Electronic Supplementary Information

Design, Synthesis and Biological Evaluation of β-Peptoid-Capped HDAC Inhibitors with Anti-Neuroblastoma and Anti-Glioblastoma Activity

Nina Reßing[a,b], Viktoria Marquardt[b,c,d,e], Christoph G. W. Gertzen[b,f], Andrea Schöler[a], Alexander Schramm[g], Thomas Kurz[b], Holger Gohlke[b,f], Achim Aigner[h], Marc Remke[c,d,e] and Finn K. Hansen[a,b]*

[a]Pharmaceutical/Medicinal Chemistry, Institute of Pharmacy, Leipzig University, Medical Faculty, Brüderstr. 34, 04103 Leipzig.
*Email: finn.hansen@uni-leipzig.de, phone: +49 341 97 36801, fax: +49 341 97 36889

[b]Institute of Pharmaceutical and Medicinal Chemistry, Heinrich-Heine-University Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf.

[c]Department of Pediatric Oncology, Hematology, and Clinical Immunology, Medical Faculty, Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf.

[d]Department of Neuropathology, Medical Faculty, Heinrich-Heine-University, Moorenstr. 5 40225 Düsseldorf.

[e]Division of Pediatric Neuro-Oncogenomics, German Cancer Research Center (DKFZ), Moorenstr. 5, 40225 Düsseldorf.

[f]John von Neumann Institute for Computing (NIC), Jülich Supercomputing Centre (JSC), and Institute for Complex Systems - Structural Biochemistry (ICS-6), Forschungszentrum Jülich GmbH, Wilhelm-Johnen-Straße, 52425 Jülich.

[g]Department of Pediatric Oncology and Hematology, University Children's Hospital Essen, University of Duisburg-Essen, Hufelandstr. 55, 45122 Essen.

[h]Rudolf-Boehm-Institute for Pharmacology and Toxicology, Independent Division for Clinical Pharmacology, Leipzig University, Medical Faculty Immunology, Medical Faculty, Härtelstr. 16-18, 04107 Leipzig. Heinrich-Heine-University, Moorenstr. 5, 40225 Düsseldorf.

Table of Contents

1. Biological evaluation

Cell Lines and Cell Culture

The human neuroblastoma cell lines CHP-134, IMR-32, NB-1 and SK-N-AS were kindly provided by Dr. Alexander Schramm (Department of Pediatric Oncology, University Hospital Essen) and were grown at 37 °C under humidified air supplemented with 5 % CO_2 in RPMI 1640 containing 10 % fetal calf serum. The glioma cell line G55T2 was a kind gift from Dr. Katrin Lamszus (Uniklinikum Eppendorf) and was grown under the same conditions in IMDM containing 10 % fetal calf serum + 1 % glutamine.

CellTiter-Glo Luminescent Cell Viability Assay

CHP-134, IMR-32, NB-1 and SK-N-AS were seeded at the respective optimal density of 4500, 4000, 6000, 2500 cells/well in white 384-well plates (Corning) and incubated with increasing concentrations of the test compounds (12 dilutions from 0.005-25 µM). After 72 h the plates were equilibrated at room temperature for 30 minutes, 30 µL Celltiter Glo reagent (Promega) was added, and after shaking the plates for 2 min and a subsequent incubation time of 10 min the luminescent signals were read on a Spark 10M microplate reader (Tecan).

WST8 colorimetric assay

G55T2 cells were seeded at 1,500 cells / well in 96-well plates. After 24 h, the medium was aspirated and 150 µl fresh medium was added, containing the test compounds (coming from a 10 mM stock solution in DMSO) at final concentrations between 100 µM and 0.01 µM. The corresponding volumes of DMSO served as negative control. After 72 h, the medium was aspirated and viable cells were quantitated in triplicate wells per concentration using the WST8 colorimetric assay (Dojindo Laboratories, Munich, Germany) according to manufacturer's protocol. Absorption at 450 nm was measured after 1 h using a multiplate reader.

***In vitro* testing on HDAC1 and HDAC6**

The in vitro inhibitory activity of compounds **6a-k**, vorinostat, and ricolinostat against human HDAC1 and HDAC6 were measured using a previously published protocol.[1] OptiPlate-96 black microplates (Perkin Elmer) were used with an assay volume of 50 µL. 5 µL test compound or control, diluted in assay buffer (50 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 0.1 mg/mL BSA), were incubated with 35 µL of the fluorogenic substrate ZMAL (Z-Lys(Ac)-AMC) (21.43 µM in assay buffer) and 10 µL of human recombinant HDAC1 (BPS Bioscience, Catalog# 50051) or HDAC6 (BPS Bioscience, Catalog# 50006) at 37 °C. After an incubation time of 90 min, 50 µL of 0.4 mg/mL trypsin in trypsin buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl) were added, followed by further incubation at 37 °C for 30 min. Fluorescence was measured with an excitation wavelength of 355 nm and an emission wavelength of 460 nm using a Fluoroskan Ascent microplate reader (Thermo Scientific). All compounds were evaluated in duplicate in at least two independent experiments.

Cell cycle analysis

For cell cycle analysis, $1x10^4$ G55T2 cells were seeded into 24-well plates and treated with inhibitors as indicated in the figure / in the text. After 72 h, cells were harvested by trypsinization, washed with PBS and fixed for 30 min with 70% ethanol at 4°C. Prior to addition of 50 µg/ml propidium iodide (Sigma-Aldrich), cells were incubated with 50 µg/ml RNase A for 30 min at 37°C on a rocker platform and subsequently analyzed by flow cytometry using an Attune® Acoustic Focusing Cytometer (Life Technologies, Darmstadt, Germany).

2. Chemistry

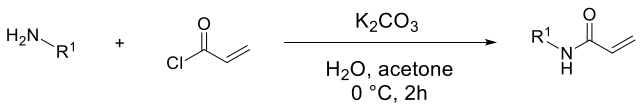
General information

All reagents and solvents were purchased from commercial sources and used without further purification. Gradient flash column chromatography was performed on a Teledyne ISCO Combiflash R_f system using prepacked silica cartridge and specified solvent systems. Thin layer chromatography was carried out using Macherey-Nagel pre-coated aluminium foil sheets which were visualised using UV light (254 nm) and, in the case of hydroxamic acids, stained with a 1% solution of iron(III) chloride in methanol.

^{1}H-NMR and ^{13}C-NMR spectra were recorded at room temperature on Bruker Avance III spectrometers operating at either 300 or 600 MHz. Chemical shifts (δ) are quoted in parts per million (ppm). All spectra were standardised in accordance with the signals of the deuterated solvents ($CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm; DMSO-d_6: δ_H = 2.50 ppm, δ_C = 39.5 ppm, MeOH-d_4: δ_H = 4.87 ppm). Coupling constants (*J*) are reported in Hertz (Hz). ^{1}H signals marked with an asterisk (*) correspond to peaks assigned to the major rotamer conformation. Mass-spectra were measured by the Heinrich-Heine-Universität Mass Spectrometry Service, using electrospray ionisation (ESI) on a Bruker Daltonics UHR-QTOF maXis 4G. The uncorrected melting points were determined using a Stuart SMP11 apparatus.

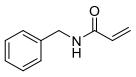
Analytical HPLC analysis were carried out using a Knauer Azura P 6.1L system equipped with P 6.1L (pumps), a Smartline UV detector 2600 and a Phenomenex Luna 5u C18(2) 1.8 µm particle (250 mm x 4.6 mm) column, supported by Phenomenex Security Guard Cartridge Kit C18 (4.0 mm x 3.0 mm). UV absorption was detected at 254 nm with a linear gradient of 10% B to 100% B within 20 minutes. HPLC-grade water (solvent A) and HPLC-grade acetonitrile (solvent B) were used for elution at a flow rate of 1 mL/min. Both solvents were enriched with 0.1% TFA. The purity of all final compounds was at least 95.0%.

