Supporting Information

Title: Use of the NOC-Click Reaction for Molecular Probe Generation – A New Class of Isoform Selective Histone Deacetylase Inhibitors (HDACIs) Showing Picomolar Activity at HDAC6

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Synthesis. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker spectrometer at 300/400 MHz and 75/100 MHz respectively with TMS as an internal standard. Standard abbreviation indicating multiplicity was used as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintuplet, m = multiplet and br = broad. HRMS experiment was performed on Q-TOF-2TM (Micromass). TLC was performed with Merck 250-mm 60F254 silica gel plates. Preparative TLC was performed with Analtech 1000-mm silica gel GF plates. Column chromatography was performed using Merck silica gel (40-60 mesh).

HDAC Inhibition Assays:

Purified HDACs were incubated with 1 mm carboxyfluorescein (FAM)-labeled acetylated peptide substrate and test compound for 17 h at 25 °C in HDAC assay buffer containing 100 mm HEPES (pH 7.5), 25 mm KCl, 0.1% BSA, and 0.01% Triton X-100. Reactions were terminated by the addition of buffer containing 0.078% SDS for a final SDS concentration of 0.05%. Substrate and product were separated electrophoretically using a Caliper LabChip 3000 system with blue laser excitation and green fluorescence detection (CCD2). The fluorescence intensity in the substrate and product peaks was determined using the Well Analyzer software on the Caliper system. The reactions were performed in duplicate for each sample. IC50 values were automatically calculated using the IDBS XLFit version 4.2.1 plug-in for Microsoft Excel and the XLFit 4-Parameter Logistic Model (sigmoidal dose–response model): ((A+ ((B_A)/1+((C/x)D)))), in which x is compound concentration, A and B are respectively the estimated minimum and maximum of percent inhibition, C is the inflection point, and D is the Hill slope of the sigmoidal curve. The standard errors of the IC50 values were automatically calculated using the IDBS XLFit version 4.2.1 plug-in for Microsoft Excel and the formula xf4_FitResultStdError().

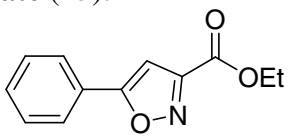
Cytotoxicity Assays:

The pancreatic cancer cell lines BxPc-3, HupT3, Mia Paca-2, Panc 04.03, and SU 86.86 were obtained from ATCC (Rockville, MD, USA) and were grown in medium (DMEM or RPMI) containing 10% fetal calf serum and l-glutamine. Pancreatic cancer cells were plated out in duplicate into 6 wells of a 96-well microtiter plate at 2.5–4P103 cells per well. Four hours post plating, individual wells were treated with diluent (DMSO) or varying concentrations of SAHA or the indicated HDACIs from a concentration of 1 nm to 50 mm. Cytotoxicity was measured at time “0”, and 72 h post treatment using the colorimetric MTT assay according to the

manufacturer's suggestions (Promega, Madison, WI, USA). The IC50 values were calculated using XLfit (IDBS Limited, Guildford, UK).

Experimentals

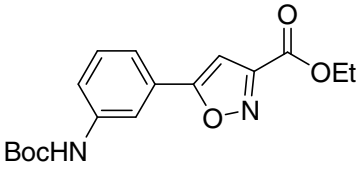
Ethyl 5-phenyl-3-isoxazolecarboxylate (**15**):



Phenylacetylene **12** (3.0 g, 29.4 mmol) and ethyl 2-chloro-2(hydroxyimino)acetate (9.8 g, 64.7 mmol) were mixed in 60 mL of anhydrous THF and triethylamine (10.3 mL, 73.5 mmol) was added slowly over 6 h by syringe pump. The mixture was stirred for 16 h and then diluted with 200 mL of ethyl acetate and 50 mL of 1 M HCl. The two layers were separated, and the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water (100 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in *vacuo*. Purification of the crude reaction mixture by column chromatography (SiO_2, 15-20 % ethyl acetate/hexane) afforded the title compound (3.2, 50 %) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (m, 2H), 7.49 (m, 3H), 6.93 (s, 1H), 4.48 (q, J = 7 Hz, 2 H), 1.44 (t, J = 7 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.2, 160.5, 157.4, 131.3, 129.6, 127.1, 126.4, 100.4, 62.7, 14.6. MS (ESI) *m/z*: 240 $[M + Na]^+$. ESI-HRMS: calc. for $C_{12}H_{12}NO_3$: 218.0812, found: 218.1298.

