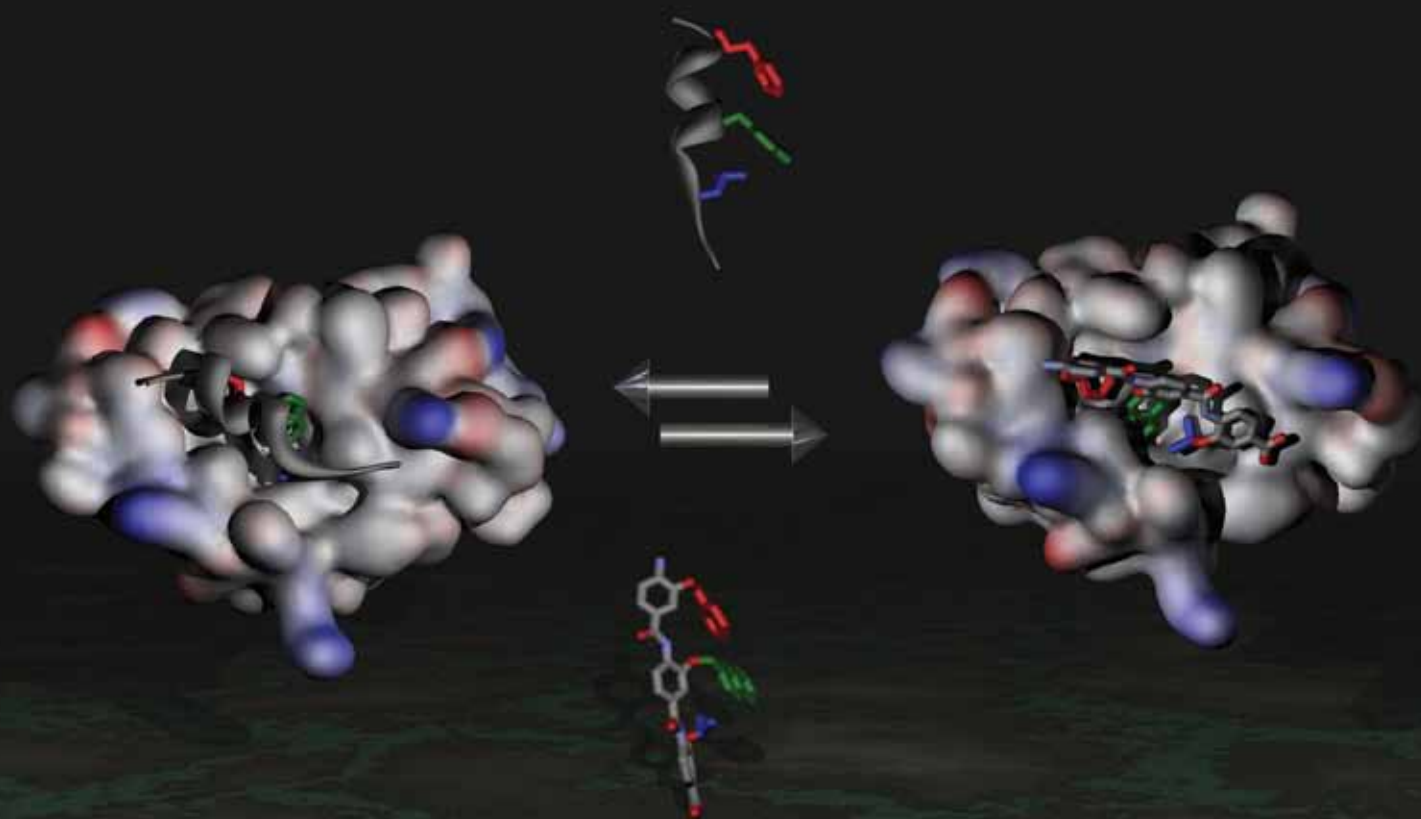


ChemComm

Chemical Communications

www.rsc.org/chemcomm

Number 34 | 14 September 2009 | Pages 5045–5184



ISSN 1359-7345

COMMUNICATION

Jeffrey P. Plante, Thomas Burnley, Barbora Malkova, Michael E. Webb,
Stuart L. Warriner, Thomas A. Edwards and Andrew J. Wilson
Oligobenzamide proteomimetic inhibitors of the p53-*h*DM2 protein-protein
interaction