General procedure for the synthesis of compounds 2a-2c



1 **2a-c**

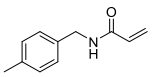
A suspension of potassium carbonate (2.0 eq) in a mixture of acetone and water (4:1) was cooled to 0 °C and acryloyl chloride (2.0 eq) followed by the appropriate amine **1** (1.0 eq) were added dropwise over 30 min. The resulting mixture was then stirred at 0 °C for 2 h before the precipitate was removed by filtration. The filtrate was evaporated and the residue was diluted with water (20 mL), extracted with CH_2Cl_2 (3 x 50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to afford the desired products **2a-2c.**



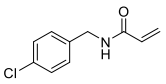
***N*-Benzylacrylamide (2a).** Synthesized using potassium carbonate (8.28 g, 60.0 mmol), acryloyl chloride (4.8 mL, 60,0 mmol) and benzylamine (3.27 mL, 30.0 mmol) in acetone (60 mL) and water (15 mL). Purification by column chromatography (1:1 *n*-hexane/EtOAC) afforded **2a** as a white solid (4.65 g, 28.8 mmol, 96%); mp 60-65 °C; R_f 0.64 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.68 – 8.50 (m, 1H), 7.36 – 7.23 (m, 5H), 6.29 (dd, *J* = 17.1, 10.2 Hz, 1H), 6.14 (dd, *J* = 17.1, 2.2 Hz, 1H), 5.62 (dd, *J* = 10.2, 2.2 Hz, 1H), 4.36 (d, *J* = 6.0 Hz, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 164.58, 139.27, 131.65, 128.32, 127.37, 126.85, 125.39, 42.18 ppm.

Spectroscopic data matched those reported in the literature.[2]

N-(4-Methylbenzyl)acrylamide (2b). Synthesis using potassium carbonate (8.28 g, 60.0 mmol), acryloyl chloride (4.8 mL, 60.0 mmol) and 4-methylbenzylamine

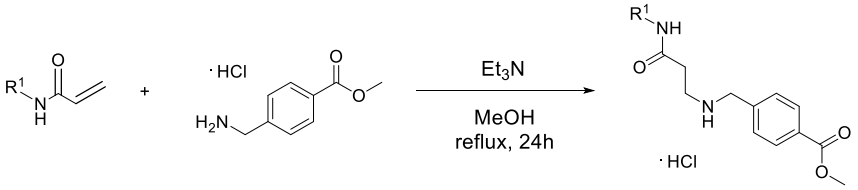


(4.3 mL, 34.0 mmol) in acetone (60 mL) and water (15 mL) afforded **2b** as a white solid (5.79 g, 33.0 mmol, 97%); mp 102-108 °C; R_f 0.72 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H = 7.24 – 7.08 (m, 4H), 6.31 (dd, *J* = 17.0, 1.5 Hz, 1H), 6.10 (dd, *J* = 17.0, 10.2 Hz, 1H), 5.96 – 5.78 (m, 1H), 5.65 (dd, *J* = 10.2, 1.5 Hz, 1H), 4.47 (d, *J* = 5.7 Hz, 2H), 2.33 (s, 3H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 165.52, 137.36, 135.11, 130.84, 129.46, 128.00, 126.70, 43.52, 21.19 ppm; HRMS (ESI$^+$): found: 176.1071; $C_{11}H_{13}NO$ (MH^+) requires 176.1070.
Spectroscopic data matched those reported in the literature.[2]



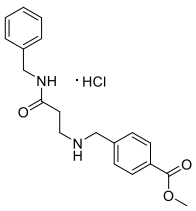
***N*-(4-Chlorobenzyl)acrylamide (2c).** Synthesized using potassium carbonate (2.04 g, 14.8 mmol), acryloyl chloride (1.15 mL, 14.1 mmol) and (4-chlorophenyl)methanamine (0.86 mL, 7.06 mmol) in acetone (20 mL) and water (5 mL). Purification by flash column chromatography (1:1 *n*-hexane/EtOAC) afforded 2c as a white solid (1.09 g, 5.06 mmol, 79%); mp 97-100 °C; R_f 0.68 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H = 7.32 – 7.27 (m, 2H), 7.24 – 7.20 (m, 2H), 6.32 (dd, *J* = 17.0, 1.4 Hz, 1H), 6.11 (dd, *J* = 16.9, 10.3 Hz, 1H), 5.92 (s, 1H), 5.68 (dd, *J* = 10.3, 1.4 Hz, 1H), 4.48 (d, *J* = 5.9 Hz, 2H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 165.71, 136.75, 133.38, 130.60, 129.22, 128.88, 127.06, 42.97ppm; HRMS (ESI$^+$): found: 196.0522; $C_{10}H_{10}ClNO$ (MH^+) requires 196.0524.

General procedure for the synthesis of compounds 3a-d

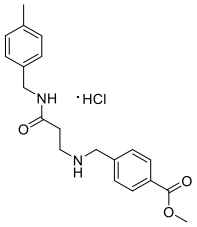


2a-c **3a-d**

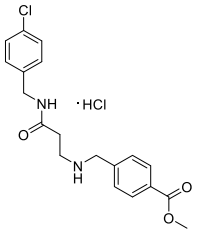
To a mixture of methyl 4-(aminomethyl)benzoate hydrochloride (1.0 eq) and the appropriate acryl amide **2** (1.2 eq) in MeOH was added triethylamine (3.0 eq) and the resulting mixture was refluxed for 24h. Upon completion of the reaction, the solvent and remains of triethylamine were removed *in vacuo*. The residue was dissolved in MeOH (20 mL), treated with a sat. solution of HCl in diethyl ether (4 mL) and allowed to crystallise at −18 °C for 18 h. Filtration of the precipitate, washing with cold diethyl ether (30 mL) and ethyl acetate (10 mL) and drying *in vacuo* afforded the desired products **3a-3c**



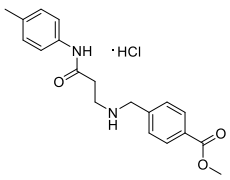
Methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl) hydrochloride (3a). Synthesis using methyl 4-(aminomethyl)benzoate hydrochloride (2.10 g, 10.3 mmol), *N*-benzylacrylamide (**2a**) (2.00 g, 12.4 mmol) and triethylamine (5.00 mL, 35.9 mmol) in MeOH (100 mL) afforded **3a** as a white solid (3.43 g, 9.45 mmol, 92%); mp 237-240 °C; R_f 0.37 (9:1 CH_2Cl_2/MeOH + 0.1% NEt_3); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 9.38 (s, 2H), 8.68 (t, *J* = 6.0 Hz, 1H), 8.03 – 7.97 (m, 2H), 7.72 – 7.67 (m, 2H), 7.65 – 7.62 (m, 0H), 7.35 – 7.29 (m, 2H), 7.29 – 7.19 (m, 3H), 4.28 (d, *J* = 5.9 Hz, 2H), 4.23 (s, 2H), 3.87 (s, 3H), 3.13 (t, *J* = 7.4 Hz, 2H), 2.68 (t, *J* = 7.4 Hz, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 169.04, 165.92, 139.17, 137.29, 130.39, 129.99, 129.38, 129.23, 128.34, 127.36, 126.90, 52.35, 49.48, 42.87, 42.18, 30.98 ppm; HRMS (ESI$^+$): found: 327.1703; $C_{19}H_{22}N_2O_3$ (MH^+) requires 327.1703.



Methyl 4-(((3-((4-methylbenzyl)amino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (3b). Synthesis using 4-(aminomethyl)benzoate hydrochloride (403 mg, 2.00 mmol), *N*-(4-methylbenzyl)acrylamide (**2b**) (387 mg, 2.40 mmol) and triethylamine (0.83, 6.00 mmol) in MeOH (10 mL) afforded **3b** as a white solid (609 mg, 1.68 mmol, 84%); mp 241-241 °C; R_f 0.40 (9:1 CH_2Cl_2/MeOH + 0.1% NEt_3); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 9.46 (s, 2H), 8.64 (t, *J* = 5.9 Hz, 1H), 8.04 – 7.98 (m, 2H), 7.71 (d, *J* = 7.9 Hz, 2H), 7.12 (q, *J* = 8.1 Hz, 4H), 4.23 – 4.21 (m, 4H), 3.87 (s, 3H), 3.11 (t, *J* = 7.4 Hz, 2H), 2.67 (t, *J* = 7.4 Hz, 2H), 2.26 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 168.90, 165.86, 137.31, 136.10, 135.91, 130.32, 129.93, 129.34, 129.30, 129.18, 128.82, 127.33, 52.30, 49.43, 42.83, 41.89, 30.95, 20.67 ppm; HRMS (ESI$^+$): found: 341.1860; $C_{20}H_{24}N_2O_3$ (MH^+) requires 341.1860.

