

# CHEMISTRY

## A European Journal

A Journal of



### Accepted Article

**Title:** Unveiling the TThree Finger Pharmacophoreerrequired for p53-MDM2 Inhibition by Saturation Transfer Difference NMR Initial Growth Rates Approach

**Authors:** Jesus Angulo; Sarah A Goffin; Daivik Gandhi; Mark Searcey; Lesley Ann Howell

This manuscript has been accepted after peer review and the authors have elected to post their Accepted Article online prior to editing, proofing, and formal publication of the final Version of Record (VoR). This work is currently citable by using the Digital Object Identifier (DOI) given below. The VoR will be published online in Early View as soon as possible and may be different to this Accepted Article as a result of editing. Readers should obtain the VoR from the journal website shown below when it is published to ensure accuracy of information. The authors are responsible for the content of this Accepted Article.

**To be cited as:** Chem. Eur. J. 10.1002/chem.201600114

**Link to VoR:** <http://dx.doi.org/10.1002/chem.201600114>

Supported by  
**ACES**

WILEY-VCH

# Unveiling the “Three Finger Pharmacophore” required for p53-MDM2 Inhibition by Saturation Transfer Difference NMR Initial Growth Rates Approach

Jesus Angulo\*,<sup>[a]</sup> Sarah A. Goffin,<sup>[a]</sup> Daivik Gandhi,<sup>[a]</sup> Mark Searcey<sup>[a,b]</sup> and Lesley A. Howell\*<sup>[a]</sup>

**Abstract:** Inhibitors of the p53-MDM2 protein-protein interaction are emerging as a novel and validated approach to treating cancer. In this work we describe the synthesis and inhibitory evaluation of a series of isoquinolin-1-one analogues, and highlight the utility of an initial growth rates STD NMR approach supported by protein-ligand docking to investigate p53-MDM2 inhibition. The approach is illustrated by the study of compound 1, providing key insights into the binding mode of this kind of MDM2 ligands and, more importantly, readily unveiling the previously proposed three finger pharmacophore requirement for p53-MDM2 inhibition.

Protein-protein interactions (PPIs) had been considered “undruggable” primarily due to large surface areas and their flat, featureless and hydrophobic nature.<sup>[1–3]</sup> However, the success of small molecule inhibitors such as the Nutlins,<sup>[4]</sup> p53-MDM2 inhibitors and Navitoclax,<sup>[5,6]</sup> a dual inhibitor of Bcl-2 and Bcl-xL (both of which are currently in clinical trials) have defied this view point. PPIs still pose a considerable challenge to the medicinal chemistry community, but they are an attractive drug target due to their ability to modulate outcomes within cells, hence allowing greater control than classical drug targets such as enzymes or receptors.<sup>[7,8]</sup> The most widely studied PPI is the p53-MDM2 paradigm.<sup>[9]</sup> The tumour suppressor protein p53 is a key transcription factor involved in regulating the cell cycle and apoptosis.<sup>[10]</sup> It is often referred to as the guardian of the genome<sup>[11]</sup> and plays a crucial role in cancer; nearly all tumours show a defect in the p53 gene itself or in other negative regulatory proteins such as MDM2 or MDMX.<sup>[12]</sup> In cancer cells with wt-p53, over expression of either MDM2 and/or MDMX suppresses p53 activity and disrupts the apoptosis pathway.<sup>[13]</sup> Therefore the restoration of the p53 pathway, by inhibiting the p53-MDM2 interaction with small molecules, represents an attractive and viable approach to treating cancer.

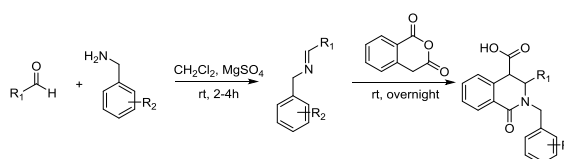
NMR is one of the most powerful spectroscopic techniques to study biomolecular interactions and has been applied to the

discovery of protein-protein inhibitors,<sup>[14]</sup> some of them focused on the MDM2-p53 interaction.<sup>[15–17]</sup> In particular, Holak and co-workers recently devised a smart 2D <sup>1</sup>H,<sup>15</sup>N NMR based method to test inhibition of PPIs, called AIDA, that they also applied to the MDM2-p53 interaction.<sup>[18,19]</sup> However, although that method provides relevant information about the protein residues of MDM2 involved in binding, the information about the bioactive conformation and the mode of binding of the ligand is lost. This kind of structural information is attainable by saturation transfer difference (STD) NMR spectroscopy,<sup>[20–22]</sup> if a thorough quantitative analysis of the experiments is carried out by means of the STD build-up curves.<sup>[23–25]</sup>

