Rational Design of Topographical Helix Mimics as Potent Inhibitors of Protein-Protein Interactions

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Supporting Information

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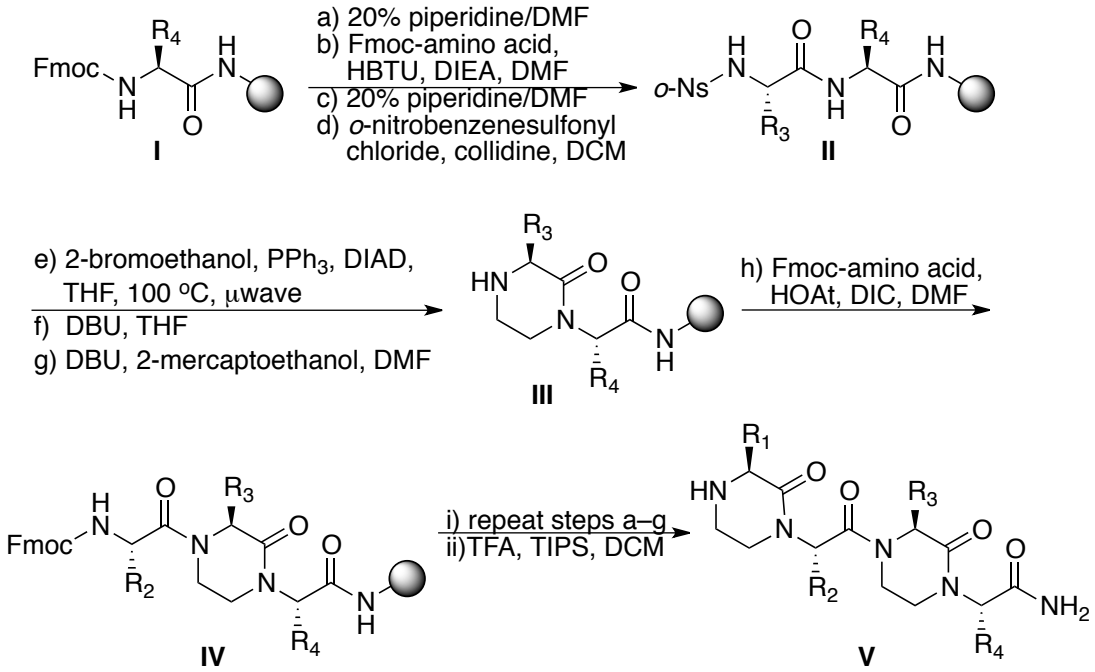


Scheme S1. Solid-phase synthesis of oxopiperazine dimers.

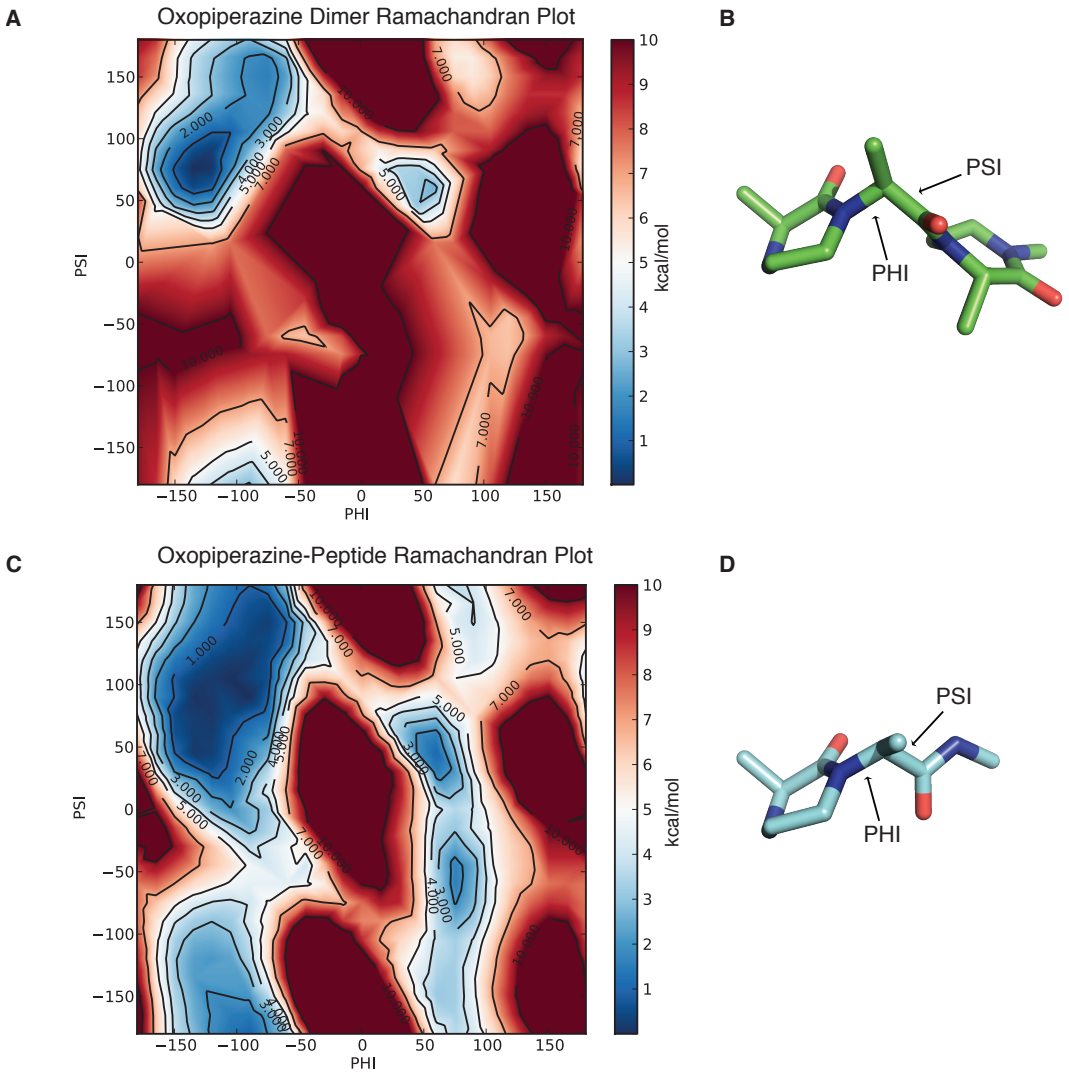


Figure S1: QM Ramachandran plots of oxopiperazine dimer and oxopiperazine-amino acid. A) Oxopiperazine dimer Ramachandran plot shows a constrained molecule with a single low energy well near ϕ and ψ values of -135° and 75°, respectively. B) Low energy conformation of oxopiperazine dimer model used in QM calculations. C) Oxopiperazine-amino acid Ramachandran plot shows a more flexible molecule than the oxopiperazine dimer with a very broad low energy well. D) Low energy conformation of oxopiperazine-amino acid model with ϕ and ψ values of -75° and 150°, respectively. The comparison between plots in A and C provides additional support for our hypothesis that the oxopiperazine dimer is a conformationally stable scaffold.

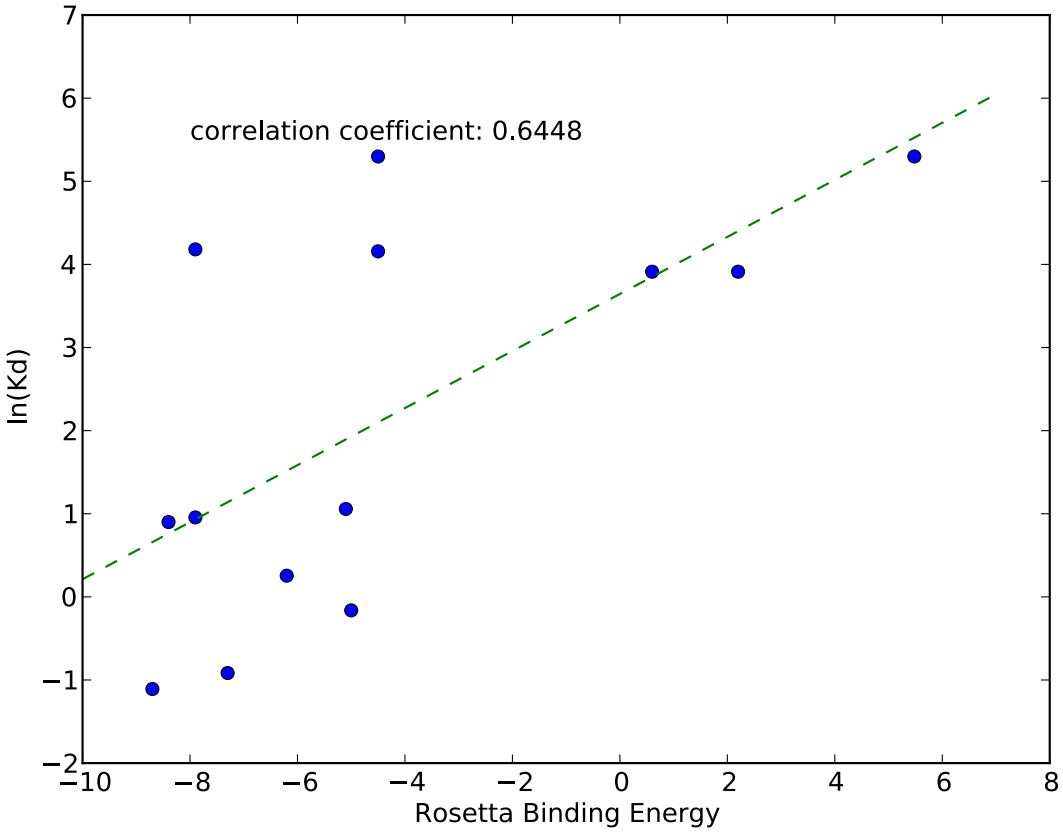


Figure S2. Correlation of Rosetta binding energy predictions with experimental K_d for Mdm2 ligands. Data points are taken from Table S2, and correlation coefficient was calculated using Python's scipy.stats.stats.pearsonr function. Rosetta binding energy (x axis) approximates free energy of binding which is proportional to ln(Kd). Explicitly: deltaG = RTln(Kd) where R = ideal gas constant and T = temperature.

