Activity-Directed Synthesis of Inhibitors of the p53/hDM2 Protein-Protein Interaction

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1. General Experimental

Solvents were removed under reduced pressure using a Büchi rotary evaporator with a Vacuubrand PC2001 Vario diaphragm pump, or under N\textsubscript{2} blowdown at 40 °C. Dry solvents and reagents were purchased from commercial suppliers and used without further purification. Rhodium catalysts were purchased from Sigma-Aldrich and used as supplied. Flash column chromatography was carried out using silica gel 60 (35-70 μm particles) supplied by Merck. Thin-layer chromatography was conducted with Macherey-Nagel Polygram SIL G/UV254 0.2mm silica gel 60 with fluorescent indicator plates.

Analytical LC-MS was performed using a system comprising an Ultimate3000 HPLC instrument with a Brucker Amazon Speed MS detector with electrospray ionisation. The system ran with a positive and negative switching mode and UV diode array detector using a Phenomenex Kinetex C18 (50 mm × 2.1 mm × 2.6 μm) column and gradient elution with two binary solvent systems: MeCN/H\textsubscript{2}O or MeCN/H\textsubscript{2}O plus 0.1% formic acid. Accurate mass spectrometry was performed using electrospray ionisation on a Brucker MaXis Impact spectrometer.

NMR analysis was conducted using a Bruker AV-400 spectrometer (\textsuperscript{1}H = 400 MHz, \textsuperscript{13}C = 100 MHz and \textsuperscript{19}F = 376 MHz C-F decoupled), Bruker AV-500(Cyroprobe) spectrometer (\textsuperscript{1}H = 500 MHz and \textsuperscript{13}C = 125 MHz), JEOL ECA600ii 14.1 T spectrometer (\textsuperscript{1}H = 600 MHz and \textsuperscript{13}C = 150 MHz), 750 MHz Oxford Magnet spectrometer (TCI-Cyroprobe, \textsuperscript{1}H optimized triple resonance NMR ‘inverse’ probe) (\textsuperscript{1}H = 750 MHz and \textsuperscript{15}N = 76 MHz) or a 600 MHz Oxford Magnet spectrometer (QCI-P-Cyroprobe, \textsuperscript{1}H optimized quadruple resonance NMR ‘inverse’ probe) (\textsuperscript{1}H = 600 MHz and \textsuperscript{15}N = 61 MHz) using an internal deuterium lock. Chemical shifts are quoted in parts per million (ppm) and coupling constants are given in Hz. Splitting patterns have been abbreviated as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). NMR data is reported in the format: ppm (number of protons, splitting pattern, coupling constant). Infrared spectra were recorded on a Bruker Alpha ATR FR-IR spectrometer; absorptions are reported in wavenumber (cm\textsuperscript{-1}).

The human homologue of MDM2 is referred to as hDM2 throughout. The p53/hDM2 fluorescence anisotropy assay was assembled and performed as described by Wilson et. al.\textsuperscript{1} hDM2 protein (residues 17 to 125, with L33E mutation; referred to as hDM2\textsubscript{17-125}) and p53\textsubscript{15-31}-fluorescein (Ac-SQETFSDLWKLPPENVC(Flu)-NH\textsubscript{2}) peptide tracer, in which the fluorophore was linked to the C-terminal cysteine thiol through a maleimide, were used for all biological screening.

Total product concentration was used to standardise the effective screening concentrations for the high-throughput screening of reaction mixtures and is defined as the concentration of the limiting reactant in each well before the reaction took place (here, the diazo reactant).
2. Synthesis of diazo compounds

CAUTION: All diazo compounds (excluding those isolated as solid material) described below appear to be volatile at room temperature under reduced pressure. Gradual loss of mass was observed when left under high vacuum. Diazo compounds are potentially explosive on contact and should be treated with caution, although no adverse advents occurred during this study. Compounds D1, D4, D5, D7, D8 and 2-diazo-1-(pyrrolidin-1-yl)ethenone were synthesised as previously reported. Enantiomerically pure compounds (S)-D4 ([α]_D^{20} = −35) and (R)-D4 ([α]_D^{20} = +28) were prepared by the same procedure as racemic D4. Compound D2 was prepared as previously reported. Compound D8 was prepared as previously reported.

2.1 General procedure for the scale-up of ADS hits, A

A crimp vial (10 or 20 mL) was sequentially charged with solutions of Rhodium(II) catalyst in DCM (240 µL, 12.5 mM) and co-substrate in DCM (240 µL, 6.25 M) and stirred. A solution of diazo in DCM (240 µL, 1.25 M) was added and the vial capped. After 24 hours 900 mg of Quadrapure TU resin was added, followed by a further 720 µL DCM. After a further 24 hours the resin was removed by filtration and the solvent evaporated under reduced pressure to yield the crude reaction product.

2.2 General procedure for the scale-up of ADS hits/analogues using a syringe pump, B

A 20 mL vial was charged with Rhodium(II) catalyst (1 mol%) and degassed under N₂ atmosphere, followed by the addition of co-substrate (6.25 M) in DCM. Diazo (1.25 M) in DCM was then added dropwise to the stirred solution over 6 hours using a syringe pump. After 24 hours Quadrapure TU™ resin was then added and the reaction left for a further 24 hours. The resin was then removed by filtration and the solvent removed under reduced pressure to give a crude product.

Cyclobutylmethyl 2-(4-chlorophenyl)-2-diazoacetate, D9

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\text{N,N-diisopropylethylamine (2.9 mL, 17 mmol) was added to a stirred suspension of EDC.HCl (882 mg, 4.6 mmol) and 4-dimethylaminopyridine (51 mg, 0.40 mmol) in DCM (50 mL), followed by the sequential addition of cyclobutane methanol (0.4 mL, 4.6 mmol) and 4-chlorophenyl acetic acid (717 mg, 4.2 mmol). After 20 hours half the solvent was removed under vacuum and the reaction mixture
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washed with 10% w/v aqueous citric acid (2 x 50 mL), brine (1 x 50 mL), 10% v/v aqueous NaHCO₃ (2 x 50 mL), brine (1 x 50 mL) and distilled water (3 x 100 mL). The combined organic layer was then passed through a phase separation frit and concentrated under reduced pressure to give a crude oil. The oil was then dissolved in acetonitrile (42 mL, 0.1 M) and cooled to 0 °C using an ice-bath, 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.9 mL, 1.4 eq) was then added followed by the portion-wise addition of 4-Acetamidobenzenesulfonyl azide (1.1 g, 1.2 eq). After 16 hours, the solvent was removed under reduced pressure to give a crude product that was dissolved in diethyl ether (40 mL) and washed sequentially with 10% w/w citric acid (2 x 10 mL), brine (2 x 10 mL), 10% w/v ammonium chloride (2 x 10 mL) and brine (2 x 10 mL). The organics were passed through a phase separation frit and concentrated under reduced pressure to give a crude material that was purified by flash column chromatography eluting 20:1 Pentane/Et₂O to give the diazo D9 as an orange oil (1.1 g, 99%), Rᵣ 0.46 (20:1 Pentane/Et₂O); δ̃ₜ (500 MHz, Acetone-d₆) 7.57 (2H, d, J 8.7, Ar-2H and -6H), 7.43 (2H, d, J 8.7, Ar-3H and -5H), 4.24 (2H, d, J 6.6, cyclobutylmethyl-4H₂), 2.71 (1H, dt, J 14.9 and 7.3, cyclobutylmethyl-3H₁₂), 2.12 – 2.06 (2H, m, cyclobutylmethyl-1H₁₂) and 1.97 – 1.81 (4H, m, cyclobutylmethyl-2H₂ and -2''H₂). δ̃ₖ (125 MHz) 165.3, 131.6, 129.7, 126.2, 126.0, 69.1, 63.7, 35.1, 25.1 and 18.9. IR νmax (CH₂Cl₂ film)/cm⁻¹ 2084 (diazo), 1682 and 1490. HRMS (ESI): C₁₃H₁₃ClN₂O₂ requires [2M+H -N₂]⁺, calculated 501.1348, found 501.1343.

N-[3-(4-Chlorophenyl)-1,2-oxazol-5-yl]-2-diazoacetamide, D10

2-[(4-Methylbenzenesulfonamido)imino]acetic acid (823 mg, 3.4 mmol) was suspended in Toluene (12.5 mL) and stirred. Thionyl chloride (0.5 mL, 6.8 mmol) was added and the reaction heated to 90 °C for 3 hours, then the solvent removed under vacuum to give an orange solid. The solid was then dissolved in DCM (20 mL) and cooled to 0 °C using an ice-bath. A solution of 3-(4-chlorophenyl)isoxazole-5-amine (668 mg, 3.4 mmol) and N,N-dimethylaniline (0.5 mL, 3.7 mmol) in DCM (5 mL) was subsequently added drop-wise to the stirred solution over 5 minutes. After 1 hour triethylamine (2.3 mL, 17 mmol) was added and the reaction allowed to warm to room temperature overnight. The organics were then washed sequentially with 10% w/w citric acid (2 x 20 mL), brine (2 x 20 mL), 10% w/v ammonium chloride (2 x 20 mL) and brine (2 x 20 mL), passed through a phase
separation frit and concentrated under reduced pressure to give a crude material that was purified by flash column chromatography eluting 9:1 DCM/Et₂O to afford diazo **D10** as a bright orange oil (165 mg, 19%), Rf 0.09 (9:1 DCM/Et₂O); δ_H (500 MHz, Acetonitrile-d₉) 9.14 (1H, diazacetamide-NH), 7.82 (2H, d, J 8.7, phenyl-3H and -5H), 7.49 (2H, d, J 8.7, phenyl-2H and -6H), 6.65 (1H, s, oxazolyl-4H) and 5.31 (1H, s, diazacetamide-2H). δ_C (125 MHz) 163.3, 163.2, 163.1, 136.5, 130.0, 129.2, 129.1, 86.3 and 49.9. IR ν_max (CH₂Cl₂ film)/cm⁻¹ 2994, 2113 (diazo), 1678 and 1364. HRMS (ESI): C₃₃H₂₆ClN₄O₂ requires [M+H]⁺, calculated 563.0336, found 563.0323.

