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Benzimidazole-2-one: A novel anchoring principle for antagonizing p53-Mdm2

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ABSTRACT

Herein we propose the benzimidazole-2-one substructure as a suitable tryptophan mimic and thus a reasonable starting point for the design of p53 Mdm2 antagonists. We devise a short multicomponent reaction route to hitherto unknown 2-(2-oxo-2,3-dihydro-*1H*-benzo[*d*]imidazol-1-yl)acetamides by reacting mono N-carbamate protected phenylenediamine in a Ugi-3CR followed by base induced cyclisation. Our preliminary synthesis and screening results are presented here. The finding of the benzimidazolone moiety as a tryptophan replacement in mdm2 is significant as it offers access to novel scaffolds with potentially higher selectivity and potency and improved biological activities. Observing low μM affinities to mdm2 by NMR and fluorescence polarization we conclude that the 2-(2-oxo-2,3-dihydro-*1H*-benzo[*d*]imidazol-1-yl)acetamide scaffold might be a good starting point to further optimize the affinities to Mdm2.

1. Introduction

The protein protein interaction (PPI) between the transcription factor p53 and the oncogene Mdm2 is of major recent interest as a novel non genotoxic anti cancer target.[1] Although PPIs are generally believed to be difficult to treat by small molecules, p53-Mdm2 is an exception and several potent compounds have been recently disclosed.[2] Additionally one compound RG-7112 currently undergoes clinical trials and interim results are promising.[3]

As part of our ongoing interest in the rational design of small molecular weight PPI antagonists we describe here the discovery and screening of novel p53-Mdm2 antagonists based on a new variation of the three component Ugi reaction leading to substituted 1-oxo-benzimidazoles.[2,4]

In his seminal work on the 'structure of the mdm2 oncoprotein bound to the p53 tumor suppressor transactivation domain' Pavletich described the 'hot spot' of this protein–protein interaction (PPI).[5] The interface of this PPI is formed by a small α-helical part of the N-terminal p53 interacting with the deep and spatially confined binding site on the N-terminal Mdm2 (Fig. 1).

Thus p53-Mdm2 differs from many other PPI in several respects: (1) the binding site is deep and concave as opposed to flat and convex or planar; the hot spot amino acids F19W23L26 are deeply buried in the Mdm2 receptor, thus enabling the accommodation of a small molecule. (2) The dimension of the binding site is of similar size as typically seen in small molecules; the distance between the C-α's of F19 and L26 is 12 Å. (3) Additionally, the interaction is highly hydrophobic in nature, thus potentially facilitating the transport of the small molecule to the intracellular and -nuclear target site. The characteristics of benign PPIs for discovering drug-like small molecule inhibitors have been recently discussed by Fry.[6]

Several potent small molecules have been recently structurally characterized in Mdm2.[4m,n,7] Currently, all well characterized small molecules have been shown to competitively inhibit the PPI and in fact bind into the same p53 Mdm2 site. Thus most known p53-Mdm2 antagonizing small molecule scaffolds clearly mimic the hot-spot amino acids F19Y23L26. Amongst the hot-spot amino acids, Y23 is the most buried and hydrophobic. Presumably much binding energy can be gained for the small molecules by properly mimicking this moiety. Additionally, the indole-NH of p53-W23 is forming a hydrogen bond to the backbone carbonyl of Mdm2-L54. Currently there are several p53-W23 mimics used in small molecules (Fig. 2). The *para*-halo phenyl residue is the most popular used in the majority of active compounds (**1**, **2**, **5**) to mimic W23. Remarkably, in these compounds the p53-W23 Mdm2-L54 hydrogen bond is not reconstituted suggesting that it is not needed to achieve high affinity to the receptor. Compound **3** is mimicking W23 with an oxindole moiety. A 6-chloro

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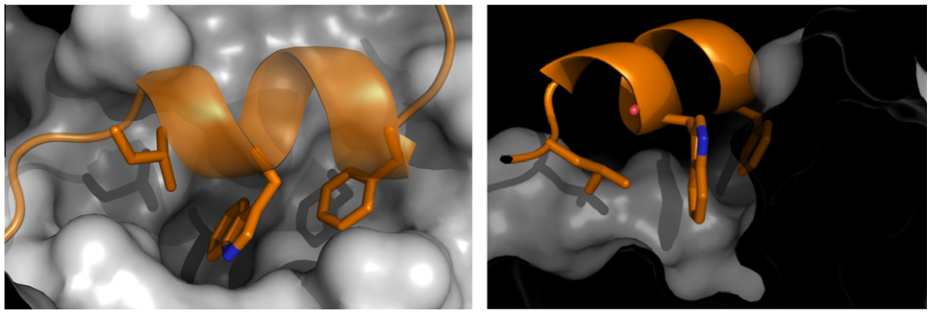


Figure 1. The hot spot interaction of the transcription factor p53 and the oncogene Mdm2 (PDB ID 1YCR). Left: Frontal view of the p53 amino acids F19W23L26 (orange sticks) mounted on the amphipathic α-helix are deeply buried in the Mdm2 receptor (grey surface); Right: Cut away view to show the hydrophobic floor below the anchor amino acid ^{p53}W23, which is typically filled with bulky halogen atoms in the inhibitor molecules.

substituent, similarly to all other compounds considerably enhances the affinity to the receptor by filling a hydrophobic pocket at the bottom of the W23. Compounds **4** and **6** most closely mimic the W23 by providing a 6-chloroindole substructure. Both compounds were discovered by an approach that makes use of the most buried amino acid side chain of a PPI, serving as a focal point for synthesis. [4k] A very large virtual library containing indole substructures based on multicomponent reaction chemistry was screened using the freeware ANCHOR.QUERY.[8] In the following we want to introduce the benzimidazole-2-one substructure as a new anchor moiety useful for the design of p53 mdm2 antagonists.

2. Results

We envisioned that a 1-oxo-benzimidazole moiety might function in a similar way to tryptophan exhibiting similar size, shape, hydrophobicity and H-bond donor capacity and bind into the W23 pocket of Mdm2. To test this idea and efficiently access sample compounds we screened the synthetic literature for short and convergent access to the benzimidazole-2-one moiety. However all syntheses of complex molecules incorporating this moiety were of considerable length involving several sequential steps or were leading to compounds of insufficient diversity.[9] Therefore we decided to investigate convergent and short multicomponent reaction (MCR) approaches towards the benzimidazole-2-one structure. In fact Hulme et al. reported an elegant and versatile MCR towards hydantoins and cyclic ureas using mono protected bifunctional building blocks and CO_2 in methanol in an Ugi 5-CR followed by cyclisation (Scheme 1).[10] Unfortunately when using *o*-phenylenediamine the expected product could not be isolated.

We therefore designed a straightforward and general MCR approach involving the two step sequence Ugi 3-CR of mono carbamate protected o-phenylenediamine followed by base induced cyclisation (Scheme 2). The required mono carbamate protected phenylenediamines can be readily accessed by reduction of the corresponding nitro aniline precursor.[11]

There are many experimental conditions described to run the U-3CR including a very recent improvement by List et al. using toluene as a solvent and phenylphosphinic acid as an acid catalyst under refluxing conditions.[12] These variations of the U-3CR, however, in our hands did not work and we had to adapt it to our starting materials by changing the solvent and reaction temperature. Screening several reaction conditions with the three components methyl 2-amino-5-chlorophenylcarbamate (**S1**), phenylacetaldehyde (**S3**) and phenylethylisocyanide (**S12**), we found that the combination of the solvent THF and the catalyst phenylphosphinic acid works well for the U-3CR of our starting materials. The cyclisation can be performed in good to acceptable yields using triazabicyclodecene (TBD) as a strongly basic catalyst (Table 1).

With this short 2-step reaction sequence in hand we designed a small compound library of 2-(2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)acetamides based on modeling virtual compounds into the p53/Mdm2 PDB structure 1YCR using the software Moloc.[13] In order to obtain compounds with good affinity to Mdm2 it is well established that indoles with a chlorine in 6-position can be used to fortunately fill a void and very hydrophobic subpocket at the bottom of p53's Trp23.[14] Thus we included a chlorine at the 6-position of our phenylenediamine starting material (Fig. 3, **S1**). Next in order to fill the crucial Leu26 and Phe19 pockets suitable substituents in the isocyanide and aldehyde part had to be chosen. Based on the pseudo symmetry of the p53 binding pocket in Mdm2 we included into our design bulky aliphatic side chains such as tert-butyl, cyclohexyl, iso-butyl and aromatic side chains such as benzyl and phenylethyl (Fig. 3, **S12**–**S16**).[2b]

In order to physically measure the interaction of our small molecules to the Mdm2 receptor we performed a fluorescence polarization (FP) assay as previously described by us. [4n] The compounds' affinities screened as racemic mixtures are between no binding (>60 μM) and 16 μM (Table 1).

For the most active compound **10** we separated the enantiomers using preparative supercritical CO_2 HPLC. The separation was fast and very efficient (Fig. 4 1 and 2). The slower dextrorotatory ($[\alpha]_D^{20.5}$ −69.6 c = 0.98 g/100 ml, MeOH) enantiomer **10 B** was more active in FP (4 μM) then the faster levorotatory enantiomer **10 A** ($[\alpha]_D^{20.5}$ +66.54, c = 0.98 g/100 ml, MeOH) (>60 μM) and the separated enantiomers served for further binding studies using orthogonal physicochemical screening systems. A NMR-based competition assay for the discovery of PPI antagonists was recently introduced by us (AIDA_NMR for antagonist induced dissociation assay-NMR). [4b-d,i] In this one dimensional experiment the antagonist is titrated to the preformed p53-peptide Mdm2 complex. The release of p53 can be observed in a concentration dependent manner by observing the indole-NH of W23 of p53 (∼10 ppm, Fig. 4.4). According to this AIDA experiments enantiomer **10 B** has a Ki of ∼2 μM whereas enantiomer **10 A** is inactive (>60 μM). As a third screening system we performed the well-known heteronuclear single quantum coherence (HSQC) experiment where the enantiomer **10 B** is titrated to the ^{15}N labeled Mdm2 and the ligand-induced perturbations in NMR chemical shifts are observed resulting in a K_i of 5 μM.[15] Dependent on the binding site of the small molecule on the receptor the NMR cross peaks are shifted in a concentration dependent manner. Moreover, the strong binding of compounds to Mdm2, with K_D of less than 1 μM (and a slow chemical exchange), is indicated by signals doubling. [16,4k,n]

Enantiomer **10 B** is showing binding to Mdm2 in all three screening systems and gratifyingly the binding constants are comparable (K_i ∼2–5 μM). We also screened the compound for Mdm4 activity by FP but could not find any binding. Based on the different