5-(3-*tert*-Butoxycarbonylaminophenyl)isoxazole-3-carboxylic acid ethyl ester (**16**):

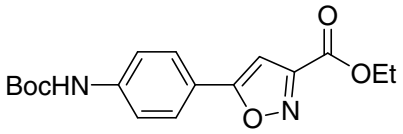


3-Ethynylaniline (3 g, 25.6 mmol) was dissolved in anhydrous THF (100 mL) and Boc-anhydride (6.7 mL, 28.16 mmol) was added and the resulting reaction mixture was stirred under reflux for another 16 h and cooled and concentrated in *vacuo*, and the crude reaction mixture was subjected to the next step without further purification.

The title compound was synthesized following the same procedure as used to prepare **15** to afford the desired product in 50% yield (4.2 g) as a white solid.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.92 (s, 1H), 7.49 (m, 3H), 6.95 (s, 1H), 6.72 (s, 1H), 4.48 (q, J = 7 Hz, 2H), 1.54 (s, 9H), 1.44 (t, J = 7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1, 159.6, 156.5, 152.2, 138.9, 129.4, 126.9, 120.2, 119.9, 99.89, 99.6, 80.6, 61.8, 27.9, 13.8. MS (ESI) *m/z*: 333 $[M + H]^+$. ESI-HRMS: calc. for $C_{17}H_{21}N_2O_5$: 333.1445, found: 333.1432.

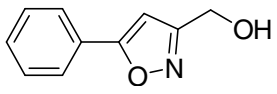
5-(4-*tert*-Butoxycarbonylaminophenyl)isoxazole-3-carboxylic acid ethyl ester (**17**):



The title compound was synthesized from 4-ethynylaniline **14** (3 g, 25.6 mmol) following the same procedure as used to prepare **16** to afford the desired product in 50% yield (4.2 g) as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J = 9 Hz, 2H), 7.53 (d, J = 9 Hz, 2H), 6.83 (m, 3H), 4.47 (q, J = 7 Hz, 2H), 1.53 (s, 9H), 1.45 (t, J = 7 Hz, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.9, 160.6, 157.4, 152.8, 141.3, 127.4, 121.6, 118.9, 99.4, 81.6, 62.7, 28.7, 14.6. MS (ESI) *m/z*: 333 $[M + H]^+$. ESI-HRMS: calc. for $C_{17}H_{21}N_2O_5$: 333.1445, found: 333.1439.

5-phenyl-3-isoxazolemethanol (**18**):

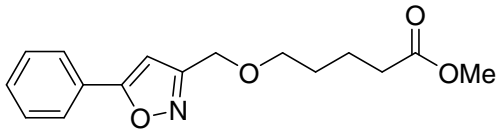


Compound **15** (800 mg, 3.7 mmol) was dissolved in 15 mL of anhydrous MeOH and the resulting solution was cooled to 0 °C. $NaBH_4$ (840 mg, 22.2 mmol) was then added portionwise. After completion of the addition, the reaction mixture was allowed to warm to rt and the stirred for 2 h. The reaction mixture was again cooled to 0 °C, and the excess sodium borohydride was quenched with a saturated aqueous NH_4Cl solution. This mixture was diluted with 200 mL of ethyl acetate, washed with water (20 mL), brine (10 mL) and dried over anhydrous Na_2SO_4 and

concentrated in *vacuo*. Purification of the crude reaction mixture by column chromatography (SiO_2, 50-80 % EtOAc/hexane) yielded the title compound (342 mg, 52 %) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 7.88 (m, 2H), 7.53 (m, 3H), 6.99 (s, 1H), 5.55 (t, J = 8 Hz, 1H), 4.55 (d, J = 8 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.5, 164.4, 130.5, 129.2, 127.4, 126.0, 98.5, 57.2. MS (ESI) *m/z*: 176 $[M + H]^+$. ESI-HRMS: calc. for $C_{10}H_{10}NO_2$: 176.0706, found: 176.0703.

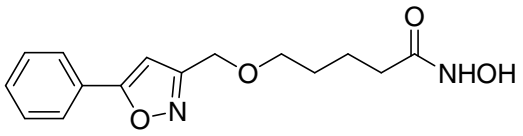
Methyl 5-(5-phenyl-3-isoxazolylmethoxy)pentanoate (**19**):



Compound **18** (200 mg, 1.1 mmol) was dissolved in 2 mL of anhydrous DMF and the resulting solution was cooled to 0 °C. NaH (91 mg, 2.3 mmol, 60 % dispersion in mineral oil) was added and the reaction mixture was stirred at the same temperature for another 30 min and then warmed to room temperature. After 1 h at room temperature again reaction mixture cooled to 0 °C and methyl 5-bromovalerate (0.2 mL, 1.7 mmol) was added. The resultant reaction mixture was warmed at 70 °C for 12 h. Excess sodium hydride was quenched with water and then diluted with 100 mL of ethyl acetate. The two layers are separated and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layer was washed with water (20 mL), brine (10mL) and dried (Na_2SO_4) and concentrated in *vacuo*. Purification of the crude reaction mixture by column chromatography (SiO_2, 20 % EtOAc/Hexane) afforded the title compound (88 mg, 26 %) as a colorless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (m, 2H), 7.49 (m, 3H), 6.58 (s, 1H), 4.61 (s, 2H), 3.66 (s, 3H), 3.56 (t, J = 6.0 Hz, 2H), 2.37 (t, J = 7.0 Hz, 2H), 1.76 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.9, 170.2, 162.3, 130.2, 129.0, 127.5, 125.9, 98.8, 70.4, 64.2, 51.5, 33.7, 29.0, 21.7. MS (ESI) *m/z*: 290 $[M + H]^+$. ESI-HRMS: calc. for $C_{16}H_{20}NO_4$: 290.1387, found: 290.1381.

