Supporting Information

Improved Selective Class I HDAC and Novel Selective HDAC3 Inhibitors: Beyond Hydroxamic Acids and Benzamides

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1. Synthetic procedures for compounds 6, 8-15, 19-22

General experimental details

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Silica gel chromatography purifications were performed on prepacked cartridges on a Biotage system. UPLC-MS analyses were performed on a Waters Acquity UPLC™, equipped with a diode array and a ZQ mass spectrometer, using an X-Terra C18 column (5 μm, 4.6 x 50mm) or a BEH C18 column (1.7 μm, 2.1 x 50 mm). Mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (A), and MeCN containing 0.1% formic acid (B). The linear gradient used is: (A): 90% (0.1 min), 90%-0% (2.6 min), 0% (0.3 min), 0%-90% (0.1 min) with a 0.5 mL/min flow. ^1H, ^{13}C NMR spectra were at 400 and 101 MHz on a 400 MHz Bruker spectrometer. Chemical shift (δ) are reported in parts per million downfield to tetramethylsilane using DMSO-d_6 as a solvent unless otherwise noted. Coupling constants (*J*) are reported in Hertz (Hz). Multiplicities are reported as singlet (s), broad (br), doublet (d), doublet of doublets (dd), doublet of doublets of doublets (ddd), triplet (t), doublet of triplet (dt) or multiplet (m). Unless indicated, spectra were acquired at 300 K. Temperatures are expressed in degrees Celsius (°C) and are uncorrected. The reported yields are the actual isolated yields of purified material and are not optimized. High resolution molecular ion determinations (HRMS) were performed using a Dionex

Ultimate 3000 RS UHPLC system coupled to an Orbitrap™ Q-Exactive high-resolution mass spectrometer (Thermo Scientific) operating in ESI positive full scan (*m/z* range 100e-1000 at resolution 140,000 FWHM at 200 *m/z*). Mass error was within 2.2 ppm accuracy.

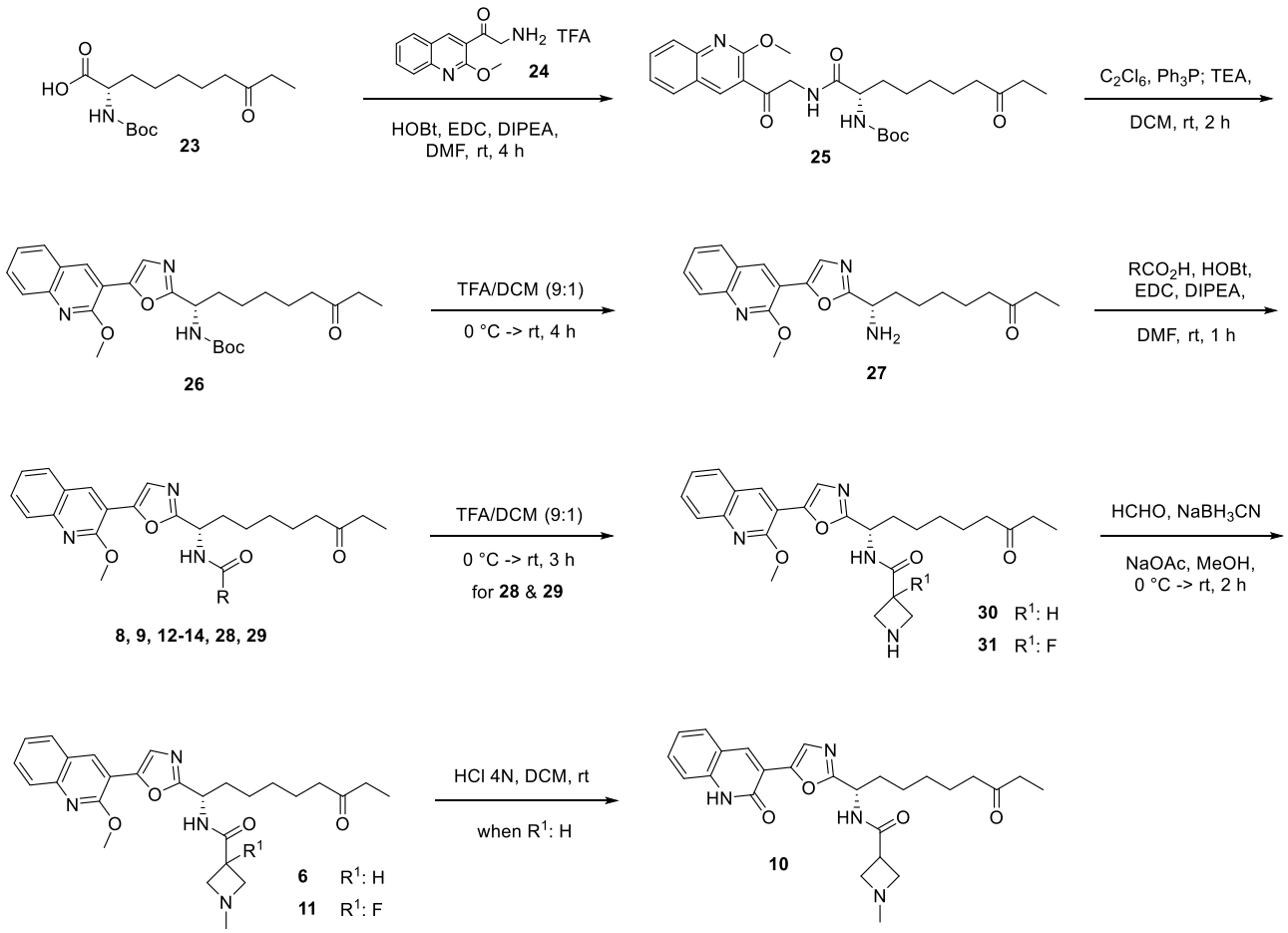
The purity of final compounds was assessed in a Waters Acquity UPLC™, equipped with a diode array and a ZQ mass spectrometer a BEH C18 column (1.7 μm, 2.1 x 50 mm). Mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (A), and MeCN containing 0.1% formic acid (B). The linear gradient of B used are:

Method A: 90% (0.1 min), 90%-0% (2.5 min), 0% (0.3 min), 0%-90% (0.1 min) with a 0.5 mL/min flow. Run time: 3 min.

Method B: 95% (0.7 min), 95%-0% (3.1 min), 0% (1.2 min), with a 0.5 mL/min flow. Run time: 5 min.

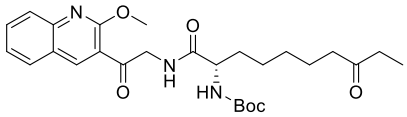
The synthesis and characterization of compounds **2-5**, **7**, and **16-18** have been previously reported.[1, 2, 3] The synthesis for new compounds **6**, **8-15** and **19-22** are reported herein. Compounds **19-20** were prepared exclusively in a library format for which the intermediates and procedures are described.

Synthetic method for compounds 6, 8-14



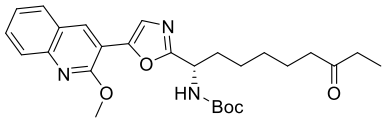
((*S*)-3-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl)-1-methylazetidin-1-ium) L-tartrate (6):

Step 1: (*S*)-*tert*-butyl (1-((2-(2-methoxyquinolin-3-yl)-2-oxoethyl)amino)-1,8-dioxodecan-2-yl)carbamate (**25**).



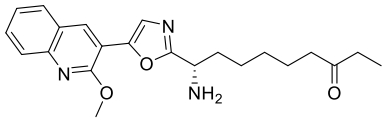
A solution of (*S*)-2-((*tert*-butoxycarbonyl)amino)-8-oxodecanoic acid **23**[4] (2.38 g, 7.92 mmol), EDC.HCl (1.95 g, 10.2 mmol) and HOBt (1.39 g, 10.2 mmol) in DMF (13.2 mL) was stirred for 10 min. A solution of **24**[5] (3.52 g, 7.92 mmol) and DIEA (5 mL, 30.6 mmol) in DMF (13.2 mL) was added and the mixture was stirred for 2 h before being diluted with DCM. The organic layer was separated, washed with brine. The dried organic phase was filtered and concentrated *in vacuo* to give a residue that was purified on silica gel (eluting with 10-80% ethyl acetate in petroleum ether) to give the title compound (2.10 g, 53%) as a white solid. LRMS (ES^+) *m/z* calcd. for $C_{27}H_{37}N_3O_6$ 499.27, found 500 $(M+H)^+$.

Step 2: (*S*)-tert-butyl (1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamate (**26**).



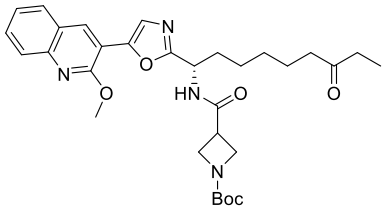
A solution of Ph_3P (2.64 g, 10.1 mmol) and C_2Cl_6 (2.39 g, 10.1 mmol) in DCM (12 mL) was stirred then treated with a solution of **25** (2.52 g, 5.04 mmol) and Et_3N (2.81 mL, 20.18 mmol) in DCM (12 mL). The mixture was stirred for 2 h then concentrated *in vacuo*. The residue was taken up with ethyl acetate and washed with saturated aqueous $NaHCO_3$ and brine. The dried organics were concentrated and the residue was purified on silica gel (eluting with 5-60% EtOAc in petroleum ether) to give the title compound (1.07 g, 44%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.51 (s, 1H), 7.99 (d, *J* = 8.5 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.64 (s, 1H), 7.59-7.48 (m, 2H), 4.72 (br d, *J* = 6.1 Hz, 1H), 4.16 (s, 3H), 2.46-2.31 (m, 4H), 1.96-1.71 (m, 2H), 1.51-1.24 (m, 6H), 1.42 (s, 9H), 0.90 (t, *J* = 7.5 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{27}H_{35}N_3O_5$ 481.26, found 482 $(M+H)^+$.

Step 3: (*S*)-9-amino-9-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)nonan-3-one (**27**).



