

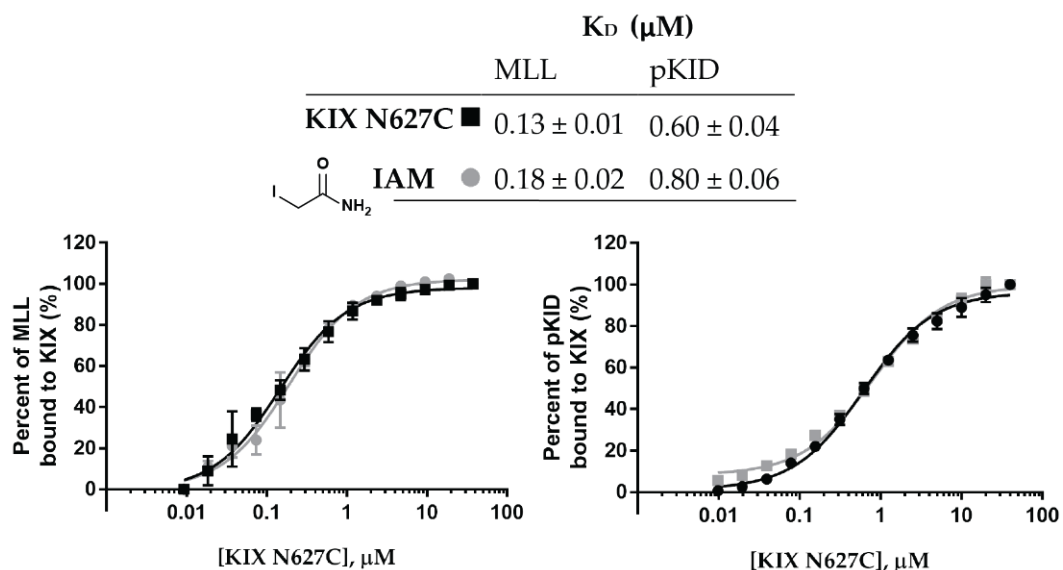
CHEMBIOCHEM

Supporting Information

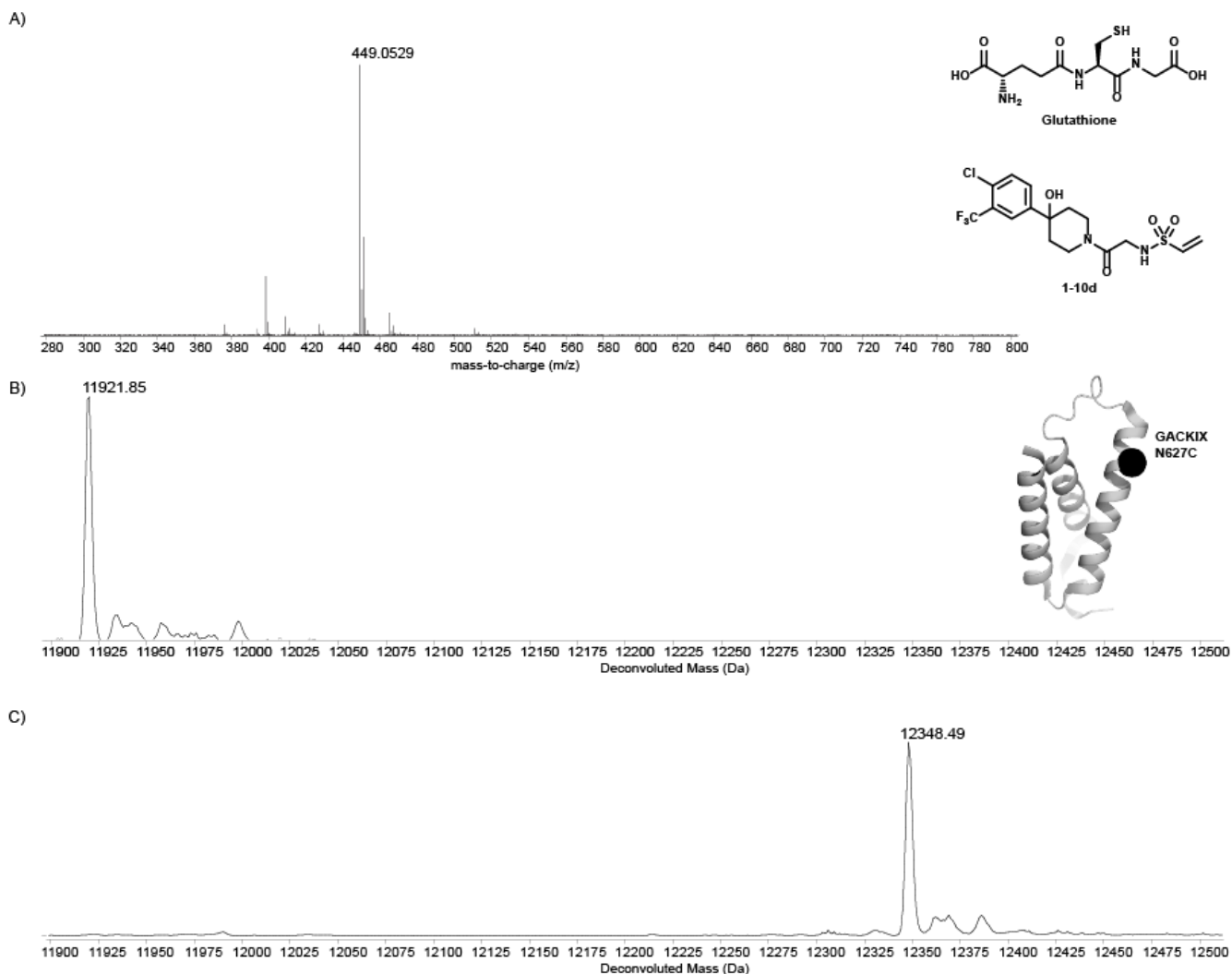
Covalent Chemical Cochaperones of the p300/CBP GACKIX Domain