### 2.3 Synthesis of Co-Substrates

**2-Cyclopropyl-1-(1,2,3,6-tetrahydropyridin-1-yl)ethan-1-one, S5**

![Cyclopropyl-1-(1,2,3,6-tetrahydropyridin-1-yl)ethan-1-one](image)

Cyclopropylacetic acid (0.50 g, 5.0 mmol) and carbonylimidazole (0.81 g, 5.0 mmol) were dissolved in THF (20 mL) and stirred for 30 minutes, followed by dropwise addition of 1,2,3,6-tetrahydropyridine (0.42 g, 5.0 mmol) in THF (4 mL). After 16 hours 1M HCl (10 mL) was added and the mixture stirred vigorously for 10 minutes. The solvent was then reduced to a minimum under vacuum and partitioned with DCM (30 mL). The organics were washed sequentially with 20% v/v NaHCO₃ (1 x 20 mL) and brine (1 x 20 mL), passed through a phase separation filter, and dried under vacuum to afford **amide S5** as a colourless oil (0.71 g, 86%). δ_H (500 MHz, Chloroform-d) 5.89 – 5.77 (1H, m, *THP-5H), 5.70 – 5.61 (1H, m, THP-4H), 4.05 – 3.90 (2H, m, THP-6H₂), 3.67 (1H, t, J 5.8, THP-2H₂), 3.49 (1H, t, J 5.8, THP-2H₂), 2.26 (2H, dd, J 6.8 and 12.0, ethanone-2H₂), 2.18 – 2.11 (2H, m, THP-3H₂), 1.07 – 1.01 (1H, m, cyclopropyl-1H), 0.56 – 0.51 (2H, m, cyclopropyl-2H₂ and 2’H₂) and 0.18 – 0.13 (2H, m, cyclopropyl-2H₂ and 2’H₂). δ_C (125 MHz) 171.5 (rot-A), 171.5 (rot-B), 126.8 (rot-A), 125.1 (rot-B), 124.6 (rot-A), 123.4 (rot-B), 45.1 (rot-A), 42.7 (rot-A), 42.0 (rot-B), 39.1 (rot-B), 38.3 (rot-B), 38.3 (rot-B), 26.0 (rot-A), 25.0 (rot-B), 7.4 (rot-A), 7.2 (rot-B), 4.6 (rot-A), 4.5 (rot-B). IR ν_max (CH₂Cl₂ film)/cm⁻¹ 3079, 2918, 1622 and 1431. HRMS (ESI): C₁₀H₁₅NO requires [M+H]⁺, calculated 166.1231, found 166.1226. *THP = tetrahydropyridin-1-yl.
3. Synthesis of hDM2 Ligands

2-[(5-Chloro-2,3-dihydro-1H-inden-1-yl)oxy]-1-(3-phenylpyrrolidin-1-yl)ethenone, P2a and P2b

According to general procedure A, Rh₂piv₄ (2.8 mg, 4.6 µmol), 2-diazo-1-(3-phenylpyrrolidin-1-yl)ethan-1-one (100 mg, 0.46 mmol) and 5-chloro-2,3-dihydro-1H-inden-1-ol (391 mg, 2.32 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et₂O to afford ether P2 as a colourless oil (127 mg, 78%), R₆ 0.18 (100% Et₂O); δ_H (600 MHz, d⁶-DMSO, 1:1 mixture of diastereomers; 1:1 mixture of rotamers): 7.48 – 7.45 (1H, m, Ar-4H), 7.35 – 7.23 (7H, m, Ar), 4.97 – 4.93 (1H, m, inden-1-yloxy-1H), 4.21 – 4.14 (2H, m, ethenone-2H), 3.89 – 3.83 (1H, m, pyrrolidinyl-3H), 3.66 – 3.59 (1H, m, pyrrolidinyl-2Hₐ), 3.50 – 3.39 (1H, m, pyrrolidinyl-2Hₐ), 3.37 – 3.21 (2H, m, pyrrolidinyl-5H), 3.00 – 2.74 (2H, m, pyrrolidinyl-4H), 2.33 – 2.219 (2H, m, 2,3-dihydroindenyl-3H) and 2.03 – 1.87 (2H, m, 2,3-dihydroindenyl-2H). δ_C (150 MHz, d⁶-DMSO): 167.3 (broad s, major), 167.3 (rot-A, minor), 167.2 (rot-B, minor), 146.3 (broad s, major), 146.3 (broad s, minor), 141.6 (broad s, major), 141.6 (broad s, minor), 141.2 (broad s, major), 141.1 (broad s, minor), 132.9 (broad s, major+minor), 128.5 (broad s, major+minor), 127.1 (major), 127.0 (minor), 126.8 (major), 126.8 (minor), 126.7 (broad s, major), 126.6 (broad s, minor), 126.2 (broad s, major), 126.2 (broad s, minor), 124.7 (broad s, major+minor), 82.0 (broad s, major), 81.9 (broad s, minor), 67.7 (rot-A, major), 67.7 (rot-B, major), 67.6 (broad s, minor), 51.6 (broad s, major), 51.2 (broad s, minor), 45.4 (broad s, major), 45.0 (broad s, minor), 43.7 (broad s, major), 41.6 (broad s, minor), 33.0 (broad s, major), 33.0 (broad s, minor), 32.0 (broad s, major), 32.0 (broad s, minor), 30.8 (broad s, major) and 30.0 (broad s, minor). HRMS (ESI): C₂₁H₂₂ClNO₂ requires [M+H]⁺, calculated 356.1417, found 356.1425. The diastereomeric ratio was determined by analysis of the ¹³C chemical shift for the inden-1-yloxy-C1 carbon.

2-[(1R)-5-Chloro-2,3-dihydro-1H-inden-1-yl]oxy]-1-[(3S)-3-phenylpyrrolidin-1-yl]ethenone, P2a

According to general procedure A, Rh₂piv₄ (0.6 mg, 1.0 µmol), 2-diazo-1-((S)-3-phenylpyrrolidin-1-yl)ethan-1-one (21.5 mg, 0.1 mmol) and (1R)-5-chloro-2,3-dihydro-1H-inden-1-ol (84.0 mg, 0.5 mmol)
gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et₂O to afford ether P2a as a colourless oil (24 mg, 67%), \(R_f\) 0.48 (100% Et₂O); \(\delta_\text{H}\) (500 MHz, Chloroform-d): 7.32 – 7.09 (16H, m, Ar, rot-A and rot-B), 4.93 (1H, ddd, \(J\) 17.6, 6.4 and 3.7, inden-1-yloxy-1H, rot-A and rot-B), 4.11 – 4.09 (2H, m, ethenone-2H\(_a\), rot-A and rot-B), 3.98 – 3.93 (1H, m, pyrrolidinyl-2H\(_a\), rot-A), 3.80 – 3.72 (2H, m, pyrrolidinyl-3H, rot-A and rot-B), 3.65 – 3.60 (1H, m, pyrrolidinyl-2H\(_a\), rot-B), 3.50 – 3.25 (6H, m, pyrrolidinyl-2H\(_a\) and -5H\(_a\), rot-A and rot-B), 3.01 – 2.96 (2H, m, 2,3-dihydroindenyl-3H\(_a\), rot-A and rot-B), 2.74 – 2.71 (2H, m, 2,3-dihydroindenyl-3H\(_b\), rot-A and rot-B), 2.33 – 2.20 (4H, m, pyrrolidinyl-4H\(_b\), rot-A and rot-B), 2.13 – 2.05 (2H, m, 2,3-dihydroindenyl-2H\(_a\), rot-A and rot-B) and 2.00 – 1.88 (2H, m, 2,3-dihydroindenyl-2H\(_b\), rot-A and rot-B). \(\delta_\text{C}\) (125 MHz, Chloroform-d): 168.3 (rot A), 168.2 (rot B), 146.4 (rot A), 146.4 (rot B), 141.0 (rot-A and rot-B), 140.8 (rot A), 140.6 (rot B), 134.6 (rot-A and rot-B), 128.9 (rot A), 128.8 (rot B), 127.2 (rot A), 127.1 (rot B), 126.8 (rot A), 126.7 (rot B), 126.6 (rot A), 126.6 (rot B), 125.3 (rot-A and rot-B), 83.1 (rot-A and rot-B), 68.6 (rot A), 68.5 (rot B), 52.6 (rot A), 52.1 (rot B), 46.1 (rot A), 46.0 (rot B), 44.7 (rot-A and rot-B), 42.3 (rot-A and rot-B), 33.8 (rot-A and rot-B), 32.6 (rot-A and rot-B), 31.3 (rot-A and rot-B) and 30.3 (rot-A and rot-B). HRMS (ESI): \(\text{C}_{21}\text{H}_{22}\text{ClNO}_2\) requires [M+Na]⁺, calculated 378.1237, found 378.1237.

2-\{[(1S)-5-Chloro-2,3-dihydro-1H-inden-1-yl]oxy\}-1-\{[(3R)-3-phenylpyrrolidin-1-yl]ethenone, ent-P2a\}

According to general procedure A, Rh\(_2\)piv\(_4\) (0.6 mg, 1.0 \(\mu\)mol), 2-diazo-1-\{(R)-3-phenylpyrrolidin-1-yl\}ethan-1-one (21.5 mg, 0.1 mmol) and (1S)-5-chloro-2,3-dihydro-1H-inden-1-ol (84.0 mg, 0.5 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et₂O to afford ether ent-P2a as a colourless oil (19 mg, 54%), \(R_f\) 0.32 (100% Et₂O); spectroscopically identical to compound P2a.
2-[(1S)-5-Chloro-2,3-dihydro-1H-inden-1-yl]oxy]-1-[(3S)-3-phenylpyrrolidin-1-yl]ethanone, P2b

According to general procedure A, Rh₂piv₄ (0.6 mg, 1.0 µmol), 2-diazo-1-((S)-3-phenylpyrrolidin-1-yl)ethan-1-one (21.5 mg, 0.1 mmol) and (1S)-5-chloro-2,3-dihydro-1H-inden-1-ol (84.0 mg, 0.5 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et₂O to afford ether P2b as a colourless oil (28 mg, 79%), Rf 0.47 (100% Et₂O); δ H (500 MHz, Chloroform-d): 7.33 – 7.08 (16H, m, rot-A and rot-B), 4.94 (2H, ddd, J 12.4, 6.5 and 3.7, inden-1-yloxy-1H, rot-A and rot-B), 4.14 – 4.09 (4H, m, ethenone-2H₂, rot-A and rot-B), 3.97 – 3.93 (1H, m, pyrrolidinyl-2H₉, rot-A), 3.83 – 3.72 (2H, m, pyrrolidinyl-3H, rot-A and rot-B), 3.65 – 3.61 (1H, m, pyrrolidinyl-2H₉, rot-B), 3.48 – 3.24 (6H, m, pyrrolidinyl-2H₉ and -5H₂, rot-A and rot-B), 3.02 – 2.95 (2H, m, 2,3-dihydroindenyl-3H₉, rot-A and rot-B), 2.76 – 2.68 (2H, m, 2,3-dihydroindenyl-3H₉, rot-A and rot-B), 2.32 – 2.18 (4H, m, pyrrolidinyl-4H₂, rot-A and rot-B), 2.11 – 2.06 (2H, m, 2,3-dihydroindenyl-2H₂, rot-A and rot-B) and 2.01 – 1.84 (2H, m, 2,3-dihydroindenyl-2H₂, rot-A and rot-B). δ C (125 MHz, Chloroform-d): 168.3 (rot A), 168.3 (rot B), 146.4 (rot-A and rot-B), 141.0 (rot A), 140.7 (rot-A and rot-B), 140.6 (rot B), 134.6 (rot-A and rot-B), 128.9 (rot A), 128.8 (rot B), 127.2 (rot-A and rot-B), 127.1 (rot A), 127.1 (rot B), 126.8 (rot A), 126.7 (rot B), 126.6 (rot A), 126.6 (rot B), 125.3 (rot A), 125.3 (rot B), 83.2 (rot A), 83.1 (rot B), 68.7 (rot A), 68.5 (rot B), 52.6 (rot A), 52.1 (rot B), 46.1 (rot A), 46.0 (rot B), 44.7 (rot-A and rot-B), 42.3 (rot-A and rot-B), 33.7 (rot A), 32.6 (rot A and rot-B), 31.3 (rot B), 30.3 (rot-A) and 30.3 (rot B). HRMS (ESI): C₂₂H₂₂ClNO₂ requires [M+Na]⁺, calculated 378.1237, found 378.1230.