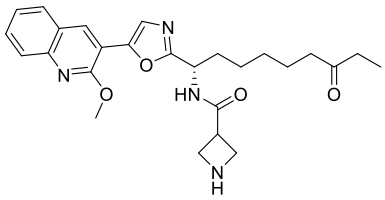
A solution of (*S*)-tert-butyl (1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamate **26** (990 mg, 2.05 mmol) in 1:1 mixture of DCM/TFA (22.6 mL) was stirred at 0 °C for 3 h. The mixture was diluted with toluene and concentrated *in vacuo*. The residue was taken up in DCM and washed with saturated aqueous $NaHCO_3$ and brine. The dried organics were concentrated *in vacuo* to give the title compound as an orange oil (750 mg, 96%) that was used directly in the subsequent step. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.41 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.83 (d, *J* = 8.3 Hz, 1H), 7.68-7.62 (m, 1H), 7.60 (s, 1H), 7.47-7.40 (m, 1H), 4.24 (s, 3H), 4.21-4.15 (m, 1H), 2.48-2.36 (m, 4H), 2.08-1.97 (m, 1H), 1.94-1.83 (m, 1H), 1.68-1.56 (m, 2H), 1.54-1.32 (m, 4H), 1.05 (t, *J* = 7.5 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{22}H_{27}N_3O_3$ 381.21, found 382 $(M+H)^+$.

Step 4: (*S*)-*tert*-butyl 3-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl) azetidine-1-carboxylate (**28**).



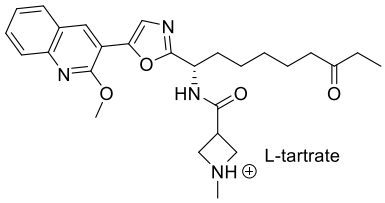
To a solution of **27** (413 mg, 1.08 mmol) in DMF (5.7 mL) were added HOBt (511 mg, 3.79 mmol), EDC.HCl (726 mg, 3.79 mmol), 1-(*tert*-butoxycarbonyl)azetidine-3-carboxylic acid (283 mg, 1.41 mmol) and DIEA (670 mL, 3.94 mmol). The resulting mixture was stirred for 1 h then diluted with DCM. The organic solution was washed with saturated aqueous $NaHCO_3$ and brine. The dried organics were concentrated to give a residue that was purified on silica gel (eluting with 30-100% EtOAc in petroleum ether) to give the title compound (567 mg, 93%) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.40 (s, 1H), 7.87 (d, *J* = 8.8 Hz, 1H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.67 (t, *J* = 7.7 Hz, 1H), 7.60 (s, 1H), 7.49-7.42 (m, 1H), 6.62 (br d, *J* = 8.8 Hz, 1H), 5.41 (q, *J* = 7.5 Hz, 1H), 4.24 (s, 3H), 4.21-4.05 (m, 4H), 3.36-3.24 (m, 1H), 2.45-2.35 (m, 4H), 2.11-1.98 (m, 1H), 2.02-1.89 (m, 1H), 1.64-1.55 (m, 2H), 1.46 (s, 9H), 1.48-1.36 (m, 4H), 1.05 (t, *J* = 7.2 Hz, 3H); LRMS (ES^+) *m/z* calcd. for $C_{31}H_{40}N_4O_6$ 564.29, found 565 $(M+H)^+$.

Step 5: (*S*)-*N*-(1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)azetidine-3-carboxamide (**30**).



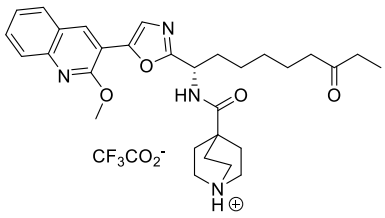
A solution of (*S*)-*tert*-butyl 3-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl) azetidine-1-carboxylate **28** (564 mg, 1 mmol) in a 9:1 mixture of DCM/TFA (25 mL) was prepared at 0 °C and allowed to warm to 20 °C over 3 h. The mixture was diluted with Et_2O and concentrated *in vacuo*. The residue was taken up in DCM and washed with saturated aqueous $NaHCO_3$ and brine. Concentration of the dried organics gave the title compound (446 mg, 96%) as an oil that was used directly in the subsequent step. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.76 (br d, *J* = 6.1 Hz, 1H), 8.54 (s, 1H), 8.03 (d, *J* = 7.3 Hz, 1H), 7.82 (d, *J* = 8.5 Hz, 1H), 7.72 (t, *J* = 7.3 Hz, 1H), 7.66 (s, 1H), 7.51 (t, *J* = 7.3 Hz, 1H), 5.10 (dd, *J* = 7.3, 14.6 Hz, 1H), 4.16 (s, 3H), 4.08-3.93 (m, 4H), 3.69-3.58 (m, 1H), 2.46-2.31 (m, 4H), 2.04-1.92 (m, 1H), 1.89-1.75 (m, 1H), 1.53-1.38 (m, 2H), 1.38-1.23 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H); LRMS (ES^+) *m/z* calcd. for $C_{26}H_{32}N_4O_4$ 464.24, found 465 $(M+H)^+$.

Step 6: ((*S*)-3-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl)-1-methylazetidin-1-ium) *L*-tartrate (**6**).



To a solution of **30** (276 mg, 0.59 mmol) in MeOH (5.8 mL) were added formaldehyde (37% aqueous solution, 0.153 mL, 2.07 mmol), NaOAc (145 mg, 1.77 mmol) and $NaBH_3CN$ (111 mg, 1.77 mmol). The mixture was stirred for 3 h then concentrated *in vacuo* to give a residue that was purified by automated RP-HPLC. Lyophilization of the appropriate fractions afforded the trifluoracetate salt of **6** (238 mg) that was partitioned between DCM and saturated aqueous $NaHCO_3$. The organic phase was separated, dried and concentrated under reduced pressure and the resulting oil was dissolved in a mixture 1:1 of $MeCN/H_2O$ and treated with L-tartaric acid (24 mg, 0.16 mmol). The resulting solution was lyophilized to give the title compound (186 mg, 57%) as a solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.63 (d, *J* = 8.3 Hz, 1H), 8.53 (s, 1H), 8.02 (d, *J* = 7.9 Hz, 1H), 7.82 (d, *J* = 8.3 Hz, 1H), 7.72 (ddd, *J* = 1.3, 7.0, 8.3 Hz, 1H), 7.66 (s, 1H), 7.51 (ddd, *J* = 1.3, 7.0, 8.3 Hz, 1H), 5.16-5.03 (m, 1H), 4.16 (s, 3H), 3.94 (s, 1H from tartrate counteranion), 3.77-3.66 (m, 2H), 3.52-3.44 (m, 2H), 3.44-3.32 (m, 1H), 2.45-2.34 (m, 7H), 2.01-1.89 (m, 1H), 1.89-1.76 (m, 1H), 1.47-1.39 (m, 2H), 1.39-1.24 (m, 4H), 0.90 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 211.2, 174.9, 171.8, 164.8, 158.0, 146.5, 145.6, 134.1, 131.2, 129.4, 128.2, 127.4, 125.9, 125.4, 113.5, 72.2, 58.7, 58.6, 54.8, 47.9, 44.9, 35.8, 34.5, 33.5, 29.0, 26.0, 24.0, 8.6; LRMS (ES^+) *m/z* calcd. for $C_{27}H_{34}N_4O_4$ 478.26, found 479 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{27}H_{35}N_4O_4$ $[M+H]^+$ 479.2653, found, 479.2647; HPLC t_R 1.38 min (method A, peak area 100%), 2.35 min (method B, peak area 100%).

(*S*)-4-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl)quinuclidin-1-ium 2,2,2-trifluoroacetate (8):

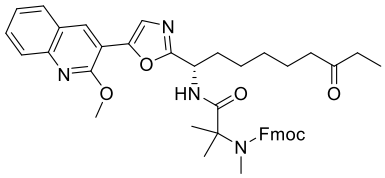


A solution of (*S*)-9-amino-9-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)nonan-3-one **27** (44 mg, 0.115 mmol) in DMF (1.5 mL) was treated with HOBt (18.6 mg, 0.138 mmol) and EDC.HCl (26.5 mg, 0.138 mmol). The appropriate carboxylic acid, 1-azabicyclo[2.2.2]octane-4-carboxylic acid

hydrochloride (26.4 mg, 0.138 mmol) and DIEA (0.048 mL, 0.28 mmol) were added then the mixture was stirred for 18 h. The solution was concentrated *in vacuo* and the residue was directly purified by automated RP-HPLC using water (+ 0.1% TFA) and MeCN (+ 0.1% TFA) as eluents (C_{18} column). Fractions containing product were identified by LCMS and were lyophilized to give the title compound (23.9 mg, 33%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.21 (br s, 1H), 8.85 (br d, *J* = 8.5 Hz, 1H), 8.27 (s, 1H), 7.86-7.77 (m, 2H), 7.56 (br t, *J* = 7.3 Hz, 1H), 7.39 (br d, *J* = 8.5 Hz, 1H), 7.30-7.23 (m, 1H), 5.14-5.04 (m, 1H), 4.30 (s, 3H), 4.20 (br s, 2H), 4.05 (br s, 2H), 3.63-3.52 (m, 1H), 2.92 (s, 1H), 2.80 (s, 3H), 2.73-2.64 (m, 1H), 2.44-2.29 (m, 4H), 2.02-1.87 (m, 1H), 1.82 (br d, *J* = 7.3 Hz, 1H), 1.53-1.43 (m, 2H), 1.39-1.20 (m, 4H), 0.90 (br t, *J* = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{30}H_{38}N_4O_4$ 518.29, found 519 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{30}H_{39}N_4O_4$ $[M+H]^+$ 519.2966, found 519.2956; HPLC t_R 1.40 min (method A, peak area 98%), 2.36 min (method B, peak area 100%).

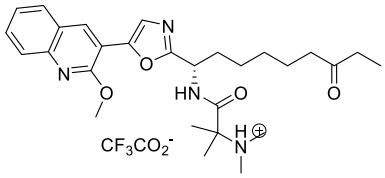
<u>*N*-(1-(((*S*)-1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)amino)-2-methyl-1-oxopropan-2-yl)-N-methylmethyliumaminium 2,2,2-trifluoroacetate (9):</u>

Step 1: (9*H*-fluoren-9-yl)methyl (*S*)-(1-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)amino)-2-methyl-1-oxopropan-2-yl)(methyl)carbamate (**9a**).