Herein we report the synthesis of a series of isoquinolin-1-one analogues, their p53-MDM2 inhibitory activity as determined using a fluorescence polarisation assay, and a STD NMR initial growth rates approach to identify the binding mode of one of the lead compounds. Notably, the results suggest that STD NMR is an easy and powerful tool to verify the proposed three finger pharmacophore requirement for p53-MDM2 inhibition, providing in addition key structural information regarding its interactions with the hydrophobic groove of MDM2, for future lead optimisation.

Our previous work on p53-MDM2 inhibitors has involved the natural product chlorofusin<sup>[26,27]</sup> and more recently the identification of a novel small molecule inhibitor inspired by our studies of this natural product.<sup>[28]</sup> Of late we have been working on producing a library of isoquinolin-1-one analogues. Isoquinoline-1-ones have been shown to inhibit the p53-MDM2 PPI with low micromolar activity.<sup>[29]</sup> We sought to develop a procedure by which a relatively large number of diverse compounds could be synthesised quickly with minimum purification to identify potent small molecule inhibitors of the p53-MDM2 interaction.

A modified Castagnoli reaction was employed to synthesise the isoquinolin-1-ones.<sup>[30]</sup> The reaction involves the condensation of a Schiff base with an acid anhydride (scheme 1). Briefly a stoichiometric amount of a benzyl amine and an aldehyde were reacted together under anhydrous conditions in



sulphate to form the corresponding

**Scheme 1** Synthesis of isoquinolin-1-ones

[a] Dr J. Angulo\*, S. A. Goffin, D. Gandhi, Prof. M Searcey, Dr L. A. Howell\*  
School of Pharmacy  
University of East Anglia  
Norwich Research Park, Norwich, NR4 7TJ, UK.  
E-mail: [j.angulo@uea.ac.uk](mailto:j.angulo@uea.ac.uk), [l.howell@uea.ac.uk](mailto:l.howell@uea.ac.uk)

[b] Prof. Mark Searcey  
School of Chemistry  
University of East Anglia  
Norwich Research Park, Norwich, NR4 7TJ, UK.

imine. The reactions reached completion in 2-4 hours (as shown by TLC), after which the solutions were filtered. The filtrate was then added to 1 equivalent of homophthalic anhydride and the reaction left overnight at room temperature. The resultant precipitate was filtered, washed with hot ethyl acetate and dried *in vacuo*. Those compounds that did not precipitate were evaporated and purified via flash column chromatography. A total of 60 compounds were prepared in this manner (see ESI). The reaction can produce either the *cis* or *trans* isomers or a mixture of both with the major diastereoisomer expected to be the more thermodynamically stable *trans* product.<sup>[31]</sup>

We had originally assumed the reaction would proceed racemically and initially proposed to evaluate the compounds as racemic mixtures. However, on further investigation we found the reaction did indeed exhibit some stereocontrol as the compounds were found to be optically active. Looking into the literature there is some precedent for stereocontrol for this class of reaction: the condensation of *o*-anisylidenemethylamine with glutaric anhydride yields the *trans* piperidone product as the major diastereoisomer with the aromatic ring occupying the axial position essentially fixing the stereochemistry at this carbon.<sup>[32]</sup> This is a result of the planar nature of the amide bond. Presumably this is further enhanced in our molecules due to the additional planar benzene fused to the piperidone resulting in only one enantiomer formed as the major product. An alternative explanation for the observation of only one enantiomer is preferential crystallisation, also known as resolution by entrainment, whereby the resolution is performed without the use of a resolving agent.<sup>[33]</sup>

