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Supporting Information

69451 Weinheim, Germany

Robust Generation of Lead Compounds for Protein–Protein Interactions by Computational and MCR Chemistry: p53/Hdm2 Antagonists**

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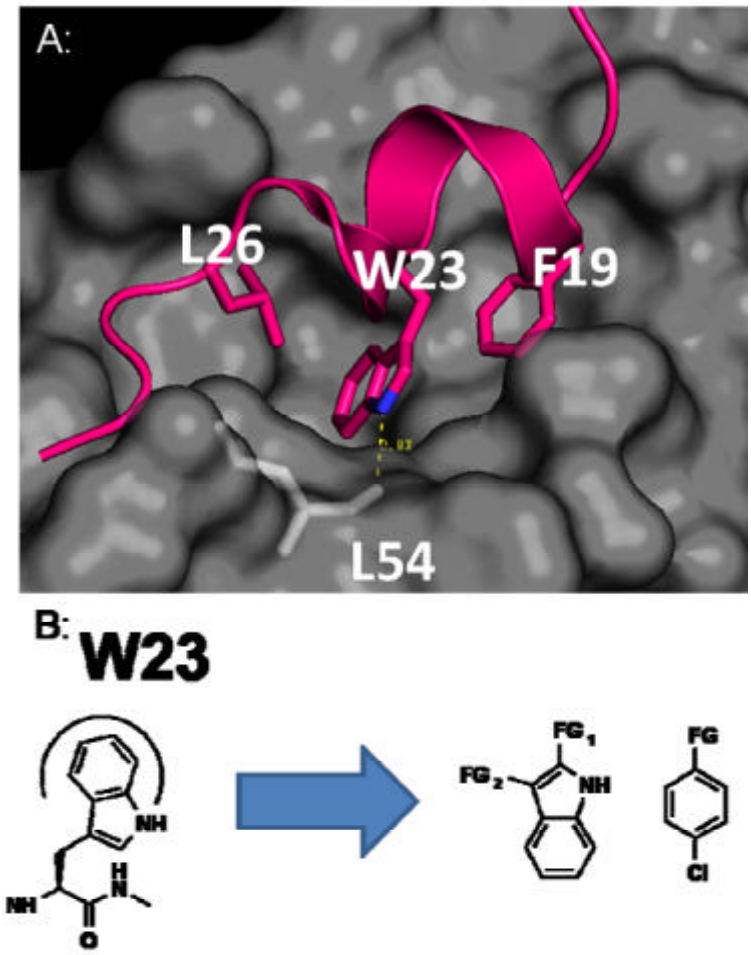
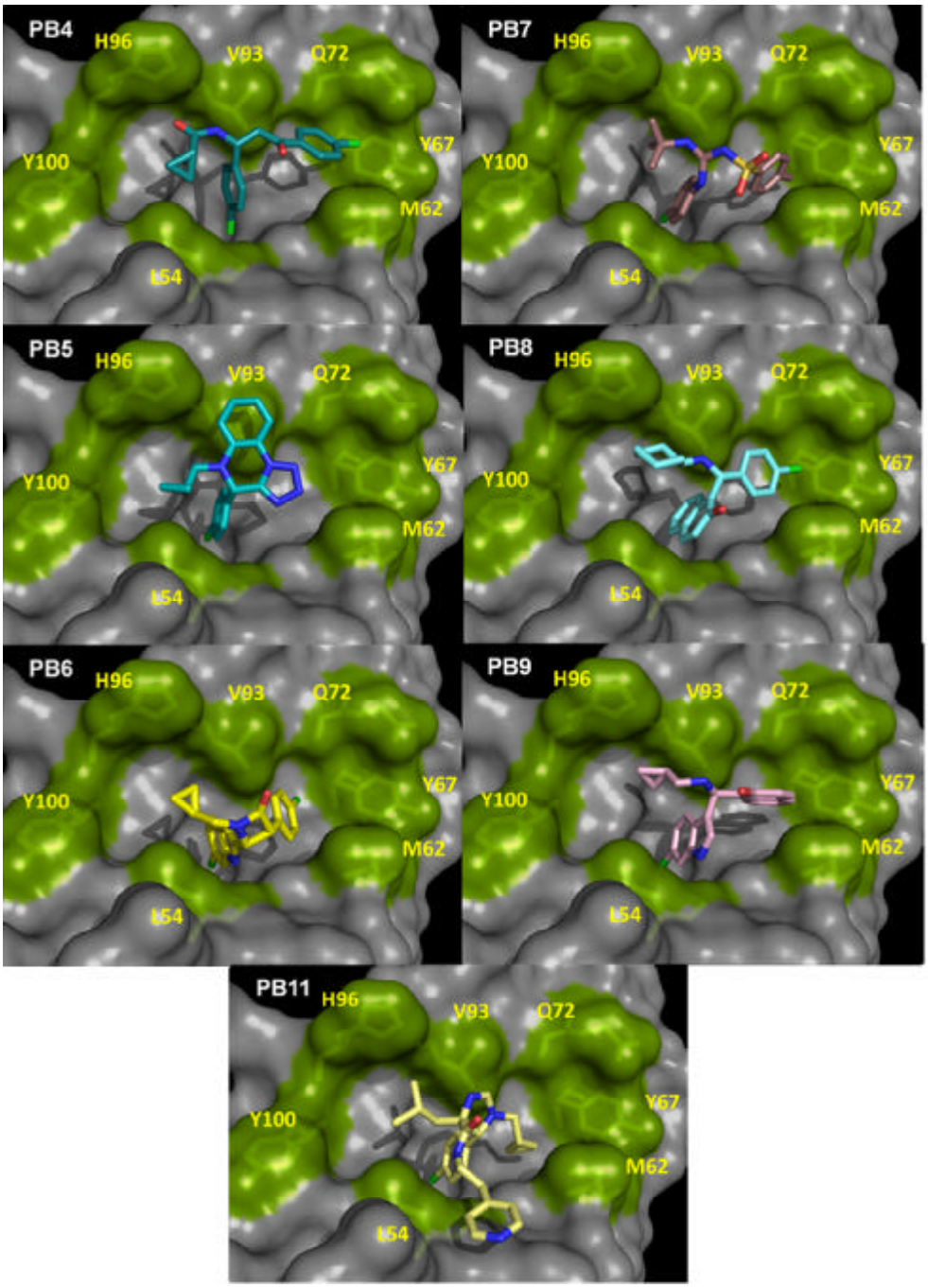
Supplemental Data

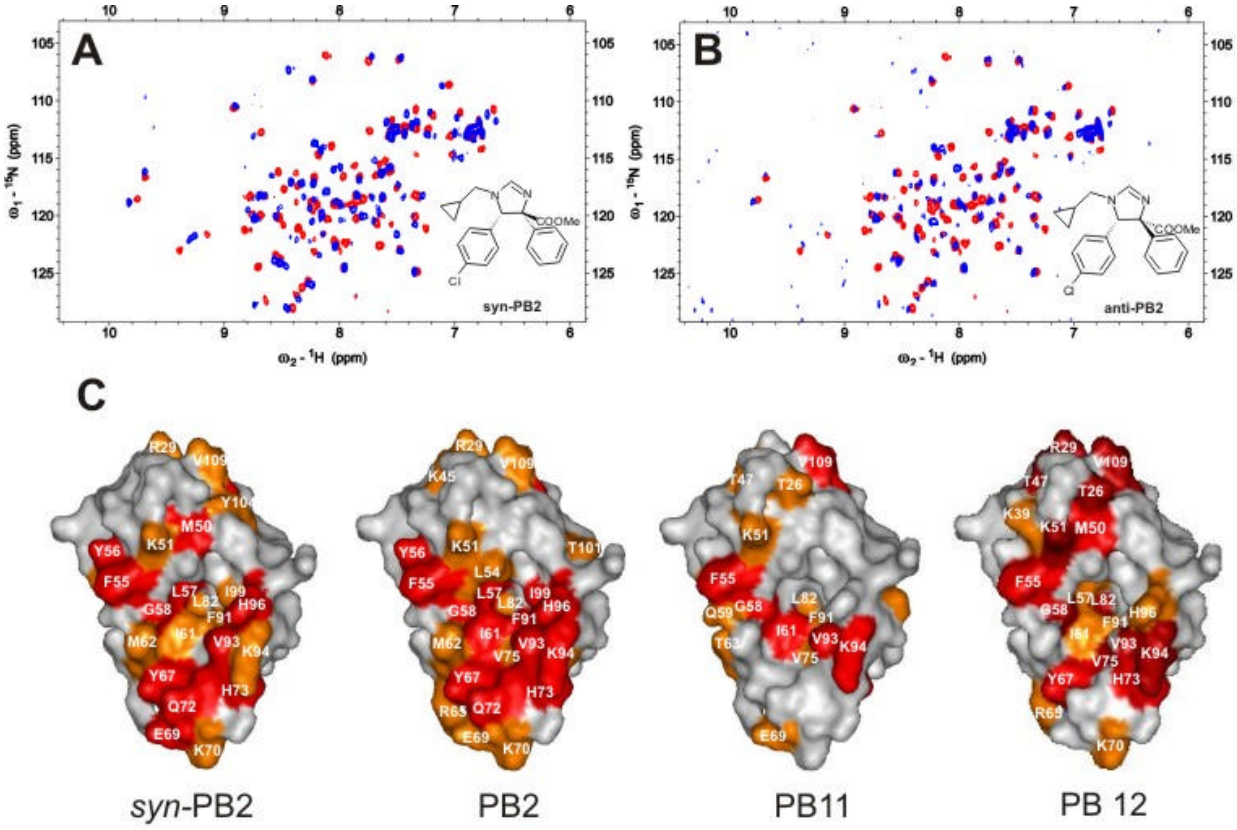


SI Figure 1. Schematic representation of the "ANCHOR" generation process from the p53 hot spot template amino acid Trp23.