2-[(1R)-5-Chloro-2,3-dihydro-1H-inden-1-yl]oxy]-1-[(3R)-3-phenylpyrrolidin-1-yl]ethanone, ent-P2b

According to general procedure A, Rh₂piv₄ (0.6 mg, 1.0 µmol), 2-diazo-1-((R)-3-phenylpyrrolidin-1-yl)ethan-1-one (21.5 mg, 0.1 mmol) and (1R)-5-chloro-2,3-dihydro-1H-inden-1-ol (84.0 mg, 0.5 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et₂O
to afford ether ent-P2b as a colourless oil (18 mg, 51%), Rf 0.59 (100% Et2O); spectroscopically identical to compound P2b.

2-(Cyclopentyloxy)-1-(3-phenylpyrrolidin-1-yl)ethenone, SI1

![Structure of 2-(Cyclopentyloxy)-1-(3-phenylpyrrolidin-1-yl)ethenone]

According to general procedure B, Rh2(piv)4 (1.4 mg, 2.5 µmol), 2-diazo-1-(3-phenylpyrrolidin-1-yl)ethan-1-one (50 mg, 0.25 mmol) and cyclopentanol (105 µL, 1.2 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et2O to afford ether 10 as a colourless oil (34 mg, 50%), Rf 0.29 (100% Et2O); δH (500 MHz, CDCl3): 7.29 – 7.16 (10H, m, Ar, rot-A and rot-B), 4.00 (4H, d, J 11.4, ethenone-2H), 3.98 – 3.86 (4H, m, cyclopentyloxy-1H and pyrrolidinyl-2H), 3.74 (2H, ddd, J 10.9, 8.2 and 2.9, pyrrolidinyl-2H), 3.68 (1H, ddd, J 10.9, 8.2 and 2.9, pyrrolidinyl-2H), 3.68 – 3.54 (6H, m, pyrrolidinyl-3H and -5H), 2.33 – 2.19 (2H, m, pyrrolidinyl-4H), 2.04 – 1.87 (2H, m, pyrrolidinyl-4H), 1.67 – 1.62 (16H, m, cyclopentyloxy-2H and -3H), 1.62 (1H, d, J 8.0, indenyl-oxy-7H), 1.62 (1H, br. s, indenyl-oxy-4H), 1.62 – 1.58 (1H, m, indenyl-oxy-6H), 4.99 (1H, dd, J 6.5 and 3.7, indenyl-oxy-1H), 4.14 (2H, m, ethenone-1H), 3.49 (2H, app. t, J 6.9, pyrrolidinyl-2H), 3.45 – 3.38 (2H, H2, H4).

HRMS (ESI): C17H23NO2 requires [M+H]+, calculated 274.1807, found 274.1803.

2-[(5-Chloro-2,3-dihydro-1H-inden-1-yl)oxy]-1-(pyrrolidin-1-yl)ethenone, SI2

![Structure of 2-[(5-Chloro-2,3-dihydro-1H-inden-1-yl)oxy]-1-(pyrrolidin-1-yl)ethenone]

According to general procedure B, Rh2(piv)4 (2.2 mg, 3.6 µmol), 2-diazo-1-(pyrrolidin-1-yl)ethenone (50 mg, 0.36 mmol) and 5-chloro-2,3-dihydro-1H-inden-1-ol (120 mg, 0.72 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 100% Et2O to afford ether 11 as a colourless oil (51 mg, 50%), Rf 0.09 (100% Et2O); δH (500 MHz, CDCl3): 7.37 (1H, d, J 8.0, indenyl-oxy-7H), 7.22 (1H, broad s, indenyl-oxy-4H), 7.18 – 7.16 (1H, m, indenyl-oxy-6H), 4.99 (1H, dd, J 6.5 and 3.7, indenyl-oxy-1H), 4.14 (2H, m, ethenone-1H), 3.49 (2H, app. t, J 6.9, pyrrolidinyl-2H), 3.45 – 3.38 (2H, H2, H4).
m, pyrrolidinyl-2H), 3.09 – 3.03 (1H, m, indenyloxy-3H), 2.81 – 2.76 (1H, m, indenyloxy-3H), 2.35 (1H, ddt, J 13.0, 8.5 and 6.4, indenyloxy-2H), 2.15 (1H, dddd, J 13.3, 8.4, 4.8 and 3.8, indenyloxy-2H), 1.95 – 1.90 (2H, m, pyrrolidinyl-3H) and 1.86 – 1.81 (2H, m, pyrrolidinyl-3H). δ_C (125 MHz, CDCl_3) 168.3, 146.4, 140.7, 134.5, 126.7, 126.6, 125.2, 83.0, 68.5, 46.2 (rot-A), 46.1 (rot-B), 32.5, 30.3, 26.3 (rot-A) and 24.0 (rot-B). HRMS (ESI): C_{15}H_{16}ClNO_2 requires [M+Na]^+, calculated 302.0924, found 302.0926.

2,2,2-Trifluoroethyl 2-(6-chloro-1H-indol-3-yl)-2-(4-chlorophenyl)acetate, P1

According to general procedure B, Rh_2pfb_4 (3.8 mg, 3.6 µmol), 2,2,2-trifluoroethyl 2-(4-chlorophenyl)-2-diazoacetate (100 mg, 0.36 mmol) and 6-chloroindole (272 mg, 1.8 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 pentane/Et_2O to afford indole P1 as a colourless oil (20 mg, 14%), R_f 0.19 (3:1 Pentane/Et_2O); δ_H (500 MHz, CDCl_3): 8.10 (1H, s, 6-chloroindolyl-NH), 7.30 (1H, d, J 1.8, 6-chloroindolyl-4H), 7.28 – 7.24 (4H, m, 4-chlorophenyl-2H and -3H), 7.21 (1H, d, J 8.5, 6-chloroindolyl-2H), 7.14 (1H, dd, J 2.5 and 0.8, 6-chloroindolyl-7H), 6.99 (1H, dd, J 8.5 and 1.8, 6-chloroindolyl-5H), 5.24 (1H, s, acetate-2H) and 4.49 (2H, qq, J 12.7 and 8.4, trifluoroethyl-1H). δ_C (125 MHz, CDCl_3): 170.9, 136.8, 135.8, 133.9, 129.8, 129.1, 128.8, 124.9, 124.0, 121.8, 121.0, 119.9, 112.5, 111.5, 61.0 (q, J_{C-F} 36.7), 47.9. HRMS (ESI): C_{19}H_{12}ClF_3NO_2 requires [M+H]^+, calculated 402.0275, found 402.0270.
According to general procedure A, Rh₂pfb₄ (2.8 mg, 2.6 µmol), 6-chloro-3-diazo-1H-indol-2-one (50 mg, 0.26 mmol) and 6-chloroindole (254 mg, 1.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 DCM/Et₂O to afford oxindole P6 as a colourless oil (44 mg, 53%), Rf 0.17 (9:1 DCM/Et₂O); δᵢ (500 MHz, CDCl₃): 8.54 (1H, broad s, biindol-2-one-NH), 8.29 (1H, broad s, biindol-NH), 7.31 (1H, d, J 1.8 Hz, biindol-4H), 7.14 (1H, d, J 8.4, biindol-2H), 7.06 – 7.04 (2H, m, biindol-2-one-4H and -7H), 6.99 (2H, td, J 8.4 and 1.8, biindol-2-one-5H and biindol-5H), 6.94 (1H, d, J 1.8, biindol-7H) and 4.83 (1H, s, biindol-2-one-3H). δₓ (125 MHz, CDCl₃): 178.5, 142.4, 137.1, 134.2, 128.7, 127.8, 126.1, 124.7, 122.9, 121.0, 111.5, 110.7, 110.4 and 44.5. HRMS (ESI): C₁₆H₁₀Cl₂N₂O requires [M+H]⁺, calculated 317.0248, found 317.0226.