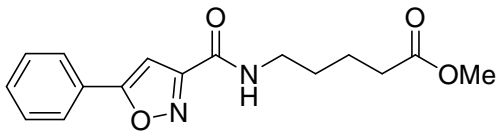
Compound 1:



To a stirred solution of compound **19** (50 mg, 0.2 mmol) in 3 mL of methanol, freshly prepared NH_2OH (prepared from 5.2 g of $NH_2OH{\cdot}HCl$ and 4.7 g of KOH in 20 mL of MeOH) was added in excess through filtration followed by the addition of excess KOH until reaction was over from TLC monitoring. After 30 min, the reaction mixture was diluted with 100 mL of ethyl acetate and 30 mL of water and separated. The aqueous layer was extracted again with ethyl acetate (2 × 30 mL) and the combined organic layer was washed with 1M HCl (20 mL), water (20 mL) and brine (10 mL) and concentrated in *vacuo*. The reaction mixture was purified by HPLC to give the pure title compound (11 mg, 20 %) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H), 8.67 (s, 1H), 7.90 (m, 2H), 7.54 (m, 3H), 7.06 (s, 1H), 4.55 (s, 2H), 3.47 (t, J = 6 Hz, 2H), 1.96 (t, J = 6 Hz, 2H), 1.53 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.2, 168.9, 162.3, 130.4, 129.2, 126.8, 125.6, 99.8, 69.8, 63.3, 31.9, 28.5, 21.8. MS (ESI) *m/z*: 290 $[M]^+$. ESI-HRMS: calc. for $C_{15}H_{19}N_2O_4$: 291.1339, found: 291.1335.

Methyl 5-(5-phenyl-3-isoxazolylcaboxamido)pentanoate (**20**):

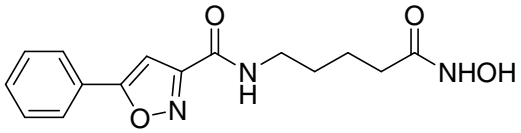


Compound **15** (500 mg, 2.3 mmol) was dissolved in THF-MeOH-H_2O system (5 mL, 3:1:1) and LiOH (193 mg, 4.6 mmol) was added. After 1 h the reaction mixture was acidified with 1M HCl solution and diluted with 100 mL of EtOAc and separated. The aqueous layer was extracted with EtOAc (2 × 20 mL) and the combined organic layer was washed with water (20 mL), brine (10 mL) and dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. The crude acid was used in the next step without further purification.

The crude acid was dissolved in 4 mL of anhydrous DMF and cooled to 0 °C, HOBT (311 mg, 2.3 mmol) and EDCI (441 mg, 2.3 mmol) were added and stirred at the same temperature for another 15 min. Methyl 5-aminopentanoate [generated from its hydrochloride salt (578.3 mg, 3.5 mmol) by adding diisopropylethyl amine (3 mL, 7.3 mmol) dissolved in 1 mL of DMF was added and the resultant reaction mixture was stirred at the room temperature for 12 h. 10 mL of H_2O was added and the resultant reaction mixture was diluted with 200 mL of ethyl acetate and another 30 mL of H_2O, and separated. The aqueous layer was extracted with ethyl acetate (2 × 50 mL) and the combined organic layer was washed with 1M HCl (20 mL), water (20 mL), saturated aqueous $NaHCO_3$ solution (20 mL), water (30 mL), brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. Purification of crude reaction mixture by column chromatography (SiO_2, 50-70 % EtOAc/Hexane) gave the title compound (487 mg, 70 %) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.80 (m, 2H), 7.49 (m, 3H), 6.96 (s, 1H), 6.92 (m, 1H), 3.69 (s, 3H), 3.52 (q, J = 7 Hz, 2 H), 2.41 (t, J = 6 Hz, 2H), 1.72 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.9, 171.7, 130.9, 129.3, 126.9, 126.1, 99.3, 51.8, 39.2, 33.7, 29.1, 22.3. MS (ESI) *m/z*: 303 $[M + H]^+$. ESI-HRMS: calc. for $C_{16}H_{19}N_2O_4$: 303.1339, found: 303.1334.