(**A**): Architecture of the "hot spot" in the p53/Hdm2 interface (PDB Identifier: 1YCR). The triad of amino acid side chains, Phe19, Trp23 and Leu26 of the interacting p53 α-helix make strong van der Waals contacts to the Hdm2 receptor. Trp23 additionally forms a hydrogen bond to Hdm2's Leu54 backbone carbonyl. The Hdm2 surface is shown in grey. The hot spot amino acid side chains of p53 are shown as pink sticks and the p53 α-helix in cartoon format. (**B**): The backbone of Trp23 was excised and the resulting indole or the bioisosteric 4-chlorophenyl moiety was attached to different functional groups (FG), e.g., -COOH, -NH_2, -CHO, and –CN, as dictated by the requirements of the MCR chemistry.

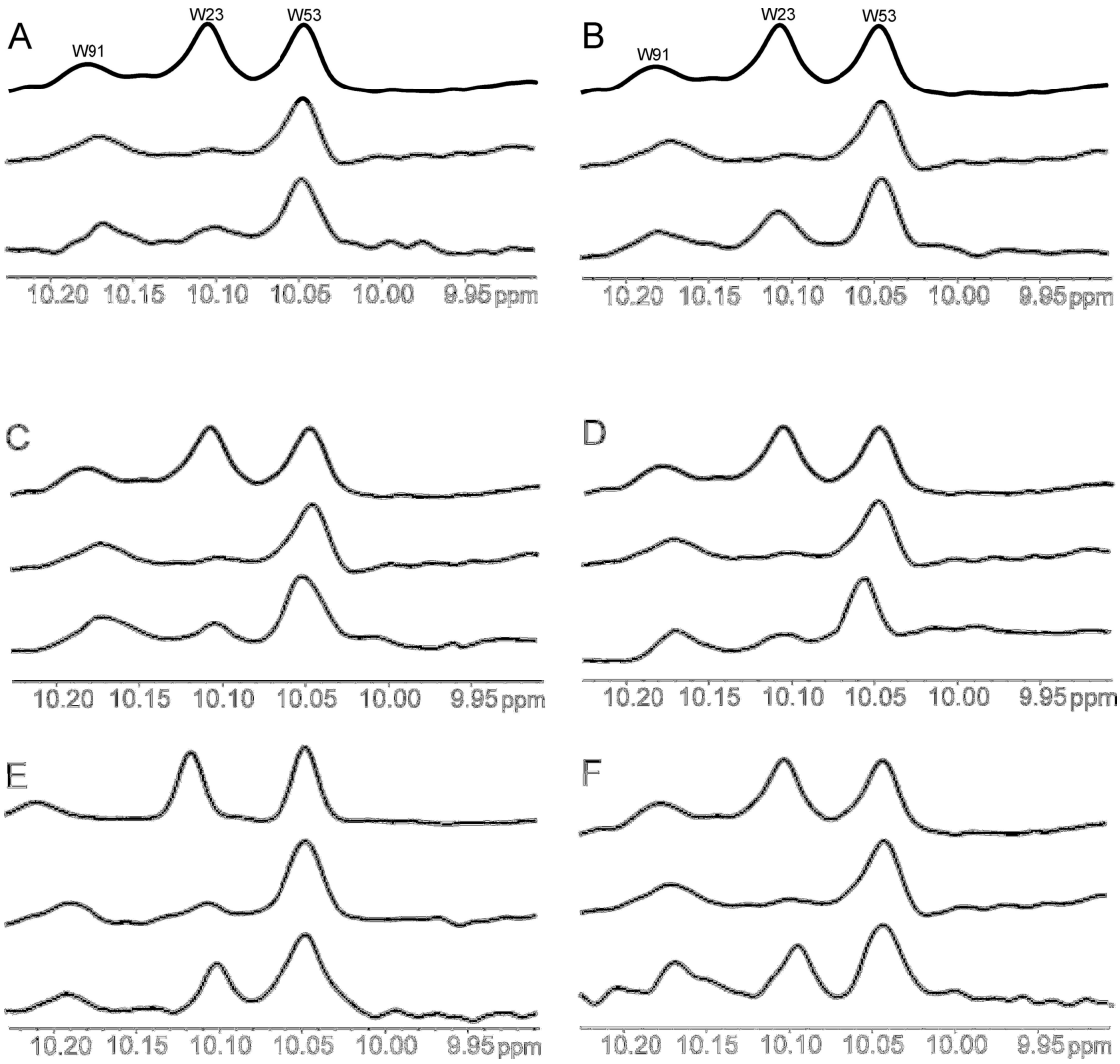


SI Figure 2 Docking poses for the Hdm2-p53 antagonists PB4-PB9, PB11 (sticks) into the Hdm2 receptor (PDB ID 1YCR). Key Hdm2 receptor residues in the rim region (green surface) determining the shape of the binding site are enumerated in yellow. The figures are rendered using PYMOL (DeLano, W. L. The PyMOL Molecular Graphics System; DeLano Scientific: Palo Alto, CA; http://www.pymol.org).



SI Figure 3. NMR-based screening of p53/Hdm2 antagonists.

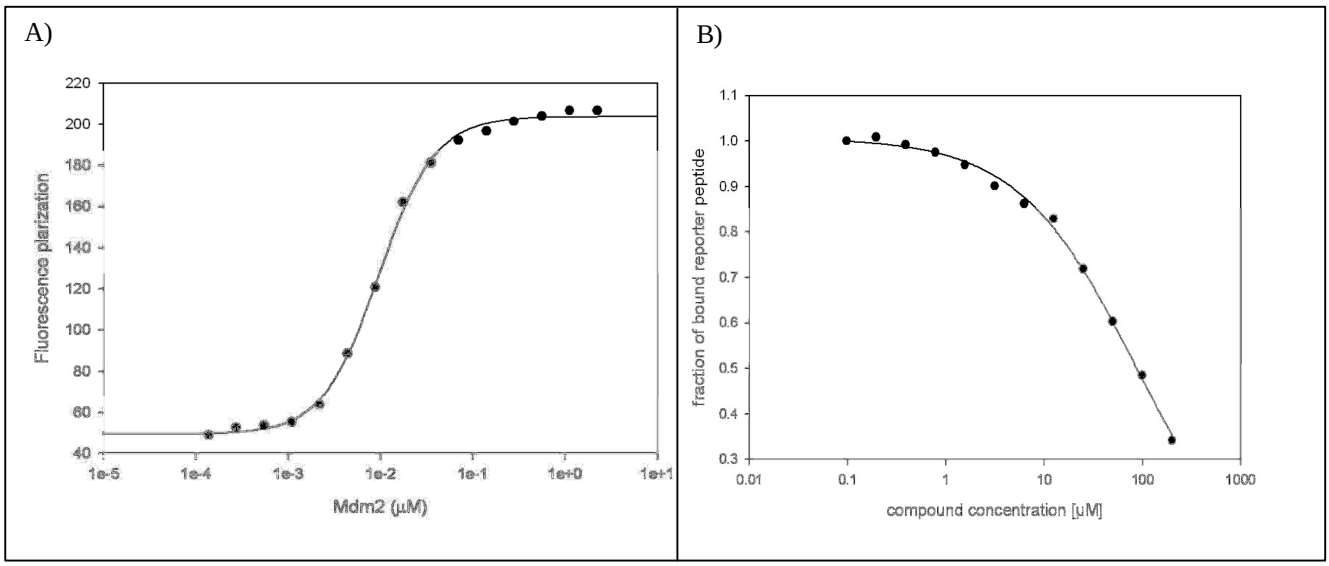
(**A)**: The HSQC perturbation spectra of Hdm2. The spectrum of free Hdm2 (red), and Hdm2 plus *syn*-**PB2** (blue). The final ratio of Hdm2 to *syn*-**PB2** was 1:5. (**B**): *anti*-**PB2**, the spectrum of free Hdm2 (red), and Hdm2 plus *anti*-**PB2** (blue). The final ratio of Hdm2 to *anti*-**PB2** was 1:2. (**C**): Contact surfaces of Hdm2 for the ligands *syn*-**PB2**, **PB2** (diastereomeric mixture), **PB11,** an optimized derivative of the Orru MCR series, and **PB12**. Residues which show significant induced NMR chemical shifts upon complexation with compounds are highlighted in orange and red for observed vectorial shifts of 0.09-0.15 and greater than 0.15 ppm, respectively. The residues of **PB12**, that show the slow chemical exchange has been highlighted in dark red.



