SUPPORTING INFORMATION

A Novel Series of Potent and Selective Ketone Histone Deacetylase Inhibitors with Anti-tumor Activity *in vivo.*

Philip Jones,* Sergio Altamura, Raffaele De Francesco, Odalys Gonzalez Paz, Olaf Kinzel, Giuseppe Mesiti, Edith Monteagudo, Giovanna Pescatore, Michael Rowley, Maria Verdirame and Christian Steinkühler.

IRBM/Merck Research Laboratories, Via Pontina km 30,600, 00040 Pomezia, Italy.

Table of Contents

Experimental section and synthetic schemes

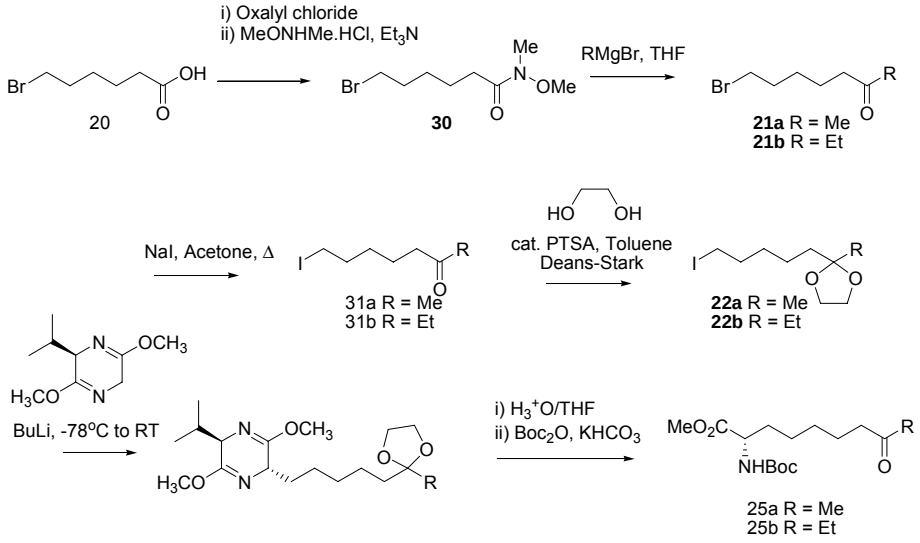
General Experimental Protocols

Solvents and reagents were obtained from commercial suppliers and were used without further purification. Flash chromatography purifications were performed on Merck silica gel (200-400 mesh) as the stationary phase or were conducted using prepacked cartridges on a Biotage system, eluting with petroleum ether/ethyl acetate mixtures. HPLC-MS and UPLC-MS analyses were performed on either a Waters Alliance 2795 apparatus, equipped with a diode array and a ZQ mass spectrometer using an X-Terra C18 column (5μM, 4.6x50mm) or on a Acquity UPLC™

equipped with a BEH C18 1.7 μm, 2.1 x 50 mm column. Mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (solvent A) and MeCN containing 0.1% formic acid (solvent B).

Nuclear magnetic resonance spectra (^1H NMR recorded at 500, 400 or 300 MHz, ^{13}C NMR recorded at 125, 100 or 75 MHz) were obtained on Bruker AMX spectrometers and are referenced in ppm relative to TMS. Unless indicated, spectra were acquired at 300 K.

High resolving power accurate mass measurement electrospray (ES) and atmospheric pressure chemical ionization (APCI) mass spectral data were acquired by use of a Bruker Daltonics 7T Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). External calibration was accomplished with oligomers of polypropylene glycol.



6-Bromo-*N*-methoxy-*N*-methylhexanamide **(30)**

To a solution 6-bromohexanoic acid (20 g, 0.10 mol) in DCM (200 mL) a solution of oxalyl chloride (2.0 M in DCM, 77 mL, 0.15 mol) was added dropwise over 15 min. After addition of the first 10 mL, DMF (50 μL) was added and after gas evolution initiated, the remainder of the solution was added slowly. Upon complete addition the mixture was stirred at RT for a further hour. Additional DMF (50 μL) was added and after a further 30 min of stirring, the solution was concentrated under reduced pressure.

The residue was taken up in DCM (50 mL) and added dropwise over 5 min to a stirred solution of *N*,*O*-dimethyl hydroxylamine.HCl (12 g, 0.123 mol) and Et_3N (42.9 mL, 0.3 mol) in DCM (400 mL) and the mixture was stirred for 1 h. The mixture was diluted with DCM (200

mL) and washed with H_2O (200 mL), 1 M HCl solution (250 mL), 1M NaOH (250 mL), and brine (100 mL). The resulting solution was dried (Na_2SO_4), and concentrated under reduced pressure to yield the desired material (23.7 g, 97%) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.70 (3H, s), 3.47 (2H, t, J = 7 Hz), 3.19 (3H, s), 2.47 (2H, t, J = 7 Hz), 1.91 (2H, app. q, J = 7 Hz), 1.68 (2H, app. quintet, J = 7 Hz), 1.53 (2H, app. quintet, J = 7 Hz).

7-Bromoheptan-2-one (21a)

To a stirred solution of **30** (61.2 g, 0.26 mmol) in THF (700 mL) at 0 ^oC under N_2 a solution of MeMgBr (3.0 M in Et_2O, 128 mL, 0.38 mol) was added dropwise over 15 min. The mixture was stirred for 3 hours at 0 ^oC during which time a white precipitate develops. [TLC: 15% EtOAc/petroleum ether, staining anisaldehyde showed complete reaction]. Poured cautiously into 1M HCl solution (400 mL) at 0 ^oC and the THF was then removed under reduced pressure. The organics were extracted with EtOAc (3 x 400 mL), washed with brine (200 ml). The resulting solution was dried (Na_2SO_4), and concentrated under reduced pressure to yield the desired ketone (48.4 g, 98%) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.42 (2H, t, J = 7 Hz), 2.45 (2H, t, J = 7 Hz), 2.16 (3H, s), 1.86 (2H, app. q, J = 7 Hz), 1.63 (2H, app. quintet, J = 7 Hz), 1.46 (2H, app. quintet, J = 7 Hz).

7-Iodoheptan-2-one (31a)

A mixture of **21a** (48.4 g, 0.25 mol), NaI (113 g, 0.75 mol) in acetone (1 L) was heated at reflux for 18 hours. The resulting mixture was concentrated under reduced pressure and was then taken up in Et_2O (1 L), filtered, and the filter cake was washed with Et_2O (500 mL). The combined Et_2O filtrates were washed with H_2O (2 x 300 mL), sodium thiosulfate solution (250 mL) and brine (100 mL). The resulting solution was dried (Na_2SO_4), and concentrated under reduced pressure to yield the desired material (55.5 g, 87%) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.21 (2H, t, J = 7 Hz), 2.47 (2H, t, J = 7 Hz), 2.18 (3H, s), 1.85 (2H, app. q, J = 7 Hz), 1.63 (2H, app. quintet, J = 7 Hz), 1.43 (2H, app. quintet, J = 7 Hz).

2-(5-Iodopentyl)-2-methyl-1,3-dioxolane (22a)

A mixture of **31a** (55.5 g, 0.22 mol), ethylene glycol (20.3 g, 0.33 mol) and PTSA (2.0 g, 5 mol%) in toluene (1 L) was heated at reflux in the presence of a Deans-Stark trap for 36 hours. The resulting mixture was diluted with Et_2O (600 mL), and washed with 0.5 N NaOH solution (2

x 300 mL), and brine (150 mL). The resulting solution was dried (Na_2SO_4), and concentrated under reduced pressure to yield the desired material (67.8 g, 99%) was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.96-3.88 (4H, m), 3.21 (2H, t, J = 7 Hz), 1.88 (2H, app. quin., J = 7 Hz), 1.70-1.62 (2H, m), 1.48-1.38 (4H, m), 1.32 (3H, s).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-8-oxononanoate (25a)

To a stirred solution of (2*R*)-2-isopropyl-3,6-dimethoxy-2,5-dihydropyrazine (29.2 g, 0.158 mmol) in THF (700 mL) at -78 ^{o}C a solution of BuLi (1.6 N in hexanes, 99 mL, 0.158 mol) was added dropwise over 15 min, and then stirring was continued at -78 ^{o}C for 45 min. A pre-cooled solution of **22a** (45.0 g, 0.158 mol) in THF (150 mL) was then added by cannula over 10 min and the reaction stirred for 24 h, slowly warming to RT.

The reaction was quenched by the addition of aqueous NH_4Cl solution (600 mL). The THF layer was decanted off and concentrated under reduced pressure. Meanwhile, the aqueous mixture was extracted with EtOAc (3 x 400 mL). The combined EtOAc extracts were used to redissolve the oily THF residue and this solution was washed with brine (250 mL), dried (Na_2SO_4) and concentrated under reduced pressure.

The crude (2*R*,5*S*)-2-isopropyl-3,6-dimethoxy-5-[5-(2-methyl-1,3-dioxolan-2-yl)pentyl]-2,5-dihydropyrazine was dissolved in THF (800 mL) and 1M HCl (792 mL, 0.79 mol) was added and the mixture was stirred for 4 hours. The mixture was cooled to 0 ^{o}C and quenched with 2M NaOH (400 mL). The THF was removed under reduced pressure and the resulting aqueous phase was extracted with EtOAc (3 x 500 mL). The combined EtOAc extracts were washed with brine (200 mL), dried (Na_2SO_4), and then concentrated under reduced pressure.

The crude methyl (2*S*)-2-amino-8-oxodecanoate was dissolved in 1,4-dioxane/water (1:1, 1.2 L), then $NaHCO_3$ (53.3 g, 0.63 mol) and Boc_2O (69.1 g, 0.32 mol) were added and the mixture was stirred overnight at RT. The 1,4-dioxane was removed under reduced pressure and the aqueous phase was extracted with Et_2O (3 x 500 mL). The combined Et_2O extracts were washed with water (2 x 200 mL) and brine (200 mL), dried (Na_2SO_4) and concentrated under reduced pressure. The oil was purified by column chromatography on silica gel eluting with 30-60% Et_2O/petroleum ether to obtain the titled compound (33.1 g, 69%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.99 (1H, br. d, J = 7.3 Hz), 4.29 (1H, br. q, J = 6.6 Hz), 3.74 (3H, s), 2.42 (2H, t, J = 7.3 Hz), 2.14 (3H, s), 1.85-1.75 (1H, m), 1.68-1.52 (5H, m), 1.45 (9H, s),

1.40-1.25 (4H, m). MS (ES) $C_{15}H_{27}NO_3$ requires: 301, found: 324 $(M+Na)^+$. $[\alpha]^{20}_D$ = -13 (c=0.178, MeOH).

8-Bromooctan-3-one (21b)

The reaction was carried out as described for **21a** using **30** (21.31 g, 89.6 mmol) and EtMgBr to yield the desired ketone (17.81 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.46 (2H, t, J = 7 Hz), 2.51-2.41 (4H, m), 1.86 (2H, app. q, J = 7 Hz), 1.62 (2H, app. quintet, J = 7 Hz), 1.47 (2H, app. quintet, J = 7 Hz), 1.07 (2H, t, J = 7 Hz).