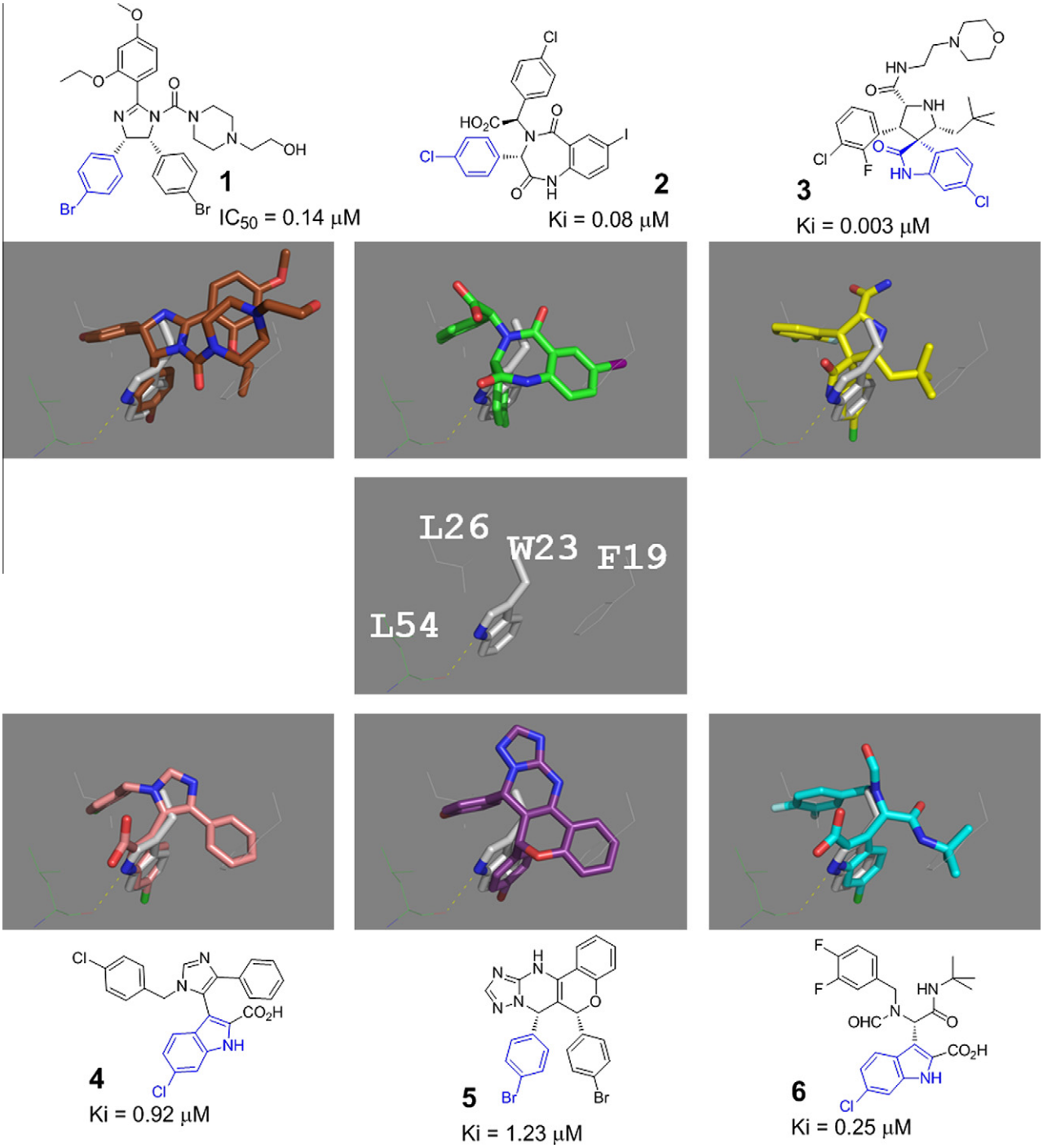


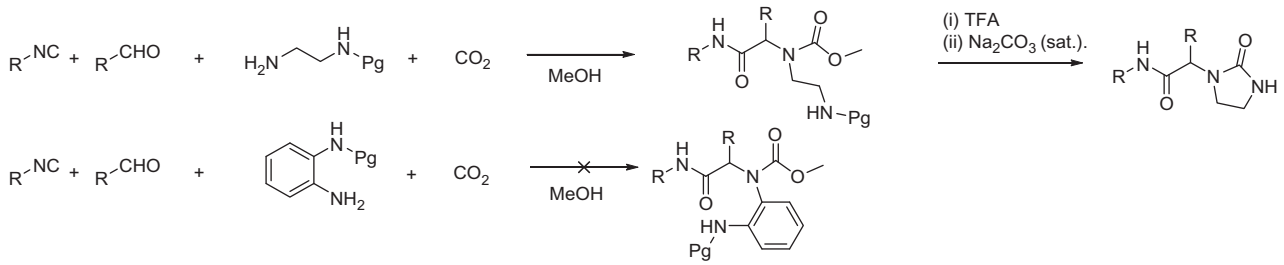
Figure 2. The W23-mimicking anchors of different cocrystallized p53 Mdm2 antagonists based on six chemotypes shown as structural alignments of the indicated PDBs. In 2D the anchor residues are marked in blue. Center: Anchor residue p53-W23 shown as grey sticks, p53-L26 and –F19 shown as red lines, Mdm2-L54 shown as green lines, hydrogen bond indicated with yellow dotted line (1YCR). First row left: nultin derivative **1** as brown sticks (1RV1); middle: benzodiazepindione **2** as green sticks (1T4E); right: spirooxindole **3** as yellow sticks (3LBL, the morpholinoethyl side chain has no electron density due to flexibility); lowest row right: imidazole **4** as pink sticks (3LBK); chromenotriazolopyrimidine **5** as purple sticks (3JZK); α-aminoacylamide **6** as cyan sticks (3TU1).

biophysical screening results and the structurally well characterized p53 Mdm2 structure we docked the most potent compound **10** into the Mdm2 binding site using the Moloc software (Fig. 5).[13]

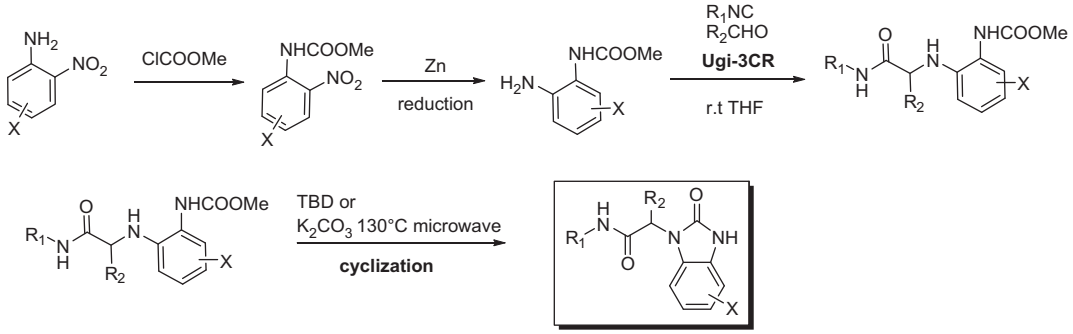
According to this model the benzimidazolone moiety binds into the W27 binding site displaying a very similar size, shape and hydrophobicity as the indole ring. The benzimidazolidinone NH forms a similar hydrogen bond to the rim L54 carbonyl. In addition the 6-chloro substituent fills the hydrophobic subpocket at the bottom of W23 as seen in all co crystal structures (Figs. 1 and 2). The cyclohexyl substituent resulting from the isocyanide input in the Ugi reaction mimics the F19 and fills the hydrophobic pockets exhaustively. The benzyl group introduced via the phenylacetaldehyde is pointing deeply into the L26 pocket.

3. Discussion

Amongst all, the largest and most hydrophobic amino acid tryptophan plays an extraordinary role in PPIs and is the most



Scheme 1.



Scheme 2.

abundant interface amino acid.[17] Typically tryptophan it is highly buried and the indole substructure is anchored in deep hydrophobic pockets and can thus form a formidable starting point for the design of small molecular weight antagonists. Our ongoing interest in the rational design of small molecule antagonists of the p53 Mdm2 and Mdm4 interactions has prompted us to design scaffolds with novel tryptophan mimicking features.[4k] While Wang et al. introduced the oxindole anchor, we introduced indole and others are using *p*-substituted phenyl groups, we wanted to explore here the benzimidazolidinone moiety as a tryptophan mimicking moiety.[18] For reasons of resources we restricted our search of suitable benzimidazolidinone scaffolds to multicomponent reactions as opposed to sequential multi step syntheses. We developed a novel Ugi 3-CR variant using N-methylcarbamate monoprotected o-phenylenendiamines followed by a base-induced cyclisation to efficiently access the target scaffold in great diversity and only two steps from abundantly accessible starting materials. These reactions suited us well to investigate initial binding activities to the oncogene Mdm2 by using two NMR assays and the fluorescence polarization assay. The use of several orthogonal screening systems is of high importance to avoid false positives especially when screening protein protein interactions with strongly hydrophobic interfaces.[19] Initially found activities ranged from >60 μM (inactive) to 16 μM for racemic compound **10** in a small set of synthesized compounds. We note on the side that the compounds of this series—although synthesized as racemic mixtures—can be conveniently and fast separated on a preparative scale using supercritical fluid CO_2 HPLC. The more active enantiomer of compound **10** revealed similar K_i's of 2–5 μM in the three convergent binding assay.

Strikingly, a very large subset of PPIs is mediated by hydrophobic interactions involving an amphipathic α-helix.[20] Typically, a string of hydrophobic amino acids side chains (i, i + 3, i + 7 and higher) of the donor protein interacts with a suitably formed hydrophobic binding groove of the receptor protein. Based on the shire number of α-helix mediated PPIs it is likely that PPIs with similar shape and hydrophobicity characteristics exist. A FxxxW(F)xxL sequence motif query in the PDB, for example, results almost 5000 hits and many of them are parts of α-helix motifs (query Dec. 2011). The question of selectivity of the small molecules addressing such interaction thus arises.[21] We hypothesize that small molecules interacting with their target exclusively based on shape and hydrophobicity features might be ambiguous and show binding to a range of similar hydrophobic binding sites. On the other hand it is well established that hydrogen bonds are spatially directed, while van der Waals interactions are not.[22] Whereas several currently pursued p53 Mdm2 antagonizing scaffolds rely on exploration of shape and hydrophobic characteristics (e.g. **1**, **2**, **5**), we believe that a mix of hydrogen bonds and hydrophobic interactions with the receptor increases the likelihood for more selective compounds (e.g. **3**, **4**, **6**). On the other hand the purposely design of polypharmacological features can be beneficial as often seen in the kinase field.[23] This however requires a detailed structural and biochemical target class understanding which is certainly currently not given in the PPI field. Therefore we believe that novel tryptophan mimicking anchors in addition to the established indole and oxindole moieties will advance the field of design of specific and potent small molecular weight PPI inhibitors for p53 Mdm2 and Mdm4 specifically, but also generally for other tryptophan mediated PPIs. Although the herein disclosed benzimidazolidinones show weak initial activity they are suitable starting points for future optimization.