SI Figure 4. PB compounds disrupt preformed p53/Hdm2 complex.

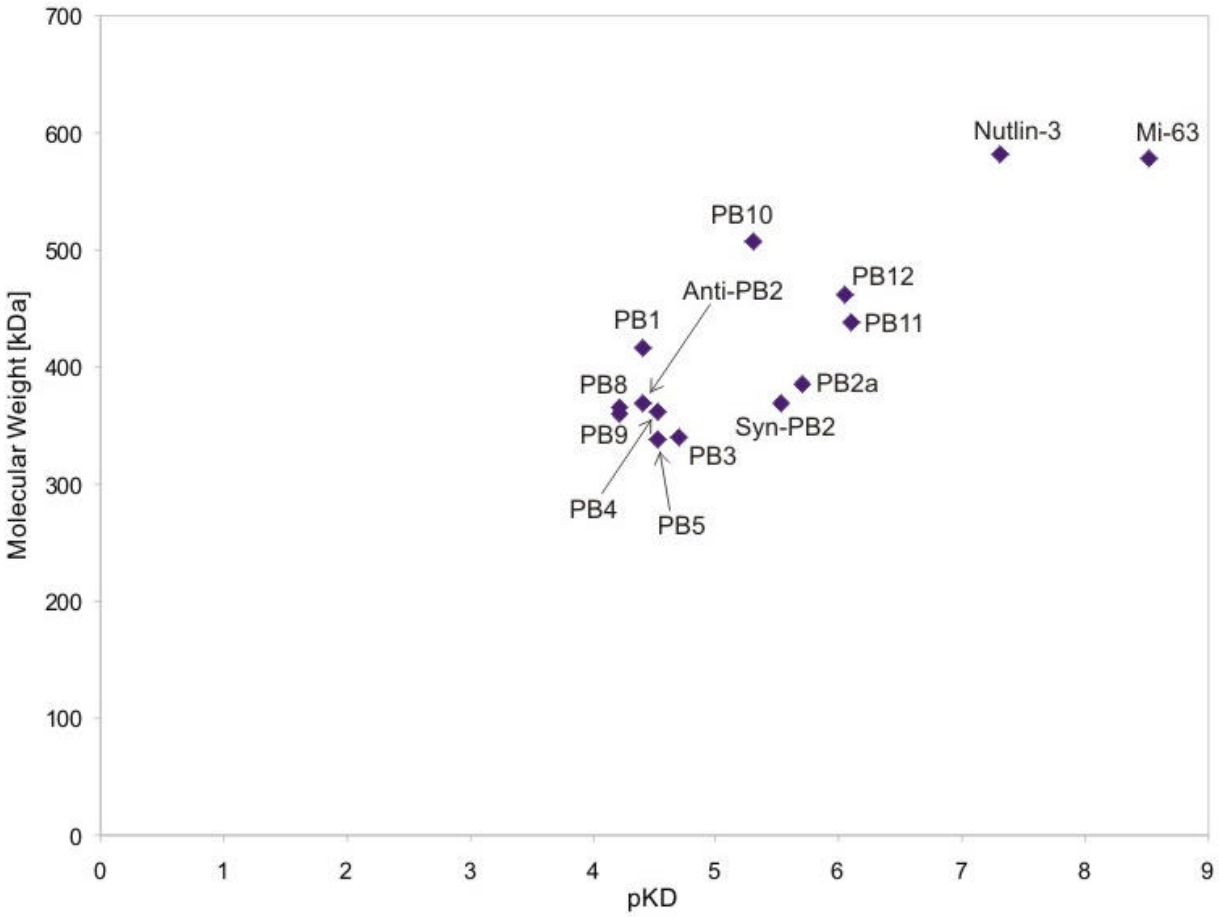
1D AIDA-NMR analysis with **PB** compounds. Upper trace: spectrum of p53 (residues 1-321). The three peaks are tryptophans of p53: Trp91, Trp23 and Trp53. Middle trace: the spectrum of the complex of p53 (res. 1-321) + Hdm2 (res. 1-125). Tryptophans 53 and 91 are not sensitive to the binding to Hdm2. Trp23 is in the binding site and therefore disappears on binding to Hdm2. Lower trace: addition of **PB** compounds to the complex releases p53 as seen by the reappearance of the tryptophan Trp23. (**a**): *anti*-**PB2**. (**b**): *syn*-**PB2**, (**c**): **PB3**, (**d**): **PB5,** (**e**): **PB10**, (**f**): **PB11.**

SI Figure 5 The reference FP binding curve of Hdm2 titrated with P5-FAM peptide [17] (A); Competitive displacement of the p53 FAM-P5 peptide from Hdm2 by compound **PB10** (B). The fluorescence polarization binding assay is described in section 11 of the Supplemental Data.

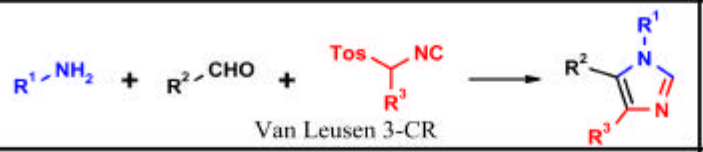
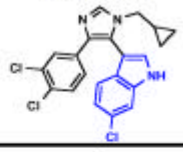
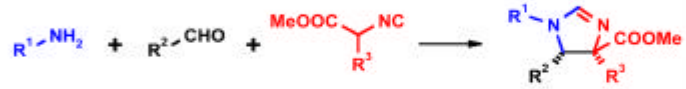
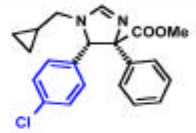
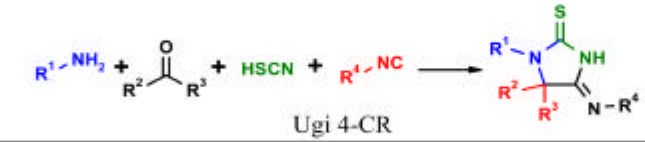
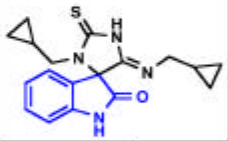
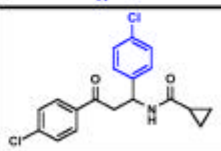
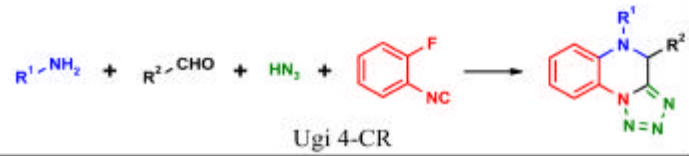
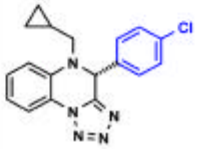
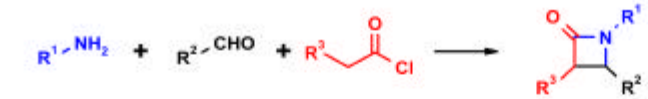
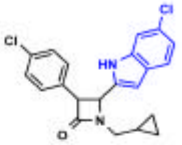
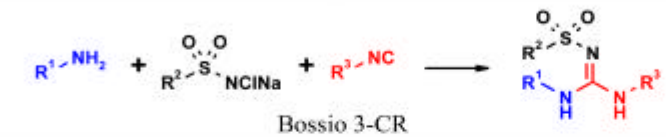
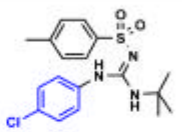
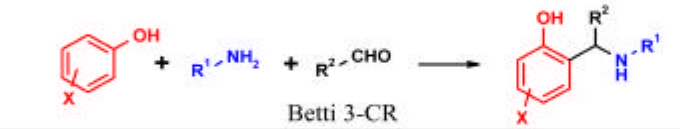
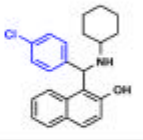
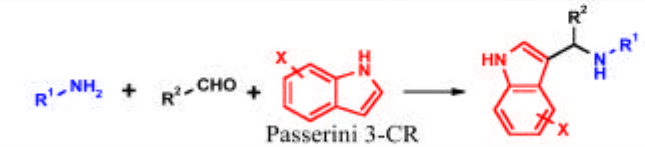
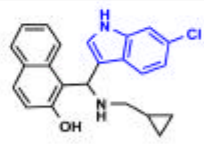
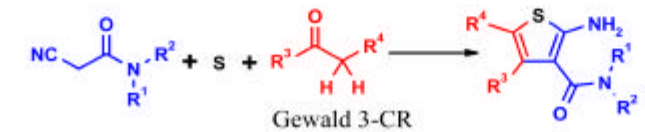
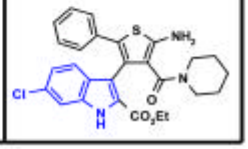


SI Figure 6 Plot of pK_D values (defined as the negative $base_{10}$ logarithm of the K_D value expressed in molar units) vs. molecular weight (MW) for the compounds in Table 1.