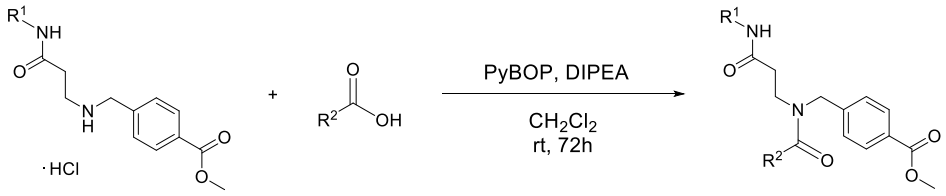


Methyl 4-(((3-((4-chlorobenzyl)amino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (3c). Synthesis using 4-(aminomethyl)benzoate hydrochloride (514 mg, 2.55 mmol), *N*-(4-chlorobenzyl)acrylamide (**2c**) (600 mg, 3.10 mmol) and triethylamine (1.00 mL, 7.23 mmol) in MeOH (15 mL) afforded **3c** as a white solid (626 mg, 1.58 mmol, 62%); mp 244-248°C; R_f 0.39 (9:1 CH_2Cl_2/MeOH + 0.1% NEt_3), ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 9.53 (s, 2H), 8.76 (t, *J* = 6.0 Hz, 1H), 8.03 – 7.98 (m, 2H), 7.75 – 7.69 (m, 2H), 7.40 – 7.34 (m, 2H), 7.31 – 7.24 (m, 2H), 4.25 (d, *J* = 6.0 Hz, 2H), 4.22 (s, 2H), 3.86 (s, 3H), 3.11 (t, *J* = 7.5 Hz, 2H), 2.70 (t, *J* = 7.4 Hz, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 169.05, 165.85, 138.27, 137.27, 131.38, 130.33, 129.93, 129.33, 129.18, 128.21, 52.30, 49.42, 42.75, 41.46, 30.94 ppm; HRMS (ESI$^+$): found: 361.1311; $C_{19}H_{21}ClN_2O_3$ (MH^+) requires 361.1313.



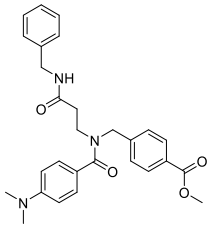
Methyl 4-(((3-oxo-3-(*p*-tolylamino)propyl)amino)methyl)benzoate hydrochloride (3d). Synthesis using 4-(aminomethyl)benzoate hydrochloride (403 mg, 2.00 mmol), *N*-(*p*-tolyl)acrylamide (378 mg, 2.40 mmol) and triethylamine (1.00 mL, 7.23 mmol) in MeOH (10 mL) afforded **3d** as a white solid (609 mg, 1.68 mmol, 84%); mp 225-229 °C; R_f 0.31 (9:1 CH_2Cl_2/MeOH + 0.1% NEt_3); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 10.27 (s, 1H), 9.63 (s, 2H), 8.09 – 7.89 (m, 2H), 7.80 – 7.65 (m, 2H), 7.59 – 7.36 (m, 2H), 7.09 (d, *J* = 8.1 Hz, 2H), 4.26 (s, 2H), 3.86 (s, 3H), 3.18 (t, *J* = 7.3 Hz, 2H), 2.88 (t, *J* = 7.3 Hz, 2H), 2.23 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 167.68, 165.86, 137.26, 136.43, 132.17, 130.39, 129.94, 129.32, 129.27, 129.19, 129.05, 119.18, 52.29, 49.51, 42.54, 32.01, 20.44 ppm; HRMS (ESI$^+$): found: 327.1705; $C_{19}H_{22}N_2O_3$ (MH^+) requires 327.1703.

General procedure for the synthesis of compounds 5b-f and 5h

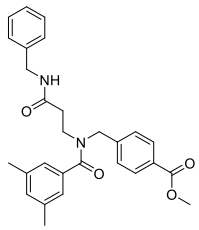


3a-d **4** **5b-f, h**

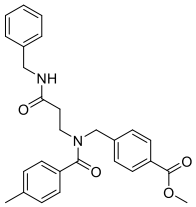
To a suspension of the respective ammonium salt **2** (1.0 eq) and the appropriate carboxylic acid (1.1 eq) in CH_2Cl_2 were added PyBOP (1.1 eq) and DIPEA (3.0 eq), successively. The resulting solution was then stirred at rt for 72h before being concentrated *in vacuo*. Purification of the residue by flash column chromatography (prepacked silica cartridge, *n*-hexane/EtOAc gradient 90:10 to 0:100 in 30 min) afforded the desired products **5b-f and 5h**.



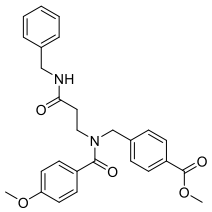
Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-(dimethylamino)benzamido)-methyl)benzoate (5b). Synthesized using methyl 4-(((3-(benzylamino)-3 oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (304 mg, 0.85 mmol), 4-(dimethylamino)benzoic acid (153 mg, 0.92 mmol), PyBOP (486 mg, 0.92 mmol) and DIPEA (0.45 mL, 2.72 mmol) in CH_2Cl_2 (9 mL). Purification by flash column chromatography afforded **5b** as a white solid (220 mg, 0.46 mmol, 55%); mp 105-108 °C; R_f 0.64 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): 8.53 – 8.38 (m, 1H), 8.01 – 7.92 (m, 2H), 7.46 – 7.20 (m, 9H), 6.74 – 6.62 (m, 2H), 4.66 (s, 2H), 4.24 (d, J = 5.9 Hz, 2H), 3.85 (s, 3H), 3.53 (t, J = 7.2 Hz, 2H), 2.92 (s, 6H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.50, 170.08, 166.05, 151.07, 143.73, 139.36, 130.91, 129.51, 128.99, 128.43, 128.35, 128.26, 127.33, 127.22, 126.78, 122.53, 111.03, 110.74, 52.09, 42.11, 39.73 ppm; HRMS (ESI$^+$): found: 474.2394; $C_{28}H_{31}N_3O_4$ (MH^+) requires 474.2387.



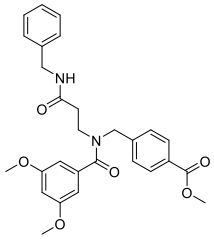
Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-3,5-dimethylbenzamido)-methyl)benzoate (5c). Synthesized using methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (303 mg, 0.83 mmol), 3,5-dimethylbenzoic acid (150 mg, 1.00 mmol), PyBOP (522 mg, 1.00 mmol) and DIPEA (0.41 mL, 2.49 mmol) in CH_2Cl_2 (9 mL). Purification by repeated flash column chromatography afforded **5c** as a colourless oil (312 mg, 0.68 mmol, 82%); R_f 0.69 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.53/8.39 (2 x s, 1H), 8.01 – 7.92 (m, 2H), 7.48 – 6.85 (m, 10H), 4.72/4.47 (2 x s, 2H), 4.28/4.19 (2 x s, 2H), 3.85 (s, 3H), 3.54/3.39 (2 x s, 2H), 2.52/2.39 (2 x s, 2H), 2.30*/2.21 (2 x s, 6H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.42, 170.55, 169.82, 166.25, 166.13, 143.59, 143.13, 139.51, 139.23, 137.78, 136.40, 130.79, 129.65, 129.59, 128.74, 128.49, 128.39, 127.69, 127.46, 127.36, 127.09, 126.94, 123.97, 52.41, 52.24, 46.92, 45.27, 42.27, 42.21, 41.88, 40.06, 39.94, 34.65, 33.49, 20.90 ppm; HRMS (ESI$^+$): found: 459.2280; $C_{28}H_{30}N_2O_4$ (MH^+) requires 459.2278.