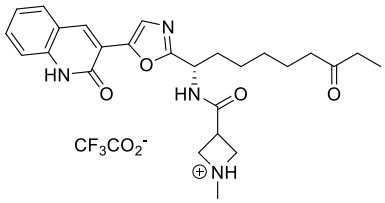
To a solution of **27** (50 mg, 0.13 mmol) in DMF (1.3 mL) were added, HOBt (22.9 mg, 0.17 mmol), EDC.HCl (32.6 mg, 0.17 mmol), 2-((((9H-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-2-methylpropanoic acid (26.4 mg, 0.17 mmol) and DIEA (0.045 mL, 0.26 mmol). The mixture was stirred for 18 h then concentrated *in vacuo*. The residue was taken up in DCM and washed with saturated aqueous $NaHCO_3$ and brine. The dried organics were concentrated and the residue was purified on silica gel (eluting with 30% EtOAc in petroleum ether) to give the title compound (80 mg, 88%) as an orange oil that was used directly in the subsequent reaction. LRMS (ES$^+$) *m/z* calcd. for $C_{42}H_{46}N_4O_6$ 702.34, found 703 $(M+H)^+$.

Step 2: *N*-(1-(((*S*)-1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)amino)-2-methyl-1-oxopropan-2-yl)-N-methylmethyliumaminium 2,2,2-trifluoroacetate (**9**).



A solution of the preceding compound **9a** (80 mg, 0.11 mmol) in THF (4.2 mL) was treated with Et_2NH (1.4 mL, 13.3 mmol). The mixture was stirred for 1 h then concentrated *in vacuo*. The residue filtered through silica gel (eluting with 50% EtOAc in petroleum ether then with 10% MeOH in DCM) to give yellow oil (37 mg) that which was taken up in MeOH (1.5 mL). Formaldehyde (37% aqueous solution, 0.034 mL, 0.46 mmol), NaOAc (32 mg, 0.39 mmol) and $NaBH_3CN$ (24.4 mg, 0.39 mmol) were added and the resulting mixture was stirred for 18 h. Removal of the volatiles afforded a residue that was purified directly by automated RP-HPLC. Fractions containing product were identified by LCMS and were lyophilized to give the title compound (11 mg, 16%) as a colorless oil. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.72 (br s, 1H), 8.86 (br s, 1H), 8.48 (s, 1H), 8.00 (d, *J* = 7.3 Hz, 1H), 7.82 (d, *J* = 8.2 Hz, 1H), 7.72 (t, *J* = 7.4 Hz, 1H), 7.67 (s, 1H), 7.51 (t, *J* = 7.3 Hz, 1H), 5.11 (br d, *J* = 4.9 Hz, 1H), 4.16 (s, 3H), 2.77-2.60 (m, 6H), 2.46-2.38 (m, 4H), 2.12-2.05 (m, 1H), 2.01-1.92 (m, 1H), 1.60-1.21 (m, 12H), 0.91 (t, *J* = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{28}H_{38}N_4O_4$ 494.29, found 495 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{28}H_{39}N_4O_4$ $[M+H]^+$ 495.2966, found, 495.2959; HPLC t_R 1.38 min (method A, peak area 100%), 2.34 min (method B, peak area 99%).

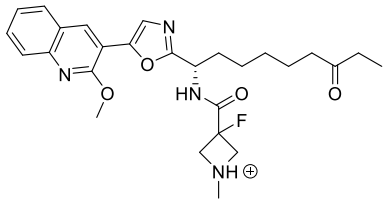
(*S*)-1-methyl-3-((7-oxo-1-(5-(2-oxo-1,2-dihydroquinolin-3-yl)oxazol-2-yl)nonyl)carbamoyl)azetidin-1-ium 2,2,2-trifluoroacetate (10):



To a solution of **6** (50 mg, 0.084 mmol) in DCM (2 mL) was added HCl (4 N in dioxane, 0.4 mL, 1.6 mmol). The mixture was stirred for 1 h then concentrated *in vacuo* to give a residue that was purified by automated RP-HPLC. Fractions containing product were identified by LCMS and were lyophilized to give the title compound (13.8 mg, 28%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.21 (br s, 1H), 8.85 (d, *J* = 8.5 Hz, 1H), 8.27 (s, 1H), 7.84 (d, *J* = 8.5 Hz, 1H), 7.79 (s, 1H), 7.56 (t, *J* = 7.3 Hz, 1H), 7.39 (d, *J* = 8.5 Hz, 1H), 7.26 (t, *J* = 7.3 Hz, 1H), 5.15-5.04 (m, 1H), 4.20 (br s, 2H), 4.05 (br s, 2H), 3.66-3.51 (m, 1H), 2.78 (br s, 3H), 2.44-2.35 (m, 4H), 2.01-1.87 (m, 1H), 1.92-1.85 (m, 1H), 1.53-1.40 (m, 2H), 1.40-1.21 (m, 4H), 0.90 (t, *J* = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{26}H_{32}N_4O_4$ 464.24, found 465 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated

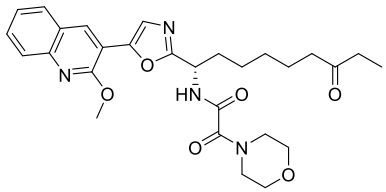
for $C_{26}H_{33}N_4O_4$ $[M+H]^+$, 465.2496, found 465.2492; HPLC t_R 1.10 min (method A, peak area 97%), 1.98 min (method B, peak area 98%).

(*S*)-3-fluoro-3-((1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)carbamoyl)-1-methylazetidin-1-ium 2,2,2-trifluoroacetate (11):



Following the general procedure described for **6** elaboration of **27** (60 mg, 0.15 mmol) with 1-(*tert*-butoxycarbonyl)-3-fluoroazetidine-3-carboxylic acid (41.4 mg, 0.19 mmol) gave an initial product that was purified by preparative RP-HPLC, using water (+ 0.1% TFA) and MeCN (+ 0.1% TFA) as eluents (C_{18} column). Lyophilization of the fractions give the title compound (19.5 mg, 21% over three steps) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 10.45 (br s, 1H), 9.23 (br d, J = 7.3 Hz, 1H), 8.53 (s, 1H), 8.02 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 8.5 Hz, 1H), 7.66 (s, 1H), 7.51 (t, J = 7.3 Hz, 1H), 5.19-5.08 (m, 1H), 4.44 (br d, J = 17.1 Hz, 4H), 4.16 (s, 3H), 2.87 (br s, 3H), 2.44-2.37 (m, 4H), 2.14-2.02 (m, 1H), 2.01-1.89 (m, 1H), 1.54-1.43 (m, 2H), 1.42-1.22 (m, 4H), 0.91 (t, J = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{27}H_{33}FN_4O_4$ 496.25, found 497 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{27}H_{34}FN_4O_4$ $[M+H]^+$ 497.2559, found 497.2560; HPLC t_R 1.41 min (method A, peak area 99%), 2.37 min (method B, peak area 94%).

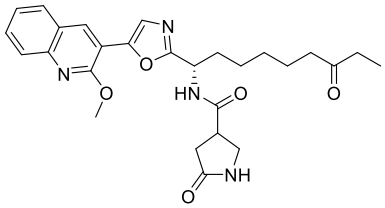
(*S*)-*N*-(1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)-2-morpholino-2-oxoacetamide (12):



Following the general procedure described for **8** treatment of **27** (18 mg, 0.047 mmol) with 2-morpholino-2-oxoacetic acid (15 mg, 0.094 mmol) gave the title compound (3.5 mg, 14%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.45 (d, J = 7.3 Hz, 1H), 8.52 (s, 1H), 8.01 (d, J = 7.3 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.76-7.69 (m, 1H), 7.67 (s, 1H), 7.55-7.48 (m, 1H), 5.14-5.04 (m, 1H), 4.16 (s, 3H), 3.67-3.57 (m, 4H), 3.54-3.44 (m, 4H), 2.46-2.35 (m, 4H), 2.05-1.85 (m, 2H), 1.54-1.41 (m, 2H), 1.41-1.23 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{28}H_{34}N_4O_6$ 522.25, found 523 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{28}H_{35}N_4O_6$ $[M+H]^+$

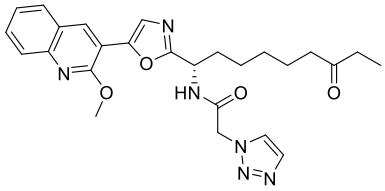
523.2551, found 523.2551; HPLC t_R 2.01 min (method A, peak area 99%), 2.99 min (method B, peak area 100%).