Jean M. Lodge, Chinmay Y. Majmudar, James Clayton, and Anna K. Mapp*^[a]

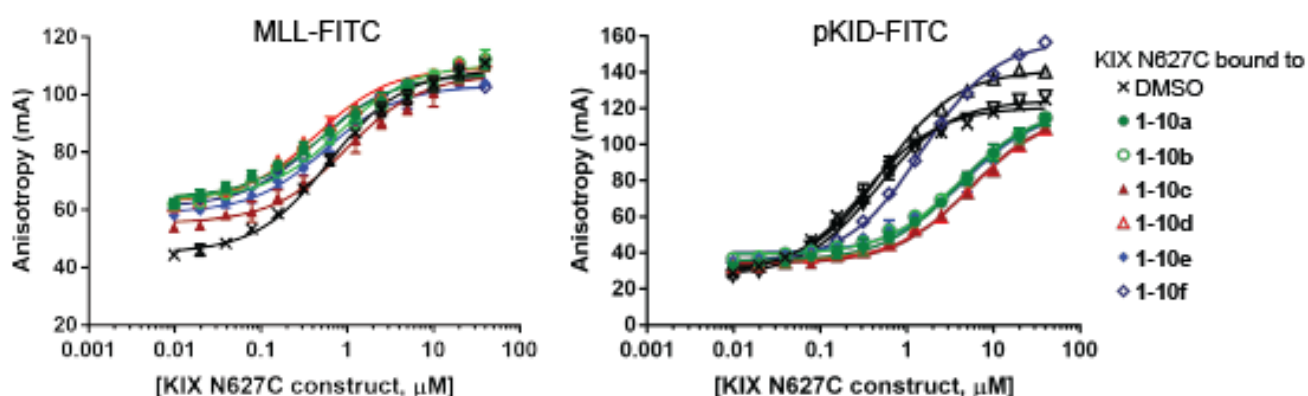
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Supplementary Figure 1 The interactions for unlabeled KIX N627 and KIX N627C labeled by iodoacetamide (IAM) were compared against the binding partners, MLL and pKID using fluorescence polarization (FP) direct binding assays. These interactions were not significantly impacted by the presence of IAM at the MLL-binding site while some distal effects occurred with pKID binding. All measurements were performed in triplicated. The dissociation constants are reported with the standard error (SE). The FP direct binding curves are shown for the MLL and pKID tracers. The values represent the average and the error bars represent the standard deviation (SD). Data analysis was performed in GraphPad Prism 7.0.