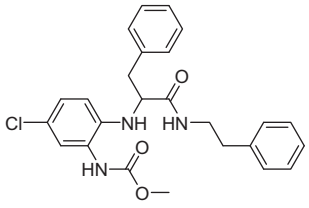
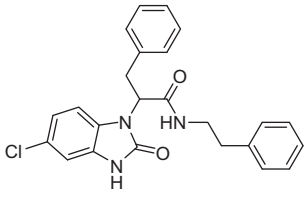
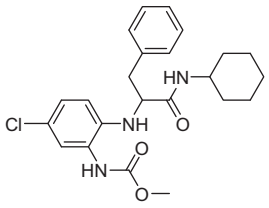
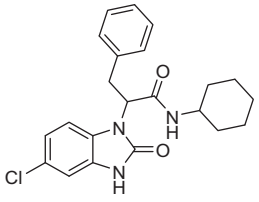
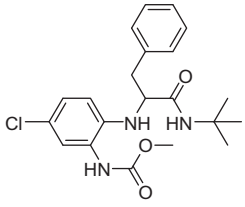
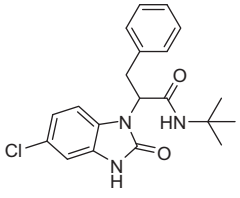
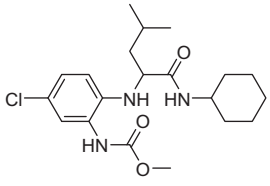
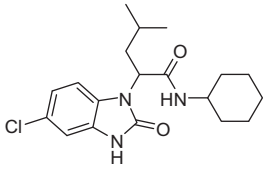
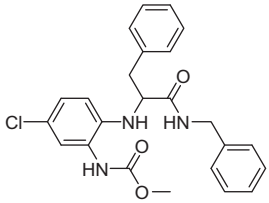
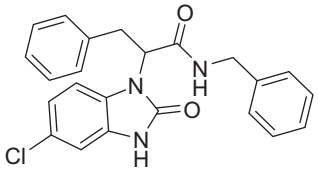
4. Experimental

4.1. Synthesis

All reagents were purchased from commercial sources and used without further purification. The reactions were conducted under air atmosphere unless otherwise indicated. Analytical thin-layer chromatography (TLC) was performed on SiO_2 plates 250 μm on Alumina from Whatman. Visualization was

accomplished by UV irradiation at 254 nm. The purification was conducted using Preparative Silica gel TLC plates (1000 μm, 20 × 20 cm). Proton and carbon NMR spectra were determined on Bruker 400 or 600 MHz NMR spectrometer. Chemical shifts are reported as δ values in parts per million (ppm) as referenced to residual solvent. ^{1}H NMR spectra are tabulated as follows: chemical shift, number of protons, multiplicity (s = singlet, br s = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constant. High Resolution Mass spectra were obtained at the University of Pittsburgh, Mass Spectrometry facility. LC–MS analysis was performed on an SHIMADZU instrument, using an analytical C18 column (Dionex Acclaim 120 Å, 2.1 × 50 mm, 3.0 μm, 0.2 ml/min). Acetonitrile/water mixtures were used as mobile phase for reverse-phase HPLC coupled to electrospray ionization-mass spectrometry (ESI-MS). The enantiomers of **10** were separated by chiral separation via Supercritical Fluid Chromatography (SFC). Experiments were performed using the analytical SFC-MS Resolution System (Waters 2998 Photodiode Array Detector, Waters 3100 Mass Detector), and the preparative TharSFC System (Waters 2998 Photodiode Array Detector). Both analytical and preparative SFC columns were operating at 40 °C in Analytical-2-Prep Column Oven. Carbon dioxide supplied by BDS 500 Gas Delivery System was used as the primary mobile phase for SFC. The optical rotation of **10**

Table 1
Yields and bioactivities of the synthesized structures

No.	Starting materials	Structure Ugi products	Yields[a]	Structure cyclized Products	Yields[a]	K_i[b]	$c\log P$[c]
1	S1, S3, S12	 **7**	66%	 **8**	65%	60	4.93
2	S1, S3, S13	 **9**	67%	 **10**	49%	16	4.71
3	S1, S3, S14	 **11**	65%	 **12**	61%	43	3.97
4	S1, S4, S13	 **13**	80%	 **14**	44%	n.i.[d]	4.31
5	S1, S3, S15	 **15**	60%	 **16**	36%	33	4.64

(*continued on next page*)

Table 1 (*continued*)

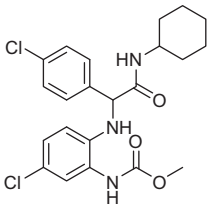
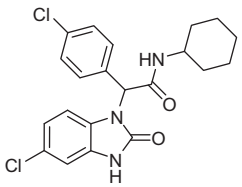
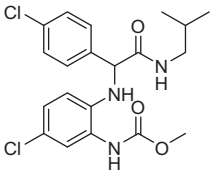
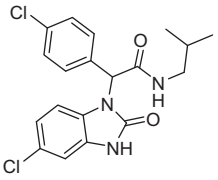
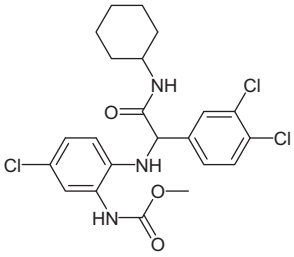
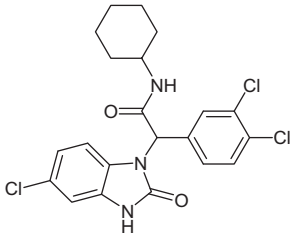
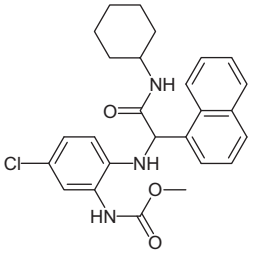
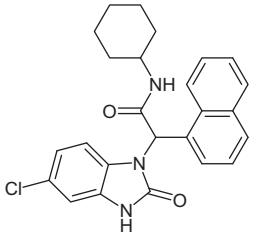
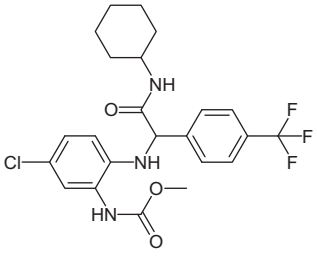
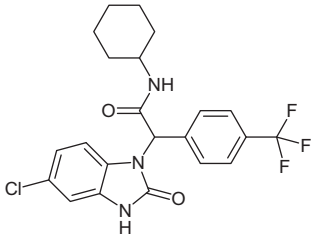
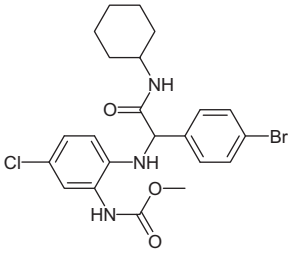
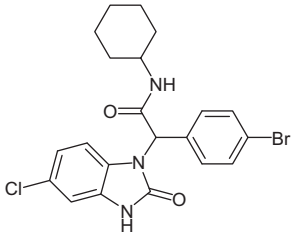
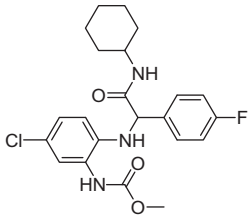
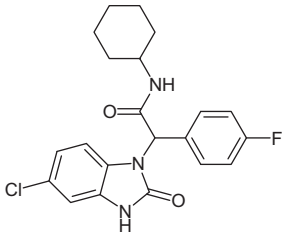
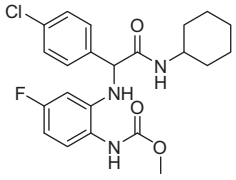
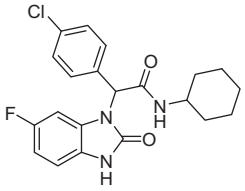
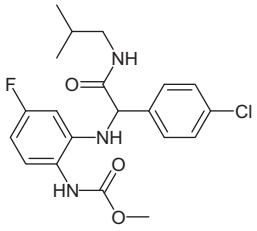
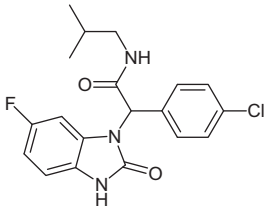
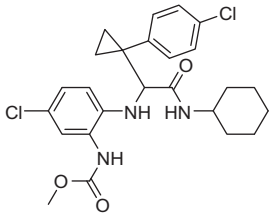
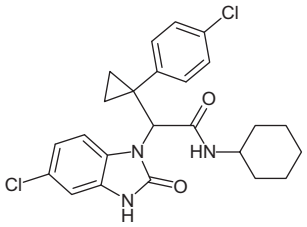
No.	Starting materials	Structure Ugi products	Yields[a]	Structure cyclized Products	Yields[a]	K_i[b]	*c*Log *P*[c]
6	S1, S5, S13	 **17**	45%	 **18**	31%	n.i.	5.03
7	S1, S5, S12	 **19**	52%	 **20**	45%	n.i.	4.47
8	S1, S6, S13	 **21**	42%	 **22**	41%	n.i.	5.63
9	S1, S7, S13	 **23**	63%	 **24**	43%	n.i.	5.41
10	S1, S8, S13	 **25**	41%	 **26**	41%	35	5.30
11	S1, S9, S13	 **27**	48%	 **28**	32%	54	5.19

Table 1 (*continued*)

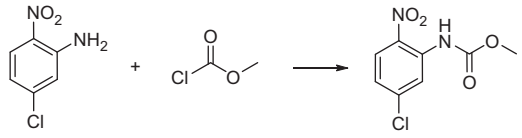
No.	Starting materials	Structure Ugi products	Yields[a]	Structure cyclized Products	Yields[a]	K_i[b]	*c*Log *P*[c]
12	S1, S10, S13	 **29**	52%	 **30**	31%	n.i.	4.57
13	S2, S5, S13	 **31**	43%	 **32**	30%	f[e]	4.57
14	S1, S5, S16	 **33**	45%	 **34**	33%	n.i.	4.01
15	S1, S11, S13	 **35**	48%	 **36**	40%	n.i.	5.60

[a] Yields after chromatographic isolation of the products are given for both reactions (Ugi, cyclisation) seperately.
[b] K_i based on fluorescence polarisation.
[c] *c*Log *P* are predicted by Instant J. Chem 2.4.3.1, 2011, ChemAxon (http://www.chemaxon.com).
[d] n.i.-no interaction.
[e] f-fluorescent.

was measured on the Schmidt & Haensch Polartronic MH8 using a 1 dm qs cuvette at $T = 20.5$ °C in the solvent methanol.