The dashed line shows the plot expected for the best, minimal leads of p53/Hdm2 antagonists. The known p53/Hdm2 antagonists Nutlin-3 [1] and MI-219 [2] are included for reference.

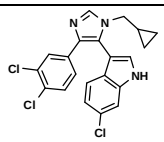
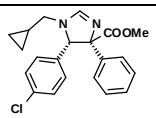
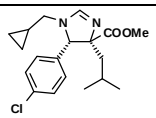
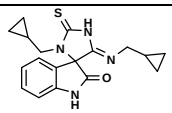
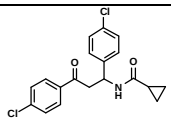
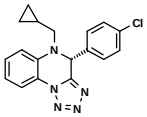
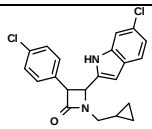
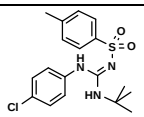
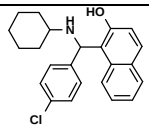
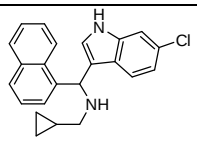


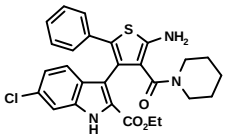
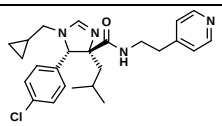
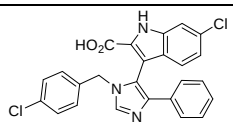
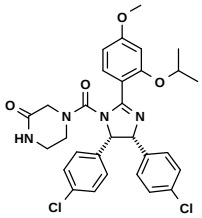
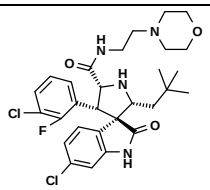
SI Table 1. MCRs used to produce representative p53/Hdm2 antagonists. The different classes of starting materials are color-coded and represent the variation of the MCR. The ANCHOR moiety in column three is marked in blue. **PB** = Pittsburgh. **PB12** [G. M. Popowicz, A. Czarna, S. Wolf, K. Wang, W. Wang, A. Dömling, T. A. Holak, *Cell Cycle* **2010**, *8*, 1176-1184.] is synthesized via vL-3CR (analogously to **PB1**). **PB2a** and **PB11** are synthesized analogously to **PB2**.

no	reaction scheme	representative antagonist*
PB1	 Van Leusen 3-CR	
PB2	 Orru 3-CR	
PB3	 Ugi 4-CR	
PB4	 α-amido-alkylation 3-CR	
PB5	 Ugi 4-CR	
PB6	 Staudinger 3-CR	
PB7	 Bossio 3-CR	
PB8	 Betti 3-CR	
PB9	 Passerini 3-CR	
PB10	 Gewald 3-CR	

SI Table 2 Drug-like characteristics of PB compounds according to the Pfizer rules.

Violations of the rules are indicated in red. (MW, molecular weight; TPSA, total polar surface area; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; clogP, calculated logarithm of the 1-octanol-water partition coefficient; nrotb, number of rotatable bonds)

No	Structure	MW [g/mol]	TPSA [Å^2]	HBD	HBA	clogP	nrotb	Pfizer rules
PB1		416.74	33.617	1	3	**6.298**	4	**yes**
PB2		368.86	41.907	0	4	4.701	6	**yes**
PB2a		348.88	41.907	0	4	4.76	7	**yes**
PB3		340.45	56.727	2	5	2.703	4	**yes**
PB4		362.25	46.169	1	3	3.743	6	**yes**
PB5		337.81	46.848	0	5	3.925	3	**yes**
PB6		385.30	36.1	1	3	**5.838**	4	**yes**
PB7		379.91	70.56	2	5	4.711	6	**yes**
PB8		365.9	32.255	2	2	**6.957**	4	**yes**
PB9		360.88	27.82	2	2	**5.79**	5	**yes**

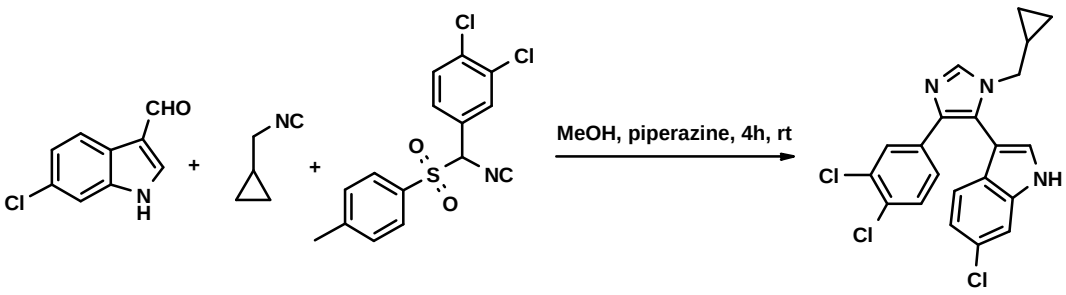
PB10		**507.14**	88.428	3	6	**5.901**	6	**no**
PB11		439.0	57.59	1	5	4.518	9	**yes**
PB12		462.33	70.91	2	5	4.90	4	**yes**
Nutlin3		**581.5**	83.477	1	8	**5.713**	6	**no**
MI-63		**577.53**	82.69	3	7	4.54	7	**yes**

8. Chemical Synthesis

The reactions have been not optimized for yields but standard procedures from literature was followed.

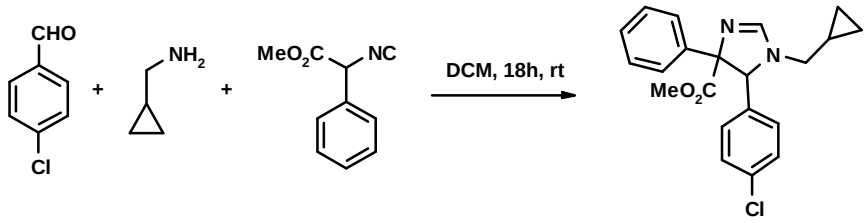
General experimental methods: Standard syringe techniques were applied for transfer of air sensitive reagents. Dry solvents and all purchased chemicals were purchased from Aldrich, Fisher Scientific, Acros Organics or Alfa Aesar and used as received. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance II Ultrashield Plus 600 at 600 and 150 MHz, respectively. Chemical shift values are in ppm relative to residual solvent signal. Abbreviations used are s = singlet, brs = broad singlet, d = doublet, brd = broad doublet, m = multiplet; data in parenthesis are given in the following order: multiplicity, number of protons and coupling constants in Hz. Flash chromatography was performed with the indicated solvent mixture on silica gel, MP Silitech 32-63 D, 60 Å, Bodman. Chromatotron chromatography was performed on Harrison Research Chromatotron, Ser. no. 65F with the indicated solvent mixture using silica gel, Merck, TLC grade 7749, with gypsum binder and fluorescent indicator, Sigma-Aldrich. Thin layer chromatography was performed using Whatmann flexible-backed TLC plates on aluminum with fluorescence indicator. Compounds on TLC were visualized by UV-detection. HPLC-MS measurements were done on a Shimadzu prominence HPLC equipped with a dual wavelength UV detector and an API 2000 LC-MS/MS system, Applied Biosystems MDS SCIEX, (MS) using a Dionex Acclaim 120 column (C18, 3µm, 120 Å, 2.1 x 150 mm) using a mobile phase of water and acetonitrile, both containing 0.1% acetic acid and the following gradient: 5-90% acetonitrile in 7 min, injection volume: 5 µL, detection wavelength 254 nm. HRMS measurements were performed at the Department of Chemistry, University of Pittsburgh with a Waters/Micromass Q-Tof spectrometer, ionization mode: ESI. Microwave reactions were performed on the Emrys Optimizer system from Personal Chemistry.