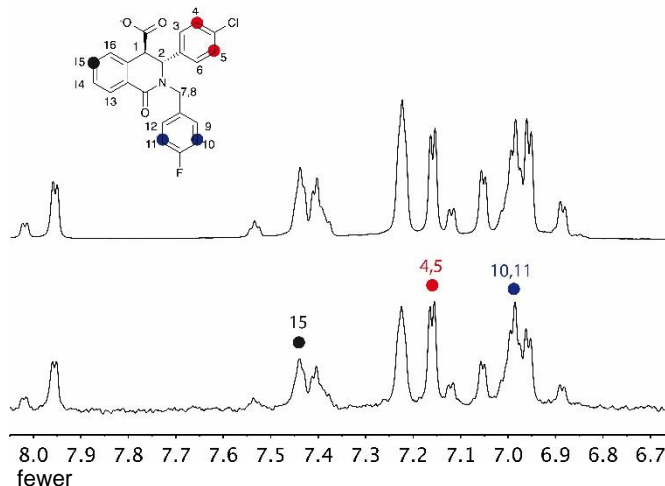
The compounds were screened using a fluorescence polarisation (FP) assay described previously.<sup>[28]</sup> Human MDM2 protein (17-125) was used in the polarisation assay and the wildtype p53 peptide (residues 15–27) was used as a positive control and had an  $IC_{50}$  of 14.45  $\mu$ M and  $K_i$  of 1.82  $\mu$ M. Screening the isoquinolin-1-ones at 100  $\mu$ M concentration revealed seven hits which were further evaluated over a wider concentration range to determine their  $IC_{50}$ 's (Table 1). Interestingly all seven compounds were found to be the *trans* isomer and all were substituted with halogens indicating the importance of halides for MDM2 binding ligands. Compound **7** was the most active with low micromolar activity with compounds **2**, **5** and **6** exhibiting similar activity to wild type p53.

We chose compound **1** as our model compound for the NMR binding study. Due to the structural similarity of **1-7**, comparable binding modes for all could be foreseen, and we then decided to test one of the weakest ligands, as STD NMR requires a binding kinetics falling within the so-called fast exchange conditions ( $K_i$  values in Table 1 suggest these molecules might fall at the limits of the technique). Within the weakest binders (**1**, **3**, and **4**) we selected **1**, as it was the most potent inhibitor (lowest  $K_i$ ). The binding of **1** to human MDM2 in solution was confirmed by STD NMR (Figure 1). Strong saturation transfer signals were observed in the difference spectrum (Figure 1, bottom). Besides providing a proof of binding, structural information was obtained by a complete kinetics study of the evolution of STD intensities with the saturation time of the protein. Briefly, the STD intensities were determined at different saturation times (STD build-up curves), and the initial growth rates of each curve were obtained by

mathematical fitting; those initial slope values were then used to map out the ligand epitope (see ESI).<sup>[24]</sup> The presence of aromatic rings in combination with saturated residues (e.g. aliphatics) in most of the structures of protein-protein inhibitors (as in the case of **1**) makes this approach very suitable, as "aromatic ring – protein" contacts can be overstated if a single large saturation time is employed, instead of a whole build-up analysis.<sup>[34]</sup>

The initial growth rates of the curves were determined using a monoexponential model (see ESI) and their relative distribution among the protons of **1** was calculated to map out the binding epitope of the ligand for binding to human MDM2 (normalised STD values in Figure 2). The binding epitope of **1** (Figure 2) revealed significant structural information about the molecular recognition of this kind of compound by human MDM2. The three aromatic residues of **1** constitute the main spatial contacts with the protein in the bound state, with the chloride substituted phenyl ring ( $R_1$  in scheme 1) showing the closest contacts. In contrast, the six-membered ring tethering the aromatic residues together showed the lowest STD intensities, along with the methylene bridge protons linking the fluoride substituted phenyl ring (Figure 2, in blue). The differences in relative STD values, although small, are significant, as they correspond to a slow kinetics (low micromolar) binder, for the standards of STD NMR, and for that reason the criterion to split STD intensities in strong and weak was raised up to a level of 80% relative STD (Figure 2). The firmness of the interpretation of the binding epitope of **1** relies on the accuracy of the STD initial growth rates approach followed on this work (see ESI).