Compound 2:

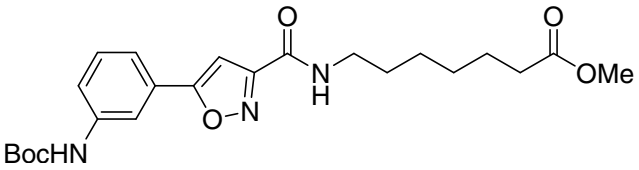


The title compound was obtained from compound **20** (300 mg, 1.0 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 30 % yield (91 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H), 8.84 (t, J = 5 Hz, 1H), 8.68 (s, 1H), 7.94 (m, 2H), 7.56 (m, 3H), 7.35 (s, 1H), 3.26 (q, J = 5 Hz, 2H), 1.98 (t, J = 5 Hz, 2H), 1.51 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 168.9, 159.7, 158.4, 130.8, 129.3, 126.3, 125.7, 99.8,

38.6, 31.9, 28.5, 22.6. MS (ESI) *m/z*: 304 $[M + H]^+$. ESI-HRMS: calc. for $C_{15}H_{18}N_3O_4$: 304.1292, found: 304.1289.

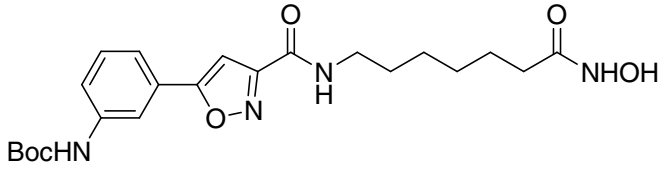
Methyl 7-[5-(3-*tert*Butoxycarbonylaminophenyl)-3-isooxazolecarboxamido]heptanoate (**21**):



The title compound was synthesized from **16** (1.0 g, 3.0 mmol) and 7-aminoheptanic acid methyl ester hydrochloride (883 mg, 4.5 mmol) following the same procedure as used to prepare **20** to provide the desired compound in 70% yield (936 mg) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (s, 1H), 7.46 (m, 3H), 6.97 (s, 1H), 6.86 (t, J = 7 H, 1H), 6.66 (s, 1H), 3.67 (s, 3H), 3.48 (q, J = 7 Hz, 2H), 2.34 (t, J = 8 Hz, 2H), 1.63 (m, 4H), 1.53 (m, 9H), 1.40 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.1, 171.3, 159.2, 158.8, 152.5, 139.3, 129.8, 127.5, 120.5, 120.4, 115.6, 99.5, 81.1, 51.5, 39.4, 33.9, 29.3, 28.7, 28.3, 26.5, 24.8. MS (ESI) *m/z*: 446 $[M + H]^+$. ESI-HRMS: calc. for $C_{23}H_{32}N_3O_6$: 446.2286, found: 446.2278.

Compound 3:

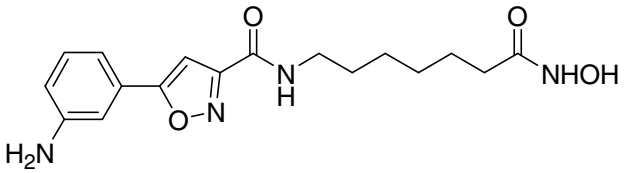


The title compound was obtained from compound **21** (400 mg, 0.9 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 30% yield (121 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.37 (s, 1H), 9.66 (s, 1H), 8.84 (t, J = 6 Hz, 1H), 8.67 (s, 1H), 8.11 (s, 1H), 7.54 (d, J = 9 Hz, 2H), 7.45 (m, 1H), 7.24 (s, 1H), 3.27 (q, J = 7 Hz, 2H), 2.01 (t, J = 7 Hz, 2H), 1.49 (m, 13H), 0.87 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 169.2, 159.7, 158.4, 152.8, 140.5, 129.8, 126.7, 120.4, 119.8, 114.6, 99.7, 79.5, 62.8, 32.2, 28.8, 28.3,

28.1, 26.2, 25.1. MS (ESI) *m/z*: 469 $[M + Na]^+$. ESI-HRMS: calc. for $C_{22}H_{30}N_4O_6Na$: 469.2058, found: 469.2039.

Compound 5:



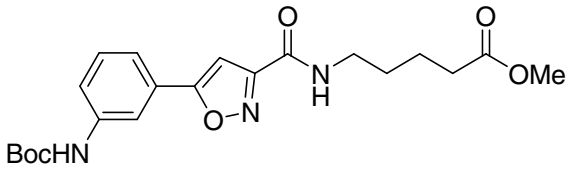
Compound **21** (300 mg, 0.7 mmol) was dissolved in 4 mL of anhydrous CH_2Cl_2 and cooled to 0 °C and 1 mL of trifluoroacetic acid (TFA) was added and the resultant reaction mixture was stirred at room temperature for 2 h. The excess TFA was evaporated under vacuum and the brown residue was dissolved in 2 mL of methanol and the resulting solution was neutralized with excess saturated aqueous $NaHCO_3$ solution. EtOAc (100 mL) was added and the organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (20 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The crude free amino compound was subjected to next reaction without further purification.