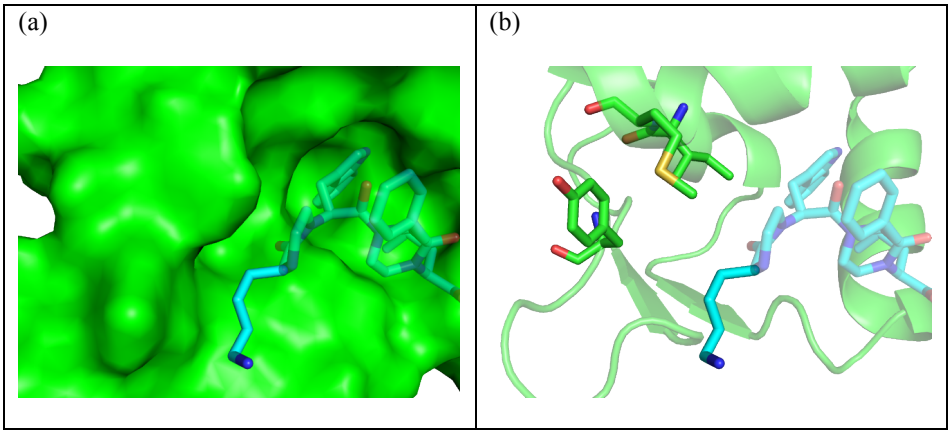
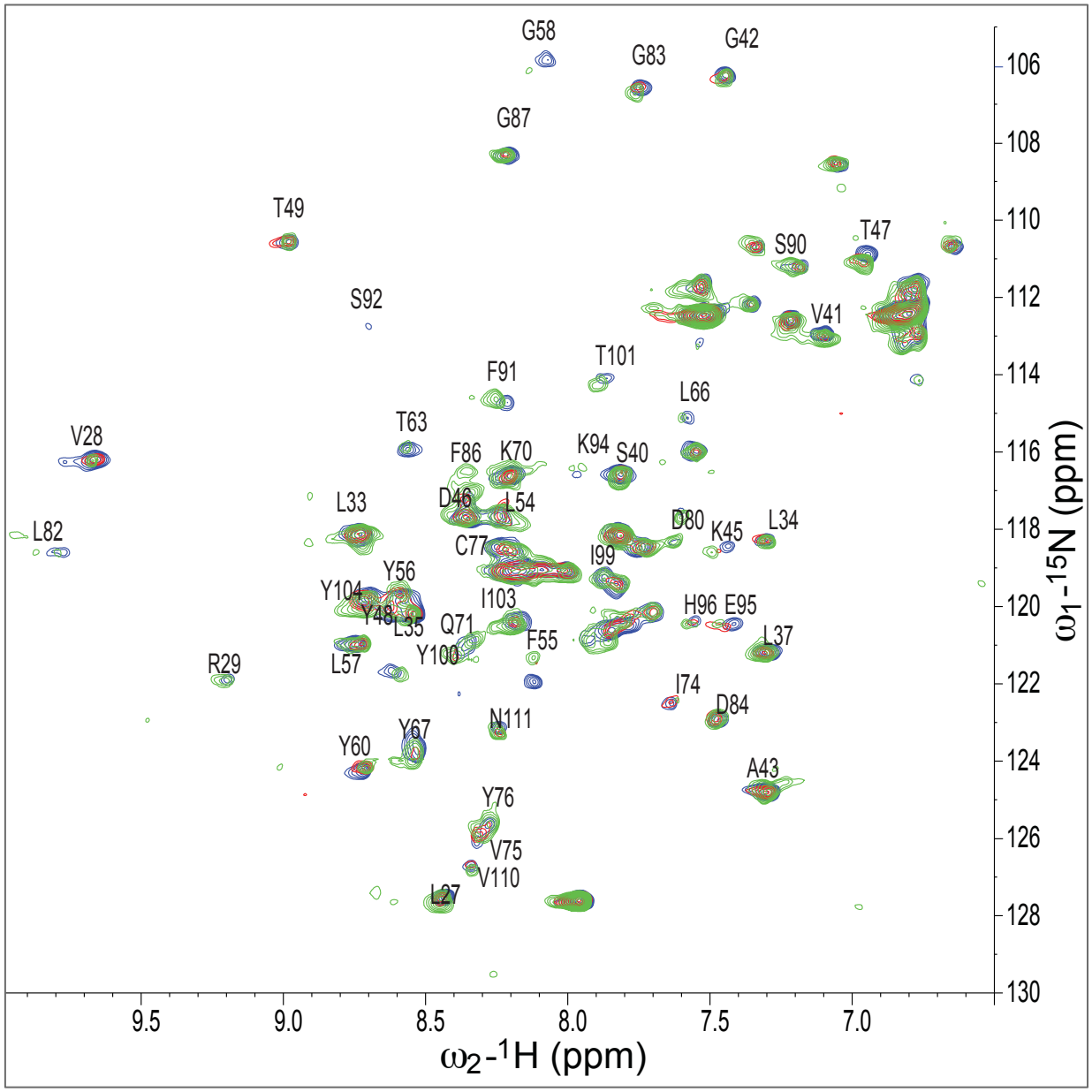


Figure S3: Predicted conformation of oxopiperazine **7 (**KWFL) in complex with MDM2. Upon manual inspection of the KWFL design, the lysine in the 1st position of the oxopiperazine does not occupy the same pocket as the p53 Phe19. This suggests oxopiperazine 7 is a poor competitor with p53 and provides a clear explanation of the discrepancy between the predicted Rosetta binding energy and the experimental K_d. A) surface and B) ribbon representation.

(a)


G58
G83
G42
G87
T49
S90
T47
S92
V41
T101
F91
L66
T63
V28
F86
K70
K94
S40
L33
D46
L54
D80
K45
L34
L82
C77
I99
Y56
Y104
I103
H96
E95
Y48
L35
Q71
L37
F55
R29
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I74
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N111
Y67
Y60
A43
Y76
V75
V110
L27
9.5
9.0
8.5
8.0
7.5
7.0
ω2-1H (ppm)
106
108
110
112
114
116
118
120
122
124
126
128
130
ω1-15N (ppm)

(b)

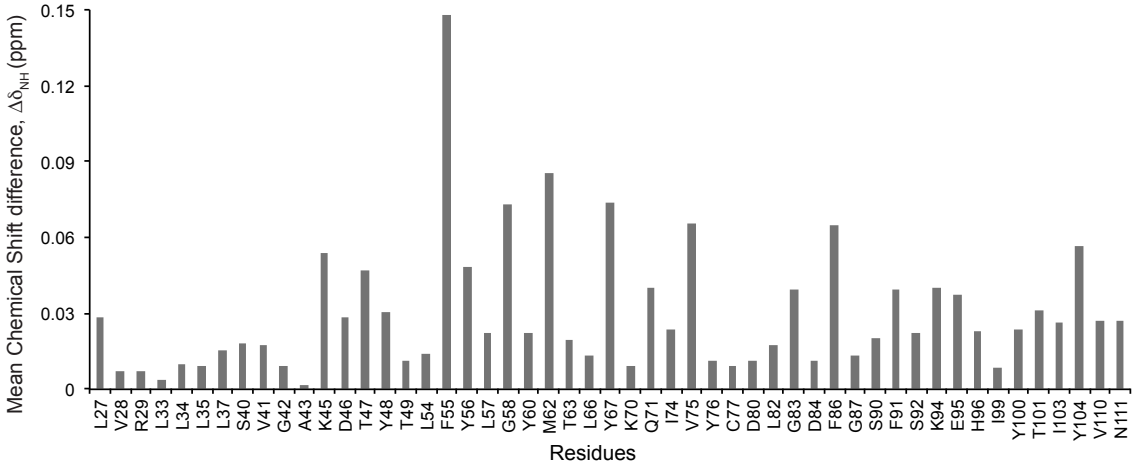


Figure S4. ^{1}H-^{15}N HSQC titration spectra. (a) Spectra of MDM2 (blue), Mdm2:F(3-Cl)WFL **18** (1:0.2, red), and Mdm2:F(3-Cl)WFL **18** (1:0.5, green) are overlaid. Mdm2 assignments are previously described in the literature.[1,2] (**b**) Mean chemical shift difference (ΔδNH) plot depicting changes in residues.[3]

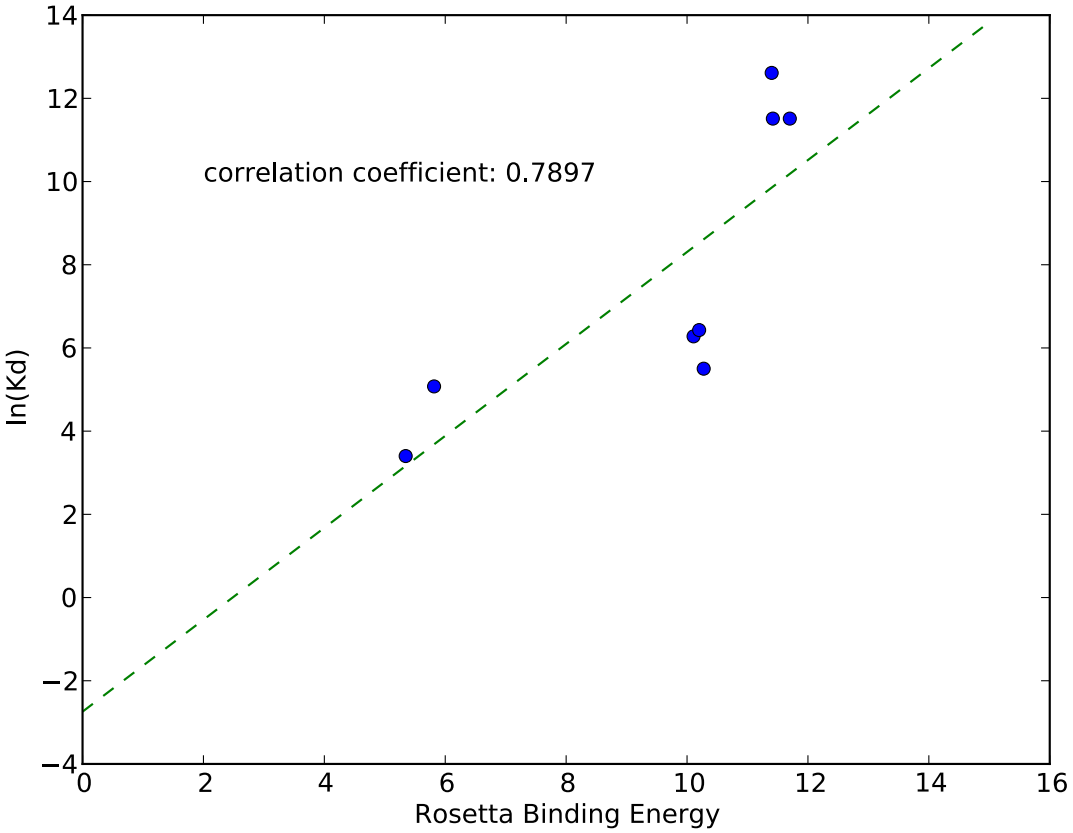


Figure S5. Correlation of Rosetta binding energy predictions with experimental K_d for p300–CH1 ligands. Data points are taken from Table S4, and correlation coefficient was calculated using Python's scipy.stats.stats.pearsonr function. Rosetta binding energy (x axis) approximates free energy of binding which is proportional to ln(Kd). Explicitly: deltaG = RTln(Kd) where R = ideal gas constant and T = temperature.

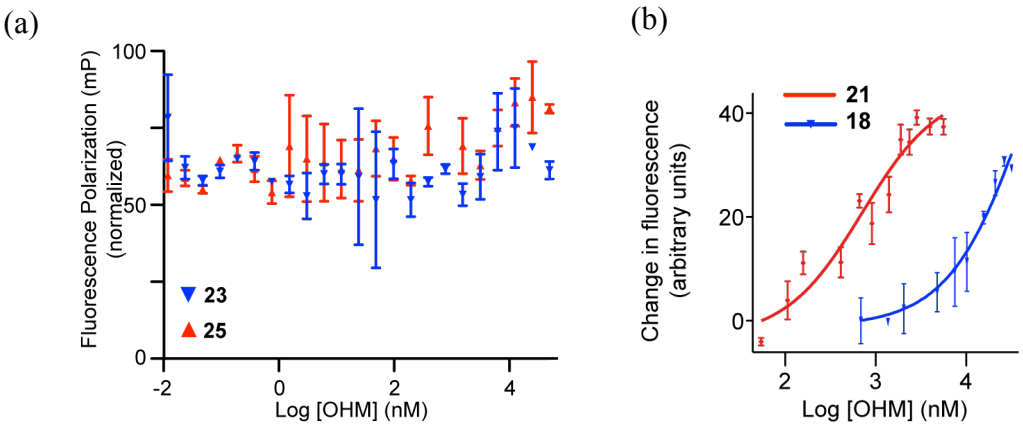


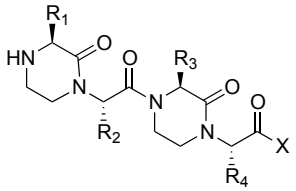
Figure S6. Determination of oxopiperazine analog binding to (a) His_6-tagged Mdm2 by a fluorescence-polarization assay and (b) p300–CH1 by a tryptophan fluorescence spectroscopy.