In solution, **1** binds human MDM2 mainly through the apolar aromatic moieties, which is compatible with the largely hydrophobic character of the amino acid residues lining the p53-binding site of human MDM2. The most polar part of **1** (the central six-membered ring and the methylene bridge) makes

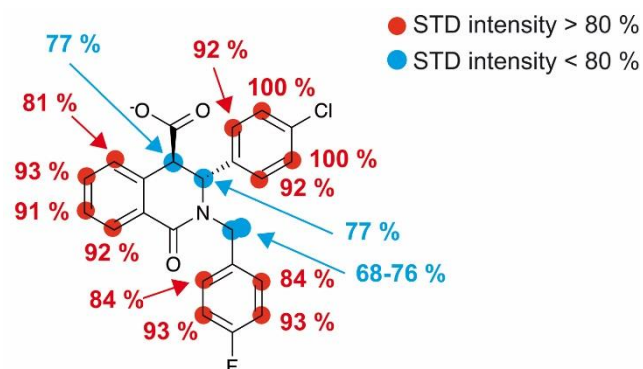


**Figure 1.** Expanded view of the aromatic region of the STD NMR (298 K, 800 MHz, 1 s saturation time) of a sample containing an excess of **1** (1 mM concentration) over the protein MDM2 (20  $\mu$ M). The top spectrum corresponds to the equilibrium intensities of **1** (1D reference  $^1$ H NMR spectrum) whereas the bottom one shows the difference spectrum, in which the intensities corresponds to transfer of magnetization from the protein upon binding (most intense STD signals highlighted in the spectrum and on the chemical formula of **1**, inset at the top).

contacts with the protein and is accordingly more solvent exposed in the bound state. This mode of binding is reminiscent of that of some previously described MDM2-p53 inhibitors, strongly supporting that the initial growth rates STD NMR approach can be used a simple technique to unveil the so-called "three finger pharmacophore" requirement for MDM2-p53 inhibition (Figure 2).<sup>[9]</sup>

To get a 3D molecular model of the interaction of **1** with human MDM2 we carried out docking calculations using the program Glide.<sup>[35,36]</sup> We used the published Cartesian coordinates of the protein (pdb entry: 1T4E) in complex with a benzodiazepinedione.<sup>[37]</sup> The original ligand was removed and docking calculations were run with ligand **1**. Although we knew the ligand sample (without MDM2) had optical activity, by NMR it was not possible to elucidate which enantiomer was in excess. For that reason we carried out the docking calculations with both enantiomers. Interestingly, the 3*R*,4*R*-enantiomer led to the energetically most favourable docking solution, which in addition was in excellent agreement with the experimental STD NMR data (Figure 3). For the 3*S*,4*S* enantiomer, the best scored docking solution was much higher in energy (above 17 kcal/mol), and gave very poor agreement with the experimental STD NMR data (see ESI). In this way, the combined protocol of STD initial growth rates and docking calculations allowed us to identify the 3*R*,4*R*-enantiomer as the most active for MDM2 in solution. The best-scored docking pose for the 3*R*,4*R*- enantiomer was further energy minimized and the solution is shown in Figure 3.

A comparison of Figures 2 and 3 demonstrates that the 3D docking molecular model of **1** bound to human MDM2 agrees remarkably well with the experimental NMR results in solution state. The three aromatic residues of **1** make close contacts with the protein in the bound state, with a higher predominance of contacts with amino acids with hydrophobic side chains (see map of protein-ligand contacts in the ESI). The chlorine substituted phenyl ring is buried in an internal cavity between GLY58 and LEU54, explaining the largest amount of saturation transferred from the protein in the NMR spectra. The carboxylate group as well as the methylene bridge are pointing towards the solvent, which agrees very well with the observed lower STD intensities.



**Figure 2** Binding epitope of **1** to the protein MDM2 as obtained by STD NMR. The values indicate normalized STD values for each proton of **1**. The highest values results from very close contacts of the ligand to the MDM2 surface in the bound state (red), whereas the smallest ones indicate regions of **1** being solvent exposed.

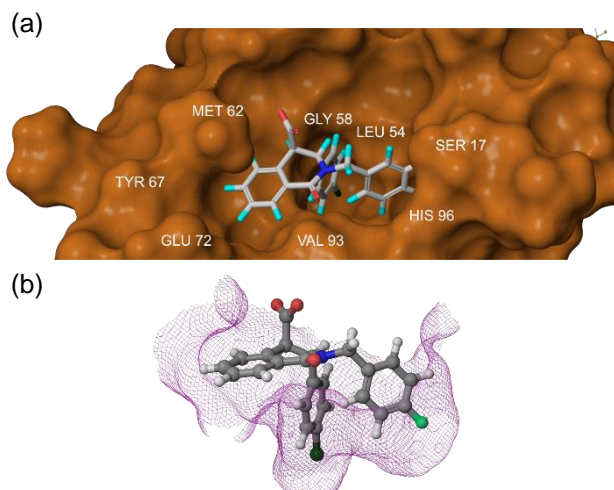
**Table 1.** Binding of compounds **1-7** to MDM2 using the fluorescence polarisation assay.