To the stirred solution of the above free amino compound in 3 mL of methanol, freshly prepared NH_2OH (prepared from 5.2 g of $NH_2OH{\cdot}HCl$ and 4.7 g of KOH in 20 mL of MeOH) was added in excess through filtration followed by the addition of excess KOH until reaction was over from TLC monitoring. After 30 min, the reaction mixture was diluted with 100 mL of ethyl acetate and 30 mL of water and separated. The aqueous layer was extracted again with ethyl acetate (2 × 30 mL) and the combined organic layer was washed with water (20 mL) and brine (10 mL) and concentrated in *vacuo*. The reaction mixture was purified by HPLC to give the pure title compound (24 mg, 10 %) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.33 (s, 1H), 8.81 (t, J = 9 Hz, 1H), 8.66 (s, 1H), 8.11 (s, 1H), 7.14 (m, 4H), 6.71 (d, J = 7 Hz, 1H), 5.42 (s, 2H), 3.25 (m, 2H), 1.96 (t, J = 8 Hz, 2H), 1.50

(m, 4H), 1.27 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 169.1, 159.6, 158.4, 149.4, 129.8, 126.8, 116.2, 113.3, 110.1, 99.0, 32.2, 28.8, 28.8, 28.3, 26.1, 25.1. MS (ESI) *m/z*: 347 [M + H]$^+$. ESI-HRMS: calc. for $C_{17}H_{22}N_4O_4$: 347.1714, found: 347.1704.

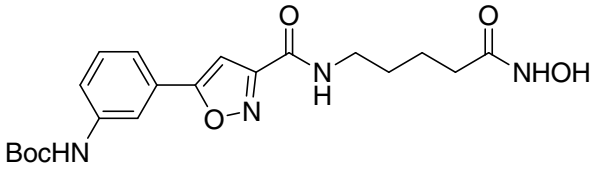
Methyl 5-[5-(3-*tert*Butoxycarbonylaminophenyl)-3-isooxazolecarboxamido]pentanoate (**22**):



The title compound was synthesized from **16** (500 mg, 1.5 mmol) and 5-aminopentanic acid methyl ester hydrochloride (377 mg, 2.3 mmol) following the same procedure as used to prepare **20** to provide the desired compound in 65% yield (407 mg) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.61 (s, 1H), 8.83 (t, J = 8 Hz, 1H), 8.09 (s, 1H), 7.54 (d, J = 7 Hz, 2H), 7.45 (m, 1H), 7.23 (s, 1H), 3.58 (s, 3H), 3.27 (q, J = 5 Hz, 2H), 2.34 (t, J = 6 Hz, 2H), 1.55 (m, 4 H), 1.49 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.2, 170.3, 159.6, 158.3, 152.7, 140.4, 129.7, 126.7, 119.7, 114.5, 99.7, 79.5, 51.2, 32.8, 28.2, 28.1, 21.8. MS (ESI) *m/z*: 418 [M + H]$^+$. ESI-HRMS: calc. for $C_{21}H_{28}N_3O_6$: 418.1973, found: 418.1967.

Compound 4:

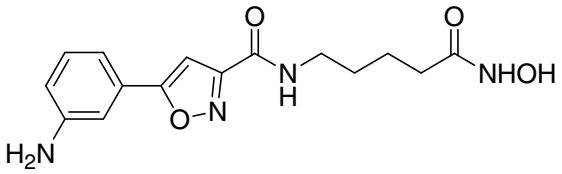


The title compound was obtained from compound **22** (200 mg, 0.5 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 15% yield (31 mg) as a white solid after HPLC purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 9.61 (s, 1H), 8.82 (t, J = 7 Hz, 1H), 8.68 (s, 1H), 8.08 (s, 1H), 7.53 (d, J = 6 Hz, 2H), 7.44 (m, 1H), 7.23 (s, 1H), 3.24 (m, 2H), 1.97 (t, 2H), 1.49 (m, 13H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 168.9, 159.7, 158.3, 152.7, 140.4,

129.8, 126.6, 120.3, 119.8, 114.5, 99.7, 79.5, 38.6, 31.9, 28.5, 28.1, 22.6. MS (ESI) *m/z*: 417 [M - H]$^+$. ESI-HRMS: calc. for $C_{20}H_{27}N_4O_6$: 419.1925, found: 419.1919.