5. General procedures

5.1. N-Protection



5-Chloro-2-nitroaniline (1.0 g, 5.8 mmol) added into 5 ml methyl chloroformate, the reaction mixture was refluxed for 9 h. Evaporated and the residue purified by recrystallization with EtOAc and hexane. Methyl 5-chloro-2-nitrophenylcarbamate was obtained as solid (1.2 g, 90%).[24]

5.2. Methyl 5-chloro-2-nitrophenylcarbamate

$C_8H_7ClN_2O_4$, Mw: 230.60 g/mol; HRMS (ESI-TOF) *m*/*z* (calcd): 230.0094, (found) $[M+H]^+$: 230.0102; ^1H NMR (600 MHz, $CDCl_3$): 9.87 (s, 1H), 8.55 (d, $J = 2.4$ Hz, 1H), 8.10 (d, $J = 9.0$ Hz, 1H), 7.03 (dd, $J = 2.4$, 9.0 Hz, 1H), 3.80 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): 151.13, 142.61, 136.26, 133.93, 127.06, 122.48, 119.93, 53.01

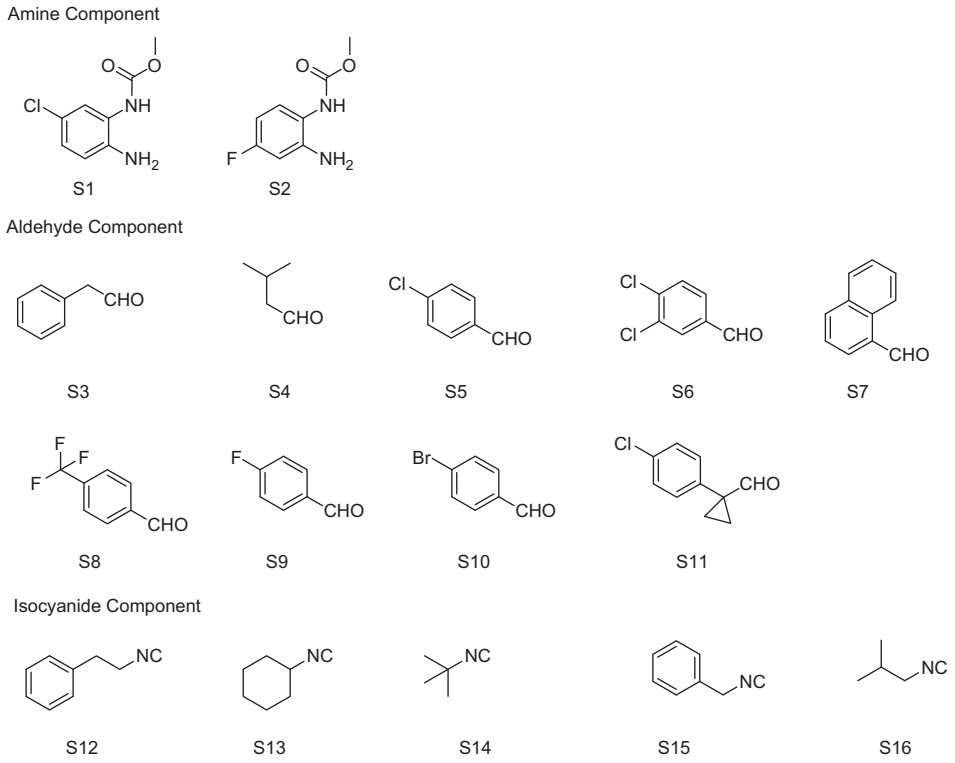
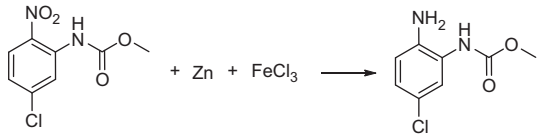


Figure 3. Starting materials used in Ugi 3-CR reaction.

5.3. Reduction

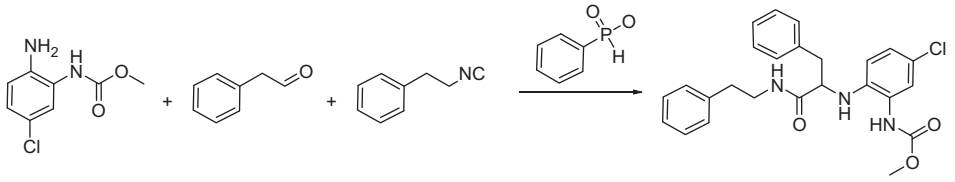


A mixture of ferric chloride (2.8 g, 172 mmol), Zinc dust (3.4 g, 52.4 mmol) and methyl 5-chloro-2-nitrophenylcarbamate (1.2 g, 52 mmol) was added to the mixed solvent dimethyl formamide and water (1: I, 80 ml) and heated in water-bath (100 °C). After completion of the reaction (0.5 h, monitored by TLC), the reaction mixture filtered and filtrate was diluted with water (80 ml) and basified by adding saturated solution of sodium carbonate. It was extracted with dichloromethane (3 × 140 ml). The combined organic layer was dried and concentrated. The residue was purified by column chromatography to afford methyl 2-amino-5-chlorophenylcarbamate (0.88 g, 84%).

5.4. Methyl 2-amino-5-chlorophenylcarbamate

$C_8H_9ClN_2O_2$, Mw: 200.62 g/mol; HRMS (ESI-TOF) *m*/*z* (calcd): 200.0353, (found) $[M+Na]^+$: 200.0361; ^1H NMR (600 MHz, $CDCl_3$): 7.39 (br, 1H), 6.98 (dd, *J* = 1.8, 8.4 Hz, 1H), 6.71 (d, *J* = 8.4 Hz, 1H), 6.48 (br, 1H), 3.79 (s, 3H), 3.69 (br, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 154.81, 137.98, 126.10, 125.33, 124.35, 118.63, 52.78

5.5. Ugi 3-CC reaction



To a solution of methyl 2-amino-5-chlorophenylcarbamate (20 mg, 0.1 mmol), 2-phenylacetaldehyde (12 mg, 0.1 mmol), phenylphosphinic acid (7 mg, 0.05 mmol) in 0.5 ml THF was added (2-isocyanoethyl)benzene (13 mg, 0.1 mmol). After stirring at room temperature for 24 h, 4 ml water was added the reaction mixture. The mixture was neutralized with 20% NaOH. The solution was extracted with ether (3 × 5 ml). The combined organic layer was washed with brine, dried and concentrated. The residue was purified by column chromatography to afford methyl 5-chloro-2-(1-oxo-1-(phenethylamino)-3-phenylpropan-2-ylamino)phenylcarbamate (20 mg, 66%).

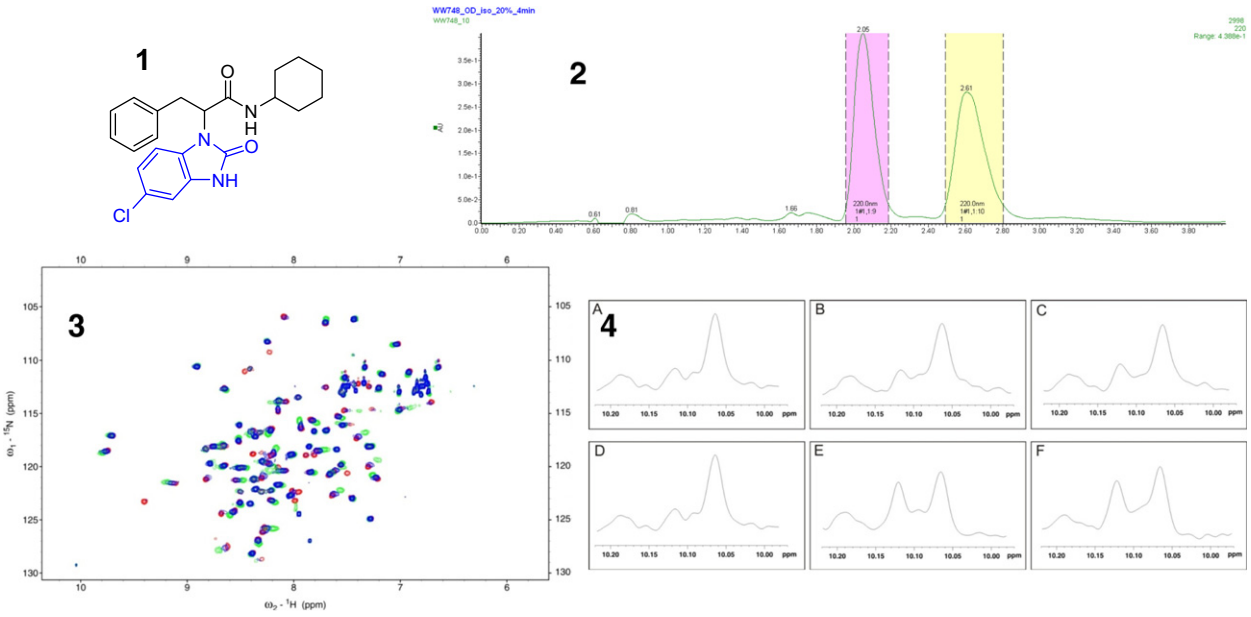
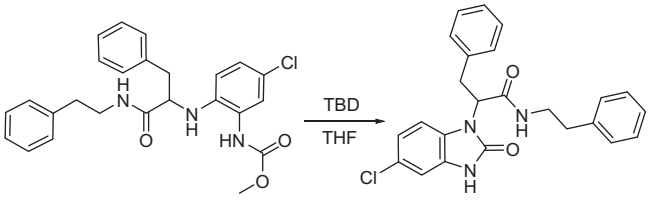


Figure 4. (1): Structure of benzimidazolone **10**; (2): preparative SFC trace (UV 220 nM) of the separation of **10** on the RegisCell (#784104, OD) column with baseline separation after 3 min; (3): Superposition of NMR HSQC spectra of ^{15}N-labeled Mdm2 titrated against enantiomer **10 B**. The spectrum of free Mdm2 is shown in red. The spectrum of Mdm2–**10 B** (intermediate ratio, 1: 7.56, respectively) is shown in blue, and the spectrum of Mdm2–**10 B** (final ratio, 1:2) is shown in green; (4): AIDA NMR of the enantiomers of compound **10**. (A and D) p53-Mdm2 reference, (B) enantiomer **10 A** ratio 1:2, (C) enantiomer **10 A** ratio 1:8, (E) enantiomer **10 B** ratio 1:2, (F) enantiomer **10 B** ratio 1:8.

5.6. Cyclization

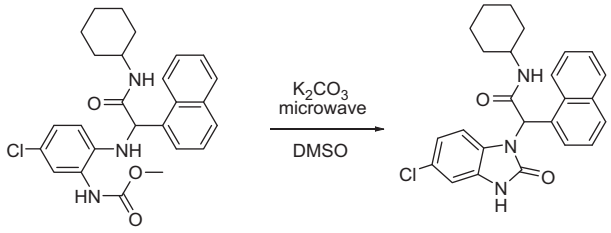
Two methods:

A: For cyclization of Ugi products **7**, **9**, **11**, **13**, **15**, **17**, **19**



20 mg Methyl 5-chloro-2-(1-oxo-1-(phenethylamino)-3-phenylpropan-2-ylamino)phenylcarbamate and 1 mg of triazabicyclodecene in 1 ml THF were refluxed for 4 h. 12 mg (65%) of **8** was obtained after preparative TLC separation.