RSC Publishing

Oligobenzamide proteomimetic inhibitors of the p53–hDM2 protein–protein interaction†

Jeffrey P. Plante,^{ab} Thomas Burnley,^b Barbora Malkova,^b Michael E. Webb,^{ab} Stuart L. Warriner,^{ab} Thomas A. Edwards^{*b} and Andrew J. Wilson^{*ab}

Received (in Cambridge, UK) 24th April 2009, Accepted 10th June 2009

First published as an Advance Article on the web 24th June 2009

DOI: 10.1039/b908207g

Oligobenzamide inhibitors of the p53–hDM2 protein–protein interaction are described.

Although many cellular processes depend on enzymatic reactions, protein–protein interactions (PPIs) mediate many regulatory pathways—the explosion of interest in their study mirrors a key role in diseased states.¹ Small molecules that selectively target PPIs are therefore urgently required.^{2,3} What is not clear is how to achieve inhibition using a small molecule, given that it must cover ~800–1100 Å² of a protein surface and complement the discontinuous projection of hydrophobic and charged domains on a relatively shapeless surface.⁴ In the *proteomimetic*⁵ approach a scaffold is used to project binding functionality in an identical spatial orientation to mimic that presented by a given secondary structure (commonly an α -helix) involved in the interaction.⁶ In addition to Hamilton's terphenyl,^{5,7} a number of proteomimetic scaffolds have been described.^{8–15} Similarly, foldamers,¹⁶ if suitably designed, function as inhibitors of PPIs.^{17–19} Aromatic oligoamides are particularly attractive as foldamers because they exhibit predictable folding patterns controlled largely by the preferred conformation of the Aryl–NHCO–Aryl bond and adjacent *ortho* interactions,^{20,21} however, such foldamers have rarely been shown to act as α -helix mimicking antagonists of PPIs.^{10,22,23}

During our studies on aromatic oligoamides,^{24,25} we reported a modular synthesis of rod shaped aromatic oligobenzamides that incorporate different side chains *via* an *O*-alkyl substituent.²⁵ Elsewhere, trimers of this type have been proposed to act as α -helix mimetics^{23,26,27} and shown to act as inhibitors of PPIs.²³ We now report that such compounds act as low μ M affinity inhibitors of the p53–hDM2 protein–protein interaction. p53 is the major tumour suppressor in humans and is regulated by binding to hDM2.²⁸ Over-expression of hDM2 prevents p53 from exerting its pro-apoptotic activity and so this PPI is a major target for cancer chemotherapy, with several examples of small molecule inhibitors reported.^{29–33} The interaction between p53 and hDM2 involves three key hydrophobic residues Phe19,

Trp23 and Leu26 from p53 binding in a helical conformation to a hydrophobic cleft on hDM2 (Fig. 1).³⁴

Our design was driven by two criteria: (i) the scaffold should maintain sufficient flexibility so as to optimise its conformation for maximum binding affinity and (ii) mimetic synthesis should be amenable to library generation. The scaffold should also position side chains to mimic the key residues at *i*, *i* + 4 and *i* + 7 of the p53 helix (Fig. 2a). Shown in Fig. 2b is the minimum energy conformation of one such trimeric benzamide as identified by a *Monte Carlo* search in MacroModel using the *MMFFs* force field (see ESI† for details). Pleasingly, the *O*-alkyl substituents mimic the spatial orientation of the *i*, *i* + 4 and *i* + 7 residues of the p53 α -helix reasonably well. Shown in Fig. 2c is mimetic **1aec** superimposed upon the p53 α -helix—the RMSD = (0.1245 Å) indicates a good geometrical match between the oxygen of the scaffold and the α -carbons of the helix. For this amino-terminated tribenzamide, the *O*-alkyl substituents all reside on the same face—in contrast to the results obtained when a nitro group is at the N-terminus of the tribenzamide as described earlier.²⁵ However, conformational analysis in solution reveals these compounds also adopt an extended conformation with intramolecular hydrogen-bonding fixing rotation about the Ar–NH bond and free rotation about the CO–Ar bond (see ESI†).