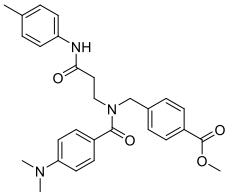
Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-methylbenzamido)-methyl)benzoate (5d). Synthesized using methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (300 mg, 0.83 mmol), 4-methylbenzoic acid (147 mg, 1.08 mmol), PyBOP (526 mg, 1.08 mmol) and DIPEA (0.41 mL, 2.49 mmol) in CH_2Cl_2 (9 mL). Purification by flash column chromatography followed by recrystallization from EtOAC (5 mL) and *n*-hexane (30 mL) afforded **5d** as a white solid (328 mg, 0.74 mmol, 89%); mp 128-132 °C; R_f 0.63 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.54/8.39 (2 x s, 1H), 7.94 (m, 2H), 7.50 – 7.12 (m, 11H), 4.73/4.49 (2 x s, 2H), 4.27/4.20 (2 x s, 2H), 3.85 (s, 3H), 3.54/3.42 (2 x s, 2H), 2.53/2.41 (2 x s, 2H), 2.32 (m, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.18, 166.02, 138.99, 133.45, 129.56, 129.50, 128.90, 128.26, 127.54, 127.35, 126.80, 126.43, 126.40, 54.91, 52.26, 52.11, 48.61, 46.96, 46.87, 45.30, 42.17, 41.84, 34.46, 33.27, 20.89 ppm; HRMS (ESI$^+$): found: 445.2122; $C_{27}H_{28}N_2O_4$ (MH^+) requires 445.2122.



Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-methoxybenzamido)-methyl)benzoate (5e).
Synthesized using methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (350 mg, 0.96 mmol), 4-methoxybenzoic acid (161 mg, 1.06 mmol), PyBOP (551 mg, 1.06 mmol) and DIPEA (0.47 mL, 2.88 mmol) in CH_2Cl_2 (10 mL). Purification by flash column chromatography followed by recrystallization from EtOAC (5 mL) and *n*-hexane (30 mL) afforded **5e** as a white solid (389 mg, 0.85 mmol, 88%); mp 95-98 °C; R_f 0.62 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.45 (s, 1H), 7.99 – 7.90 (m, 2H), 7.55 – 7.14 (m, 9H), 7.02 – 6.84 (m, 2H), 4.70/4.56 (2 x s, 2H), 4.23 (s, 2H), 3.85 (s, 3H), 3.77 (s, 3H), 3.49 (s, 3H), 2.48 – 2.40 (m, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 170.92, 166.03, 165.27, 160.04, 143.33, 139.28, 136.09, 129.51, 128.46, 128.37, 128.27, 127.35, 126.95, 126.81, 113.68, 55.22, 52.11, 42.12, 40.05 ppm; HRMS (ESI$^+$): found: 461.2069; $C_{27}H_{28}N_2O_5$ (MH^+) requires 461.2071.

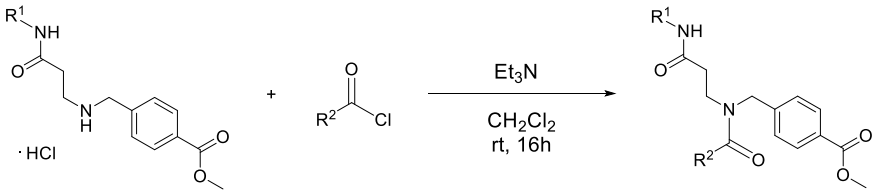


Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-3,5-dimethoxybenzamido)-methyl)benzoate (5f). Synthesized using methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (350 mg, 0.96 mmol), 3,5-dimethoxybenzoic acid (193 mg, 1.06 mmol), PyBOP (551 mg, 1.06 mmol) and DIPEA (0.47 mL, 2.88 mmol) in CH_2Cl_2 (10 mL). Purification by repeated flash column chromatography afforded **5f** as a colourless oil (315 mg, 0.64 mmol, 67%); R_f 0.67 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.54/8.40 (2 x s, 1H), 8.01 – 7.92 (m, 2H), 7.51 – 7.41 (m, 1H), 7.36 – 7.11 (m, 5H), 6.61 – 6.36 (m, 3H), 4.72/4.46 (2 x s, 2H), 4.27/4.19 (2 x s, 2H), 3.85 (s, 3H), 3.77/3.65 (2 x s, 6H), 3.55/3.40 (2 x s, 2H), 2.54/2.42 (2 x s, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 170.69, 170.62, 170.50, 170.47, 169.78, 166.22, 166.12, 160.50, 160.44, 143.39, 143.13, 139.45, 139.22, 138.39, 138.26, 129.61, 128.42, 128.37, 128.32, 127.71, 127.63, 127.38, 127.04, 126.92, 104.31, 104.28, 101.11, 55.50, 55.36, 52.22, 46.94, 45.12, 42.24, 42.01, 40.05, 39.94, 34.65, 33.46 ppm; HRMS (ESI$^+$): found: 491.2178; $C_{28}H_{30}N_2O_6$ (MH^+) requires 491.2177.



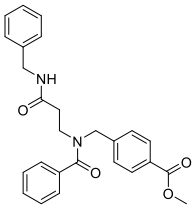
Methyl 4-((4-(dimethylamino)-*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamido)-methyl)benzoate (5h). Synthesized using methyl 4-(((3-oxo-3-(*p*-tolylamino)-propyl)amino)methyl)benzoate hydrochloride (**3d**) (400 mg, 1.12 mmol), 4-(dimethylamino)benzoic acid (201 mg, 1.22 mmol), PyBOP (640 mg, 1.22 mmol) and DIPEA (0.60 mL, 3.58 mmol) in CH_2Cl_2 (12 mL). Purification by flash column chromatography afforded **5h** as an off-white solid (477 mg, 1.00 mmol, 89%); mp 142-147 °C; R_f 0.72 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 9.88 (s, 1H), 8.04 – 7.87 (m, 2H), 7.46 – 7.34 (m, 4H), 7.31 – 7.24 (m, 2H), 7.12 – 7.04 (m, 2H), 6.72 – 6.64 (m, 2H), 4.71 (s, 2H), 3.85 (s, 3H), 3.58 (t, *J* = 7.2 Hz, 2H), 2.92 (s, 6H), 2.64 (t, *J* = 7.0 Hz, 2H), 2.23 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.58, 166.04, 151.09, 143.74, 136.52, 132.04, 129.52, 129.03, 128.98, 128.52, 128.44, 128.34, 127.19, 122.49, 119.13, 111.05, 110.74, 59.76, 52.09, 39.71, 34.83, 20.76, 20.43, 14.09 ppm, HRMS (ESI$^+$): found: 474.2390; $C_{28}H_{31}N_3O_4$ (MH^+) requires 474.2387.

General procedure for the synthesis of compounds 5a, 5g, 5i-k

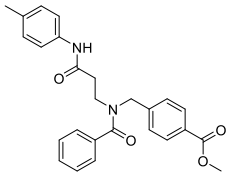


3a-d **4** **5a, g, i-k**

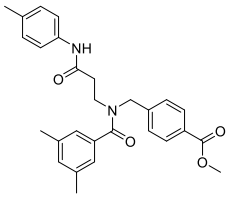
To a mixture of the respective ammonium salt **2** (1.0 eq) and trimethylamine (2.0 eq) in CH_2Cl_2 at 0 °C was added the appropriate carboxylic acid chloride (1.08 eq) dropwise over 5 min. Upon complete addition, the solution was allowed to warm to rt and stirred for 16 h. The mixture was then diluted with CH_2Cl_2 (10 mL), washed with 1M aq. HCl (2 x 3 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification of the residue by flash column chromatography (prepacked silica cartridge, *n*-hexane/EtOAc gradient 90:10 to 0:100 in 30 min) afforded the desired products **5a, 5g, 5i-k.**



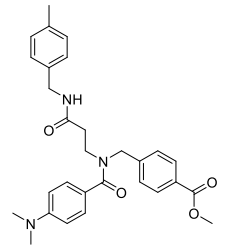
Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)benzamido)methyl)benzoate (5a). Synthesized using methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (304 mg, 0.85 mmol), triethylamine (0.28 mL, 2.02 mmol) and benzoyl chloride (0.11 mL, 0.92 mmol) in CH_2Cl_2 (4 mL). Purification by flash column chromatography afforded **5a** as a yellow oil (157 mg, 0.36 mmol, 43%); R_f 0.67 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H = 8.55/8.39 (2 x s, 1H), 8.02 – 7.85 (m, 2H), 7.52 – 7.13 (m, 12H), 4.75/4.47 (2 x s, 2H), 4.28/4.20 (2 x s, 2H), 3.85 (s, 3H), 3.57/3.41 (2 x s, 2H), 2.55/2.41 (2 x s, 2H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 171.06, 170.99, 170.26, 169.54, 166.09, 166.02, 143.40, 142.93, 139.47, 139.16, 136.31, 129.47, 129.37, 128.43, 128.27, 127.58, 127.41, 127.37, 127.29, 126.93, 126.80, 126.37, 126.28, 52.27, 52.11, 46.91, 45.24, 45.20, 42.09, 41.93, 34.44, 33.39, 21.82, 18.10, 16.72 ppm; HRMS (ESI$^+$): found: 431.1969; $C_{26}H_{26}N_2O_4$ (MH^+) requires 431.1965.