B: For cyclization of Ugi products **21**, **23**, **25**, **27**, **29, 31, 33, 35**



46 mg of methyl 5-chloro-2-(2-(cyclohexylamino)-1-(naphthalen-1-yl)-2-oxoethylamino)phenyl carbamate and 13 mg of K_2CO_3 were heated under 130 °C for 30 min. Next it was diluted with 5 ml EtOAc and washed with 5 ml water, the organic layer was collected, dried and evaporated. The residue was purified by preparative TLC to give product **22** 19 mg (43%).

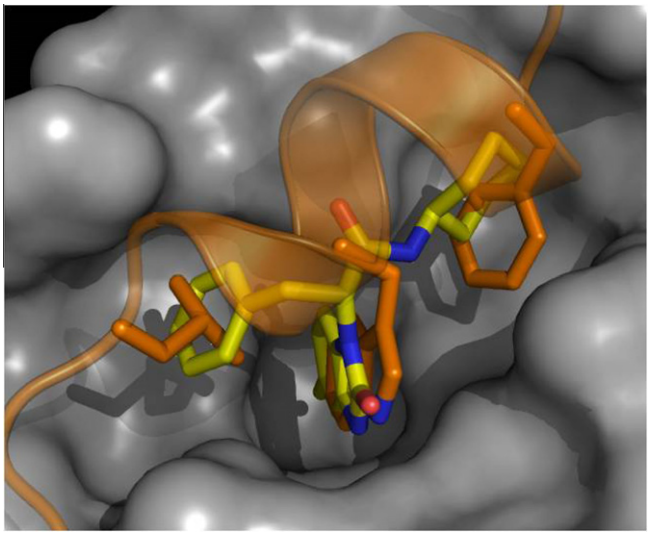


Figure 5. Docking pose of *(S)*-**10** into the superimposed p53 Mdm2 structure suggesting the binding of the 1-oxo-benzimidazole, the cyclohexyl and the benzyl moieties into the W23, F19 and L26 pockets, respectively (PDB ID 1YCR).

Analytical Data of Ugi products and cyclization products:

5.7. Methyl 5-chloro-2-(1-oxo-1-(phenethylamino)-3-phenylpropan-2-ylamino)phenylcarbamate (7)

$C_{25}H_{26}ClN_3O_3$, Mw:451.95 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 449.1273, (found) $[M+Na]^+$:474.1581; ^{1}H NMR (600 MHz, $CDCl_3$): 7.25–7.32 (m, 3H), 7.14–7.21 (m, 6H), 7.02 (dd, J = 1.8, 9.0 Hz,

1H), 6.99 (d, J = 6.6 Hz, 2H), 6.86 (br, 1H), 6.49 (d, J = 9.0 Hz, 1H), 6.45 (br, 1H), 4.11 (s, 1H), 4.02 (s, 1H), 3.66 (s, 3H), 3.48–3.50 (m, 1H), 3.37–3.42 (m, 1H), 3.17–3.20 (m, 1H), 3.11–3.12 (m, 1H), 2.65–2.71 (m, 2H), ^{13}C NMR (150 MHz, $CDCl_3$): 172.21, 154.90, 139.67, 138.66, 136.29, 129.22, 128.81, 128.63, 128.44, 127.57, 127.23, 126.30, 125.89, 124.38, 123.36, 114.17, 58.97, 52.87, 40.45, 38.83, 35.32

5.8. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-phenethyl-3-phenylpropanamide (8)

$C_{24}H_{22}ClN_3O_2$, Mw: 419.90 g/mol; HRMS (ESI-TOF), m/z (calcd):419.1401, (found) $[M+Na]^+$: 442.1263; ^{1}H NMR (600 MHz, $CDCl_3$): 9.80 (s, 1H), 7.10–7.19 (m, 7H), 7.06 (dd, J = 1.8, 8.4 Hz, 1H), 7.01 (d, J = 7.2 Hz, 2H), 6.99 (d, J = 1.8 Hz, 1H), 6.97 (d, J = 7.2 Hz, 2H), 6.42 (t, J = 6.0 Hz, 1H), 5.10 (dd, J = 6, 10.2 Hz, 1H), 3.54–3.60 (m, 2H), 3.43–3.48 (m, 1H), 3.35 (dd, J = 4.2, 14.4 Hz, 1H), 2.73 (t, J = 6.6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 168.39, 155.28, 138.34, 136.50, 128.87, 128.59, 128.52, 127.72, 127.20, 126.95, 126.50, 121.83, 111.33, 110.24, 57.98, 40.84, 35.31, 34.49.

5.9. Methyl 5-chloro-2-(1-(cyclohexylamino)-1-oxo-3-phenylpropan-2-ylamino)phenylcarbamate (9)

$C_{23}H_{28}ClN_3O_3$, Mw:429.94 g/mol; HRMS (ESI-TOF), m/z (calcd): 429.1819, (found) $[M+Na]^+$: 452.1707; ^{1}H NMR (600 MHz, $CDCl_3$): 7.38–7.25 (m, 3H), 7.19 (d, J = 7.2 Hz, 2H), 7.11 (s, 1H), 7.03 (d, J = 9.0 Hz, 1H), 6.85 (br, 1H), 6.73 (br, 1H), 6.51 (d, J = 9.0 Hz, 1H), 4.20 (s, 1H), 4.12 (s, 1H), 3.68–3.69 (m, 1H), 3.64 (s, 3H), 3.13–3.20 (m, 2H), 1.74 (d, J = 10.8 Hz, 1H), 1.54–1.64 (m, 4H), 1.21–1.29 (m, 2H), 0.99–1.06 (m, 2H), 0.84–0.88 (m, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): 171.06, 154.98, 139.87, 136.16, 129.40, 128.70, 127.43, 127.16, 126.04, 124.24, 122.88, 113.86, 58.63, 52.81, 48.16, 38.65, 32.58, 32.50, 25.31, 24.83, 24.69.

5.10. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-3-phenylpropanamide (10)

$C_{22}H_{24}ClN_3O_2$, Mw: 397.90 g/mol; HRMS (ESI-TOF), m/z (calcd): 397.1557, (found) $[M+Na]^+$: 420.1466; ^{1}H NMR (600 MHz, $CDCl_3$): 9.95 (s, 1H), 7.26 (t, J = 7.2 Hz, 1H), 7.13 (t, J = 7.2 Hz, 2H), 7.06–7.09 (m, 2H), 7.00–7.02 (m, 3H), 6.23 (d, J = 7.8 Hz, 1H), 5.13 (dd, J = 6.0, 10.2 Hz, 1H), 3.74–3.81 (m, 1H), 3.60 (dd, J = 6.0, 14.4 Hz, 1H), 3.35 (dd, J = 10.2, 14.4 Hz, 1H), 1.79–1.84 (m, 2H), 1.57–1.67 (m, 3H), 1.29–1.36 (m, 2H), 0.99–1.13 (m, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): 167.45, 155.48, 136.55, 128.90, 128.59, 128.48, 127.70, 127.32, 126.93, 121.75, 111.49, 110.27, 58.33, 48.75, 34.83, 32.80, 32.71, 25.36, 24.74, 24.67.

5.11. Methyl 2-(1-(*tert*-butylamino)-1-oxo-3-phenylpropan-2-ylamino)-5-chlorophenylcarbamate (11)

$C_{21}H_{26}ClN_3O_3$, Mw: 403.90 g/mol; HRMS (ESI-TOF), m/z (calcd): 403.1663, (found) $[M+Na]^+$: 426.1546; ^{1}H NMR (600 MHz, $CDCl_3$): 7.31 (t, J = 7.2 Hz, 2H), 7.25–7.28 (m, 1H), 7.20 (d, J = 6.6 Hz, 2H), 7.16 (s, 1H), 7.04 (dd, J = 1.2, 9.0 Hz, 1H), 6.69 (br, 1H), 6.53 (d, J = 9.0 Hz, 1H), 6.37 (br, 1H), 4.20 (s, 1H), 3.82–3.85 (m, 1H), 3.66 (s, 3H), 3.14 (m, 2H), 1.22 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$): 171.34, 154.82, 139.55, 136.47, 129.39, 128.72, 128.53, 127.14, 125.66, 124.75, 123.28, 114.37, 59.92, 52.73, 51.13, 38.82, 28.38.

5.12. *N*-*tert*-Butyl-2-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-3-phenylpropanamide (12)

$C_{20}H_{22}ClN_3O_2$, Mw: 371.86 g/mol; HRMS (ESI-TOF), m/z (calcd): 371.1401, (found) $[M+Na]^+$: 394.1288; ^{1}H NMR (600 MHz, $CDCl_3$): 8.50 (s, 1H), 7.24 (d, J = 8.4 Hz, 1H), 7.12–7.18 (m, 3H), 7.08 (dd, J = 1.8, 8.4 Hz, 1H), 7.02–7.03 (m, 3H), 5.95 (s, 1H), 5.05 (dd, J = 6.0, 9.6 Hz, 1H), 3.56 (dd, J = 6.0, 13.8 Hz, 1H), 3.31 (dd, J = 10.2, 13.8 Hz, 1H), 1.29 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$): 167.53, 154.72, 136.59, 128.94, 128.50, 128.18, 127.62, 127.45, 126.92, 121.88, 111.44, 109.94, 51.78, 34.67, 30.96, 28.53.

5.13. Methyl 5-chloro-2-(1-(cyclohexylamino)-4-methyl-1-oxopentan-2-ylamino)phenylcarbamate (13)

$C_{20}H_{30}ClN_3O_3$, Mw: 395.92 g/mol; HRMS (ESI-TOF) m/z (calcd): 395.1976, (found) $[M+Na]^+$: 418.1908; ^{1}H NMR (600 MHz, $CDCl_3$): 7.38 (br, 1H), 7.17 (s, 1H), 7.02 (dd, J = 2.4, 9.0 Hz, 1H), 6.95 (br, 1H), 6.51 (d, J = 9.0 Hz, 1H), 4.23 (d, J = 4.8 Hz, 1H), 3.77 (s, 3H), 3.62–3.65 (m, 1H), 1.72–1.78 (m, 3H), 1.51–1.65 (m, 5H), 1.17–1.27 (m, 3H), 1.02–1.11 (m, 2H), 0.95 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): 172.98, 155.43, 140.01, 127.20, 125.79, 124.33, 122.75, 113.62, 57.29, 52.87, 48.02, 42.68, 32.67, 32.61, 25.34, 25.05, 24.81, 24.67, 23.19, 21.60.