Supplementary Figure 2 A) The reactivity of **1-10d** toward glutathione was tested by incubating 50 μM of **1-10d** with 500 μM of glutathione in 10 mM sodium phosphate buffer, 100 mM NaCl, pH 6.8 for 45 minutes at room temperature. The reaction was injected onto the 1290 Agilent liquid chromatography system equipped with a Proshell 300 SB-C8 column and analyzed with the 6545 Agilent quadrupole time-of-flight (Q-TOF) mass spectrometer. The mass spectrum is shown with a prominent peak at m/z of 449.0529 corresponding to the **1-10d** sodium adduct (calculated $(\text{M}+\text{H}+\text{Na})^+$ 449.0526 m/z). Free glutathione eluted off the column first with an observed m/z 308.0912 (calculated $(\text{M}+\text{H})^+$ 308.0838 m/z) and 330.0731 m/z (calculated $(\text{M}+\text{H}+\text{Na})^+$ 330.0736). B) Deconvoluted mass spectrum shows the unbound KIX N627C at 11,921.85 Da. C) KIX N627C (5 μM) was mixed with 50 μM of **1-10d** and 500 μM of glutathione for 45 minutes and the reaction was analyzed on the Q-TOF LC-MS (Agilent). The mass spectra were deconvoluted using the Agilent MassHunter Bioconfirm software (Agilent) and maximum entropy algorithm. KIX N627C was fully labeled by **1-10d** with an observed deconvoluted mass of 12348.49 Da (calculated mass 12348.69 Da).



Supplementary Figure 3 The anisotropy binding curves of the GACKIX N627C bound and unbound complexes. The K_D values are shown in the supplementary Table 1. All measurements were performed in triplicate and analyzed in GraphPad Prism 7.0. The symbols represent the average and the error bars represent the SD error.

Supplementary Table S1 The K_D values of for MLL and pKID with the unbound (DMSO) and labeled GACKIX N627C complexes. The error reflects the standard error from three FP experiments.

Compound	K_D (μM)	
	MLL	pKID
DMSO	0.32 ± 0.02	0.60 ± 0.04
1-10a	3.9 ± 0.4	0.52 ± 0.07
1-10b	4.1 ± 0.2	0.8 ± 0.1
1-10c	5.4 ± 0.3	0.9 ± 0.1
1-10d	5.6 ± 0.4	0.40 ± 0.04
1-10e	4.6 ± 0.5	0.52 ± 0.04
1-10f	1.47 ± 0.06	0.40 ± 0.04

General Compound Information

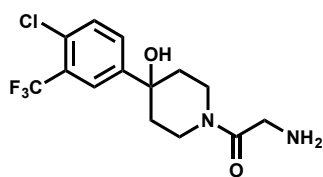
NMR spectra were recorded on a Varian MR400 or VNMRS 500 spectrometer. The chemical shifts (δ) are reported in ppm downfield of tetramethylsilane. Coupling constants (J) are reported in hertz (Hz) rounded to 0.5 Hz and were calculated using ACD Labs 1D NMR processor version 12.01. Splitting patterns are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), or a combination of these. Solvents were used as internal standards when assigning NMR spectra (δ H: CDCl_3 7.27 ppm; δ C: CDCl_3 77.0 ppm; CD_3OD 3.35, 4.78 ppm; δ C: CD_3OD 49.3 ppm; $\text{DMSO}-d_6$ 2.50 ppm; δ C: $\text{DMSO}-d_6$ 39.4 ppm).

ES mass spectra were recorded on a Micromass LCT Time-of-Flight mass spectrometer. High resolution mass spectra (HRMS, EI and ES) were recorded on an Agilent Technologies 6520 Accurate-Mass Q-TOF HPLC-MS mass spectrometer.

Infrared spectra were recorded on a Perkin Elmer Spectrum BX FT-IR spectrometer as a film on a sodium chloride plate. Only absorption maxima of interest are reported and are quoted as wavenumbers (cm^{-1}).

CH_2Cl_2 and THF were dried using a solvent system equipped with activated alumina columns. Unless otherwise noted, all other solvents and commercially obtained reagents were used as received or purified using standard procedures.

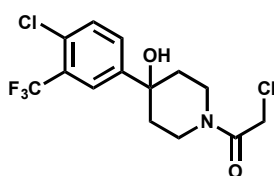
Thin layer chromatography (TLC) was performed using commercially available pre-coated glass plates (EMD Chemicals Inc. TLC Silica Gel 60 F_{254}) and visualised with UV light at 254 nm. Flash chromatography was carried out with E. Merck Silica Gel 60 (230-400 mesh).

Intermediate 1 – 2-Amino-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)ethanone

Triethylamine (0.45 mL, 3.42 mmol) was added to a solution of EDCI·HCL (284 mg, 1.48 mmol), HOBt (185 mg, 1.37 mmol) and Boc-Gly-OH (200 mg, 1.14 mmol) in DMF (11 mL) at room temperature. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-4-piperidinol (287 mg, 1.03 mmol) was added, and the reaction was stirred at room temperature for 3 hours. The reaction was quenched with water (10 mL) and extracted with diethyl ether (40 mL). The organic layer was washed with saturated aqueous NH₄Cl, brine and dried over MgSO₄. The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 50 % gradient of [1 %:9 %:90 %, NH₄OH:MeOH:CH₂Cl₂] in CH₂Cl₂) to give a colourless oil with R_f 0.59. This oil was dissolved in CH₂Cl₂ (10 mL) and TFA (0.5 mL) was added. The solution was stirred at room temperature for 3 hours at which point complete Boc deprotection was observed by TLC. The solvent was removed under reduced pressure, the residue was re-dissolved in CH₂Cl₂ and washed with 3 M NaOH (0.25 mL) the layers were separated and the organic layer was dried over MgSO₄. The solvent was removed under reduced pressure to give **Intermediate 1** (214 mg, 56 %) as a colourless solid.