_N_-((_S_)-1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)-5-oxopyrrolidine-3-carboxamide (13):



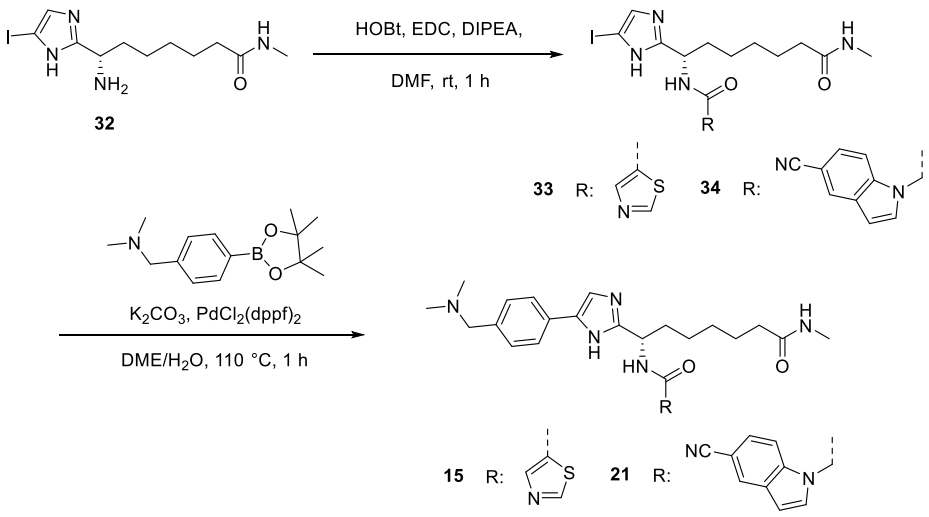
Following the general procedure described for **8** treatment of **27** (14 mg, 0.037 mmol) with 5-oxopyrrolidine-3-carboxylic acid (6 mg, 0.046 mmol) gave the title compound (3.2 mg, 18%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.67 (d, J = 8.5 Hz, 1H), 8.51 (s, 1H), 8.09-7.93 (m, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.75-7.63 (m, 2H), 7.63-7.55 (m, 1H), 7.50 (br t, J = 7.9 Hz, 1H), 5.13-4.99 (m, 1H), 4.16 (s, 3H), 3.34-3.07 (m, 2H), 2.45-2.29 (m, 7H), 2.04-1.88 (m, 1H), 1.88-1.75 (m, 1H), 1.62-1.40 (m, 2H), 1.40-1.13 (m, 4H), 0.90 (t, J = 6.7 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{27}H_{32}N_4O_5$ 492.24, found 493 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{27}H_{33}N_4O_5$ [M+H]$^+$ 493.2445, found 493.2444; HPLC t_R 1.77 min (method A, peak area 100%), 2.72 min (method B, peak area 100%).

(_S_)-_N_-(1-(5-(2-methoxyquinolin-3-yl)oxazol-2-yl)-7-oxononyl)-2-(1_H_-1,2,3-triazol-1-yl)acetamide (14):



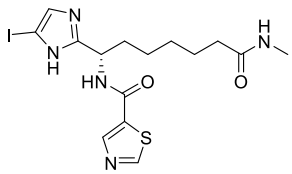
Following the general procedure described for **8** treatment of **27** (43 mg, 0.113 mmol) with 2-(1*H*-1,2,3-triazol-1-yl)acetic acid (17.2 mg, 0.135 mmol) gave the title compound (34 mg, 62%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.03 (d, J = 7.3 Hz, 1H), 8.57 (s, 1H), 8.03 (d, J = 8.5 Hz, 1H), 7.86-7.77 (m, 2H), 7.77-7.63 (m, 2H), 7.51 (t, J = 7.3 Hz, 1H), 5.76 (s, 1H), 5.27 (s, 2H), 5.08 (q, J = 7.3 Hz, 1H), 4.17 (s, 3H), 2.46-2.33 (m, 4H), 2.05-1.92 (m, 1H), 1.92-1.81 (m, 1H), 1.55-1.41 (m, 2H), 1.41-1.21 (m, 4H), 0.90 (t, J = 7.3 Hz, 3H); LRMS (ES$^+$) *m/z* calcd. for $C_{26}H_{30}N_6O_4$ 490.56, found 491 (M+H)$^+$; HRMS (ES$^+$) *m/z* calculated for $C_{26}H_{31}N_6O_4$ [M+H]$^+$ 491.2401, found 491.2396; HPLC t_R 2.01 min (method A, peak area 99%), 2.99 min (method B, peak area 100%).

Synthetic method for compounds 15 and 21



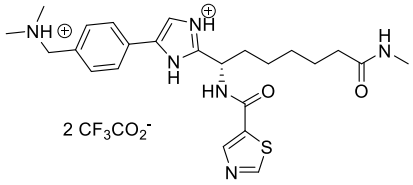
(*S*)-5-(4-((dimethylammonio)methyl)phenyl)-2-(7-(methylamino)-7-oxo-1-(thiazole-5-carboxamido)heptyl)-1*H*-imidazol-3-ium 2,2,2-trifluoroacetate (15):

Step 1. (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide (**33**).



To a solution of (*S*)-7-amino-7-(5-iodo-1*H*-imidazol-2-yl)-*N*-methylheptanamide **32**[3] (1.08 g, 3.10 mmol) in DMF (1.5 mL) were added, HOBt (0.63 g, 4.64 mmol), EDC.HCl (0.89 g, 4.64 mmol), thiazole-5-carboxylic acid (0.6 g, 4.64 mmol) and DIEA (1.2 mL, 6.89 mmol). The mixture was stirred 1 h then diluted with DCM and saturated aqueous $NaHCO_3$. The organic phase was separated and washed with brine, then dried. Removal of the volatiles gave a residue that was purified on silica gel (eluting with 50-80% EtOAc in petroleum ether) to furnish the title compound (1.13 g, 79%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.24 (br s, 1H), 9.23 (s, 1H), 9.02 (br d, J = 8.3 Hz, 1H), 8.58 (s, 1H), 7.65 (br d, J = 4.4 Hz, 1H), 7.23 (s, 1H), 5.04 (dt, J = 6.4, 8.4 Hz, 1H), 2.55 (d, J = 5.2 Hz, 3H), 2.02 (t, J = 7.6 Hz, 2H), 2.10-1.89 (m, 1H), 1.88-1.75 (m, 1H), 1.54-1.46 (m, 2H), 1.36 - 1.23 (m, 4H); LRMS (ES^+) *m/z* calcd. for $C_{15}H_{20}IN_5O_2S$ 461.04, found 462 $(M+H)^+$.

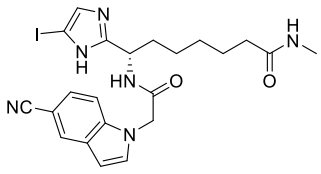
Step 2. (*S*)-5-(4-((dimethylammonio)methyl)phenyl)-2-(7-(methylamino)-7-oxo-1-(thiazole-5-carboxamido)heptyl)-1*H*-imidazol-3-ium 2,2,2-trifluoroacetate (**15**).



A microwave vial was charged with (*S*)-*N*-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)thiazole-5-carboxamide **33** (105 mg, 0.23 mmol), *N*,*N*-dimethyl-1-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methanamine hydrochloride (136 mg, 0.46 mmol), $PdCl_2(dppf)_2$ (33.8 mg, 0.046 mmol) and K_2CO_3 (94.4 mg, 0.68 mmol). A degassed solution of DME/H_2O (1:1) (4.6 mL) was added and the suspension was degassed for a further 10 min then heated to 110 °C and kept at this temperature for 1 h. After cooling, the mixture was concentrated *in vacuo* and the residue was dissolved in MeOH and passed through an SPE cartridge containing SCX resin (5 g) and washed with MeOH. After treatment of the resin with NH_3 in MeOH (7 N) and concentration *in vacuo* the residue obtained was purified by automated RP-HPLC. Fractions containing product were identified by LCMS and were lyophilized to give the title compound (11 mg, 10%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.74 (br s, 1H), 9.27 (s, 1H), 9.18 (br s, 1H), 8.62 (s, 1H), 7.98-7.81 (m, 3H), 7.71-7.60 (m, 1H), 7.60-7.47 (m, 2H), 5.24-5.07 (m, 1H), 4.39-4.22 (m, 2H), 2.75 (br d, *J* = 3.9 Hz, 6H), 2.57-2.53 (m, 3H), 2.13-1.95 (m, 4H), 1.54-1.27 (m, 6H); LRMS (ES^+) *m/z* calcd. for $C_{24}H_{32}N_6O_2S$ 468.23, found 469 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{24}H_{33}N_6O_2S$ $[M+H]^+$ 469.2380, found 469.2370; HPLC t_R 0.73 min (method A, peak area 100%), 1.24 min (method B, peak area 100%).

(*S*)-5-(4-((dimethylammonio)methyl)phenyl)-2-(1-(2-(5-cyano-1*H*-indol-1-yl)acetamido)-7-(methylamino)-7-oxoheptyl)-1*H*-imidazol-3-ium 2,2,2-trifluoroacetate (21):

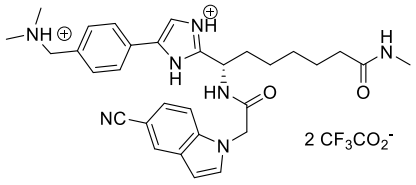
Step 1. (*S*)-7-(5-iodo-1*H*-imidazol-2-yl)-7-(2-(5-cyano-1*H*-indol-1-yl)acetamido)-*N*-methylheptanamide (**34**).



To a solution of (*S*)-7-amino-7-(5-iodo-1*H*-imidazol-2-yl)-*N*-methylheptanamide **32**[3] (115 mg, 0.33 mmol) in DMF (2.1 mL) were added HOBt (49.1 mg, 0.36 mmol), EDC.HCl (69.6 mg, 0.36 mmol), 5-cyanoindole-1-acetic acid (69.4 mg, 0.35 mmol) and DIEA (0.12 mL, 0.66 mmol). The mixture

was stirred for 18 h then diluted with DCM and washed with saturated aqueous $NaHCO_3$, brine, dried with Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified on silica gel chromatography eluting with 25-100% EtOAc/Petroleum ether to give the title compound as a white solid (110 mg, 62%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 12.20 (br s, 1H), 8.76 (br d, *J* = 8.3 Hz, 1H), 8.09 (d, *J* = 0.9 Hz, 1H), 7.65 (br d, *J* = 3.9 Hz, 1H), 7.61-7.44 (m, 3H), 7.24 (s, 1H), 6.60 (d, *J* = 3.5 Hz, 1H), 5.00 (d, *J* = 16 Hz, 1H), 4.93 (d, *J* = 16 Hz, 1H), 4.89-4.81 (m, 1H), 2.55 (d, *J* = 4.4 Hz, 3H), 2.00 (t, *J* = 7.5 Hz, 2H), 1.92-1.76 (m, 1H), 1.76-1.57 (m, 1H), 1.53-1.36 (m, 2H), 1.35-1.11 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{22}H_{25}IN_6O_2$ 532.11, found 533 $(M+H)^+$.