5.14. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-4-methylpentanamide (14)

$C_{19}H_{26}ClN_3O_2$, Mw: 363.88 g/mol; HRMS (ESI-TOF), m/z (calcd):363.1714, (found) $[M+Na]^+$: 386.1647; ^{1}H NMR (600 MHz, $CDCl_3$): 10.23 (s, 1H), 7.22 (d, J = 9.0 Hz, 1H), 7.12 (s, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.25 (s, 1H), 4.99 (dd, J = 5.4, 10.2 Hz, 1H), 3.73–3.79 (m, 1H), 2.15–2.17 (m, 1H), 2.03–2.07 (m, 1H), 1.88–1.93 (m, 1H), 1.78–1.80 (m, 1H), 1.57–1.69 (m, 3H), 1.30–1.39 (m, 3H), 1.02–1.14 (m, 3H), 0.96 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): 168.38, 155.58, 128.70, 127.69, 127.37, 121.82, 111.44, 110.20, 55.27, 48.61, 37.34, 32.80, 25.39, 24.98, 24.73, 24.65, 22.99.

5.15. Methyl 2-(1-(benzylamino)-1-oxo-3-phenylpropan-2-ylamino)-5-chlorophenylcarbamate (15)

$C_{24}H_{24}ClN_3O_2$, Mw: 437.92 g/mol; HRMS (ESI-TOF), m/z (calcd): 437.1506, (found) $[M+Na]^+$: 460.1408; ^{1}H NMR (600 MHz, $CDCl_3$): 7.05–7.25 (m, 12H), 6.56 (d, J = 7.8 Hz, 1H), 4.30–4.42 (m, 2H), 4.11–4.16 (m, 1H), 3.60 (s, 3H), 3.21 (s, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): 172.17, 154.91, 139.81, 137.92, 136.11, 129.30, 128.82, 128.55, 128.40, 127.67, 127.38, 127.17, 126.00, 124.31, 123.41, 114.40, 59.00, 52.84, 43.17, 38.74.\

5.16. *N*-Benzyl-2-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-3-phenylpropanamide (16)

$C_{23}H_{20}ClN_3O_2$, Mw: 405.88 g/mol; HRMS (ESI-TOF), m/z (calcd): 405.1244, (found) $[M+Na]^+$: 428.1143; ^{1}H NMR (600 MHz, $CDCl_3$): 8.68 (s, 1H), 7.25–7.26 (m, 3H), 7.21 (d, J = 8.4 Hz, 1H), 7.12–7.17 (m, 5H), 7.03–7.07 (m, 3H), 6.96 (s, 1H), 6.70 br, 1H), 5.17 (dd, J = 6.0, 10.2 Hz, 1H), 4.38–4.52 (m, 2H), 3.63 (dd, J = 6.0, 14.4 Hz, 1H), 3.39 (d, J = 4.2, 13.8 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): 168.35, 154.83, 137.49, 136.35, 128.91, 128.70, 128.55, 128.18, 127.73, 127.67, 127.62, 127.40, 126.98, 121.90, 111.31, 110.04, 58.25, 43.76, 34.63.

5.17. Methyl 5-chloro-2-(2-(cyclohexylamino)-1-(1-(3-fluorophenyl)cyclopropyl)-2-oxoethylamino)phenyl carbamate (17)

$C_{22}H_{25}Cl_2N_3O_3$, Mw: 450.36 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 449.1273, (found) $[M+H]^+$: 472.1166; HPLC-MS rt: 11.41, *m*/*z* $[M]^+$ 450.2. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.95 (1H, m), 1.14–1.20 (2H, m), 1.22–1.36 (2H, m), 1.60 (2H, m), 1.71 (2H, m), 1.89 (2H, m), 3.76 (1H, m), 3.80 (3H, m), 4.76 (1H, s), 6.47 (1H, s), 6.57 (2H, d, *J* = 7.2 Hz), 7.04 (1H, dd, *J*1 = 2.4 Hz, *J*2 = 8.8 Hz), 7.32 (1H, s), 7.38 (4H, s). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.6, 24.7, 25.3, 32.6, 32.8, 48.5, 52.9, 62.7, 114.8, 124.1, 125.4, 126.8, 128.7, 129.4, 134.6, 136.7, 138.8, 155.0, 169.3.

5.18. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-2-(4-chlorophenyl)-*N*-cyclohexylacetamide (18)

$C_{21}H_{21}Cl_2N_3O_2$, Mw: 418.32 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 417.1011, (found) $[M+H]^+$: 440.0908; HPLC-MS rt: 11.18, *m*/*z* $[M+H]^+$ 418.1. ^{1}H NMR (MeOD, 400 MHz): δ = 1.2–1.42 (6H, m), 1.65 (1H, d, *J* = 12.4 Hz), 1.78 (2H, m), 1.87 (1H, d, *J* = 11.2 Hz), 1.99 (1H, d, *J* = 12 Hz), 3.78 (1H, m), 6.26 (1H, s), 6.72 (1H, d, *J* = 8.4 Hz), 6.87 (1H, d, *J* = 8.8 Hz), 7.06 (1H, s), 7.32 (2H, d, *J* = 8.4 Hz), 7.41 (2H, d, *J* = 8 Hz). 13C-NMR (MeOD, 100 MHz): δ = 24.7, 25.2, 47.8, 58.1, 109.1, 112.2, 120.4, 126.8, 128.0, 128.6, 129.4, 129.5, 133.6, 134.0, 155.2, 167.4.

5.19. Methyl 5-chloro-2-(1-(4-chlorophenyl)-2-(isobutylamino)-2-oxoethylamino)phenylcarbamate (19)

$C_{20}H_{23}Cl_2N_3O_3$, Mw: 424.32 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 423.1116, (found) $[M+Na]^+$: 446.1006; HPLC-MS rt: 11.06, *m*/*z* $[M]^+$424.1. ^{1}H NMR (MeOD, 400 MHz): δ = 0.87 (6H, d, *J* = 6 Hz), 1.01 (rotamer, d, *J* = 6.8 Hz), 1.80 (1H, m), 3.00 (1H, m), 3.14 (1H, m), 3.85 (3H, s), 5.04 (1H, s), 6.58 (1H, d, *J* = 8.8 Hz), 7.06 (1H, d, *J* = 4.8 Hz), 7.31 (1H, s), 7.45 (2H, d, *J* = 8 Hz), 7.58 (2H, d, *J* = 8 Hz), 8.42 (1H, s). ^{13}C-NMR (MeOD, 100 MHz): δ = 18.9, 28.2, 47.1, 51.7, 61.2, 113.5, 122.2, 125.4, 1256.0, 128.5, 128.6, 133.7, 137.3, 139.5, 156.3, 171.8.

5.20. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-2-(4-chlorophenyl)-*N*-isobutylacetamide (20)

$C_{19}H_{19}Cl_2N_3O_2$, Mw: 392.28 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 391.0854, (found) $[M+Na]^+$: 414.0746; HPLC-MS rt: 10.84, *m*/*z* $[M+H]^+$392.. ^{1}H NMR (MeOD, 400 MHz): δ = 0.93 (6H, d, *J* = 6.8 Hz), 1.85 (1H, m), 3.12 (2H, m), 6.29 (1H, s), 6.74 (1H, d, *J* = 8.4 Hz), 6.89 (1H, dd, *J*1 = 2 Hz, *J*2 = 10.4 Hz), 7.07 (1H, d, *J* = 2 Hz), 7.34 (2H, d, *J* = 8.4 Hz), 7.41 (2H, d, *J* = 8.4 Hz). 13C NMR (MeOD, 100 MHz): δ = 19.1,19.1, 28.1, 58.3, 109.2, 111.9, 120.5, 126.9, 128.0, 129.4, 129.7, 133.4, 134.1, 155.2, 168.4.

5.21. Methyl 5-chloro-2-(2-(cyclohexylamino)-1-(3,4-dichlorophenyl)-2-oxoethylamino)phenylcarbamate (21)

$C_{22}H_{24}Cl_3N_3O_3$, Mw: 484.80 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 483.0883, (found) $[M+Na]^+$: 506.0785; HPLC-MS rt: 11.78, *m*/*z* $[M+H]^+$484.1. ^{1}H NMR (DMSO, 400 MHz): δ = 1.05–1.25 (5H, m), 1.52–1.58 (3H, m), 1.67 (1H, m), 1.78 (1H, m), 3.50 (1H, m), 3.70 (3H, s), 5.04 (1H, d, *J* = 7.6 Hz), 5.81 (1H, d, *J* = 7.6 Hz), 6.28 (1H, d, *J* = 8.8 Hz), 6.97 (1H, dd, *J*1 = 2.4 Hz, *J*2 = 8.8 Hz), 7.24 (1H, s), 7.52 (1H, dd, *J*1 = 2 Hz, *J*2 = 8.4 Hz), 7.63 (1H, d, *J* = 8.4 Hz), 7.80 (1H, d, *J* = 1.6 Hz), 8.28 (1H, d, *J* = 8 Hz), 9.22 (1H, s). ^{13}C NMR (DMSO, 100 MHz): δ = 24.6, 24.8, 25.5, 32.4, 32.6, 48.2, 52.5, 59.2, 113.1, 120.4, 125.7, 127.8, 129.2, 130.7, 131.1, 131.6, 141.0, 155.5, 168.7.

5.22. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-2-(3,4-dichlorophenyl)acetamide (22)

$C_{21}H_{20}Cl_3N_3O_2$, Mw: 452.76 g/mol; HRMS (ESI-TOF), v (calcd): 451.0621, (found) $[M+Na]^+$: 474.0525; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.11–1.17 (3H, m), 1.20–1.40 (3H, m), 1.61–1.74 (3H, m), 1.89 (d, 1H, *J* = 12 Hz), 2.0 (d, 1H, *J* = 12 Hz), 3.88 (s, 1H), 6.11 (s, 1H), 6.39 (1H, d, *J* = 7.6 Hz), 6.99 (1H, d, *J* = 8.4 Hz), 7.08 (1H, s), 7.19 (1H, d, *J* = 8.4 Hz), 7.45 (1H, t, *J* = 6.4 Hz), 9.55 (1H, s). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 24.7, 32.7, 32.8, 49.2, 58.6, 110.3, 111.8, 122.0, 127.1, 127.3, 128.0, 128.5, 130.0, 130.8, 133.0, 133.2, 134.3, 135.1, 165.7.

5.23. Methyl 5-chloro-2-(2-(cyclohexylamino)-1-(naphthalen-1-yl)-2-oxoethylamino)phenylcarbamate (23)

$C_{26}H_{28}ClN_3O_3$, Mw: 465.97 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 465.1819, (found) $[M+H]^+$: 466.1903; HPLC-MS rt: 11.53, *m*/*z* $[M+H]^+$466.0. ^{1}H NMR (MeOD, 400 MHz): δ = 1.15–1.19 (2H, m), 1.34–1.39 (3H, m), 1.64-1.68 (3H, m), 1.78 (1H, m), 1.95 (1H, m), 3.67 (3H, s), 3.79 (1H, m), 5.72 (1H, s), 6.74 (1H, d, *J* = 8.8 Hz), 7.09 (1H, dd, *J*1 = 2.8 Hz, *J*2 = 8.4 Hz), 7.26 (1H, d, *J* = 1.6 Hz), 7.50 (1H, dd, *J*1 = 7.2 Hz, *J*2 = 8 Hz), 7.53 (2H, m), 7.60 (1H, dd, *J*1 = 0.8 Hz, *J*2 = 7.2 Hz), 7.92 (1H, d, *J*1 = 8.4 Hz), 7.93 (1H, dd, *J*1 = 1.2 Hz, *J*2 = 7.2 Hz), 8.11 (1H, d, *J* = 7.2 Hz). 13C NMR (MeOD, 100 MHz): δ = 24.5, 24.6, 25.1, 32.0, 32.2, 48.7, 51.6, 58.9, 122.8, 122.2, 122.9, 124.96, 125.0, 125.6, 126.4, 128.6, 128.8, 131.6, 133.7, 124.2, 156.2, 171.6.