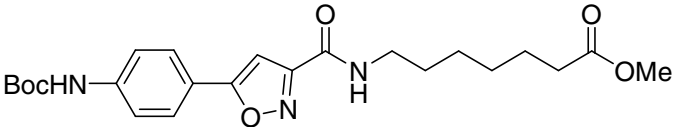
Compound 6:



The title compound was synthesized from **22** (300 mg, 0.7 mmol) following the same procedure as used to prepare **5** to provide the desired compound in 20 % yield (45 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.35 (s, 1H), 8.79 (t, J = 6 Hz, 1H), 8.67 (s, 1H), 8.09 (s, 1H), 7.14 (m, 4H), 6.71 (d, J = 7 Hz, 1H), 5.40 (s, 2H), 3.24 (q, J = 5 Hz, 2H), 1.97 (t, 2H), 1 51 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.1, 168.9, 159.5, 158.4, 149.3, 129.8, 126.8, 116.2, 113.3, 110.1, 98.9, 38.5, 31.9, 28.5, 22.6. MS (ESI) *m/z*: 319 [M + H]$^+$. ESI-HRMS: calc. for $C_{15}H_{19}N_4O_4$: 319.1401, found: 319.1396.

Methyl 7-[5-(4-*tert*Butoxycarbonylaminophenyl)-3-isooxazolecarboxamido]heptanoate (23):

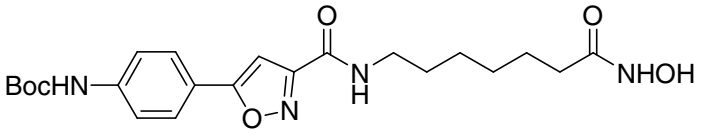


The title compound was synthesized from **17** (1.0 g, 3.0 mmol) and 7-aminoheptanic acid methyl ester hydrochloride (883 mg, 4.5 mmol) following the same procedure as used to prepare **20** to provide the desired compound in 70 % yield (936 mg) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 9.70 (s, 1H), 8.76 (t, J = 6 Hz, 1H), 7.83 (d, J = 8 Hz, 2H), 6.86 (t, J = 7 H, 1H), 7.63 (d, J = 8 Hz, 2H), 7.16 (s, 1H), 3.57 (s, 3H), 3.22 (q, J = 7 Hz, 2H), 2.29 (t, J = 7 Hz, 2H), 1.49 (m, 13H), 1.28 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3, 170.3, 159.6,

158.5, 152.6, 141.8, 126.5, 119.9, 118.1, 98.4, 79.6, 51.1, 33.2, 28.6, 28.1, 26.0, 24.4. MS (ESI) *m/z*: 446 $[M + H]^+$. ESI-HRMS: calc. for $C_{23}H_{31}N_3O_6Na$: 468.2105, found: 468.2085.

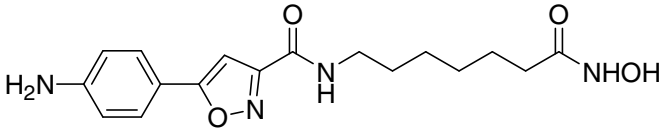
Compound 7:



The title compound was obtained from compound **23** (400 mg, 0.9 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 30 % yield (121 mg) as a white solid after HPLC purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.34 (s, 1H), 9.71 (s, 1H), 8.76 (t, J = 6 Hz, 1H), 8.66 (s, 1H), 7.83 (d, J = 9 Hz, 2H), 7.63 (d, J = 9 Hz, 2H), 7.17 (s, 1H), 3.24 (q, J = 7 Hz, 2H), 1.96 (t, J = 7 Hz, 2H), 1.49 (m, 13H), 1.27 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 169.1, 159.6, 158.4, 152.5, 141.9, 126.5, 119.9, 118.1, 98.4, 79.6, 32.2, 28.7, 28.3, 28.0, 26.1, 25.1. MS (ESI) *m/z*: 447 $[M + H]^+$. ESI-HRMS: calc. for $C_{22}H_{30}N_4O_6Na$: 469.2058, found: 469.2040.

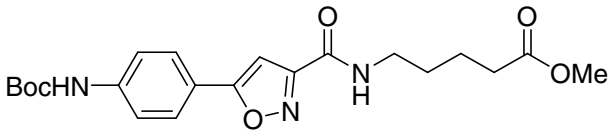
Compound 9:



The title compound was obtained from compound **23** (300 mg, 0.7 mmol) following the same procedure as used to prepare **5** to provide the desired compound in 10 % yield (24 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.33 (s, 1H), 8.69 (t, J = 9 Hz, 1H), 7.57 (d, J = 9 Hz, 2H), 6.89 (s, 1H), 6.66 (d, J = 9 Hz, 2H), 5.78 (s, 2H), 3.26 (q, J = 7 Hz, 2H), 1.91 (t, J = 8 Hz, 2H), 1.49 (m, 4H), 1.27 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 169.1, 159.4, 158.7, 151.3, 127.1, 113.6, 95.8, 32.2, 28.7, 28.3, 26.1, 25.1. MS (ESI) *m/z*: 347 $[M + H]^+$. ESI-HRMS: calc. for $C_{17}H_{22}N_4O_4Na$: 369.1534, found: 369.1521.