| Compound | R <sub>1</sub> | R <sub>2</sub> | IC <sub>50</sub> (μM) <sup>[a]</sup> | K <sub>i</sub> (μM) <sup>[b]</sup> |
|----------|----------------|----------------|--------------------------------------|------------------------------------|
| 1        | 4-ClPh         | 4-F            | 56.6                                 | 7.13                               |
| 2        | 4-BrPh         | 4-F            | 19.8                                 | 2.49                               |
| 3        | 4-IPh          | 4-F            | 57.7                                 | 7.29                               |
| 4        | 4-ClPh         | 4-Cl           | 61.2                                 | 7.71                               |
| 5        | 4-BrPh         | 4-Cl           | 21.3                                 | 2.68                               |
| 6        | 4-ClPh         | 4-Br           | 27.1                                 | 2.36                               |
| 7        | 4-BrPh         | 4-Br           | 6.6                                  | 0.83                               |

[a] Concentration of substrate required to decrease polarization fluorescence by 50%. Experiments performed in triplicate. [b] Apparent inhibition constant. Experiments performed in triplicate.

The STD NMR initial growth rates approach and the modelling study of **1** has provided very relevant structural information about the molecular recognition of this series of ligands by the human MDM2 receptor. In this way, further improvement of human MDM2 ligands should exploit this information; e.g. modifications must be on those parts of the ligands making fewer contacts with the protein (carboxylate and methylene bridge). Indeed an analogue substituted on the methylene bridge with a methyl alcohol inhibited the PPI with an IC<sub>50</sub> of 15.01 μM. This is also in agreement with the previous study by Holak and co-workers, as they showed that changing the carboxylate of similar compounds to an amide spacer did not affect the affinity.<sup>[29]</sup> Interestingly, the comparison of the binding mode of **1** with the published structure of the complex with the benzodiazepinedione ligand (see ESI), further highlights the two pending halogenated aromatic residues as being the most important elements for molecular recognition. In the case of the benzodiazepinedione ligand,<sup>[37]</sup> the fused aromatic ring bears a bulky iodine atom that pushes it farther from the protein surface than in the case of **1**, so that the fused aromatic ring of **1** falls in a significantly shifted position, compared to that ligand, which supports that the molecular recognition is not specific for that moiety. Yet, the matching of the two halogenated aromatic rings of **1** with the published benzodiazepinedione structure is excellent (see ESI).

In conclusion we have reported the synthesis of a library of isoquinolin-1-ones as potential inhibitors of the p53-MDM2 protein-protein interaction. Seven compounds were identified with IC<sub>50</sub>'s in the low micromolar range. One of the compounds, **1**, was explored further using STD NMR to determine its binding epitope for the hydrophobic groove of human MDM2. These studies have shown the power of the initial growth rates STD NMR approach applied to biologically relevant PPIs. In the particular case of the p53-MDM2 interaction, the approach has



**Figure 3** 3D molecular model of the complex of **1** with human MDM2. (a) Bound conformation (best docking pose) of **1** in the p53-binding pocket of MDM2. (b) Mesh representation of the MDM2 surface closest to non-polar hydrogens of **1** in the bound state (those observable in STD NMR). The energetically most favourable docking solution (enantiomer 3*R*,4*R*) qualitatively gives an excellent agreement with the observed STD NMR intensities.

demonstrated to be very useful for verifying the three finger pharmacophore requirement of small molecules for inhibition, without the need for isotopic labelling of the protein, and using small sub-stoichiometric amounts of the receptor. The analysis of the whole STD build-up curve (initial growth rates) is mandatory as typical PPI inhibitors contain protons with different relaxation properties that could lead to misinterpreted epitopes if a single “one-saturation time” experiment were used. Recently, a novel type of p53-MDM2 inhibitor, based on 6-chloroindole scaffolds, has demonstrated that the “plasticity” of the p53-binding site on MDM2 allows some small molecules to show an extended four-point pharmacophore model;<sup>[38]</sup> the STD NMR approach followed here will be a powerful and simple method for distinguishing between both (3- or 4-finger) pharmacophore modes of binding for novel generations of MDM2 ligands. We envisage an increased use of the STD NMR initial growth rates approach to the design of protein-protein inhibitors, to verify the pharmacophore, and to determine the structural requirements for molecular recognition, extremely valuable information for the improvement of the small molecule candidates to inhibit PPIs.