8-Iodooctan-3-one (31b)

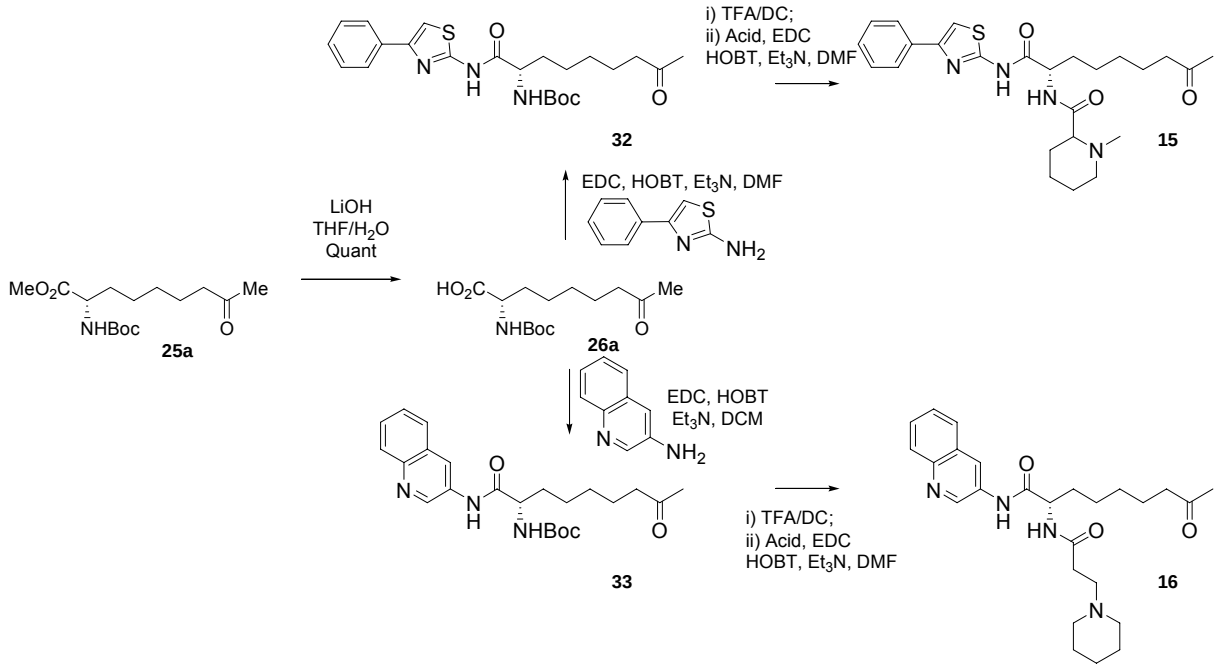
The reaction was carried out as described for **31a** using **21b** (19.7 g, 95 mmol) to yield the desired material (23.7 g, 98%) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.22 (2H, t, J = 7 Hz), 2.47-2.41 (4H, m), 1.86 (2H, app. q, J = 7 Hz), 1.62 (2H, app. quintet, J = 7 Hz), 1.43 (2H, app. quintet, J = 7 Hz), 1.08 (2H, t, J = 7 Hz),

2-Ethyl-2-(5-iodopentyl)-1,3-dioxolane **(22b)**

The reaction was carried out as described for **22a** using **31b** (23.68 g, 93 mmol) to yield the desired material (28.37 g, 98%) which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.85 (4H, br. s), 3.22 (2H, t, J = 7 Hz), 1.78 (2H, app. quin., J = 7 Hz), 1.58-1.45 (4H, m), 1.38-1.25 (4H, m), 0.83 (3H, t, J = 7 Hz).

Methyl (2*S*)-2-[(*tert*-butoxycarbonyl)amino]-8-oxodecanoate **(25b)**

The reaction was carried out as described for **25a** using **22b** (32.4 g, 0.11 mol.) to obtain the title compound (20.8 g, 61%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.98 (1H, br. d, J = 7.5 Hz), 4.28 (1H, br. q, J = 5.3 Hz), 3.74 (3H, s), 2.46-2.36 (4H, m), 1.84-1.73 (1H, m), 1.67-1.52 (5H, m), 1.45 (9H, s), 1.40-1.25 (4H, m). MS (ES) $C_{16}H_{29}NO_5$ requires: 315, found: 338 $(M+H)^+$. $[\alpha]^{20}_D$ = -10 (*c*=0.491, MeOH).



(2S)-2-[(*tert*-Butoxycarbonyl)amino]-8-oxononanoic acid (26a)

25a (5.20 g, 17.3 mmol) was dissolved in a mixture of THF and water (1:1, 300 mL) and LiOH hydrate (0.83 g, 34.6 mmol) was added and the mixture was stirred for 60 min. The mixture was cooled to 0 ^{o}C and quenched by addition of 0.5M HCl solution (70 mL). The organics were extracted with EtOAc (3 x 200 mL) and the combined EtOAc extracts were washed with brine (100 mL), dried (Na_2SO_4) and concentrated under reduced pressure to yield the desired compound (5.20 g, 99%) as a colourless oil. ^{1}H NMR (400 MHz, d6-DMSO) δ: 12.36 (1H, br. s), 7.02 (1H, d, J = 8.1 Hz), 3.88-3.74 (1H, m), 2.40 (1H, t, J = 7.3 Hz), 1.99 (3H, s), 1.67-1.48 (2H, m), 1.46-1.36 (2H, m), 1.38 (9H, s), 1.34-1.15 (4H, m). MS (ES) $C_{14}H_{25}NO_5$ requires: 287, found: 286 $(M-H)^-$.

***tert*-Butyl ((1*S*)-7-oxo-1-{[(4-phenyl-1,3-thiazol-2-yl)amino]carbonyl}octyl)carbamate (32)**

A mixture of **26a** (250 mg, 0.87 mmol), HOBt (147 mg, 0.96 mmol), EDCI (183 mg, 0.96 mmol) and Et_3N (0.30 mL, 2.2 mmol) in DMF (10 mL) was stirred at RT for 15 minutes and then 4-phenyl-1,3-thiazol-2-amine (307 mg, 1.74 mmol) was added. The resulting solution was left to stir at RT for 14 hours and was then concentrated under reduced pressure azeotroping with xylene (50 mL). The residue was dissolved in EtOAc (100 mL) and washed sequentially with 0.5 N HCl solution (2 x 50 mL), sat. aq. $NaHCO_3$ solution (50 mL) and brine (50 mL). The solution was dried (Na_2SO_4) and concentrated under reduced pressure. The oily residue was

purified by column chromatography on silica eluting with 30-35% EtOAc/Petroleum ether to yield the desired compound (44 mg, 11%) as a colourless oil ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.81 (1H, br. s), 7.82 (2H, d, J = 8.1 Hz), 7.42 (2H, app. t, J = 8 Hz), 7.33 (1H, t, J = 7.4 Hz), 7.15 (1H, s), 5.00 (1H, d, J = 7.4 Hz), 4.35 (1H, br. s), 2.43 (2H, t, J = 7.2 Hz), 2.14 (3H, s), 2.08-1.90 (1H, m), 1.72-1.10 (7H, m), 1.48 (9H, s). MS (ES) $C_{23}H_{31}N_3O_4S$ requires: 445, found: 446 $(M+H)^+$.

1-Methyl-2-{[((1S)-7-oxo-1-{[(4-phenyl-1,3-thiazol-2-yl)amino]carbonyl}octyl)amino] carbonyl}piperidinium trifluoroacetate (15)

A mixture of **32** (44 mg, 0.1 mmol) in DCM (4 mL) and TFA (1 mL) was stirred at RT for 90 min. The solvents were removed under reduced pressure, azeotroping with toluene (10 mL) to yield (2*S*)-1,8-dioxo-1-[(4-phenyl-1,3-thiazol-2-yl)amino]nonan-2-aminium trifluoro acetate. MS (ES) $C_{18}H_{23}N_3O_2S$ requires: 345, found: 346 $(M+H)^+$.

A mixture of 1-methylpiperidine-2-carboxylic acid hydrochloride (72 mg, 0.4 mmol), HOBT (61 mg, 0.4 mmol), EDCI (77 mg, 0.4 mmol) and Et_3N (70 uL, 0.5 mmol) in DMF (4 mL) was stirred at RT for 90 min and then added to a stirred solution of the amine.TFA salt in DMF (2 mL). The resulting solution was left to stir at RT for 12 h and was then concentrated under reduced pressure. The residue was taken up in EtOAc (50 mL), washed with sat. aq. $NaHCO_3$ solution (20 mL) and brine (20 mL), then dried and concentrated under reduced pressure. The crude was purified by RP-HPLC and the desired fractions lyophilized to yield the desired product (35 mg, 60%) as a white powder. UPLC purity: > 98 % by diode array. H NMR (300 MHz, d6-DMSO) δ: 12.54 (1H, br. s), 9.65 (1H, br. s), 9.05 (1H, d, J = 6.6 Hz), 7.89 (2H, d, J = 7.4 Hz), 7.64 (1H, s), 7.43 (2H, t, J = 7.4 Hz), 7.33 (1H, t, J = 7.4 Hz), 4.60-4.45 (1H, m), 3.85-3.65 (1H, m), 3.36 (1H, d, J = 11.5 Hz), 3.15-2.96 (1H, m), 2.70 (3H, s), 2.40 (2H, t, J = 7.2 Hz), 2.06 (3H, s), 1.90-1.15 (14H, m). MS (ES) $C_{25}H_{34}N_4O_3S$ requires: 470, found: 471 $(M+H)^+$. $[\alpha]^{20}_D$ = -16 (*c*=0.144, MeOH). HRMS $C_{25}H_{35}N_4O_3S$ requires: 471.2424, found: 471.2423 $(M+H)^+$.

***tert*-Butyl {(1*S*)-7-oxo-1-[(quinolin-3-ylamino)carbonyl]octyl}carbamate (33)**

A mixture of **26a** (50 mg, 0.17 mmol), HOBT (40 mg, 0.26 mmol), EDCI (50 mg, 0.26 mmol) in DCM (10 mL) was stirred at RT for 10 min and then a solution containing 3-aminoquinoline (38 mg, 0.26 mmol) in DCM (0.5 mL) was added to the mixture and left to stir O/N. The mixture was diluted with DCM (30 mL) and washed with 1 M HCl (10 mL), 1 M

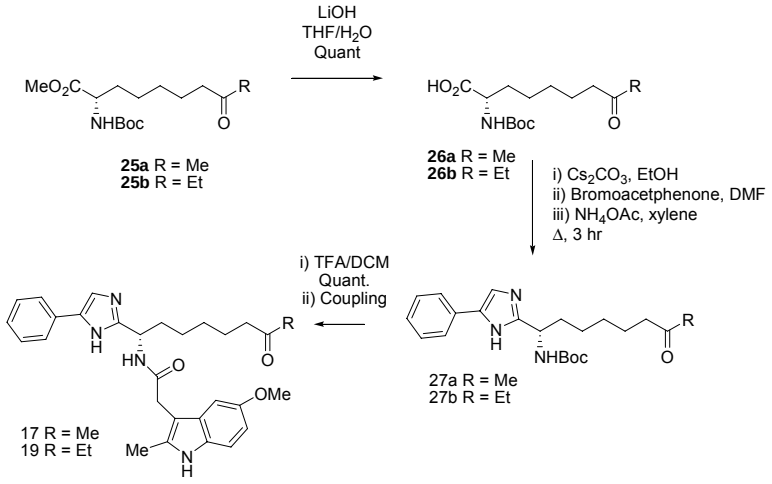
NaOH (10 mL) and brine (10 mL). The resulting solution was dried (Na_2SO_4) and concentrated under reduced pressure to yield **33** which was used directly in the next step without purification. MS (ES) $C_{23}H_{31}N_3O_4$ requires: 413, found: 414 $(M+H)^+$.

