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2,3'-Bis(1'*H*-indole) Heterocycles: New p53/MDM2/MDMX Antagonists

Constantinos G. Neochoritis^a, Kan Wang^b, Natalia Estrada-Ortiz^a, Eberhardt Herdtweck^c, Katarzyna Kubica^d, Aleksandra Twarda^{e,f}, Krzysztof M. Zak^e, Tad A. Holak^{d,e}, and Alexander Dömling^{a,b,*}

^aDepartment of Drug Design, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands ^bDepartment of Chemistry, University of Pittsburgh, 3501 Fifth Avenue, Pittsburgh, PA 15261, USA ^cDepartment Chemie, Technische Universität München, Lichtenbergstrasse 4, D-85747 Garching bei München, Germany ^dDepartment of Organic Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow, Poland ^eMalopolska Centre of Biotechnology, Jagiellonian University, Gronostajowa 7a, 30-387 Krakow, Poland ^fFaculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland

Abstract

The protein-protein interaction of p53 and MDM2/X is a promising non genotoxic anticancer target. A rapid and efficient methodology was developed to synthesize the 2,3'-bis(1'*H*-indole) heterocyclic scaffold **2** as ester, acid and amide derivatives. Their binding affinity with MDM2 was evaluated using both fluorescence polarization (FP) assay and HSQC experiments, indicating good inhibition and a perfect starting point for further optimizations.

Graphical abstract

*Corresponding author. Tel.: +3150-3633307.; fax: +3150-3637582; a.s.s.domling@rug.nl.

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Supplementary data

Experimental procedures for the synthesis of compounds, characterization of compounds, crystal data, as well FP assay and NMR HSQC are provided in the supporting information.

Keywords

p53; MDM2; MDMX; Protein-protein interaction; Cancer

The transcription factor and tumor suppressor p53 has multiple roles in stabilizing the genome by preventing mutations.^{1,2} However p53, our genome guardian, is the most mutated protein in human cancer with >50% of cancers not showing functional p53.³ The remainder group of cancers also exhibit reduced p53 pathway activity due to the negative regulation through the protein-protein interaction (PPI) with inhibitors as MDM2/X or viral proteins. MDM2 is a key regulator of p53 activity via a complex regulatory feedback system that involves all levels of expression control including transcription, mRNA translation and protein degradation. MDM2 inhibits the N-terminal trans-activation domain (TAD) of p53, and promotes p53 degradation through the ubiquitin-proteasome system (E3 ligase activity).^{4a,b} Similarly to MDM2, the negative regulation of p53 of MDMX (90% homology with MDM2) is displayed via the inhibition of the TAD domain and the formation of heterodimers with MDM2 which can increase the rates of ubiquitinylation of p53 by MDM2.^{4c,d} Therefore, blocking the interaction between wild-type p53 and its negative regulators MDM2 and MDMX has become an important target in oncology to restore the anti-tumor activity of p53.⁵⁻⁷

The discovery of novel p53-MDM2/X inhibitors was one of the highlights in anti-tumor agents.⁸ Since the disclosure of Nutlin-3,⁹ many scaffolds have been prepared and evaluated, including indo-imidazole, imidazoline, benzodiazepinedione and spirooxindole scaffolds.¹⁰ Thus, antagonizing the p53-MDM2 or/and MDMX interaction is a promising anticancer strategy and several compounds are presently undergoing early clinical evaluations.¹¹⁻¹⁴ However, the discovery of new p53/MDM2/MDMX scaffolds is still of high interest due to low single agent activity currently seen in clinical trials and insufficient PKPD properties.