According to general procedure B, Rh₂pfb₄ (2.8 mg, 2.6 µmol), 6-chloro-3-diazo-1H-indol-2-one (50 mg, 0.26 mmol) and 5-chloroindole (254 mg, 1.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 DCM/Et₂O to afford oxindole 12 as a colourless oil (31.2 mg, 38%), Rf 0.17 (9:1 DCM/Et₂O); δᵢ (500 MHz, DMSO-d₆): 11.27 (1H, s, biindol-2-one-NH), 10.71 (1H, s, biindol-NH), 7.39 (1H, dd, J 8.6 and 0.5, biindol-2-one-4H), 7.31 (1H, d, J 2.5, biindol-4H), 7.11 – 7.04 (3H, m, biindol-2-one-5H, biindol-2H and -7H), 6.98 – 6.95 (2H, m, biindol-2-one-7H and biindol-7H) and 4.98 (1H, s, biindol-2-one-3H). δₓ (125 MHz, DMSO-d₆): 177.4, 144.0, 134.9, 132.1, 128.9, 127.1, 126.2, 125.9, 123.3, 121.3, 121.2, 117.6, 113.3, 109.4, 109.4 and 43.6. HRMS (ESI): C₁₆H₁₀Cl₂N₂O requires [M+H]⁺, calculated 317.0248, found 317.0237.
(1S*,3R*)-6’-Chloro-3-(4-chlorophenyl)-1’H-spiro[cyclopropane-1,3’-indol]-2’-one, P5

According to general procedure A, Rh₂piv₄ (1.6 mg, 2.6 µmol), 6-chloro-3-diazo-1H-indol-2-one (50 mg, 0.26 mmol) and 4-chlorostyrene (156 µL, 1.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 DCM/Et₂O to afford oxindole P5 as a colourless oil (46 mg, 58%), R₇ 0.17 (9:1 DCM/Et₂O); δ_H (500 MHz, CDCl₃): 8.57 (1H, broad s, indolone-NH), 7.28 (2H, d, J 8.2, 4-chlorophenyl-3H₂), 7.11 (2H, d, J 8.2, 4-chlorophenyl-2H₂), 6.96 (1H, d, J 1.8, indol-2-one-7H), 6.69 (1H, dd, J 8.1 and 1.9, indol-2-one-5H), 5.84 (1H, d, J 8.1, indol-2-one-4H), 3.28 (1H, appt., J 8.6, cyclopropane-1H), 2.23 (1H, dd, J 9.2 and 4.8, cyclopropane-2Hₐ), 1.97 (1H, dd, J 8.0 and 4.8, cyclopropane-2Hₐ). δ_C (125 MHz, CDCl₃): 178.3, 142.0, 133.7, 133.3, 132.8, 131.4, 128.9, 126.0, 121.9, 121.8, 110.5, 35.7, 33.5 and 22.9. HRMS (ESI): C₁₆H₁₁Cl₂NO requires [M+H]⁺, calculated 304.0296, found 304.0286.

(1S*,3R*)-6’-Chloro-3-(3-chlorophenyl)-1’H-spiro[cyclopropane-1,3’-indol]-2’-one, SI4

According to general procedure B, Rh₂piv₄ (1.6 mg, 2.6 µmol), 6-chloro-3-diazo-1H-indol-2-one (50 mg, 0.26 mmol) and 3-chlorostyrene (165 µL, 1.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 DCM/Et₂O to afford oxindole 13 as a colourless oil (39 mg, 49%), R₇ 0.38 (9:1 DCM/Et₂O); δ_H (500 MHz, CDCl₃): 8.42 (1H, br s, indol-2-one-NH), 7.27 – 7.22 (3H, m, 3-chlorophenyl-4H, -5H and -6H), 7.04 (1H, broad dd, J 7.3 and 0.6, 3-chlorophenyl-2H), 6.96 (1H, d, J 1.8, indol-2-one-7H), 6.69 (1H, dd, J 8.1 and 1.9, indol-2-one-5H), 5.87 (1H, d, J 8.1, indol-2-one-4H), 3.29 (1H, apppt., J 8.6, cyclopropane-1H), 2.23 (1H, dd, J 9.2 and 4.8, cyclopropane-2Hₐ) and 1.99 (1H, dd, J 8.0 and 4.8, cyclopropane-2Hₐ). δ_C (125 MHz, CDCl₃): 178.0, 142.0, 136.9, 134.6, 132.9,
According to general procedure A, Rh₂piv₄ (1.9 mg, 3.0 µmol) and N,2-bis(4-chlorophenyl)-2-diazo-N-methylacetamide (100 mg, 0.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography initially eluting 100% DCM, which gave a mixture of products, and the oil was re-purified eluting 8:2 pentane/EtOAc to afford oxindole P₃ as a colourless oil (3.5 mg, 4%), Rₜ 0.15 (8:2 pentane/EtOAc); δₕ (500 MHz, CDCl₃): 7.33 – 7.31 (3H, m, 4-chlorophenyl-3H₂ and indol-2-one-4H), 7.14 – 7.11 (3H, m, 4-chlorophenyl-2H₂ and indol-2-one-5H), 6.82 (1H, d, J 8.3, indol-2-one-4H), 4.57 (1H, s, indol-2-one-3H) and 3.24 (3H, s, indol-2-one-NCH₃). δₜ (125 MHz, CDCl₃): 175.2, 143.2, 134.4, 134.0, 130.0, 129.9, 129.3, 128.8, 128.4, 109.4, 51.5 and 26.8. HRMS (ESI): C₁₅H₁₁Cl₂NO requires [M+H]^+, calculated 292.029, found 292.0279.

N,2-Bis(4-chlorophenyl)-N-methyl-2-oxoacetamide, P₄

According to general procedure A, Rh₂piv₄ (1.9 mg, 3.0 µmol) and N,2-bis(4-chlorophenyl)-2-diazo-N-methylacetamide (100 mg, 0.3 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography initially eluting 100% DCM, which gave a mixture of products, and the oil was re-purified eluting 8:2 pentane/EtOAc to afford oxoacetamide P₄ as a colourless oil (3.6 mg, 4%), Rₜ 0.38 (8:2 pentane/EtOAc); δₕ (500 MHz, CDCl₃): 7.81 (2H, d, J 8.4, acetamide Ar-3H), 7.44 (2H, d, J 8.4, acetamide Ar-2H), 7.24 (2H, d, J 8.5, oxo Ar-3H), 7.06 (2H, d, J 8.5, oxo Ar-2H) and 3.45 (3H, s, oxoacetamide-NCH₃). δₜ (125 MHz, CDCl₃): 189.3, 166.6, 141.3, 139.8, 134.3, 131.9, 130.9, 130.0,
129.5, 128.2 and 36.5. HRMS (ESI): C_{13}H_{14}ClNO_{2} requires [M+Na]^+, calculated 330.0064, found 330.0059.

3-(Ethoxymethyl)-1'H-spiro[cyclopropane-1,3'-indol]-2'-one, P7

According to general procedure A, Rh_{2}piv_{4} (1.3 mg, 2.1 µmol), 6-chloro-3-diazo-1H-indol-2-one (40 mg, 0.21 mmol) and allyl methyl ether (113 µL, 1.0 mmol) gave an orange oil. The crude oil was then purified by flash column chromatography eluting 9:1 DCM/Et_{2}O to afford oxindole P7 as a colourless oil (7.3 mg, 14%), R_{f} 0.08 (9:1 DCM/Et_{2}O); δ_{H} (500 MHz, CDCl_{3}): 8.31 (1H, s, indol-2-one-NH), 6.98 (1H, dd, J 8.0 and 1.9, indol-2-one-5H), 6.94 (1H, d, J 1.5, indol-2-one-7H), 6.90 (1H, d, J 8.0, indol-2-one-4H), 3.77 (1H, dd, J 11.2 and 5.5, methyl-H_{a}), 3.64 (1H, dd, J 11.2 and 7.6, methyl-H_{b}), 3.45 (2H, q, J 7.0, ethoxy-1H_{2}), 2.24 (1H, dtd, J 13.1, 7.6 and 5.5, cyclopropane-3H), 1.95 (1H, dd, J 9.4 and 4.5, cyclopropane-2H_{a}), 1.57 (1H, dd, J 7.8 and 4.5, cyclopropane-2H_{b}) and 1.16 (3H, t, J 7.0, ethoxy-2H_{3}). δ_{C} (125 MHz, CDCl_{3}): 178.3, 142.3, 132.7, 126.9, 122.2, 121.8, 110.6, 67.4, 66.3, 32.0, 31.6, 22.0 and 15.2. HRMS (ESI): C_{18}H_{34}ClNO_{2} requires [M+H]^+, calculated 252.0791, found 252.0777.
4. Implementation of high-throughput chemistry for Activity-Directed Synthesis Reaction Arrays

Activity-Directed Synthesis reactions were carried out in 0.75 mL shell vials (Chemglass CV-2100-0830) equipped with a teflon-coated stir bar (Biotage 0.2-0.5 mL magnetic stir bar #355545) and sealed using either a Freeslate 96-well reaction block or a Sigma-Aldrich Katalysis 24-well reaction block (Z742107 Aldrich). Prior to the assembly of each reaction array the following stock solutions were made: diazo reaction solvent (1.25 M); catalyst in THF (25 mM); and co-substrate in DCM (6.25 M). Each reaction vial was charged with catalyst stock (8 µL) and the solvent allowed to evaporate to dryness, then DCM (84 µL) was added and the reaction block placed on a magnetic stirring plate.

Each reaction vial was then sequentially charged with co-substrate stock (8 µL) and diazo stock (8 µL), then the plate sealed using a Teflon film and stirred. After 24 hours Quadrapure TU resin (30 mg) was added to each vial and left overnight to scavenge the catalyst. The solvent was then evaporated under a stream of nitrogen gas and the crude material dissolved in molecular biology grade DMSO (200 µL) to create a biological screening master stock (50 mM total product concentration) that was passed through a 96-well filter plate (Agilent Technologies: #200933-100) and stored at –20 °C.

Example 96-well reaction plate layout:

|   | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | Catalyst   |
|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------------|
| A | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D1 Control | Rh₃Piv₄ Control | PIV |
| B | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D2 Control | BLANK | PIV |
| C | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D3 Control | BLANK | PIV |
| D | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D4 Control | BLANK | PIV |
| E | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D1 Control | Rh₃Pfb₃ Control | PFB |
| F | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D2 Control | BLANK | PFB |
| G | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D3 Control | BLANK | PFB |
| H | S1  | S2  | S3  | S4  | S5  | S6  | S7  | S8  | S9  | S10  | D4 Control | BLANK | PFB |

Example 384-well biological assay plate layout:
5. Fluorescence Anisotropy Assay for the inhibition of the p53/hDM2 protein-protein interaction

5.1 hDM2 protein expression

The pet28a His\textsubscript{10} – hDM2 (17-125) L33E construct\textsuperscript{1} was over-expressed in the *E.coli* strain Rosetta 2. An overnight starter culture (10 mL) was used to inoculate 2xYT medium (1 L) containing Kanamycin (50 µg/ml). Cultures were grown at 37 °C until the optical density of the cell suspension reached OD\textsubscript{600} = 0.6 – 0.8, then the temperature was switched to 18 °C and protein expression induced by the addition of IPTG (1 mM). Induced cultures were grown at 18 °C overnight before harvesting by centrifugation for 10 minutes at 8655 xg. Cells were resuspended in lysis buffer (20 mM TRIS pH 8.0, 500 mM NaCl, 15 mM imidazole) and lysed by sonication in the presence of 10 µL of 1 U/ml\textsuperscript{1} DNase per liter of over-expression culture, protease inhibitor cocktail tablet (Roche) and lysozyme. The cell lysate was cleared by centrifugation (Sorvall SS34 rotor, 17,000 rpm, 45 min, 8 °C) and the supernatant was filtered (0.22 µm syringe filter) before loaded onto a 5 ml HisTrap that had previously been equilibrated with lysis buffer. The HisTrap was washed with 10 column volumes (CV) of a wash buffer containing 20 mM TRIS pH 8.0, 500 mM NaCl and 15 mM imidazole, followed by 6 CV two further wash buffers containing 20 mM TRIS pH 8.0, 500 mM NaCl and 50 mM imidazole, and 6 CV 20 mM TRIS pH 8.0, 500 mM NaCl and 100 mM imidazole. The His-hDM2 fusion protein was then eluted from the HisTrap with an elution buffer containing 20 mM TRIS pH 8.0, 500 mM NaCl and 300 mM imidazole. The His-hDM2 fusion protein was dialysed overnight at 4 °C against 20 mM TRIS pH 8.0, 250 mM NaCl in the presence of TEV protease to remove the tag. To remove any uncleaved hDM2, the cleaved tag and the protease, the sample was reapplied to a HisTrap in 20 mM TRIS pH 8.0, 250 mM NaCl and the flow through containing the cleaved hDM2 was collected then concentrated (Amicon Ultra centrifugal filter, MWCO 10,000) to approximately 10 ml. The sample was then filtered before being loaded onto a Superdex 75 column (GE healthcare) equilibrated with 20 mM TRIS pH 8.0, 250 mM NaCl, 0.5 mM DTT, 2.5% Glycerol. The purified protein was concentrated and stored at −80 °C.