## Acknowledgements

We thank the School of Pharmacy (UEA) for a studentship for SAG and for start-up funds to JA and the Swansea EPSRC Mass Spectrometry Service for high resolution mass spectra. This work was supported by the Wellcome Trust [102201/Z/13/Z] a vacation scholarship for DG. We also thank G. Richard Stephenson for helpful discussions.

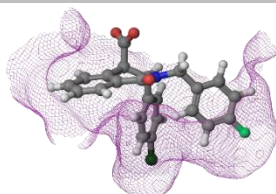
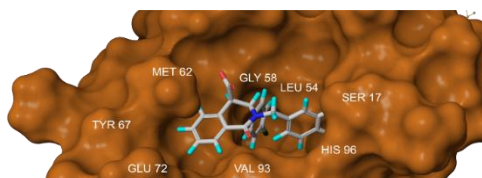
**Keywords:** saturation-transfer difference NMR • p53-MDM2 • cancer • protein-protein interactions • molecular modelling

- [1] S. Jones, J. M. Thornton, *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 13–20.
- [2] O. Keskin, A. Gursoy, B. Ma, R. Nussinov, *Chem. Rev.* **2008**, *108*, 1225–1244.
- [3] A. J. Wilson, *Chem. Soc. Rev.* **2009**, *38*, 3289–3300.
- [4] L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, et al., *Science* **2004**, *303*, 844–848.
- [5] M. Bruncko, T. K. Oost, B. A. Belli, H. Ding, M. K. Joseph, A. Kunzer, D. Martineau, W. J. McClellan, M. Mitten, S.-C. Ng, et al., *J. Med. Chem.* **2007**, *50*, 641–662.
- [6] K. Tse, A. R. Shoemaker, J. Adickes, M. G. Anderson, J. Chen, S. Jin, E. F. Johnson, K. C. Marsh, M. J. Mitten, P. Nimmer, et al., *Cancer Res.* **2008**, *68*, 3421–3428.
- [7] V. Azzarito, K. Long, N. S. Murphy, A. J. Wilson, *Nat. Chem.* **2013**, *5*, 161–173.
- [8] J. A. Wells, C. L. McClendon, *Nature* **2007**, *450*, 1001–1009.
- [9] K. Houry, A. Dömling, *Curr. Pharm. Des.* **2012**, *18*, 4668–4678.
- [10] K. H. Vousden, X. Lu, *Nat. Rev. Cancer* **2002**, *2*, 594–604.
- [11] D. P. Lane, *Nature* **1992**, *358*, 15–16.
- [12] M. Hollstein, D. Sidransky, B. Vogelstein, C. C. Harris, *Science* **1991**, *253*, 49–53.
- [13] M. Wade, Y.-C. Li, G. M. Wahl, *Nat. Rev. Cancer* **2013**, *13*, 83–96.
- [14] E. Barile, M. Pellecchia, *Chem. Rev.* **2014**, *114*, 4749–4763.
- [15] O. Schon, A. Friedler, M. Bycroft, S. M. V. Freund, A. R. Fersht, *J. Mol. Biol.* **2002**, *323*, 491–501.
- [16] D. C. Fry, S. D. Emerson, S. Palme, B. T. Vu, C.-M. Liu, F. Podlaski, *J. Biomol. NMR* **2004**, *30*, 163–173.
- [17] C. Riedinger, M. E. Noble, D. J. Wright, F. Mulks, I. R. Hardcastle, J. A. Endicott, J. M. McDonnell, *Chem. Biol. Drug Des.* **2011**, *77*, 301–308.
- [18] L. D’Silva, P. Ozdowdy, M. Krajewski, U. Rothweiler, M. Singh, T. A. Holak, *J. Am. Chem. Soc.* **2005**, *127*, 13220–13226.
- [19] M. Krajewski, U. Rothweiler, L. D’Silva, S. Majumdar, C. Klein, T. A. Holak, *J. Med. Chem.* **2007**, *50*, 4382–4387.
- [20] J. Angulo, P. M. Nieto, *Eur. Biophys. J. EBJ* **2011**, *40*, 1357–1369.
- [21] M. Mayer, B. Meyer, *Angew. Chem. Int. Ed.* **1999**, *38*, 1784–1788.
- [22] M. Mayer, B. Meyer, *J. Am. Chem. Soc.* **2001**, *123*, 6108–6117.
- [23] J. Angulo, P. M. Enriquez-Navas, P. M. Nieto, *Chem. Weinh. Bergstr. Ger.* **2010**, *16*, 7803–7812.
- [24] M. Mayer, T. L. James, *J. Am. Chem. Soc.* **2004**, *126*, 4453–4460.
- [25] J. C. Muñoz-García, E. Chabrol, R. R. Vivès, A. Thomas, J. L. de Paz, J. Rojo, A. Imberty, F. Fieschi, P. M. Nieto, J. Angulo, *J. Am. Chem. Soc.* **2015**, *137*, 4100–4110.
- [26] J. P. Malkinson, M. Zloh, M. Kadom, R. Errington, P. J. Smith, M. Searcey, *Org. Lett.* **2003**, *5*, 5051–5054.
- [27] E. C. Y. Woon, M. Arcieri, A. F. Wilderspin, J. P. Malkinson, M. Searcey, *J. Org. Chem.* **2007**, *72*, 5146–5151.
- [28] M. M. D. Cominetti, S. A. Goffin, E. Raffel, K. D. Turner, J. C. Ramoutar, M. A. O’Connell, L. A. Howell, M. Searcey, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4878–4880.
- [29] U. Rothweiler, A. Czarna, M. Krajewski, J. Ciombor, C. Kalinski, V. Khazak, G. Ross, N. Skobeleva, L. Weber, T. A. Holak, *ChemMedChem* **2008**, *3*, 1118–1128.
- [30] M. Cushman, J. Gentry, F. W. Dekow, *J. Org. Chem.* **1977**, *42*, 1111–1116.
- [31] M. Cushman, T.-C. Choong, J. T. Valko, M. P. Koleck, *J. Org. Chem.* **1980**, *45*, 5067–5073.
- [32] M. Cushman, N. Castagnoli, *J. Org. Chem.* **1973**, *38*, 440–448.
- [33] A. Collet, M. J. Brienne, J. Jacques, *Chem. Rev.* **1980**, *80*, 215–230.
- [34] J. Yan, A. D. Kline, H. Mo, M. J. Shapiro, E. R. Zartler, *J. Magn. Reson. San Diego Calif* **1997** **2003**, *163*, 270–276.
- [35] R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, et al., *J. Med. Chem.* **2004**, *47*, 1739–1749.
- [36] T. A. Halgren, R. B. Murphy, R. A. Friesner, H. S. Beard, L. L. Frye, W. T. Pollard, J. L. Banks, *J. Med. Chem.* **2004**, *47*, 1750–1759.
- [37] 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, et al., *J. Med. Chem.* **2005**, *48*, 909–912.
- [38] M. Bista, S. Wolf, K. Houry, K. Kowalska, Y. Huang, E. Wrona, M. Arciniega, G. M. Popowicz, T. A. Holak, A. Dömling, *Structure* **2013**, *21*, 2143–2151.