PB1: 6-Chloro-3-[3-cyclopropylmethyl-5-(3,4-dichlorophenyl)-3*H*-imidazol-4-yl]-1*H*-indole [5]



6-Chloro-1*H*-indole3-carbaldehyde (180 mg, 1 mmol) were dissolved in 2 mL of MeOH, and 85.6 µL (1 mmol) cyclopropylmethyl amine was added dropwise. The reaction mixture was stirred for 4 h at room temperature and 1,2-dichloro-4-[isocyano(toluene-4-sulfonyl)methyl]benzene (340 mg, 1 mmol) and piperazine (86 mg, 1 mmol) were added and stirred over night at room temperature. The solvent was evaporated and the crude product purified by chromatography on silica with a gradient of 3:1 to 2:1 heptane/ethyl acetate to yield 6-chloro-3-[3-cyclopropylmethyl-5-(3,4-dichlorophenyl)-3*H*-imidazol-4-yl]-1*H*-indole (**PB1**) 356 mg (86%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.17 (d, J = 4.80 Hz, 2H), 0.54 (d, *J* = 7.86 Hz, 2H), 0.99-1.03 (m, 1H), 3.59 (d, *J* = 6.90 Hz, 2H), 7.07-7.12 (m, 2H), 7.17-7.21 (m, 2H), 7.25-7.26 (m, 1H), 7.46 (m, 1H), 7.74-7.75 (m, 1H), 7.86 (s, 1H), 9.63 (s, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 4.0, 11.2, 49.8, 104.5, 111.4, 120.0, 131.2, 131.6, 124.8, 125.4, 125.6, 127.5, 128.5, 129.5, 129.7, 131.8, 134.5, 136.3, 136.6, 137.1; HPLC-MS (ESI): r_t = 9.53 min, *m/z* 416 $[M+H]^+$; HRMS (ESI-TOF) $C_{21}H_{17}Cl_3N_3$ *m/z* calcd $[M+H]^+$ 416.0488, found 416.0498.

PB2: Methyl 5-(4-chlorophenyl)-1-cyclopropylmethyl-4-phenyl-4,5-dihydro-1*H*-imidazole-4-carboxylate [6]



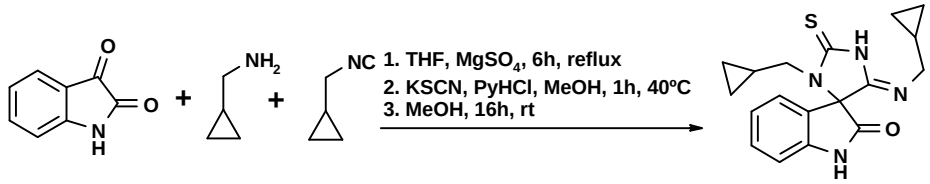
p-Chlorobenzaldehyde (422 mg, 3 mmol) was solubilized in 20 ml dry dichloromethane. Cyclopropylmethylamine (257 µL, 3mmol) and methyl isocyanophenylacetate (525 mg, 3mmol) were added and the mixture was allowed to stir over night at room temperature. Isolation of the mixture of the two diasteromers by column chromatography on silica gel with a gradient of 3:1 to 1:5 petroleum ether/ethyl acetate gradient yielded 893 mg (81%) of methyl 5-(4-chlorophenyl)-1-cyclopropylmethyl-4-phenyl-4,5-dihydro-1*H*-imidazole-4-carboxylate (PB2) as a yellow oil as a mixture of diastereomers (33:17). The mixture of the two diastereomers (760 mg) was separated by column chromatography on neutral alumia with ethyl acetate to give 260 mg of pure major diasteromer and 374 mg of the mixture of two diastereomers. ^{1}H-NMR for the major diastereomer ($CDCl_3$, 600 MHz): δ 0.05-0.06 (m, 2H), 0.47-0.51 (m, 1H), 0.59-0.62 (m, 1H), 0.88-0.91 (m, 1H), 2.55-2.59 (m, 1H), 3.09-3.12 (m, 1H), 3.79 (s, 3H), 5.64 (s, 1H), 6.90-6.91 (m, 4H), 7.04-7.06 (m, 5H), 7.44 (s, 1H); ^{13}C-NMR for the major diastereomer ($CDCl_3$, 150 MHz): δ 2.4, 4.5, 9.1, 50.0, 52.7, 68.7, 84.1, 126.2, 126.8, 127.3, 127.5, 132.7, 134.1, 136.9, 156.3, 173.8; ^{1}H-NMR for the minor diastereomer ($CDCl_3$, 600 MHz): δ 0.02-0.04 (m, 2H), 0.44-0.46 (m,

1H), 0.54-0.56 (m, 1H), 0.82-0.84 (m, 1H), 2.61-2.65 (m, 1H), 3.09-3.13 (m, 1H), 3.29 (s, 3H), 5.09 (s, 1H), 7.31-7.43 (m, 8H), 7.76-7.77 (m, 2H); ^{13}C-NMR for the minor diastereomer ($CDCl_3$, 150 MHz): δ 2.5, 4.3, 9.3, 49.7, 51.7, 72.8, 85.0, 126.3, 127.4, 128.0, 128.3, 129.0, 133.8, 135.3, 143.2, 155.1, 170.7; HPLC-MS (ESI): r_t =12.13 min *m/z* 369 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{22}ClN_2O_2$ $[M+H]^+$ 369.1370, found 369.1365.

PB2a: 5-(4-Chlorophenyl)-1-cyclopropylmethyl-4-isobutyl-4,5-dihydro-1*H*-imidazole-4-carboxylic acid methyl ester

preparation according to **PB2** and as described in [16]

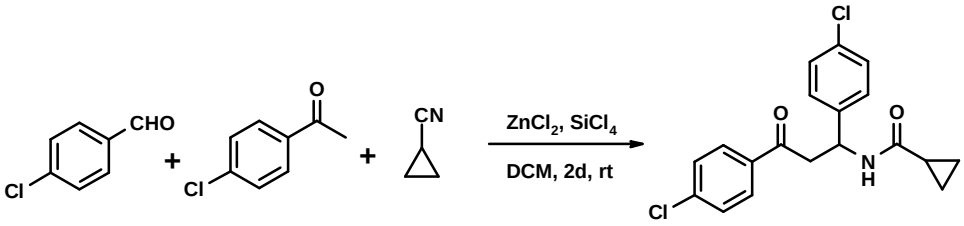
PB3:(*Z*)-3-(cyclopropylmethyl)-5-(cyclopropylmethylimino)-2-thioxospiro(imidazolidine-4,3'-indolin)-2'-one [7]



Isatin (908 mg, 5 mmol) and cyclopropylmethylamine (431 µL, 5 mmol) were dissolved in THF. A small amount $MgSO_4$ was added and the reaction mixture was refluxed for 6h, filtered and evaporated to give the precondensed Schiff base. A solution of KSCN (485 mg, 5 mmol) and pyridium hydrochloride (528 mg, 5 mmol) in 15 mL of MeOH was heated at 40 ^{o}C for 1h, then cooled with ice-water and filtered. The Schiff base (1.0 g, 5 mmol) was added to the solution and isocyanomethylcyclopropane (405 mg, 5 mmol) was added drop wise. The reaction mixture was stirred at room temperature overnight. The solvent was evaporated and the residue purified by column chromatography to yield (*Z*)-3-(cyclopropylmethyl)-5-(cyclopropylmethylimino)-2-thioxospiro(imidazolidine-4,3'-indolin)-2'-one (PB3) 350 mg (25%); ^{1}H-NMR for the major diastereomer (MeOD, 600 MHz): δ –0.10 (m, 1H), –0.01 (m, 1H), 0.22 (m, 2H), 0.27 (m, 1H), 0.35 (m, 1H), 0.46 (m, 2H), 0.81 (m, 1H), 1.03 (m, 1H), 3.18 (dd, *J* = 7.2, 14.4 Hz, 1H), 3.23 (dd, *J* = 1.2, 6.6 Hz, 2H), 3.70 (dd, *J* = 6.6, 14.4 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 7.14 (m, 2H), 7.45 (m, 1H); ^{13}C-NMR (MeOD, 150 MHz): δ 2.3, 2.4, 3.2, 4.2, 9.5, 9.6, 49.4, 76.9, 111.3, 123.2, 123.3,

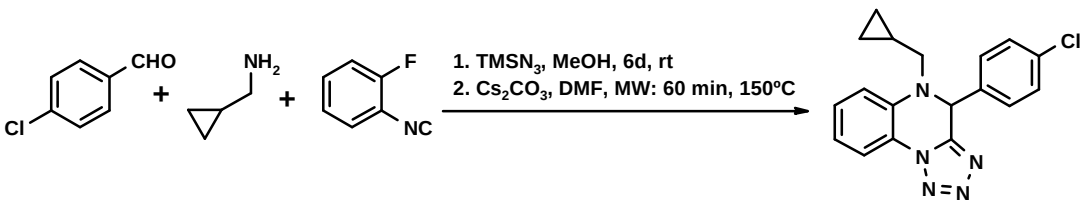
124.9, 131.4, 143.3, 171.3, 173.4, 197.7; HPLC-MS (ESI): r_t = 8.25 min *m/z* 341 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{20}N_4OS$ 340.1357, found 340.1366.