Table S1: Noncanonical amino acid design library for Rosetta applied to MDM2 target

Position R_1 Rosetta code: noncanonical amino acid name
NC A12: 2.4-dimethyl-phenylalanine
NC A31: 2-amino-5-phenyl-pentanoic_acid
NC A34: 2-aminomethyl-phenylalanine
NC A43: 2-hydroxy-phenylalanine
NC A48: 2-methyl-phenylalanine
NC A68: 3-aminomethyl-phenylalanine
NC A69: 3-amino-tyrosine
NC A78: 3-hydroxy-phenylalanine
NC A80: 3-hydroxy-tyrosine
NC A84: 3-methyl-phenylalanine
NC A94: 4-aminomethyl-phenylalanine
NC APA: 4-amino-phenylalanine
NC B12: 4-carboxy-phenylalanine
NC B27: 4-methyl-phenylalanine
NC B30: 4-phenyl-phenylalanine
NC B31: 4-tert-butyl-phenylalanine
NC B96: beta.beta-diphenyl-alanine
NC C43: phenyl-serine
NC B67: beta-(1-naphthyl)-alanine
NC B92: beta-beta-dicyclohexyl-alanine_boat_boat
NC B93: beta-beta-dicyclohexyl-alanine_boat_chair
NC B94: beta-beta-dicyclohexyl-alanine_chair_boat
NC B95: beta-beta-dicyclohexyl-alanine_chair_chair
NC B99: beta-cyclohexyl-alanine_boat
NC C00: beta-cyclohexyl-alanine_chair
NC C01: beta-cyclopentyl-alanine
NC C02: beta-cyclopentyl-alanine_puck
NC C11: cyclohexyl-glycine_boat
NC C12: cyclohexyl-glycine_chair
NC B21: 4-hydroxy-phenylglycine
NC B44: 9-anthryl-alanine
NC B67: beta-(1-naphthyl)-alanine
NC B74: beta-(2-naphthyl)-alanine
NC C15: diphenylglycine
NC C42: phenylglycine
NC C95: 3-chloro-phenylalanine

Position R_4 Rosetta code: noncanonical amino acid name
NC A30: 2-amino-4-bromo-4-pentenoic_acid
NC A91: 4.5-dehydro-leucine
NC B47: allo-isoleucine
NC C91: fluoro-leucine_ent1
NC C92: fluoro-leucine_ent2
NC C93: hexafluoro-leucine
NC C61: trifluoro-leucine
NC C94: trifluoro-leucine_ent2
NC HLU: homoleucine
NC A20: 2-allyl-glycine
NC ABA: amino-butyric acid
NC NLU: norleucine
NC NVL: norvaline

Table S2: Rosetta predicted binding energy vs. experimental K_d (μM) for MDM2 target



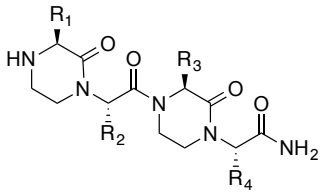
Mimetic	R_1	R_2	R_3	R_4	K_d (μM)[a]	Rosetta binding energy
18	Phe(3-Cl)	Trp	Phe	Leu	0.3 ± 0.04	-8.7
13	Phe	Trp	Phe	Nle	2.5 ± 0.5	-8.4
1	Phe	Trp	Ala	Leu	65 ± 8	-7.9
19	Phe(3-Me)	Trp	Phe	Leu	2.6 ± 0.04	-7.9
16	Tyr	Trp	Phe	Leu	0.4 ± 0.05	-7.3
20	Phe(4-Cl)	Trp	Phe	Leu	1.3 ± 0.1	-6.2
5	Phe	Trp	Phe	Leu	2.9 ± 0.1	-5.1
15	Nap	Trp	Phe	Leu	0.9 ± 0.1	-5.0
7	Lys	Trp	Phe	Leu	≥200	-4.5
8	Phe	Ala	Phe	Leu	64 ± 7	-4.5
23	Leu	Nle	Ala	Gln	>50	0.6
25	Hle	Hle	Ala	Gln	>50	2.2
6	Phe	Trp	Phe	Lys	≥200	5.5

Table S3: Noncanonical amino acid design library for Rosetta applied to p300 target

Positions R_1 and **R_2** Rosetta code: noncanonical amino acid name	
NC A04: 1-amino-cyclopentane-carboxylic_acid_puck1	NC B96: beta.beta-diphenyl-alanine
NC A05: 1-amino-cyclopentane-carboxylic_acid_puck2	NC B97: beta-chloro-alanine
NC A12: 2.4-dimethyl-phenylalanine	NC B99: beta-cyclohexyl-alanine_boat
NC A20: 2-allyl-glycine	NC C00: beta-cyclohexyl-alanine_chair
NC A24: 2-amino-2-phenylbutyric_acid	NC C01: beta-cyclopentyl-alanine
NC A30: 2-amino-4-bromo-4-pentenoic_acid	NC C02: beta-cyclopentyl-alanine_puck
NC A31: 2-amino-5-phenyl-pentanoic_acid	NC C03: beta-fluoro-alanine
NC A33: 2-amino-heptanoic_acid	NC C05: beta-iodo-alanine
NC A34: 2-aminomethyl-phenylalanine	NC C11: cyclohexyl-glycine_boat
NC A44: 2-indanyl-glycine_puck1	NC C12: cyclohexyl-glycine_chair

NC A45: 2-indanyl-glycine_puck2 NC A48: 2-methyl-phenylalanine NC A84: 3-methyl-phenylalanine NC A91: 4.5-dehydro-leucine NC B04: 4-amino-tetrahydrothiopyran-4-carboxylic_acid_boat1 NC B05: 4-amino-tetrahydrothiopyran-4-carboxylic_acid_boat2 NC B06: 4-amino-tetrahydrothiopyran-4-carboxylic_acid_chair1 NC B07: 4-amino-tetrahydrothiopyran-4-carboxylic_acid_chair2 NC C90: 4-fluoro-tryptophan NC B27: 4-methyl-phenylalanine NC B28: 4-methyl-tryptophan NC B30: 4-phenyl-phenylalanine NC B31: 4-tert-butyl-phenylalanine NC C80: 5-bromo-tryptophan NC C81: 5-chloro-tryptophan NC B35: 5-fluoro-tryptophan NC B38: 5-methyl-tryptophan NC C83: 6-bromo-tryptophan NC C84: 6-chloro-tryptophan NC C85: 6-fluoro-tryptophan NC B40: 6-methyl-tryptophan NC C86: 7-azatryptophan NC C87: 7-bromo-tryptophan NC C88: 7-methyl-tryptophan NC B44: 9-anthryl-alanine NC ABA: aminobutyric acid NC B47: allo-isoleucine NC B57: alpha-methyl-leucine NC B58: alpha-methyl-phenylalanine NC B60: alpha-methyl-tryptophan NC B62: alpha-methyl-valine NC B67: beta-(1-naphthyl)-alanine NC B74: beta-(2-naphthyl)-alanine NC B92: beta-beta-dicyclohexyl-alanine_boat_boat NC B93: beta-beta-dicyclohexyl-alanine_boat_chair NC B94: beta-beta-dicyclohexyl-alanine_chair_boat NC B95: beta-beta-dicyclohexyl-alanine_chair_chair	NC C15: diphenylglycine NC C16: dipropyl-glycine NC C20: ethionine NC C91: fluoro-leucine_ent1 NC C92: fluoro-leucine_ent2 NC C93: hexafluoro-leucine NC HLU: homoleucine NC C26: homocysteine NC C27: homophenylalanine NC MAL: MAL NC C12: MPA NC C36: n-in-methyl-tryptophan NC NLU: norleucine NC NVL: norvaline NC C41: penicillamine NC C42: phenylglycine NC C53: tert-butyl-alanine NC C54: tert-butyl-cysteine NC C55: tert-butyl-glycine NC C60: trifluoro-alanine NC C61: trifluoro-leucine NC C94: trifluoro-leucine_ent2

Table S4: Rosetta predicted binding energy vs. experimental K_d (nM) for p300 target



Mimetic	R_1	R_2	R_3	R_4	K_d (μM)[a]	
23	Leu	Nle	Ala	Gln	0.03 ± 0.01	5.34
25	Hle	Hle	Ala	Gln	0.16 ± 0.06	5.80
30	Leu	Hle	Ala	Gln	ND	6.42
31	Hle	Nle	Ala	Gln	ND	9.63
21	Leu	Leu	Ala	Gln	0.53 ± 0.14	10.1
26	Leu	Leu	Ala	Ala	0.62 ± 0.27	10.2
24	Met	Met	Ala	Gln	0.24 ± 0.04	10.3
28	Hle	Leu	Ala	Gln	ND	10.4
22	Leu	Ala	Ala	Gln	>10	11.4
18	Phe(3-Cl)	Trp	Phe	Leu	>30	11.4
27	Ala	Ala	Ala	Ala	>10	11.7
29	Met	Leu	Ala	Gln	ND	11.9

*ND = not determined

Synthesis of oxopiperazine dimers. Commercial grade solvents and reagents were used without further purification. Fmoc amino acids and peptide synthesis reagents were purchased from Novabiochem. Molecular biology grade salts and buffers were obtained from Sigma. An Fmoc amino acid linked to Knorr Rink Amide resin was extended to a dipeptide using standard Fmoc solid phase peptide synthesis methods in a solid phase reaction vessel.[4] The resultant dipeptide was deprotected with 20% piperidine/dimethylformamide (DMF) and resin was washed sequentially with DMF, dichloromethane (DCM), methanol (MeOH), and diethyl ether and dried under vacuum. *o*-Nitrobenzenesulfonyl chloride (Ns-Cl, 10 eq) and collidine (10 eq) were dissolved in dry DCM and added to the reaction vessel. The mixture was shaken for 2 hours at 23°C to obtain **II**. The resin was washed with sequentially with DMF, DCM, MeOH, and diethyl ether and dried for 12 hours under vacuum. The resin was transferred to a glass microwave tube (CEM). Triphenylphosphine (PPh_3, 10 eq) was added and the tube was flushed with nitrogen gas for 30 minutes. Tetrahydrofuran (THF), diisopropyl azodicarboxylate (DIAD, 10 eq), and 2-bromoethanol (10 eq) were added and the reaction mixture was subjected to microwave irradiation (200 watts, 250 psi) for 10 minutes at 100°C. Resin was washed sequentially with THF, DMF, and DCM. The resin was transferred to a solid phase vessel and treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in THF for 2 hours. Resin was washed with THF, DMF, DCM, and diethyl ether, allowed to dry for 30 minutes, and then treated with DBU and 2-mercaptoethanol in DMF for 2 hours. Compound **III** was then washed with DMF, DCM, MeOH and diethyl either and dried. The desired pre-activated Fmoc-amino acid was added to the resin and the mixture was shaken at 23°C for 12 hours affording **IV**. Nosyl protection and the ring formation steps were repeated to obtain oxopiperazine dimers **V** after

cleavage from the resin with 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisopropylsilane (TIPS).

Mdm2 Protein Expression and Purification. Competent BL21 DE3 pLySS E. coli cells were transformed by the heat shocking the bacteria at 42° C for 1 min in media containing a pET-14B vector containing a His6-tagged $MDM2_{25\text{-}117}$ fusion protein. Cells were grown on ampicillin containing agar places (50 mg/mL), and a single culture was used to inoculate a 100 mL overnight culture of LB media containing ampicillin (50 mg/mL). 500 mL of terrific broth (4L flask) was seeded with 25 mL of overnight culture and incubated at 30° C for 1.5 hours before induction of protein expression with 0.4 mM IPTG. The flask was incubated at 30° C for an additional 4.5 hours. The cells were harvest by centrifugation at 6000 g for 20 minutes and the supernatant was discarded. The cells were resuspended in 50 mL binding buffer (0.5 M NaCl, 20 mM Tris-HCl, 5 mM imidazole, pH 7.9), and lysed by sonication in ice (10 x 10 seconds pulses over 2 minutes). The cells were again centrifuged at 15,000 g for 20 minutes, and the resulting supernatant containing the desired MDM2 fusion protein was purified using a His-Bind® column affinity purification kit (Novagen). The resulting protein was dialyzed in 10 mM PBS with 5 mM EDTA and 0.5 mM DTT and characterized by SDS-PAGE analysis.