5.24. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-2-(naphthalen-1-yl)acetamide (24)

$C_{25}H_{24}ClN_3O_2$, Mw: 433.93 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 433.1557, (found) $[M+Na]^+$: 456.1487; ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 1.19–1.38 (2H, m), 1.40–1.0 (3H, m), 1.64 (1H, m), 1.76 (1H, m), 1.91 (1H, d, *J* = 2.0 Hz), 2.05 (1H, d, *J* = 2.0 Hz), 3.84 (1H, m), 6.52 (1H, d, *J* = 8.4 Hz), 6.66 (1H, dd, *J*1 = 2 Hz, *J*2 = 8.4 Hz), 6.97 (1H, d, *J* = 2 Hz), 7.49 (1H, m), 7.59 (1H, dd, *J*1 = 7.6 Hz, *J*2 = 8.4 Hz), 7.77 (1H, d, *J* = 7.2 Hz), 7.90–7.99 (3H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 24.6, 25.2, 32.1, 32.2, 48.9, 56.8, 109.0, 111.8, 120.3, 122.4, 124.4, 126.0, 126.3, 126.8, 127.0, 126.7, 128.7, 129.2, 129.7, 130.3, 131.8, 134.1, 155.0, 168.7.

5.25. Methyl 5-chloro-2-(2-(cyclohexylamino)-2-oxo-1-(4-(trifluoromethyl)phenyl)ethylamino)phenyl carbamate (25)

$C_{23}H_{25}ClF_3N_3O_3$, Mw: 483.91 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 483.1537, (found) $[M+H]^+$: 484.1602; HPLC-MS rt: 11.55, *m*/*z* $[M+H]^+$484.2. ^{1}H NMR (DMSO, 400 MHz): δ = 1.08–1.25 (5H, m), 1.51–1.60 (3H, m), 1.63–1.67 (1H, m), 1.78 (1H, m), 3.5 (1H, m), 3.69 (3H, s), 5.13 (1H, d, *J* = 7.6 Hz), 5.85 (1H, d, *J* = 7.6 Hz), 6.27 (1H, d, *J* = 8.8 Hz), 6.96 (1H, dd, *J* = 2.4 Hz, *J* = 8.4 Hz), 7.24 (1H, s), 7.72 (2H, d, *J* = 8.8 Hz), 7.75 (2H, d, *J* = 8.8 Hz), 8.32 (1H, d, *J* = 8 Hz), 9.25 (1H, s). ^{13}C-NMR (DMSO, 100 MHz): δ = 24.6, 24.8, 25.5, 32.4, 32.7, 48.2, 52.5, 60.0, 113.1, 120.4, 123.3, 125.7, 125.8, 126.0, 128.1, 128.5, 128.8, 139.3, 144.4, 155.6, 168.8.

5.26. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-2-(4-(trifluoromethyl)phenyl) acetamide (26)

$C_{22}H_{21}ClF_3N_3O_2$, Mw: 451.87 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 451.1274, (found) $[M+Na]^+$: 474.1148; HPLC-MS rt:

11.36, *m*/*z* [M+H]$^+$ 452.2. ^{1}H NMR (MeOD, 400 MHz): δ = 1.2–1.5 (6H, m), 1.65–1.90 (4H, m), 2.02 (1H, d, *J* = 12.4 Hz), 3.81 (1H, m), 6.37 (1H, s), 6.77 (1H, d, *J* = 8.4 Hz), 6.89 (1H, dd, *J*1 = 2 Hz, *J*2 = 8.4 Hz), 7.09 (1H, d, *J* = 2 Hz), 7.52 (2H, d, *J* = 8.4 Hz), 7.72 (2H, d, *J* = 8.4 Hz). 13C NMR (MeOD, 100 MHz): δ = 23.2, 23.7, 23.8, 30.6, 30.7, 3108, 47.5, 56.8, 107.7, 110.5, 119.0, 121.2, 123.8, 125.4, 126.4, 127.0, 127.9, 128.5, 128.8, 137.8, 153.7, 165.5.

5.27. Methyl 2-(1-(4-bromophenyl)-2-(cyclohexylamino)-2-oxoethylamino)-5-chlorophenylcarbamate (27)

$C_{22}H_{25}BrClN_3O_3$, Mw: 494.81 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 493.0768, (found) [M+Na]$^+$: 516.0669; HPLC-MS rt: 11.49, *m*/*z* [M+H]$^+$ 494.0. ^{1}H NMR (DMSO, 400 MHz): δ = 1.10–1.28 (5H, m), 1.54–1.80 (3H, m), 1.80–1.82 (2H, m), 3.36 (1H, s), 3.52 (1H, m), 3.72 (3H, s), 5.02 (1H, d, *J* = 7.6 Hz), 5.79 (1H, d, *J* = 7.6 Hz), 6.30 (1H, d, *J* = 8.8 Hz), 7.00 (1H, dd, *J* = 2.4 Hz, *J*2 = 8.4 Hz), 7.26 (1H, s), 7.50 (2H, d, *J* = 8.4 Hz), 7.70 (2H, d, *J* = 8.4 Hz), 8.26 (1H, d, *J* = 7.6 Hz), 9.25 (1H, s). ^{13}C NMR (DMSO, 100 MHz): δ = 24.7, 24.8, 25.6, 32.4, 32.7, 48.1, 52.4, 59.7, 113.1, 120.2, 121.2, 125.6, 129.5, 131.7, 139.0, 155.5, 169.1.

5.28. 2-(4-Bromophenyl)-2-(5-chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexylacetamide (28)

$C_{21}H_{21}BrClN_3O_2$, Mw: 462.77 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 461.0506, (found) [M+Na]$^+$: 484.0356; HPLC-MS rt: 11.3, *m*/*z* [M+H]$^+$462.1. ^{1}H NMR (MeOD, 400 MHz): δ = 1.2–1.5 (6H, m), 1.65–1.90 (4H, m), 2.01 (1H, d, *J* = 12.4 Hz), 3.78 (1H, m), 6.24 (1H, s), 6.72 (1H, d, *J* = 8.8 Hz), 6.87 (1H, dd, *J*1 = 2 Hz, *J*2 = 8.4 Hz), 7.06 (1H, d, *J* = 2 Hz), 7.25 (2H, d, *J* = 8 Hz), 7.56 (2H, d, *J* = 8.4 Hz). 13C-NMR (MeOD, 100 MHz): δ = 24.7, 24.7, 25.2, 32.1, 32.2, 49.0, 58.2, 109.1, 112.2, 120.4, 122.0, 126.8, 129.0, 129.4, 129.8, 131.6, 134.1, 155.2, 167.3

5.29. Methyl 5-chloro-2-(2-(cyclohexylamino)-1-(4-fluorophenyl)-2-oxoethylamino)phenylcarbamate (29)

$C_{22}H_{25}ClFN_3O_3$, Mw: 433.90 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 433.1568, (found) [M+H]$^+$: 434.1653; ^{1}H NMR (DMSO, 400 MHz): δ = 1.07–1.25 (5H, m), 1.51–1.54 (3H, m), 1.60–1.82 (2H, m), 3.49 (1H, s), 4.04 (3H, s), 5.00 (1H, d, *J* = 7.6 Hz), 5.75 (1H, d, *J* = 7.6 Hz), 6.28 (1H, d, *J* = 8.8 Hz), 6.95 (1H, dd, *J* = 2.4 Hz, *J*2 = 8.4 Hz), 7.15–7.23 (3H, m), 7.55 (2H, dd, *J* = 5.2 Hz, *J* = 8.8 Hz), 8.21 (1H, d, *J* = 7.6 Hz), 9.22 (1H, s). ^{13}C-NMR (DMSO, 100 MHz): δ = 24.7, 24.8, 25.6, 32.4, 32.7, 48.0, 52.4, 59.6, 113.1, 115.6 (d, *J* = 21 Hz), 120.1, 125.6, 129.3 (d, *J* = 9 Hz), 135.7, 155.5, 169.4 .

5.30. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-cyclohexyl-2-(4-fluorophenyl)acetamide (30)

$C_{21}H_{21}ClFN_3O_2$, Mw: 401.86 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 401.1306, (found) [M+H]$^+$: 402.1419; HPLC-MS rt: 10.83, *m*/*z* [M+H]$^+$ 402.2. ^{1}H NMR (DMSO, 400 MHz): δ = 1.1–1.4 (5H, m), 1.56 (1H, d, *J* = 12.8 Hz), 1.69 (3H, m), 1.86 (1H, d, *J* = 11.2 Hz), 3.66 (1H, m), 6.18 (1H, s), 6.66 (1H, d, *J* = 8.8 Hz), 6.84 (1H, dd, *J*1 = 2 Hz, *J*2 = 8.4 Hz), 6.98 (1H, d, *J* = 2 Hz), 7.20 (1H, m), 7.28 (1H, m), 8.49 (1H, d, *J* = 4.8 Hz), 11.21 (1H, s). ^{13}C-NMR (DMSO, 100 MHz): δ = 25.0, 25.6, 32.6, 32.7, 48.5, 57.7, 109.1, 112.6, 115.9, 116.1, 120.3, 125.4, 128.7, 130.0, 130.4, 130.5, 132.3, 154.6, 161.5, 166.7.

5.31. Methyl 2-(1-(4-chlorophenyl)-2-(cyclohexylamino)-2-oxoethylamino)-4-fluorophenylcarbamate (31)

$C_{22}H_{25}ClFN_3O_3$, Mw: 433.90 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 433.1568, (found) [M+H]$^+$: 434.1653; HPLC-MS rt: 11.12, *m*/*z* [M+H]$^+$434.1. ^{1}H NMR (MeOD, 400 MHz): δ = 1.24–1.17 (2H, m), 1.35–1.45 (3H, m), 1.68–1.76 (3H, m), 1.84 (1H, d, *J* = 12.4 Hz), 1.95 (1H, d, *J* = 11.2 Hz), 3.70 (1H, m), 3.83 (1H, s), 5.03 (1H, s), 6.29 (1H, d, *J* = 11.2 Hz), 6.47 (1H, t, *J* = 8.4 Hz), 7.16 (1H, t, *J* = 7.2 Hz), 7.45 (2H, d, *J* = 8 Hz), 7.57 (2H, d, *J* = 8 Hz). 13C-NMR (MeOD, 100 MHz): δ = 24.5, 24.6, 25.1, 31.9, 32.2, 51.6, 60.4, 99.4 (*d* = 27 Hz), 103.1 (*d* = 23 Hz), 119.5 (*d* = 2 Hz), 128.4, 128.5, 133.7, 137.1, 143.3 (*d* = 11 Hz), 157, 161.5 (*d* = 340 Hz), 170.4.