The quality and purity of the preparation was assessed by mass spec and circular dichroism spectroscopy. The activity of the protein was verified by testing its binding to fluorescently labelled p53 peptide in a fluorescence anisotropy assay (Figure S1). For \textsuperscript{15}N labeled protein the expression was carried out using the same method but using M9 minimal media supplemented with \textsuperscript{15}NH\textsubscript{4}Cl as nitrogen source.
5.2 Fluorescence anisotropy assays

The fluorescein-labelled p53\textsubscript{15-31 \text{Flu}} transactivation domain peptide (Ac-SQETFSDLWKLPPENVC(Flu)-NH\textsubscript{2}) was purchased from Peptide Synthetics. The assay was carried out using Perkin-Elmer 384-well Opti-plate assay plates (6007270). Fluorescence anisotropy assays were performed in a buffer containing 40 mM phosphate pH 7.5, 200 mM NaCl and 0.02 mg/mL bovine serum albumin (BSA). Results were collected using a Perkin-Elmer Envision 2103 Multilabel Reader using a 431 nm mirror, 480(104) nm excitation filter, and 535(208) and 535(209) nm emission filters after 2.5 or 24 hours of incubation at room temperature. Test well anisotropy values were then calculated using the blank corrected S and P channel values using the following formula:

Eq. 1: \[ \text{Intensity} = (2 \times P_{\text{corrected}} \times G \text{ factor}) + S_{\text{corrected}} \]

Eq. 2: \[ \text{Anisotropy} = \frac{S_{\text{corrected}} - G \times P_{\text{corrected}}}{\text{Intensity}} \]

The fraction of bound tracer was calculated using the following formula:

Eq. 3: \[ \text{Fraction Bound} = \frac{(r-r_{\text{min}})}{(\lambda(r-r_{\text{max}})-(r-r_{\text{min}}))} \]

Where \( \lambda \) is the intensity of bound/unbound tracer (\( \lambda = I_{\text{bound}}/I_{\text{unbound}} \)) and \( r \) is anisotropy.

The fraction of ligand bound was then multiplied by the concentration of p53\textsubscript{15-31 \text{Flu}} and fit to the model in equation 4 to obtain \( K_d \).

Eq. 4: \[ y = \frac{K_d + x + [FL] - \sqrt{(K_d + x + [FL])^2 - 4x[FL]}}{2} \]

Where \( y \) is the fraction bound of p53\textsubscript{15-31 \text{Flu}} multiplied by 54.5 (p53\textsubscript{15-31 \text{Flu}} concentration, nM), [FL] is p53\textsubscript{15-31 \text{Flu}}, and \( x \) is the concentration of hDM2. The observed \( K_d \) for the p53\textsubscript{15-31 \text{Flu}}:hDM2 binding was 180 \pm 30 nM.

5.3 Binding of p53\textsubscript{15-31 \text{Flu}} to hDM2

A serial dilution of hDM2 (0.0006 \( \mu \text{M} \) to 20.75 \( \mu \text{M} \), final concentration) was added to a fixed concentration of p53\textsubscript{15-31 \text{Flu}} (54.5 nM) and PBSA buffer (20 \( \mu \text{L} \)), to give a 60 \( \mu \text{L} \) total volume per assay well. Each dilution was performed in triplicate and the measured intensity of each well calculated using equation 1, then anisotropy was calculated using equation 2. The fraction bound of the tracer could also be determined using equation 3.
Figure S1. Fluorescence anisotropy titration of hDM2 (0.0006 µM to 20.75 µM) into fixed concentration the fluorescein-labelled p53 tracer (54.5 nM) in aqueous phosphate buffer (pH 7.5, 40 mM phosphate, 200 mM NaCl and 0.02 mg/mL Bovine Serum Albumin). FB = Fraction bound.
5.4 Inhibition of the p53<sub>15-31 Flu</sub>/hDM2 protein-protein interaction with Nutlin-3a

Nutlin-3a was serially diluted in DMSO and then diluted 33-fold in PBSA to give effective concentrations between 73 µM and 0.4 nM in 3% DMSO/PBSA. Each serial dilution was repeated in triplicate. An aliquot of each point (20 µL) was then added to a 384-well assay plate, followed by hDM2 (150 nM) and p53<sub>15-31 Flu</sub> (25 nM), to give final concentrations of Nutlin-3a between 24 µM and 0.08 nM (Figure S2).

EC<sub>50</sub> values were determined and curves were fit in Origin Pro 2019b using a non-linear curve fitting with the dose response fitting procedure (equation 5) and Levenberg Marquardt iteration algorithm.

Eq. 5:  
\[ y = \frac{r_{\text{min}} + (r_{\text{max}} - r_{\text{min}})}{1 + 10^{(EC_{50} - x) \times p}} \]

Where \( p \) is the Hill slope and EC<sub>50</sub> is the concentration for half-maximal response from the baseline.

![Figure S2. Dose response of Nutlin-3a (positive control) in the p53/hDM2 fluorescence anisotropy assay in pH 7.5 aqueous phosphate buffer (40 mM phosphate, 200 mM NaCl and 0.02 mg/mL Bovine Serum Albumin). Observed EC<sub>50</sub>: 95.2 ± 1.6 nM, reported EC<sub>50</sub>: 90 nM.](image-url)
5.5 Procedure for screening reaction mixtures at 20 µM:

An aliquot from the master stock of each reaction mixture (2 µL, 50 mM) was diluted into 48 µL DMSO to create a 2 mM total product concentration intermediate screening stock that was used for all subsequent reaction mixture screening. An aliquot of each 2 mM reaction mixture stock (4.9 µL) was then diluted into 155.1 µL PBSA buffer (pH 7.5, 40 mM phosphate, 200 mM NaCl and 0.02 mg/mL Bovine Serum Albumin, PBSA) to create a 60 µM 3% DMSO screening stock. 20 µL of each screening stock was then added to its corresponding well in a 384-well PerkinElmer Opti-plate (see example plate layouts in section 4). Each test well was then charged sequentially with 20 µL 450 nM hDM2 in pH 7.5 PBSA buffer and 20 µL 75 nM p53-tracer in pH 7.5 PBSA buffer. Each blank well was then charged with 20 µL 450 nM hDM2 in pH 7.5 PBSA buffer and 20 µL pH 7.5 PBSA buffer. The total volume of each well was 60 µL and the final concentrations of each reagent were:

- Reaction mixture: 20 µM (Total Product Concentration)
- hDM2: 150 nM
- p53-tracer: 25 nM

Percentage inhibition values were then calculated using Nutlin-3a (10 µM) as the positive control reference and a 1% DMSO blank well containing 150 nM hDM2 and 25 nM p53-tracer as the negative control reference.

\[
\% \text{ Inhibition relative to 10 µM Nutlin – 3a} = \frac{\text{DMSO Control Anisotropy} – \text{Sample Anisotropy}}{\text{Negative Control Anisotropy} – \text{Positive Control Anisotropy}} \times 100
\]
Round 1 HTS at 20 µM total product concentration:

Reaction array 1 controls for individual reagents:
Round 2 HTS at 20 µM:

Reaction array 2 controls for individual reagents:
5.6 Determining IC₅₀ values for isolated compounds

Pure compounds (P1 – P7 and SI1 – SI4) were serially diluted in 100% DMSO (using 12 two-fold dilution steps) to achieve the correct effective concentrations (15.8 – 0.007 mM), then diluted 33-fold in pH 7.5 aqueous phosphate buffer (40 mM phosphate, 200 mM NaCl and 0.02 mg/mL Bovine Serum Albumin) to achieve a 3% DMSO intermediate stock solution (480 – 0.23 µM). The assay was then implemented similarly to the examples above to give final compound concentrations between 160 – 0.08 µM.

IC₅₀ values were determined and curves were fit in Origin Pro 2019b using a non-linear curve fitting with the dose response fitting procedure and Levenberg Marquardt iteration algorithm.

P1:
P2:

P2a and ent-P2a, P2b and ent-P2b, SI1 and SI2:
Table S1. Summary of measured IC$_{50}$ values for P2 diastereomers and analogues SI1 and SI2.

| No. | R$^1$ | R$^2$ | Isomer | FA IC$_{50}$ (µM) |
|-----|-------|-------|--------|-------------------|
| P2a | Ph    |       | S,R    | 28.2 ± 3.0        |
| P2b | Ph    |       | S,S    | 36.5 ± 3.5        |
| ent-P2a | Ph |       | R,S    | 26.0 ± 3.8        |
| ent-P2b | Ph |       | R,R    | 28.6 ± 2.9        |
| SI1 | Ph    |       | -      | >200              |
| SI2 | H     |       | -      | >200              |
Due to poor compound solubility a full dose-response curve could not be obtained.

Due to poor compound solubility a full dose-response curve could not be obtained.
Due to poor compound solubility a full dose-response curve could not be obtained.

**IC₅₀**: 15.8 ± 0.4 μM

Due to poor compound solubility a full dose-response curve could not be obtained.

**IC₅₀**: 48.4 ± 28.3 μM
Due to poor compound solubility a full dose-response curve could not be obtained. Curve fitting failed and EC\textsubscript{50} could not be determined.