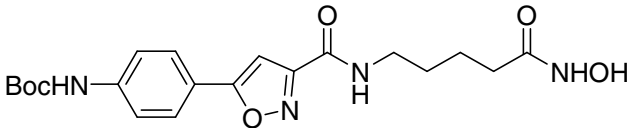
Methyl 5-[5-(4-*tert*-butoxycarbonylaminophenyl)-3-isooxazolecarboxamido]pentanoate (24):



The title compound was synthesized from **17** (1 g, 3.0 mmol) and 5-aminopentanic acid methyl ester hydrochloride (377 mg, 2.3 mmol) following the same procedure as used to prepare **20** to provide the desired compound in 70 % yield (877 mg) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6): δ 9.71 (s, 1H), 8.79 (t, J = 5, 1H), 7.83 (d, J = 8, 2H), 7.63 (J =, 2H), 7.17 (s, 1H), 3.58 (s, 3H), 3.26 (q, J = 6 Hz, 4H), 2.34 (t, J = 6 Hz, 2H), 1.54 (m, 4H), 1.49 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 173.2, 170.3, 159.6, 158.5, 152.5, 141.9, 126.5, 119.9, 118.0, 98.4, 79.6, 51.2, 32.8, 28.2, 28.0, 21.8. MS (ESI) *m/z*: 418 $[M + H]^+$. ESI-HRMS: calc. for $C_{21}H_{28}N_3O_6$: 418.1973, found: 418.1960.

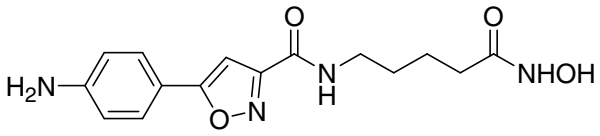
Compound 8:



The title compound was obtained from compound **24** (200 mg, 0.5 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 15 % yield (31 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.37 (s, 1H), 9.71 (s, 1H), 8.79 (t, 1H), 8.68 (s, 1H), 7.83 (d, J = 6 Hz, 2H), 7.63 (d, J = 7 Hz, 2H), 7.17 (s, 1H), 3.23 (q, 2H), 1.97 (t, 2H), 1.49 (m, 13H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.3, 168.9, 159.6, 158.4, 152.6, 141.9, 126.5, 119.9, 118.1, 98.4, 79.6, 38.6, 31.9, 28.5, 28.0, 22.6. MS (ESI) *m/z*: 419 $[M + H]^+$. ESI-HRMS: calc. for $C_{20}H_{26}N_4O_6Na$: 441.1745, found: 441.1739.

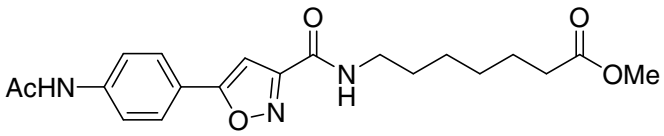
Compound 10:



The title compound was synthesized from **24** (300 mg, 0.7 mmol) following the same procedure as used to prepare **5** to provide the desired compound in 20 % yield (45 mg) as white solid after HPLC purification.

^{1}H NMR (400 MHz, DMSO-d_6): δ 10.38 (s, 1H); 8.70 (d, J = 7 Hz, 2H), 7.56 (d, J = 9 Hz, 2H), 6.90 (s, 1H), 6.66 (d, J = 8 Hz, 2H), 5.79 (s, 2H), 3.22 (q, 2H), 1.98 (t, 2H), 1.49 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 171.5, 168.9, 159.4, 158.8, 151.3, 127.2, 113.6, 113.5, 95.8, 38.5, 31.9, 28.5, 22.6. MS (ESI) *m/z*: 319 $[M + H]^+$. ESI-HRMS: calc. for $C_{15}H_{18}N_4O_4$: 319.1401, found: 319.1396.

Methyl 7-[5-(4-acetylaminophenyl)-3-isooxazolecarboxamido]heptanoate (**25**):



Compound **17** (500 mg, 1.5 mmol) was dissolved in 4 mL of anhydrous CH_2Cl_2 and cooled to 0 °C and 1 mL of trifluoroacetic acid (TFA) was added and the resultant reaction mixture was stirred at room temperature for 2 h. The excess TFA was evaporated under vacuum and the brown gummy residue was dissolved in 2 mL of methanol and the resulting solution was neutralized with excess saturated aqueous $NaHCO_3$ solution. EtOAc (100 mL) was added and the organic layer was separated and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with water (20 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The intermediate free amino compound was subjected to next reaction without further purification.

