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MDM2-p53 protein–protein interaction inhibitors: A-ring substituted isoindolinones

Anna F. Watson [a], Junfeng Liu [b], Karim Bennaceur [b], Catherine J. Drummond [b], Jane A. Endicott [c], Bernard T. Golding [a], Roger J. Griffin [a], Karen Haggerty [a], Xiaohong Lu [b], James M. McDonnell [c], David R. Newell [b], Martin E.M. Noble [c], Charlotte H. Revill [a], Christiane Riedinger [c], Qing Xu [b], Yan Zhao [b], John Lunec [b,*], Ian R. Hardcastle [a,*]

[a] Newcastle Cancer Centre at the Northern Institute for Cancer Research and School of Chemistry, Bedson Building, Newcastle University, Newcastle, NE1 7RU, UK
[b] Newcastle Cancer Centre at the Northern Institute for Cancer Research, Paul O'Gorman Building, Medical School, Framlington Place, Newcastle University, Newcastle, NE2 4HH, UK
[c] Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU, UK

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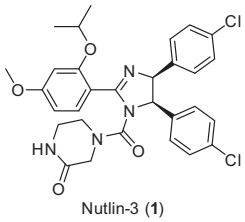
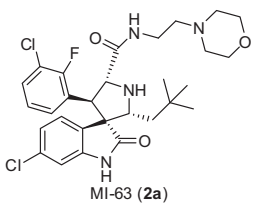
ABSTRACT

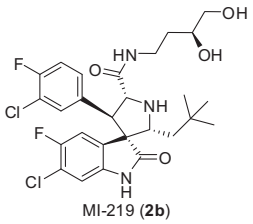
Structure–activity relationships for the MDM2-p53 inhibitory activity of a series of A-ring substituted 2-*N*-benzyl-3-(4-chlorophenyl)-3-(1-(hydroxymethyl)cyclopropyl)methoxy)isoindolinones have been investigated, giving rise to compounds with improved potency over their unsubstituted counterparts. Isoindolinone A-ring substitution with a 4-chloro group for the 4-nitrobenzyl, 4-bromobenzyl and 4-cyanobenzyl derivatives (**10a–c**) and substitution with a 6-*tert*-butyl group for the 4-nitrobenzyl derivative (**10j**) were found to confer additional potency. Resolution of the enantiomers of **10a** showed that potent MDM2-p53 activity resided in the (−)-enantiomer ((−)-**10a**; IC_{50} = 44 ± 6 nM). The cellular activity of key compounds has been examined in cell lines with defined p53 and MDM2 status. Compounds **10a** and (−)-**10a** increase p53 protein levels, activate p53-dependent MDM2 and p21 transcription in MDM2 amplified cells, and show improved selectivity for growth inhibition in wild type p53 cell lines over the parent compound.

The tumour suppressor protein p53 functions as a molecular sensor in diverse signalling pathways resulting from cellular stresses, such as hypoxia, DNA damage and oncogene activation.[1] The MDM2 protein, the gene for which is a transcriptional target of p53, downregulates p53 in a negative feedback loop.[2,3] MDM2 binds to the p53 transactivation domain and ubiquitylates the MDM2-p53 complex, resulting in export from the nucleus and proteasomal degradation.[4,5] Amplification of the *MDM2* gene, resulting in overexpression of MDM2 and inactivation of p53, has been reported in around 11% of all tumours.[6,7]

The investigation of small-molecule inhibitors of protein–protein interactions as potential therapeutic agents has received considerable interest, and has been reviewed recently.[8–10] The MDM2-p53 binding interaction has many attractive features for small-molecule inhibition, as it consists of a relatively deep binding groove on the surface of the MDM2 protein into which an amphipathic helix of p53 binds.[11] The X-ray crystal structures of MDM2 with a number of peptide and small-molecule ligands bound have been reported, demonstrating the prevalent key shape-filling and hydrophobic interactions.[12–14] Potent MDM2-p53 inhibitors, such as Nutlin-3 (**1**)[12] and the spirooxindoles, for example, MI-63 and MI-219 (**2a** and **2b**),[15,16] have demonstrated cellular activity consistent with inhibition of MDM2-p53 binding and have shown in vivo antitumor activity.[12,17]


Nutlin-3 (**1**)


MI-63 (**2a**)


MI-219 (**2b**)

* Corresponding authors.
E-mail address: i.r.hardcastle@ncl.ac.uk (I.R. Hardcastle).