6. NMR Measurements for K\textsubscript{d} estimation by \textsuperscript{15}N HSQC NMR

NMR titrations were performed by recording a series of \textsuperscript{1}H/\textsuperscript{15}N-HSQC experiments on a 750 MHz Oxford Magnet spectrometer (TCl-Cyroprobe, \textsuperscript{1}H optimized triple resonance NMR ‘inverse’ probe) (\textsuperscript{1}H = 750 MHz and \textsuperscript{15}N = 76 MHz) in pH 7.5 aqueous phosphate buffer containing 100 mM phosphate, 1 mM DTT and 2.5% glycerol with 50 \mu M \textsuperscript{15}N-labelled hDM2\textsubscript{17-125}, 10\% D\textsubscript{2}O and 1\% DMSO. Temperature was maintained at 298 K throughout the experiments. Pure compounds were titrated into the \textsuperscript{15}N-hDM2\textsubscript{17-125} sample in 0.5-, 1-, 1.5- and 2-molar equivalents relative to \textsuperscript{15}N- hDM2\textsubscript{17-125} as standard and further molar equivalents of 4- and 6-times compound-to- hDM2\textsubscript{17-125} were added if the protein was not fully saturated. Data was processed using Topspin and analysed with Sparky.\textsuperscript{6}

K\textsubscript{d} values were obtained by plotting the observed chemical shift perturbation (csp) of the reporter peaks L54, L57, G58, M62, V75, V93, K94, H96 and K98 against the molar ratio of ligand. The csp of each reporter peak was calculated as the deviation from the free protein resonances using equation 6.

Eq.6:  
\[ \text{csp} = \sqrt{(\omega_2 \text{free} - \omega_2 \text{bound})^2 + \frac{(\omega_1 \text{free} - \omega_1 \text{bound})^2}{10}} \]
Where $\omega_1$ is the $^{15}$N chemical shift and $\omega_2$ is the $^1$H chemical shift corresponding to the observed HSQC cross-peak for a given reporter residue.

$K_d$ values for each reporter peak were then obtained by solving equation 7.

Eq. 7:  
$$
\Delta = \Delta_o \frac{(K_d + [L] + [P]) - \sqrt{(K_d + [L] + [P])^2 - 4[P][L]}}{2[P]}
$$

Where $\Delta$ is the observed csp, $\Delta_o$ is the maximum csp, and $[P]$ and $[L]$ are the protein and ligand concentrations respectively. The global $K_d$ was then obtained from the average $K_d$ for the combined reporter peaks as shown in equation 8.

Eq. 8:  
$$
Global K_d = \frac{\sum\log_{10}(iK_d)}{n}
$$

Where $i$ is the reporter peak and $n$ is the number of reporter peaks.

6.1 Spectra and fitting

P1 – due to intermediate and slow exchange chemical shift perturbation $K_d$ could not be estimated using the reporter peaks outlined above.
NMR $K_K$: Significantly lower than MDM2 concentration

Chemical Shift Perturbation

Molar Ratio

$\omega_1 - ^{15}N$ (ppm)

$\omega_2 - ^1H$ (ppm)
NMR $K_d$: Significantly lower than MDM2 concentration

Chemical Shift Perturbation

Molar Ratio

$\omega_1 - ^1\text{H} (\text{ppm})$

$\omega_2 - ^1\text{H} (\text{ppm})$
P3 – weak chemical shift perturbation observed up to a 6:1 molar ratio of P3/\textsuperscript{15}N-hDM2
P7 – no chemical shift perturbation was observed up to a 6:1 molar ratio of P7/¹⁵N-hDM2

Figure S3. ¹⁵N-H HSQC chemical shift perturbation of assigned peaks for 50 µM ¹⁵N-labelled hDM2 on addition of Nutlin-3a (100 µM). Unassigned residues are highlighted in grey.
6.2 Counter-screening of P1, P2, P5 and P6 against MCL-1

$^{15}$N-labelled MCL-1 was expressed using the procedure reported by Wilson et. al.\textsuperscript{1e,1f} using M9 minimal media enriched with $^{15}$NH\textsubscript{4}Cl as nitrogen source.

Single point NMR screens were performed by recording two $^1$H/$^{15}$N-HSQC experiments on a 600 MHz Oxford Magnet spectrometer (QCI-P-Cyroprobe, $^1$H optimized quadruple resonance NMR ‘inverse’ probe) ($^1$H = 600 MHz and $^{15}$N = 61 MHz) in pH 7.4 aqueous buffer containing 100 mM phosphate, 1 mM DTT and 2.5% glycerol, with 50 µM $^{15}$N-labelled MCL-1, 10% D\textsubscript{2}O and 1% DMSO. Temperature was maintained at 298 K throughout the experiments. Pure compounds were added into the $^{15}$N- MCL-1 sample as one 6 molar equivalent relative to $^{15}$N- MCL-1 as standard. Data was processed using Topspin and analysed with Sparky.\textsuperscript{5}

P1 – Black cross peaks: free $^{15}$N- MCL-1 and red cross peaks: 6:1 molar ratio of P1/$^{15}$N- MCL-1.
P2 – Black cross peaks: free $^{15}$N- MCL-1 and red cross peaks: 6:1 molar ratio of P2/$^{15}$N- MCL-1.

P5 – Black cross peaks: free $^{15}$N- MCL-1 and red cross peaks: 6:1 molar ratio of P5/$^{15}$N- MCL-1.
P6 – Black cross peaks: free $^{15}$N- MCL-1 and red cross peaks: 6:1 molar ratio of $^\text{P6}/^{15}\text{N}-\text{MCL-1}$. 
7. LC-MS Analysis of Reaction Mixtures

All 154 reactions from the round 1 reaction array were analysed by LC-MS to investigate how many combinations had produced a desired product (Figure S4). All samples were diluted to 1 mg/mL concentrations from the original 50 mM DMSO master stock, with respect to the initial diazo starting concentration. Reaction wells containing diazo and substrate were analysed for intermolecular product(s) and blank control wells were analysed for intramolecular product(s). Dark green squares indicate clear m/z for the desired product(s) and a clear corresponding UV peak(s). Light green squares indicate m/z for the desired product(s) and either weak or no corresponding UV peak(s). Blank squares indicate that no m/z was observed for the desired product(s).

Figure S4. LC-MS heatmap for reaction array one.