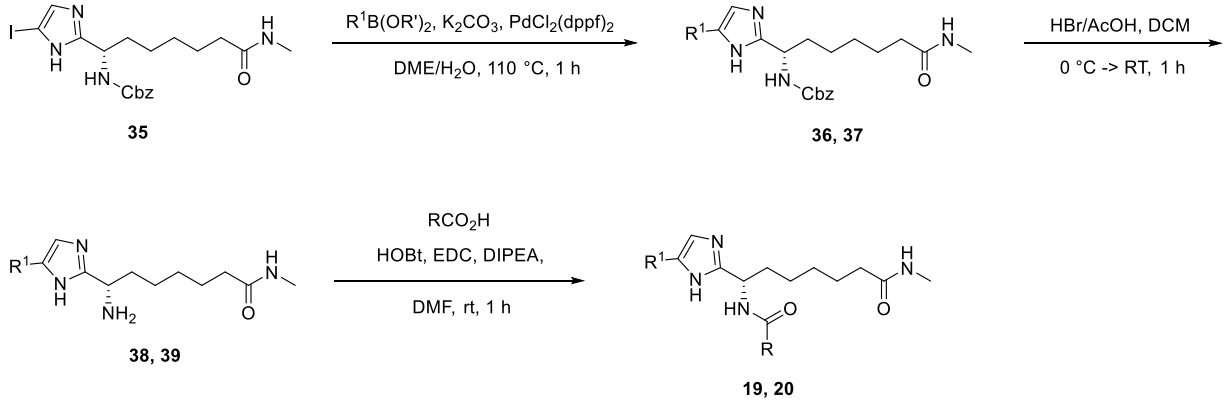
Step 2. *General procedure for cross coupling of iodo-imidazole intermediates.* (*S*)-5-(4-((dimethylammonio)methyl)phenyl)-2-(1-(2-(5-cyano-1*H*-indol-1-yl)acetamido)-7-(methylamino)-7-oxoheptyl)-1*H*-imidazol-3-ium 2,2,2-trifluoroacetate (**21**).



A microwave vial was charged with (*S*)-7-(5-iodo-1*H*-imidazol-2-yl)-7-(2-(5-cyano-1*H*-indol-1-yl)acetamido)-*N*-methylheptanamide **34** (107 mg, 0.20 mmol), *N*,*N*-dimethyl-1-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methanamine hydrochloride (105 mg, 0.40 mmol), $PdCl_2(dppf)_2$ (29.8 mg, 0.040 mmol) and K_2CO_3 (83.3 mg, 0.60 mmol). A degassed 1:1 mixture of DME/H_2O (4.6 mL) was added and the suspension was degassed for further 10 min, heated to 110 °C and maintained at this temperature for a further 1 h. After cooling, the mixture was diluted with EtOAc and filtered through a pad of solka-floc. The organic phase was washed with saturated aqueous NaHCO3 and brine then dried. Concentration of the solvents *in vacuo* gave a residue that was purified, in the present example by direct automated RP-HPLC. Fractions containing product were identified by LCMS and were lyophilized to give the title compound (31.5 mg, 20%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.77 (br s, 1H), 8.94 (br s, 1H), 8.07 (d, *J* = 0.9 Hz, 1H), 7.96-7.88 (m, 1H), 7.85 (d, *J* = 8.3 Hz, 2H), 7.66 (br d, *J* = 3.9 Hz, 1H), 7.61-7.49 (m, 4H), 7.41 (dd, *J* = 1.5, 8.6 Hz, 1H), 6.59 (d, *J* = 3.1 Hz, 1H), 5.07 (d, *J* = 16 Hz, 1H), 5.03-4.89 (m, 2H), 4.33-4.23 (m, 2H), 2.85-2.63 (m, 6H), 2.54 (d, *J* = 4.4 Hz, 3H), 2.02 (t, *J* = 7.5 Hz, 2H), 1.98-1.81 (m, 2H), 1.53-1.41 (m, 2H), 1.39-1.20 (m, 4H); ^{13}C-NMR (101 MHz, DMSO-d_6) δ 172.4, 167.5, 158.4 (q, *J* = 32.3 Hz, 1C, CF_3CO_2H), 149.1, 138.0, 134.5, 132.6, 131.8, 131.5, 129.8, 127.9, 125.9, 125.3, 123.8, 120.6, 115.6 (m, 1C, CF_3), 111.2, 101.9, 101.3, 72.3, 60.2, 59.7, 59.3, 59.7, 48.5, 46.8, 41.7, 35.2, 32.7, 28.1, 25.4, 25.1, 25.0; LRMS (ES$^+$) *m/z* calcd. for $C_{31}H_{37}N_7O_2$ 539.30, found

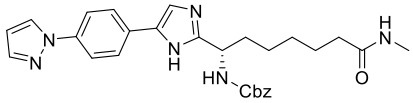
540 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{31}H_{38}N_7O_2$ $[M+H]^+$ 540.3081, found 540.3070; HPLC t_R 0.76 min (method A, peak area 100%), 1.58 min (method B, peak area 100%).

Synthetic method for compounds 19-20



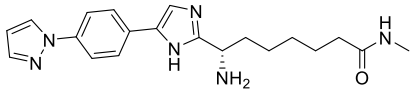
(*S*)-7-(3-(1*H*-indol-3-yl)propanamido)-7-(5-(4-(1*H*-pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (19):

Step 1. (*S*)-(1-(5-(4-(1*H*-pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (**36**).



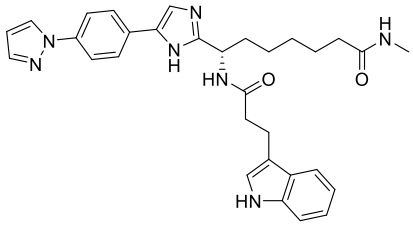
Following the general procedure described for **21** (step 2) treatment of benzyl (*S*)-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate **35**[3] (300 mg, 0.62 mmol) with (4-(1*H*-pyrazol-1-yl)phenyl)boronic acid (230 mg, 1.24 mmol) gave a residue that was purified on silica gel (eluting with 10-100% EtOAc in petroleum ether) to give the title compound (160 mg, 52%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.92 (br s, 1H), 8.48 (d, *J* = 2.4 Hz, 1H), 7.92-7.72 (m, 5H), 7.69-7.59 (m, 2H), 7.55 (s, 1H), 7.42-7.27 (m, 5H), 6.54 (br s, 1H), 5.09 (d, *J* = 12 Hz, 1H), 5.04 (d, *J* = 12 Hz, 1H), 4.73-4.58 (m, 1H), 2.55 (d, *J* = 4.4 Hz, 3H), 2.09-2.01 (m, 2H), 1.95-1.84 (m, 1H), 1.84-1.69 (m, 1H), 1.55-1.42 (m, 2H), 1.35-1.23 (m, 4H); LRMS (ES^+) *m/z* calcd. for $C_{28}H_{32}N_6O_3$ 500.25, found 501 $(M+H)^+$.

Step 2. (*S*)-7-(5-(4-(1*H*-pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-7-amino-N-methylheptanamide (**38**).



A solution of (*S*)-(1-(5-(4-(1*H*-pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate **36** (160 mg, 0.31 mmol) in DCM (4 mL) was cooled to 0 °C and treated with HBr (33% solution in AcOH, 2.1 mL, 40.2 mmol). The mixture was stirred at 0 °C for 2 h then allowed to warm to 20 °C of 1 h. The mixture was concentrated *in vacuo* and the residue was taken up in toluene. The volatiles were removed *in vacuo* and the resulting residue was dissolved in MeOH, passed through a SCX resin (5 g) and washed with MeOH. After treatment of the resin with NH_3 in MeOH (7 N) and concentration *in vacuo* the title compound was obtained as a pale brown solid (113 mg, 96%) which was used as such in the next step. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.82 (br s, 1H), 8.49 (s, 1H), 7.82 (br d, *J* = 9.7 Hz, 4H), 7.73 (s, 1H), 7.66 (br d, *J* = 3.7 Hz, 1H), 7.53 (br s, 1H), 6.54 (s, 1H), 3.85 (t, *J* = 7.3 Hz, 1H), 2.55 (d, *J* = 4.4 Hz, 3H), 2.47-2.11 (m, 2H), 2.03 (t, *J* = 7.3 Hz, 2H), 1.89-1.68 (m, 1H), 1.66-1.54 (m, 1H), 1.54-1.42 (m, 2H), 1.40-1.16 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{20}H_{26}N_6O$ 366.22, found 367 $(M+H)^+$.

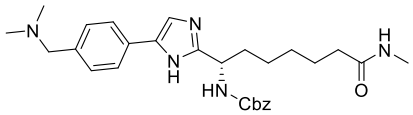
(*S*)-7-(3-(1*H*-indol-3-yl)propanamido)-7-(5-(4-(1*H*-pyrazol-1-yl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (**19**).



A solution of 3-(1*H*-indol-3-yl)propanoic acid (3.8 mg, 0.02 mmol) and HOBt (3.1 mg, 0.023 mmol) in DCM (1.0 mL) was treated with PS-carbodiimide (0.9 mmol/g, 30.3 mg, 0.026 mmol) and stirred for 20 min. A solution of **38** (5.0 mg, 0.014 mmol) in DMF (0.5 mL) was added and the mixture was stirred for 24 h before the addition of a suspension of MP-trisamine (2 mmol/g, 68.2 mg, 0.13 mmol) in DCM (2 mL). After a further 24 h the mixture was diluted with a 1:1 mixture of DCM/DMF and filtered through a fitted syringe. The filtrate was concentrated using a Genevac to give the title compound (4.1 mg, 35%) as an oil. LRMS (ES$^+$) *m/z* calcd. for $C_{31}H_{35}N_7O_2$ 537.29, found 538 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{31}H_{36}N_7O_2$ $[M+H]^+$ 538.2925, found 538.2923; HPLC t_R 1.11 min (method A, peak area 95%), 1.99 min (method B, peak area 97%).

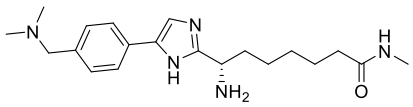
(*S*)-7-(3-(1*H*-indol-3-yl)propanamido)-7-(5-(4-((dimethylamino)methyl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (20):

Step 1. Benzyl (*S*)-(1-(5-(4-((dimethylamino)methyl)phenyl)-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (**37**).