Expression of ^{15}N-Mdm2. The pET-14B vector containing a His6-tagged $MDM2_{25\text{-}117}$ fusion protein was transformed into BL21(DE3) competent *E.coli* (Novagen) in M9 minimal media with $^{15}NH_4Cl$ as the main nitrogen source. Protein production was induced with 0.4 mM IPTG at O.D.600 and incubated for 16 hours at 15 °C. Bacteria were harvested and resuspended in the lysis buffer with 20 mM Phosphate buffer (Research Products International, Corp.), 100 µM DTT (Fisher), 1 mM EDTA (Sigma), 0.5% TritonX 100 (Sigma), 1 mg/mL Pepstatin A

(Research Products International, Corp.), 10 mg/mL Leupeptin A (Research Products International, Corp.), 500 μM PMSF (sigma), and 0.5% glycerol at pH 8.0. Pellets were lysed by sonication and centrifuged at 4 ^{o}C 20,000 rpm for 20 min. Fusion protein was collected from the bacterial supernatant and the resulting supernatant containing the desired MDM2 fusion protein was purified using a His-Bind® column affinity purification kit (Novagen). The resulting protein was dialyzed in 10 mM PBS with 5 mM EDTA and 0.5 mM DTT and characterized by SDS-PAGE analysis.

p300-CH1 Plasmids. The DNA sequence of human p300 CH1 domain (amino acid residues 323-423) was subcloned into a pUC57 plasmid by Genscript, Inc. After transformation of JM109 bacteria (Promega) with the plasmid, it was amplified and purified. Then the gene of interest was subcloned between *Bam*HI and *Eco*RI restriction sites of pGEX-4T-2 expression vector (Amersham).

p300–CH1 Protein Expression and Purification. The pGEX-4T-2-p300 fusion vector was transformed into BL21(DE3) competent *E.coli* (Novagen) in M9 minimal media with $^{15}NH_4Cl$ as the primary nitrogen source. Protein production was induced with 1 mM IPTG at OD_{600} of 1 for 16 hours at 15 °C. Production of the desired p300-CH1-GST fusion product was verified by SDS-PAGE. Bacteria were harvested and resuspended in the lysis buffer with 20 mM Phosphate buffer (Research Products International, Corp.), 100 μM DTT (Fisher), 100 μM $ZnSO_4$ (Sigma), 0.5% TritonX 100 (Sigma), 1 mg/mL Pepstatin A (Research Products International, Corp.), 10 mg/mL Leupeptin A (Research Products International, Corp.), 500 μM PMSF (Sigma), and 0.5% glycerol at pH 8.0. Pellets were lysed by sonication and centrifuged at 4 ^{o}C and 20,000 rpm for

20 min. Fusion protein was collected from the bacterial supernatant and purified by affinity chromatography using glutathione Sepharose 4B beads (Amersham) prepared according to the manufacturer's directions. GST-tag was cleaved by thrombin and protein was eluted from resin. Collected fractions were assayed by SDS-PAGE gel; pooled fractions were treated with protease inhibitor cocktail (Sigma) and against a buffer containing 10 mM Tris, 50 mM NaCl, 2mM DTT (Fisher), 3 equivalents $ZnSO_4$ at pH 8.0 to ensure proper folding (*vide supra*).

^{1}H-^{15}N HSQC NMR Spectroscopy. Protein samples were prepared as described above. Uniformly ^{15}N-labeled N-terminal His_6-tagged $Mdm2_{25\text{-}117}$ was concentrated to 50 μM in NMR buffer (10 mM PBS pH 7.4, 5 mM EDTA, 0.5 mM DTT) using 3 kDa MWCO Amicon Ultra centrifugal filter (Millipore) and supplemented with 5% D_2O. For HSQC titration experiments, data was collected on a 600 MHz Bruker four-channel NMR system at 25 ºC and analyzed with the TopSpin software (Bruker).

For the HSQC titration experiments, 0.2 and 0.5 molar equivalents of compound **18** (F(3-Cl)WFL) in DMSO were added to ^{15}N-labelled Mdm2, and the data was collected as described above. Mean chemical shift difference ($\Delta\delta_{NH}$) observed for ^{1}H and ^{15}N nuclei of various resonances were calculated as described,[3] where α is the range of H ppm shifts divided by the range of NH ppm shifts (α = 1/9).

$$d = \sqrt{\frac{1}{2}[\delta_H^2 + (\alpha \cdot \delta_N^2)]} \qquad \textbf{(1)}$$

Fluorescence Polarization Assay. The relative affinity of peptides for ^{15}N-labeled N-terminal His_6-tagged $MDM2_{25-117}$ was determined using fluorescence polarization binding assay with fluoresceine-tagged p53. The polarization experiments were performed with a DTX 880 Multimode Detector (Beckman) at 25° C, with excitation and emission wavelengths of 485 and 525 nm, respectively. Addition of an increasing concentration (0 nm to 3.5 μM) of MDM2 protein to a 15 nM solution of fluorescein labeled p53 peptide in 10 mM PBS pH 7.4, 5 mM EDTA, 0.5 mM DTT, and 0.1% pluronic F-68 (Sigma) in 96 well plates afforded the IC_{50} value, which was fit into equation (**2)** to calculate the dissociation constant (K_D) for the Mdm2/p53 complex.[5] The binding affinity (K_D) reported for each peptide is the average of three individual experiments, and were determined by fitting the experimental data to a sigmoidal dose-response nonlinear regression model on GraphPad Prism 5.0.

$$K_D = (R_T \times (1\text{-}F_{SB}) + L_{ST} \times F_{SB}^2)/F_{SB}\text{-}L_{ST} \qquad (\mathbf{2})$$

where,

R_T = Total concentration of Mdm2

L_{ST} = Total concentration of fluorescent peptide

F_{SB} = Fraction of bound fluorescent peptide

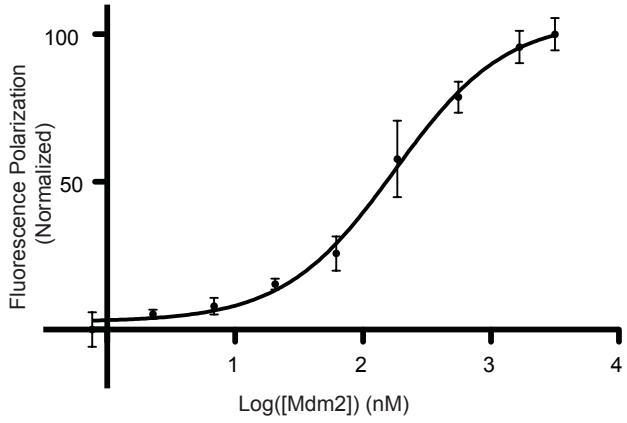


Figure S7: Direct binding of Flu-p53 to Mdm2.

The K_D of Flu-p53 was determined to be 169 ± 7 nM. For competitive inhibition experiments, a solution of 250 nM Mdm2 and 15 nM Flu-p53 in buffer (10 mM PBS pH 7.4, 5 mM EDTA, 0.5 mM DTT, and 0.1% pluronic F-68) was incubated at 25 ºC in a 96 well plate. After 30 minutes appropriate concentrations of the oxopiperazine were added to the Mdm2/Flu-p53 solution and the resulting mixtures were incubated at 25° C for 30 minutes before measuring the degree of dissociation of Flu-p53 by polarization. The EC_{50} was fit into equation (**2**) to calculate the K_{D2} value of the oxopiperazine**.** The inhibition curves are shown in the main manuscript.

$$K_{D2} = K_D*F_{SB}*((L_T/(L_{ST}*F_{SB2} - (K_D + L_{ST} + R_T)*F_{SB} + R_T)) - 1/(1 - F_{SB})) \qquad (3)$$

where:

K_D = K_D of fluorescent probe Flu-p53

R_T = Total concentration of Mdm2 protein

L_{ST} = total concentration of p53 fluorescent peptide

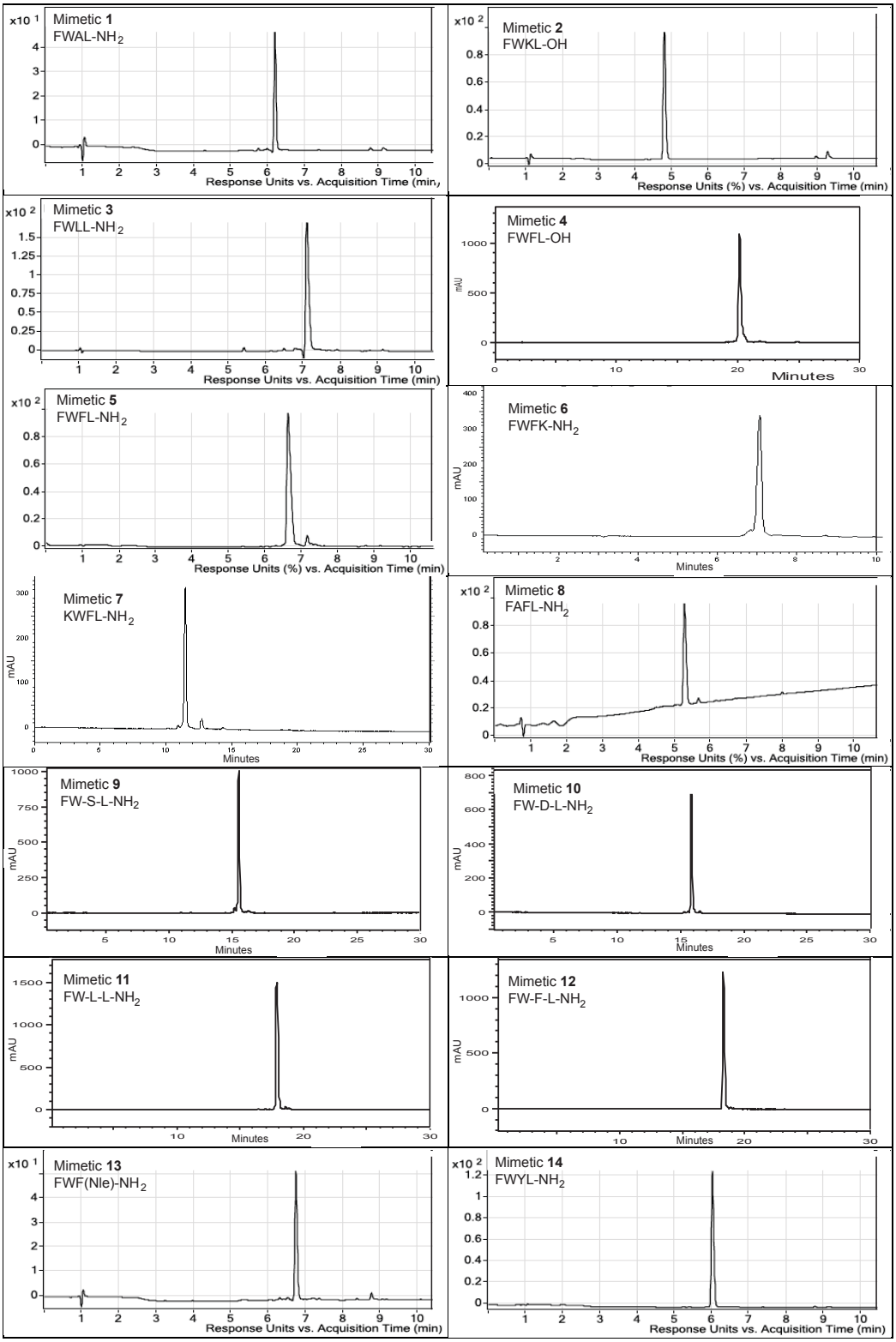
F_{SB} = Fraction of bound oxopiperazine (at EC_{50})

L_T = total concentration of oxopiperazine (EC_{50})

Tryptophan Fluorescence Binding Assay. Spectra were recorded on a QuantaMaster 40 spectrofluorometer (Photon Technology International) in a 10 mm quartz fluorometer cell at 25°C with 4 nm excitation and 4 nm emission slit widths from 200 to 400 nm at intervals of 1 nm/s. Samples were excited at 295 nm and fluorescence emission was measured from 200-400 nm and recorded at 335 nm. Peptides stock solutions were prepared in DMSO. Aliquots containing 1 μL DMSO stocks were added to 400 μL of 1 μM p300-CH1 in 50 mM Tris and 100 mM NaCl (pH 8.0). After each addition, the sample was allowed to equilibrate for 5 minutes before UV analysis. Background absorbance and sample dilution effects were

corrected by titrating DMSO into p300-CH1 in an analogous manner. Final fluorescence is reported as the absolute value of $[(F_1-F_0)/F_1]*100$, where F_1 is the final fluorescence upon titration, and F_0 is the fluorescence of the blank DMSO titration. EC_{50} values for each peptide were determined by fitting the experimental data to a sigmoidal dose-response nonlinear regression model in GraphPad Prism 5.0, and the dissociation constants, K_D, were obtained from equation (**2)**.