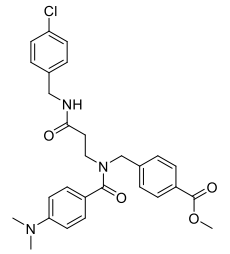
Methyl 4-((*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamido)methyl)benzoate (5g). Synthesized using methyl 4-(((3-oxo-3-(*p*-tolylamino)-propyl)amino)methyl)benzoate hydrochloride (**3d**) (400 mg, 1.12 mmol), triethylamine (0.37 mL, 2.70 mmol) and benzoyl chloride (0.14 mL, 1.22 mmol) in CH_2Cl_2 (4 mL). Purification by flash column chromatography afforded **5g** as a white solid (367 mg, 0.85 mmol, 76%); mp 105-107 °C; R_f 0.71 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 9.96/9.81 (2 x s, 1H), 8.06 – 7.86 (m, 2H), 7.54 – 7.19 (m, 9H), 7.19 – 6.96 (m, 2H), 4.79/4.58 (2 x s, 2H), 3.85 (s, 3H), 3.63/3.49 (2 x s, 2H), 2.71/2.55 (2 x s, 2H), 2.23 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.10, 169.13, 168.31, 165.97, 143.44, 142.92, 136.55, 136.36, 132.08, 129.57, 129.41, 129.04, 127.58, 127.53, 127.01, 126.97, 126.38, 126.24, 119.05, 52.33, 52.28, 52.10, 47.02, 45.10, 41.72, 35.34, 34.35, 20.43 ppm; HRMS (ESI$^+$): found: 431.1969; $C_{26}H_{26}N_2O_4$ (MH^+) requires 431.1965.



Methyl 4-((3,5-dimethyl-*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamido)-methyl)benzoate (5i). Synthesized using methyl 4-(((3-oxo-3-(*p* tolylamino)propyl)amino)methyl)benzoate hydrochloride (**3d**) (100 mg, 0.28 mmol), 3,5-dimethylbenzoic acid chloride (51.3 mg, 0.30 mmol) and triethylamine (0.08 mL, 0.57 mmol) in CH_2Cl_2 (4 mL). Purification by flash column chromatography afforded **5i** as a colourless oil (105 mg, 0.22 mmol, 82%); R_f0.78 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H = 9.14 (s, 1H), 8.11 – 7.99 (m, 2H), 7.50 – 7.43 (m, 2H), 7.33 – 7.23 (m, 2H), 7.15 – 6.98 (m, 5H), 4.85/4.70* (2 x s, 2H), 3.96 (s, 3H), 3.83 – 3.74*/3.72 – 3.65 (2 x m, 2H), 2.83*/2.49 (2 x s, 2H), 2.40 – 2.21 (m, 9H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 173.39, 169.51, 166.70, 142.18, 138.41, 135.78, 135.58, 133.59, 131.48, 130.07, 129.55, 129.31, 127.02, 124.16, 120.05, 77.51, 54.11, 52.17, 42.65, 35.57, 21.17, 20.87 ppm; HRMS (ESI$^+$): found: 459.2284; $C_{28}H_{30}N_2O_4$ (MH^+) requires 459.2278.

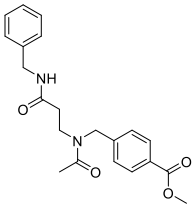


Methyl 4-((4-(dimethylamino)-*N*-(3-((4-methylbenzyl)amino)-3-oxopropyl) benzamido)methyl)benzoate (5j). Synthesized using methyl 4-(((3-((4-methylbenzyl)amino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3b**) (250 mg, 0.73 mmol), 4-(dimethylamino)benzoic acid chloride (40.0 mg, 0.78 mmol) and triethylamine (0.25 mL, 1.79 mmol) in CH_2Cl_2 (4 mL). Purification by flash column chromatography afforded **5j** as a colourless oil (206 mg, 0.42 mmol, 58%); R_f0.72 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H = 8.00 – 7.95 (m, 2H), 7.25 – 7.04 (m, 10H), 6.55 – 6.49 (m, 2H), 4.67 (s, 2H), 4.32 (d, *J* = 5.8 Hz, 2H), 3.89 (s, 3H), 3.64 (t, *J* = 6.7 Hz, 2H), 2.94 (s, 6H), 2.56 (s, 2H), 2.30 (s, 3H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 173.16, 170.93, 166.83, 151.49, 142.97, 136.88, 135.54, 131.78, 130.08, 129.35, 129.27, 128.74, 127.92, 127.02, 122.33, 111.23, 110.68, 77.37, 52.17, 43.29, 40.16, 34.86, 21.17 ppm; HRMS (ESI$^+$): found: 488.2546; $C_{29}H_{33}N_3O_4$ (MH^+) requires 488.2544.



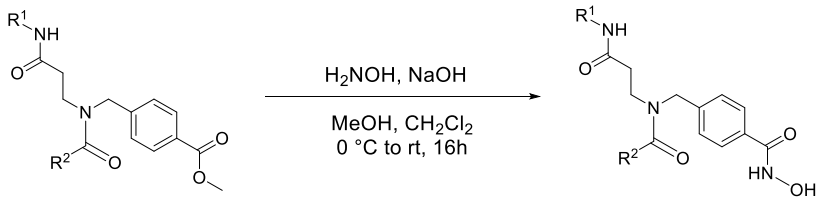
Methyl 4-((*N*-(3-((4-chlorobenzyl)amino)-3-oxopropyl)-4-(dimethylamino) benzamido)methyl)benzoate (5k). Synthesized using methyl 4-(((3-((4-chlorobenzyl)amino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3c**) (100 mg, 0.25 mmol), 4-(dimethylamino)benzoic acid chloride (50.0 mg, 0.28 mmol) and triethylamine (0.07 mL, 0.51 mmol) in CH_2Cl_2 (2 mL). Purification by flash column chromatography afforded **5k** as a white solid (108 mg, 0.21 mmol, 85%); mp 152-156 °C; R_f0.82 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H

= 8.02 – 7.96 (m, 2H), 7.27 – 7.12 (m, 11H), 6.59 – 6.52 (m, 2H), 4.67 (s, 2H), 4.34 (d, *J* = 5.9 Hz, 2H), 3.91 (s, 3H), 3.66 (t, *J* = 6.6 Hz, 2H), 2.96 (s, 6H), 2.60 (s, 2H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 173.12, 171.09, 169.03, 166.86, 151.58, 142.77, 137.27, 133.12, 130.23, 129.57, 129.37, 128.80, 128.77, 127.08, 111.38, 52.29, 42.89, 40.30, 34.92, 31.45 ppm; HRMS (ESI$^+$): found: 508.2001; $C_{28}H_{30}ClN_3O_4$ (MH^+) requires 508.1998.