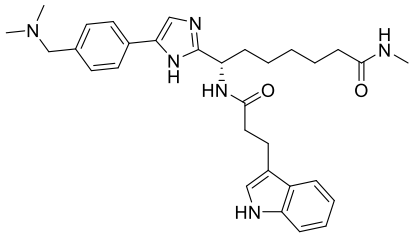
Following the general procedure described for compound **21** (step 2) coupling between (*S*)-(1-(5-iodo-1*H*-imidazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate **35**[3] (850 mg, 1.76 mmol), and *N*,*N*-dimethyl-1-[4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]methanamine hydrochloride (783 mg, 2.63 mmol) gave a residue that was purified on silica gel (eluting with 10-20% MeOH in DCM) to give the title compound (530 mg, 62% yield) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 11.85 (br s, 1H), 7.69 (br d, *J* = 7.3 Hz, 2H), 7.61 (br d, *J* = 8.5 Hz, 2H), 7.45 (br s, 1H), 7.39-7.27 (m, 5H), 7.24 (br d, *J* = 8.5 Hz, 2H), 5.08 (d, *J* = 12 Hz, 1H), 5.03 (d, *J* = 12 Hz, 1H), 4.69-4.60 (m, 1H), 3.37 (br s, 2H under signal of water), 2.55 (d, *J* = 4.4 Hz, 3H), 2.15 (br s, 6H), 2.03 (br t, *J* = 7.3 Hz, 2H), 1.96-1.84 (m, 1H), 1.82-1.70 (m, 1H), 1.52-1.41 (m, 2H), 1.35-1.19 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{28}H_{37}N_5O_3$ 491.29, found 492 $(M+H)^+$.

Step 2. (*S*)-7-amino-7-(5-(4-((dimethylamino)methyl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (**39**).



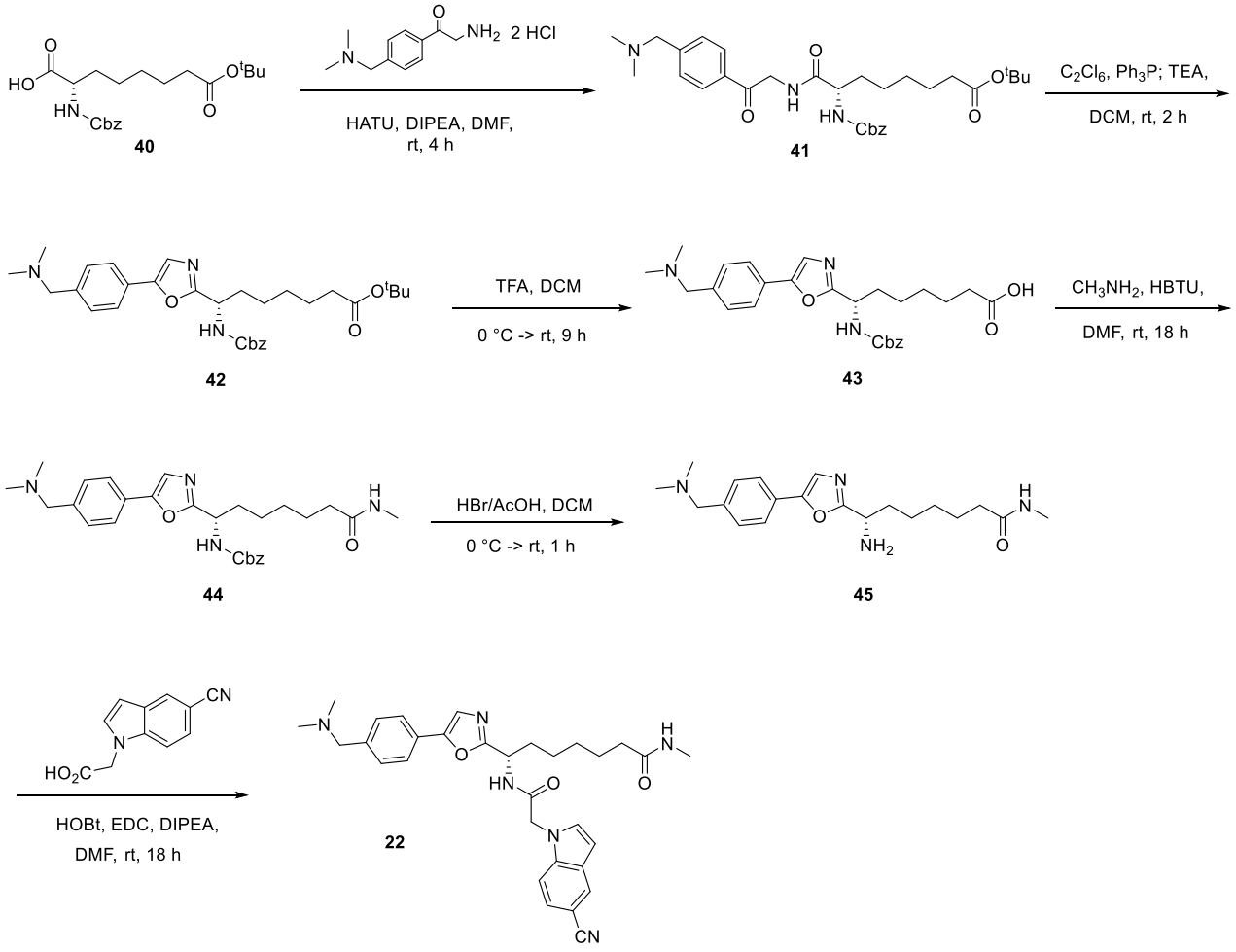
A solution of **37** (45 mg, 0.091 mmol) in DCM (1.2 mL) was cooled to 0 °C and treated with + HBr (33% solution in AcOH, 0.6 mL, 11.5 mmol). The mixture was stirred 0 °C for 1 h then allowed to warm to 20 °C prior to removal of the volatiles *in vacuo*. The residue was taken up in toluene and evaporated to dryness. The residue was dissolved in MeOH and loaded onto an SPE cartridge containing SCX resin (5 g). The cartridge was washed with MeOH before release of the product by elution with NH_3 in MeOH (7 N). Removal of the volatiles gave the title compound (25 mg, 77%) as a pale solid that was used directly in the subsequent reaction. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.62 (d, *J* = 8.5 Hz, 2H), 7.28 (d, *J* = 8.5 Hz, 2H), 7.20 (s, 1H), 6.56 (br d, *J* = 3.7 Hz, 1H), 5.00 (br s, 2H), 4.18 (br t, *J* = 6.7 Hz, 1H), 3.44 (br s, 2H), 2.73 (d, *J* = 4.4 Hz, 3H), 2.31-2.21 (m, 6H), 2.09 (br t, *J* = 7.3 Hz, 2H), 1.97-1.83 (m, 1H), 1.81-1.69 (m, 1H), 1.58-1.47 (m, 2H), 1.36-1.26 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{20}H_{31}N_5O$ 357.25, found 358 $(M+H)^+$.

Step 3. (*S*)-7-(3-(1*H*-indol-3-yl)propanamido)-7-(5-(4-((dimethylamino)methyl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide (**20**).



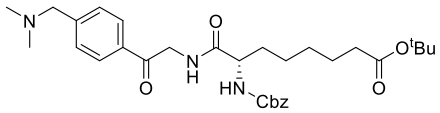
Reaction between (*S*)-7-amino-7-(5-(4-((dimethylamino)methyl)phenyl)-1*H*-imidazol-2-yl)-*N*-methylheptanamide **39** (5.0 mg, 0.014 mmol) and 3-(1*H*-indol-3-yl)propanoic acid (3.8 mg, 0.02 mmol) following the procedure described for 19 (step 3) gave the title compound (5.3 mg, 72%) as an oil. LRMS (ES^+) *m/z* calcd. for $C_{31}H_{40}N_6O_2$ 528.32, found 529 $(M+H)^+$; HRMS (ES^+) *m/z* calculated for $C_{31}H_{41}N_6O_2$ $[M+H]^+$ 529.3286, found 529.3288; HPLC t_R 0.74 min (method A, peak area 100%), 1.55 min (method B, peak area 100%).

Synthetic method for compound 22



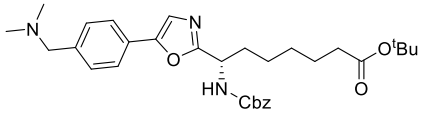
(*S*)-1-(4-(2-(1-(2-(5-isocyano-1*H*-indol-1-yl)acetamido)-7-(methylamino)-7-oxoheptyl)oxazol-5-yl)phenyl)-*N*,*N*-dimethylmethanaminium 2,2,2-trifluoroacetate (22):

Step 1: (*S*)-7-(((benzyloxy)carbonyl)amino)-8-((2-(4-((dimethylamino)methyl)phenyl)-2-oxoethyl)amino)-8-oxooctanoate (**41**).



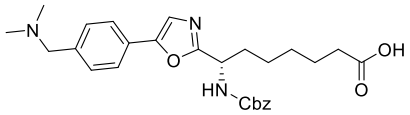
A solution of (*S*)-2-(((benzyloxy)carbonyl)amino)-8-(*tert*-butoxy)-8-oxooctanoic acid **40**[x] (2 g, 5.27 mmol), HATU (2.2 g, 5.8 mmol), 2-(4-((dimethylammonio)methyl)phenyl)-2-oxoethan-1-aminium di chloride (1.4 g, 5.27 mmol), and DIEA (2.76 mL, 15.8 mmol) in DMF (23 mL) was stirred for 1 h and concentrated *in vacuo*. The residue was diluted with dichloromethane and the organic phase was washed with saturated aqueous $NaHCO_3$ and brine then dried. Concentration *in vacuo* gave a residue that was purified on silica gel (eluting with 0-10% MeOH in DCM) to give the title compound (1.9 g, 65% yield) as an oil. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.37 (br s, 1H), 8.21 (br t, J = 5.3 Hz, 1H), 7.97 (br d, J = 7.9 Hz, 1H), 7.62-7.39 (m, 2H), 7.39-7.26 (m, 6H), 5.08-4.98 (m, 2H), 4.70-4.48 (m, 2H), 4.18-4.02 (m, 2H), 3.89-3.75 (m, 1H), 3.60 (br s, 3H), 3.14 (br s, 3H), 2.20-2.13 (m, 2H), 1.74-1.60 (m, 1H), 1.60-1.52 (m, 1H), 1.50-1.30 (m, 6H), 1.40 (s, 9H); LRMS (ES^+) *m/z* calcd. for $C_{31}H_{43}N_3O_6$ 553.32, found 554 $(M+H)^+$.