We prepared a series of compounds (Fig. 3) and initially tested compound **1aec** in a fluorescence anisotropy assay described previously.³⁵ The assay involves displacement of a fluorescein labelled analogue of p53 : p53_{15–31Flu}, from hDM2 with concomitant loss of anisotropy. In our hands, direct titration of p53_{15–31Flu} with hDM2 resulted in the expected increase in fluorescence anisotropy and a dissociation constant of 74 nM was determined based on a 1 : 1 isotherm (Fig. S2, ESI†). Upon titration with mimetic **1aec**, the expected

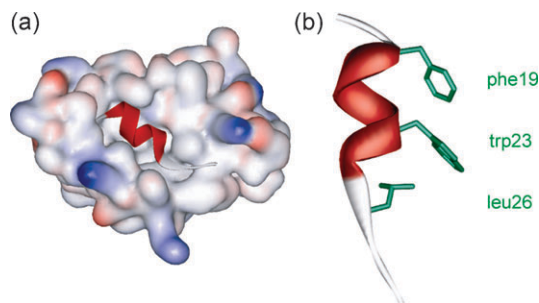


Fig. 1 (a) Crystal structure of hDM2 in complex with a p53 peptide (PDB ID: 1YCR) and (b) the p53 peptide showing side chains key for binding.

^a School of Chemistry, University of Leeds, Woodhouse Lane, Leeds, UK LS29JT. E-mail: A.J.Wilson@leeds.ac.uk; Fax: +44 (0)113 3431409; Tel: +44 (0)113 3436565

^b Astbury Centre for Structural Molecular Biology, University of Leeds, Woodhouse Lane, Leeds, UK LS29JT. E-mail: T.A.Edwards@leeds.ac.uk

† Electronic supplementary information (ESI) available: Synthetic procedures, protein expression and FP assays. See DOI: 10.1039/b908207g

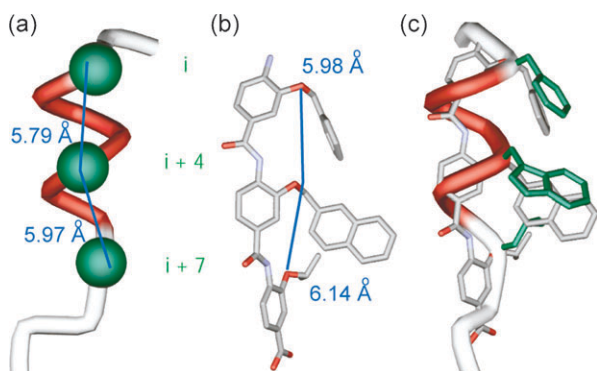


Fig. 2 (a) p53 helix depicting key side chains (in green); (b) minimised structure of an aromatic oligoamide with $R^3 = \text{Bn}$, $R^2 = \text{Me-2-Naph}$ and $R^1 = \text{iPr}$ (carbon in grey, oxygen in red and nitrogen in light purple); (c) p53 α -helix superimposed onto minimised aromatic oligoamide.

decrease in anisotropy was observed and an IC_{50} of $1.0 \mu\text{M}$ was determined (Fig. 4 and Table 1). Similarly, a decrease in anisotropy was observed for the positive control; unlabelled p53 peptide (**p53_{15-31Flu}**) gave a IC_{50} of $1.2 \mu\text{M}$. We then tested a further series of mimetics **1** and **2** containing different side chains to probe for side chain specificity. Compounds **2aa** and **2ba** are negative controls, which do not possess a sufficient number of side chains to mimic two turns of an α -helix. Compounds **1aaa**, **1bca**, **1acd**, **1ace** and **1adc** contain a selection of side chains matched and mismatched to the sequence of p53 (naphthyl acting as a mimetic of indole). The results of our screening are summarised in Table 1 (see also Fig. S4, ESI†). As predicted, compounds **2aa** and **2ba** were both inactive under the conditions of our assay. The remaining compounds were found to inhibit the interaction with low μM affinity: compounds with more and larger aromatic groups tended to be more potent although this effect was only subtle. We could not perform a titration with a nitro terminated compound because our synthetic approach results in hydrolysis of the 4-nitro benzamide terminus rather than the methyl ester.²⁵