R_f: 0.31 (0.5 %:4.5 %:95 %, NH₄OH:MeOH:CH₂Cl₂), v_{max}(film)/cm⁻¹ 3364 (OH), 2926 (CH), 1649 (C=O);

MS m/z (ES+) 337 (100 %, (M+H)⁺); **HRMS**: found 337.1083, (M+H)⁺, C₁₄H₁₇ClF₃N₂O₂ requires 337.0930; **¹H NMR** (CD₃OD, 400 MHz) δ 7.91 (d, J 2 Hz, 1H), 7.68 (dd, J 8.5, 2 Hz, 1H), 7.55 (d, J 8.5 Hz, 1H), 4.47 (dt, J 13, 2 Hz, 1H), 3.71 (dt, J 13, 2 Hz, 1H), 3.60 - 3.45 (m, 3H), 3.13 (t, J 13 Hz, 1H), 2.02 (dt, J 14, 4 Hz, 1H), 1.93 (dt, J 13.5, 4.5 Hz, 1H) 1.72 (d, J 13 Hz, 2H); **¹³C NMR** (CD₃OD, 100 MHz) δ 152.4, 135.1, 133.9, 133.7, 131.2, 128.3, 127.8, 125.7, 74.3, 44.3, 42.0, 41.6, 41.0.

Alkylator 1 – 2-chloro-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)ethanone

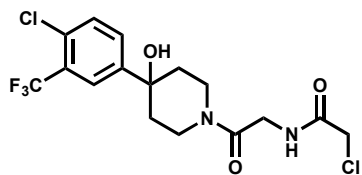
Triethylamine (100 μL, 0.72 mmol) was added to a solution of EDCI·HCL (100 mg, 0.54 mmol) and chloroacetic acid (51 mg, 0.54 mmol) in DMF (10 mL) at room temperature. 4-(4-Chloro-3-(trifluoromethyl)phenyl)-4-piperidinol (100 mg, 0.36 mmol) was added, and the reaction was stirred at room temperature for 3 hours. The reaction was quenched with water (10 mL) and extracted with diethyl ether (30 mL). The organic layer was washed with saturated aqueous NH₄Cl, brine and dried over MgSO₄. The solvent was removed under reduced pressure and the residue was purified using flash chromatography (20 % to 60 % gradient of EtOAc in Hexane) to give **Alkylator 1** (18.9 mg, 14 %) as a colourless oil that solidified on standing.

R_f: 0.51 (1 %:9 %:90 %, NH₄OH:MeOH:CH₂Cl₂), v_{max}(film)/cm⁻¹ 3446 (OH), 2923 (CH), 1643 (C=O);

MS m/z (ES+) 337.9 (100 %, (M-OH)⁺); **HRMS**: found 356.0434, (M+H)⁺, C₁₄H₁₅Cl₂F₃NO₂ requires 356.0431;

¹H NMR (CDCl₃, 400 MHz) δ 7.81 (d, J 2 Hz, 1H), 7.54 (dd, J 8, 2 Hz, 1H), 7.48 (d, J 8 Hz, 1H), 4.53 (dt, J 13, 2 Hz, 1H), 4.13 (d, J 12 Hz, 1H), 4.05 (d, J 12 Hz, 1H), 3.79 (dt, J 13, 2 Hz, 1H), 3.65 (td, J 13, 2 Hz, 1H), 3.14 (td, J 13, 2 Hz, 1H), 2.07 - 1.93 (m, 2H), 1.87 - 1.74 (m, 2H) 1.62 (bs, 1H); **¹³C NMR** (CDCl₃, 100 MHz) δ 165.1, 146.5, 131.61, 129.0, 123.9, 71.2, 42.5, 41.0, 38.6, 38.2, 37.5.