Overall, 112 out of 154 reactions (73%) showed the presence of an expected product m/z, by LC-MS, of which 78 reactions also showed distinct UV peaks. For all 70 combinations of diazo and co-substrate, excluding blank intramolecular controls, only 8 combinations out of 70 (11%) failed to give detectable product m/z when considering reactions across both catalysts. This demonstrates considerable sampling of the available chemical space across the first reaction array.
| Diazo/Substrate | Catalyst | Formula   | Adduct | Expected | Found | Peak Intensity | UV peak? |
|----------------|----------|-----------|--------|----------|-------|----------------|----------|
| D1S1           | Rh2piv4  | C19H16Cl2N2O2 | -      | 374.06   | -     | -              | N        |
| D1S2           | Rh2piv4  | C17H15Cl2N2O3 | -      | 351.04   | -     | -              | N        |
| D1S3           | Rh2piv4  | C25H22Cl3NO2 | -      | 473.07   | -     | -              | N        |
| D1S4           | Rh2piv4  | C18H21Cl2N2O4 | -      | 364.12   | -     | -              | N        |
| D1S5           | Rh2piv4  | C21H25Cl2N2O3 | H      | 389.16   | 389.16 | 5x10^6         | N        |
| D1S6           | Rh2piv4  | C20H18Cl2N3O3 | -      | 355.10   | -     | -              | N        |
| D1S7           | Rh2piv4  | C22H23Cl2N2O4 | H      | 383.15   | 383.15 | 1x10^7         | N        |
| D1S8           | Rh2piv4  | C20H19Cl2N3O3 | H      | 392.07   | 392.08 | 5x10^6         | N        |
| D1S9           | Rh2piv4  | C20H17Cl2N2O2 | H      | 374.06   | 374.07 | 5x10^6         | N        |
| D1S10          | Rh2piv4  | C15H18Cl2N4O5 | H      | 344.07   | 344.07 | 3x10^6         | N        |
| D1blank        | Rh2piv4  | C11H10Cl2N2O2 | 2M + 2H | 448.10   | 448.64 | 7.5x10^7       | Y        |
| D1S1           | Rh2pf4   | C19H16Cl2N2O2 | H      | 375.06   | 375.07 | 1x10^7         | N        |
| D1S2           | Rh2pf4   | C17H15Cl2N2O3 | H      | 352.04   | 352.24 | 4x10^7         | Y        |
| D1S3           | Rh2pf4   | C25H22Cl3N2O4 | Na     | 496.06   | 498.1  | 4x10^7         | Y        |
| D1S4           | Rh2pf4   | C18H21Cl2N2O4 | H      | 365.12   | 365.03 | 1x10^7         | Y        |
| D1S5           | Rh2pf4   | C21H25Cl2N2O4 | -      | 388.16   | -     | -              | N        |
| D1S6           | Rh2pf4   | C20H18Cl2N2O3 | -      | 355.10   | -     | -              | N        |
| D1S7           | Rh2pf4   | C22H23Cl3N2O4 | -      | 382.15   | -     | -              | N        |
| D1S8           | Rh2pf4   | C20H19Cl2N3O3 | Na     | 414.06   | 413.97 | 3x10^6         | Y        |
| D1S9           | Rh2pf4   | C20H17Cl2N2O2 | H      | 374.06   | 374.07 | 2x10^7         | N        |
| D1S10          | Rh2pf4   | C15H18Cl2N4O5 | -      | 343.07   | -     | -              | N        |
| D1blank        | Rh2pf4   | C11H10Cl2N2O2 | -      | 223.04   | -     | -              | N        |
| D2S1           | Rh2piv4  | C18H12Cl2F3NO2 | H      | 402.02   | 401.98 | 3x10^6         | N        |
| D2S2           | Rh2piv4  | C16H11Cl2F3O3 | H      | 379.00   | 379.01 | 7.5x10^6       | N        |
| D2S3           | Rh2piv4  | C24H18Cl3F3O2 | -H     | 499.02   | 498.86 | 7.5x10^7       | Y        |
| D2S4           | Rh2piv4  | C17H17Cl2F3NO4 | H      | 392.08   | 392.02 | 3x10^7         | Y        |
| D2S5           | Rh2piv4  | C20H21Cl2F3NO3 | -H     | 414.11   | 414.11 | 6x10^7         | Y        |
| D2S6           | Rh2piv4  | C19H14Cl2F3O3 | Na     | 405.04   | 404.80 | 1x10^7         | Y        |
| D2S7           | Rh2piv4  | C21H19Cl2F3NO2 | H      | 409.11   | 410.02 | 7.5x10^8       | Y        |
| D2S8           | Rh2piv4  | C19H15Cl2F3O3 | Na     | 441.02   | 440.97 | 6x10^6         | Y        |
| D2S9           | Rh2piv4  | C19H13Cl2F3O2 | H      | 401.02   | 400.93 | 2x10^6         | N        |
| D2S10          | Rh2piv4  | C14H14Cl2F3O4S | -H     | 369.01   | 368.85 | 2x10^7         | Y        |
| D2blank        | Rh2piv4  | C20H12Cl2F6O4 | -H     | 498.99   | 498.99 | 1x10^6         | N        |
| D2S1           | Rh2pf4   | C18H12Cl2F3NO2 | -H     | 400.01   | 399.92 | 1x10^8         | Y        |
| D2S2           | Rh2pf4   | C16H11Cl2F3O3 | Na     | 400.99   | 400.89 | 1.5x10^6       | N        |
| D2S3           | Rh2pf4   | C24H18Cl3F3O2 | -      | 500.03   | -     | -              | N        |
| D2S4           | Rh2pf4   | C17H17Cl2F3NO4 | H      | 392.08   | 392.09 | 5x10^7         | Y        |
| D2S5           | Rh2pf4   | C20H21Cl2F3NO3 | -      | 415.12   | -     | -              | N        |
| D2S6           | Rh2pf4   | C19H14Cl2F3O3 | -      | 382.06   | -     | -              | N        |
| D2S7           | Rh2pf4   | C21H19Cl2F3NO2 | H      | 410.11   | 410.02 | 7.5x10^8       | Y        |
| D2S8           | Rh2pf4   | C19H15Cl2F3O3 | Na     | 441.02   | 440.96 | 2x10^6         | Y        |
| D2S9 | Rh$_2$pf$_4$ | C19H13Cl$_2$F3O$_2$ | H | 401.02 | 400.92 | 1x10$^7$ | Y |
| D2S10 | Rh$_2$pf$_4$ | C14H14ClF3O4S | - | 370.03 | - | - | N |
| D2blank | Rh$_2$pf$_4$ | C20H12Cl$_2$F6O$_4$ | - | 500.00 | - | - | N |
| D3S1 | Rh$_2$pf$_4$ | C23H17Cl$_2$N$_2$O | H | 445.04 | 444.93 | 2.5x10$^7$ | Y |
| D3S2 | Rh$_2$pf$_4$ | C21H16Cl$_2$N$_2$O$_2$ | H | 422.02 | 421.90 | 2x10$^8$ | Y |
| D3S3 | Rh$_2$pf$_4$ | C29H23Cl$_4$NO | - | 541.05 | - | - | N |
| D3S4 | Rh$_2$pf$_4$ | C22H22Cl$_2$N$_2$O$_3$ | H | 433.10 | 432.98 | 2x10$^8$ | Y |
| D3S5 | Rh$_2$pf$_4$ | C25H26Cl$_2$N$_2$O$_2$ | K | 495.10 | 495.07 | 1.25x10$^7$ | N |
| D3S6 | Rh$_2$pf$_4$ | C24H19Cl$_2$NO$_2$ | - | 423.08 | - | - | N |
| D3S7 | Rh$_2$pf$_4$ | C26H24Cl$_2$N$_2$O$_2$ | H | 451.13 | 451.13 | 2x10$^7$ | N |
| D3S8 | Rh$_2$pf$_4$ | C24H28Cl$_2$N$_2$O$_2$ | - | 459.06 | - | - | N |
| D3S9 | Rh$_2$pf$_4$ | C24H18Cl$_3$NO | - | 441.05 | - | - | N |
| D3S10 | Rh$_2$pf$_4$ | C19H19Cl$_2$N$_2$O$_3$ | -H | 410.05 | 410.04 | 2x10$^5$ | N |
| D3blank | Rh$_2$pf$_4$ | C15H11Cl$_2$NO | -H | 290.01 | 289.71 | 5x10$^6$ | Y |
| D3S1 | Rh$_2$pf$_4$ | C23H17Cl$_3$N$_2$O | H | 445.04 | 444.95 | 7.5x10$^7$ | Y |
| D3S2 | Rh$_2$pf$_4$ | C21H16Cl$_3$N$_2$O$_2$ | - | 419.02 | - | - | N |
| D3S3 | Rh$_2$pf$_4$ | C29H23Cl$_4$NO | - | 541.05 | - | - | N |
| D3S4 | Rh$_2$pf$_4$ | C22H22Cl$_2$N$_2$O$_3$ | H | 433.10 | 432.97 | 4x10$^8$ | Y |
| D3S5 | Rh$_2$pf$_4$ | C25H26Cl$_2$N$_2$O$_2$ | - | 456.14 | - | - | N |
| D3S6 | Rh$_2$pf$_4$ | C24H19Cl$_2$NO$_2$ | - | 423.08 | - | - | N |
| D3S7 | Rh$_2$pf$_4$ | C26H24Cl$_2$N$_2$O$_2$ | - | 450.13 | - | - | N |
| D3S8 | Rh$_2$pf$_4$ | C24H20Cl$_3$N$_2$O | - | 459.06 | - | - | N |
| D3S9 | Rh$_2$pf$_4$ | C24H18Cl$_3$NO | - | 441.05 | - | - | N |
| D3S10 | Rh$_2$pf$_4$ | C19H19Cl$_2$N$_2$O$_3$ | - | 411.05 | - | - | N |
| D3blank | Rh$_2$pf$_4$ | C15H11Cl$_2$NO | H | 292.02 | 292.02 | 4x10$^7$ | Y |
| D3S1 | Rh$_2$pf$_4$ | C20H19Cl$_3$N$_2$O | H | 339.12 | 339.02 | 1x10$^8$ | Y |
| D3S2 | Rh$_2$pf$_4$ | C18H18Cl$_3$N$_2$O$_2$ | H | 316.10 | 315.96 | 1.5x10$^8$ | Y |
| D3S3 | Rh$_2$pf$_4$ | C26H25Cl$_2$NO | - | 437.13 | - | - | N |
| D4S4 | Rh$_2$pf$_4$ | C19H24N$_2$O$_3$ | NH$_4$ | 347.18 | 347.08 | 1x10$^7$ | Y |
| D4S5 | Rh$_2$pf$_4$ | C22H28N$_2$O$_2$ | - | 352.21 | - | - | N |
| D4S6 | Rh$_2$pf$_4$ | C21H21NO$_2$ | H | 320.16 | 320.01 | 7x10$^7$ | Y |
| D4S7 | Rh$_2$pf$_4$ | C23H26N$_2$O | - | 346.21 | - | - | N |
| D4S8 | Rh$_2$pf$_4$ | C21H22Cl$_2$NO | 2M + Na | 733.26 | 733.19 | 2x10$^8$ | Y |
| D4S9 | Rh$_2$pf$_4$ | C21H20ClNO | H | 338.12 | 338.03 | 1.7x10$^8$ | Y |
| D4S10 | Rh$_2$pf$_4$ | C16H21NO$_3$S | H | 308.12 | 307.97 | 2x10$^7$ | Y |
| D4blank | Rh$_2$pf$_4$ | C24H26N$_2$O$_2$ | H | 375.20 | 375.1 | 3x10$^8$ | Y |
| D4S1 | Rh$_2$pf$_4$ | C20H19Cl$_3$N$_2$O | H | 339.12 | 339.01 | 1x10$^8$ | Y |
| D4S2 | Rh$_2$pf$_4$ | C18H18Cl$_3$N$_2$O | - | 315.10 | - | - | N |
| D4S3 | Rh$_2$pf$_4$ | C26H25Cl$_2$NO | Na | 460.12 | 460.23 | 1x10$^7$ | Y |
| D4S4 | Rh$_2$pf$_4$ | C19H24N$_2$O$_3$ | H | 329.18 | 329.19 | 3x10$^7$ | N |
| D4S5 | Rh$_2$pf$_4$ | C22H28N$_2$O$_2$ | -H | 351.22 | 351.21 | 5x10$^5$ | N |
| D4S6 | Rh$_2$pf$_4$ | C21H21NO$_2$ | H | 320.16 | 320.05 | 8x10$^6$ | Y |
| D4S7 | Rh$_2$pf$_4$ | C23H26N$_2$O | - | 346.20 | - | - | N |
| D4S8 | Rh$_2$pf$_4$ | C21H22Cl$_2$NO | 2M + Na | 733.26 | 733.21 | 2x10$^7$ | N |
| D4S9  | Rh2pfba | C21H20CINO | H     | 338.12 | 338.04 | 4x10^7 | Y     |
| D4S10 | Rh2pfba | C16H21NO3S | H     | 308.12 | 308.13 | 1x10^7 | N     |
| D4blank| Rh2pfba | C24H26N2O2 | H     | 375.20 | 375.08 | 3x10^8 | Y     |
| D5S1  | Rh2piv4 | C16H19CINO2 | H     | 307.11 | 306.94 | 1x10^8 | Y     |
| D5S2  | Rh2piv4 | C14H18CINO3 | H     | 284.10 | 283.88 | 3x10^7 | Y     |
| D5S3  | Rh2piv4 | C22H25Cl2NO2 | H   | 406.13 | 406.13 | 5x10^6 | N     |
| D5S4  | Rh2piv4 | C15H24N2O4 | H     | 297.17 | 297.18 | 4x10^6 | N     |
| D5S5  | Rh2piv4 | C18H28N2O3 | NH4   | 338.24 | 338.23 | 1x10^7 | N     |
| D5S6  | Rh2piv4 | C17H21NO3 | H     | 288.15 | 287.94 | 1x10^7 | Y     |
| D5S7  | Rh2piv4 | C19H26N2O2 | -     | 314.30 | -      | -      | N     |
| D5S8  | Rh2piv4 | C17H22CINO3 | 2M + Na | 669.25 | 669.24 | 1x10^8 | Y     |
| D5S9  | Rh2piv4 | C17H20CINO2 | H     | 306.12 | 305.94 | 3x10^7 | Y     |
| D5S10 | Rh2piv4 | C12H21NO4S | -     | 275.12 | -      | -      | N     |
| D5blank| Rh2piv4 | C8H13NO2 | -      | 155.09 | -      | -      | N     |
| D6S1  | Rh2piv4 | C16H19CINO2 | H     | 307.11 | 306.92 | 3x10^7 | Y     |
| D6S2  | Rh2piv4 | C14H18CINO3 | H     | 284.10 | 283.86 | 2x10^7 | Y     |
| D6S3  | Rh2piv4 | C22H25Cl2NO2 | Na  | 428.12 | 428.22 | 2x10^6 | Y     |
| D6S4  | Rh2piv4 | C15H24N2O4 | H     | 297.17 | 297.18 | 5x10^6 | N     |
| D6S5  | Rh2piv4 | C18H26N2O3 | -     | 320.21 | -      | -      | N     |
| D6S6  | Rh2piv4 | C17H21NO3 | H     | 288.15 | 288.16 | 5x10^6 | N     |
| D6S7  | Rh2piv4 | C19H26N2O2 | H     | 315.20 | 315.21 | 1x10^7 | N     |
| D6S8  | Rh2piv4 | C17H22CINO3 | 2M + Na | 669.25 | 669.24 | 5x10^7 | Y     |
| D6S9  | Rh2piv4 | C17H20CINO2 | H     | 306.12 | 305.93 | 2.5x10^6 | Y     |
| D6S10 | Rh2piv4 | C12H21NO4S | -     | 275.12 | -      | -      | N     |
| D6blank| Rh2piv4 | C8H13NO2 | 2M + H | 311.20 | 311.04 | 1.5x10^7 | N     |
| D6S1  | Rh2piv4 | C17H14CINO2 | -H    | 298.07 | 298.06 | 1x10^6 | N     |
| D6S2  | Rh2piv4 | C15H13ClO3 | H     | 277.06 | 277.06 | 3x10^6 | N     |
| D6S3  | Rh2piv4 | C23H20Cl2O2 | -    | 398.08 | -      | -      | N     |
| D6S4  | Rh2piv4 | C16H19NO4 | H     | 290.13 | 290.14 | 5x10^6 | N     |
| D6S5  | Rh2piv4 | C19H24NO3 | K     | 353.14 | 353.05 | 2x10^7 | Y     |
| D6S6  | Rh2piv4 | C18H16O3 | H     | 281.11 | 281.12 | 7.5x10^6 | N     |
| D6S7  | Rh2piv4 | C20H22NO2 | H     | 309.17 | 309.17 | 1.5x10^8 | Y     |
| D6S8  | Rh2piv4 | C18H17ClO3 | H     | 317.09 | 317.06 | 3x10^7 | Y     |
| D6S9  | Rh2piv4 | C18H15ClO2 | H     | 299.08 | 299.08 | 7.5x10^6 | N     |
| D6S10 | Rh2piv4 | C13H16O4S | H     | 269.08 | 269.08 | 3x10^7 | N     |
| D6blank| Rh2piv4 | C18H16O4 | H     | 297.11 | 297.11 | 7.5x10^7 | Y     |
| D6S1  | Rh2pfba | C17H14CINO2 | H     | 300.07 | 299.94 | 1x10^7 | Y     |
| D6S2  | Rh2pfba | C15H13ClO3 | -     | 276.06 | -      | -      | N     |
| D6S3  | Rh2pfba | C23H20Cl2O2 | -   | 398.08 | -      | -      | N     |
| D6S4  | Rh2pfba | C16H19NO4 | H     | 290.13 | 290.14 | 5x10^6 | N     |
| D6S5  | Rh2pfba | C19H24NO3 | K     | 353.14 | 353.11 | 1x10^7 | N     |
| D6S6  | Rh2pfba | C18H16O3 | -     | 280.11 | -      | -      | N     |
| D6S7  | Rh2pfba | C20H22NO2 | H     | 309.17 | 309.17 | 1.5x10^8 | Y     |
| D6S8  | Rh2pfba | C18H17ClO3 | 2M + Na | 655.16 | 655.05 | 6x10^7 | Y     |
|   | Formula          | Molecular Weight | LogP | MW   | IC50 (nM) | ECFP4 Tanimoto Sim | Tanimoto Sim | 50% PDBID  |
|---|-----------------|------------------|------|------|-----------|--------------------|--------------|------------|
|  | D6S9 Rh2pfba    | C18H15ClO2       | H    | 299.08 | 299.08 | 4x10^7 | N          |            |
|  | D6S10 Rh2pfba   | C13H16O4S        | H    | 269.08 | 269.00 | 3x10^7 | Y          |            |
|  | D6blank Rh2pfba | C18H16O4         | -    | 296.10 |          | N                  |              |            |
|  | D7S1 Rh2pfiv    | C14H12CIN3O2     | H    | 290.06 | 289.93 | 2x10^7 | Y          |            |
|  | D7S2 Rh2pfiv    | C12H11CIN2O3     | H    | 267.05 | 266.89 | 6x10^6 | Y          |            |
|  | D7S3 Rh2pfiv    | C20H18C12N2O2    | H    | 389.08 | 389.08 | 6x10^6 | Y          |            |
|  | D7S4 Rh2pfiv    | C13H17N3O4       | Na   | 302.11 | 302.01 | 2x10^6 | Y          |            |
|  | D7S5 Rh2pfiv    | C16H21CIN3O3     | -H   | 302.16 | 302.01 | 1x10^6 | Y          |            |
|  | D7S6 Rh2pfiv    | C15H14N2O3       | H    | 271.10 | 270.93 | 3x10^7 | Y          |            |
|  | D7S7 Rh2pfiv    | C17H20N3O2       | NH4  | 316.19 | 316.10 | 2x10^6 | N          |            |
|  | D7S8 Rh2pfiv    | C15H15CIN2O3     | H    | 307.08 | 306.96 | 5x10^7 | Y          |            |
|  | D7S9 Rh2pfiv    | C15H13CIN2O2     | H    | 289.07 | 288.93 | 4x10^7 | Y          |            |
|  | D7S10 Rh2pfiv   | C10H14N2O4S      | H    | 259.07 | 258.87 | 6x10^6 | Y          |            |
|  | D7blank Rh2pfiv | C12H12N4O4       | H    | 276.09 | 276.95 | 8x10^6 | Y          |            |
|  | D7S1 Rh2pfiv    | C14H12CIN3O2     | H    | 290.06 | 289.92 | 1.5x10^7 | Y     |
|  | D7S2 Rh2pfiv    | C12H11CIN2O3     | H    | 267.05 | 266.89 | 1x10^7 | Y          |            |
|  | D7S3 Rh2pfiv    | C20H18C12N2O2    | H    | 389.07 | 389.06 | 1.5x10^7 | Y     |
|  | D7S4 Rh2pfiv    | C13H17N3O4       | -H   | 278.12 | 278.11 | 4x10^6 | N          |            |
|  | D7S5 Rh2pfiv    | C16H21CIN3O3     | H    | 304.16 | 304.17 | 4x10^6 | N          |            |
|  | D7S6 Rh2pfiv    | C15H14N2O3       | H    | 271.10 | 270.91 | 1x10^7 | Y          |            |
|  | D7S7 Rh2pfiv    | C17H19N3O2       | H    | 298.15 | 298.06 | 1.5x10^7 | Y     |
|  | D7S8 Rh2pfiv    | C15H15CIN2O3     | 2M+Na| 365.14 | 365.04 | 1x10^7 | Y          |            |
|  | D7S9 Rh2pfiv    | C15H13CIN2O2     | H    | 289.07 | 288.92 | 1.5x10^7 | Y     |
|  | D7S10 Rh2pfiv   | C10H14N2O4S      | H    | 259.07 | 258.91 | 3x10^6 | Y          |            |
|  | D7blank Rh2pfiv | C12H12N4O4       | H    | 277.09 | 276.95 | 7x10^7 | Y          |            |