Step 2: (*S*)-7-(((benzyloxy)carbonyl)amino)-7-(5-(4-((dimethylamino)methyl)phenyl)oxazol-2-yl)heptanoate (**42**).



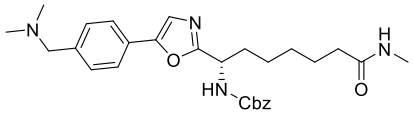
A solution of Ph_3P (2.7 g, 10.3 mmol) and C_2Cl_6 (2.43 g, 10.3 mmol) in DCM (12 mL) was stirred for 10 min then treated with a solution of **41** (1.9 g, 3.4 mmol) and Et_3N (2.87 mL, 20.6 mmol) in DCM (12 mL). The mixture was stirred 10 min then concentrated *in vacuo* to give a residue that was taken up in EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The dried organics were concentrated to give a residue that was purified on silica gel (eluting with 0-20% MeOH in DCM) to give the title compound (420 mg, 23%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.99 (br d, J = 8.3 Hz, 1H), 7.64 (br d, J = 7.9 Hz, 2H), 7.58 (s, 1H), 7.48-7.28 (m, 6H), 5.07 (s, 2H), 4.80-4.67 (m, 1H), 3.73-3.45 (m, 2H), 2.38-2.20 (m, 6H), 2.17 (br t, J = 7.3 Hz, 2H), 1.94-1.74 (m, 2H), 1.52-1.23 (m, 6H), 1.39 (s, 9H); LRMS (ES^+) *m/z* calcd. for $C_{31}H_{41}N_3O_5$ 535.30, found 536 $(M+H)^+$.

Step 3. (*S*)-7-(((benzyloxy)carbonyl)amino)-7-(5-(4-((dimethylamino)methyl)phenyl)oxazol-2-yl)heptanoic acid (**43**).



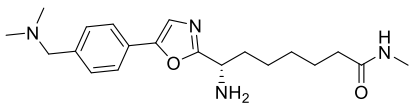
A solution of **42** (420 mg, 0.78 mmol) in a 4:1 mixture of DCM/TFA (10 mL) was stirred at 0 °C for 10 min then concentrated *in vacuo*. The residue was taken up in DCM and loaded onto an SPE cartridge containing SAX resin (10g). The column was washed with DCM and then product was eluted using a 1:1 mixture of DCM/MeOH. The filtrates were concentrated *in vacuo* and the residue was triturated with petroleum ether to give a precipitate that was filtered and dried to furnish the title compound (376 mg, 99%) as a white solid that was used directly in the subsequent reaction step. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.80 (br s, 1H), 8.00 (br d, J = 8.3 Hz, 1H), 7.76 (d, J = 8.3 Hz, 2H), 7.68 (s, 1H), 7.59 (d, J = 8.3 Hz, 2H), 7.45-7.29 (m, 5H), 5.07 (s, 2H), 4.80-4.70 (m, 1H), 4.34 (d, J = 4.0 Hz, 2H), 2.78 (s, 3H), 2.74 (s, 3H), 2.19 (br t, J = 7.2 Hz, 2H), 1.97-1.74 (m, 2H), 1.57-1.42 (m, 2H), 1.41-1.23 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{27}H_{33}N_3O_5$ 479.24, found 480 $(M+H)^+$.

Step 4. Benzyl (*S*)-(1-(5-(4-((dimethylamino)methyl)phenyl)oxazol-2-yl)-7-(methylamino)-7-oxoheptyl)carbamate (**44**).



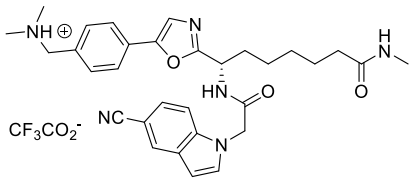
To a solution of **43** (350 mg, 0.73 mmol) in DMF (3.5 mL) were added HBTU (415 mg, 1.1 mmol) and $MeNH_2$ (2.0 M solution in THF, 1.82 mL, 3.65 mmol). The mixture was stirred for 1 h prior to addition of more HBTU (415 mg, 1.1 mmol) and $MeNH_2$ (2.0 M solution in THF, 1.82 mL, 3.65 mmol). Stirring was continued for 18 h then the mixture was concentrated *in vacuo* to give a residue that was purified on silica gel (eluting with DCM) to furnish the title compound (360 mg, 100%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.04-7.91 (m, 1H), 7.73-7.59 (m, 4H), 7.57 (s, 1H), 7.45-7.34 (m, 6H), 5.07 (s, 2H), 4.73 (br d, J = 6.1 Hz, 1H), 3.51 (br s, 2H), 2.82 (s, 3H), 2.80 (s, 3H), 2.55 (d, J = 4.4 Hz, 3H), 2.03 (br t, J = 7.5 Hz, 2H), 1.94-1.84 (m, 1H), 1.84-1.73 (m, 1H), 1.58-1.32 (m, 4H), 1.38-1.21 (m, 2H); LRMS (ES$^+$) *m/z* calcd. for $C_{28}H_{36}N_4O_4$ 492.27, found 493 $(M+H)^+$.

Step 5. (*S*)-7-amino-7-(5-(4-((dimethylamino)methyl)phenyl)oxazol-2-yl)-*N*-methylheptanamide (**45**).



A solution of **44** (360 mg, 0.73 mmol) in DCM (4 mL) was treated at 0 °C with HBr (33% solution in AcOH, 2 mL, 38.3 mmol). The mixture was stirred for 20 min then the volatiles were removed *in vacuo* and the residue was taken up in toluene. Concentration *in vacuo* gave a residue that was dissolved in MeOH and loaded onto an SPE cartridge containing SCX resin (2 g). The column was washed with MeOH then product was eluted with NH_3 in MeOH (7 N). Concentration *in vacuo* gave the title compound (260 mg, 100%) as a pale yellow powder that was used directly in the subsequent step. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 7.65 (d, *J* = 8.3 Hz, 2H), 7.52 (s, 1H), 7.38 (d, *J* = 8.3 Hz, 2H), 3.91 (br t, *J* = 6.8 Hz, 1H), 3.40 (s, 2H), 2.55 (d, *J* = 4.4 Hz, 3H), 2.15 (s, 6H), 2.02 (br t, *J* = 7.5 Hz, 2H), 1.83-1.72 (m, 1H), 1.72-1.62 (m, 1H), 1.54-1.41 (m, 2H), 1.37-1.20 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{20}H_{30}N_4O_2$ 358.24, found 359 $(M+H)^+$.

Step 6. (*S*)-1-(4-(2-(1-(2-(5-cyano-1*H*-indol-1-yl)acetamido)-7-(methylamino)-7-oxoheptyl)oxazol-5-yl)phenyl)-*N*,*N*-dimethylmethanaminium 2,2,2-trifluoroacetate (**22**).



Following the general procedure described for 8 reaction between **45** (25 mg, 0.070 mmol) and 5-cyanoindole-1-acetic acid (18.1 mg, 0.091 mmol) gave after RP-HPLC purification the title compound (4.7 mg, 12%) as a white solid. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.73-9.61 (m, 1H), 9.01 (br d, *J* = 7.9 Hz, 1H), 8.10 (s, 1H), 7.78-7.61 (m, 3H), 7.64-7.49 (m, 3H), 7.40 (dd, *J* = 1.5, 8.6 Hz, 1H), 6.64-6.60 (m, 1H), 5.08-4.95 (m, 2H), 4.32 (br d, *J* = 5.3 Hz, 1H), 3.49 (br s, 2H under signal of water), 2.76 (s, 3H), 2.75 (s, 3H), 2.56 (d, *J* = 4.4 Hz, 3H), 2.03 (br t, *J* = 7.5 Hz, 2H), 1.99-1.80 (m, 2H), 1.55-1.43 (m, 2H), 1.42-1.21 (m, 4H); LRMS (ES$^+$) *m/z* calcd. for $C_{31}H_{36}N_6O_3$ 540.28, found 541 $(M+H)^+$; HRMS (ES$^+$) *m/z* calculated for $C_{31}H_{37}N_6O_3$ $[M+H]^+$ 541.2922, found 541.2913; HPLC t_R 0.96 min (method A, peak area 90%), 1.81 min (method B, peak area 93%).

2. Biological Assay Procedures

Human HDAC1 activity assay

Human HDAC1 (Enzo, BML-SE456, USA) was diluted to 1 nM in the assay buffer (TBS + 1 mM $MgCl_2$ + 0.1% BSA). 15 µL of the diluted enzyme mix was transferred to each well of compound containing microplates (Thermo, 4316, USA). After 10 minutes incubation at room temperature, 5 µL of assay buffer diluted substrate (Enzo, BML-KI104, USA) were added to each well to a final concentration of 80 µM. The reaction was incubated for one hour at room temperature. To develop

the reaction signal, 15 µL of 600x assay buffer diluted developer solution (Enzo, BML-KI105, USA) are transferred to each well with the addition of 3 µM final concentration dacinostat (Selleckchem, S1095, USA) to stop the reaction. After 10 minutes incubation at room temperature the reaction signal is red at 360 nm excitation, 460 nm emission on a suitable spectrophotometer.