PB4: Cyclopropanecarboxylic acid [1,3-bis-(4-chlorophenyl)-3-oxopropyl]amide [8]



141 mg (1 mmol) 4-Chlorobenzaldehyde, 132.6 µL (1 mmol) 1-(4-chlorophenyl)ethanone and 75.6 µL (1 mmol) cyclopropanecarbonitrile were combined in dry DCM. Zinc chloride (273 mg, 2 eq, 2 mmol) and silicon tetrachloride (458.3 µL, 4eq, 4 mmol) were added and the reaction mixture was allowed to stir for 2 days at room temperature. The reaction mixture was purified by chromatography on silica gel with 4:1 heptane/ethyl acetate to yield cyclopropanecarboxylic acid [1,3-bis-(4-chlorophenyl)-3-oxopropyl]amide (PB4) 43 mg (12%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.77-0.79 (m, 2H), 0.98-1.00 (m, 2H), 1.40-1.45 (m, 1H), 3.39-3.43 (m, 1H), 3.73-3.77 (m, 1H), 5.53-5.56 (m, 1H), 6.80 (d, 1H), 7.28-7.33 (m, 4H), 7.44-7.47 (m, 2H), 7.86 (d, 2H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 7.4, 7.5, 14.9, 43.1, 49.5, 127.9, 128.8, 129.1, 129.5, 133.3, 134.8, 139.4, 140.2, 173.1, 197.2; HPLC-MS (ESI): r_t = 11.30 min *m/z* 362 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{19}H_{17}Cl_2NO_2Na$ $[M+Na]^+$ 384.0534, found 384.0550.

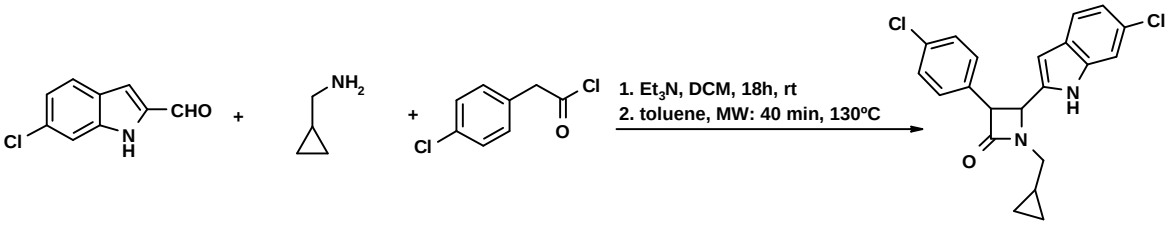
PB5:4-(4-Chlorophenyl)-5-cyclopropylmethyl-4,5-dihydro-1,2,3,5,9b-pentaazacyclopenta[*a*]naphthalene [9]



4-Chlorobenzaldehyde (422 mg, 3 mmol) and cyclopropylmethylamine (262.6 µL, 3mmol) were dissolved in 3 mL of MeOH and stirred for 5 h at room temperature. 1-Fluoro-2-isocyanobenzene (472 mg, 1.3 eq, 3.9 mmol) was added and the reaction mixture was allowed to stir for 6 days at room temperature. The solvent was evaporated and the residue dissolved in ethyl acetate and washed with water and brine. The

organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by chromatography on silica gel with a 9:1 to 4:1 gradient of heptane/ethyl acetate to yield 1074 mg of {(4-chlorophenyl)-[1-(2-fluorophenyl)-1*H*-tetrazol-5-yl]methyl}cyclopropylmethylamine. {(4-Chlorophenyl)-[1-(2-fluorophenyl)-1*H*-tetrazol-5-yl]methyl}cyclopropylmethylamine (100 mg, 0.28 mmol) was dissolved in 4 mL of dry DMF and baked Cs_2CO_3 (182 mg, 2eq, 0.56 mmol) was added and the reaction mixture was heated in the microwave for 60 min at 150 ºC. The solvent was evaporated and the residue dissolved in ethyl acetate and extracted with water and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by chromatography on silica gel with 4:1 heptane/ethyl acetate to yield 4-(4-chlorophenyl)-5-cyclopropylmethyl-4,5-dihydro-1,2,3,5,9b-pentaazacyclopenta[*a*]naphthalene (PB5) 19 mg (20%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.15-0.23 (m, 2H), 0.53-0.57 (m, 1H), 0.65-0.68 (m, 1H), 1.04-1.06 (m, 1H), 3.01-3.05 (m, 1H), 3.57-3.60 (m, 1H), 6.50 (s, 1), 7.00-7.05 (m, 2H), 7.22-7.31 (m, 4H), 7.37-7.40 (m, 1H), 7.99-7.00 (d, 1H); HPLC-MS (ESI): r_t = 12.29 min *m/z* 337 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{16}ClN_5$ 337.1094, found 337.1093.

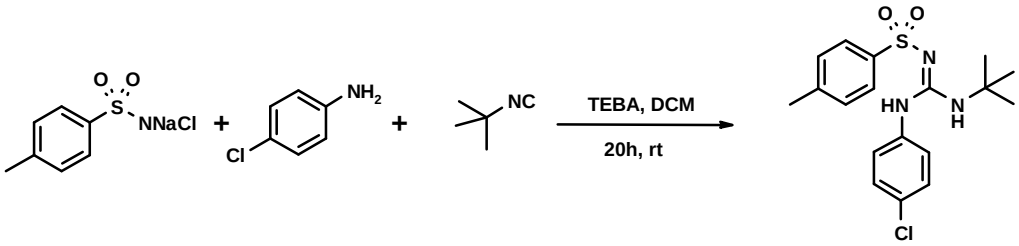
PB6: 4-(6-Chloro-1*H*-indol-2-yl)-3-(4-chlorophenyl)-1-cyclopropylmethylazetidin-2-one [10]



6-Chloro-1*H*-indole-2-carbaldehyde (180 mg, 1 mmol) and cyclopropylmethylamine (85.6 mL, 1 mmol) were dissolved in DCM. A small amount $MgSO_4$ was added and the mixture was stirred over night. The salt was filtered off and the filtrate concentrated under reduced pressure. The residue was dissolved in toluene, and triethylamine (669 µL, 4.8 mmol) and (4-chlorophenyl)acetylchloride (251.6 µL, 1.72 mmol) were added simultaneously. The reaction mixture was heated in the microwave for 40 min at 130 ºC. After the mixture cooled to room temperature the solid was filtered off and the filtrate was evaporated. The residue was dissolved in ethyl acetate and extracted with water and brine. The organic layer was dried over $MgSO_4$, filtered and evaporated. The crude product was purified by chromatography on silica gel with 4:1 heptane/ethyl acetat to yield 4-(6-chloro-1*H*-indol-2-yl)-3-(4-chlorophenyl)-1-

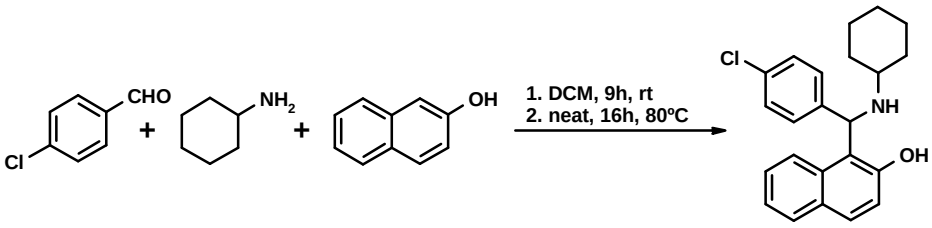
cyclopropylmethylazetidin-2-one (PB6) 66 mg (18%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.43-0.45 (m, 2H), 0.51-0.52 (m, 2H) 0.92-0.95 (m, 1H), 2.65-2.69 (m, 1H), 3.52-3.56 (m, 1H), 4.43 (s, 1H), 4.79 (s, 1H), 6.52 (s, 1H), 7.07-7.08 (m, 1H), 7.24-7.48 (m, 6H), 9.98 (s, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 3.0, 4.2, 9.3, 45.9, 58.2, 62.2, 103.5, 111.3, 120.8, 121.3, 126.3, 128.5, 128.6, 129.3, 133.0, 133.9, 134.6, 137.5, 168.1; HPLC-MS (ESI): r_t =12.54 min *m/z* 384 $[M-H]^-$; HRMS (ESI-TOF) *m/z* calcd for $C_{21}H_{18}Cl_2N_2O$ 384.0796, found 384.07977.