A three finger pharmacophore model is now widely accepted to be responsible for the binding of small molecules to the MDM2 and recently an extended four finger model was experimentally shown by co-crystallization.^{15,16} The corresponding amino acid residues in p53 are tryptophan (Trp23), leucine (Leu26) and phenylalanine (Phe19). Initially, indole fragment was taken as starting point to mimic tryptophan residue, where an important

hydrogen bond was observed. Studies by Garcia-Echeverria et al. on a p53-derived linear octapeptide showed that a Trp23 to (6-Cl) Trp substitution gave rise to a 63-fold increase in affinity for MDM2.¹⁷ The starting point for our antagonist discoveries was the anchoring side chain of tryptophan embedded in a deep hydrophobic pocket formed by the residues Leu57, Phe86 and Ile99 using our pharmacophore based virtual screening platform ANCHOR.QUERY.^{7,15,18,25} This had led to several scaffolds potentially antagonizing p53-MDM2. Amongst them we could also solve representative co-crystal structures of the imidazoloindole derivative **1** binding to MDM2 (PDB ID: 3LBK) and the close relative MDMX (PDB ID: 3LBJ) confirming the initial binding hypothesis.¹⁸ Based on these findings, a new class of 1,2,3-trisubstituted bis(indoles) heterocycles (Figure 1) was designed. In this paper, we report the novel synthesis and preliminary biophysical evaluation of this first of its kind antagonists bisindoles **2**. It should be noted that the class of p53-MDM2 antagonistic imidazoloindole has also discovered independently by another group.^{18a}

Our hypothesis is that the additional phenyl annulated ring in scaffold **2** will make additional hydrophobic interactions with the ^{mdm2}Val93,¹⁰ mimicking ^{p53}Leu22 (Figure 2). To test this speculation we developed a short synthesis towards scaffold **2**.

Our retrosynthetic plan of the designed derivatives **2** is based on the indole acetyl derivative **3** and hydrazine **4** which can react in a Fischer indole synthesis (Scheme 1).

After some optimization, derivative **3a** was easily synthesized by selective acylation at the 3-position of the 6-chloroindole derivative **5a** in high yields in the presence of Lewis acids, such as SnCl₄ (Scheme 2).^{19–21} Compound **4a** was derived by the direct alkylation of the secondary amine of the phenylhydrazine **7a**, with no need of protecting groups.^{22,23} Finally, refluxing derivatives **3a** and **4a** in acetic acid, gave the targeted bisindole **2a** in good to very good yields in a just 3-step sequence.

The synthesis of bisindoles **2**, offers many possibilities for SARs since both of the main components (**3** and **4**) could easily be modified (Figure 3). Different halogens as R¹, R² and R³ and different sizes in the linkers of the two aromatic groups were tested. Moreover, the importance of carboxylate moiety was taken into account, as found in previous MDM2 binders.^{15,16,18} Using the ethyl esters, the corresponding acids and various amides could be synthesized in order to optimize some physicochemical properties (Figure 3).

The 3-alkylation of the 6-chloroindole derivatives proceeded smoothly affording the corresponding compounds in excellent yields as shown in table 1.²¹ The crystal structure obtained of the indole derivative **3c**, is described in supporting information (CCDC 981828).

The hydrazine derivatives **4a–c** were synthesized by the regioselective alkylation of the secondary amine, thus different hydrazines and benzyl halides were utilized in order to initially explore the aforementioned interactions in the MDM2 pocket (Table 2).

Next, the synthesis of the desired bisindoles **2a–h** was performed. Combining the two moieties, the indole derivatives **3** and hydrazines **4** into a Fischer indole synthesis produced

a series of active compounds (Scheme 3). All the combinations and compounds that were synthesized are shown below.