3-({(2*S*)-8-Oxo-2-[(3-piperidinium-1-ylpropanoyl)amino]nonanoyl}amino)quinolinium dichloride (16)

The crude **33** (0.174 mmol) was dissolved in a mixture of DCM:TFA (9:1, 10 mL) and stirred at RT for 1 h. The solvents were removed under reduced pressure to yield a residue which was used in the next step without purification. MS (ES) $C_{18}H_{23}N_3O_2$ requires: 313, found: 314 $(M+H)^+$.

The residue was dissolved in DMF (10 mL), 1-piperidine propionic acid (40 mg, 0.26 mmol), HOBT (40 mg, 0.26 mmol) and EDCI (50 mg, 0.26 mmol) were added, and then the reaction mixture was stirred at RT O/N. After concentrating the solvent, the residue was purified by reverse phase HPLC and the desired fractions were freeze dried to yield the desired product **2** as a TFA salt (30 mg, 25% over 3 steps).

The TFA salt was converted quantitatively into the free amine via IST ISOLUTE® SPE column SCX (loading in MeOH; eluting with 3N NH_3 in MeOH) and transformed directly into the corresponding chloride salt (37%, 0.053 mmol) by reducing under reduced pressure a solution of the free amine in HCl/CH_3CN solution. UPLC purity: 98 % by diode array. ^{1}H NMR (DMSO-d6, 500 MHz, 300K) δ: 9.12 (1H, br. s), 8.82 (1H, br. s), 8.60 (1H, d, J = 6.0 Hz), 8.04 (1H, d, J = 7.0 Hz), 8.00 (1H, d, J = 7.0 Hz), 7.76-7.68 (2H, m), 7.64 (1H, t, J = 6.0 Hz), 4.50-4.40 (1H, m), 3.40-3.30 (2H, m), 3.30-3.20 (2H, m), 2.90-2.80 (2H, m), 2.80-2.70 (2H, m), 2.40 (2H, t, J = 6.0 Hz), 2.05 (3H, s), 1.80-1.70 (5H, m), 1.70-1.60 (2H, m), 1.50-1.40 (2H, m), 1.40-1.30 (3H, m), 1.30-1.20 (2H, m). ^{13}C NMR (DMSO-d6, 125 MHz, 300K) δ: 209.3, 172.7, 170.1, 153.8, 146.4, 144.1, 133.7, 129.9, 128.9, 128.8, 128.6, 125.6, 54.7, 52.9, 52.8, 43.5, 32.5, 30.6, 30.2, 29.1, 26.2, 23.9, 23.3, 22.2, 19.9. MS (ES) $C_{26}H_{36}N_4O_3$ requires: 452, found: 453 $(M+H)^+$. $[\alpha]^{20}_D$ = -28 (c=0.14, MeOH). HRMS $C_{26}H_{37}N_4O_3$ requires: 453.2860, found: 453.2858 $(M+H)^+$.



tert-Butyl[(1*S*)-7-oxo-1-(5-phenyl-1*H*-imidazol-2-yl)octyl] carbamate (27a)

A mixture of **26a** (5.20 g, 17.3 mmol) and Cs_2CO_3 (2.81 g, 8.62 mmol) in EtOH (100 mL) was stirred for 30 min at RT and was then concentrated under reduced pressure. DMF (50 mL) was then added followed by 2-bromoacetophenone (3.44 g, 17.3 mmol) and the mixture was stirred for 1 h at RT under N_2. The DMF was removed by azeotroping with xylene (2 x 100 mL). EtOAc (100 mL) was added, the mixture was filtered and the residue was washed with more EtOAc (100 mL). The combined filtrates were concentrated under reduced pressure. A solution of the resulting oil and NH_4OAc (26.6 g, 0.35 mol) in xylene (200 mL) was heated at reflux for 90 min in the presence of a Deans Stark trap. The mixture was cooled to RT, diluted with EtOAc (500 mL) and washed with water (2 x 200 mL), sat. aq. $NaHCO_3$ solution (200 mL), more water (2 x 200 mL) and brine (200 mL). The solution was dried (Na_2SO_4), concentrated under reduced pressure. The resulting brown oil was purified by chromatography on silica gel eluting with 60-70% EtOAc/petroleum ether to obtain the imidazole (4.82 g, 72%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) (mixture of rotamers) δ: 10.70 (0.35H, br. s), 10.25 (0.65H, br. s), 7.76 (0.65H, br. s), 7.43 (0.35 H, br. s), 7.38 (2H, t, J = 8 Hz), 7.25-7.10 (2H, m), 5.32 (1H, br. s), 4.65 (1H, br. s), 2.39 (2H, t, J = 7.2 Hz), 2.20-2.06 (1H, m), 2.10 (3H, s), 1.99-1.80 (1H, m), 1.60-1.50 (2H, m), 1.43 (9H, s), 1.40-1.27 (4H, m). MS (ES) $C_{22}H_{31}N_3O_3$ requires: 385, found: 386 $(M+H)^+$.

2-((1*S*)-1-{[(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetyl]amino}-7-oxooctyl)-5-phenyl-1*H*-imidazol-1-ium chloride (17)

A mixture of **27a** (4.82 g, 12.5 mmol) in DCM (50 mL) and TFA (20 mL) was stirred at RT for 2 hours. The solvents were removed under reduced pressure, azeotroping with toluene (100 mL). The residue was taken up in EtOAc (400 mL) and washed with 0.3 M NaOH solution (2 x 150 mL) and brine (100 mL). The solvent was removed under reduced pressure to yield (8*S*)-8-amino-8-(5-phenyl-1*H*-imidazol-2-yl)octan-2-one (3.24 g, 91%). MS (ES) $C_{17}H_{23}N_3O$ requires: 285, found: 286 $(M+H)^+$.

A mixture of (5-methoxy-2-methyl-1*H*-indol-3-yl)acetic acid (2.75 g, 12.5 mmol), HOBt (1.69 g, 12.5 mmol), EDCI (2.39 g, 12.5 mmol) and Et_3N (3.49 mL, 25 mmol) in DMF (75 mL) was stirred at RT for 15 minutes and was then added to the above amine. The resulting solution was stirred at RT for 2 hours and was then concentrated under reduced pressure azeotroping with xylene (2 x 100 mL). The residue was taken up in EtOAc (400 mL) and was washed with sat. aq. $NaHCO_3$ solution (2 x 150 mL), water (2 x 150 mL) and brine (100 mL). The solution was dried (Na_2SO_4), and then concentrated under reduced pressure. The resulting oil was purified by chromatography on silica gel eluting with 90-100% EtOAc/petroleum ether to obtain the imidazole (4.90 g, 81%). This material was dissolved in MeCN (100 mL) and 0.1 M HCl solution (100 mL) was added, the mixture was then frozen and lyophilized to obtain the desired HCl salt as a white solid. UPLC purity: > 98 % by diode array. ^{1}H NMR (400 MHz, d6-DMSO) δ: 15.3-14.2 (2H, br. s), 10.63 (1H, s), 8.82 (1H, d, J = 7.3 Hz)m 8.06 (1H, s), 7.86 (1H, d, J = 7.3 Hz), 7.51 (2H, t, J = 7.3 Hz), 7.43 (1H, t, J = 7.3 Hz), 7.08 (1H, d, J = 8.6 Hz), 7.04 (1H, d, J = 2.2 Hz), 6.58 (1H, dd, J = 8.5, 2.2 Hz), 5.10 (1H, q, J = 7.6 Hz), 3.69 (3 H, s), 3.59 (1H, d, J = 15.1 Hz), 3.49 (1H, d, J = 15.1 Hz), 2.35-2.25 (5H, m), 2.03 (3H, s), 1.97-1.85 (2H, m), 1.40-1.25 (3H, m), 1.22-1.08 (3H, m). ^{13}C NMR (75 MHz, d6-DMSO) δ: 11.53, 22.85, 24.97, 27.78, 29.56, 31.33, 32.78, 42.45, 45.86, 55.38, 100.32, 104.28, 109.47, 110.75, 114.76, 125.36, 126.97, 128.71, 129.05, 130.05, 132.50, 133.87, 149.02, 152.98, 171.38, 208.29. MS (ES) $C_{29}H_{34}N_4O_3$ requires: 486, found: 487 $(M+H)^+$. $[\alpha]^{20}_D$ = -16 (*c*=0.268, MeOH). HRMS $C_{29}H_{35}N_4O_3$ requires: 487.2704, found: 487.2698 $(M+H)^+$. Chiral SFC: chiralcel AS-H (1 x 25 cm) column, 10 mL/min, T_{col} = 35°C, P_{col} = 100 bar, modifier MeOH+0.2% DEA, gradient 20% for 1 min increasing to 60% over 5.71 min then constant for 2 min. *S*-enantiomer T = 4.23 min. *R*-enantiomer T = 4.53 min, 84%ee.

(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-8-oxodecanoic acid (26b)

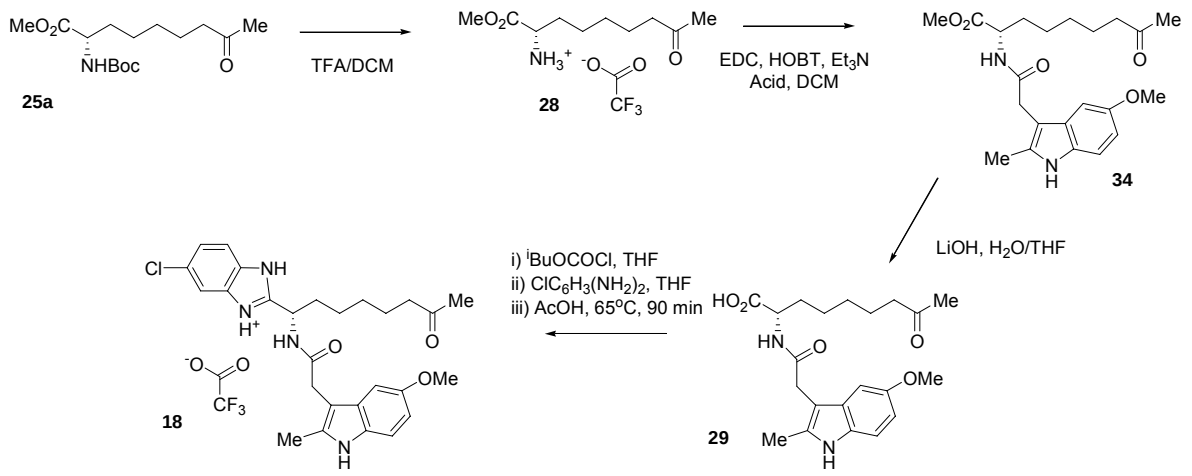
The reaction was carried out as described for **26a** using **25b** (1.05 g, 3.33 mmol) to yield the desired compound (1 g, 99%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) (mixture of rotamers) δ: 5.88 (0.22 H, br. s), 5.01 (0.78 H, m), 4.28 (0.78 H, m), 4.12 (0.22 H, br. s), 2.46-2.36 (4H, m), 1.91-1.76 (1H, m), 1.73-1.52 (3H, m), 1.50-1.20 (13H, m), 1.04 (3H, t, J = 7.3 Hz). MS (ES) $C_{15}H_{27}NO_5$ requires: 301, found: 302 $(M+H)^+$.