Alkylator 2 – 2-chloro-*N*-(2-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-2-oxoethyl)acetamide

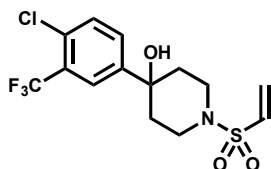


Triethylamine (15 μ L, 0.11 mmol) was added to a solution of 2-Amino-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)ethanone (**Intermediate 1**) (17 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) and the solution was cooled to 0 $^\circ\text{C}$ in an ice bath. Chloroacetyl chloride (5 μ L, 0.06 mmol) was added after 10 minutes the ice bath was removed and the solution was allowed to reach room

temperature over 50 minutes. The reaction was quenched with saturated aqueous NH_4Cl (5 ml) and extracted with CH_2Cl_2 . The organic layer was washed with saturated aqueous NH_4Cl , brine and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 50 % gradient of [1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$] in CH_2Cl_2) to give **Alkylator 2** (15.8 mg, 76 %) as a colourless oil that solidified on standing.

R_f 0.44 (1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3335 (OH), 2929 (CH_2), 1676 (C=O), 1636 (C=O); **MS m/z** (ES+) 434.9 (90 %, (M+Na)⁺); **HRMS**: found 413.0635, (M+H)⁺, $\text{C}_{16}\text{H}_{18}\text{Cl}_2\text{F}_3\text{N}_2\text{O}_3$ requires 413.0646; **$^1\text{H NMR}$** ($\text{DMSO}-d_6$, 400 MHz) δ 8.23 (t, J 5 Hz, 1H), 7.91 (d, J 2 Hz, 1H), 7.75 (dd, J 8.5, 2 Hz, 1H), 7.64 (d, J 8.5 Hz, 1H), 5.47 (s, 1H), 4.30 (bd, J 12 Hz, 1H), 4.16 (s, 2H), 4.07 (dd, J 17, 5 Hz, 1H), 3.99 (dd, J 17, 5 Hz, 1H), 3.66 (bd, J 12 Hz, 1H), 3.37 (bt, J 12 Hz, 1H), 2.95 (dt, J 11, 2 Hz, 1H), 2.01 (dt, J 13, 4 Hz, 1H), 1.77 (dt, J 13, 5 Hz, 1H), 1.57 (d, J 13 Hz, 1H); **$^{13}\text{C NMR}$** ($\text{DMSO}-d_6$, 125 MHz) δ 167.3, 167.1, 150.6, 132.6, 132.1, 120.2, 125.5, 123.2, 71.3, 43.7, 42.2, 41.7, 39.0, 38.6, 38.4.

Alkylator 3 – 4-(4-chloro-3-(trifluoromethyl)phenyl)-1-(vinylsulfonyl)piperidin-4-ol



Triethylamine (33 μ L, 0.24 mmol) was added to a solution of 4-(4-Chloro-3-(trifluoromethyl)phenyl)-4-piperidinol (34 mg, 0.12 mmol) in CH_2Cl_2 (10 mL) and the solution was cooled to 0 $^\circ\text{C}$ in an ice bath. 2-Chloroethanesulfonyl chloride (13 μ L, 0.13 mmol) was added, the ice bath was removed after 1 hour and the solution was allowed to reach room temperature over 2 hours.

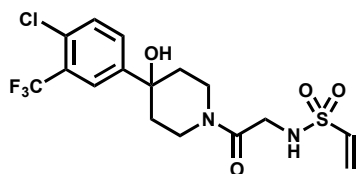
The reaction was quenched with saturated aqueous NH_4Cl (5 ml) and extracted with CH_2Cl_2 . The organic layer was washed with saturated aqueous NH_4Cl , brine and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 75 % gradient of [1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$] in CH_2Cl_2) to give **Alkylator 3** (15.5 mg, 39 %) as a colourless oil that solidified on standing.

R_f 0.38 (1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3490 (OH), 1316 (S=O), 1148 (S=O); **MS m/z** (ES+) 369.9 (100 %, (M+H)⁺); **HRMS**: found 370.0490, (M+H)⁺, $\text{C}_{14}\text{H}_{16}\text{ClF}_3\text{NO}_3\text{S}$ requires 370.0491;

$^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ 7.81 (d, J 2 Hz, 1H), 7.54 (dd, J 8, 2 Hz, 1H), 7.48 (d, J 8 Hz, 1H), 6.46 (dd, J 16.5, 10 Hz, 1H), 6.24 (d, J 16.5 Hz, 1H), 6.05 (d, J 10 Hz, 1H), 3.66 (dt, J 12, 2 Hz, 2H), 3.09 (td, J 12, 2 Hz, 2H), 2.21 (td, J 13.5, 4 Hz, 2H), 1.82 (bs, 1H, OH), 1.80 (d, J 12 Hz, 2H); **$^{13}\text{C NMR}$** (CDCl_3 , 100 MHz) δ 146.6, 132.4, 131.6, 128.9, 128.8, 124.2, 123.8, 123.9, 121.4, 70.5, 41.6, 37.7.