Compound Characterization



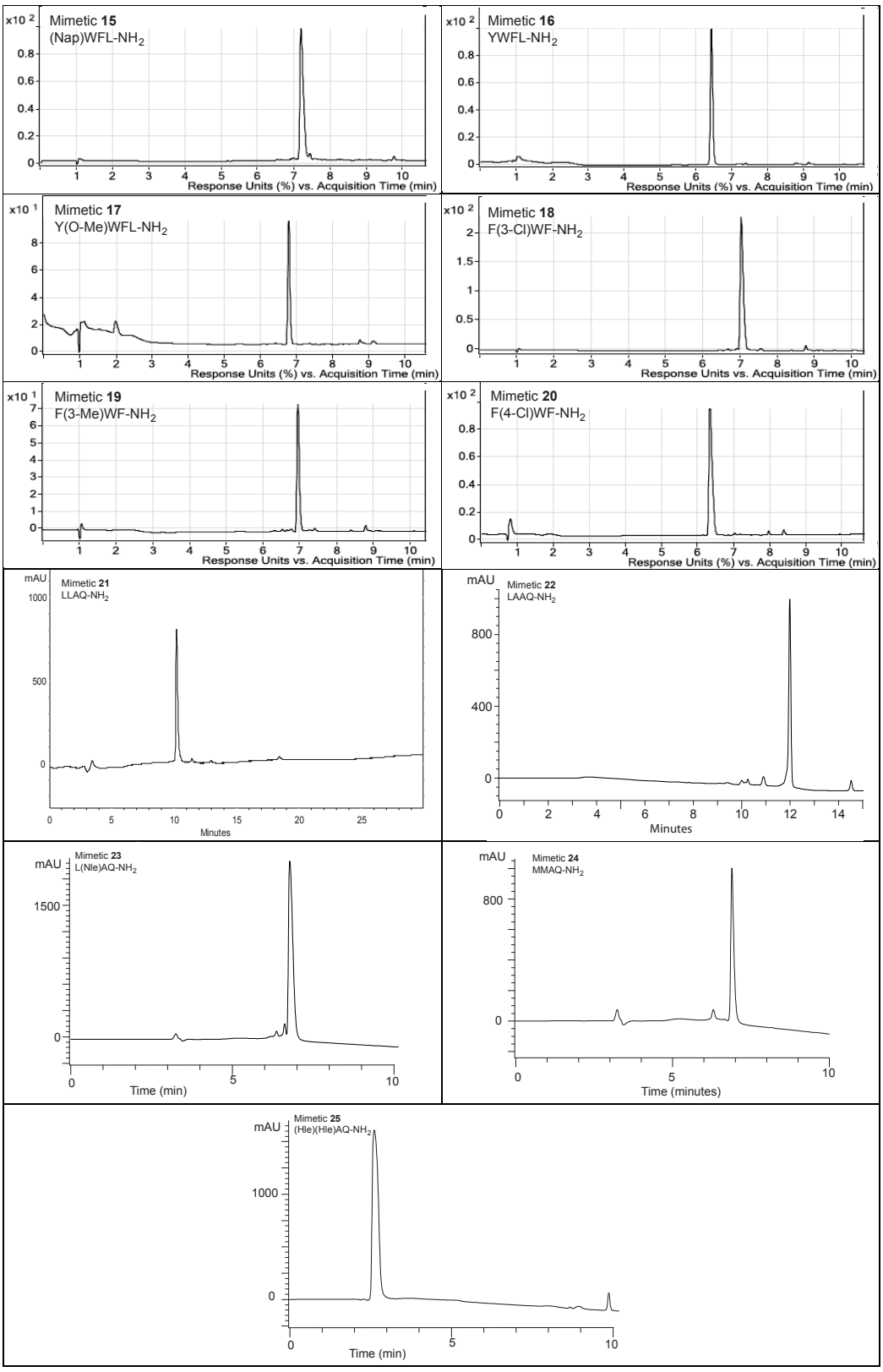
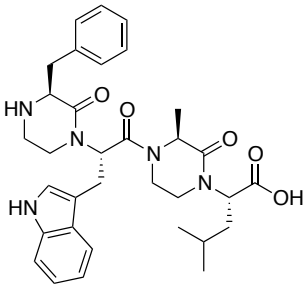


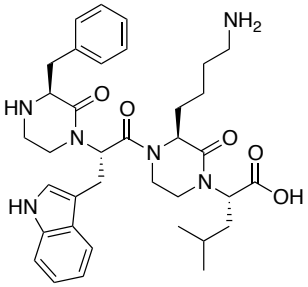
Figure S6: Analytical HPLC traces of oxopiperazines and monomer-peptides. HPLC conditions: Compounds **1-3, 5**,**13-20** : 5% to 95% acetonitrile in water (0.1% formic acid) in 10 minutes. UV trace at 280 nm.

Compounds **4**, **7**, **9-12**: 5% to 95% acetonitrile in water (0.1% trifluoroacetic acid) in 30 minutes. UV trace at 280 nm.
Compounds **6**: 5% to 95% acetonitrile in water (0.1% trifluoroacetic acid) in 10 minutes. UV trace at 280 nm.
Compounds **8** : 5% to 95% acetonitrile in water (0.1% formic acid) in 10 minutes. UV trace at 220 nm.
Compound **21**: 5% to 95% acetonitrile in water (0.1% formic acid) in 30 minutes. UV trace at 220 nm.
Compound **22**: 5% to 95% acetonitrile in water (0.1% trifluoroacetic acid) in 10 minutes; 95% to 100% from 10-15 minutes. UV trace at 230 nm.
Compound **23-25**: 5% to 95% acetonitrile in water (0.1% trifluoroacetic acid) in 10 minutes. UV trace at 220 nm.

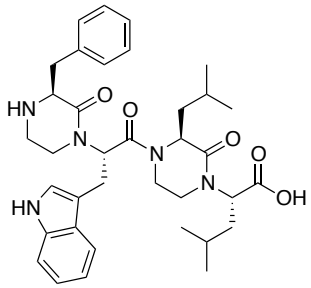
Experimentals



Oligooxopiperazine **1**- FWAL-OH
^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.93 (s, 0.3H), 10.89 (s, 0.7H), 9.35 (br, 1.0H), 8.23 (br, 1.0H), 7.64 (d, *J* = 7.86, 0.8H), 7.60 (d, *J* = 7.92, 0.2H), 7.37-7.14 (m, 5.0H), 7.13-6.97 (m, 3.0H), 5.72 (dd, *J* = 8.58, 6.48, 0.8H), 5.59 (app. t, *J* = 7.62, 0.2H), 4.97 (dd, *J* = 11.46, 7.02, 0.8H), 4.95-4.92 (m, 0.2H), 4.63 (q, *J* = 6.86, 1.0H), 4.15 (br, 1.0H), 3.80 (dt, *J* =13.60, 3.60, 1.0H), 3.59 (br, 1.0H), 3.51-3.44 (m, 1.0H), 3.27-3.15 (m, 3.0H), 3.14-3.07 (m, 1.0H), 3.05-2.95 (m, 1.0H), 2.58-2.53 (m, 4.0H), 1.80-1.68 (m, 1.0H), 1.66-1.52 (m, 1.0H), 1.42-1.32 (m, 1.0H), 1.29 (d, *J* = 7.02, 2.0H), 1.01 (d, *J*= 6.55, 0.6H), 0.92 (d, *J* = 6.55, 0.4H), 0.89 (d, *J* = 6.55, 2.1H), 0.87 (d, *J* = 6.55, 0.9H), 0.83 (d, *J* = 6.55, 2.1H), 0.81 (d, *J* = 6.55, 0.9H). HRMS (ESI) $C_{33}H_{41}N_5O_5$ $[M+H]^+$ calc'd= 588.3108; found = 588.3311

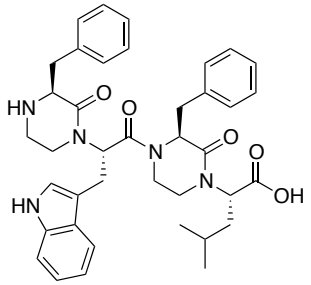


Oligooxopiperazine **2-** FWKL-OH
^{1}H-NMR (400 MHz, MeOD) (data for the major rotamer) δ 7.70 (d, *J* = 8.90, 1.0H), 7.60 (d, *J* = 7.8, 0.5H), 7.50 (d, *J* = 7.4, 0.5H), 7.40-7.20 (m, 4.0H), 7.20-6.90 (m, 4.0H), 5.80-5.67 (m, 2.0H), 5.12-5.00 (m, 2.0H), 4.49-4.40 (m, 1.6H), 4.34 (app d, *J* = 11.90, 1.4H), 4.24-4.08 (m, 2.5H), 3.82-3.51 (m, 5.5H), 2.98-2.75 (m, 5.0H), 2.71-2.58 (m, 2.0H), 2.26 (t, *J* = 7.90, 1.7H), 1.80-1.56 (m, 4.3H), 1.50-1.31 (m, 2.8H), 1.29-1.17 (m, 2.3H), 1.15 (s, 0.6H), 0.88 (d, *J* = 6.70, 3.0H), 0.82 (d, *J* = 6.10, 3.0H). HR-MS (ESI) $C_{36}H_{48}N_6O_5$ $[M+H]^+$ calc'd= 645.3686; found= 645.4044



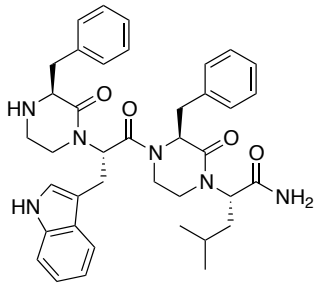
Oligooxopiperazine **3**- FWLL

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.89 (br s, 1.0H), 9.34 (br d, 1.0H), 7.62 (d, *J* = 7.84, 1.4H), 7.60 (d, *J* = 7.84, 0.6H), 7.34 (d *J* = 7.84, 2.0H), 7.30 (br s, 1.0H), 7.22-7.11 (m, 2.0H), 7.11-7.06 (m, 1.0H), 7.01 (q, *J* = 7.53, 1.5H), 6.53 (s, 0.5H), 5.73 (t, *J* = 7.40, 0.6H), 5.60-5.51 (m, 0.6H), 4.96 (dd, *J* = 11.85, 7.50, 0.4H), 4.90 (dd, *J* = 11.74, 7.08, 0.6H), 4.77 (t, *J* = 6.69, 0.6H), 4.52 (br, 0.4H), 3.84 (br, 1.0H), 3.56 (br, 1.5H), 3.23-3.17 (m, 3.5H), 3.09 (br, 1.0H), 3.02 (br, 1.0H), 2.87 (br, 1.0H), 1.78-1.69 (m, 1.5H), 1.66- 1.61 (m, 0.5H), 1.60-1.50 (m, 4.0H), 1.36-1.25 (m, 1.0H), 1.23 (s, 0.5H), 1.12 (br, 0.5H), 0.92-0.86 (m, 9.0H), 0.80 (d, *J* = 6.48, 1.0H) 0.78 (d, *J* = 6.48, 1.0H), 0.64 (d, *J*= 6.48, 1.0H), 0.56 (br, 0.7H). HRMS (ESI) $C_{36}H_{47}N_5O_5$ $[M+H]^+$ calc'd= 630.3577; found= 630.4002



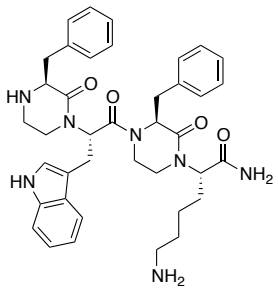
Oligooxopiperazine **4**- FWFL-OH

^{1}H-NMR (400 MHz, MeOD) δ 7.51 (d, *J*= 7.88, 0.4H), 7.40 (d, *J* = 7.88, 0.6H), 7.34-7.20 (m, 5.0H), 7.16-6.97 (m, 8.4H), 6.79 (s, 0.6H), 5.85 (app. t, *J* = 7.90, 0.4H), 5.23 (t, *J* = 7.56, 0.6H), 5.20 (dd, *J* = 11.20, 6.08, 0.6H), 4.97 (t, *J* = 5.67, 0.4H), 4.93-4.87 (m, 1.0H), 4.42 (br, 0.6H), 4.21 (dd, *J* = 9.36, 4.84, 0.4H), 4.09 (dd, *J*= 9.88, 5.88, 0.6H), 3.78 (dt, *J* = 13.49, 3.63, 0.4H), 3.67-3.55 (m, 1.0H), 3.46 (app. t, *J* = 5.05, 1.3H), 3.44-3.39 (m, 0.5H), 3.35-3.30 (m, 0.5H), 3.29 (app. t, *J* = 3.72, 0.7H), 3.20-3.04 (m, 4.0H), 3.00-2.93 (m, 1.5H), 2.85 (dd, *J* = 14.9, 9.38, 0.4H), 2.69-2.62 (m, 1.0H), 2.50 (dd, *J* = 14.89, 9.79, 0.6H), 2.46-2.38 (m, 0.5H), 1.80-1.64 (m, 1.5H), 1.60-1.36 (m, 2.0H), 1.28-1.07 (m, 1.5H), 0.91 (d, *J* = 6.96, 2.0H), 0.89 (d, *J* = 6.76, 2.0H), 0.84 (d, *J* = 6.40, 1.0H), 0.80 (d, *J* = 6.64, 1.0H). LRMS $C_{39}H_{45}N_5O_5$ $[M+H]^+$ calc'd= 664.3; found= 664.2