IC_{50} values from competition assays have been used to extract a direct measure of binding affinity (K_i) for a given target.³⁶ Upon careful analysis of our data, we observed that in the competition experiment, anisotropy values for ligands **1** and **p53₁₅₋₃₁** decreased below the original value observed at the

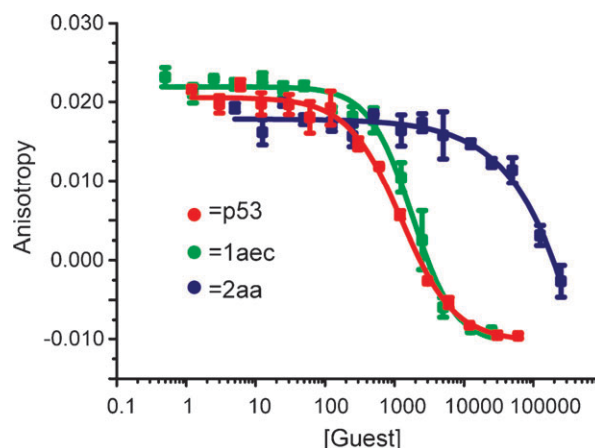


Fig. 4 Representative FA competition titration data (40 mM sodium–potassium phosphate buffer pH 7.5, 54 nM **p53_{15-31Flu}**, 42 nM *hDM2*).

Table 1 Dissociation constants determined by FP displacement assay^a

Compound	$\text{IC}_{50}/\mu\text{M}$
p53_{15-31Flu}	0.074 (± 0.004) ^b
p53_{15-31Flu}	1.2 (± 0.04)
2aa	2700 (± 1950)
2ac	50.0 (± 20.0)
1aaa	10.0 (± 3.8)
1bca	4.7 (± 0.53)
1acd	2.4 (± 0.31)
1ace	1.6 (± 0.25)
1aec	1.0 (± 0.11)
1acc	5.1 (± 0.44)

^a Conditions as indicated in Fig. 4. ^b K_d .

start of the direct titration of *hDM2* into **p53_{15-31Flu}**. This points to a more complex equilibrium that precludes determination of K_i . One possibility is that **p53_{15-31Flu}** is present in an aggregated/dimeric form before titration with *hDM2* and then becomes bound to the competitor upon displacement. Indeed, direct titration of **p53_{15-31Flu}** with both **p53₁₅₋₃₁** and our compounds resulted in a decrease in anisotropy value comparable to that observed in the competition experiments where *hDM2* is also present. Data fitting to a 1 : 1 association model provided K_d values in the μM range (see ESI†) for the

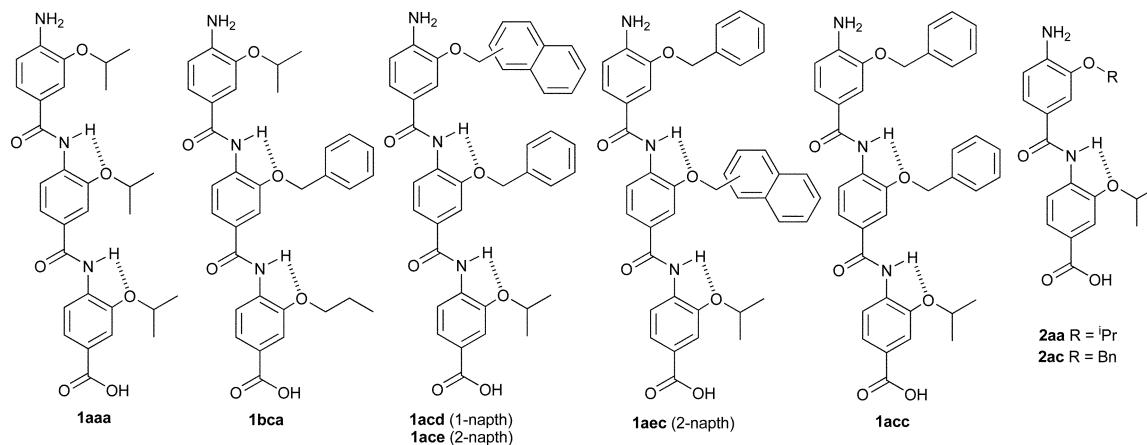


Fig. 3 Compounds tested.