Alkylator 4 –

N-(2-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-2-oxoethyl)ethanesulfonamide

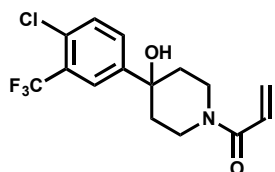


Triethylamine (17 μ L, 0.12 mmol) was added to a solution of 2-Amino-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)ethanone (**Intermediate 1**) (21 mg, 0.06 mmol) in CH_2Cl_2 (1 mL), the solution was cooled to 0 $^\circ\text{C}$ in an ice bath. 2-Chloroethanesulfonyl chloride (10 μ L, 0.09 mmol) was added after 10 minutes the ice bath was removed and the solution was allowed to reach room

temperature over 50 minutes. The reaction was quenched with saturated aqueous NH_4Cl (5 ml) and extracted with CH_2Cl_2 . The organic layer was washed with saturated aqueous NH_4Cl , brine and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 20 % gradient of [1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$] in CH_2Cl_2) to give **Alkylator 4** (3.7 mg, 13 %) as a colourless solid.

R_f: 0.25 (0.5 %:4.5 %:95 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$); **v_{max}(film)/cm⁻¹** 3402 (OH), 2924 (CH), 1637 (C=C), 1600 (C=O), 1137 (S=O); **MS m/z** (ES+) 450.9 (100 %, (M+Na)⁺); **HRMS**: found 427.0705, (M+H)⁺, $\text{C}_{16}\text{H}_{20}\text{ClF}_3\text{N}_2\text{O}_4\text{S}$ requires 427.0706; **¹H NMR** (CD_3OD , 400 MHz) δ 7.91 (d, *J* 2 Hz, 1H), 7.68 (dd, *J* 8, 2 Hz, 1H), 7.55 (d, *J* 8 Hz, 1H), 6.67 (d, *J* 16.5, 10 Hz, 1H), 6.15 (d, *J* 16.5 Hz, 1H), 5.94 (d, *J* 10 Hz, 1H), 4.42 (dt, *J* 13, 2 Hz, 1H), 3.99 (d, *J* 16 Hz, 1H), 3.88 (d, *J* 16 Hz, 1H), 3.75 (dt, *J* 13, 2 Hz, 1H), 3.52 (d, *J* 13, 2 Hz, 1H), 3.12 (d, *J* 25, 13 Hz, 1H), 2.05 (td, *J* 13, 5 Hz, 1H), 1.94 (d, *J* 13, 5 Hz, 1H), 1.76 – 1.66 (m, 2H); **¹³C NMR** (CD_3OD , 125 MHz) δ 166.8, 148.4, 136.1, 131.1, 129.8, 125.3, 124.2, 123.9, 123.8, 122.0, 70.3, 43.6, 40.8, 38.2, 37.6, 37.0.

Alkylator 5 – 1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)prop-2-en-1-one

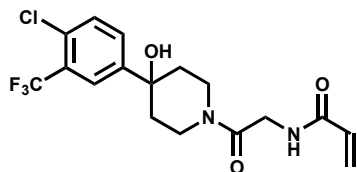


Triethylamine (62 μ L, 0.45 mmol) was added to a solution of 4-(4-Chloro-3-(trifluoromethyl)phenyl)-4-piperidinol (50 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) and the solution was cooled to 0 $^\circ\text{C}$ in an ice bath. Acryloyl chloride (20 μ L, 0.26 mmol) was added after 10 minutes the ice bath was removed and the solution was allowed to reach room temperature over 50 minutes. The reaction was quenched with saturated aqueous NH_4Cl (5 ml) and

extracted with CH_2Cl_2 . The organic layer was washed with brine and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 50 % gradient of [1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$] in CH_2Cl_2) to give **Alkylator 5** (32 mg, 54%) as a colourless oil that solidified on standing.