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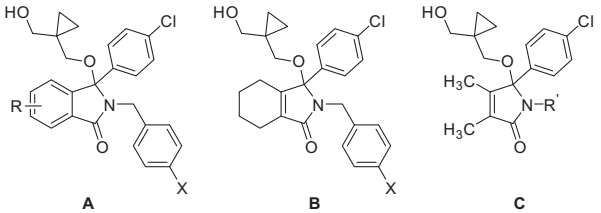
Table 1
MDM2-p53 inhibitory activity of isoindolinones **10a–n** and 1,5-dihydro-3,4-dimethylpyrrol-2-ones **11a–b**



Compound	Scaffold	X	R	R′	IC_{50}[a] (nM)
3	A	NO_2	H	—	225 ± 9
4	A	Br	H	—	1200 ± 600
5	A	CN	H	—	1800 ± 700
10a	A	NO_2	4-Cl	—	94 ± 10[b]
10b	A	Br	4-Cl	—	169 ± 3
10c	A	CN	4-Cl	—	185 ± 17
10d	A	SO_2CF_3	4-Cl	—	9000 ± 950
10e	A	NO_2	4-Me	—	274 ± 35
10f	A	NO_2	5-F	—	295 ± 65
10g	A	NO_2	5-Me	—	492 ± 35
10h	A	NO_2	5-^{t}Bu	—	733 ± 29
10i	A	NO_2	5-Br	—	902 ± 71
10j	A	NO_2	6-^{t}Bu	—	152 ± 27
10k	A	NO_2	6-F	—	852 ± 90
10l	A	NO_2	6-Br	—	1030 ± 40
10m	A	NO_2	5,6-Cl_2	—	3670 ± 1150
10n	B	NO_2	—	—	3470 ± 10
11a	C	—	—	CH_2Ph	3450 ± 90
11b	C	—	—	C_3H_7	8900[c]

[a] Values are the mean of three determinations ± SE.
[b] $n = 13$.
[c] $n = 1$.

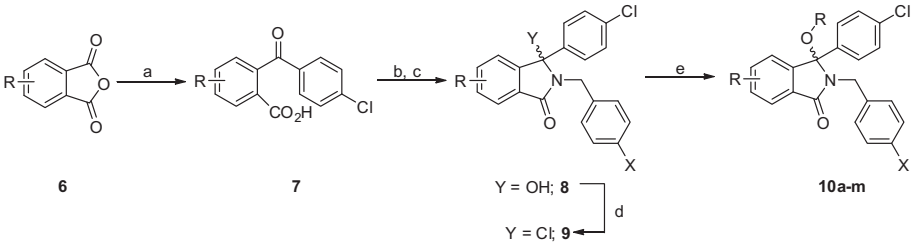
Previously, we have reported MDM2-p53 inhibitors based on an isoindolinone scaffold.[18,19] Structure–activity relationship (SAR) studies guided by NMR binding experiments have demonstrated the improved potency of 4-nitrobenzyl isoindolinones substituted with sterically hindered 3-hydroxypropoxyl groups, for example, Table 1 (**3–5**).[20] In this paper, we describe SAR studies around the isoindolinone aromatic or A-ring, leading to potent MDM2-p53 inhibitors. The choice of A-ring substitution was limited by the availability of the starting phthalic anhydrides for the chosen route, thus the 3-chloro, 3-methyl, 4-fluoro, 4-*tert*-butyl, 4-bromo, and 4,5-dichlorophthalic anhydrides were employed to investigate SARs.