The structures of the above compounds were unambiguously characterized and confirmed by the crystal structures of the compounds **2a** and **2h** (SI, CCDCs 981829 and 981827 respectively). Due to the fact that ester groups are readily cleaved in cells by esterases and are sometimes metabolically unstable, we hydrolyzed, under basic conditions specific derivatives **2** into the corresponding acids **9** in order to compare and evaluate the activity. Additionally we know from our previous studies on scaffold with the indole anchor, that the free –COOH is always 1–2 orders of magnitude more potent towards MDM2 protein.^{7,15–17,24,25}

Apart from poor absorption-distribution-metabolism-excretion-toxicology (ADMET) properties, insufficiently water-soluble compounds often lead to poor reproducibility and unreliable results or even false positive hits during *in vitro* screening. In order to potentially improve the properties of our compounds, we converted the ester group of the derivatives **2** into the corresponding better water soluble amides **10** with a one-step TBD-catalyzed amidation procedure (Scheme 5).²⁶ In our previous studies, amidation of the indole-2-carboxylate gave often improved water solubility.²⁴

Binding affinities were determined by the fluorescence polarization method (FP) towards MDM2 and MDMX (SI).²⁵ Representatives from each class compounds, esters, acids and an amide, were evaluated as inhibitors of the PPI. The results are summarized in the table 3. Noticeably, the acids **9a** and **9d** comparing with the corresponding esters **2a** and **2e** were found highly active towards both MDM2 and MDMX as previously reported by the indole anchor.^{7,15–17,24,25} In addition, compounds **9a,d** provided some initial dual activity (MDM2/X) which is an interesting factor that most of the current inhibitors fail to combine.¹⁴ Moreover comparing the binding affinities of scaffold **1** (WK23)¹⁸ which was 0.9 μ M and 36 μ M for MDM2 and MDMX respectively, the novel scaffold **9**, is more potent towards MDMX without significant loss of activity in MDM2 (**9a**) or even slight improvements (**9d**).

FP-based screening of protein-protein interactions often gives a high fraction of false positives especially with hydrophobic molecules and therefore it is advisable to run a second orthogonal biophysical assay. As a second, orthogonal screening system we performed the well-known heteronuclear single quantum coherence (HSQC) experiment where the ¹⁵N labeled MDM2 is titrated with compound **9d**. The expected ligand-induced perturbations in NMR chemical shifts are indeed observed (Figure 4).²⁷ Since all cross peaks in the MDM2 spectrum were assigned to particular amino acid residues before^{28,29} it is possible to analyze the way of interaction in the MDM2/**9d** complex. For example the interaction of **9d** with the MDM2 tryptophan subpocket is expressed by the movement of the peak assigned to V93 (Figure 4). Chemical shift changes of T101 and M62 indicate interaction with the leucine and phenylalanine subpocket, respectively. Both the FP assay and HSQC test indicate that the acid derivatives are the active species as p53-MDM2/X inhibitors.

Our modelling (Figure 5) based on the HSQC binding data and known co-crystal structures and using MOLOC software³⁰ revealed the nice alignment of the 6-chloro-indole moiety of compound **9d** with the anchoring ^{p53}Trp23, whereas the two phenyl rings occupy the hydrophobic pockets mimicking ^{p53}Phe19 and ^{p53}Leu26. Moreover, the additionally introduced phenyl-annulated ring of the second indole moiety is predicted to cover ^{mdm2}Val93 through hydrophobic interactions. Then we rationalized the tight receptor ligand interaction using a small world network approach using Scorpion software (Figure 5).³¹ His96 is forming a pi-pi interaction with the p-Cl phenyl fragment and a hydrogen bond is formed between the Leu57 backbone carbonyl and the indole NH. Multiple van der Waals interactions can be used to rationalize the tight interactions. Amongst the major contributors of the interaction, shown as red balls, are F37-Tyr67, F27-Met62, C20-Met62, N7-Leu57. C110 contributes to an extended network including Phe86, Ile99, Ile103 and Leu57. Less strong contributors are C13-Val93, C3-, C1-, C6- of the buried indole moiety and C31-Leu57 and C136-Ile99. Interestingly small world network analysis of WK23 (PDB ID 3LBK) reveals no direct contribution of the scaffold imidazole to the tight interaction, whereas in **9d** C13 interacts with Val93, supporting our hypothesis.