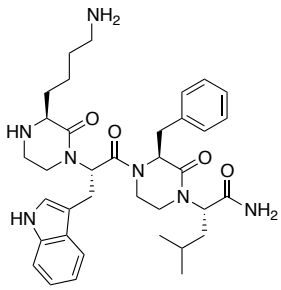
Oligooxopiperazine **5**- FWFL-NH_2

^{1}H-NMR (400 MHz, d_6-DMSO) δ 10.89 (app. d, *J* = 1.74, 0.6H), 10.88 (s, 0.4H), 9.26 (br d, 2.0H), 7.57 (d, *J* =7.76, 0.7H), 7.45 (d, *J* = 4.95, 0.7H), 7.42 (d, *J* = 4.06, 1.0H) 7.38-7.25 (m, 4.0H), 7.24-7.18 (m, 3.2H), 7.13-7.07 (m, 2.0H), 7.05-6.94 (m, 2.0H), 6.94-6.88 (m, 1.0H), 5.68 (dd, *J* = 8.72, 6.36, 0.6H), 5.32 (t, *J* =7.44, 0.4H), 4.99 (dd, *J* = 10.17, 5.85, 4.32, 1.0H), 4.93 (t, *J* = 6.30, 0.6H), 4.78-4.72 (m, 0.4H), 4.20 (br, 0.5H), 4.17 (br, 0.5H), 3.75 (br, 0.5H), 3.71 (br, 0.5H), 3.26-3.04 (m, 7.0), 3.00-2.88 (m, 3.0H), 2.85 (br, 0.3H), 2.84 (br, 0.2H), 2.81-2.73 (m, 1.8H), 1.67-1.58 (m, 1.0H), 1.56-1.47 (m, 2.0H), 1.32 (br, 1.0H), 1.24 (br, 0.5H), 1.16 (br, 1.5H), 0.95-0.86 (m, 4.0H), 0.84 (d, *J* = 6.55, 2.0H). HRMS (ESI) $C_{39}H_{46}N_6O_4$ $[M+H]^+$ calc'd= 663.3581; found= 663.3917



Oligoxopiperazine **6**- FWFK-NH_2

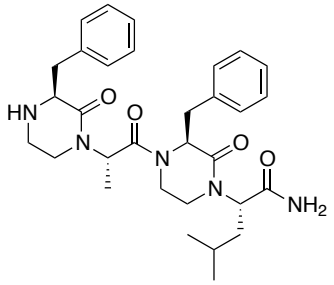
^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.88 (br, 1.0H), 8.46 (br, 0.7H), 9.30 (br d, 2.0H), 7.65 (br, 2.0H), 7.55 (d, *J*= 8.10, 0.3H), 7.52 (br, 0.3H), 7.49 (br, 0.3H), 7.40 (br, 1.0H), 7.39-7.25 (m, 4.0H), 7.24-7.06 (m, 6.0H), 7.04-6.98 (m, 1.0H), 6.92 (br, 0.7H), 6.76 (br, 0.3H), 6.53 (br, 2.0H), 5.88 (br, 0.4H), 5.69 (dd, *J*= 9.00, 6.42, 0.6H), 5.30 (br, 0.5H), 4.99 (br, 1.0H), 4.94-4.85 (m, 1.0H), 4.76 (br, 0.5H), 4.25 (br, 2.0H), 3.21-3.05 (m, 4.0H), 3.04-2.88 (m, 3.0H), 2.82-2.72 (m, 3.0H), 2.09 (s, 0.2H), 1.88-1.74 (m, 1.0H), 1.69-1.60 (m, 1.0H), 1.59-1.43 (m, 3.0H), 1.28-1.0 (m, 3.0H). HRMS (ESI) $C_{39}H_{47}N_7O_4$ $[M+H]^+$ cal'd= 678.3846; found= 678.3861



Oligooxopiperazine **7**- KWFL-NH_2

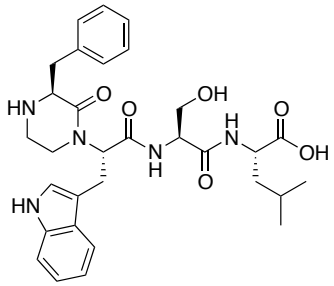
^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.78 (br, 1.0H), 7.99 (br, 2.0H), 7.75 (br, 1.0H), 7.42 (t, *J* = 6.63, 2.0H), 7.38-7.27 (m, 3.0H), 7.20 (d, *J* = 7.40, 1.5H), 7.08 (t, *J* = 7.17, 1.0H), 7.04-6.97 (m,

1.5H), 6.76 (s, 1.0H), 5.18-5.08 (m, 1.0H), 4.94-4.82 (m, 2.0H), 4.82-4.74 (m, 1.0H), 3.55-3.44 (m, 1.5H), 3.43-3.14 (m, 5.0H), 2.97 (t, *J*= 12.06, 1.0H), 2.87 (br, 1.0H), 2.69 (br, 1.5H), 2.19-2.14 (m, 1.0H), 1.74-1.62 (m, 3.0H), 1.50 (br, 2.0H), 1.34-1.22 (m, 4.0H), 0.99 (d, *J*= 5.55, 6.0H), 0.92-0.77 (m, 4.0H), 0.72 (br, 1.5H), 0.56 (br, 0.5H). HRMS (ESI) $C_{36}H_{49}N_7O_4$ $[M+H]^+$ calc'd= 644.3746; found= 644.4091



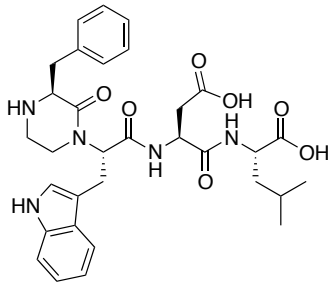
Oligooxopiperazine **8-** FAFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 9.40 (br, 2.0H), 7.46 (s, 0.5H), 7.40-7.27 (m, 5.0H), 7.26-7.19 (m, 2.5H), 7.14-7.11 (m, 1.0H), 7.10-7.04 (m, 1.0H), 6.56 (br, 1.0H), 5.36 (dd, *J* = 13.89, 6.99, 0.6H), 5.06 (q, *J* = 5.32, 0.6H), 5.02 (q, *J* = 5.55, 0.4H), 4.92 (t, *J*= 5.40, 0.6H), 4.79-4.74 (m, 0.4H), 4.73 (t, *J* = 6.74, 0.4H), 4.37 (br, 0.5H), 4.25 (br, 0.3H), 4.10 (br, 0.5H), 3.72 (dt, *J* = 13.75, 3.63, 0.7H), 3.52 (br, 1.0H), 3.47-3.42 (m, 1.0H), 3.41-3.36 (m, 1.5H), 3.30-3.23 (m, 1.5H), 3.20-3.13 (m, 2.4H), 3.06-2.97 (m, 2.6H), 2.87-2.82 (m, 1.0H), 1.72-1.65 (m, 1.0H), 1.60-1.49 (m, 2.3H), 1.42 (br, 0.7H), 1.23 (d, *J*= 6.9, 2.0H), 1.18 (br, 1.0H), 0.94-0.89 (m, 3.0H), 0.86 (d, *J* = 6.55, 2.0H), 0.66 (d, *J* = 6.55, 1.0H). HRMS (APCI) $C_{31}H_{41}N_5O_4$ $[M+H]^+$ calc'd= 548.3159; found= 548.3493



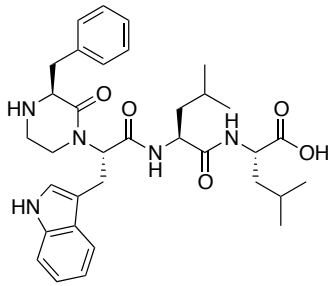
Monomer peptide **9**- FWSL-OH

^{1}H-NMR (400 MHz, d_6-MeOD) δ 8.08 (q, *J*= 7.74, 0.6H), 7.58 (d, *J*= 7.84, 1.0H), 7.30 (d, *J*= 8.08, 1.0H), 7.26-7.17 (m, 3.0H), 7.12-7.03 (m, 3.4H), 7.01-6.96 (m, 1.0H), 5.36 (dd, *J*= 10.5, 5.98, 1.0H), 4.48-4.36 (m, 2.0H), 4.12 (dd, *J*= 9.75, 5.28, 1.0H), 3.81 (d, *J* = 4.92, 0.3H), 3.79 (d, *J* = 4.92, 0.7H), 3.77 (d, *J* = 6.30, 0.3H), 3.74 (d, *J* = 6.30, 0.7H), 3.52 (dd, *J*= 7.20, 3.80, 2.0H) 3.44-3.28 (m, 3.0H), 3.14 (dt, 13.04, 3.68, 1.0H), 3.05-2.96 (m, 1.0H), 2.68 (dd, *J*= 11.96, 9.64, 1.0H), 1.73-1.63 (m, 1.0H), 1.62-1.57 (m, 2.0H), 0.90 (d, *J*= 6.50, 3.0H), 0.87 (d, *J*= 6.50, 3.0H). LRMS $C_{31}H_{39}N_5O_6$ $[M+H]^+$ calc'd= 578.3; found= 578.2



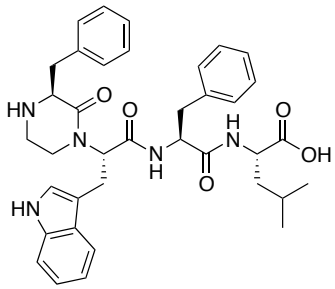
Monomer peptide **10**- FWDL-OH

^{1}H-NMR (400 MHz, d_6-MeOD) δ 8.07 (d, *J*= 8.04, 0.3H), 7.64 (d, *J*= 7.84, 0.7H), 7.38 (d, *J*= 8.04, 1.0H), 7.34-7.25 (m, 3.0H), 7.22-7.11 (m, 4.0H), 7.06 (t, *J*= 7.40, 1.0H), 5.30 (dd, *J*= 9.26, 6.62, 1.0H), 4.49-4.41 (m, 1.0H), 4.14 (dd, *J*= 9.56, 5.36, 1.0H), 3.65-3.54 (m, 1.0H), 3.53-3.36 (m, 4.0H), 3.21 (dt, *J*= 6.24, 3.36, 1.0H), 3.05-2.96 (m, 1.0H), 2.95 (d, *J* = 4.69, 0.4H), 2.91 (d, *J*= 4.6, 0.6H), 2.84-2.74 (m, 2.0H), 1.82-1.73 (m, 1.0H), 1.72-1.63 (m, 2.0H), 0.98 (d, *J*= 6.44, 3.0H), 0.94 (d, *J*= 6.44, 3.0H). LRMS $C_{32}H_{39}N_5O_7$ $[M+H]^+$ calc'd= 606.3; found= 606.2