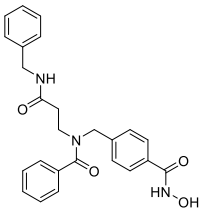
Methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)acetamido)methyl)benzoate (5l). To a solution of methyl 4-(((3-(benzylamino)-3-oxopropyl)amino)methyl)benzoate hydrochloride (**3a**) (303 mg, 0.83 mmol) in acetonitrile (12 mL) were added acetic anhydride (1.25 mL, 13.2 mmol) and triethylamine (1.25 mL, 9.03 mmol), successively. The resulting mixture was stirred at rt for 43h before being concentrated *in vacuo*. Purification of the residue by flash column chromatography (prepacked silica cartridge, *n*-hexane/EtOAc gradient 90:10 to 0:100 in 30 min) afforded **5l** as a white solid (247 mg, 0.67 mmol, 80%), mp 110-113 °C; R_f 0.54 (9:1 CH_2Cl_2/MeOH); ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 8.45*/8.41 (2 x t, *J* = 5.8/6.1 Hz, 1H), 7.98 – 7.94/7.93 – 7.87 (2 x m, 2H), 7.33 – 7.19 (m, 7H), 4.62/4.55* (2 x s, 2H), 4.25*/4.23 (2 x d, *J* = 5.8/5.9 Hz, 2H), 3.85/3.84* (2 x s, 3H), 3.51*/3.46 (2 x t, *J* = 7.0/7.2 Hz, 2H), , 2.44*/2.39 (2 x t, *J* = 7.0/7.2 Hz, 2H), 2.13*/1.96 (2 x s, 3H) ppm; ^{13}C-NMR (151 MHz, $CDCl_3$): δ_C = 171.84, 171.21, 170.82, 169.70, 166.91, 166.74, 143.28, 142.08, 138.40, 137.77, 130.39, 130.04, 129.77, 129.40, 128.93, 128.81, 128.06, 128.02, 127.94, 127.87, 127.64, 126.25, 53.13, 52.32, 52.24, 48.76, 44.97, 43.98, 43.73, 35.78, 35.17, 21.81, 21.62 ppm; HRMS (ESI$^+$): found: 369.1812; $C_{21}H_{24}N_2O_4$ (MH^+) requires 369.1809.

General procedure for the synthesis of compounds 6a-k

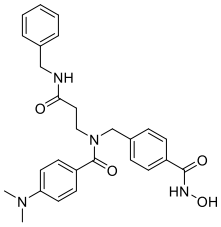


5a-k **6a-k**

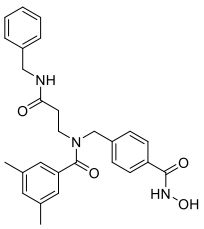
To a cooled (0 °C) solution of the respective ester **3** (1.0 eq) in MeOH and CH_2Cl_2 was added a 50% aq. solution of hydroxylamine (30.0 eq). The resulting mixture was stirred at 0 °C for 10 min before NaOH (10.0 eq) was added in one batch. The mixture was then allowed to warm to rt and stirred for 16h. Upon completion of the reaction, the solvent was evaporated *in vacuo* and the residue was suspended in water (25 mL). The mixture was neutralised using 1M aq. HCl and allowed to crystallize at 6 °C for 16h before the precipitate was isolated by filtration and washed with cold diethyl ether (15 mL). The solid thus obtained was subsequently purified by recrystallization from MeOH (5 mL) and diethylether (40 mL) and/or flash column chromatography (prepacked silica cartridge, CH_2Cl_2/MeOH gradient 100:0 to 70:30 in 30 min) to afford the desired products **6a-k.**



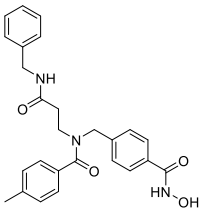
***N*-(3-(Benzylamino)-3-oxopropyl)-*N*-(4-(hydroxycarbamoyl)benzyl)benzamide (6a).** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)benzamido)methyl)benzoate (**5a**) (128 mg, 0.29 mmol), 50% aq. solution of hydroxylamine (0.48 mL, 7.84 mmol) and NaOH (110 mg, 2.75 mmol) in MeOH (4 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6a** as a white solid (58.0 mg, 0.14 mmol, 48%); mp 155-159 °C; R_f 0.26 (9:1 CH_2Cl_2/MeOH); t_R: 11.72 min, purity: 95.1%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.21 (s, 1H), 9.05 (s, 1H), 8.60 – 8.32 (m, 1H), 7.89 – 7.69 (m, 2H), 7.54 – 7.04 (m, 12H), 4.71/4.43 (2 x s, 2H), 4.28/4.20 (2 x s, 2H), 3.57 – 3.40 (m, 2H), 2.55/2.41 (2 x s, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 179.67, 171.08, 170.51, 169.66, 164.27, 164.01, 141.06, 140.54, 139.53, 139.26, 136.39, 131.88, 131.65, 129.49, 128.55, 128.38, 127.51, 127.39, 126.92, 126.70, 126.42, 99.61, 52.21, 46.71, 45.13, 42.18, 41.78, 34.52, 33.44 ppm; HRMS (ESI$^+$): found: 432.1922; $C_{25}H_{25}N_3O_4$ (MH^+) requires 432.1918.



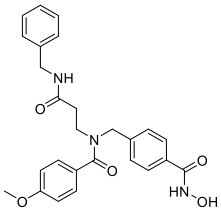
***N*-(3-(Benzylamino)-3-oxopropyl)-4-(dimethylamino)-*N*-(4-(hydroxycarbamoyl)-benzyl)benzamide (6b).** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-(dimethylamino)benzamido)methyl)benzoate (**5b**) (155 mg, 0.33 mmol), 50% aq. solution of hydroxylamine (0.62 mL, 10.1 mmol) and NaOH (140 mg, 3.50 mmol) in MeOH (4 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6b** as an off-white solid (81.0 mg, 0.17 mmol, 53%); mp 108-112 °C; R_f 0.31 (9:1 CH_2Cl_2/MeOH); t_R: 13.55 min, purity: 95.1%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H 11.18 (s, 1H), 9.14 – 8.91 (m, 1H), 8.51 – 8.37 (m, 1H), 7.79 – 7.68 (m, 2H), 7.45 – 7.10 (m, 9H), 6.76 – 6.55 (m, 2H), 4.61 (s, 2H), 4.24 (d, J = 5.9 Hz, 2H), 3.49 (t, J = 7.1 Hz, 2H), 2.93 (s, 6H), 2.51 – 2.46 (m, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 179.91, 171.50, 170.15, 164.05, 159.75, 151.09, 141.22, 139.38, 131.64, 128.38, 128.30, 127.34, 127.23, 126.82, 122.61, 111.06, 42.13, 39.77 ppm; HRMS (ESI$^+$): found: 475.2339; $C_{27}H_{30}N_4O_4$ (MH^+) requires 475.2340.



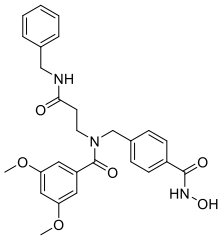
***N*-(3-(Benzylamino)-3-oxopropyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-3,5-dimethylbenzamide (6c)** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-3,5-dimethylbenzamido)methyl)benzoate (**5c**) (190 mg, 0.41 mmol), 50% aq. solution of hydroxylamine (0.47 mL, 7.67 mmol) and NaOH (110 mg, 2.75 mmol) in MeOH (4.0 mL) and CH_2Cl_2 (1.5 mL). Purification by flash column chromatography afforded **6c** as an off-white solid (140 mg, 0.30 mmol, 74%); mp 94-98 °C; R_f 0.37 (9:1 CH_2Cl_2/MeOH); t_R: 13.18 min, purity: 98.4%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 10.89 (br s, 1H), 9.26 (br s, 1H), 8.64 – 8.40 (m, 1H), 7.82 – 7.65 (m, 2H), 7.39 – 6.84 (m, 10H), 4.67/4.42 (2 x s, 2H), 4.28/4.20 (2 x s, 2H), 3.59 – 3.29 (m, 2H), 2.59 – 2.36 (m, 2H), 2.28/2.23 (2 x s, 6H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.22, 170.41, 169.66, 164.14, 163.93, 141.03, 140.58, 139.48, 139.22, 137.66, 136.42, 131.82, 131.59, 128.30, 127.53, 127.28, 127.19, 126.73, 126.68, 123.92, 52.14, 46.59, 45.88, 44.95, 42.09, 41.55, 34.56, 33.38, 25.92, 20.84 ppm; HRMS (ESI$^+$): found: 460.2238; $C_{27}H_{29}N_3O_4$ (MH^+) requires 460.2231.