In conclusion, the designed 2,3'-bis(1'*H*-indole) scaffold **9** is active as dual action inhibitor of the p53-MDM2/X interactions with initial sub- μ M affinities. Our hypothesis, that the extra phenyl ring in scaffold **2** makes additional hydrophobic interactions with the p53Val93 comparing with the derivatives **1**, is suggested by 2D NMR and modeling studies. Further studies are ongoing to introduce more 'drug-like' properties into this scaffold and to investigate cellular mechanism-based anti-cancer behaviors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Protein-protein interaction of p53 peptide (orange α -helix with sticks) with MDM2 (grey surface) (PDB ID 1YCR). Key amino acids Leu22 (marine sticks), Phe19 (green sticks), Trp23 (blue sticks) and Leu26 (red sticks) are mounted onto the orange p53 α -helix. Leu26 is embedded into the hydrophobic receptor amino acids Ile99, Ile103, Leu54 and Val93. The Trp23 pocket is formed by Leu57, Phe86 and Ile99. The Phe19 pocket consists of Val93, Gln72, Val75, Tyr67, Met62, Ile261 and Gly58. Val93 undergoes hydrophobic interactions with p^{53} Leu22.

Figure 2.
Conversion of central imidazole scaffold **1** into indole scaffold **2** to address p^{53} Leu22.

Figure 3.
SAR potential of bisindoles.

Figure 4. Superposition of NMR HSQC spectra of the ^{15}N -labeled MDM2 titrated against **9d**. The spectrum of free MDM2 is shown in blue. The spectrum of **9d**-MDM2 (ratio 1:1, respectively) is shown in red.

Figure 5.

above: Modeled binding pose of the most potent compound **9d** (green sticks) in the MDM2 pocket (grey surface presentation, PDB ID 1YCR) and alignment with the hot-spot triade F19W23L26 (blue sticks). L57 and V93 are shown as grey sticks; below: Small network analysis of **9d** in the MDM2 receptor. π - π , hydrogen bonding and van der Waals interactions are shown in red, blue and magenta dotted lines, respectively. Each ligand non-H atom is shown as colored ball according to its importance of contribution to the network

(descending importance: red, purple, grey). Atom numbering of **9d** is given in the right corner.

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Scheme 1.
Retrosynthesis of scaffold **2**

Scheme 2.
3-step sequence for preparing 2,3'-bis(1'H-indole) heterocycles.

Scheme 3.
Library of the indole derivatives **2**

Scheme 4. Hydrolysis of derivatives **2** to the corresponding acids **9**.

Scheme 5.
TBD-catalyzed amidation of the bisindoles **2**

Table 1

3-Acylation of 6-chloroindole derivatives

Entry	Y	X	Compound (yield %)
1	-CO ₂ Et	Ph	3a (99)
2	-CO ₂ Et	4-Cl-C ₆ H ₄	3b (97)
3	-CO ₂ Et	4-F-C ₆ H ₄	3c (95)
4	-CO ₂ Et	4-Cl-C ₆ H ₄ CH ₂	3d (95)
5	H	Ph	3e (99)

Table 2Alkylation of the secondary amine of hydrazines **7**.

Entry	R ¹	R ²	Compound (yield %)
1	H	H	4a (40)
2	H	Cl	4b (60)
3	Cl	Cl	4c (42)

Table 3

Results of the evaluation of inhibitory activity of compounds 2,3'-bis(1'*H*-indole) Heterocycles towards MDM2/MDMX as determined by FP.

Compound	K_i (μ M)	
	MDM2	MDMX
2a	17	NA
2c	10	NA
2d	>22	NA
2e	6.7	5.0
2f	NA	NA
9a	1.8	0.2
9d	0.7	1.5
10d	NA	6,09

WK23

WK23 (1)	0.92	36
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NA: not active, $K_i > 60 \mu$ M.