interaction between **p53_{15-31Flu}** and **p53₁₅₋₃₁**/ligand. We therefore derived a model to extract a binding constant for interaction of the competitors with *h*DM2 (see ESI†). Not surprisingly, as the K_d values for **p53_{15-31Flu}** binding to competitors are in the μM range and the concentration of competitor is far in excess of all other components in the experiment (μM vs. nM), no meaningful data can be extracted and it is not possible to confirm that the compounds bind to *h*DM2. We therefore derivatised one of the more soluble compounds (**1acc**) with a fluorescein label to give **FITC-Gly-1acc** and performed a direct binding experiment with *h*DM2—this afforded a dissociation constant of 760 (± 140) nM and confirmed strong interaction with the target protein.

In summary, we have described the design, synthesis and binding studies of potent μM inhibitors of the p53–*h*DM2 protein–protein interaction and highlighted complications associated with interpretation of data from competition experiments. The generality of the syntheses reported by us and others²⁵ represents a powerful starting point for generation of libraries for screening against a plethora of α -helix mediated PPIs. Our own investigations will focus on optimising the current compounds for binding to *h*DM2 and targeting other PPIs.

This work was supported by EPSRC (EP/D077842/1) and the Wellcome Trust (080709/Z/06/Z) through a PhD studentship (BM). We would also like to thank John Robinson (University of Zurich) for providing the *h*DM2 plasmid.