## Entry for the Table of Contents (Please choose one layout)

Layout 2:

## COMMUNICATION



Protein-protein interactions play a significant role in cancer and tumour growth, in particular the ability of tumours to overcome apoptosis. Inhibitors of the p53-MDM2 protein-protein interaction are emerging as a novel and validated approach to treating cancer. In this work we describe the synthesis and inhibitory evaluation of a series of isoquinolin-1-one analogues, and highlight the utility of an initial growth rates STD NMR approach supported by protein-ligand docking to investigate p53-MDM2 inhibition.

Jesus Angulo\*, Sarah A. Goffin, Daivik Gandhi, Mark Searcey and Lesley A. Howell\*

Page No. – Page No.

**Unveiling the “Three Finger Pharmacophore required for p53-MDM2 Inhibition by Saturation Transfer Difference NMR Initial Growth Rates Approach**

- [a] Dr J. Angulo\*, S. A. Goffin, D. Gandhi, Prof. Howell\*  
School of Pharmacy  
University of East Anglia  
Norwich Research Park, Norwich, NR4 7TJ,  
E-mail: [j.angulo@uea.ac.uk](mailto:j.angulo@uea.ac.uk), [l.howell@uea.ac.uk](mailto:l.howell@uea.ac.uk)
- [b] Prof. Mark Searcey  
School of Chemistry  
University of East Anglia  
Norwich Research Park, Norwich, NR4 7TJ,

Supporting information for this article is give the document.