PB7: *N*-[1-*tert*-Butylamino-1-(4-chlorophenylamino)meth-(*Z*)-ylidene]-4-methylbenzene-sulfonamide [11]



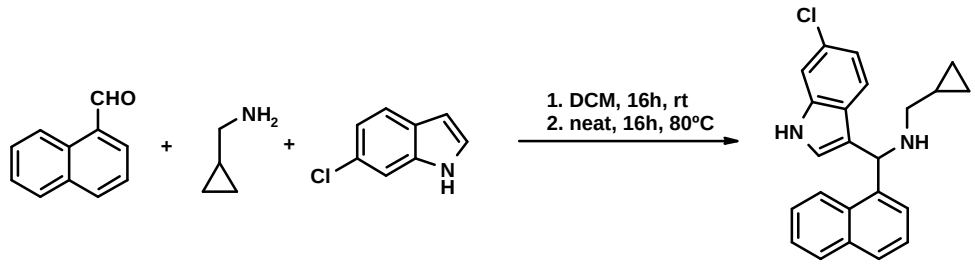
A solution of chloramine T (228 mg, 1 mmol), 4-chlorophenylamine (128 mg, 1 mmol) and *tert*-butylisocyanide (83 mg, 1 mmol) in 5 mL of dry DCM was treated with benzyltriethylammonium chloride (5 mg) and stirred for 20 h at room temperature. The reaction was quenched with water and the organic layer was separated, dried over Na_2SO_4, filtered and evaporated. The crude product was purified by chromatography on silica with 2:1 petroleum ether/ethyl acetate to yield *N*-[1-*tert*-butylamino-1-(4-chlorophenylamino)meth-(*Z*)-ylidene]-4-methylbenzenesulfonamide (PB7) 94 mg (24%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 1.32 (s, 9H), 2.43 (s, 3H), 6.62 (d, 1H), 7.07-7.10 (m, 2H), 7.28 (d, 2H), 7.38 (d, 2H), 7.84 (d, 2H), 8.81 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 21.5, 29.2, 52.8, 116.3, 125.9, 126.4, 127.0, 129.1, 129.3, 130.3, 134.4, 140.7, 142.1, 145.1, 152.7; HPLC-MS (ESI-TOF): r_t = 11.75 min *m/z* 380 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{18}H_{23}ClN_3O_2S$ $[M+H]^+$ 380.1200, found 380.1189.

PB8: 1-[(4-chlorophenyl)(cyclohexylamino)methyl]naphthalen-2-ol [12]



4-Chlorobenzaldehyde (337 mg, 1.2 eq, 2.4 mmol) and cyclohexylamine (239.9 µL, 1.05 eq, 2.1 mmol) were diluted in DCM and stirred for 9h at room temperature. The solvent was evaporated and the precondensed Schiff base was combined with naphthalen-2-ol (288 mg, 1 eq, 2 mmol) and heated to 80 ºC for 15h. The reaction mixture was purified by chromatography on silica gel with 4:1 heptane/ethyl acetate to yield 1-[(4-chlorophenyl)cyclohexylaminomethyl]naphthalen-2-ol (PB8) 343 mg (47%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.71-0.82 (m, 2H), 1.06-1.22 (m, 3H), 1.50-1.51 (m, 1H), 1.51-1.53 (m, 1H), 1.58-1.61 (m, 1H), 1.86-1.88 (m, 1H), 2.15-2.17 (m, 1H), 2.61(bs, 1H), 5.76 (s, 1H), 7.06-7.07 (d, 1H), 7.15-7.19 (m, 3H), 7.25-7.28 (m, 3H), 7.56-7.58 (d, 1H), 7.64-7.66 (m, 2H), 13.88 (bs, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 24.6, 24.7, 25.5, 32.4, 33.2, 55.5, 59.6, 113.2, 120.1, 120.6, 122.2, 126.3, 128.3, 128.7, 129.5, 131.9, 133.5, 140.4, 157.2; HPLC-MS (ESI-TOF): r_t = 10.43 min *m/z* 366 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{23}H_{24}ClNO$ 365.1546, found 365.1549.

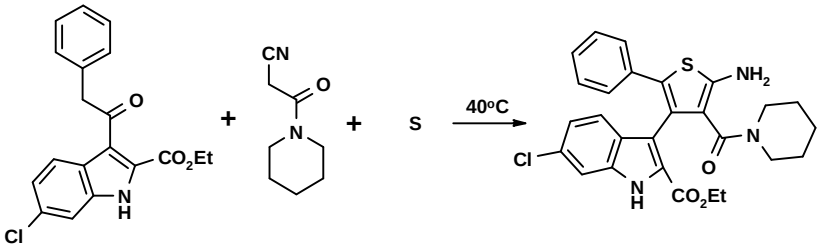
PB9: [**(6-Chloro-1*H*-indol-3-yl)naphthalen-1-yl-methyl]cyclopropylmethylamine** [13]



Naphthalene-1-carbaldehyde (327 µL, 1.2 eq, 2.4 mmol) and cyclopropylmethylamine (180 µL, 1.05 eq, 2.1 mmol) were diluted in DCM and stirred over night at room temperature. The solvent was evaporated and the precondensed Schiff base was combined with 6-chloro-1*H*-indole (303 mg, 1 eq, 2 mmol) and heated to 80 ºC for 15 h. The reaction mixture was purified by chromatography on silica gel with 4:1 heptane/ethyl acetate to yield [(6-chloro-1*H*-indol-3-yl)naphthalen-1-ylmethyl]cyclopropylmethylamine (PB9) 270 mg (37%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 0.08 (m, 2H), 0.45 (m, 2H), 1.03-1.05 (m, 1H), 2.44 (bs, 1H), 2.57-2.64 (m, 2H), 5.95 (s, 1H), 6.66 (s, 1H), 7.03 (d, 1H), 7.16 (s, 1H), 7.37-7.45 (m, 3H), 7.55

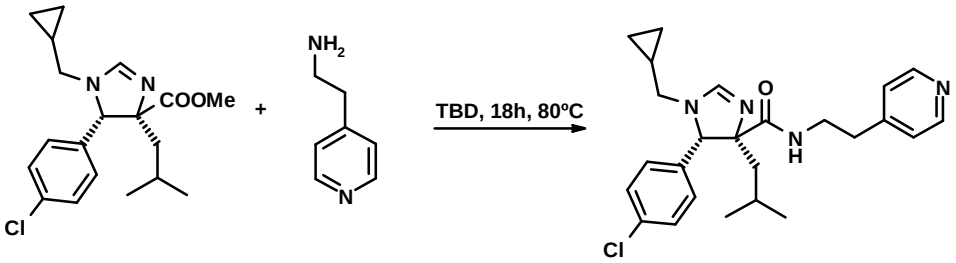
(d, 1H), 7.74-7.75 (m, 2H), 7.76 (d, 1H), 7.85 (d, 1H), 8.06 (s, 1H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 3.5, 3.7, 11.4, 53.7, 55.1, 111.3, 118.9, 119.9, 120.3, 123.3, 123.9, 124.4, 125.1, 125.5, 125.6, 126.0, 127.9, 128.9, 131.5, 134.1, 136.8, 138.3; HPLC-MS (ESI-TOF): r_t = 9.34 min *m/z* 359 $[M]^-$; HRMS (ESI-TOF) *m/z* calcd for $C_{23}H_{21}ClN_2$ 360.1393, found 360.1401.