***tert*-Butyl [(1*S*)-7-oxo-1-(5-phenyl-1*H*-imidazol-2-yl)nonyl]carbamate (27b)**

The reaction was carried out as described for **27a** using **26b** (0.2 g, 0.66 mmol) to yield the desired compound (0.2 g, 75%) as a pale yellow oil. ^{1}H NMR (300 MHz, d6-DMSO) δ: 12.28-11.55 (1H, m), 7.80-7.62 (2H, m), 7.43 (1H, s), 7.35 (2H, m), 7.22-7.10 (1H, m), 7.09-6.94 (1H, m), 4.68-4.42 (1H, m), 2.44-2.32 (4H, m), 1.90-1.61 (2H, m), 1.54-1.32 (11H, m), 1.31-1.15 (4H, m), 0.90 (3H, t, J = 7.3 Hz). MS (ES) $C_{23}H_{33}N_3O_3$ requires: 399, found: 400 $(M+H)^+$.

2-((1*S*)-1-{[(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetyl]amino}-7-oxononyl)-5-phenyl-1*H*-imidazol-1-ium trifluoroacetate (19)

The reaction was carried out as described for **17** using **27b** (0.045 g, 0.15 mmol) and purified preparative RP-HPLC, using water (0.1% TFA) and MeCN (0.1% TFA) as eluents (column: C18) and the desired product fractions were lyophilized to afford the titled compound (0.018 g, 19%) as a pale yellow solid. UPLC purity: > 98 % by diode array. ^{1}H NMR (400 MHz, d6-DMSO) δ: 15.2-13.7 (2H, br. s), 10.62 (1H, s), 8.60 (1H, d, J = 6.2 Hz), 8.03 (1H, s), 7.77 (1H, d, J = 7.3 Hz), 7.57-7.38 (3H, m), 7.10 (1H, d, J = 8.6 Hz), 6.97 (1H, m), 6.65-6.56 (1H, m), 5.01 (1H, q, J = 7.4 Hz), 3.68 (3H, s), 3.55-3.41 (2H, m, partially hidden by water), 2.43-2.26 (7H, m), 1.98-1.80 (2H, m), 1.48-1.12 (6H, m), 0.90 (3H, t, J = 7.2 Hz). ^{13}C NMR (150 MHz, CD_3OD) δ: 6.88, 10.39, 23.31, 25.46, 28.29 (broad 2xC), 31.63, 32.43, 35.33, 41.52, 55.18, 100.19, 103.52, 110.05, 110.94, 115.00, 125.40, 125.48, 128.86, 128.89, 129.07, 131.17, 134.45, 148.98, 154.03, 174.15, 213.19. MS (ES) $C_{30}H_{36}N_4O_3$ requires: 500, found: 501 $(M+H)^+$. $[\alpha]^{20}_D$ = -14 (*c*=0.1, MeOH). HRMS $C_{30}H_{37}N_4O_3$ requires: 501.2860, found: 501.2850 $(M+H)^+$.



(2*S*)-1-Methoxy-1,8-dioxononan-2-aminium trifluoroacetate (28)

To a stirred solution of **25a** (1.0 g, 3.32 mmol) in DCM (10 mL) at 0°C was added TFA (10 mL), the mixture was stirred for 2 h at RT and then the solvents were removed under reduced pressure. The remaining oil was left under high vacuum (0.3 mbar) for 2h then used in the next step without further purification. ^{1}H NMR (300 MHz, d6-DMSO) δ: 8.35 (3H, s), 4.11-3.95 (1H, m), 3.76 (3H, s), 2.41 (2H, t, J=7.1 Hz), 2.07 (3H, s), 1.83-1.64 (2H, m), 1.49-1.12 (6H, m), MS (ES) $C_{10}H_{19}NO_3$ requires: 201, found: 202 $(M+H)^+$.

Methyl (2*S*)-2-{[(5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl]amino}-8-oxononanoate (34)

To a stirred solution of **28** (360 mg, 1.14 mmol) and Et_3N (0.16 mL, 1.14 mmol) in DCM (5 mL) was added a solution of EDC (263 mg, 1.37 mmol), HOBT (185 mg, 1.37 mmol) and (5-methoxy-2-methyl-1*H*-indol-3-yl)acetic acid (300 mg, 1.2 mmol) in DCM (10 mL). The resulting mixture was stirred for 1.5 h at RT, then diluted with DCM and washed sequentially with sat. aq. $NaHCO_3$, 0.1 M HCl and brine. The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by chromatography on silica gel eluting with 30-70% EtOAc/petroleum ether to obtain the title compound as a colourless oil (180 mg, 39%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.89 (1H, s), 7.17 (1H, d, J = 8.6 Hz), 6.89 (1H, d, J = 2.2 Hz), 6.77 (1H, dd, J = 8.6, 2.2 Hz), 6.04 (1H, d, J = 8.2 Hz), 4.60-4.46 (1H, m), 3.81 (3H, s), 3.63 (5H, s), 2.38 (3H, s), 2.28 (2H, t, J = 7.3Hz), 2.08 (3H, s), 1.76-1.58 (1H, m), 1.52-1.31 (3H, m), 1.20-1.00 (4H, m), MS (ES) $C_{22}H_{30}N_2O_5$ requires: 402, found: 403 $(M+H)^+$.

(2*S*)-2-{[(5-Methoxy-2-methyl-1*H*-indol-3-yl)acetyl]amino}-8-oxononanoic acid (29)

To a stirred solution of **34** (180 mg, 0.45 mmol) in a mixture of water and THF (2 mL, 1:1) was added LiOH (42 mg, 0.49 mmol). Stirring was continued for 80 min and then the mixture was partitioned between 0.1 M HCl (10 mL) and DCM (10 mL). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford the title compound as a colourless oil (160 mg, 92%). ^{1}H NMR (300 MHz, CDCl) δ: 8.03 (1H, s), 7.16 (1H, d, J = 8.6 Hz), 6.83 (1H, d, J = 2.2 Hz), 6.77 (1H, dd, J = 8.6, 2.2 Hz), 6.10 (1H, d, J = 7.5 Hz), 4.51-4.48 (1H, m), 3.79 (3H, s), 3.65 (2H, s), 2.35 (3H, s), 2.29 (2H, t, J = 7.3Hz), 2.18 (3H, s), 1.78-1.62 (1H, m), 1.58-1.31 (3H, m), 1.20-1.00 (4H, m). MS (ES) $C_{21}H_{28}N_2O_5$ requires: 388, found: 389 $(M+H)^+$.

5-Chloro-2-((1*S*)-1-{[(5-methoxy-2-methyl-1*H*-indol-3-yl)acetyl]amino}-7-oxooctyl)-1*H*-benzimidazol-3-ium trifluoroacetate (18)

To a stirred solution of **29** (80 mg, 0.21 mmol) in anhydrous THF (1 mL) at -10°C was added ^{i}BuOCOCl (27 µL, 0.21 mmol), stirring was continued at -10 °C for 10 min and then a solution of 4-chlorobenzene-1,2-diamine (29 mg, 0.21 mmol) in anhydrous THF (0.5 mL) was added. The mixture was allowed to warm to RT and stirring was continued for a further hour. The solvent was removed under reduced pressure and the residue was partitioned between water and EtOAc. The organic phase was washed with 5% $NaHCO_3$ sol., dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oily residue was dissolved in AcOH (4 mL) and the solution was warmed to 65 °C for 90 min with stirring. After cooling to RT, the solvent was removed under reduced pressure and the product was purified by preparative RP-HPLC (C18), using water (0.1% TFA) and MeCN (0.1% TFA) as eluents. The pooled product fractions were lyophilized to afford the title compound as a white amorphous solid (7 mg, 6%). UPLC purity: 98 % by diode array. ^{1}H NMR (300 MHz, CD_3CN) δ: 8.92 (1H, s), 7.75-7.58 (2H, m), 7.56 (1H, d, J = 8.6Hz), 7.33 (1H, d, J = 8.6 Hz), 7.15 (1H, d, J = 8.6Hz), 6.92 (1H, d, J = 2.0 Hz), 6.66 (1H, dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz), 5.23-5.11 (1H, m), 3.70 (3H, s), 3.67-3.50 (2H, m), 2.34 (3H, s), 2.30 (2H, t, J = 7.3 Hz), 2.02 (3H, s), 1.95-1.80 (2H, m), 1.44-1.12 (6H, m), ^{13}C NMR (DMSO-d6, 100 MHz, 300K) δ: 208.3, 171.3, 158.4, 158.1, 156.7, 152.9, 133.8, 130.1, 128.7, 127.9, 123.9, 115.7, 114.3, 110.7, 109.4, 104.5, 100.3, 55.3, 47.0, 42.5, 32.4, 31.3, 29.6, 28.0, 25.1, 22.9, 11.5. MS (ES) $C_{27}H_{31}ClN_4O_3$ requires: 494/496, found: 495/497 $(M+H)^+$, $[\alpha]_D$ = -5° (c=0.48, EtOH). HRMS $C_{27}H_{32}{}^{35}ClN_4O_3$ requires 495.2157, found: 495.2152 $(M+H)^+$.

HPLC analysis of the final compounds

The stationary phases used were: Acquity UPLC™ BEH C18 1.7 μm, 2.1 * 50 mm column with mobile phase comprised a linear gradient of binary mixtures of H_2O containing 0.1% formic acid (solvent A) and MeCN containing 0.1% formic acid (solvent B), flow 0.5 mL/min.

Method A: 10% solvent B (0.1 min) to 100% solvent B over 2.6 min, then isocratic.

Method B: 5% solvent B (0.1 min) to 50% solvent B over 2.1 min, then isocratic.

Compound	Method A	Method B
15	1.26	1.83
16	1.06	1.58
17	1.14	1.65
18	1.44	2.08
19	1.40	2.20

HDAC Enzyme Assays

HDAC 1, 2, and 3 Assays

Working Reagents:

HDAC1+2: *C*-terminally Flag tagged HDAC1/2 was obtained and purified from mammalian cells

HDAC3: *C*-terminally Flag tagged HDAC3 and DAD co-activator complex were obtained and purified from mammalian cells

TSA Stock: TSA is provided as a 10mM stock solution in 100% dimethylsulfoxide (DMSO).

Assay Buffer: 25mM Tris/HCl pH8, 137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$, 0.1mg/ml BSA

Diluted Substrate Solution: The commercial 50mM Fluor-de-Lys substrate (KI-104) is diluted to 150uM with HDAC Assay Buffer prior to each use. The final concentration in the assay is 30uM.

Diluted Developer Solution: The commercial 20X Developer Concentrate (KI-105) is diluted 1:167 into HDAC Assay Buffer. 2uM [final] TSA to this solution increases its ability to stop the reaction.

HDAC-X Working Solution: The HDAC-X enzyme is diluted in assay buffer prior to each use from a fresh aliquot of enzyme. The final concentration in the assay is 1-2 nM.

Experimental Design:

The reaction is performed in 96-well microplate in a final volume of 50ul/well, as following:

- Add 5ul of DMSO/compound solution

- Add 35ul of HDAC1+3 or 40 uL of HDAC2 in buffer (or 35/40ul assay buffer in the controls)
- Incubate 10' at room temperature
- Start the reaction by adding 10ul (HDAC1+3) or 5uL (HDAC2) of the 150uM substrate solution
- Incubate 1h at 37°C
- Stop by adding 50ul of Developer/4uM TSA solution
- Incubate 10 min at room temperature
- Measure the fluorescence at Ex.360nM and Em.460nM

HDAC 4WT, 5, 7 and 8 assays

Working Reagents:

His-tagged HDAC catalytic domains were expressed in *E.coli* and purified by Nickel-Chelation affinity chromatography, anion exchange (MonoQ) chromatography and gel filtration

HDAC 4WT - T653-L1084; HDAC 5WT - T678-L1122; HDAC 7WT – T515-L952

TSA Stock: TSA is provided as a 10mM solution in 100% DMSO.