Human HDAC3 activity assay

Human HDAC3 (Enzo, BML-SE507, USA) was diluted to 1 nM in the assay buffer (TBS + 1 mM $MgCl_2$ + 0.1% BSA). 15 µL of the diluted enzyme mix was transferred to each well of compound containing microplates (Thermo, 4316, USA). After 10 minutes incubation at room temperature, 5 µL of assay buffer diluted substrate (Enzo, BML-KI104, USA) were added to each well to a final concentration of 80 µM. The reaction was incubated for one hour at room temperature. To develop the reaction signal, 15 µL of 600x assay buffer diluted developer solution (Enzo, BML-KI105, USA) are transferred to each well with the addition of 3 µM final concentration dacinostat (Selleckchem, S1095, USA) to stop the reaction. After 10 minutes incubation at room temperature the reaction signal is red at 360 nm excitation, 460 nm emission on a suitable spectrophotometer.

HeLa Class I HDAC inhibition assay

The buffer used in the assay is TBS + 0.25 mM $MgCl_2$ + 0.02% BSA. HeLa cells are plated in DMEM without phenol red (Thermo, 11880, USA + 10% FBS + 1X PenStrep + 1X Gln) to a density of 10000 cells per well in a 384 well plate (Thermo, 4334-11, USA) and let recover for four hours at 37 °C, 5% CO_2 in a humidified atmosphere. After the recovery, compounds are transferred to assay plates as per compound preparation method. Then, 5 µL of assay buffer diluted substrate (Enzo, BML-KI104, USA) are added to each well to a final concentration of 400 µM. The reaction is incubated four hours 37 °C, 5% CO_2 in a humidified atmosphere. To develop the reaction signal, 15 µL of 27x assay buffer diluted developer solution (Enzo, BML-KI105, USA) are transferred to each well with the addition of 3% NP40 and 6 µM final concentration dacinostat (Selleckchem, S1095, USA) to stop the reaction. After 10 minutes incubation at room temperature the reaction signal is red at 360 nm excitation, 460 nm emission on a suitable spectrophotometer

3. In vitro and in vivo assay protocols

Human Ether-a-go-go-Related Gene (hERG) inhibition assay procedure

The assay employs a membrane fraction containing hERG channel protein (Invitrogen's Predictor™ hERG Fluorescence Polarization Assay) and a high-affinity red fluorescent hERG

channel ligand, or "tracer" (Predictor™ hERG Tracer Red), in a fluorescence polarization (FP)-based format. The assay is based on the principle of fluorescence polarization where the redshifted fluorescent tracer is displaced from the hERG channel by compounds that bind to the channel. Lower polarization values correlate to greater displacement of the tracer, and therefore indicate binding to hERG. The decrease of fluorescence polarization was measured using a Tecan ULTRA microplate reader equipped with a fluorescence polarization module. Compound dilutions were made in 100% DMSO starting from 10 mM stock to have 3 mM in DMSO. The highest concentration of 3 mM was used to do a titration curves (10 points, 1:3 dilution). All concentration points were tested in duplicates. The positive control (compound E-4031) was used also starting at 30 µM concentration, as recommended by the manufacturer. The assay was performed in 384-well format. After incubation for 4 hours at room temperature, the fluorescence polarization was measured. The results were expressed as % of inhibition. The IC_{50} value was obtained by fitting the measured fluorescence polarization signal against 29 concentrations using nonlinear regression with XLfit 4.2 (IDBS Ltd, USA).

***In vivo* pharmacokinetic studies procedures**

Studies were performed in rats (Wistar), mice (NMRI) and minipig (Gottigen). The study protocols adhered to standards approved by the Institutional Animal Care and Use Committee for studies compounds were dosed as follows.

Compound **6** was dosed intravenously and orally to mice NMRI and rat Wistar, the compound was administered as a bolus at either 2 mg/kg of body weight (i.v.) and 20 mg/kg (p.o.) in mice and at 10 mg/kg (p.o.) in rats. For oral studies, the compound was dosed as a solution in PEG/Water 50/50. For intravenous studies, the compound was dosed as a solution in DMSO/PEG400/Water 20/60/20.

For all studies, blood samples were collected in EDTA-containing tubes at appropriate times and plasma was separated by centrifugation and stored at −70°C until analysis. Quantitation was conducted by high-performance liquid chromatography/mass spectroscopy (LC/MS/MS) following protein precipitation.

4. Summary of IC_{50} Values with Standard Deviations

Activities for new oxazole and imidazole based compounds in biochemical HDAC assays and in the class I HDAC cellular assay are reported in the table below. All

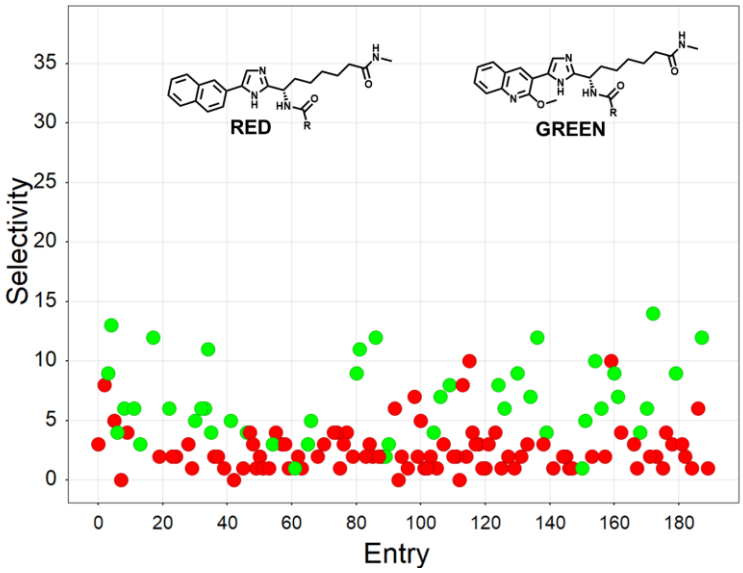
data are expressed as nanomolar concentrations, and are the arithmetic mean ± standard deviation (for n tests).

Cpd	hHDAC1 IC_{50} (nM) ± SD (n)	hHDAC2 IC_{50} (nM) ± SD (n)	hHDAC3 IC_{50} (nM) ± SD (n)	HeLa ClassI HDAC EC_{50} (nM) ± SD (n)
6	1.7 ± 0.9 (44)	2.8 ± 2.5 (2)	0.9 ± 0.6 (12)	50 ± 17 (49)
8	1.6 ± 0.7 (6)	4.7 ± 0.5 (3)	1.2 ± 1.0 (5)	43 ± 9 (6)
9	3.5 ± 1.6 (5)	5.3 ± 1.2 (4)	0.6 ± 0.2 (6)	15 ± 9 (3)
10	2.1 ± 0.5 (13)	6 ± 2 (4)	1.1 ± 0.3 (4)	94 ± 86 (9)
11	17 ± 4 (13)	30 ± 6 (3)	6.0 ± 1.2 (5)	430 ± 290 (12)
12	30.5 ± 8.4 (5)	59 ± 6 (4)	7 ± 2 (4)	610 ± 168 (4)
13	3.2 ± 0.3 (5)	4.9 ± 0.8 (4)	0.9 ± 0.4 (4)	208 ± 34 (4)
14	3 ± 1 (27)	14.9 ± 2.2 (3)	0.9 ± 0.4 (3)	138 ± 44 (28)
15	6070 ± 2970 (16)	21760 ± 3530 (4)	188 ± 84 (18)	>25000 (6)
16	325 ± 76 (3)	2460 ± 560 (4)	235 ± 144 (4)	1700 ± 570 (2)
17	198 ± 69 (17)	1220 ± 460 (3)	24 ± 14 (5)	890 ± 300 (15)
18	2490 ± 1020 (6)	>5000 (2)	223 ± 69 (6)	20490 ± 430 (2)
19	860 ± 70 (5)	2020 ± 210 (3)	42 ± 27 (6)	
20	1370 ± 630 (12)	6140 ± 614 (3)	35 ± 19 (13)	
21	1300 ± 360 (19)	4160 ± 250 (2)	26 ± 10 (17)	11450 ± 850 (2)
22	13900 ± 1750 (2)	26110 ± 830 (2)	3360 ± 1750 (5)	

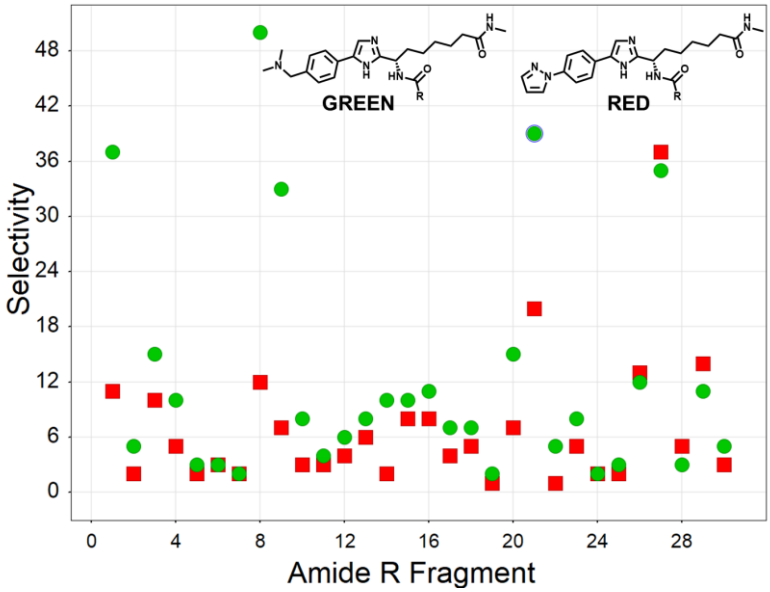
5. Supplementary plots of HDAC3 selectivity

Two further graphics are provided in support of claims made in the manuscript.

A. Very low HDAC3 selectivity is consistently found for compounds based on a 2-naphthyl substituted imidazole (red). Only marginally improved selectivity can be achieved in the presence of the 2-methoxy-3-quinoline substituted imidazole (green).



B. Summary of results for an array of 60 amides based on two alternative aryl-imidazole precursors. 4-N.N-dimethlmethanaminophenyl analogs (green) generally show somewhat improved selectivity and amides that generate high(er) selectivity (>10 fold, for example) in one series also tend to generate higher selectivity in the other.



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