Substituted 2-benzoylbenzoic acids **7** were prepared from phthalic anhydrides **6** via Friedel–Crafts acylation (Scheme 1). 3-Substituted phthalic anhydrides gave a mixture of 3- and 6-substituted-2-(4-chlorobenzoyl) benzoic acids as an inseparable mixture of regioisomers in a 20:1 ratio. Similarly, 4-substituted phthalic anhydrides gave approximately equal mixtures of 4- and 5-substituted acids in moderate to good yields. The mixtures **7** were converted into the corresponding mixture of ψ-acid chlorides and reacted with the appropriate benzylamine to give 3-hydroxyisoindolinones **8** as a separable mixture of regioisomers in moderate to good yields (42–74%). 3-Chlorination under Vilsmeier conditions gave the 3-chloro isoindolinones **9a–m** which were reacted immediately with the appropriate alcohol giving isoindolinones **10a–m**, as previously described.[19] The saturated derivative **10n** was prepared from 4,5,6,7-tetrahydroisobenzofuran-1,3-dione following the same method.

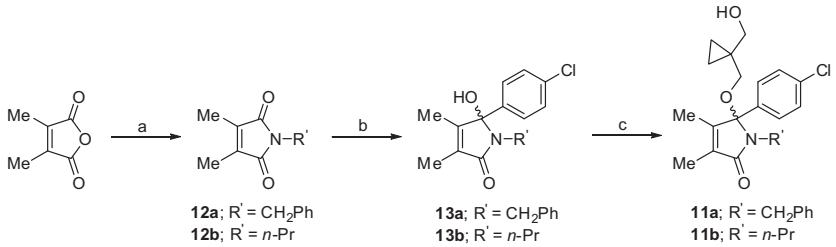
The synthesis of the 1,5-dihydro-3,4-dimethylpyrrol-2-ones **11a** and **11b** required addition of a Grignard reagent to the appropriately substituted maleimide (Scheme 2). The preparation of the 4-nitrobenzyl analogue was incompatible with the Grignard route so the *N*-propyl and *N*-benzyl compounds (**11a** and **11b**) were prepared as examples. Thus, *N*-benzyl-3,4-dimethylmaleimide **12a** and *N*-propyl-3,4-dimethylmaleimide **12b** were synthesised according to the literature method.[21] 4-Chlorophenylmagnesium bromide was added to the benzyl and propyl maleimides to give the corresponding 3-hydroxy products (**13a**,**b**) in good yields.[22] Finally, treatment with the Vilsmeier reagent followed by addition of the alcohol and base gave the benzyl and propyl 3,4-dimethyl-1,5-dihydropyrrol-2-ones **11a** and **11b** in excellent yield for both analogues.

The MDM2-p53 inhibitory activity of each final compound was determined as described previously.[19] In comparison with the parent compound **3**, the addition of a 4-chloro substituent to the A-ring resulted in a twofold improvement in potency. Interestingly, in comparison with the unsubstituted 4-bromobenzyl and 4-cyanobenzyl parent compounds (**4** and **5**) introduction of the A-ring 4-chloro substituent resulted in an approximately 10-fold improvement in potency (**10b** and **10c**). A significant improvement in potency was seen for the 4-chloro substituted trifluoromethylsulfone derivative **10d**, compared with the unsubstituted derivative (IC_{50} >50 μM),[19] however, in this series the trifluoromethylsulfone is not an effective nitro-bioisostere. The introduction of a 4-methyl group to **3** did not result in an improvement in potency. Substitution at the 5-position was not advantageous: the 5-fluoro derivative **10f** was equipotent with **3**, whereas the larger methyl, *tert*-butyl and bromo substituents resulted in a 2- to 4-fold loss of potency. The effect of substitution at the 6-position varied depending on the nature of the substituent. Introduction of the sterically demanding *tert*-butyl group (**10j**) resulted in a modest improvement in activity, whereas a fourfold loss of potency was seen for the 6-bromo compound (**10l**). Surprisingly, 6-fluoro substitution (**10k**) was not well tolerated. The 5,6-dichloro analogue (**10m**) was significantly less potent than the parent isoindolinone (**3**). The 4,5,6,7-tetrahydroisoindolinone (**10n**) was greater than 10-fold less potent than the parent, indicating a lack of tolerance for saturation in this ring. The *N*-benzyl-1,5-dihydro-3,4-dimethylpyrrol-2-ones (**11a**) showed similar potency to **10n** and the *N*-propyl analogue (**11b**) suffered a further loss in potency.