The crude amine was dissolved in 5 mL of anhydrous CH_2Cl_2 and pyridine was added (1.0 mL, 12 mmol) was added and the resulting solution was stirred for 30 min and cooled to 0 °C, acetic

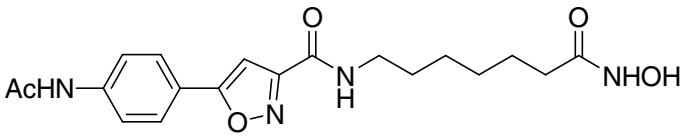
anhydride (0.86 mL, 9 mmol) was added and the reaction mixture was stirred at room temperature for another 6 h. Water and ethyl acetate was added and the organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 50 mL) and the combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in *vacuo*. The crude intermediate product was used in the next step without further characterization.

The crude intermediate as obtained above was subjected to saphonification reaction to give the intermediate acid and this acid was subjected to next reaction without further purification.

The title compound was synthesized from intermediate acid and 7-aminoheptanoic acid methyl ester hydrochloride (440 mg, 2.25 mmol) following the same procedure as used to prepare **20** to provide the desired compound in 30% yield (174 mg) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68 (d, J = 9 Hz, 2H), 7.66 (d, J = 9 Hz, 2H), 7.37 (s, 1H), 6.89 (s, 1H), 6.85 (t, J = 7 Hz, 1H), 3.46 (q, J = 7 Hz, 2H), 2.34 (t, J = 7 Hz), 2.22 (s, 3H), 1.64 (m, 4H), 1.27 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 174.5, 171.5, 168.9, 159.5, 159.3, 140.5, 127.2, 126.7, 122.8, 120.2, 98.8, 51.9, 39.8, 34.3, 29.6, 29.3, 26.8, 25.1, 25.0. MS (ESI) *m/z*: 388 $[M + H]^+$. ESI-HRMS: calc. for $C_{20}H_{25}N_3O_5Na$: 410.1687, found: 410.1675.

Compound 11:



The title compound was obtained from compound **25** (120 mg, 0.3 mmol) following the same procedure as used to prepare **1** to provide the desired compound in 30 % yield (35 mg) as a white solid after HPLC purification.

^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (s, 1H), 10.28 (s, 1H), 8.77 (s, 1H), 8.65 (t, J = , 1H), 7.87 (d, J = 9 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 7.20 (s, 1H), 3.26 (m, 2H), 2.08 (s, 3H), 1.96 (t, J = 7 Hz, 2H), 1.49 (m, 4H), 1.27 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.2, 169.1,

168.9, 141.6, 126.5, 120.8, 119.1, 98.7, 32.2, 28.7, 28.3, 26.1, 25.1, 24.1, 19.5. MS (ESI) *m/z*: 411 $[M + Na]^+$. ESI-HRMS: calc. for $C_{19}H_{24}N_4O_5Na$: 411.1639, found: 411.1626.

Table 1a. HPLC purity analysis data sheet

Compds	Gradient Method	Wavelength (nM)	t_R (min)	Purity (%)
1	B	254	14.93	99
2	B	254	13.72	99
3	A	240	11.76	99
4	B	240	11.13	99
5	B	254	9.58	99
6	B	254	7.61	99
7	A	300	11.76	99
8	B	300	16.35	99
9	B	280	10.68	99
10	B	254	2.37	99
11	B	300	12.64	99

Table 1b. HPLC purity analysis data sheet

Compds	Gradient Method	Wavelength (nM)	t_R (min)	Purity (%)
1	B	254	11.54	97.55
2	B	254	11.12	96.0
3	B	254	8.10	100.00
4	B	254	14.06	99.96
5	B	254	15.56	99.96
6	B	254	14.12	99.0
7	B	254	7.82	98.86
8	B	254	4.33	98.04
9	B	254	8.72	98.60
10	B	254	4.98	98.72
11	B	254	11.74	98.36

The HPLC analysis was carried out by using Shimadzu HPLC VP series components with a two mobile phase system [0.05 % TFA in water (solution A) and 0.05 % TFA in acetonitrile (solution B)] with a gradient.

Column1 (**Table 1a**): Inertsil 10 μC8 (250 × 4.6 mm).

Column 2 (**Table 1b**): ACE 5 AQ (250 x 4.6 mm).

Gradient A: (flow rate: 1.3 mL/ min) from 30 % solution B in solution A to 100 % solution B in 25 min.

Gradient B: (flow rate: 1.3 mL/ min) from 10 % solution B in solution A to 100 % solution B in 25 min.