### 8. Docking of ADS Ligands and similarity analysis

#### 8.1 Molecular docking

Docking of compounds into the binding site (PDB IDs: 6Q9H and 4HG7) was performed using MOE (Molecular Operating Environment) from the Chemical Computing Group (Montreal, Canada) (Figure S5). Novel molecules were docked as database and 10 conformations were generated for each molecule using the Amber10:EHT forcefield, using an aromatic pharmacophore in Leu26 and Trp23 hot spots. Among these, the conformations with the lowest docking scores were chosen to study the likely binding orientations of the ligands and each complex was assessed and ranked by the London ΔG energy scoring function.
Figure S5. P2, P4, P5 and P6 docked into the binding site (PDB: 6Q9H); the subpockets targeted by p53 hotspot residues F19 (red), W23 (blue) and L26 (green) are shown.
Figure S6. Overlay of docked poses of MDM2 binders and Nutlin-3a (PDB ID: 4HG7)
8.2 X-ray structures of AM-8735 and MI-77301

Figure S7. X-ray crystal structure of AM-8735 bound to hDM2 (PDB: 4OBA); the subpockets targeted by p53 hotspot residues F19 (red), W23 (blue) and L26 (green) are shown.\(^8\)

Figure S8. X-ray crystal structure of MI-77301 bound to hDM2 (PDB: 5TRF); the subpockets targeted by p53 hotspot residues F19 (red), W23 (blue) and L26 (green) are shown.\(^9\)
8.3 Similarity analysis

1769 compounds with annotated activity towards hDM2 (referred to as MDM2 within ChEMBL) were obtained from the ChEMBL database (accessed: 16/01/2020). Subsequent processing removed duplicate molecules leaving 1314 compounds (see accompanying Excel spreadsheet) which were then used for further analysis. The Morgan molecular fingerprint was then computed for each molecule using RDKit\textsuperscript{10} and the pairwise Tanimoto similarity scores calculated.

Table S3. Tanimoto similarity analysis comparing ADS products P1\textendash{}P6 to 1314 known hDM2 ligands from the ChEMBL database.

| Metric                  | P1 | P2 | P3 | P4 | P5 | P6 |
|-------------------------|----|----|----|----|----|----|
| Mean                    | 0.37 | 0.34 | 0.36 | 0.26 | 0.43 | 0.44 |
| Median                  | 0.38 | 0.34 | 0.36 | 0.25 | 0.44 | 0.45 |
| Minimum similarity      | 0.12 | 0.12 | 0.13 | 0.13 | 0.13 | 0.12 |
| Maximum similarity      | 0.50 | 0.48 | 0.46 | 0.37 | 0.61 | 0.51 |

Figure S9. Molecular similarities of the p53/hDM2 PPI inhibitors P2, P4, P5 and P6 and their nearest neighbour hDM2 ligands in ChEMBL.
9. Spectra

D9
ent-P2a
ent-P2b
P2b vs \textit{ent}-P2b

\begin{center}
\includegraphics[width=\textwidth]{p2b_vs_entp2b.png}
\end{center}

\textit{ent}-P2a vs \textit{ent}-P2b

\begin{center}
\includegraphics[width=\textwidth]{entp2a_vs_entp2b.png}
\end{center}
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[10] RDKit: Open-source cheminformatics; [http://www.rdkit.org](http://www.rdkit.org)