The three most potent inhibitors **10a–c** were resolved by chiral HPLC and the inhibitory activity of the enantiomers was determined (Table 2).[20,23] In each case, the fastest eluting and

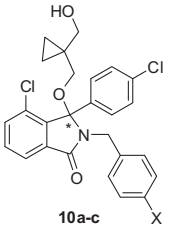


Scheme 1. Reagents and Conditions: (a) $AlCl_3$, chlorobenzene, 90 °C; (b) $SOCl_2$, DMF, THF; (c) substituted benzylamine, THF; (d) $SOCl_2$, DMF, THF; (e) ROH, THF.



Scheme 2. Reagents and conditions: (a) R′NH_2, THF, reflux, 16 h; (b) 4-chlorophenylmagnesium bromide, THF, −78 °C, 2 h; (c) (i) $SOCl_2$, DMF, THF, rt, 4 h; (ii) 1,1-bis(hydroxymethyl) cyclopropane, K_2CO_3, THF, rt, 16 h.

Table 2
MDM2-p53 inhibitory activity of enantiomers of isoindolinones **10a–c**



Compound	Rotation	X	IC_{50}[a] (nM)
10a	(+)	NO_2	763 ± 400[b]
10a	(−)	NO_2	44 ± 18[c]
10b	(+)	Br	4015 ± 285
10b	(−)	Br	137 ± 67
10c	(+)	CN	4077 ± 75
10c	(−)	CN	136 ± 38

[a] Values are the mean of three determinations ± SE.
[b] $n = 6$.
[c] $n = 7$.

laevo-rotatory enantiomer was the most potent of the pair, while the antipode displayed only modest inhibitory activity. Interestingly, the (−)-enantiomer of the 4-nitro-isoindolinone **10a** was more potent than the either the 4-bromo or 4-cyano analogues (**10b** and **10c**). To date, an X-ray structure has not been obtained for these enantiomers and so the absolute stereochemistry has not been assigned, efforts are continuing. The eutomer of the parent **3** was also the fastest eluting from the chiral HPLC column and determined to have an (*R*)-configuration, but has the opposite (+)-rotation.[20] Racemisation under the assay conditions was not observed.

The cellular activity of racemic **10a** and its enantiomers was determined by Western blotting as previously described.[20] SJSA-1

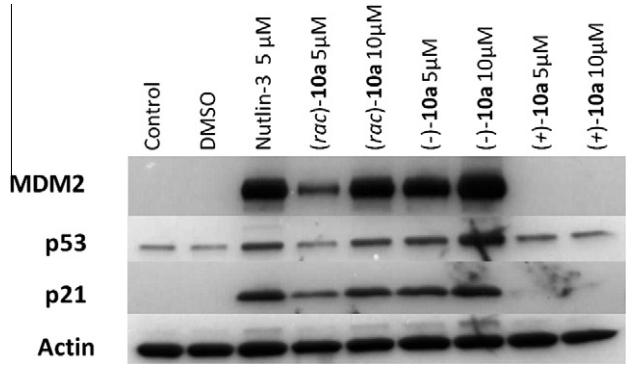


Figure 1. Western blot analysis of SJSA-1 cells treated for 6 h with (*rac*)-**10a** and enantiomers.