Assay buffer: 25mM Tris/HCl pH8, 137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$, 0.1mg/ml BSA (For HDACs5+7 also add 0.2% N-Octyl Glucoside)

Diluted substrate solution: *tert*-butyl {(1*S*)-1-{[(4-methyl-2-oxo-2*H*-chromen-7-yl)amino]carbonyl}-5-[(trifluoroacetyl)amino]pentyl}carbamate is diluted to 200µM with Tris 1mM pH 7.4 prior to each use. The final concentration in the assay is 20µM.

Diluted developer solution: The commercial 20X developer concentrate (KI-105, BioMol Research Laboratories) is diluted 1:167 into Tris 1mM pH7.4. 2µM [final] TSA to this solution increases its ability to stop the reaction.

Enzyme working solution: Enzyme is diluted in 1.25x assay buffer prior to each use from a fresh aliquot of enzyme. The final concentration in the assay is 0.2 nM (HDAC4WT) or 0.3 nM (HDAC5+7).

Experimental Design:

The reaction is performed in 96-well microplate in a final volume of 50µl/well.

- Add 5µl of DMSO/compound solution
- Add 40µl of HDAC-X enzyme in assay buffer
- Incubate 10' at RT.
- Start the reaction by adding 5µl of the 200µM substrate solution
- Incubate 1 h at 37°C.
- Stop the reaction by adding 50µl of developer/4µM TSA solution
- Incubate 30 min at RT.
- Measure the fluorescence at ex.360nM and em.460nM.

HDAC 4GOF ('gain of function') and 6 assays

Working Reagents:

HDAC6: *C*-terminally Flag tagged HDAC6 was obtained and purified from mammalian cells

HDAC4GOF: His-tagged HDAC GOF ('gain of function') (H976Y) catalytic domains (T653-L1084) was expressed in *E.coli* and purified by Nickel-Chelation affinity chromatography and anion exchange (MonoQ) chromatography

TSA stock: TSA is provided as a 10mM stock solution in 100% DMSO.

Assay buffer: 20mM Hepes pH 7.5, 137mM NaCl, 2.7mM KCl, 1 mM $MgCl_2$, 0.1mg/ml BSA

Diluted substrate solution: The 50mM Fluor-de-Lys™ substrate (KI-104, BioMol Research Laboratories) is diluted to 150µM (HDAC6) or 250µM (HDAC4GOF) with HDAC assay buffer prior to each use. The final concentration in the assay is 30µM (HDAC6) or 25µM (HDAC4GOF).

Diluted developer solution: The commercial 20X developer concentrate (KI-105, BioMol Research Laboratories) is diluted 1:167 into HDAC assay buffer. 2µM [final] TSA to this solution increases its ability to stop the reaction.

HDAC 6 working solution: The HDAC 6 enzyme is diluted in assay buffer prior to each use from a fresh aliquot of enzyme. The final concentration in the assay is 1-2 nM.

Experimental Design:

The reaction is performed in 96-well microplate in a final volume of 50µl/well.

- Add 5µl of DMSO/compound solution
- Add 35µl HDAC 6 or 40µl HDAC4GOF in buffer (or 35/40µl assay buffer in controls)
- Incubate 10' at RT.
- Start the reaction by adding 10µl of 150 µM (for HDAC6) or 5µl of 250 µM (HDAC4GOF) substrate solution
- Incubate for 1 h at 37°C.
- Stop the reaction by adding 50µl of developer/4µM TSA solution
- Incubate 10 min at RT.
- Measure the fluorescence at ex.360nM and em.460nM.

HDAC 8 Assay

Working Reagents:

HDAC8: *C*-terminally His tagged HDAC8 was obtained and purified from *E. coli* and purified by Nickel-Chelation affinity chromatography, anion exchange (MonoQ) chromatography and gel filtration

TSA Stock: TSA is provided as a 10mM stock solution in 100% dimethylsulfoxide (DMSO).

Assay Buffer: 25mM Tris/HCl pH8, 137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$, 0.1mg/ml BSA

Diluted Substrate Solution: The commercial 5mM Fluor-de-Lys substrate (KI-1178) is diluted to 1mM with Tris 1mM pH7.4. The final concentration in the assay is 100uM.

Diluted Developer Solution: The commercial 20X Developer Concentrate (KI-176) is diluted at 1X into Tris 1mM pH7.4. 2uM [final] TSA to this solution increases its ability to stop the reaction.

HDAC8 Working Solution: The HDAC-X enzyme is diluted in 1.25x assay buffer prior to each use from a fresh aliquot of enzyme. The final concentration in the assay is 25-30 nM.

Experimental Design:

The reaction is performed in 96-well microplate in a final volume of 50ul/well, as following:

- Add 5ul of DMSO/compound solution
- Add 40ul of HDAC8 or 40ul assay buffer in the controls
- Incubate 10' at room temperature
- Start the reaction by adding 5ul of the 1mM substrate solution
- Incubate 1 h at 37°C
- Stop by adding 50ul of Developer/4uM TSA solution
- Incubate 10 min at room temperature
- Measure the fluorescence at Ex.360nM and Em.460nM

Protocols for cell proliferation assays

Cell viability is estimated using CellTiter-Blue Cell Viability Assay according to the manufacturer instructions (Promega). The assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resofurin) by the cellular dehydrogenases. The amount of resofurin produced is directly proportional to the cell number in a range of cell line.

HeLa (cervical cancer, P53-): 5000 cells/well. Growth Media: DMEM with 0.11G/L Pyr.*

A549 (lung cancer, P53+): 5000 cells/well. Growth Media: Nutrient Mixture F-12 Kaighn's Modification liquid with L-Glutamine.*

HCT116 (colon carcinoma, P53+): 5000 cells/well. Growth Media: McCoy's 5A Medium with L-Glutamine, without Serum.*

G401 (kidney cancer): 10000 cells/well. Growth Media: McCoy's 5A Medium with L-Glutamine, without Serum.*

A2780 (ovarian cancer): 10000 cells/well. Growth Media: RPMI 1640 + 1.25 mL Insulin 4mg/mL.*

Human Renal Epithelia Cells: 6000 cells/well. Growth Media: REGM BulletKit (supplied from Lonza, CC-3190) which contains REBM (Renal Epithelial Basal Medium) and REGM SingleQuots (supplements and growth factors: hydrocortisone, hEGF, FBS, epinephrine, insulin, triiodothyronine, trensferrin and gentamicin/amphotericin-B).

* The medium was complemented with 10% FCS, 1% Penicillin-Streptomycin (10mg/ml) and 1% L-Glutamine 200 mM (100x).

Protocol

- Seed cells in a 96-well microplates in 100 ul complete DMEM
- Incubate 4 h at 37°C, 5% CO_2
- Add 25ul/well of the Sample/DMSO 5X solution in DMEM complete
- Incubate at 37°C, 5% CO_2 up to 72 h
- Add 20µl Celltiter Blue solution to 125µl cell suspension or monolayer in each well
- Incubate the mixture for 1 h in a humidified incubator in 5% CO_2 at 37^{o}C
- Read plate @ ex:550/20nm, em:590/20 nm on Fusion Alpha microplate reader (Packard Bioscience)

Protocol for plasma stability

Plasma stability was performed in plasma of different species: rat, dog, mouse and human. Plasma was spiked with the compound (5µM) from a stock 10 mM DMSO solution (final%DMSO = 0.5%) and incubated at 37°C. Aliquots (50 µL) were removed and quenched with MeCN (200 µL) at 0, 15, 30min and at 1, 2, 4 and 24h. After mixing, the samples were centrifuged (3000 rpm, 15 min at 4°C) and the supernatant transferred and dried under N_2. The samples were reconstituted in H_2O/MeCN (150 µL, 90/10) and then injected directly onto the LC/MS/MS system to monitor the degradation of the substrate.

LC/MS/MS conditions: HPLC was performed using an AGILENT HP1100 equipped with a CTC Analytics PAL autosampler (HTS PAL). Chromatography was performed on an ACE C_{18} Column (5µm particle, 4.6 mm x 5 cm) The gradient consisted of two mobile phases, A and B. (Mobile phase A: 0.1% formic acid in H_2O. Mobile phase B: 0.1% formic acid in MeCN). Mobile phase B was ramped from 5 to 95% between 0.0 and 2.0 min and then maintained at this concentration for a further min, followed by reduction to 5% between 3.0 and 3.1 min and then held constant for 90 sec. The flow rate = 1.5 mL/min with injection volume = 10 µL. LC/MS/MS

detection was performed using a Sciex API 3000 triple quadrupole mass spectrometer with a Turbo Ionspray ionization source operated in the positive ion mode. The spray voltage was 5500V with source temperature = 550°C. The peak areas were determined using Analyst Quantitation Wizard software version 1.4 and the computer control system was Analyst version 1.4.

Protocol rat PK studies

Rats Male Sprague-Dawley rats (250-350 g), were used for the absorption disposition studies. In each rat, an indwelling cannula was implanted in the right jugular vein for blood sampling. The surgery was performed under light anaesthesia (Ketamine-Xilazine (85 mg/kg and 2.5 mg/kg respectively i.m.) one day prior the experiment. During the kinetic study, all animals were housed individually in plastic metabolism cages, and were unrestrained throughout the experiment. Compounds (6 mg/mL) were dissolved in 20% DMSO/60% PEG400/20% Water for intravenous administration, and dissolved or suspended in 1% Methylcellulose for oral administration (2 mg/ml). After an overnight fast, the rats received an i.v. (via caudal vein) or an oral dose of compound. Blood samples were collected at different times point after dosing. Plasma was separated immediately after blood sampling by centrifugation, and the plasma samples were kept frozen (-20°C) until assayed by LC/MS/MS.

Analytical Procedures Plasma samples were extracted using Liquid Handling Robot MultiProbe Packard by protein precipitation with acetonitrile. Then the samples were centrifuged (3000 rpm x 15 min.at 4°C) and the supernatant transferred and dried under nitrogen. The samples were reconstituted in Water/Acetonitrile 90/10 and then injected directly into an HPLC column. Sample analyses were performed using a an API 3000 or / and API 2000 or / and API 4000 Mass Spectrometer interfaced via the Turbo Ion Spray (ESI)/APCI to an LC system consisting of an HTS PAL CTC autosampler and an Agilen HP 1100 Binary Pump. The results are calculated using Analyst Software linear regression with 1/x*x weighting. The Assay Precision was calculated for the Quality Controls by Watson Lims database.