PB10: Ethyl 3-(5-amino-2-phenyl-4-(piperidine-1-carbonyl)thiophen-3-yl)-6-chloro-*1H*-indole-2-carboxylate [14,15]



Titanium tetrachloride (2.0 mL, 1.0 M in toluene) was added dropwise to a solution of ethyl 6-chloro-3-(2-phenylacetyl)-1H-indole-2-carboxylate (1.0 mmol, 340 mg), 3-oxo-3-(piperidin-1-yl)propanenitrile (1.5 mmol, 228 mg) in 1 mL of THF. Then triethylamine (0.3 mL) was added dropwise, the mixture was stirring under 40 ^{o}C overnight. After work up with 10% HCl, the mixture was extracted by ethyl acetate. The combined organic layer was washed with 2 M NaOH, the dried over magnesium sulfate. The intermediate was purified by column chromatography on silica gel (petroleum ether/ ethyl acetate, 10:1 to 5:1) as yellow oil (160 mg, yield: 34%, a mixture of Z- and E- isomers). Then the isolated intermediate was treated with sulfur (32 mg), triethylamine (0.15 mL) in 1 mL of ethanol and the mixture was stirring under 50 ^{o}C for 2 days. The product was purified by column chromatography on silica gel (petroleum ether/ ethyl acetate, 5:1) as brown solids (14 mg, yield: 8%). ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 9.14 (1H, s), 7.38 (1H, s), 6.98-7.08 (7H, m), 4.73 (2H, br.s), 4.01-4.17 (2H, m), 3.06-3.18 (4H, m), 1.23-1.27 (6H, m), 1.17 (3H, t, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 14.0, 24.2, 29.3, 56.0, 61.1, 111.4, 116.8, 117.1, 122.1, 123.0, 126.5, 127.8, 128.2, 134.5, 135.7, 152.9, 160.7, 161.4; HPLC-MS (ESI-TOF): r_t = 11.46 min *m/z* 508.0 $[M+H]^+$; HRMS (ESI-TOF) *m/z* calcd for $C_{27}H_{27}N_3O_3SCl$ $[M+H]^+$, calcd 508.1462, found 508.1425.

PB11: *cis*-5-(4-Chlorophenyl)-1-(cyclopropylmethyl)-4-isobutyl-*N*-(2-(pyridin-4-yl)ethyl)-4,5-dihydro-1*H*-imidazole-4-carboxamide [16]



Synthetic procedure as recently described by us (Srivastava et al., 2009). Yield 35 mg (0.1 mmol, 79%); ^{1}H-NMR ($CDCl_3$, 600 MHz): δ -0.02-0.03 (2H, m), 0.43-0.48 (1H, m), 0.53-0.58 (1H, m), 0.66 (3H, d, J= 6 Hz), 0.76-0.83 (4H, m), 0.89 (1H, dd, J= 12 Hz & 6 Hz), 1.10 (1H, dd, J= 12 Hz & 6 Hz), 1.53-1.60 (1H, m), 2.62 (1H, dd, J= 12 Hz & 6 Hz), 2.84-2.96 (2H, m), 3.09 (1H, dd, J= 12 Hz & 6 Hz), 3.56-3.68 (2H, m), 4.84 (1H, s), 7.16-7.18 (3H, m), 7.24-7.30 (2H, m), 7.34 (2H, brd, J= 12 Hz), 8.53 (2H, brd, J= 6 Hz); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ .69, 4.77, 9.33, 23.73, 24.40, 24.61, 29.70, 35.05, 39.26, 45.05, 50.32, 70.07, 79.84, 124.13, 128.45, 133.53, 134.20, 147.94, 149.86, 155.54, 176.39; HRMS (ESI-TOF) $C_{25}H_{31}ClN_4O$ *m/z* calcd $[M+H]^+$ 439.2265, found 439.2237$[M+H]^+$.

PB12: 6-Chloro-3-(1-(4-chlorobenzyl)-4-phenyl-1H-imidazol-5-yl)-*1H*-indole-2-carboxylic acid [synthesized according to: G. M. Popowicz, A. Czarna, S. Wolf, K. Wang, W. Wang, A. Dömling, T. A. Holak, *Cell Cycle* **2010**, *9*, 1104-1111.]

10. Protein expression and purification. Recombinant human Hdm2 (residues 1-125) was obtained from an *E. coli* BL21(DE3) RIL expression system using the pET-20 vector. Cells were grown at 37°C and induced with 1 mM IPTG at an OD_{600nm} of 0.7. After induction, the cells were cultured for an additional 4.5 h at 37°C and the recombinant protein was purified from inclusion bodies. After washing with PBS containing 0.05% Triton X100 with subsequent low-speed centrifugation (12000G), the inclusion bodies were solubilized in 6 M guanidinium-HCl (GuHCl) in 100 mM Tris-HCl, pH 8.0, including 1 mM EDTA and 10 mM DTT. The protein was then dialyzed against 4 M GuHCl, pH 4.0. For renaturation, the protein was diluted (1:100) into 10 mM Tris-HCl, pH 7.0, containing 1 mM EDTA and 10 mM DTT, by adding the protein in several pulses to the refolding buffer. Refolding was performed overnight at 4°C. Ammonium sulfate was then added to a final concentration of 2 M, and the resulting

sample was mixed with 10 ml of the Butyl-Sepharose 4 Fast Flow (Pharmacia, FRG). The protein was eluted with 100 mM Tris-HCl, pH 7.2, containing 5 mM DTT, and further purified on Butyl-Sepharose FF (Amersham).

Recombinant human p53 (residues 1-321) was obtained from an *E. coli* BL21(DE3) RIL expression system using the pET-46Ek/LIC vector (Novagen). Cells were grown at 37°C and induced with 0.5 mM IPTG at OD_{600nm} of 0.6. The recombinant protein expression was carried for an additional 12 h at 20°C. The protein was purified under native conditions using Ni-NTA Agarose (Qiagen). Complexes were made by mixing p53 and Hdm2 in a ratio of 1:2. The excess Hdm2 was then removed by gel filtration.

11. Fluorescence polarization binding assays. All fluorescence experiments were performed as described by Czarna et al.[17]. 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 ($Int_{parallel}$) and perpendicular ($Int_{perpedicular}$) to the plane of excitation were measured in 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: $P = (Int_{parallel} - GInt_{perpedicular})/(Int_{parallel} + GInt_{perpedicular})$, 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 at al.[18] (the P4 peptide in Czarna et al.[17]) towards MDM2 and MDMX proteins were determined in the buffer which contained 50 mM NaCl, 10 mM Tris pH 8.0, 1 mM EDTA, 10% DMSO. Each sample contained 10 nM of the fluorescent P4 peptide and MDM2 (the MDM2 concentration used, from 0 to 1 µM and MDMX, from 0 to 10 µM) in a final volume of 50 µl. Competition binding assays were performed using the 10 nM fluorescent P4 peptide, 15 nM MDM2 or 120 nM MDMX. Plates were read at 30 min after mixing all assay components. Binding constant and inhibition curves were fitted using the SigmaPlot (SPSS Science Software).

12. NMR Methods. All NMR spectra were acquired at 300 K on a Bruker DRX 600 MHz spectrometer equipped with a cryoprobe. Typically, NMR samples contained 0.05-0.2 mM protein in 50 mM KH_2PO_4

and 50 mM Na_2HPO_4, pH 7.4., containing 150 mM NaCl and 5 mM DTT. 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;(D'Silva, et al., 2005; Popowicz, et al., 2007) for example, 500 µL of the protein sample, at a concentration of about 0.1 mM, in 10% D_2O and a 20 mM stock solution of nutin-3 (purchased from Cayman Chemical, MI) in DMSO-d_6 were used in all of the experiments. 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-^{15}N-HSQC spectra were recorded using fastHSQC pulse sequence (Mori et al., 1995). The maximum concentration of DMSO at the end of titration experiments was less than 1%.

13 Computational library generation and docking. A database of several hundred scaffolds amenable by MCR chemistry in one or two synthetic transformations served as the underlying chemical space.(Dömling, 2006) Virtual libraries of the size <=1.000 per scaffold and based on 40 different scaffolds (30.000 compounds in total) were generated using REACTOR software including scope and limitations as apparent from the literature (Pirok, et al., 2006). The fragment indole or 4-chlorophenyl was included in each scaffold in the different variable positions. The other positions of each scaffold were complemented by substitutents derived from commercially available starting materials covering a broad physicochemical property space, e.g. aliphatic, aromatic, small, bulky substitutents. All possible stereoisomers to a particular compound were created. The created SMILES libraries were converted into 3D coordinates using OMEGA software. The constrained docking including energy minimization was performed using MOLOC software using the template matching routine.(Gerber, 1998; Gerber and Müller, 1995) The resulting docking models of the virtual MCR molecules were visually inspected and ranked. A molecule was ranked high if besides the ANCHOR residue sitting in the Trp23 site also the Leu26 and Phe19 binding site was occupied by hydrophobic substitutents (electrostatic complementarity), if no sterical clashes with the Hdm2 receptor occurred, if internal strain was low and if the starting materials were commercially available or easy to synthesize. In addition we preferred to use chemistry were we had prior knowledge and experience.(Dömling, 2006) Initially 20 compounds were identified according to the above criteria and synthesized and screened. Of those 11 compounds based on 7 different MCR scaffolds were found to show a K_D <= 60 uM (Table 1, 2). A detailed method

description including virtual library generation, enumeration, conformer generation, ANCHOR placement, energy minimization, scoring and ranking and a web-based tool will be subject to future publication (Camacho and Dömling, in preparation).

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