***N*-(3-(Benzylamino)-3-oxopropyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-4-methylbenzamide (6d).** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-methylbenzamido)methyl)benzoate (**5d**) (150 mg, 0.33 mmol), 50% aq. solution of hydroxylamine (0.63 mL, 10.3 mmol) and NaOH (143 mg, 3.58 mmol) in MeOH (4 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6d** as a white solid (115 mg, 0.25 mmol, 78%); mp 127-130 °C; R_f 0.33 (9:1 CH_2Cl_2/MeOH); t_R: 12.27 min, purity: 96.3%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.24 – 11.17 (m, 1H), 9.02 (s, 1H), 8.60 – 8.36 (m, 1H), 7.78 – 7.66 (m, 2H), 7.45 – 7.11 (m, 11H), 4.69/4.45 (2 x s, 2H), 4.27/4.20 (2 x s, 2H), 3.61 – 3.31 (m, 2H), 2.59 – 2.39 (m, 2H), 2.36 – 2.24 (m, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.39, 170.62, 169.83, 164.23, 162.28, 141.12, 140.71, 139.55, 139.18, 133.55, 133.51, 131.94, 131.88, 131.74, 131.67, 129.09, 128.46, 127.46, 127.00, 126.74, 126.58, 52.27, 46.84, 45.24, 42.28, 41.80, 34.72, 33.48, 21.52, 21.05 ppm; HRMS (ESI$^+$): found: 446.2073; $C_{26}H_{27}N_3O_4$ (MH^+) requires 446.2074.

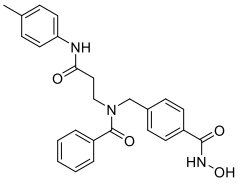


***N*-(3-(Benzylamino)-3-oxopropyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-4-methoxy-benzamide (6e).** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-4-methoxybenzamido)methyl)benzoate (**5e**) (150 mg, 0.32 mmol), 50% aq. solution of hydroxylamine (0.61 mL, 9.96 mmol) and NaOH (138 mg, 3.45 mmol) in MeOH (4.0 mL) and CH_2Cl_2 (1.5 mL). Purification by flash column chromatography afforded **6e** as a white solid (102 mg, 0.22 mmol, 69%); mp 83-88 °C; R_f 0.31 (9:1 CH_2Cl_2/MeOH); t_R: 12.00 min, purity: 96.2%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.20 (s, 1H), 9.04 (s, 1H), 8.49 (s, 1H), 7.80 – 7.67 (m, 2H), 7.50 – 7.12 (m, 9H), 7.01 – 6.89 (m, 2H), 4.65/4.51 (2 x s, 2H), 4.23 (s, 2H), 3.77 (s, 3H), 3.54 – 3.29 (m, 2H), 2.59 – 2.31 (m, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 170.92, 170.48, 164.05, 163.99, 163.88, 160.05, 140.96, 140.75, 139.33, 131.70, 129.50, 128.41, 128.31, 127.37, 127.26, 126.98, 126.84, 126.73, 126.62, 126.56, 126.52, 113.71, 113.63, 55.25, 52.24, 47.02, 46.96, 45.32, 42.15, 40.05, 34.47, 33.36, 29.05, 25.81 ppm; HRMS (ESI$^+$): found: 462.2022; $C_{26}H_{27}N_3O_5$ (MH^+) requires 462.2023.

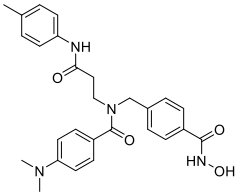


***N*-(3-(Benzylamino)-3-oxopropyl)-*N*-(4-(hydroxycarbamoyl)benzyl)-3,5-dimethoxybenzamide (6f).** Synthesized using methyl 4-((*N*-(3-(benzylamino)-3-oxopropyl)-3,5-dimethoxybenzamido)methyl)benzoate (**5f**) (76.0 mg, 0.16 mmol), 50% aq. solution of hydroxylamine (0.23 mL, 3.68 mmol) and NaOH (55.0 mg, 1.38 mmol) in MeOH (3 mL) and CH_2Cl_2 (1 mL). Purification by flash column chromatography afforded **6f** as an off-white solid (43.0 mg, 0.08 mmol, 55%); mp 83-88 °C; R_f 0.33 (9:1 CH_2Cl_2/MeOH); t_R: 12.40 min, purity: 96.6%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.19 (s, 1H), 9.03 (s, 1H), 8.64 – 8.40 (m, 1H), 7.85 – 7.62 (m, 2H), 7.42 – 7.06 (m, 7H), 6.61 – 6.37 (m, 3H), 4.67/4.41 (2 x s, 2H), 4.31 – 4.23/4.23 – 4.15 (2 x m, 2H), 3.77/3.66 (2 x s, 6H), 3.58 – 3.49/3.40 – 3.35 (2 x m, 2H), 2.53/2.43 (2 x s, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 170.39, 169.70, 164.14, 160.42, 160.37, 140.86, 140.58, 139.49, 139.44, 139.21, 138.42, 138.26, 131.62, 128.30, 127.34, 127.19, 126.82, 126.59, 104.25, 101.01, 55.43, 55.30, 52.02, 46.61, 44.85, 42.16, 41.75, 40.06, 34.56, 33.37 ppm; HRMS (ESI$^+$): found: 92.2133; $C_{27}H_{29}N_3O_6$ (MH^+) requires 492.2129.

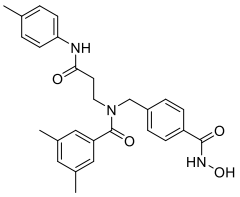
.



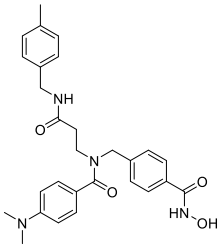
***N*-(4-(Hydroxycarbamoyl)benzyl)-*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamide (6g).** Synthesized using methyl 4-((*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamido)methyl)benzoate (**5g**) (152 mg, 0.35 mmol), 50% aq. solution of hydroxylamine (0.62 mL, 10.1 mmol) and NaOH (140 mg, 3.50 mmol) in MeOH (4.5 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6g** as a pale brown solid (88.0 mg, 0.21 mmol, 59%); mp 158-164 °C; R_f 0.32 (9:1 CH_2Cl_2/MeOH); t_R: 14.07 min, purity: 95.2%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.21 (s, 1H), 10.04 – 9.82 (m, 1H), 9.04 (s, 1H), 7.75 (s, 2H), 7.52 – 7.19 (m, 9H), 7.13 – 7.00 (m, 2H), 4.75/4.54 (2 x s, 2H), 3.61/3.64 (2 x s, 2H), 2.70/2.57 (2 x s, 2H), 2.23 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.04, 170.34, 169.15, 168.33, 163.98, 155.03, 149.90, 140.92, 140.41, 136.60, 136.36, 132.10, 129.41, 129.06, 128.47, 127.33, 127.19, 126.65, 126.33, 119.13, 59.76, 52.13, 46.75, 44.89, 41.50, 40.06, 35.32, 34.33, 20.77, 20.44, 14.10 ppm ppm; HRMS (ESI$^+$): found: 432.1922; $C_{25}H_{25}N_3O_4$ (MH^+) requires 432.1918.



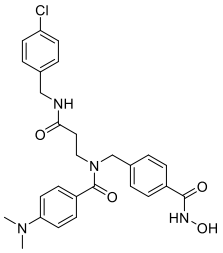
4-(Dimethylamino)-*N*-(4-(hydroxycarbamoyl)benzyl)-*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamide (6h). Synthesized using 4-((4-(dimethylamino)-*N*-(3-oxo-3-(*p* tolylamino)propyl)benzamido)methyl)benzoate (**5h**) (165 mg, 0.35 mmol), 50% aq. solution of hydroxylamine (0.62 mL, 10.1 mmol) and NaOH (140 mg, 3.50 mmol) in MeOH (4.5 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6h** as an off-white solid (84.0 mg, 0.18 mmol, 53%); mp 177-182 °C; R_f 0.32 (9:1 CH_2Cl_2/MeOH); t_R: 11.57 min, purity: 96.0%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.20 (s, 1H), 9.92 (s, 1H), 9.03 (s, 1H), 7.78 – 7.72 (m, 2H), 7.49 – 7.24 (m, 6H), 7.13 – 7.04 (m, 2H), 6.74 – 6.59 (m, 2H), 4.68 (s, 2H), 3.57 (t, J = 7.2 Hz, 2H), 2.93 (s, 6H), 2.71 – 2.63 (m, 2H), 2.24 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.55, 169.03, 168.87, 163.98, 151.11, 141.20, 136.54, 132.09, 131.65, 129.08, 128.37, 127.25, 126.90, 122.56, 119.18, 111.08, 39.76, 20.46 ppm; HRMS (ESI$^+$): found: 475.2339; $C_{27}H_{30}N_4O_4$ (MH^+) requires 475.2340.