Pharmacokinetic Analysis The plasma clearance (CLp) of compounds were calculated (using Watson PK program) as the dose divided by the area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$). The apparent half-life was estimated from the slope of the terminal phase of the log plasma concentration-time data. The volume of distribution (Vdss) was

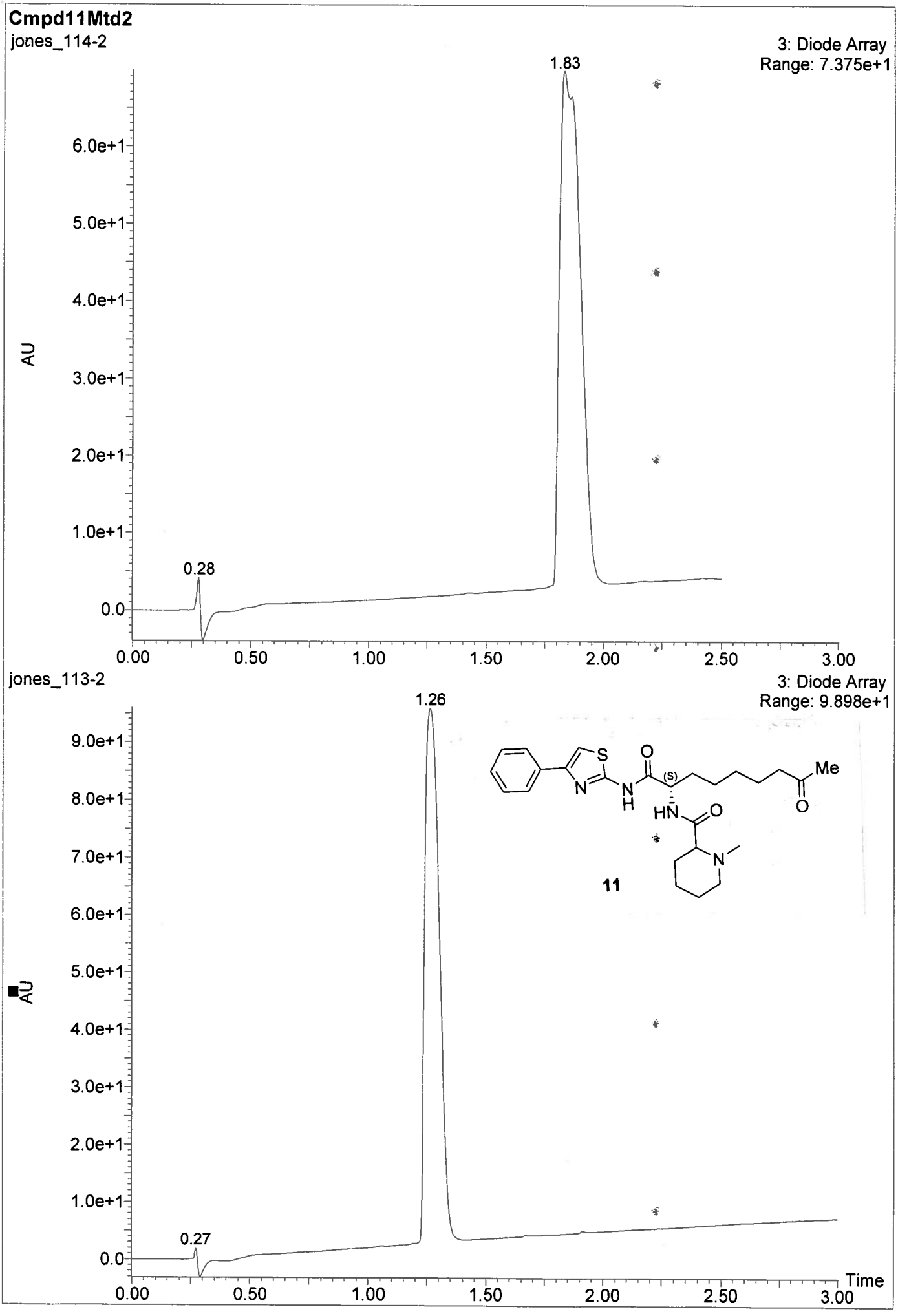
determined using the following noncompartmental method: Vdss = (Dose IV x AUMC)/($AUC_{0-\infty}$)2 where AUMC is the total area under the first moment of the drug concentrationtime curve from time zero to infinity. Bioavailability was estimated as the $AUC_{0-\infty}$ ratio following oral and intravenous administration, normalized for differences in dose.

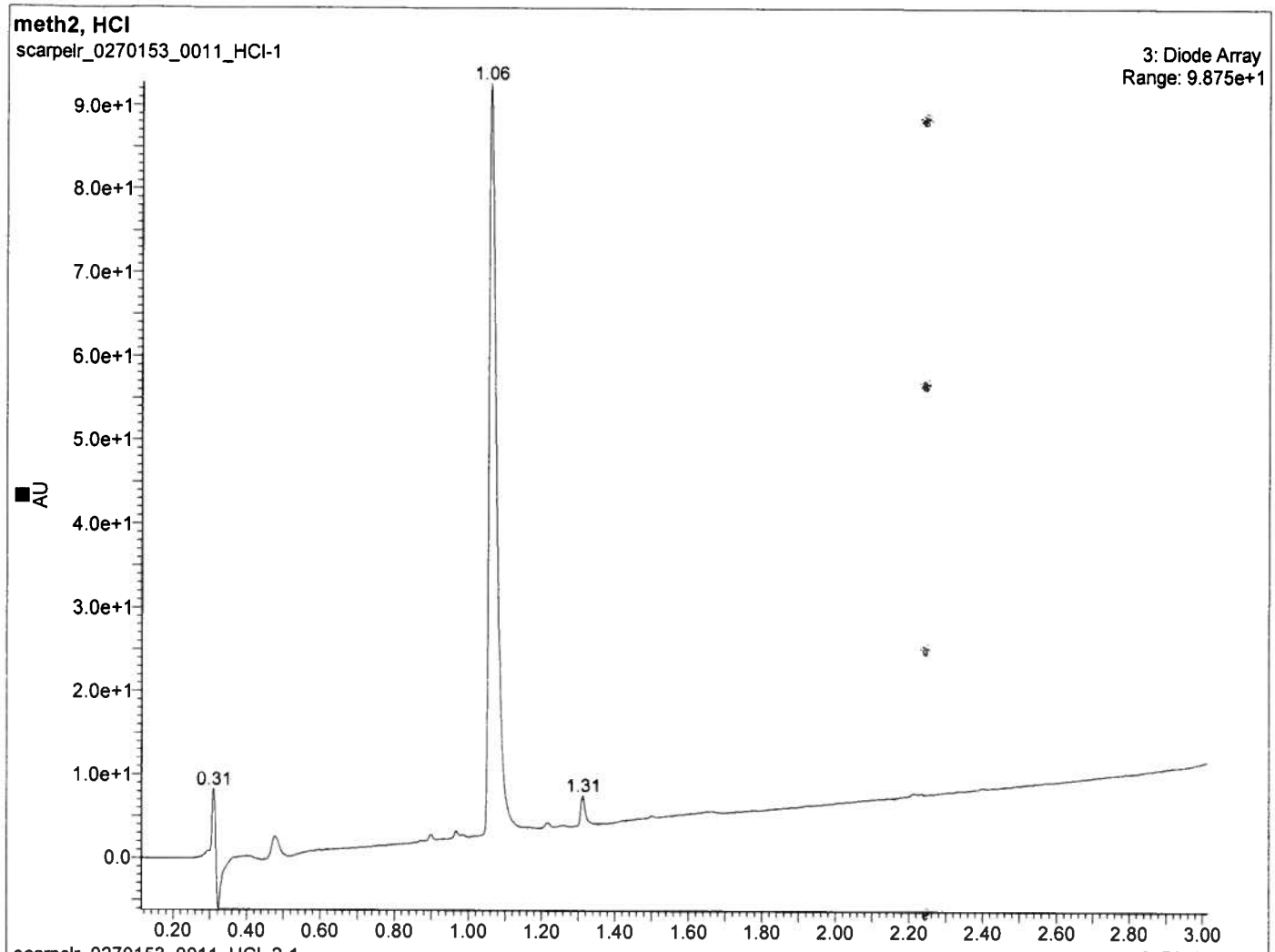
Protocol for tumor xenograft studies

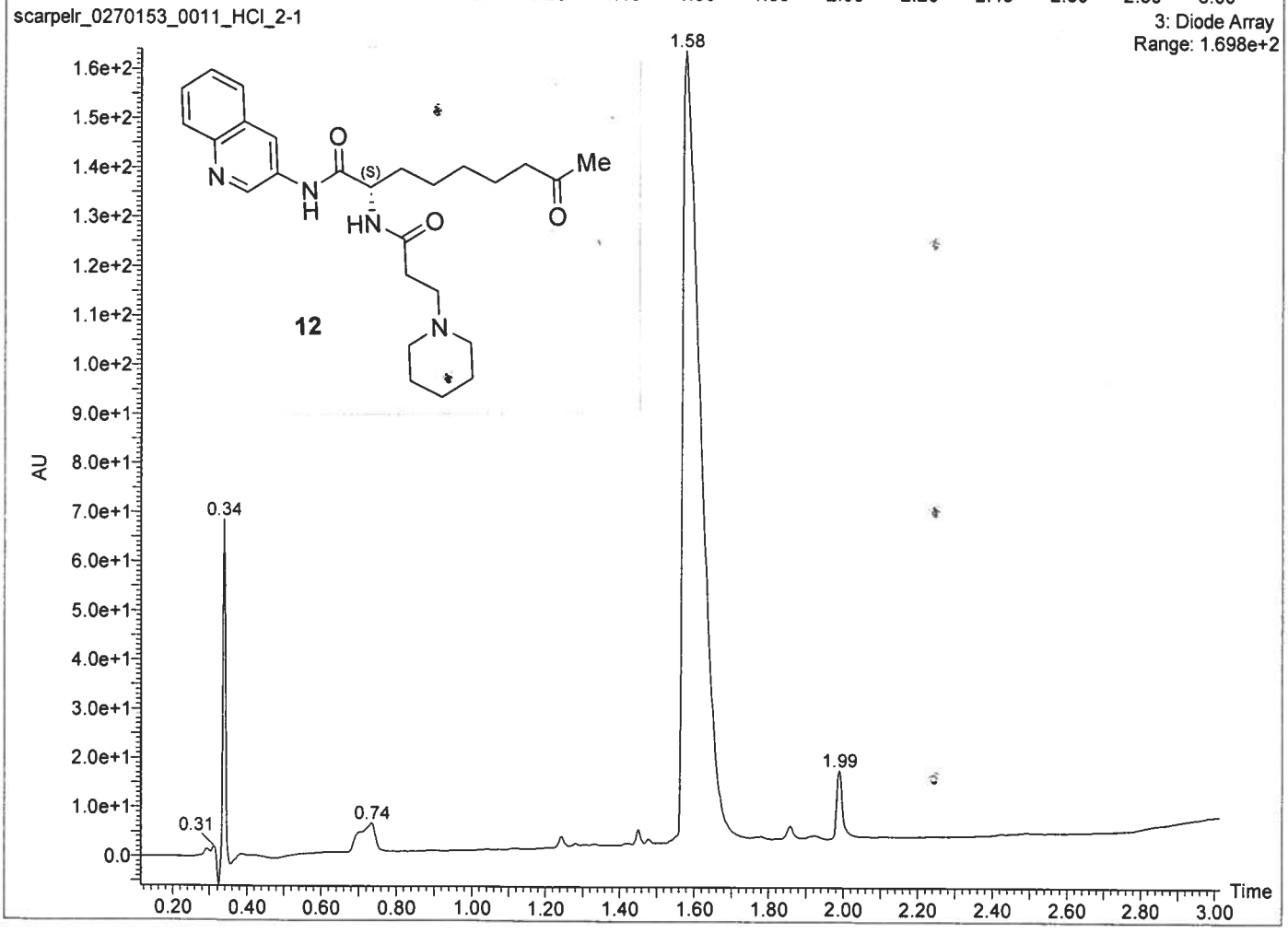
Cell Culture: Human colon adenocarcinoma HCT116 cells were purchased from ATCC. The cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 2 mM L-glutamine, penicillin 100 U/mL, and streptomycin 100 mg/mL. Cultures were maintained at 37°C in 5% CO_2.