Table 3
Growth inhibitory activity of (*rac*)-**10a** and enantiomers in MDM2 amplified cell lines

Cell Lines	SRB assay GI_{50}[a] (μM)				
	MI-63	Nutlin-3	**10a**	(−)-**10a**	(+)-**10a**
SJSA-1	0.55 ± 0.01	1.3 ± 0.2	6.0 ± 0.8	3.7 ± 0.6	12.2 ± 1.1
HCT-116	1.3 ± 0.2	2.1 ± 0.6	6.0 ± 0.7	3.7 ± 0.6	12.3 ± 3.0
HCT-116 p53 (−/−)	11.8 ± 1.4	25.6 ± 1.8	14.9 ± 1.9	12.5 ± 3.0	14.4 ± 2.8
A2780	1.19 ± 0.04	1.6 ± 0.9	4.2 ± 1.9	3.3 ± 2.2	7.7 ± 1.5
A2780 CP70	15.1 ± 2.5	20.4 ± 1.7	15.9 ± 1.4	13.6 ± 3.5	14.5 ± 2.3

[a] Values are the mean of at least three determinations ± SE.

cells (MDM2 amplified, p53 (wt)) were treated with increasing concentrations (1–20 μM) of **10a**, **(+)-10a**, and **(−)-10a**. Nutlin-3a (**1**) was included for comparison (Fig. 1). Both racemic **10a** and (−)-**10a** induced a dose-dependent increase in MDM2, p53, and p21 protein levels, consistent with cellular activation of p53. As expected, the less potent **(+)-10a** did not activate p53 at the highest concentration, suggesting that the p53 dependent cellular activity of (*rac*)-**10a** resides solely in the (+)-enantiomer.

The growth inhibitory activity of compounds **10a**, **(+)-10a**, and **(−)-10a** was investigated in cell-lines with known p53 and MDM2 status (Table 3). Nutlin-3 and MI-63 were included as positive controls for comparison. The GI_{50} values for **10a**, **(+)-10a**, and **(−)-10a** in p53 wild type, the MDM2 amplified cell line (SJSA-1) showed that the least potent enantiomer **(+)-10a** is 2- to 3-fold less growth inhibitory than the more potent **(−)-10a**. Compounds **10a** and **(−)-10a** were 2.5- to 4-fold more growth inhibitory in p53 wild-type cells (A2780 and HCT116 p53(+/+)) compared with their isogenically paired p53 mutant (A2780-derived CP70) and p53 null cell lines (HCT116 p53(−/−)), whereas a less than twofold differential was observed for the less potent **(+)-10a**. These results suggest that this series possess growth inhibitory activity which is dependent on p53-MDM2 inhibition, but also a component of p53-independent activity is observed. Importantly, all classes of MDM2-p53 inhibitors tested showed similar growth inhibitory activities in p53-null cell-lines at an equivalent potency. Isoindolinone (−)-**10a** is 3- to 6-fold less growth inhibitory than MI-63 in p53 functioning cells, consistent with the inhibitor's 10-fold lower potency. Similarly, (−)-**10a** 2- to 3-fold less growth inhibitory than Nutlin-3.

In conclusion, we have discovered that modifications to the A-ring of the isoindolinones has significant impact on their MDM2-p53 activity. In particular, introduction of a 4-chloro group results in an approximately 4-fold improvement in potency for (−)-**10a** compared with (+)-**3**. Structural studies to rationalise the observed SAR are ongoing. These results demonstrate the versatility of the isoindolinone scaffold as MDM2-p53 inhibitors and show that significant improvements in potency may be gained by modest structural modifications. Studies are ongoing to optimise the potency, selectivity and pharmaceutical properties of this series.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.084.

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23. *Chiral separations*: (**10a**) Diacel Chiralpak Ad-H, 250 × 10 mm; 80:20 hexane, ethanol, 1.6 mL/min; rt, (−) 11.1 min, (+) 15.4 min; (**10b**) Diacel Chiralpac IA, 250 × 10 mm; 85:15 heptane, propan-2-ol, 3.75 mL/min; rt, (−) 17.8 min, (+) 24.9 min; (**10c**) Diacel Chiralpac IC, 250 × 4.6 mm; 95:5 heptane, ethanol, 1.0 mL/min; rt, (−) 17.4 min, (+) 23.3 min.