Notes and references

- W. E. Stites, *Chem. Rev.*, 1997, **97**, 1233–1250.
- M. R. Arkin and J. A. Wells, *Nat. Rev. Drug Discovery*, 2004, **3**, 301–317.
- H. Yin and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2005, **44**, 4130–4163.
- A. J. Wilson, J. Hong and A. D. Hamilton, *Org. Biomol. Chem.*, 2007, **5**, 276–285.
- B. P. Orner, J. T. Ernst and A. D. Hamilton, *J. Am. Chem. Soc.*, 2001, **123**, 5382–5383.
- I. Saraogi and A. D. Hamilton, *Biochem. Soc. Trans.*, 2008, **36**, 1414–1417.
- H. Yin, G.-I. Lee, K. A. Sedey, O. Kutzki, H. S. Park, B. P. Orner, J. T. Ernst, H.-G. Wang, S. M. Sebt and A. D. Hamilton, *J. Am. Chem. Soc.*, 2005, **127**, 10191–10196.
- H. Yin, G.-i. Lee, K. A. Sedey, J. M. Rodriguez, H.-G. Wang, S. M. Sebt and A. D. Hamilton, *J. Am. Chem. Soc.*, 2005, **127**, 5463–5468.
- I. C. Kim and A. D. Hamilton, *Org. Lett.*, 2006, **8**, 1751–1754.
- J. M. Rodriguez and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2007, **46**, 8641–8617.
- J. Becerril and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2007, **46**, 4471–4473.
- S. M. Biro, L. Moisan, E. Mann, A. Carella, D. Zhai, J. C. Reid and J. Rebek, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4641–4645.
- P. Maity and B. König, *Org. Lett.*, 2008, **10**, 1473–1476.
- J. M. Rodriguez, L. Nevola, N. T. Ross, G.-i. Lee and A. D. Hamilton, *ChemBioChem*, 2009, **10**, 829–833.
- C. G. Cummings, N. T. Ross, W. P. Katt and A. D. Hamilton, *Org. Lett.*, 2009, **11**, 25–28.
- S. H. Gellman, *Acc. Chem. Res.*, 1998, **31**, 178–190.
- O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay and A. Schepartz, *J. Am. Chem. Soc.*, 2005, **127**, 13126–13127.
- J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H.-S. Lee, N. Umezawa, Z. Nikolovska-Coleska, S. Wang, D. C. S. Huang, Y. Tomita and S. H. Gellman, *J. Am. Chem. Soc.*, 2007, **129**, 139–154.
- E. A. Harker, D. S. Daniels, D. A. Guarracino and A. Schepartz, *Bioorg. Med. Chem.*, 2009, **17**, 2038–2046.
- I. Huc, *Eur. J. Org. Chem.*, 2004, 17–29.
- Z.-T. Li, J.-L. Hou and H.-P. Yi, *Chem. Asian J.*, 2006, **1**, 766–778.
- J. T. Ernst, J. Becerril, H. S. Park, H. Yin and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2003, **42**, 535–550.
- During preparation of this manuscript another group elegantly described libraries of aromatic oligoamides that act as inhibitors of the p53–*h*DM2 interaction using an ELISA assay, see: A. Shaginian, L. R. Whitby, S. Hong, I. Hwang, B. Farooqi, M. Searcey, J. Chen, P. K. Vogt and D. L. Boger, *J. Am. Chem. Soc.*, 2009, **131**, 4904–4916.
- F. Campbell, J. Plante, C. Carruthers, M. J. Hardie, T. J. Prior and A. J. Wilson, *Chem. Commun.*, 2007, 2240–2242.
- J. Plante, F. Campbell, B. Malkova, C. Kilner, S. L. Warriner and A. J. Wilson, *Org. Biomol. Chem.*, 2008, **1**, 138–146.
- J.-M. Ahn and S.-Y. Han, *Tetrahedron Lett.*, 2007, **48**, 3543–3547.
- I. Saraogi, C. D. Incarvito and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2008, **47**, 9691–9694.
- L. Romer, C. Klein, A. Dehner, H. Kessler and J. Buchner, *Angew. Chem., Int. Ed.*, 2006, **45**, 6440–6460.
- S. Patel and M. R. Player, *Expert Opin. Invest. Drugs*, 2008, **17**, 1865–1882.
- L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi and E. A. Liu, *Science*, 2004, **303**, 844–848.
- B. L. Grasberger, T. Lu, C. Schubert, D. J. Parks, T. E. Carver, H. K. Koblish, M. D. Cummings, L. V. LaFrance, K. L. Milkiewicz, R. R. Calvo, D. Maguire, J. Lattanze, C. F. Franks, S. Zhao, K. Ramachandren, G. R. Bylebyl, M. Zhang, C. L. Manthey, E. C. Petrella, M. W. Pantoliano, I. C. Deckman, J. C. Spurlino, A. C. Maroney, B. E. Tomczuk, C. J. Molloy and R. F. Bone, *J. Med. Chem.*, 2005, **48**, 909–912.
- H. Yin, G.-I. Lee, H. S. Park, G. A. Payne, J. M. Rodriguez, S. M. Sebt and A. D. Hamilton, *Angew. Chem., Int. Ed.*, 2005, **44**, 2704–2707.
- S. Shangary, D. Qin, D. McEachern, M. Liu, R. S. Miller, S. Qiu, Z. Nikolovska-Coleska, K. Ding, G. Wang, J. Chen, D. Bernard, J. Zhang, Y. Lu, Q. Gu, R. B. Shah, K. J. Pienta, X. Ling, S. Kang, M. Guo, Y. Sun, D. Yang and S. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3933–3938.
- P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine and N. P. Pavletich, *Science*, 1996, **274**, 948–953.
- J. A. Kritzer, J. D. Lear, M. E. Hodsdon and A. Schepartz, *J. Am. Chem. Soc.*, 2004, **126**, 9468–9469.
- Z. Nikolovska-Coleska, R. Wang, X. Fang, H. Pan, Y. Tomita, P. Li, P. P. Roller, K. Krajewski, N. G. Saito, J. A. Stuckey and S. Wang, *Anal. Biochem.*, 2004, **332**, 261–273.