R_f: 0.54 (1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$); **v_{max}(film)/cm⁻¹** 3360 (OH), 2926 (CH), 1637 (C=C), 1597(C=O); **MS m/z** (ES+) 316.0 (100 %, (M-OH)⁺); **HRMS**: found 334.0819, (M+H)⁺, $\text{C}_{15}\text{H}_{16}\text{ClF}_3\text{NO}_2$ requires 334.0821; **¹H NMR** (CDCl_3 , 500 MHz) δ 7.83 (s, 1H), 7.54 (dd, *J* 8, 2 Hz, 1H), 7.47 (d, *J* 8 Hz, 1H), 6.61 (dd, *J* 16.5, 10.5 Hz, 1H), 6.27 (dd, *J* 16.5, 2 Hz, 1H), 5.72 (dd, *J* 10.5, 2 Hz, 1H), 4.61 (d *J* 11.5 Hz, 1H), 3.93 (d, *J* 12.5 Hz, 1H), 3.61 (t, *J* 12.5 Hz, 1H), 3.15 (t, *J* 12.5 Hz, 1H), 2.69 (bs, 1H, OH), 1.84 - 1.74 (m, 4H); **¹³C NMR** (CDCl_3 , 125 MHz) δ 165.5, 146.9, 131.5, 128.2, 127.4, 124.0, 121.4, 71.2, 41.9, 38.9, 38.0, 37.58, 29.7, 28.3.

Alkylator 6 – *N*-(2-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-2-oxoethyl)acrylamide

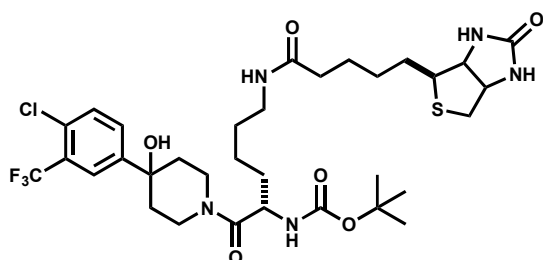


Triethylamine (30 μ L, 0.23 mmol) was added to a solution of 2-Amino-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)ethanone (**Intermediate 1**) (40 mg, 0.12 mmol) in CH_2Cl_2 (1 mL), the solution was cooled to 0 $^\circ\text{C}$ in an ice bath. Acryloyl chloride (11 μ L, 0.14 mmol) was added after 10 minutes the ice bath was

removed and the solution was allowed to reach room temperature over 50 minutes. The reaction was quenched with saturated aqueous NH_4Cl (5 ml) and extracted with CH_2Cl_2 . The organic layer was washed with saturated aqueous NH_4Cl , brine and dried over MgSO_4 . The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 30 % gradient of [1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$] in CH_2Cl_2) to give **Alkylator 6** (15.5 mg, 34 %) as a colourless oil that solidified on standing.

R_f 0.41 (1 %:9 %:90 %, $\text{NH}_4\text{OH}:\text{MeOH}:\text{CH}_2\text{Cl}_2$); $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 3318 (OH), 2923 (CH), 1643 (C=C), 1626, 1481; **MS** m/z (ES+) 413 (100 %, (M+Na) $^+$); **HRMS**: found 391.1044, (M+H) $^+$, $\text{C}_{17}\text{H}_{19}\text{ClF}_3\text{N}_2\text{O}_3$ requires 391.1036; **$^1\text{H NMR}$** (CD_3OD , 400 MHz) δ 7.95 (d, J 2 Hz, 1H), 7.72 (dd, J 8.5, 2 Hz, 1H), 7.58 (d, J 8.5 Hz, 1H), 6.37 (dd, J 17, 10 Hz, 1H), 6.26 (dd, J 17, 2 Hz, 1H), 5.71 (dd, J 10, 2 Hz, 1H), 4.48 (dt, J 13, 2 Hz, 1H), 4.29 (d, J , 17 Hz, 2H), 4.14 (d, J 17 Hz, 1H), 3.84 (dt, J 13, 2 Hz, 1H), 3.59 (dt, J 13, 2 Hz, 1H), 3.15 (dt, J 13, 3 Hz, 1H) 2.12 (dt, J 13 Hz, 4.5 H), 1.98 (t, J 13, 4.5 Hz, 1H), 1.70 - 1.79 (m, 2H); **$^{13}\text{C NMR}$** (CD_3OD , 125 MHz) δ 148.4, 131.1, 130.1, 129.8, 125.7, 123.9, 70.4, 40.8, 40.5, 38.1, 37.6, 37.1.

tert-butyl ((2*R*)-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-1-oxo-6-(5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexan-2-yl)carbamate



Biotin (0.20 g, 0.815 mmol, 1 equiv) was suspended in CH_2Cl_2 (8 ml) at room temperature, triethylamine (0.228 mL, 1.64 mmol, 2 equiv) was added the solution was stirred for 2 minutes, pivaloyl chloride (0.10 mL, 0.815 mmol, 1equiv) was added and the solution was stirred for 1 hour over which time the suspension dissolved. Separately Boc-L-lysine (0.20 g, 0.815 mmol, 1equiv) was suspended in CH_2Cl_2 (2 ml) at room temperature, triethylamine (0.25 mL, 1.80 mmol, 2.2 equiv) was added the solution was stirred for 5 minutes, and the biotin mixed anhydride solution was added with a pasture pipette and the reaction was stirred for 30 minutes at room temperature. Pivaloyl chloride ((0.10 mL, 0.815 mmol 1equiv) was added and the solution was stirred for 15 minutes. 4-(4-Chloro-3-