***N*-(4-(Hydroxycarbamoyl)benzyl)-3,5-dimethyl-*N*-(3-oxo-3-(*p*-tolylamino)-propyl)benzamide (6i).** Synthesized using methyl 4-((3,5-dimethyl-*N*-(3-oxo-3-(*p*-tolylamino)propyl)benzamido)-methyl)benzoate (**5i**) (104 mg, 0.23 mmol), 50% aq. solution of hydroxylamine (0.40 mL, 6.53 mmol) and NaOH (93.0 mg, 2.33 mmol) in MeOH (4.0 mL) and CH_2Cl_2 (1.0 mL). Recrystallization afforded **6i** as a white solid (16.0 mg, 0.103 mmol, 15%); mp 108-111 °C; R_f 0.33 (9:1 CH_2Cl_2/MeOH); t_R: 14.13 min, purity: 95.0%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.22 (s, 1H), 9.87 (s, 1H), 9.07 (s, 1H), 7.75 (s, 2H), 7.52 – 6.89 (m, 10H), 4.73/4.52 (2 x s, 2H), 3.58/3.45 (2 x s, 2H), 2.67/2.55 (2 x s, 2H), 2.30 – 2.18 (m, 9H), ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.25, 169.21, 168.45, 163.98, 140.98, 140.66, 140.59, 137.66, 136.55, 136.47, 136.38, 132.13, 131.90, 131.64, 130.67, 129.51, 129.08, 127.37, 127.27, 127.21, 126.75, 123.94, 119.21, 119.12, 52.26, 46.57, 44.87, 41.38, 40.05, 35.44, 34.40, 20.79, 20.45 ppm; HRMS (ESI$^+$): found: 460.2233; $C_{27}H_{29}N_3O_4$ (MH^+) requires 460.2231.



4-(Dimethylamino)-*N*-(4-(hydroxycarbamoyl)benzyl)-*N*-(3-((4-methylbenzyl)-amino)-3-oxopropyl)benzamide (6j). Synthesized using methyl 4-((4-(dimethylamino)-*N*-(3-((4-methylbenzyl)amino)-3-oxopropyl)benzamido)-methyl)benzoate **(6j**) (173 mg, 0.36 mmol), 50% aq. solution of hydroxylamine (0.67 mL, 10.9 mmol) and NaOH (153 mg, 3.83 mmol) in MeOH (4.0 mL) and CH_2Cl_2 (1.5 mL). Recrystallization afforded **6j** as a white solid (126 mg, 0.26 mmol, 72%); mp 165-168 °C; R_f 0.32 (9:1 CH_2Cl_2/MeOH); t_R: 10.73 min, purity: 97.7%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.19 (s, 1H), 9.03 (s, 1H), 8.45 – 8.34 (m, 1H), 7.79 – 7.67 (m, 2H), 7.37 – 7.18 (m, 4H), 7.19 – 7.03 (m, 4H), 6.73 – 6.59 (m, 2H), 4.60 (s, 2H), 4.18 (d, *J* = 5.9 Hz, 2H), 3.48 (t, *J* = 7.2 Hz, 2H), 2.92 (s, 6H), 2.47 (t, *J* = 7.4 Hz, 2H), 2.26 (s, 3H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.56, 170.17, 166.53, 164.01, 151.13, 141.21, 136.38, 135.90, 131.68, 130.05, 128.88, 128.43, 127.41, 127.27, 126.85, 122.62, 111.09, 83.40, 41.90, 33.97, 20.74, 6.68 ppm; HRMS (ESI$^+$): found: 489.2504; $C_{28}H_{32}N_4O_4$ (MH^+) requires 489.2496.



***N*-(3-((4-Chlorobenzyl)amino)-3-oxopropyl)-4-(dimethylamino)-*N*-(4-(hydroxy-carbamoyl)benzyl)benzamide (6k).** Synthesized using methyl 4-((*N*-(3-((4-chlorobenzyl)-amino)-3-oxopropyl)-4-(dimethylamino)benzamido)-methyl)benzoate (**6k**) (123 mg, 0.24 mmol), 50% aq. solution of hydroxylamine (0.45 mL, 7.35 mmol) and NaOH (102 mg, 2.55 mmol) in MeOH (4.0 mL) and CH_2Cl_2 (1.0 mL). Recrystallization afforded **6k** as a white solid (60.0 mg, 0.12 mmol, 50%); mp 170-175 °C; R_f 0.43 (9:1 CH_2Cl_2/MeOH); t_R: 10.82 min, purity: 97.0%; ^{1}H-NMR (600 MHz, DMSO-d_6): δ_H = 11.19 (s, 1H), 9.02 (s, 1H), 8.56 – 8.45 (m, 1H), 7.76 – 7.71 (m, 2H), 7.39 – 7.34 (m, 2H), 7.32 – 7.19 (m, 7H), 6.69 – 6.64 (m, 2H), 4.60 (s, 2H), 4.22 (d, *J* = 5.9 Hz, 2H), 3.48 (t, *J* = 7.2 Hz, 2H), 2.92 (s, 6H), 2.49 – 2.45 (m, 2H) ppm; ^{13}C-NMR (151 MHz, DMSO-d_6): δ_C = 171.54, 170.28, 164.11, 151.11, 141.20, 139.16, 138.55, 138.35, 131.67, 131.38, 129.28, 128.41, 128.26, 127.28, 126.84, 122.58, 118.61, 111.07, 99.57, 41.47, 33.82 ppm; HRMS (ESI$^+$): found: 509.1949; $C_{27}H_{29}N_4O_4$ (MH^+) requires 509.1950.

3. Molecular docking

For the molecular docking the *cis*- and *trans*-rotamer of compound **6i** were drawn with ChemDraw Ultra[3], converted into a 3D structure, and energy minimized with Moloc using the MAB force field.[4] The HDACi was then docked into crystal structures of HDAC1 (PDB ID: 4BKX)[5] and HDAC6 (PDB ID: 5EDU)[6] utilizing AutoDock3[7] as a docking engine and the DrugScore[8,9] distance-dependent pair-potentials as an objective function, as described in ref. 10. In the docking, default parameters were used with the exception of the clustering RMSD cutoff, which was set to 2.0 Å, to account for the flexibly connected saturated carbon cycles. Docking solutions with more than 20% of all configurations in the largest cluster were considered sufficiently converged. The configuration in the largest cluster with the lowest docking energy and with a distance < 3 Å between the hydroxamic acid oxygen and the zinc ion in the binding pocket was used for further evaluation.

4. References

1 M. K. W. Mackwitz, A. Hamacher, J. D. Osko, J. Held, A. Schöler, D. W. Christianson, M. U. Kassack and F. K. Hansen, *Org. Lett.* **2018**, *20*, 3255−3258.

2 T. Yamato J.-Y. Hu and N. Shinoda, *J. Chem. Res*. **2007**, *11*, 641−643.

3 N. Mills, *J. Am. Chem. Soc.* **2006**, *128,* 13649–13650.

4 P. R. Gerber and K. Müller. *J. Comput. Aided Mol. Des*. **1995**, *9*, 251–268.

5 C. J. Millard, P. J. Watson, I. Celardo, Y. Gordiyenko, S. M. Cowley, C. V. Robinson, L. Fairall and J. W. Schwabe*, Mol. Cell*. **2013**, *51*, 57–67.

6 Y. Hai and D.W. Christianson*, Nat. Chem. Biol.* **2016**, *12*, 741-747.

7 F. Osterberg, G. M. Morris, M. F. Sanner, A. J. Olson and D. S. Goodsell, *Proteins* **2002**. *46*, 34-40.

8 S. Radestock, M. Bohm and H. Gohlke*, J. Med. Chem.* **2005**. *48*, 5466-5479.

9 H. Gohlke, M. Hendlich and G. Klebe, *J. Mol. Biol.* **2000**. *295*, 337-356.

10 C. A. Sotriffer, H. Gohlke and G. Klebe, *J. Med. Chem.* **2002**, *45*, 1967-1970.