Monomer peptide **11**- FWLL-OH

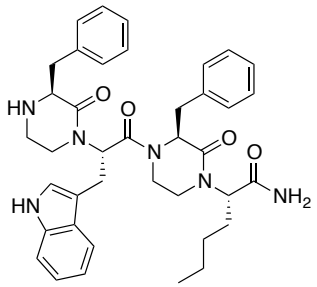
^{1}H-NMR (400 MHz, d_6-MeOD) δ 8.30 (d, *J* = 7.56, 0.6H), 8.22 (d, *J* = 7.88, 0.5H), 7.68 (d, *J* = 7.88, 0.9H), 7.39 (d, *J* = 8.08, 1.0H), 7.33-7.26 (m, 2.7H), 7.19-7.14 (m, 3.3H), 7.07 (t, *J* = 7.30, 1.0H), 5.50 (dd, *J* = 10.11, 5.37, 1.0H), 4.52-4.43 (m, 2.0H), 4.21 (dd, *J* = 9.54, 5.20, 1.0H), 3.76 (br, 1.0H), 3.63-3.55 (m, 1.0H), 3.49-3.36 (m, 3.0H), 3.22 (dt, *J*= 12.96, 3.80, 1.0H), 3.19-3.11 (m, 1.0H), 2.78 (dd, *J*= 14.92, 9.56, 1.0H), 1.82-1.71 (m, 1.0H), 1.70-1.59 (m, 5.0H), 1.02-0.91 (m, 12.0H). LRMS $C_{34}H_{45}N_5O_5$ $[M+H]^+$ calc'd= 604.3; found= 604.2



Monomer peptide **12**- FW-F-L-OH

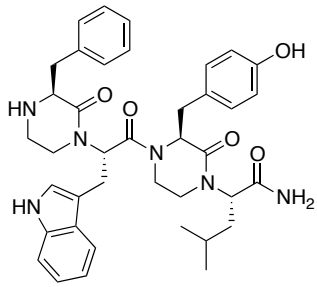
^{1}H-NMR (400 MHz, d_6-MeOD) δ 8.30 (d, *J*= 8.0, 0.4H), 8.13 (d, *J*= 7.92, 0.6H), 7.64 (d, *J*= 7.88, 1.0H), 7.40 (d, *J*= 8.12, 1.0H), 7.35-7.13 (m, 11.0H), 7.08 (t, *J*= 7.42, 1.0H), 5.41 (dd, *J*= 10.08, 6.00, 1.0H), 4.80-4.74 (m, 1.0H), 4.52-4.45 (m, 1.0H), 4.08 (dd, *J*= 9.48, 5.24, 1.0H), 3.56-3.44 (m, 2.0H), 3.43-3.40 (m, 1.0H), 3.39-3.36 (m, 1.0H), 3.29 (d, *J*= 4.84, 1.0H), 3.26 (d, *J*= 4.36, 1.0H), 3.18 (dt, 13.04, 3.56, 1.0H), 2.97-2.87 (m, 2.0H), 2.76 (dd, *J*= 14.86, 9.54, 1.0H),

1.81-1.72 (m, 1.0H), 1.72-1.65 (m, 2.0H), 0.99 (d, J = 6.35, 3.0H), 0.96 (d, J = 6.35, 3.0H). LRMS $C_{37}H_{43}N_5O_5$ $[M+H]^+$ calc'd= 638.3; found= 638.2



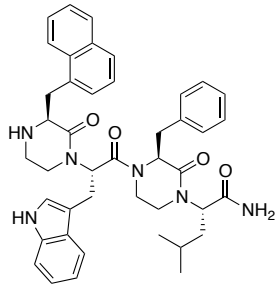
Oligooxopiperazine **13-** FWF(Nle)-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.82 (s, 0.6H), 10.76 (s, 0.4H), 7.56 (d, J = 7.58, 0.5H), 7.41 (d, J = 8.80, 1.0H), 7.32 (t, J= 8.20, 1.0H), 7.29-7.01 (m, 10.0H), 7.08-6.97 (m, 3.0H), 6.53 (br, 1.0H), 5.69 (dd, J = 8.55, 6.37, 0.6H), 5.19 (br, 0.4H), 4.98 (br, 0.6H), 4.88 (dd, J = 10.62, 5.46, 0.6H), 4.86 (dd, J = 10.62, 5.46, 0.4H), 4.72 (dd, J= 8.10, 4.14, 0.4H), 4.26 (br, 0.4H), 3.97 (t, J = 6.69, 0.2H), 3.82 (br, 0.6H), 3.29-3.25 (m, 1.0H), 3.22 (d, J = 3.96, 0.3H), 3.20-3.16 (m, 2.0H), 3.14 (t, J = 3.06, 0.4H), 3.10 (br, 0.3H), 3.09-3.05 (m, 1.7H), 3.02 (br, 0.5H), 2.98 (dt, J = 12.90, 3.57, 1.7H), 2.87 (br, 2.0H) 1.80-1.70 (m, 1.4H), 1.63-1.57 (m, 0.6H), 1.56-1.47 (m, 1.0H), 1.32-1.25 (m, 3.0H), 1.23 (s, 2.0H), 1.16-1.10 (m, 1.0H) 1.08-1.02 (m, 0.8H), 1.0-0.93 (m, 0.6H), 0.88 (app q, J= 7.38, 3.0H). HRMS (ESI) $C_{39}H_{46}N_6O_4$ $[M+H]^+$ calc'd= 663.3581; found= 663.3908



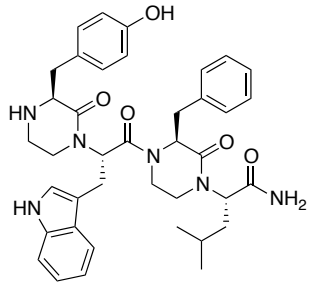
Oligooxopiperazine **14-** FWYL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.92 (d, J= 1.62, 0.4H), 10.90 (d, J= 1.85, 0.6H), 9.27 (br, 3.0H), 7.58 (d, J= 7.94, 0.6H), 7.47 (d, J= 7.48, 0.4H), 7.44 (s, 0.4H), 7.41 (s, 0.6H), 7.33-7.23 (m, 4.0H), 7.23-7.16 (m, 2.0), 7.10-6.99 (m, 3.0H), 6.88 (d, J= 8.42, 1.0H), 6.63-6.60 (m, 2.0H), 6.56 (d, J = 8.46, 1.0H), 5.67 (dd, J= 8.96, 6.03, 0.6H), 5.41 (app. t, J= 7.77, 0.4H), 5.02-4.97 (m, 1.0H), 4.85 (t, J=5.47, 0.6H), 4.70 (t, J= 6.03, 0.4H), 4.30 (br, 0.6H), 4.15 (dt, J= 13.27, 3.79, 0.4H), 4.09 (br, 0.4H), 3.70 (dt, J= 13.27, 3.70, 0.6H), 3.63-3.57 (m, 1.0H), 3.33 (br, 1.0H), 3.25-3.07 (m, 6.0H), 3.04-2.85 (m, 3.0H), 2.76-2.67 (m, 1.5H), 2.58 (dd, J = 13.85, 5.12, 0.5H), 1.62-1.55 (m, 0.4H), 1.55-1.45 (m, 1.6H), 1.33-1.25 (m, 0.5H), 1.24-1.10 (m, 0.5H), 0.88 (d, J = 6.55, 2.0H), 0.87 (d, J = 6.55, 2.0H), 0.84 (d, J = 6.55, 2.0H). HRMS (ESI) $C_{39}H_{46}N_6O_5$ $[M+H]^+$ calc'd= 679.3530; found= 679.3810



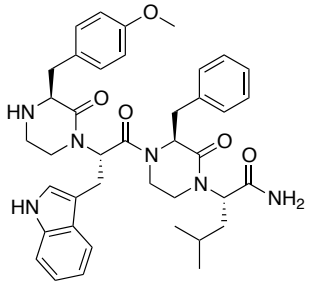
Oligooxopiperazine **15-** (Nap)WFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.92 (br. s, 1.0H), 9.20 (br, 2.0H), 8.03-7.97 (m, 1.3H), 7.90 (br, 0.7H), 7.66-7.53 (m, 2.0H), 7.49-7.38 (m, 2.0H), 7.35 (t, *J*= 7.8, 1.0H), 7.26 (s, 1.0H), 7.15-7.0 (m, 5.0H), 6.99-6.90 (m, 1.5H), 6.52 (s, 0.5H), 5.72 (br, 0.7H), 5.39 (br, 0.3H), 5.01 (dd, J= 11.40, 6.00, 1.0H), 4.96 (br, 0.7H), 4.78 (br, 0.3H), 4.33 (br, 0.5H), 4.17 (br, 0.5H), 3.82 (br, 0.3H), 3.76 (br, 1.0H), 3.64 (br, 0.7H), 3.24-3.04 (m, 7.0H), 3.04-2.96 (m, 2.5H), 2.95-2.87 (m, 1.5H), 2.86-2.74 (m, 2.0H), 1.66-1.59 (m, 0.7H), 1.57-1.47 (m, 2.0H), 1.35 (br, 0.3H), 1.24 (br, 0.5H), 1.18 (br, 0.5H), 0.89 (d, *J* = 6.55, 3.5H), 0.85 (d, *J* = 6.55, 2.5H). HRMS (ESI) $C_{43}H_{48}N_6O_4$ $[M+H]^+$ calc'd= 713.3737; found= 713.4041



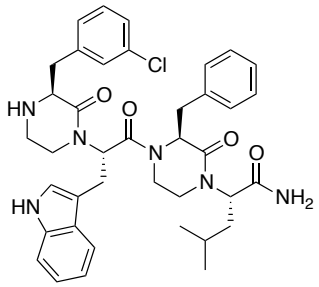
Oligooxopiperazine **16-** YWFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.88 (br, 0.6H), 10.86 (br, 0.4H), 9.37 (br, 1.0H), 9.17 (br, 1.0H), 8,97 (br, 1.0H), 7.57 (d, *J* = 8.00, 0.6H), 7.46-7.39 (m, 1.4H), 7.33 (t, *J* = 7.74, 1.0H), 7.24-7.20 (m, 2.0H), 7.18 (br, 0.5H), 7.12-7.06 (m, 3.5H), 7.05-6.97 (m, 4.0H), 6.89 (br, 1.0H), 6.71 (d, *J*= 7.92, 1.8H), 6.53 (br, 0.2H), 5.67 (dd, *J*= 8.10, 6.30, 0.6H), 5.31 (br, 0.4H), 4.99 (dd, *J*= 10.38, 5.34, 1.0H), 4.93, (t, *J* = 5.82, 0.6H), 4.74 (br, 0.4H), 4.18 (br, 1.0H), 3.99 (br, 0.4H), 3.73 (br, 0.6H), 3.55 (br, 1.0H), 3.25-3.14 (m, 3.5H), 3.13-3.03 (m, 4.0H), 3.00-2.73 (m, 3.0H), 2.79-2.74 (m, 0.5H), 1.65-1.60 (m, 0.5H), 1.56-1.47 (m, 1.5H), 1.33-1.26 (m, 0.5H), 1.22-1.10 (m, 0.5H), 0.89 (d, *J* = 6.55, 2.0H), 0.87 (app. t, *J* = 3.10, 2.0H), 0.84 (d, *J* = 6.55, 2.0H). HRMS (ESI) $C_{39}H_{45}N_5O_6$ $[M+H]^+$ calc'd= 679.3530; found= 679.3755



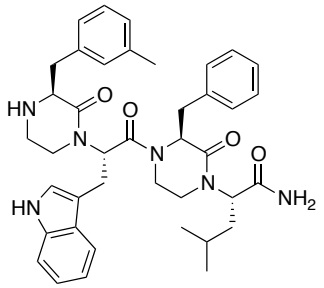
Oligooxopiperazine **17-** Y(O-Me)WFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.89 (br, 0.6H), 10.87 (br, 0.4H), 9.20 (br, 2.0H), 7.57 (d, *J* = 8.00, 0.3H), 7.8-7.39 (m, 1.0H), 7.34 (t, *J* = 8.13, 0.7H), 7.23-7.17 (m, 2.0H), 7.12-7.06 (m, 4.0H), 7.04-6.98 (m, 1.5H), 6.91 (br, 1.0H), 6.90 (d, *J* = 1.5H), 6.54 (br, 1.0H), 5.70 (dd, *J*= 8.79, 6.03, 0.6H), 5.31 (t, *J* = 6.40, 0.4H), 4.99 (dd, *J*= 10.74, 5.52, 1.0H), 4.94 (t, *J*= 5.73, 0.6H), 4.74 (dd, *J*= 7.47, 5.16, 0.4H), 4.24-4.16 (m, 0.4H), 3.73 (app. d, *J*= 2.40, 3.0H), 3.54 (br, 0.6H), 3.28-3.03 (m, 9.0H), 3.02-2.84 (m, 3.0H), 2.83-2.75 (m, 1.0H), 2.68 (br, 1.0H), 1.66-1.60 (m, 0.5H), 1.56-1.45 (m, 2.0H), 1.37-1.31 (m, 0.5H), 1.24-1.12 (m, 1.0H) 0.90-0.86 (m, 4.0H), 0.84 (d, *J* = 6.60, 2.0H) . HRMS (ESI) $C_{40}H_{47}N_5O_6$ $[M+H]^+$ calc'd= 693.3686; found= 693.3944