(trifluoromethyl)phenyl)-4-piperidinol (0.194 g, 0.69 mmol, 0.85 equiv) was added and the solution was stirred for 30 minutes, and the reaction was quenched with saturated aqueous NH₄Cl, the organic layer was extracted with CH₂Cl₂, the organic layer was washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified using flash chromatography (0 % to 100 % gradient of [1 %:9 %:90 %, NH₄OH:MeOH:CH₂Cl₂] in CH₂Cl₂ to give (0.22 g, 43%) as a colourless oil.

R_f 0.38 (1 %:9 %:90 %, NH₄OH:MeOH:CH₂Cl₂);

ν_{max}(film)/cm⁻¹ 3311 (OH), 2929 (CH), 2457, 1676 (C=C), 1623,

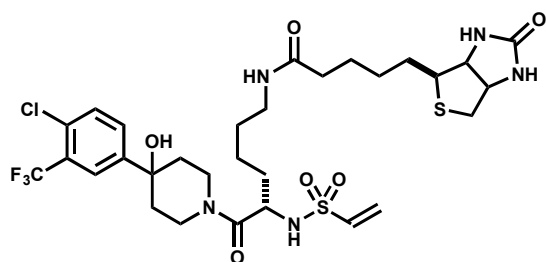
MS m/z (ES+) 734 (100 %, (M+H)⁺);

HRMS: found 734.2970 (M+H)⁺, C₃₃H₄₈ClF₃N₅O₆S requires 734.2965;

¹H NMR (CD₃OD, 400 MHz) δ 7.98 (m, 1H, Ar), 7.76 (m, 1H, Ar), 7.62 (m, 1H, Ar), 4.66-4.45 (m, 2H), 4.35 (m, 1H), 4.09-3.91 (m, 1H), 3.73-3.57 (m, 1H), 3.32-3.08 (m, 1H), 2.97 (dd, *J* 12.5, 4.5, 1H), 2.75 (dd, *J* 12.5, 4.5 Hz 1H), 2.52 (t, *J* 7 Hz, 1H), 2.33-2.18 (m, 2H), 2.13-1.92 (m, 2H), 1.90-1.54 (m, 7H), 1.49 (s, 9H, Boc).

¹³C NMR (CD₃OD, 125 MHz) δ 174.5, 172.4, 171.6, 164.6, 156.5, 148.4, 131.18, 129.9, 124.5, 121.8, 79.2, 70.4, 62.0, 60.2, 55.6, 50.2, 41.8, 41.4, 39.8, 38.7, 38.1, 37.7, 35.5, 32.5, 32.4, 31.4, 28.8, 28.6, 28.4, 28.3, 28.1, 27.5, 25.6, 25.1, 22.8.

N-((*S*)-6-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-6-oxo-5-(vinylsulfonamido)hexyl)-5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamide



tert-butyl ((2*R*)-1-(4-(4-chloro-3-(trifluoromethyl)phenyl)-4-hydroxypiperidin-1-yl)-1-oxo-6-(5-(2-oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexan-2-yl)carbamate

(85 mg, 0.115 mmol, 1 equiv) was dissolved in TFA and stirred at room temperature, TIPSH (10 μl) and water (10 μl) were added and the solution was stirred at room temperature for six hours by which time TLC indicated consumption of starting material. TFA was removed under reduced pressure. The residue was precipitated with ice cold diethyl ether, the ether was decanted and the precipitate was redissolved in DMF (2 mL) and was cooled in an ice bath. Triethylamine (80 μl, 0.58 mmol, 5 equiv) was added 2-chloroethyl sulfonyl chloride (14 μl, 1.2 equiv) was added, a precipitate formed almost immediately. The ice bath was removed and the solution was stirred for 1 hour, and quenched with NH₄Cl. The reaction was extracted with ethyl acetate, washed with brine and dried with Na₂SO₄. The

solvent was removed under reduced pressure the residue was purified using flash chromatography (0 % to 100 % gradient of [1 %:9 %:90 %, NH₄OH:MeOH:CH₂Cl₂] in CH₂Cl₂ to give 19 mg as a colourless oil. That was HPLC was purified.

HRMS: found 724.2212 (M+H)⁺, C₃₀H₄₂ClF₃N₅O₆S₂ requires 724.221716;