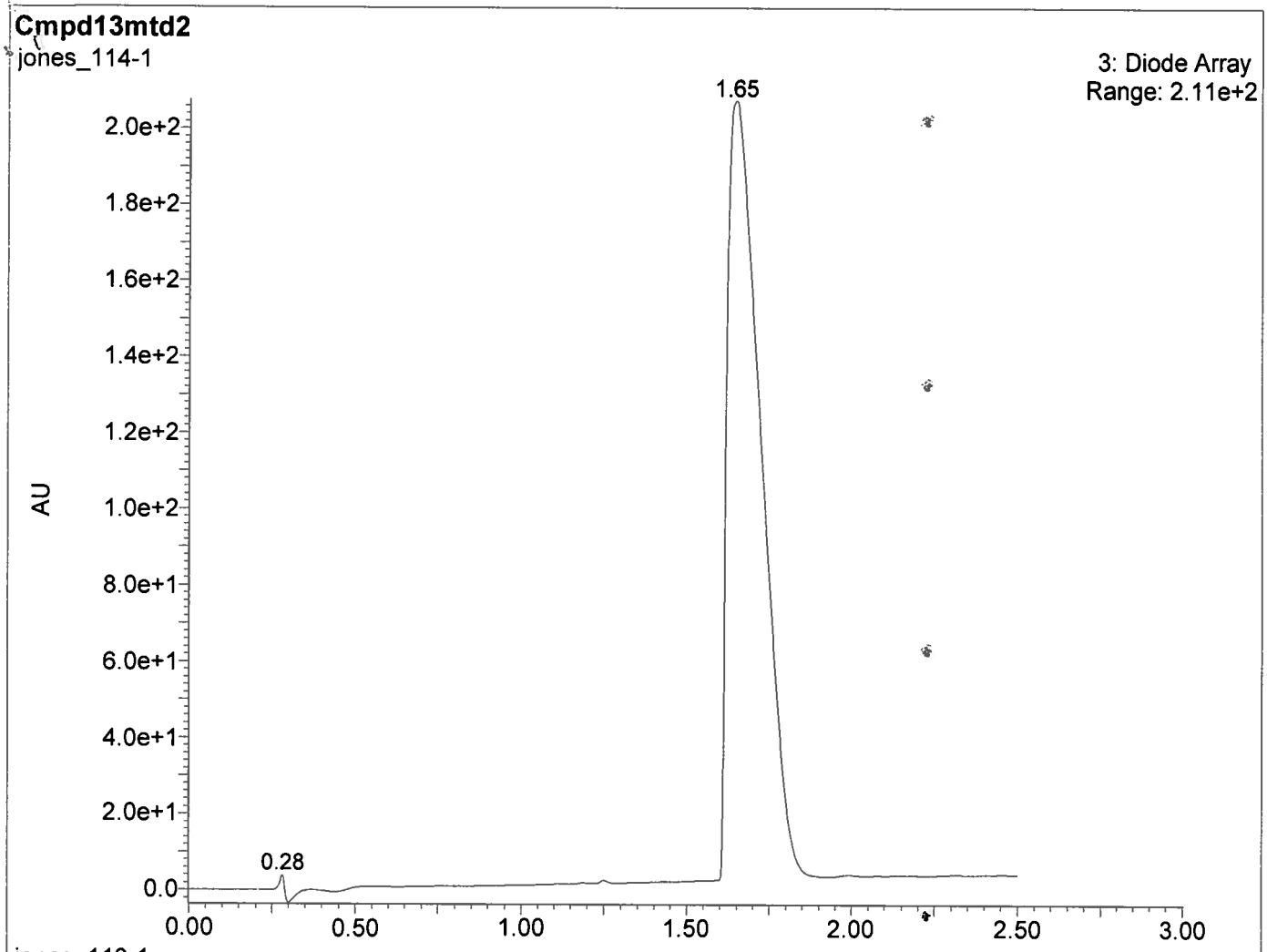
***In Vivo* Antitumor Activity:** HCT116 cell ($4x10^6$ cells from *in vitro* cultures) were xenografted s.c. into the right subaxillary region of Female BALB/c-nu/nu nude 8-10 weeks old mice (Charles River, Calco, Italy). Animals were maintained under a 12 h light/dark cycle with free access to food and water. The animals were housed and handled in compliance with European Union directive 86/609/EEC and Italian law DL 116/92 for the protection of vertebrate animals used for experimental and other scientific purposes. All animal studies were reviewed and approved by the IRBM Institutional Animal Care and Use Committee and the Italian Ministry of Health.

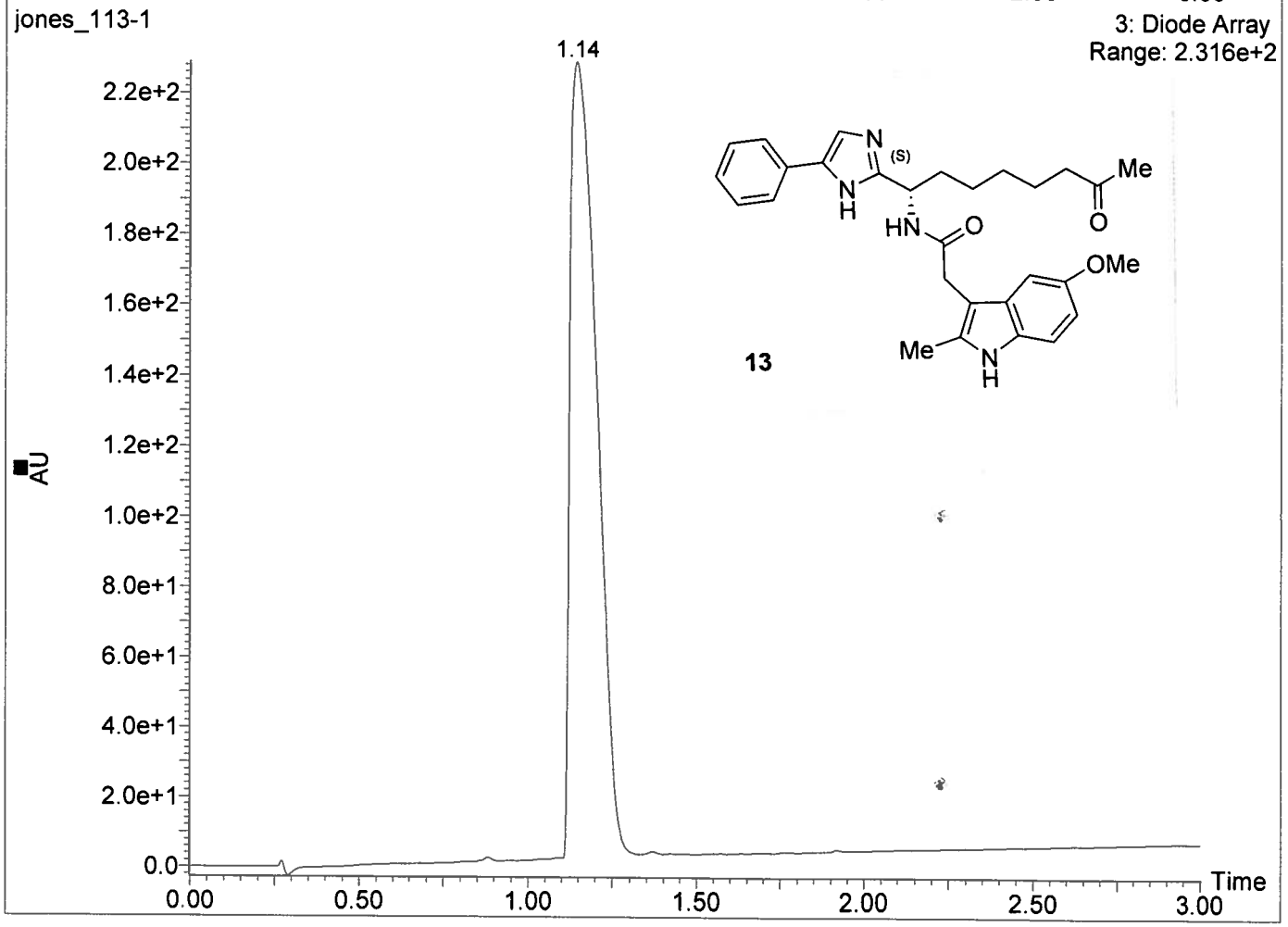
Treatment was initiated at ~2 weeks after transplantation, when tumors reached a weight of 100–200 mg. Mice bearing a tumor xenograft HCT116 were randomized into treated and control groups of 15 mice per group. Compounds were dissolved in vehicle and delivered intraperitoneal (i.p.) in a volume of 10 ml/kg body weight. Tumors were measured using a Vernier caliper, and tumor volume (V) was calculated using the equation $V = 1/2ab^2$, where a and b are the shortest and the longest diameter respectively (in mm). Drug efficacy was assessed at the end of treatment as the percentage of Tumor growth reduction in treated *versus* control mice


Cmpd11Mtd2
jones_114-2
3: Diode Array
Range: 7.375e+1
1.83
0.28
AU
6.0e+1
5.0e+1
4.0e+1
3.0e+1
2.0e+1
1.0e+1
0.0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
jones_113-2
3: Diode Array
Range: 9.898e+1
1.26
0.27
AU
9.0e+1
8.0e+1
7.0e+1
6.0e+1
5.0e+1
4.0e+1
3.0e+1
2.0e+1
1.0e+1
0.0
Time
0.00
0.50
1.00
1.50
2.00
2.50
3.00
S
N
O
N
H
(S)
HN
O
Me
O
N
11


meth2, HCl
scarpelr_0270153_0011_HCl-1
3: Diode Array
Range: 9.875e+1
AU
1.06
0.31
1.31


scarpelr_0270153_0011_HCl_2-1
3: Diode Array
Range: 1.698e+2
AU
1.58
0.34
0.31
0.74
1.99
Time
12


Cmpd13mtd2
jones_114-1
3: Diode Array
Range: 2.11e+2
1.65
0.28
AU
2.0e+2
1.8e+2
1.6e+2
1.4e+2
1.2e+2
1.0e+2
8.0e+1
6.0e+1
4.0e+1
2.0e+1
0.0
0.00
0.50
1.00
1.50
2.00
2.50
3.00