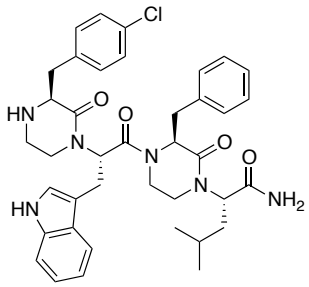
Oligooxopiperazine **18-** F(3-Cl)WFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.88 (s, 0.7H), 10.87 (s, 0.3H), 9.40 (br d, 2.0H), 7.57 (d, *J*= 7.90, 0.6H), 7.48-7.40 (m, 1.4H), 7.38-7.29 (m, 3.5H), 7.25-7.17 (m, 2.5H), 7.16-6.98 (m, 5.0H), 6.95-6.86 (m, 0.8H), 6.54 (br, 0.2H), 5.66 (dd, *J* = 8.77, 6.37, 0.6H), 5.30 (t, *J*= 7.32, 0.4H), 5.02-4.97 (m, 1.0H), 4.94 (t, *J* = 5.85, 0.6H), 4.70 (dd, *J* = 7.83, 5.33, 0.4H), 4.29 (br, 0.6H), 4.19 (dt, *J* = 13.17, 3.76, 0.4H), 4.07 (br, 0.4H), 3.70 (dt, *J* = 13.17, 3.76, 0.6H), 3.62-3.49 (m, 2.0H), 3.30-3.22 (m, 1.0H), 3.23-3.02 (m, 6.0H), 3.16-2.86 (m, 3.0H), 2.76-2.72 (m, 1.0H), 1.66-1.59 (m, 0.4H), 1.56-1.45 (m, 1.6H), 1.36-1.28 (m, 0.5H), 1.26-1.11 (m, 1.5H), 0.89-0.86 (m, 4.0H), 0.84 (d, *J* = 6.57, 2.0H). HRMS (ESI) $C_{39}H_{44}ClN_5O_5$ $[M+H]^+$ calc'd= 697.3191; found= 697.3568



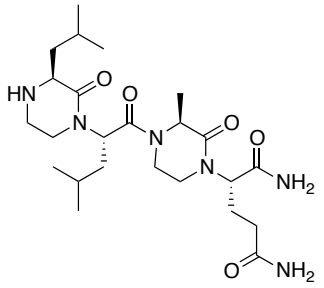
Oligooxopiperazine **19-** F(3-Me)WFL-NH_2

^{1}H-NMR (400 MHz, d_6-DMSO) δ 10.88 (br, 0.6H), 10.87 (br, 0.4H), 9.18 (br d, 2.0H), 7.57 (d, *J*= 7.65, 0.7H), 7.47-7.39 (m, 1.3H), 7.37-7.32 (m, 1.0H), 7.25-7.18 (m, 3.0H), 7.15-7.07 (m, 3.5H), 7.06-6.97 (m, 3.5H), 6.93-6.88 (m, 1.0H), 5.67 (dd, *J* = 8.82, 6.07, 0.6H), 5.32 (t, *J* = 7.34, 0.4H) 5.02-4.97 (m, 1.0H), 4.93 (t, *J* = 5.68, 0.6H), 4.77-4.71 (dd, *J* = 7.87, 5.65, 0.4H), 4.28 (br, 0.5H), 4.21-4.15 (m, 0.5H), 4.08 (br, 0.5H), 3.75-3.68 (m, 1.5H), 3.39-3.04 (m, 9.0H), 3.02-2.83 (m, 2.5H), 2.79-2.73 (m, 0.5H), 2.29 (s, 3.0H), 1.69-1.59 (m, 0.5H), 1.56-1.47 (m, 2.0H), 1.39-1.27 (m, 0.5H), 1.27-1.06 (m, 2.0H), 0.90-0.86 (m, 4.0H), 0.84 (d, *J* = 6.54, 2.0H). HRMS (ESI) $C_{40}H_{48}N_6O_4$ $[M+H]^+$ calc'd= 677.3737; found= 677.4042



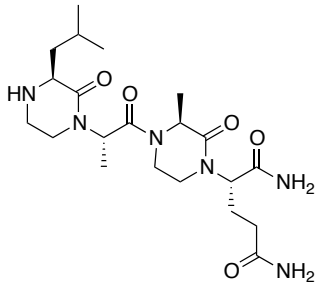
Oligooxopiperazine **20-** F(4-Cl)WFL-NH_2

^{1}H-NMR (600 MHz, d_6-DMSO) δ 10.88 (s, 0.7H), 10.87 (s, 0.3H), 9.38 (br d, 2.0H), 7.57 (d, *J*= 7.84, 0.5H), 7.45 (s, 0.5H), 7.42 (d, *J* = 8.45, 1.0H), 7.34 (t, *J* = 7.63, 2.5H), 7.24-7.20 (m, 3.0H), 7.16-7.06 (m, 4.0H), 7.05-6.98 (m, 1.5H), 6.90 (br, 0.9H), 6.55 (br, 0.1H), 5.66 (dd, *J* = 8.99, 6.25, 0.6H), 5.30 (t, *J*= 7.32, 0.4H), 5.01-4.97 (m, 1.0H), 4.94 (t, *J* = 5.85, 0.6H), 4.70 (dd, *J* = 8.11, 5.10, 0.4H), 4.25 (br, 0.5H), 4.19 (dt, *J* = 13.52, 3.83, 0.4H), 4.04 (br, 0.5H), 3.72 (dt, *J* = 13.52, 3.83, 0.6H), 3.57 (br, 1.0H), 3.28-3.25 (m, 1.0H), 3.23-3.13 (m, 3.5H), 3.10-3.03 (m, 3.0H), 3.01-2.86 (m, 2.5H), 2.85-2.70 (m, 2.0H), 1.66-1.59 (m, 0.5H), 1.57-1.45 (m, 1.8H), 1.37-1.29 (m, 0.5H), 1.24 (br, 0.2H), 1.21-1.10 (m, 1.0H), 0.90-0.86 (m, 4.0H), 0.84 (d, *J* = 6.56, 2.0H). HRMS (APCI) $C_{39}H_{44}ClN_5O_5$ $[M+H]^+$ calc'd= 697.3191; found= 697.3587



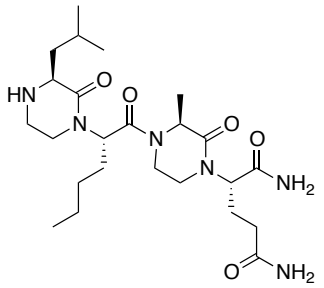
Oligooxopiperazine **21**- LLAQ

^{1}H-NMR (600 MHz, d_6-DMSO, 100 °C) δ 6.87 (br, 3H), 6.61 (br, 3H), 5.36 (t, *J* = 7.47, 1H), 4.90-4.85 (m, 1H), 4.67 (q, *J* = 6.88, 1H), 3.96 (br, 2H), 3.63-3.24 (m, 8H), 1.95-1.82 (m, 3H), 1.72-1.58 (m, 3H), 1.57-1.49 (m, 1H), 1.38 (s, 3H), 1.06-0.75 (m, 12H). HRMS (ESI) $C_{24}H_{42}N_6O_5$ $[M+H]^+$ calc'd= 494.3217; found= 495.3502



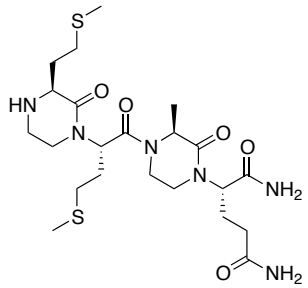
Oligooxopiperazine **22**- LAAQ

^{1}H-NMR (400 MHz, d_6-DMSO, 100 °C) δ 6.77 (br, 2H), 6.52 (br, 2H), 5.34 (q, *J* = 6.91, 1H), 4.90-4.84 (m, 1H), 4.67 (q, *J* = 6.91, 1H), 4.01-3.91 (m, 1H), 3.76-3.69 (m, 1H), 3.57-3.27 (m, 7H), 3.24-3.14 (m, 1H), 2.19-2.01 (m, 3H), 2.00-1.80 (m, 3H), 1.64-1.55 (m, 1H), 1.38 (d, *J* = 6.92, 3H), 1.27 (d, *J* = 6.92, 3H), 0.94 (t, *J* = 6.02, 6H). HRMS (ESI) $C_{21}H_{36}N_6O_5$ $[M+H]^+$ calc'd= 453.2747; found= 453.2863



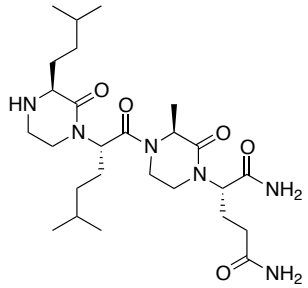
Oligooxopiperazine **23**- L(Nle)AQ

^{1}H-NMR (600 MHz, d_6-DMSO, 100 °C) δ 6.87 (br, 4H), 5.27 (t, *J* = 7.25, 1H), 4.92-4.83 (m, 1H), 4.67 (q, *J* = 7.07, 1H), 3.96 (br, 1H), 3.88 (br, 1H), 3.59-3.56 (m, 1H), 3.54-3.46 (m, 3H), 3.45-3.39 (m, 2H), 3.36-3.31 (m, 1H), 3.30-3.21 (m, 1H), 2.12-2.01 (m, 2H), 1.99-1.83 (m, 2H), 1.80-1.59 (m, 4H), 1.39-1.20 (m, 8H), 0.95 (d, *J* = 6.29, 3H), 0.94 (d, *J* = 6.19, 3H), 0.89 (t, *J* = 7.31, 3H). HRMS (ESI) $C_{24}H_{42}N_6O_5$ $[M+H]^+$ calc'd= 495.3217; found= 495.3377



Oligooxopiperazine **24**- MMAQ

^{1}H-NMR (600 MHz, d_6-DMSO, 100 °C) δ 9.33 (br, 1H), 6.90 (br, 3H), 5.46 (t, *J* = 6.91, 1H), 4.91-4.84 (m, 1H), 4.70-4.63 (m, 1H), 4.16-3.91 (m, 1H), 3.87-3.75 (m, 1H), 3.59-3.54 (m, 2H), 3.50 (t, *J* = 5.98, 2H), 3.47-3.40 (m, 1H), 3.36-3.22 (m, 3H), 2.70-2.60 (m, 3H), 2.23-2.14 (m, 1H), 2.08 (d, *J* = 2.96, 3H), 2.07-1.87 (m, 5H), 1.74-1.62 (m, 5H), 1.7 (br, 1H), 1.27 (s, 3H). HRMS (ESI) $C_{22}H_{38}N_6O_5S_2$ $[M+H]^+$ calc'd= 530.2345; found= 531.2423.



Oligooxopiperazine **25**- (Hle)(Hle)AQ

^{1}H-NMR (600 MHz, d_6-DMSO, 100 °C) δ 6.83 (br, 4H), 5.26 (t, *J* = 7.06, 1H), 4.92-4.83 (m, 1H), 4.66 (q, *J* = 6.80, 1H), 3.96 (br, 1H), 3.88 (br, 1H), 3.55-3.46 (m, 3H), 3.45-3.36 (m, 3H), 3.35-3.30 (m, 1H), 3.29-3.22 (m, 1H), 3.21 (s, 1H), 1.99-1.83 (m, 2H), 1.83-1.63 (m, 3H), 1.62-1.49 (m, 2H), 1.46-1.20 (m, 7H), 1.19-1.09 (m, 2H), 0.95-0.83 (m, 12H). HRMS (ESI) $C_{24}H_{42}N_6O_5$ $[M+H]^+$ calc'd= 523.3530; found= 523.3642

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