5.32. 2-(4-Chlorophenyl)-*N*-cyclohexyl-2-(6-fluoro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)acetamide (32)

$C_{21}H_{21}ClFN_3O_2$, Mw: 401.86 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 401.1306, (found) [M+H]$^+$: 402.1383; HPLC-MS rt: 10.86, *m*/*z* [M+H]$^+$ 402.1. ^{1}H NMR (MeOD, 400 MHz): δ = 1.2–1.43 (6H, m), 1.66 (1H, d, *J* = 12.8 Hz), 1.78 (2H, m), 1.88 (1H, d, *J* = 11.2 Hz), 2.00 (1H, d, *J* = 11.6 Hz), 3.79 (1H, m), 6.28 (1H, s), 6.55 (1H, d, *J* = 9.2 Hz), 6.78 (1H, dd, *J*1 = *J*2 = 9.2 Hz), 7.00 (1H, dd, *J*1 = 4.8 Hz, *J*2 = 8.4 Hz), 7.33 (2H, d, *J* = 8 Hz), 7.42 (2H, d, *J* = 8 Hz). 13C-NMR (MeOD, 100 MHz): δ = 24.6, 25.2, 32.1, 32.2, 48.9, 58.2, 99.3 (d, *J* = 30 Hz), 107.7 (d, *J* = 24 Hz), 109.3 (d, *J* = 10 Hz), 124.5, 128.0 (m), 128.7, 129.5, 133.5, 134.1, 155.7, 158.0 (d, *J* = 240 Hz), 167.4.

5.33. Methyl 2-(1-(4-chlorophenyl)-2-(isobutylamino)-2-oxoethylamino)-4-fluorophenylcarbamate (33)

$C_{20}H_{23}ClFN_3O_3$, Mw: 407.87 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 407.1412, (found) [M+H]$^+$: 408.1467; HPLC-MS rt: 10.78, *m*/*z* [M+H]$^+$408.3. ^{1}H NMR (DMSO, 400 MHz): δ = 0.74 (6H, d, *J* = 6.4 Hz), 0.85 (2H, d, *J* = 6.8 Hz), 1.65 (1H, m), 2.93 (2H, m), 3.66 (3H, s), 5.05 (1H, d, *J* = 6.4 Hz), 5.88 (1H, d, *J* = 7.2 Hz), 6.05 (1H, dd, *J*1 = 2.4 Hz, *J*2 = 11.6 Hz), 6.38 (1H, m), 7.07 (1H, m), 7.72 (2H, d, *J* = 6.8 Hz), 7.57 (2H, d, *J* = 6.8 Hz), 8.02 (1H, s), 8.31 (1H, s), 8.97 (1H, s). ^{13}C-NMR (DMSO, 100 MHz): δ = 20.3, 20.4, 28.5, 45.0, 46.6, 52.3, 59.4, 98.7, 98.9, 102.6, 102.8120.4, 128.3, 128.9, 129.3, 132.8, 138.5, 156.0 m, 161.4, 170.0.

5.34. 2-(4-Chlorophenyl)-2-(6-fluoro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-*N*-isobutylacetamide (34)

$C_{19}H_{19}ClFN_3O_2$, Mw: 375.82 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 375.115, (found) [M+Na]$^+$: 398.1056; ^{1}H NMR (MeOD, 400 MHz): δ = 0.91 (6H, d, *J* = 2.8 Hz), 1.84 (1H, m), 3.11 (2H, dd, *J*1 = 2.4 Hz, *J*2 = 2.4 Hz), 6.27 (1H, s), 6.55 (1H, d, *J* = 9.6 Hz), 6.74 (1H, dd, *J*1 = 9.2 Hz, *J*2 = 9.2 Hz), 6.96 (1H, dd, *J*1 = 8.4 Hz, *J*2 = 5.6 Hz), 7.33 (2H, d, *J* = 8 Hz), 7.41 (2H, t, *J* = 8 Hz), 13C NMR (MeOD, 100 MHz): δ = 19.1, 28.1, 47.8, 58.3, 99.0 (d, *J* = 0 Hz), 107.7 (d, *J* = 22 Hz), 109.3 (d, *J* = 11 Hz), 124.6, 128.6, 129.6, 129.8, 133.4, 134.2, 155.8 (d, *J* = 127 Hz), 159.3, 168.4.

5.35. Methyl 5-chloro-2-(1-(1-(4-chlorophenyl)cyclopropyl)-2-(cyclohexylamino)-2-oxoethylamino)phenyl carbamate (35)

$C_{25}H_{29}Cl_2N_3O_3$, Mw: 490.42 g/mol; HRMS (ESI-TOF), *m*/*z* (calcd): 489.1586, (found) [M+H]$^+$: 490.1665; HPLC-MS rt: 11.88, *m*/*z* [M+H]$^+$490.2. ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.79–1.05 (4H, m), 1.18–1.41 (4H, m), 1.42–1.60 (2H, m), 1.65–1.76 (3H, m),

1.93–197 (1H, m), 3.88 (3H, s), 6.45 (2H, d, J = 8.8 Hz), 7.04–7.08 (1H, m), 7.27–7.34 (4H, m). ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 11.8, 12.4, 24.7, 24.8, 25.0, 25.3, 28.3, 32.4, 33.1, 47.1, 48.1, 53.0, 65.6, 113.8, 122.8, 123.7, 126.0, 127.7, 128.2, 128.4, 129.6, 132.1, 132.3, 132.5, 133.4, 139.0, 140.0, 154.8, 160.2, 163.5, 169.5.

5.36. 2-(5-Chloro-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-1-yl)-2-(1-(4-chlorophenyl)cyclopropyl)-*N*-cyclohexylacetamide (36)

$C_{24}H_{25}Cl_2N_3O_2$, Mw: 458.38 g/mol; HRMS (ESI-TOF), m/z (calcd):457.1324, (found) $[M+H]^+$: 458.1409; ^{1}H NMR ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 0.84–1.21 (4H, m), 1.22–1.52 (4H, m), 1.52–1.80 (4H, m), 1.85–1.90 (1H, m), 3.77 (1H, m), 5.07 (1H, s), 6.35 (1H, s), 6.69 (1H, d, J = 8.4 Hz), 6.87 (1H, dd, $J1$ = 0.8 Hz, $J2$ = 8.4 Hz), 6.70 (1H, d, $J1$ = 8.4 Hz), 7.07 (1H, d, $J1$ = 8.4 Hz), 7.21 (1H, d, $J1$ = 8.4 Hz), 9.08 (1H, S). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ = 11.2, 12.4, 24.7, 24.8, 25.4, 26.3, 29.7, 32.8,48.8, 63.2, 109.6, 112.6, 121.4, 127.4, 127.8, 127.9, 128.2, 132.2, 133.1, 140.1, 155.2, 165.7.

6. Protein expression and purification

The vector pET-20b containing the MDM2 crystallization construct (residues 18–111) as well as the pET-11a vector containing the MDM2 construct (residues 1–125) used for NMR-spectroscopy and FP-assay were transformed into the Bl21 Codon Plus (DE3) RIL or BL21 Arctic Express (DE3) expression cells (Stratagene). The cells were grown until the OD_{600} reached 0.8 and were then induced with 1 mM IPTG. After 4 h post induction the cells were harvested by centrifugation at 5500g for 15 min. The pellets were re-suspended in the PBS buffer. Lysis was performed by sonication followed by centrifugation at 10,000g for 30 min. The pellets were re-suspended in 10 ml of the solubilization buffer (6 M guanidinium chloride, 100 mM Tris–HCl pH 8.0, 1 mM EDTA, 10 mM β-mercaptoethanol) and incubated for 2 h on a rotator at 4 °C. Dialysis in 4 M guanidinium chloride, 10 mM β-mercaptoethanol and pH 3.5 was performed overnight, followed by refolding at 4 °C for 8 h in 10 mM Tris–HCl pH 7.0, 1 mM EDTA, 10 mM β-mercaptoethanol. Ammonium sulfate was added to a final concentration of 2 M and incubated overnight at 4 °C. The solution was centrifuged at 11,000g for 30 min. Buthylsepharose 6 Fast Flow (GE Healthcare) was added to the supernatant and incubated for 2 h. The protein was eluted with 100 mM Tris, 10 mM β-mercaptoethanol and pH 7.0, concentrated and loaded onto the HiLoad 16/60 Superdex75 or Superdex 200 pg gel filtration columns (Amersham Biosciences). The gel filtration was run with following buffer: 50 mM KH_2PO_4, 50 mM Na_2HPO_4, 150 mM NaCl, 5 mM DTT, pH 7.4. The purity and folding of the protein was confirmed by SDS–gel electrophoresis and NMR spectroscopy.

7. Fluorescence polarization (FP) assay

All FP experiments were performed as described.[25] Briefly, the fluorescence polarization experiments were read on an Ultra Evolution 384-well plate reader (Tecan) with the 485 nm excitation and 535 nm emission filters. The fluorescence intensities parallel (Intparallel) and perpendicular (Intperpedicular) to the plane of excitation were measured in parallel perpendicular black 384-well NBS assay plates (Corning) at room temperature (~20 °C). The background fluorescence intensities of blank samples containing the references buffer were subtracted and steady-state fluorescence polarization was calculated using the equation: P = (Intparallel − Gint erpendicular)/(Intparallel + Gint erpendicular), and the correction factor G (G = 0.998 determined empirically) was introduced to eliminate differences in the transmission of vertically and horizontally polarized light. All fluorescence polarization values were expressed in millipolarization units (mP). The binding affinities of the fluorescent p53-derived peptide of Hu et al. (the P4 peptide)[25] towards MDM2 was determined in the buffer which contained 50 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 10% DMSO. Competition binding assays were performed using the 10 nM fluorescent P4 peptide and 100 nM MDM2. Binding constant and inhibition curves were fitted using the SigmaPlot (SPSS Science Software). The obtained curves and data points for all compounds are shown below.

8. NMR methods

All NMR spectra were acquired at 298 K on a Bruker DRX 600 MHz spectrometer equipped with a cryoprobe. Typically, NMR samples contained 0.1–0.2 mM protein in 50 mM KH_2PO_4 and 50 mM Na_2HPO_4, pH 7.4., containing 150 mM NaCl and 5 mM β-mercaptoethanol. Water suppression was carried out using the WATERGATE sequence. NMR data were processed using the Bruker program Xwin-NMR version 3.5. NMR ligand binding experiments were carried out in an analogous way to those previously described.[4a,i] The maximum concentration of DMSO at the end of titration experiments was less than 1%. The pH was maintained constant during the entire titration. The ^{1}H–15N-HSQC spectra were recorded using fast HSQC pulse sequence.[26] The maximum concentration of DMSO at the end of titration experiments was less than 1%.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.06.020.

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