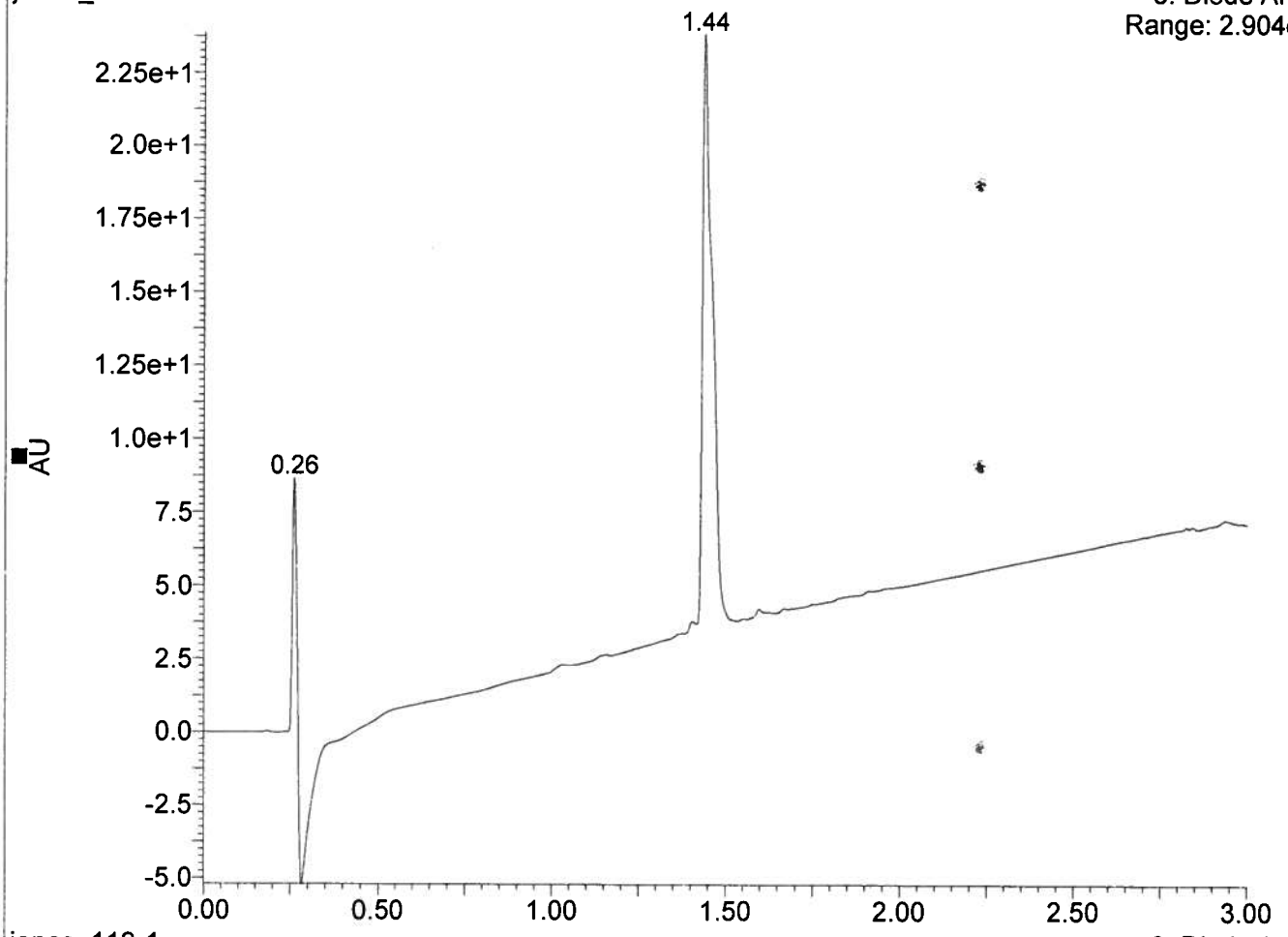

jones_113-1
3: Diode Array
Range: 2.316e+2
1.14
AU
2.2e+2
2.0e+2
1.8e+2
1.6e+2
1.4e+2
1.2e+2
1.0e+2
8.0e+1
6.0e+1
4.0e+1
2.0e+1
0.0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
Time
N
(S)
H
HN
O
Me
O
OMe
Me
N
H

13

cmpd14Mtd

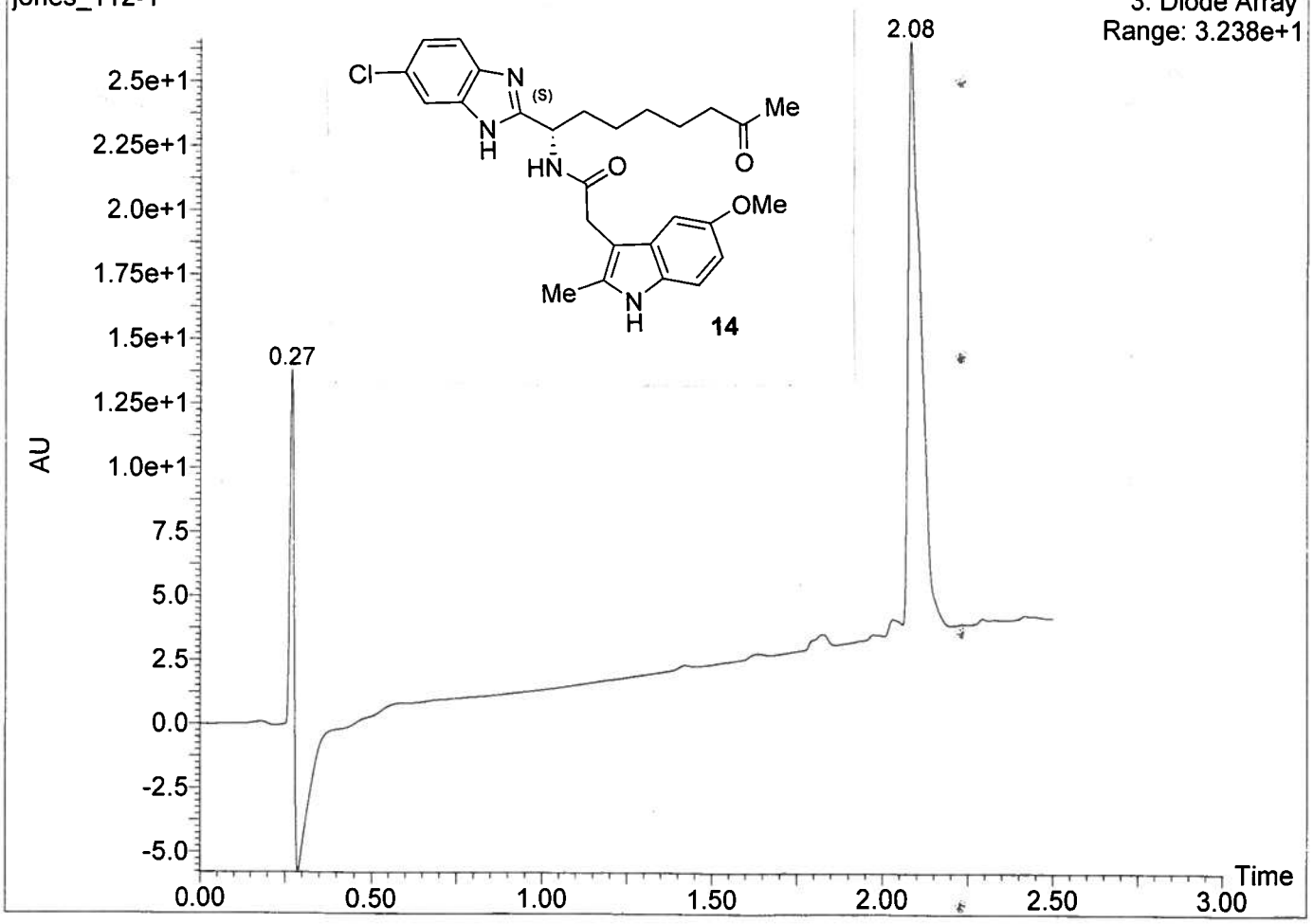
jones_110-1

3: Diode Array
Range: 2.904e+1

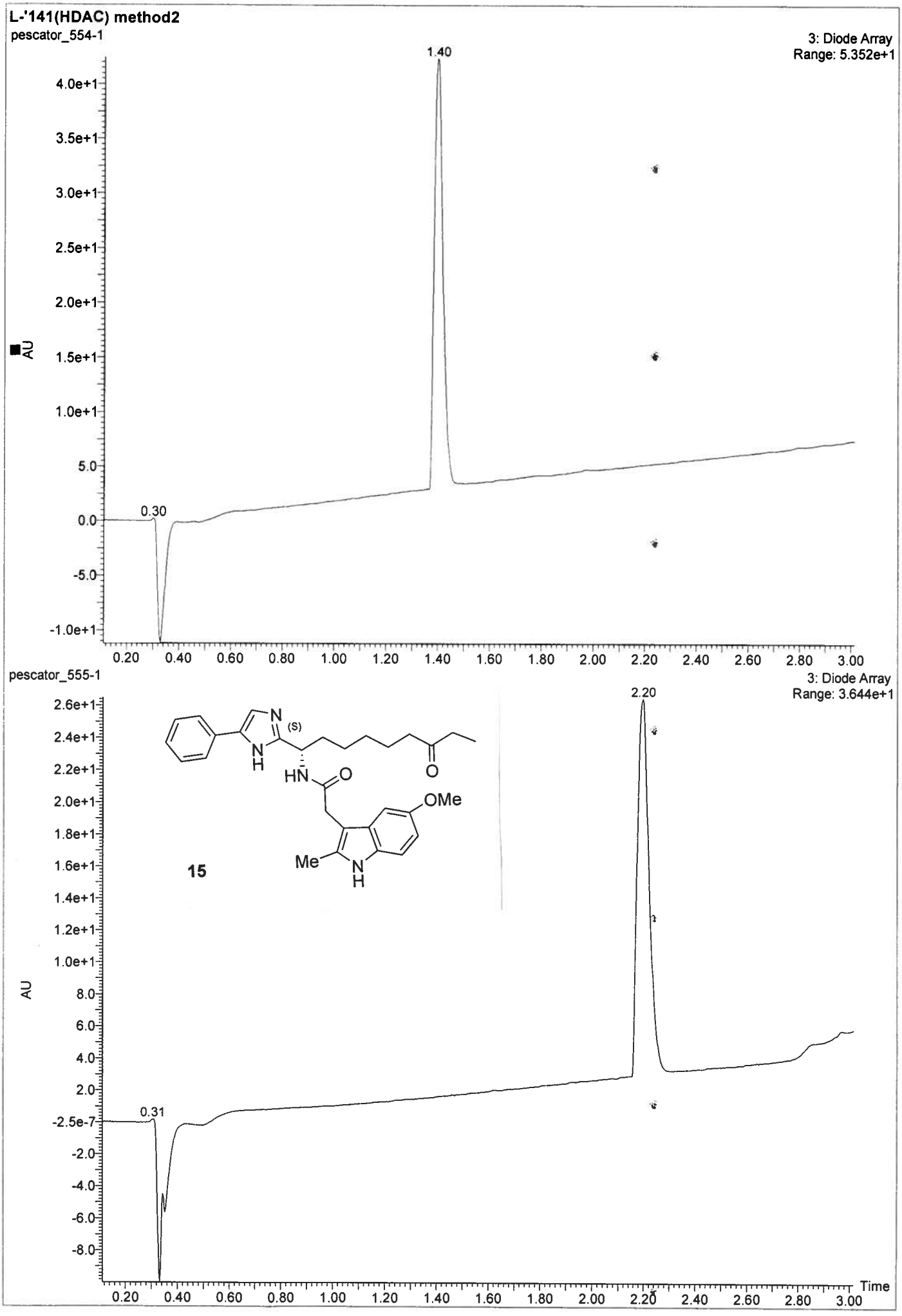


jones_112-1

3: Diode Array
Range: 3.238e+1



14


L-'141(HDAC) method2
pescator_554-1
3: Diode Array
Range: 5.352e+1
1.40
0.30
AU
4.0e+1
3.5e+1
3.0e+1
2.5e+1
2.0e+1
1.5e+1
1.0e+1
5.0
0.0
-5.0
-1.0e+1
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00
pescator_555-1
3: Diode Array
Range: 3.644e+1
2.20
0.31
AU
2.6e+1
2.4e+1
2.2e+1
2.0e+1
1.8e+1
1.6e+1
1.4e+1
1.2e+1
1.0e+1
8.0
6.0
4.0
2.0
-2.5e-7
-2.0
-4.0
-6.0
-8.0
0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40 2.60 2.80 3.00
Time
(S)
HN
O
O
OMe
Me
N
H
15